

PND17 BMDs and BMDLs and Recovery Half-Lives for the Effect of Carbofuran on Brain and Blood AChE

December 12, 2008

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1 Extract Dose-Response Data

```
> library(gdata)
> dta <- read.xls("carbofuran pnd17 che.xls")

Converting xls file to csv file... Done.
Reading csv file... Done.

> dta2 <- vector("list", 2)
> names(dta2) <- c("RBC", "brain")
> dta2[[1]] <- read.xls("carbofuran PND17 tc.xls", sheet = 1)

Converting xls file to csv file... Done.
Reading csv file... Done.

> dta2[[2]] <- read.xls("carbofuran PND17 tc.xls", sheet = 2)

Converting xls file to csv file... Done.
Reading csv file... Done.
```

```
> names(dta2[[1]]) <- names(dta2[[2]]) <- c("id", "dose", "age",
+     "time", "ache", "pct.control")
```

Rename the variables to "id", "sex", "dose", "age", "brain", and "blood", and drop sex and age, since they are all the same (17 day old males).

```
> names(dta) <- c("id", "sex", "dose", "age", "brain", "blood")
> dta <- subset(dta, select = c("id", "dose", "brain", "blood"))
> dta$time <- rep(40/60, nrow(dta))
> dta$type <- factor(rep("doseresponse", nrow(dta)), levels = c("doseresponse",
+     "timecourse"))
```

Merge the RBC and brain timecourse datasets, and rename the ache variables.

```
> dta2 <- merge(subset(dta2[["RBC"]], select = c("id", "dose",
+     "time", "ache")), subset(dta2[["brain"]], select = c("id",
+     "dose", "time", "ache")), by = c("id", "dose", "time"))
> names(dta2) <- c("id", "dose", "time", "blood", "brain")
> dta2$type <- factor(rep("timecourse", nrow(dta2)), levels = c("doseresponse",
+     "timecourse"))
> dta2$time <- dta2$time/60
```

Finally, combine dta and dta2:

```
> dta <- rbind(dta[, c("id", "dose", "time", "type", "blood", "brain")],
+     dta2[, c("id", "dose", "time", "type", "blood", "brain")])
> save(dta, file = "MoserData.RData")
```

Summarize the data set:

```
> library(lattice)
> summary(dta)
```

	id	dose	time	type
Min.	:611801	Min. :0.00	Min. : 0.2500	doseresponse:50
1st Qu.	:612205	1st Qu.:0.00	1st Qu.: 0.6667	timecourse :46
Median	:637503	Median :0.45	Median : 0.6667	
Mean	:625661	Mean :0.50	Mean : 3.8863	
3rd Qu.	:638001	3rd Qu.:1.00	3rd Qu.: 1.5000	
Max.	:638505	Max. :1.00	Max. :24.0000	
	blood	brain		
Min.	:0.0630	Min. :0.926		
1st Qu.	:0.1857	1st Qu.:2.201		
Median	:0.3120	Median :3.127		
Mean	:0.4617	Mean :3.487		
3rd Qu.	:0.7770	3rd Qu.:4.992		
Max.	:1.1730	Max. :5.855		

2 Dose and Time-Response Modeling

2.1 strategy

Use the model with simple exponential recovery (`tcmfn4()`), for consistency with other modeling efforts. We have one dose-response data set at 40 minutes, and one time course dataset at 1 mg/kg.

Fitting the model will follow these steps for each endpoint:

1. Check the extent to which the controls from the dose-response and timecourse datasets can be combined, and whether there was drift over time in the timecourse data.
2. 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.
3. Next, fit `tcmfn4()` using the parameterizations determined in the previous step, using a power variance model.

2.2 Brain

2.2.1 Controls

Are the controls in the timecourse study (at 45, 180, and 1440 minutes) homogeneous?

```
> tdata <- subset(dta, type == "timecourse" & dose == 0)
> anova(lm(brain ~ factor(time), data = tdata))
```

Analysis of Variance Table

```
Response: brain
          Df  Sum Sq Mean Sq F value Pr(>F)
factor(time)  2 0.34251 0.17126  1.8608 0.1897
Residuals    15 1.38052 0.09203
```

Are the timecourse and doseresponse controls homogeneous?

```
> tdata <- subset(dta, dose == 0)
> anova(lm(brain ~ factor(type), data = tdata))
```

Analysis of Variance Table

```
Response: brain
          Df  Sum Sq Mean Sq F value Pr(>F)
factor(type)  1 0.02604 0.02604  0.2228 0.6409
Residuals    26 3.03923 0.11689
```

Yes! So, we can use a single value for control, regardless of time or study.

2.2.2 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` and `time` to 15/60, for consistency with other analyses, and `lTr` to `log(1.5)` (it could really be anything).

```
> library(DRUtils)
> formals(tcmfn4)$delta <- 15/60
> initfile <- paste("initvals-brain-DR-1.RData", sep = "")
> if (!file.exists(initfile)) {
+   lA.start <- with(subset(dta, dose %in% 0 & !is.na(brain)),
+     mean(log(dta$brain)))
+   Start <- c(lA.start, log(0.1), -2, 0, log(2))
+   init1 <- GetInitialValues(brain ~ tcmfn4(dose, time, lA = lA,
+     tz = tz, lD = lD, lg = lg, lTr = lTr), data = subset(dta,
+     !is.na(brain)), params = list(lA ~ 1, lD ~ 1, tz ~ 1,
+     lg ~ 1, lTr ~ 1), start = Start, weights = varPower(value = 1))
+   save(init1, file = initfile)
+ } else load(initfile)
```

```

> tmp <- t(init1$Redundancy[[1]]$Eigens)
> tmp <- tmp[-grep("^1A", rownames(tmp)), ]
> round(tmp, digits = 2)

      [,1] [,2] [,3] [,4] [,5]
CondIndex 38.76 6.12 2.43 2.02 1.00
mu         0.05 0.30 0.77 0.92 1.86
1D         0.96 0.04 0.00 0.00 0.00
tz         0.88 0.12 0.00 0.00 0.00
lg         1.00 0.00 0.00 0.00 0.00
1Tr        0.01 0.13 0.09 0.75 0.02

```

2.2.3 Modeling

The above shows the results of the redundancy analysis for this model. There is a strong interrelationship among the three dose-response parameters, but it may not be too strong to get estimates. Try fitting this model, first.

```

> Start <- init1$start$beta
> idta <- subset(dta, !is.na(brain))
> drmod1 <- gnls(brain ~ tcmlfn4(dose, time, 1A = 1A, tz = tz, 1D = 1D,
+   lg = lg, 1Tr = 1Tr), data = idta, params = list(1A ~ 1, 1D ~
+   1, tz ~ 1, lg ~ 1, 1Tr ~ 1), start = Start, weights = varPower(value = 1))
> summary(drmod1)$tTable

      Value Std.Error   t-value   p-value
1A  1.6463106 0.01217487 135.2220025 1.084565e-106
1D -4.0187279 0.48606463 -8.2678879 1.065078e-12
tz -0.5401611 0.18837037 -2.8675483 5.140132e-03
lg -0.2190443 0.22517017 -0.9727944 3.332342e-01
1Tr 1.2402314 0.19670121  6.3051539 1.016677e-08

```

This model, drmod1, gives benchmark dose. The following table gives the 90% confidence intervals for the BMD:

```

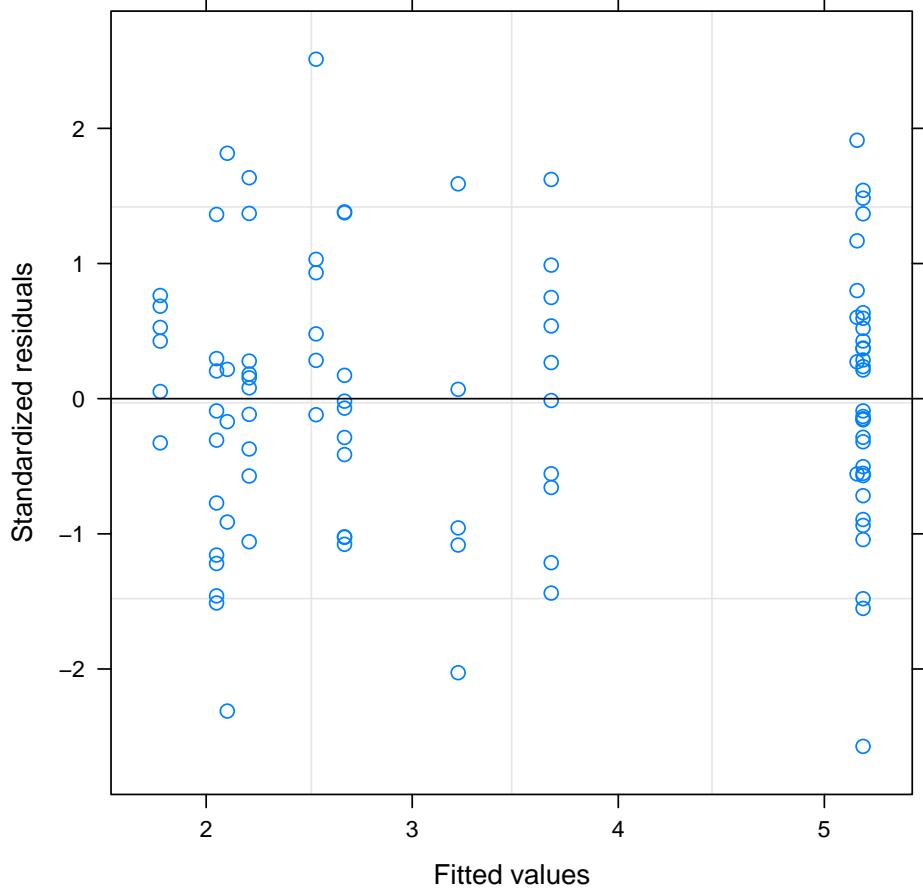
> x.BMD <- exp(intervals(drmod1, level = 0.9, which = "coef")[[1]][["1D",
+   ]])
> print(x.BMD)

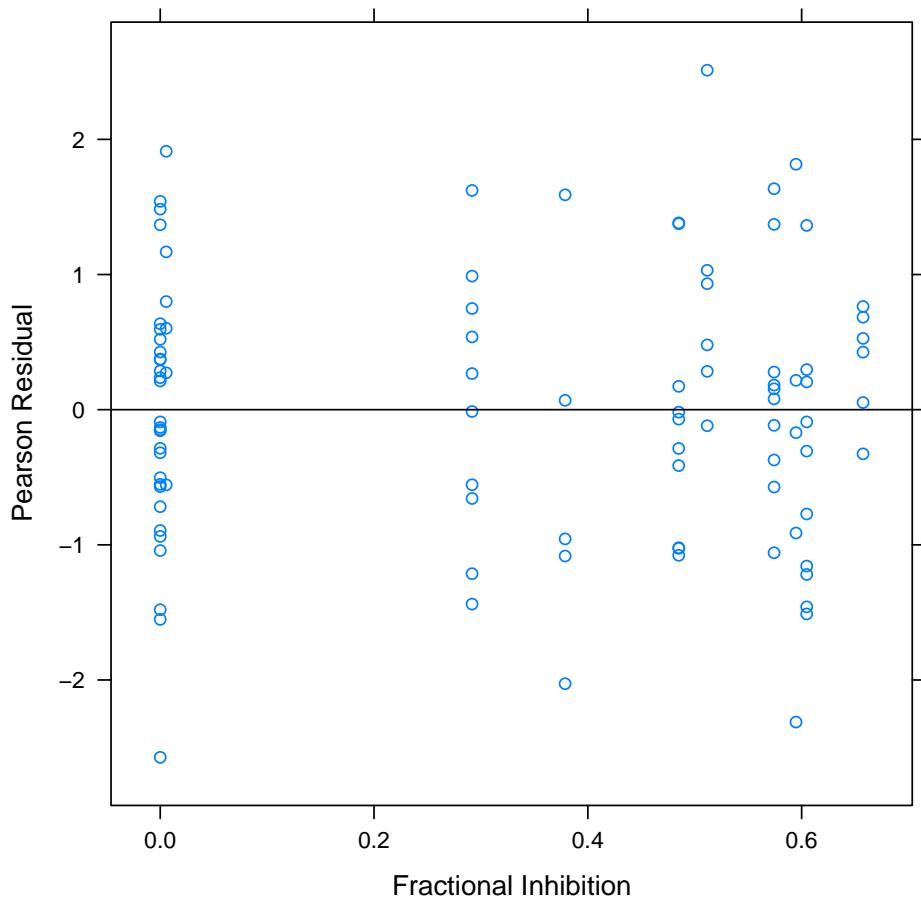
      lower       est.       upper
0.008014875 0.017975818 0.040316290

```

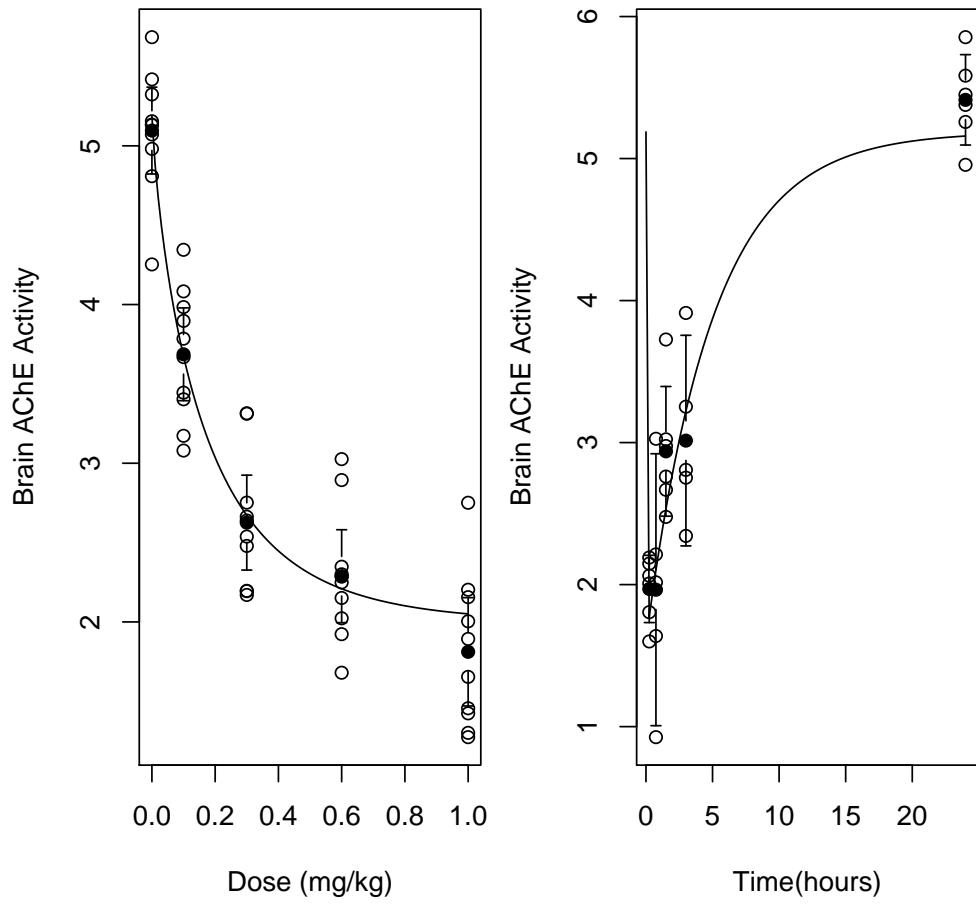
2.2.4 Goodness of Fit

How well does this model describe the data? Here are a couple of plots of residuals, the first Pearson residuals versus fitted value, and the second Pearson residual versus fraction of inhibition:





Finally, plot data and dose-response in the conventional form:



The model seems to describe the data well.

2.2.5 Results for Brain

Here is the directly calculated BMDs for the pnd17 brains, with 90% confidence intervals (so calculated so that the lower limit is a *one-sided* 95% limit for the BMD).

```
> x.BMD
```

	lower	est.	upper
	0.008014875	0.017975818	0.040316290

2.3 Blood (RBC)

This analysis very much parallels the analysis of brain AChE.

2.3.1 Controls

Are the controls in the timecourse study (at 45, 180, and 1440 minutes) homogeneous?

```
> tdata <- subset(dta, type == "timecourse" & dose == 0)
> anova(lm(blood ~ factor(time), data = tdata))
```

Analysis of Variance Table

```
Response: blood
          Df  Sum Sq Mean Sq F value Pr(>F)
factor(time)  2 0.041385 0.020692  3.6154 0.0523 .
Residuals    15 0.085850 0.005723
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Are the timecourse and doseresponse controls homogeneous?

```
> tdata <- subset(dta, dose == 0)
> anova(lm(blood ~ factor(type), data = tdata))
```

Analysis of Variance Table

```
Response: blood
          Df  Sum Sq Mean Sq F value Pr(>F)
factor(type)  1 0.016641 0.016641  1.3769 0.2513
Residuals    26 0.314228 0.012086
```

Yes! So, we can use a single value for control, regardless of time or study.

2.3.2 Initial Values

```
> formals(tcmfn4)$delta <- 15/60
> initfile <- paste("initvals-brain-DR-2.RData", sep = "")
> if (!file.exists(initfile)) {
+   lA.start <- with(subset(dta, dose %in% 0 & !is.na(blood)),
+     mean(log(dta$blood)))
+   Start <- c(lA.start, log(0.1), -2, 0, log(5))
+   init2 <- GetInitialValues(blood ~ tcmfn4(dose, time, lA = lA,
+     tz = tz, lD = lD, lg = lg, lTr = lTr), data = subset(dta,
+       !is.na(brain)), params = list(lA ~ 1, lD ~ 1, tz ~ 1,
+       lg ~ 1, lTr ~ 1), start = Start, weights = varPower(value = 1))
+   save(init2, file = initfile)
+ } else load(initfile)
> tmp <- t(init2$Redundancy[[1]]$Eigens)
> tmp <- tmp[-grep("^lA", rownames(tmp)), ]
> round(tmp, digits = 2)

 [,1] [,2] [,3] [,4] [,5]
CondIndex 15.36 2.48 2.14 1.55 1.00
mu        0.11 0.67 0.78 1.07 1.67
lD        0.99 0.01 0.00 0.00 0.00
tz        0.49 0.31 0.17 0.00 0.02
lg        0.99 0.00 0.00 0.00 0.00
lTr       0.00 0.41 0.13 0.45 0.01
```

2.3.3 Modeling

The above shows the results of the redundancy analysis for this model. Redundancy is not particularly strong among the dose-response parameters, so fit this model.

```
> Start <- init2$start$beta
> idta <- subset(dta, !is.na(blood))
```

```

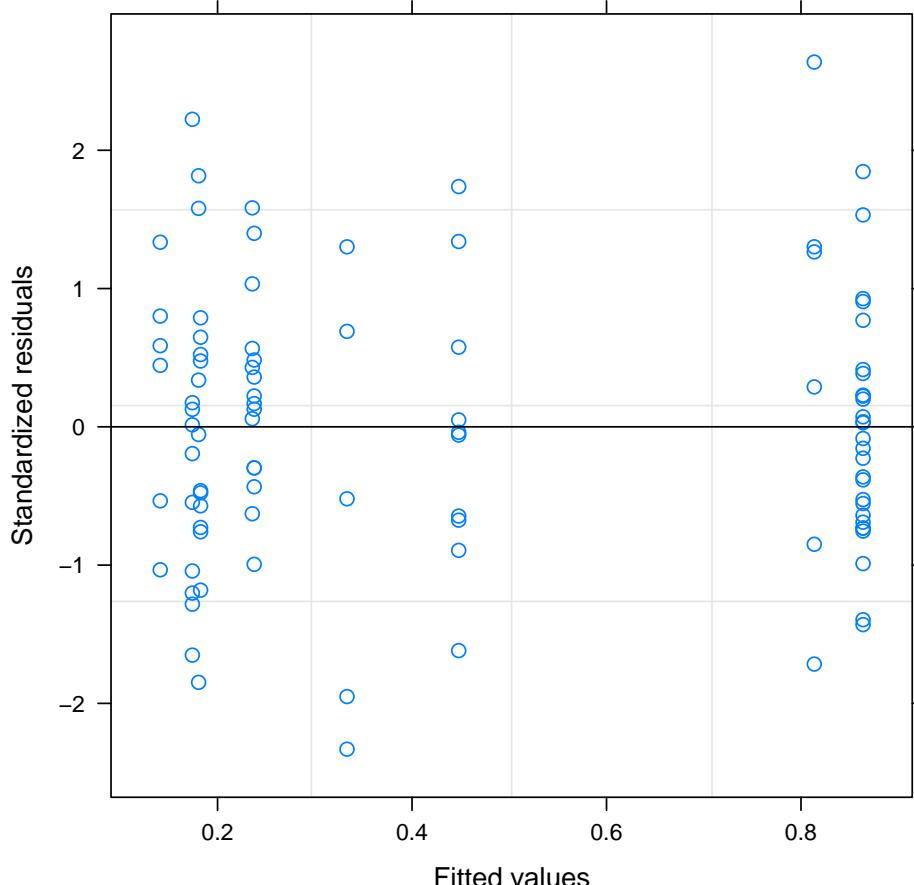
> drmod2 <- gnls(blood ~ tcfn4(dose, time, lA = lA, tz = tz, lD = lD,
+     lg = lg, lTr = lTr), data = idta, params = list(lA ~ 1, lD ~
+     1, tz ~ 1, lg ~ 1, lTr ~ 1), start = Start, weights = varPower(value = 1))
> summary(drmod2)$tTable
      Value Std.Error     t-value     p-value
lA -0.1463860 0.0294142 -4.9767133 3.044293e-06
lD -4.6055174 0.5961122 -7.7259244 1.409344e-11
tz -1.5147000 0.1384578 -10.9397991 2.832321e-18
lg -0.1505106 0.2143463 -0.7021842 4.843565e-01
lTr 1.8197984 0.2170317  8.3849444 6.078939e-13

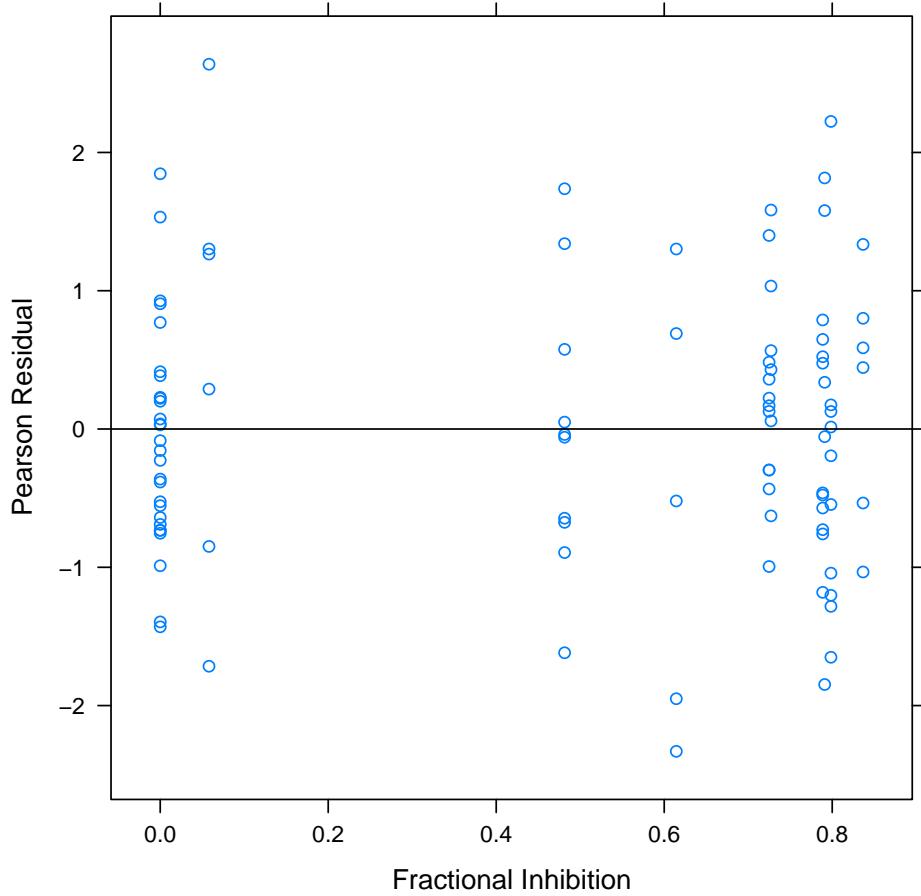
The following table gives the 90% confidence intervals for the BMD:
> xb.BMD <- exp(intervals(drmod2, level = 0.9, which = "coef")[[1]]["lD",
+   ])
> print(xb.BMD)
  lower       est.       upper
0.003712242 0.009996528 0.026919202

```

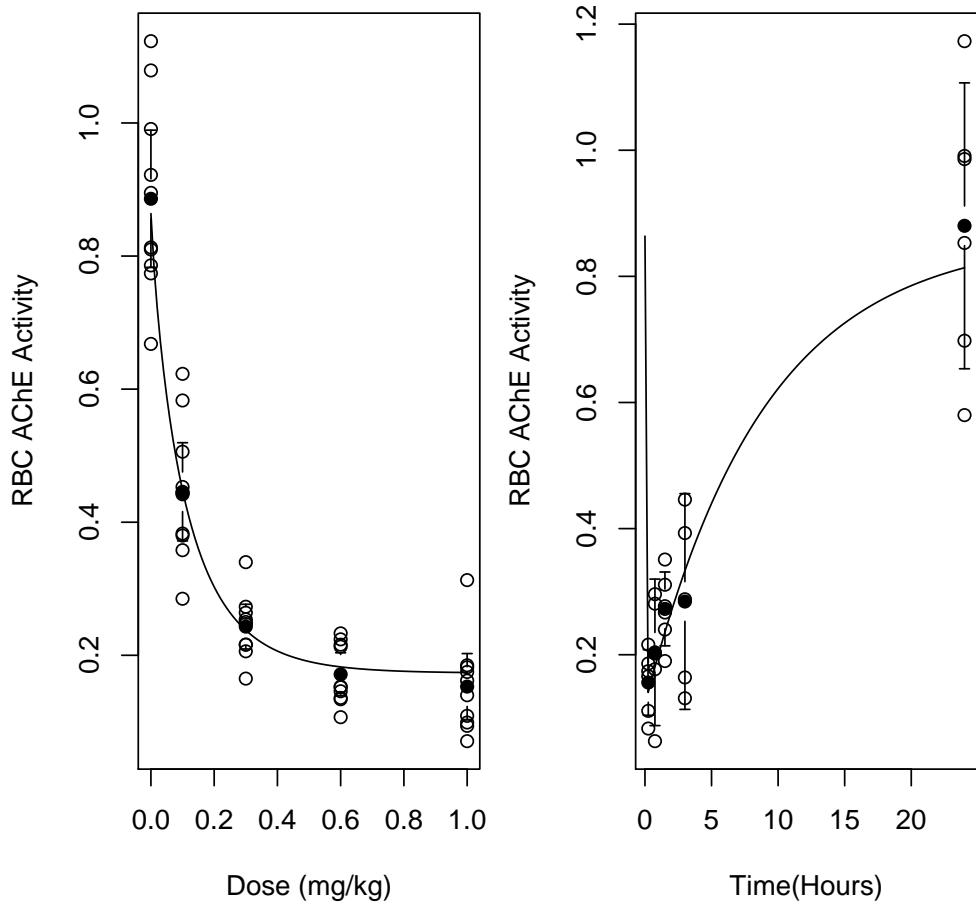
2.3.4 Goodness of Fit

How well does this model describe the data? Here are a couple of plots of residuals, the first Pearson residuals versus fitted value, and the second Pearson residual versus fraction of inhibition:





Finally, plot data and dose-response in the conventional form:



The model seems to describe the data well.

2.3.5 Results for Blood

Here are confidence intervals for the pnd17 BMD:

```
> exp(intervals(drmmod2, which = "coef")$coef["1D", ])
```

lower	est.	upper
0.003059141	0.009996528	0.032666217

3 Summary

BMD and 95% confidence intervals for 10% inhibition in brain:

```
> exp(intervals(drmmod1, which = "coef")$coef["1D", ])
```

lower	est.	upper
0.006845009	0.017975818	0.047206665

and blood:

```
> exp(intervals(drmmod2, which = "coef")$coef["1D", ])
```

```
lower      est.      upper
0.003059141 0.009996528 0.032666217
```

BMDLs (lower end of 90% confidence intervals) for brain:

```
> ints <- exp(intervals(drmmod1, which = "coef", level = 0.9)$coef["lD",
+   ])
> ints["lower"]
```

```
lower
0.008014875
```

and blood:

```
> ints <- exp(intervals(drmmod2, which = "coef", level = 0.9)$coef["lD",
+   ])
> ints["lower"]
```

```
lower
0.003712242
```

Half-lives (in Hours) and 95% CI, brain:

```
> exp(intervals(drmmod1, which = "coef")$coef["lTr", ])
lower      est.      upper
2.338497 3.456413 5.108747
```

and blood:

```
> exp(intervals(drmmod2, which = "coef")$coef["lTr", ])
lower      est.      upper
4.009601 6.170614 9.496327
```

Save everything:

```
> save.image(file = "MoserPND17Analysis.RData")
```