UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

TXR No.: 0054603
May 17, 2007

MEMORANDUM

SUBJECT: Review of Azinphos Methyl Repeated Dosing Human Volunteer Study
DP Barcode D339869, PC Code 058001

FROM: James Nguyen, Mathematical Statistician
Chemistry and Exposure Branch
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THRU: David J. Miller, Chief
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Health Effects Division (7509P)

TO: Ray Kent, Chief
Reregistration Action Branch 4
Health Effects Division (7509P)

Chemistry and Exposure Branch (CEB) was requested to provide statistical support to the HAZPOC in performing an analysis of a human cholinesterase (ChE) inhibition conducted by Inveresk Research Center. Specifically, CEB was requested to review and comment on the following study with respect to determining if statistically significant decreases in ChE (plasma and blood) were observed:

McFarlane, P. J., and S. Freestone (1999). A Randomised Double Blind Placebo Controlled Study with Azinphos-Methyl to Determine the No-Effect Level on Plasma and RBC ChE Activity after Repeat Doses. Inveresk Research, Elphinstone Research Center, Tranent, EH33 2NE, Scotland, ICR 013580, 634 p.p.. April 15, 1999, MRID 45276101.

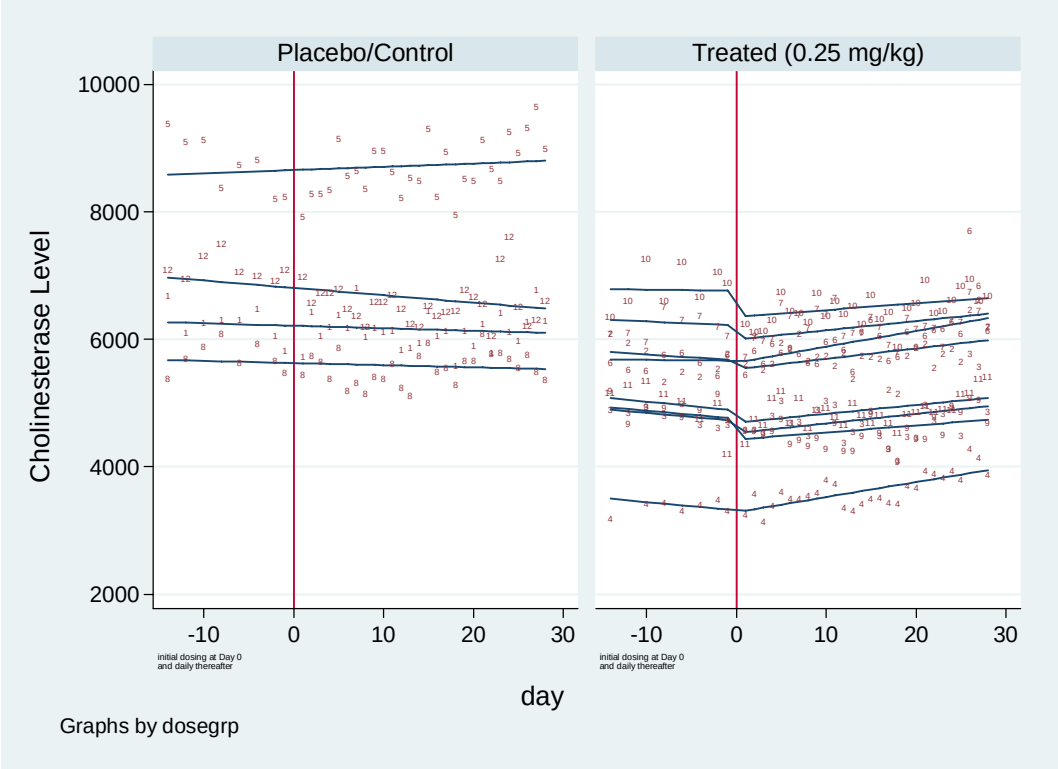
Background:

The above-identified study, conducted in 1999, was a 28-day repeated dose study. The study used a total of 12 human volunteer subjects (8 active and 4 placebo) each of whom were dosed with either 0 (control/placebo) or 0.25 mg/kg azinphos methyl commencing on the fourteenth day of the study. A total of 8 baseline (Days -14 to -1, at roughly every other day intervals) and 28 post-dosing (Days 1 to 28, collected each day during the post-dosing period) ChE measurements were taken for each subject, and the data submitted to EPA by the registrant in electronic (MS Excel) format for analysis. In general, ChE measurements during the post-dosing days were made either 4 hours following dosing (for which data are available during 12 of the 28 post dosing days) or *both* 4 hours following dosing and immediately prior to administering the next dose. Data for this latter procedure are available for all 28 of the 28 post dosing days). The statistical analyses conducted here used only the 28 post-dose ChE measurements data. Since these measurements are made immediately prior to dosing, they would be expected to be the measured time points which show maximum ChE levels (i.e., minimal depression).

Statistical analyses were conducted by fitting a series of mixed-effects models to the time series of ChE data. The modeling process was designed to provide information to determine 1) whether there was a significant decrease in ChE levels following the first dose (on Day 1) in the treated group; and 2) whether ChE levels decreased, remained the same, or increased following the first dose through Day 28 of the post dosing interval. A total of 8 models were fit (designated Models A through H) using a hierarchical approach. The models incorporated both fixed and random effects. Deviance measures and an associated Chi-Square test were used to evaluate quality of fit and determine the most appropriate model. Details regarding the models used in the statistical evaluation of plasma ChE levels are provided in Attachment 1. Similar details for RBC ChE are provided in Attachment 2. The SAS code and associated output is also provided: Attachment 3 provides this code for analysis of the plasma data while Attachment 4 provides this same information (i.e, code and output) for the RBC analysis.

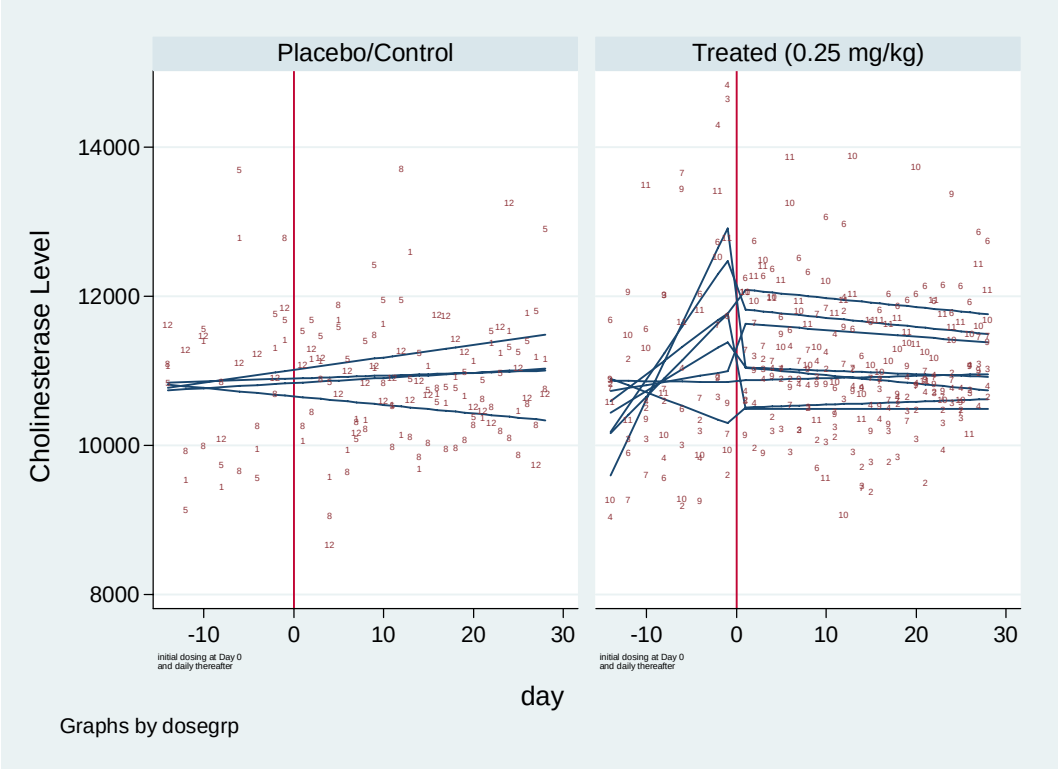
CONCLUSIONS:

For *plasma ChE*, Model F was selected as the most appropriate model, demonstrating a (i) statistically significant 3.9% decrease (p=0.0206) in plasma ChE levels as a result of initial (Day 0) dosing and (ii) a statistically significant recovery rate of 21.8 ChE IU/mL/day (p=0.0022). The time course of ChE levels and the individual ChE measurements are shown in the following graph for both the Placebo/Control group (left panel) and the Treated group (right panel):



As can be seen, the 0.25 mg/kg dose group generally displays a sharp drop in ChE concentrations between Days -1 and Day +1 (initial dosing occurred at Day 0) and an associated subsequent gradual increase in plasma ChE levels which generally recovers to near baseline values between Days 10 and 20.

For ***RBC ChE***, Model F was selected as the most appropriate model, but demonstrated no statistically significant decrease (p=0.5207) in RBC ChE levels as a result of initial (Day 0) and subsequent dosing. The time course of ChE levels for RBC is shown in the following graph for both the Placebo/Control group (left panel) and the Treated group (right panel):



Although the Treated group does appear in the right hand panel above to display sharp changes/decreases in ChE levels, these changes are not statistically significant given the wide scatter in the data. Thus, no statistically significant change in RBC ChE is apparent following repeated dosing of 0.25 mg/kg.

CEB is currently developing SAS code for a power simulation using the mixed effects model developed here. Preliminary analyses suggest a power of substantially greater than 0.9 to detect a 10% decrease in RBC ChE.

ATTACHMENT 1

Plasma_ChE

Model Selection:

Model	Fixed Effects	Variance Component + σ_ε^2	n parameters fixed	n parameters VC	Devian	Δ Deviance (df) & Comparison Model
A	Intercept, Time	Intercept, Time	2	4	6234.4	-N/A-
B	Intercept, Time, PostDose	Intercept, Time, PostDose	3	7	6212.9	A: 21.5 ** (4)
C	Intercept, Time, PostDose	Intercept, Time	3	4	6213.7	B: 0.8 (3)
D	Intercept, Time, PostDoseTime	Intercept, Time, PostDoseTime	3	7	6206.3	A: 28.1 ** (4)
E	Intercept, Time, PostDoseTime	Intercept, Time	3	4	6211.3	D: 5.0 (3)
F	Intercept, Time, PostDose, PostDoseTime	Intercept, Time, PostDose, PostDoseTime	4	11	6194.3	B: 18.6 ** (5) D: 12.0 * (5)
G	Intercept, Time, PostDose, PostDoseTime	Intercept, Time, PostDose	4	7	6197.8	F: 3.5 (4)
H	Intercept, Time, PostDose, PostDoseTime	Intercept, Time, PostDoseTime	4	7	6197.6	F: 3.3 (4)

Comments:

- Model F is chosen to perform the final analysis of plasma ChE data.

* significant at p= 0.05
** significant at p= 0.01

Terminology used in this analysis:

- Pre-dosing period: from day -14 to day 0. During this period, neither group was exposed to the chemical.

- Post-dosing period: from day 0 to day 28. During this period, subjects in treated group were exposed to the chemical every day.

Characteristics of model F:

- This mixed linear model has four advantages for analyzing repeated measures in human data:

    - The model allows study participants to have different baseline values of plasma-ChE (different intercepts or different baselines). In other words, on the first day of the pre-dosing period, each study volunteer may have a different level of plasma-ChE.

    - The model allows for all study participants to have their own unique slope during the non-dosed time period (i.e., subjects belonging to placebo group, and treatment group subjects in the pre-dosing period). For example, each study participant may have a different plasma-ChE trend during the specified period.

    - Many statistical models assume that there is the same (absolute) drop in plasma-ChE for every subject immediately following the first dosing period. However, this is a simplifying assumption used by some models and may not be appropriate to account for putative between-person differences. Model F is not limited by that assumption; specifically, Model F allows for the more reasonable assumption that different subjects can have different decreases (or increases) in plasma-ChE levels after the first dosing.

    - The model also allows for differences in change of plasma-ChE levels during the post-dosing period. For example, a subject who experiences a drop in plasma ChE after the first day of dosing might subsequently: (1) recover to the normal plasma-ChE levels, (2) maintain the depressed level of plasma-ChE, or (3) experience a continued decrease in plasma-ChE.

- In sum, model F can accommodate human study data in which different subjects may have different baseline values, have different initial (Day 0) responses to the chemical at the first dosing period, and have varying degrees or rates of recovery during the post-dosing period.

Model F:

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5774.65	372.43	11	15.51	<.0001
Time	-7.0979	3.6001	11	-1.97	0.0743
PostDose	-224.98	75.5453	7	-2.98	0.0206
PostDoseTime	21.7944	4.6416	7	4.70	0.0022

where "Intercept" is the average baseline of both groups; "Time" is the time during the study, which is valued from -14 to +28; PostDose is the average change (drop) in plasma-ChE of the treated group between day -1 and day +1; and, finally, "PostDoseTime" is a variable that cumulates time from the first day exposure to the chemical, where the coefficient represents the rate of recovery in plasma-ChE levels over time.

Explanation of results summarized in the table above:

- The slope of the plasma-ChE for the **placebo** group is *not significant* (Coefficient of Time is not significant from zero).
- The slope of the plasma-ChE for the **treated** group is *not significant* prior to dosing (Coefficient of Time is not significantly different from zero).
- There is a *significant* decrease (PostDose coefficient= -224.98; p= 0.0206) in plasma-ChE for the treated group when subjects are dosed with the chemical.
- After exposure/dosing to the chemical, the plasma-ChE for the treated group generally increases to normal levels over time during the post-dosing period (Coefficient of PostDoseTime = 21.7944, p-value = 0.002).

Conclusion:

- Based on the analysis of the study data, the plasma-ChE for the treated group has a slope not significantly different from zero prior to exposure to the chemical. After the first dosing, the plasma-ChE levels for the treated group decreases abruptly. However, the plasma-ChE returns to near normal (baseline) levels after approximately 10-20 days.

Comments on the Figures below:

- Figure 1 and Figure 2 show the plasma-ChE for all study participants (placebo and treated groups, respectively) for the duration of the study.
- Figure 3 shows the regression lines for each subjects based on the model F. Straight lines (in blue) represent for the regression lines for the 4 members of the placebo group, while the jarred lines (in red) represent regression lines for the 8 members of the treated group.

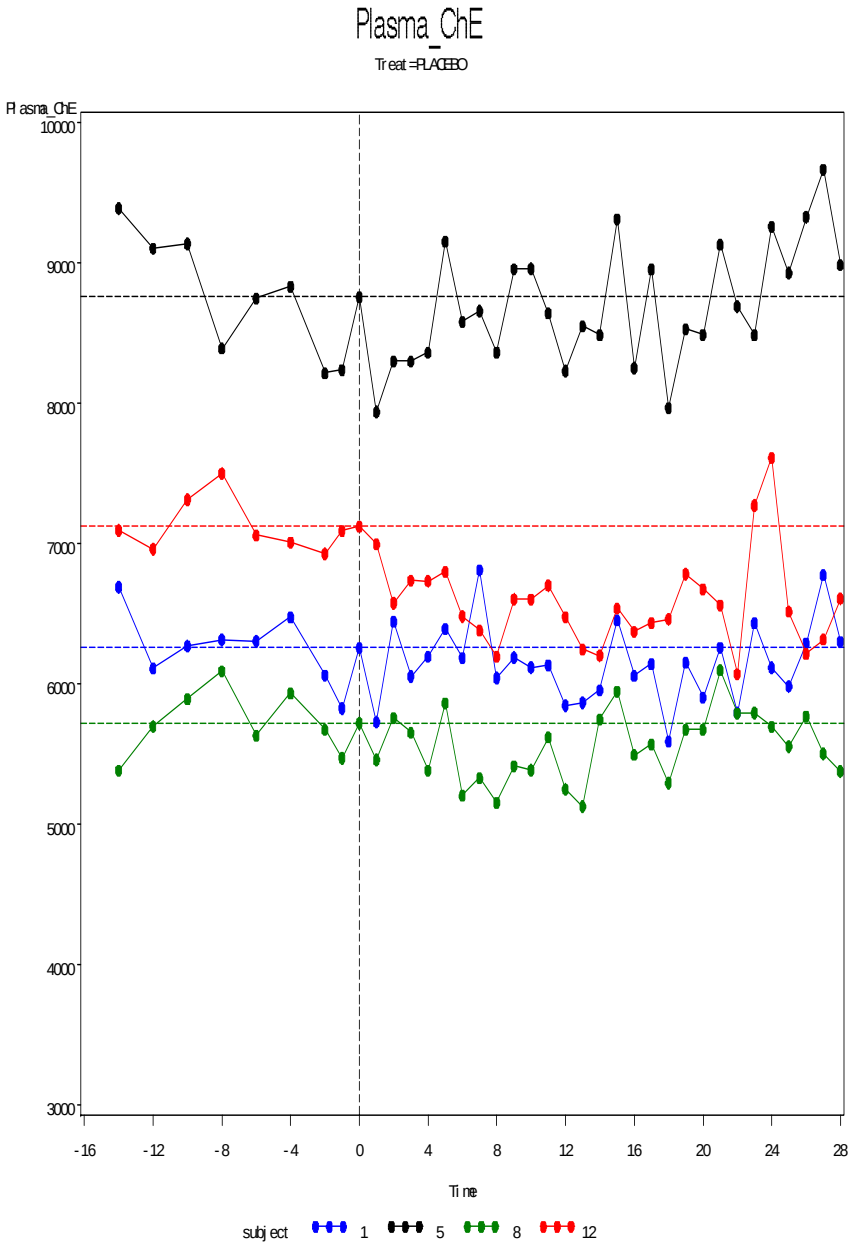


Figure 1

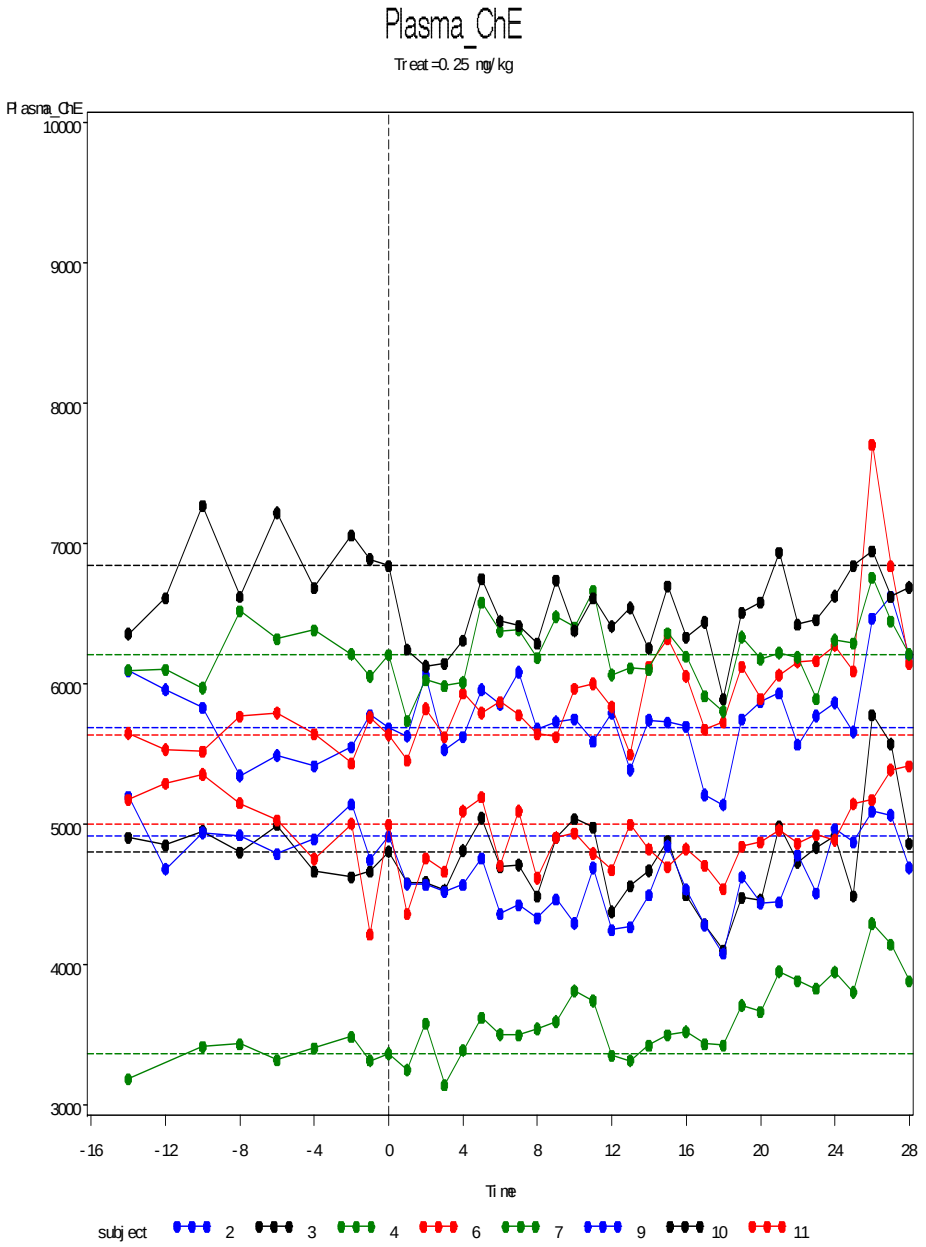
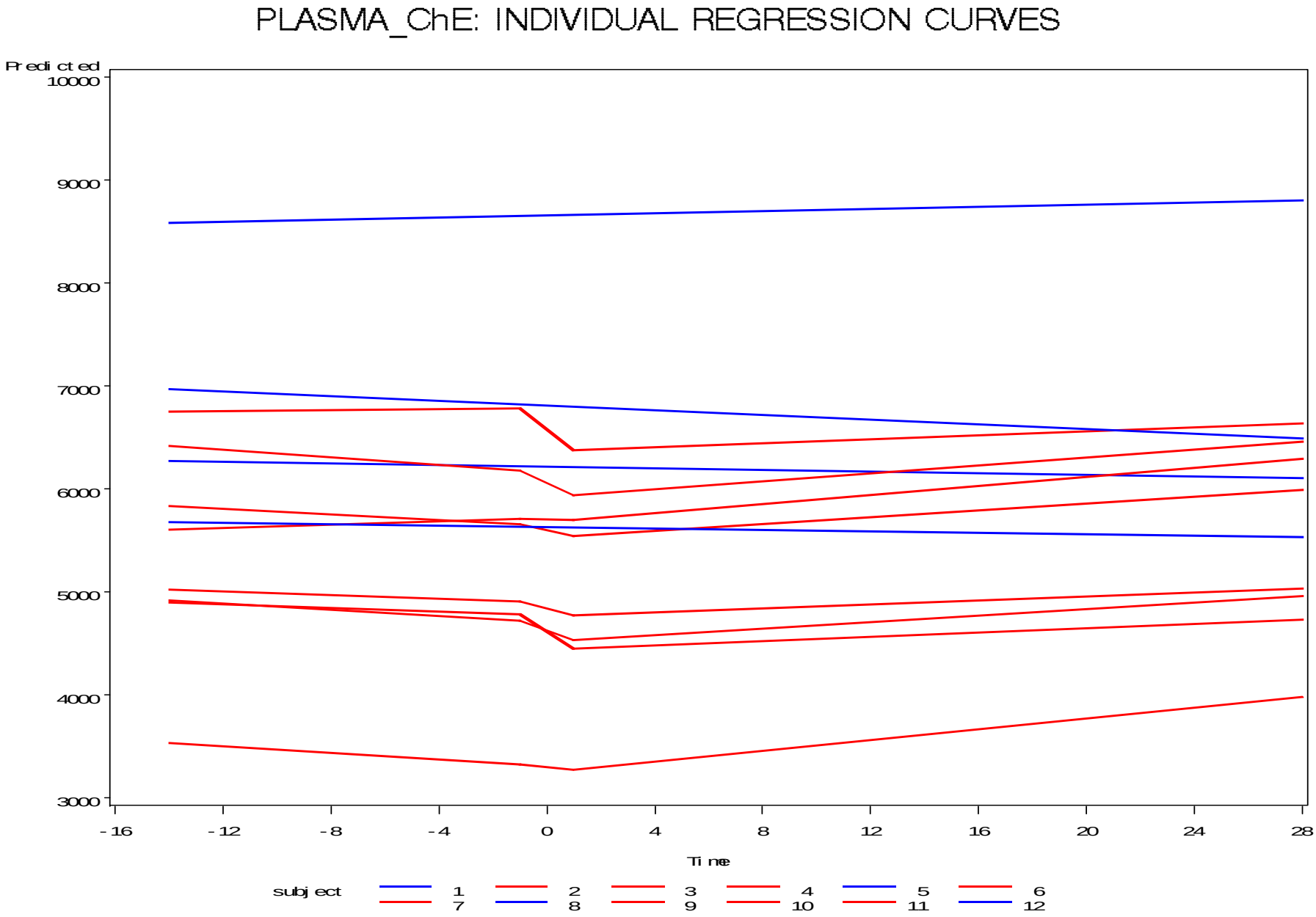


Figure 2


PLASMA_ChE: INDIVIDUAL REGRESSION CURVES
Predicted
10000
9000
8000
7000
6000
5000
4000
3000
-16
-12
-8
-4
0
4
8
12
16
20
24
28
Time
subject
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10
11
12

Figure 3

ATTACHMENT 2

RBC_ChE

Model Selection:

Model	Fixed Effects	Variance Component + σ_ε^2	n parameters fixed	n parameters VC	Devian	Δ Deviance (df)
A	Intercept, Time	Intercept, Time	2	4	7064.7	----------
B	Intercept, Time, PostDose	Intercept, Time, PostDose	3	7	7055.9	A: 8.8 (4)
C	Intercept, Time, PostDose	Intercept, Time	3	4	7064.7	B: 8.8 * (3)
D	Intercept, Time, PostDoseTime	Intercept, Time, PostDoseTime	3	7	7056.4	A: 8.3 (4)
E	Intercept, Time, PostDoseTime	Intercept, Time	3	4	7062.0	D: 5.6 (3)
F	Intercept, Time, PostDose, PostDoseTime	Intercept, Time, PostDose, PostDoseTime	4	11	7038.1	B: 17.8 ** (5) D: 18.3 ** (5) A: 26.6 ** (9)
G	Intercept, Time, PostDose, PostDoseTime	Intercept, Time, PostDose	4	7	7052.5	F: 14.4 ** (4)
H	Intercept, Time, PostDose, PostDoseTime	Intercept, Time, PostDoseTime	4	7	7055.0	F: 16.9 ** (4)

Comments:

- Model F is chosen to perform the final analysis of RBC ChE data.

* significant at p= 0.05

** significant at p= 0.01

Terminology used in this analysis:

- Pre-dosing period: from day -14 to day 0. During this period, neither group was exposed to the chemical.
- Post-dosing period: from day 0 to day 28. During this period, subjects in treated group were exposed to the chemical every day.

Characteristics of model F:

- This mixed linear model has four advantages for analyzing repeated measures in human study data:
    - The model allows study participants to have different baseline values of RBC-ChE (different intercepts or different baselines). In other words, on the first day of the pre-dosing period, each study volunteer may have a different level of RBC-ChE.
    - The model allows for all study participants to have their own unique slope during the non-dosed time period (i.e., subjects belonging to placebo group, and treatment group subjects in the pre-dosing period). For example, each study participant may have a different RBC-ChE trend during the specified period.
    - Many statistical models assume that there is the same (absolute) drop in RBC-ChE for every subject immediately following the first dosing period. However, this is a simplifying assumption used by some models and may not be appropriate to account for putative between-person differences. Model F is not limited by that assumption; specifically, Model F allows for the more reasonable assumption that different subjects can have different decreases (or increases) in RBC-ChE levels after the first dosing.
    - The model also allows for differences in change of RBC-ChE levels during the post-dosing period. For example, a subject who experiences a drop in RBC ChE after the first day of dosing might subsequently: (1) recover to the normal RBC-ChE levels, (2) maintain the depressed level of RBC-ChE, or (3) experience a continued decrease in RBC-ChE.
- In sum, model F can accommodate human study data in which different subjects may have different baseline values, have different initial (Day 0) responses to the chemical at the first dosing period, and have varying degrees or rates of recovery during the post-dosing period.

Model F is chosen:

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	11366	286.67	11	39.65	<.0001
Time	57.0893	29.3863	11	1.94	0.0781
PostDose	-220.78	326.53	7	-0.68	0.5207
PostDoseTime	-60.4456	31.0551	7	-1.95	0.0927

where "Intercept" is the average baseline of both groups; "Time" is the time during the study, which is valued from -14 to +28; PostDose is the average change (drop) in RBC-ChE of the treated group between day -1 and day +1; and, finally, "PostDoseTime" is a variable that cumulates time from the first day exposure to the chemical, where the coefficient represents the rate of recovery in RBC-ChE levels over time.

Explanation of results summarized in the table above:

- The slope of the RBC-ChE for the **placebo** group is *not significant* (Coefficient of Time is not significant from zero).
- The slope of the RBC-ChE for the **treated** group is *not significant* prior to dosing (Coefficient of Time is not significantly different from zero).
- There is a decrease but *not significant* (PostDose coefficient = -220.78; p= 0.5207) in RBC-ChE for the treated group when subjects are dosed with the chemical.
- After exposure/dosing to the chemical, the RBC-ChE for the treated group generally do not increase or decrease comparing to normal levels over time during the post-dosing period (Coefficient of PostDoseTime = -60.4456, p-value = 0.0927).

Conclusion:

- Based on the analysis of the study data, the RBC-ChE for the treated group has a slope not significantly different from zero prior to exposure to the chemical. After the first dosing, the RBC-ChE levels for the treated group do not decrease significantly and are at normal levels during the post-dosing period.

Comments on the Figures below:

- Figure 1 and Figure 2 show the RBC-ChE for all study participants (placebo and treated groups, respectively) for the duration of the study.
- Figure 3 shows the regression lines for each subjects based on the model F. Straight lines (in blue) represent for the regression lines for the 4 members of the placebo group, while the jarred lines (in red) represent regression lines for the 8 members of the treated group.

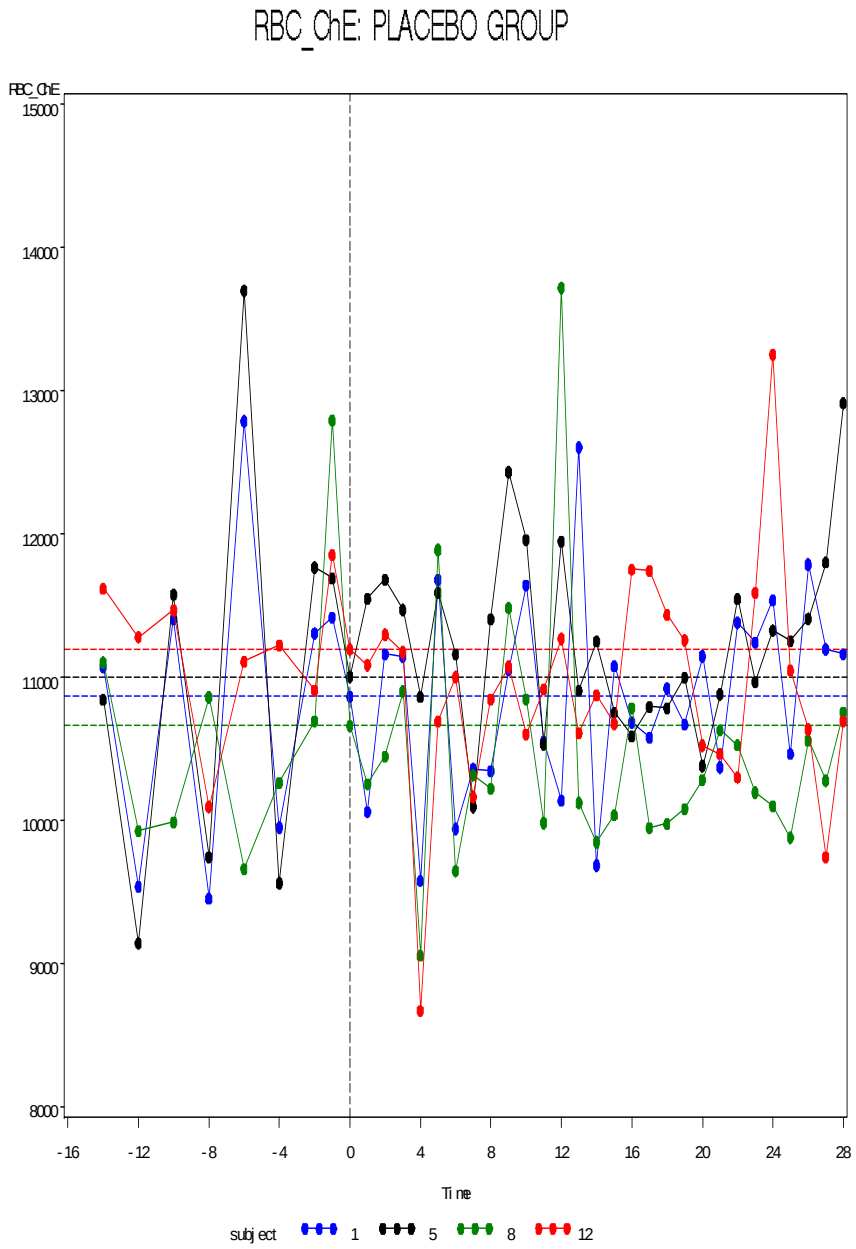

Figure 1

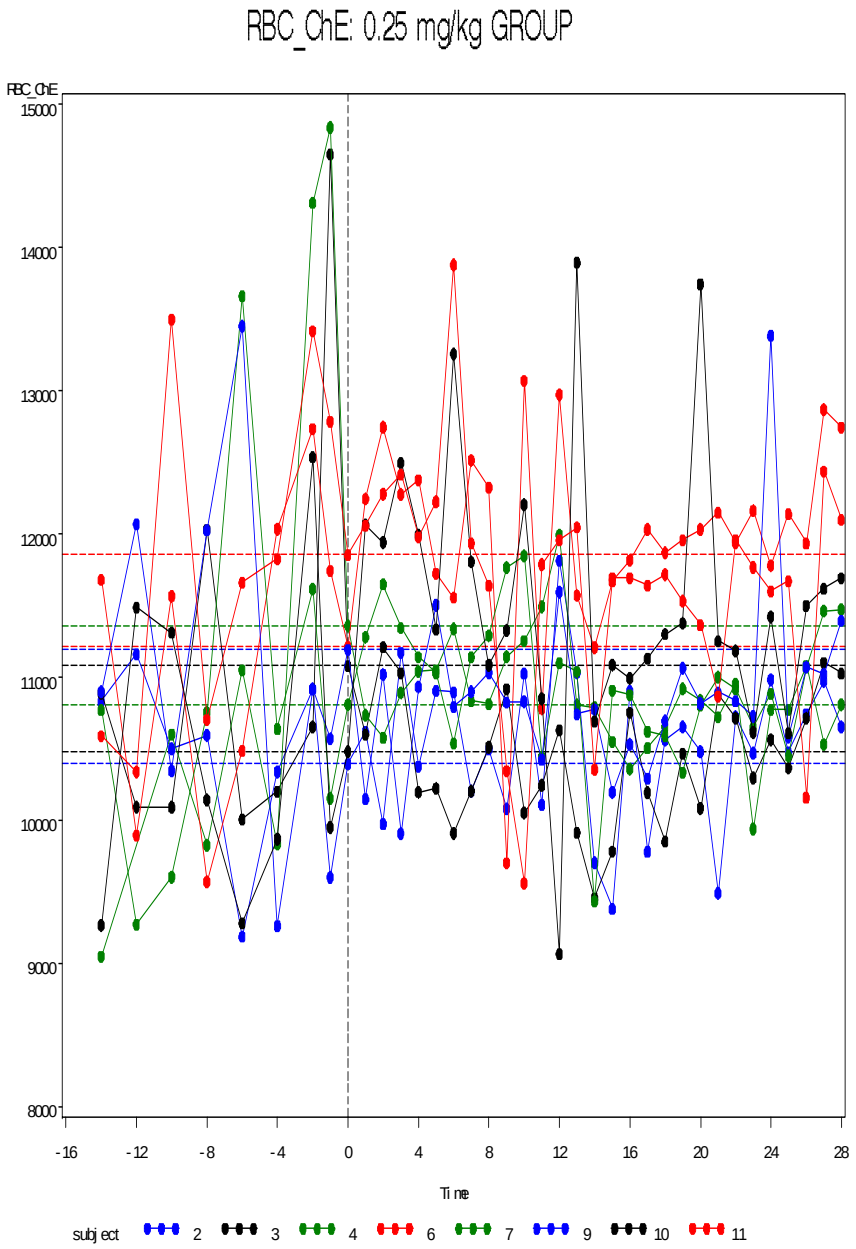

Figure 2

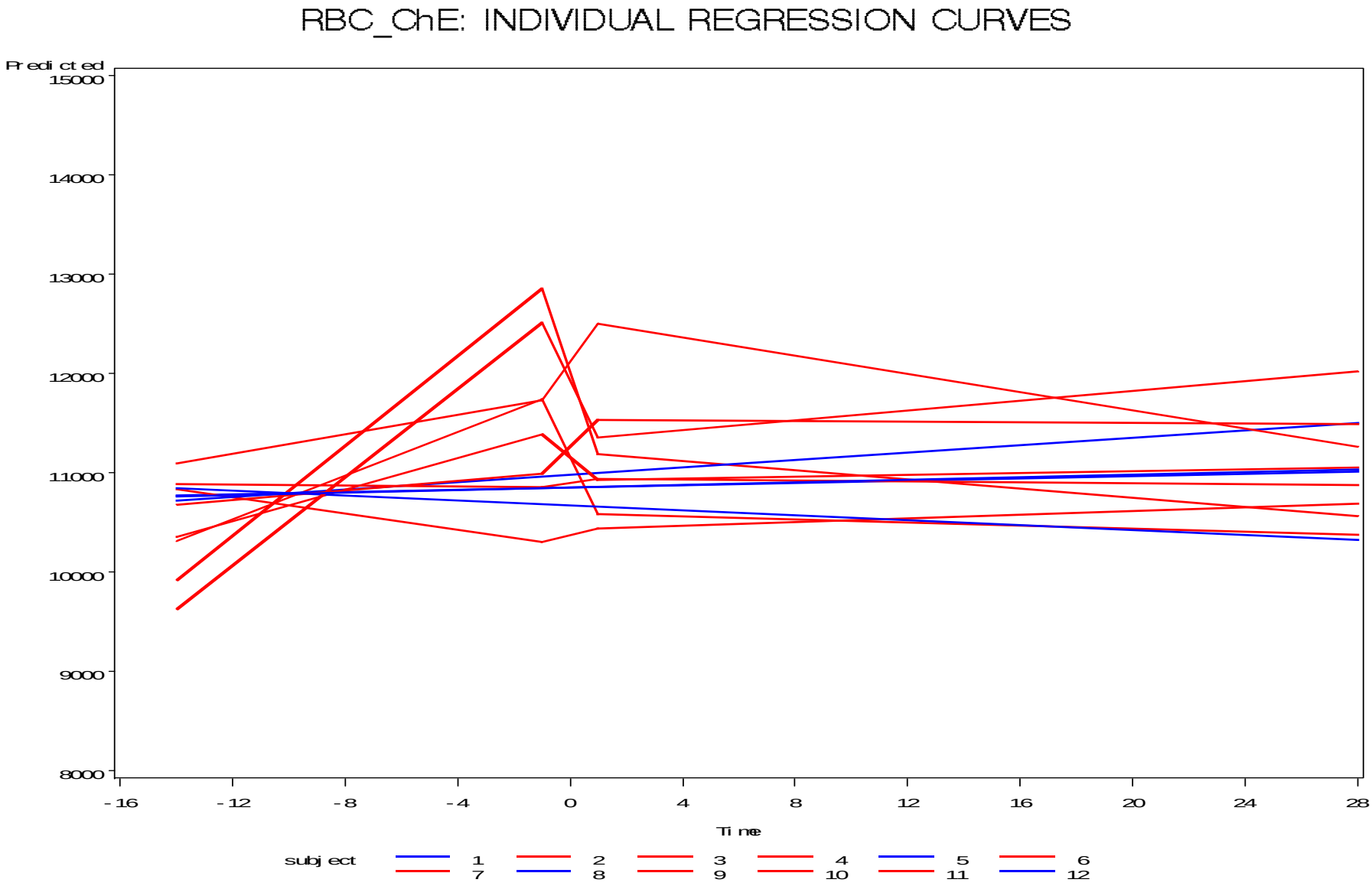
RBC_ChE: INDIVIDUAL REGRESSION CURVES
Predicted
15000
14000
13000
12000
11000
10000
9000
8000
-16
-12
-8
-4
0
4
8
12
16
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Figure 3

ATTACHMENT 3

Plasma-ChE: SAS Code and Output

Model A: VC includes: Intercept and Time (+ sigma^2)

```
proc mixed data=AZM_1 noclprint noitprint noinfo method= ml;
  Title "Model A: Plasma_ChE: Intercept and Time in VC";
  class subject;
  model Plasma_ChE = Time / solution notest outp = p;
  random intercept Time / subject=subject type=UN;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1589808
UN(2,1)	subject	-3364.86
UN(2,2)	subject	75.1825
Residual		89739

Fit Statistics

-2 Log Likelihood	6234.4
AIC (smaller is better)	6246.4
AICC (smaller is better)	6246.6
BIC (smaller is better)	6249.4

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
3	1151.25	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5771.34	364.47	11	15.83	<.0001
Time	2.4088	2.7942	11	0.86	0.4071

Model B: VC includes: Intercept, Time, and Dose (+ sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model B: Plasma_ChE: Intercept, Time, and postdose in VC";
  class subject;
  model Plasma_ChE = Time postdose /solution outp = pp;
  random intercept Time postdose /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1508542

UN(2,1)	subject	-5197.61
UN(2,2)	subject	112.58
UN(3,1)	subject	-53409
UN(3,2)	subject	280.48
UN(3,3)	subject	12881
Residual		84006

Fit Statistics

-2 Log Likelihood	6212.9
AIC (smaller is better)	6232.9
AICC (smaller is better)	6233.4
BIC (smaller is better)	6237.7

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
6	1067.51	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5870.99	355.66	11	16.51	<.0001
Time	7.7579	3.4790	11	2.23	0.0475
postdose	-312.29	73.3506	7	-4.26	0.0038

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	4.97	0.0475
postdose	1	7	18.13	0.0038

B vs. A: p-value = 0.0003

Model C: VC includes: Intercept, Time (+ sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model C: Plasma_ChE: Intercept, Time, and dose in VC";
  class subject;
  model Plasma_ChE = Time postdose /solution outp = pp;
  random intercept Time /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1487153
UN(2,1)	subject	-5623.41
UN(2,2)	subject	122.89
Residual		84653

Fit Statistics

-2 Log Likelihood	6213.7
AIC (smaller is better)	6227.7
AICC (smaller is better)	6228.0
BIC (smaller is better)	6231.1

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq

3	1066.66	<.0001	

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5872.16	353.15	11	16.63	<.0001
Time	7.8064	3.6038	11	2.17	0.0531
postdose	-295.16	62.2779	406	-4.74	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	4.69	0.0531
postdose	1	406	22.46	<.0001

C vs. B: p-value = 0.8495
Model D: VC includes: Intercept, Time, and Postdose (+ sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model D: Plasma_ChE: Intercept, Time, and postdose in VC";
  class subject;
  model Plasma_ChE = Time postdosetime /solution outp = pp;
  random intercept Time postdosetime /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1709164
UN(2,1)	subject	10038
UN(2,2)	subject	118.97
UN(3,1)	subject	-14262
UN(3,2)	subject	-41.4126
UN(3,3)	subject	9.7772
Residual		84656

Fit Statistics

-2 Log Likelihood	6206.3
AIC (smaller is better)	6226.3
AICC (smaller is better)	6226.8
BIC (smaller is better)	6231.2

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
6	1114.11	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5695.60	378.05	11	15.07	<.0001
Time	-13.5049	4.2623	11	-3.17	0.0089
postdosetime	26.8333	5.2229	7	5.14	0.0013

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F

Time	1	11	10.04	0.0089
postdosetime	1	7	26.39	0.0013

D vs. A: p-value << 0.0001
Model E: VC includes: Intercept and Time (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model E: Plasma_ChE: Intercept and Time in VC";
  class subject;
  model Plasma_ChE = Time postdosetime /solution outp = pp;
  random intercept Time /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1660338
UN(2,1)	subject	4781.79
UN(2,2)	subject	93.5306
Residual		84346

Fit Statistics

-2 Log Likelihood	6211.3
AIC (smaller is better)	6225.3
AICC (smaller is better)	6225.6
BIC (smaller is better)	6228.7

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
3	1109.08	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5701.07	372.65	11	15.30	<.0001
Time	-12.2063	4.0778	11	-2.99	0.0122
postdosetime	28.2495	5.2588	406	5.37	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	8.96	0.0122
postdosetime	1	406	28.86	<.0001

E vs. D: p-value = 0.1718
Model F: VC includes: Intercept, Time, Dose, and Postdose (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model F: Plasma_ChE: Intercept, Time, postdose, and postdosetime in
VC";
  class subject;
  model Plasma_ChE = Time postdose postdosetime /solution outp = pp;
  random intercept Time postdose postdosetime/subject = subject type = un;
```

```
run;
```


Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1653448
UN(2,1)	subject	5898.81
UN(2,2)	subject	55.1290
UN(3,1)	subject	-108672
UN(3,2)	subject	-1123.42
UN(3,3)	subject	28840
UN(4,1)	subject	-10302
UN(4,2)	subject	-14.3639
UN(4,3)	subject	1832.22
UN(4,4)	subject	3.6891
Residual		82328

Fit Statistics

-2 Log Likelihood	6194.3
AIC (smaller is better)	6224.3
AICC (smaller is better)	6225.4
BIC (smaller is better)	6231.5

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
10	1084.66	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5774.65	372.43	11	15.51	<.0001
Time	-7.0979	3.6001	11	-1.97	0.0743
postdose	-224.98	75.5453	7	-2.98	0.0206
postdosetime	21.7944	4.6416	7	4.70	0.0022

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	3.89	0.0743
postdose	1	7	8.87	0.0206
postdosetime	1	7	22.05	0.0022

F vs. B: p-value = 0.0023
F vs. D: p-value = 0.0348

Model G: VC includes: Intercept, Time, and Dose (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model G: Plasma_ChE: Intercept, Time, and postdose in VC";
  class subject;
  model Plasma_ChE = Time postdose postdosetime /solution outp = pp;
  random intercept Time postdose /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1611123
UN(2,1)	subject	1622.86
UN(2,2)	subject	36.3390
UN(3,1)	subject	-99289
UN(3,2)	subject	280.80
UN(3,3)	subject	16134
Residual		82355

Fit Statistics

-2 Log Likelihood	6197.8
AIC (smaller is better)	6219.8
AICC (smaller is better)	6220.4
BIC (smaller is better)	6225.1

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
6	1081.16	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5789.89	367.63	11	15.75	<.0001
Time	-4.9614	3.3414	11	-1.48	0.1657
postdose	-243.91	75.6767	7	-3.22	0.0146
postdosetime	21.8054	4.4223	398	4.93	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	2.20	0.1657
postdose	1	7	10.39	0.0146
postdosetime	1	398	24.31	<.0001

G vs. F: p-value = 0.4779

Model H: VC includes: Intercept, Time, and Postdose (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = reml;
  Title "Model H: Plasma_ChE: Intercept, Time, and postdosetime in VC";
  class subject;
  model Plasma_ChE = Time postdose postdosetime /solution outp = pp;
  random intercept Time postdosetime /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	1712918
UN(2,1)	subject	5663.71
UN(2,2)	subject	56.3871

UN(3,1)	subject	-12378
UN(3,2)	subject	3.6803
UN(3,3)	subject	0
Residual		83089

Fit Statistics

-2 Log Likelihood	6197.6
AIC (smaller is better)	6217.6
AICC (smaller is better)	6218.1
BIC (smaller is better)	6222.4

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
5	1081.36	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	5776.53	379.05	11	15.24	<.0001
Time	-6.9155	3.6659	11	-1.89	0.0859
postdose	-194.13	60.6731	398	-3.20	0.0015
postdosetime	20.9943	4.9826	7	4.21	0.0040

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	3.56	0.0859
postdose	1	398	10.24	0.0015
postdosetime	1	7	17.75	0.0040

H vs. F: p-value = 0.5089

ATTACHMENT 4

RBC-ChE: SAS Code and Output

Model A: VC includes: Intercept and Time (+ sigma^2)

```
proc mixed data=AZM_1 noclprint noitprint noinfo method=ml;
  Title "Model A: RBC_ChE";
  class subject;
  model RBC_ChE = Time / solution notest outp = p;
  random intercept Time / subject=subject type=UN;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	99797
UN(2,1)	subject	2814.92
UN(2,2)	subject	0
Residual		732096

Fit Statistics

-2 Log Likelihood	7064.7
AIC (smaller is better)	7074.7
AICC (smaller is better)	7074.9
BIC (smaller is better)	7077.2

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
2	58.77	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	10995	105.87	11	103.86	<.0001
Time	3.2385	3.5452	11	0.91	0.3806

Model B: VC includes: Intercept, Time, and Dose (+ sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model B: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdose /solution outp = pp;
  random intercept Time postdose /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	127666
UN(2,1)	subject	3423.65

UN(2,2)	subject	4.97E-15
UN(3,1)	subject	-168439
UN(3,2)	subject	-8055.47
UN(3,3)	subject	624008
Residual		707843

Fit Statistics

-2 Log Likelihood	7055.9
AIC (smaller is better)	7073.9
AICC (smaller is better)	7074.3
BIC (smaller is better)	7078.2

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
5	64.32	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	10991	127.25	11	86.37	<.0001
Time	2.8601	4.4655	11	0.64	0.5350
postdose	28.9264	252.31	7	0.11	0.9119

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	0.41	0.5350
postdose	1	7	0.01	0.9119

B vs A: p-value = 0.0663

Model C: VC includes: Intercept, Time (+ sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model C: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdose /solution outp = pp;
  random intercept Time /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	97966
UN(2,1)	subject	2765.82
UN(2,2)	subject	0
Residual		732166

Fit Statistics

-2 Log Likelihood	7064.7
AIC (smaller is better)	7076.7
AICC (smaller is better)	7076.9
BIC (smaller is better)	7079.6

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
2	55.52	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	10983	117.55	11	93.43	<.0001
Time	2.5754	4.5150	11	0.57	0.5799
postdose	36.3387	153.41	406	0.24	0.8129

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	0.33	0.5799
postdose	1	406	0.06	0.8129

C vs. B: Pvalue = 0.0321
Model D: VC includes: Intercept, Time, and Postdose (+ sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model D: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdosetime /solution outp = pp;
  random intercept Time postdosetime /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	220794
UN(2,1)	subject	17843
UN(2,2)	subject	1168.26
UN(3,1)	subject	-19299
UN(3,2)	subject	-1427.27
UN(3,3)	subject	1766.64
Residual		705244

Fit Statistics

-2 Log Likelihood	7056.4
AIC (smaller is better)	7076.4
AICC (smaller is better)	7077.0
BIC (smaller is better)	7081.3

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
6	66.45	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	11103	149.39	11	74.33	<.0001
Time	25.2743	12.5233	11	2.02	0.0686
postdosetime	-31.2765	16.1986	7	-1.93	0.0948

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	4.07	0.0686

postdosetime	1	7	3.73	0.0948

D vs. A: pvalue = 0.0812
Model E: VC includes: Intercept and Time (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model E: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdosetime /solution outp = pp;
  random intercept Time /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	105243
UN(2,1)	subject	3394.44
UN(2,2)	subject	15.9828
Residual		726253

Fit Statistics

-2 Log Likelihood	7062.0
AIC (smaller is better)	7076.0
AICC (smaller is better)	7076.3
BIC (smaller is better)	7079.4

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
3	60.85	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	11034	110.19	11	100.14	<.0001
Time	11.1172	5.8817	11	1.89	0.0854
postdosetime	-15.3037	8.8623	406	-1.73	0.0850

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	3.57	0.0854
postdosetime	1	406	2.98	0.0850

E vs. D: pvalue = 0.1328
Model F: VC includes: Intercept, Time, Dose, and Postdose (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model F: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdose postdosetime /solution outp = pp;
  random intercept Time postdose postdosetime/subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate

Cov Parm	Subject	Estimate
UN(1,1)	subject	803982
UN(2,1)	subject	80321
UN(2,2)	subject	8010.69
UN(3,1)	subject	-642846
UN(3,2)	subject	-66473
UN(3,3)	subject	788989
UN(4,1)	subject	-84372
UN(4,2)	subject	-8581.61
UN(4,3)	subject	69415
UN(4,4)	subject	9023.34
Residual		653585

Fit Statistics

-2 Log Likelihood	7038.1
AIC (smaller is better)	7068.1
AICC (smaller is better)	7069.2
BIC (smaller is better)	7075.4

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
10	81.47	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	11366	286.67	11	39.65	<.0001
Time	57.0893	29.3863	11	1.94	0.0781
postdose	-220.78	326.53	7	-0.68	0.5207
postdosetime	-60.4456	31.0551	7	-1.95	0.0927

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	3.77	0.0781
postdose	1	7	0.46	0.5207
postdosetime	1	7	3.79	0.0927

F vs. B: pvalue = 0.0032
F vs. D: pvalue = 0.0026
F vs. A: pvalue = 0.0016

Model G: VC includes: Intercept, Time, and Dose (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model G: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdose postdosetime /solution outp = pp;
  random intercept Time postdose /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	147839
UN(2,1)	subject	4254.39
UN(2,2)	subject	0
UN(3,1)	subject	-207577
UN(3,2)	subject	-8316.50
UN(3,3)	subject	647916

Residual	703511

Fit Statistics

-2 Log Likelihood	7052.5
AIC (smaller is better)	7072.5
AICC (smaller is better)	7073.0
BIC (smaller is better)	7077.3

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
5	67.10	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	11006	133.74	11	82.29	<.0001
Time	9.4698	5.5695	11	1.70	0.1171
postdose	265.11	288.94	7	0.92	0.3894
postdosetime	-15.3812	8.1709	398	-1.88	0.0605

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	2.89	0.1171
postdose	1	7	0.84	0.3894
postdosetime	1	398	3.54	0.0605

G vs. F: pvalue = 0.0061

Model H: VC includes: Intercept, Time, and Postdose (+sigma^2)

```
Proc mixed data = AZM_1 noclprint noitprint noinfo method = ml;
  Title "Model H: RBC_ChE";
  class subject;
  model RBC_ChE = Time postdose postdosetime /solution outp = pp;
  random intercept Time postdosetime /subject = subject type = un;
run;
```

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
UN(1,1)	subject	308187
UN(2,1)	subject	28568
UN(2,2)	subject	2493.41
UN(3,1)	subject	-32312
UN(3,2)	subject	-3034.57
UN(3,3)	subject	3716.61
Residual		691978

Fit Statistics

-2 Log Likelihood	7055.0
AIC (smaller is better)	7077.0
AICC (smaller is better)	7077.6

BIC (smaller is better)	7082.3

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
6	64.58	<.0001

Solution for Fixed Effects

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	11266	196.85	11	57.23	<.0001
Time	43.6791	19.0342	11	2.29	0.0424
postdose	-292.87	204.31	398	-1.43	0.1525
postdosetime	-44.8423	21.7677	7	-2.06	0.0784

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Time	1	11	5.27	0.0424
postdose	1	398	2.05	0.1525
postdosetime	1	7	4.24	0.0784

H vs. F: pvalue = 0.0020