GloboMax LLC Report

Simulation Study to Assess Alternative Bioavailability Calculations, Study Designs and Acceptance Criteria for Determining the Bioequivalence of Levothyroxine Sodium Tablets.

TECHNICAL REPORT

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1 GLOSSARY OF ABBREVIATIONS

Abbreviation	Definition
AUC_{0-48}, AUC0-48	Area under the plasma concentration-time curve from time = 0 to 48 hours
ANOVA	Analysis of Variance
CI	Confidence Interval
C_{max}, Cmax	Maximum observed concentration
CV%	Coefficient of Variation
dL	Deciliter
FDA	Food and Drug Administration
hr	Hour
K_{el}	First order elimination rate (λ_z) constant associated with the terminal (log-linear) portion of the plasma-concentration time curve
LS	Least square
LSMean	Least square mean
mcg	Microgram
mg	Milligram
MEM	Mixed effect model
mL	Milliliter
ms	Millisecond
NDA	New Drug Application
ng	Nanogram
N.R.	No Result
SAP	Simulation Analysis Plan
SAS	Statistical Analysis Software
SD	Standard Deviation
SE	Standard Error of the Mean
$T_{1/2}$	Half-Life
T_{max}	Time at which the maximum concentration was observed

Additional abbreviations are defined in the text at the point of use.

2 SYNOPSIS

The Food and Drug Administration (FDA) is recommending that bioequivalence studies for levothyroxine sodium oral tablets be carried out as single 600 mcg dose studies using healthy subjects and that the resulting concentration-time data for levothyroxine be analyzed without correction for the baseline, endogenous levels of levothyroxine. The data from these studies, in spite of the large 600 mcg dose, are expected to yield maximum observed (endogenous + exogenous) levothyroxine concentrations of about 14 mcg/dL, only twice the baseline, normal levothyroxine concentrations of 6 - 8 mcg/dL. It is expected that bioavailability parameters calculated from baseline uncorrected data will be much less sensitive to changes in bioavailability than is the case for bioequivalence assessment of nonendogenous compounds for which baseline levels are essentially zero.

To assess the impact of baseline levothyroxine levels on the assessment of bioequivalence, a stochastic simulation study was carried out for each of two assumptions for the behavior of endogenous levothyroxine concentrations after administration of the 600 mcg dose. One assumption was that there would be no suppression of endogenous levothyroxine production and no change from the baseline level. The second assumption was that there would be complete suppression of endogenous production and that the endogenous level decayed from the baseline level with a half-life of 7 days, the average half-life of levothyroxine.

The results provided in this report illustrate that, given very reasonable limiting assumptions about endogenous levothyroxine behavior in healthy subjects, the use of baseline uncorrected C_{max} and AUC_{0-48} values will result in an unacceptable risk of declaring two products bioequivalent when they actually differ by more than 20-25%. In fact, it is likely that products with differences in exogenous Cmax and AUC of 35% will be declared bioequivalent if baseline uncorrected parameters are used.

3 INTRODUCTION

The Food and Drug Administration (FDA) guidance for levothyroxine sodium New Drug Applications (NDA) describes two biopharmaceutic studies in healthy subjects (1). One study is a single dose, two way crossover study to compare the bioavailability of 2 x 300 mcg tablets to that of a 600 mcg dose administered as a solution. The second study is a single dose, three way crossover study to assess the dosage form proportionality of 50, 100 and 300 mcg tablets. A total of 600 mcg as a single dose was administered as 12 x 50 mcg tablets, 6 x 100 mcg tablets and 2 x 300 mcg tablets on separate occasions. The suprapharmacologic dose of 600 mcg was administered in an effort to obtain levothyroxine concentrations above those normally present in healthy individuals. The FDA instructed NDA applicants to use the baseline uncorrected data from these studies to estimate the bioavailability parameters, maximum observed concentration (C_{max}) and area under the concentration-time curve from 0 to 48 hours (AUC_{0-48}). The FDA is now recommending that this same procedure, the use of baseline uncorrected data, be applied to the assessment of bioequivalence testing for ANDA's. Abbott laboratories has performed the biopharmaceutics studies as described in the FDA guidance. The data from these studies, in spite of the large, 600 mcg dose, have yielded average maximum levothyroxine concentrations of about 14 mcg/dL, only twice the baseline, normal levothyroxine concentrations of 6 - 8 mcg/dL. Given that substantial portions of the C_{max} and AUC_{0-48} values calculated using baseline uncorrected data are related to normal, endogenous levothyroxine production, it is likely that these values will be much less sensitive to changes in bioavailability than is the case for bioequivalence assessment of nonendogenous compounds for which baseline levels are essentially zero.

To assess the impact of baseline levothyroxine levels on the assessment of bioequivalence, a stochastic simulation study was carried out for each of two assumptions for the behavior of endogenous levothyroxine concentrations after administration of the 600 mcg dose. One assumption was that there would be no suppression of endogenous levothyroxine production and no change from the baseline level. The corresponding baseline correction is designated as Method 1 . The second assumption was that there would be complete suppression of endogenous production and that the endogenous level decayed from the baseline level with a half-life of 7 days, the average half-life of levothyroxine. The corresponding baseline correction is designated as Method 2. These two methods emulate the extremes of endogenous levothyroxine autoregulation while the "true" level of suppression is likely somewhere in between.

4 OBJECTIVES

To explore via stochastic simulation the characteristics of assessing bioequivalence of levothyroxine products in studies in normal healthy volunteers with and without correction for endogenous levels. The simulations will also provide information about the behavior of alternative choices for baseline correction of levothyroxine concentration data and the use of a narrower acceptance range, 90-110%.

5 METHODS

The Simulation Plan for this project is provided in Appendix I. There were only minor deviations from this plan and these are fully described below. The minor deviations included the generation of simulated trials for more true differences than originally stated and a slightly different algorithm for converting simulated, continuous T_{max} values to discrete T_{max} values.

5.1 Baseline Corrections for C_{max} and AUC_{0-48}

5.1.1 METHOD 1 (ASSUMPTION 1)

In the biopharmaceutic NDA studies requested by the FDA and mentioned in Section 2, levothyroxine concentrations are obtained at 0.5, 0.25, and 0 hr prior to dosing. If the average of these values ($C_{baseline}$) is taken as representative of endogenous levothyroxine concentrations over the next 48 hours, baseline corrected C_{max} and AUC_{0-48} can be calculated by Equations 1 and 2-3, respectively.

$$\text{Corrected } C_{max} = \text{Observed } C_{max} - C_{baseline} \quad (1)$$

$$\text{Endogenous } AUC_{0-48} = C_{baseline} \times 48\text{hours} \quad (2)$$

$$\text{Corrected } AUC_{0-48} = \text{Observed } AUC_{0-48} - \text{Endogenous } AUC_{0-48} \quad (3)$$

This method of correction assumes that the contribution of endogenous levothyroxine to the observed levothyroxine concentration is constant.

5.1.2 METHOD 2

It is well known that levothyroxine concentrations are highly autoregulated in normal subjects. The levothyroxine concentrations resulting from the administration of the large, 600 mcg dose are likely to suppress endogenous levothyroxine production to some extent. However, even complete suppression of endogenous production would result in only a gradual decline in endogenous levothyroxine concentrations. This gradual decline is a result of the relatively long half-life for levothyroxine, about 7 days in normal subjects (2). This suggests a second approach to the correction for endogenous levothyroxine concentrations based on a half-life of 7 days. Calculation of baseline corrected C_{max} and AUC_{0-48} by Method 2 is described by Equations 4 and 5-6, respectively.

Let $\beta = \log 2/168$, the elimination rate constant corresponding to a half-life of 7 days (168 hrs).

$$\text{Corrected } C_{max} = \text{Observed } C_{max} - C_{baseline} e^{(-\beta \times \text{Observed } T_{max})} \quad (4)$$

$$\text{Endogenous } AUC_{0-48} = \frac{C_{baseline}}{\beta}(1 - \exp(-48 \times \beta)) \quad (5)$$

$$\text{Corrected} AUC_{0-48} = \text{Observed } AUC_{0-48} - \text{Endogenous } AUC_{0-48} \quad (6)$$

The quantity described in Equation 5, represents the contribution of endogenous levothyroxine to the observed AUC_{0-48}, under the assumption that production of endogenous levothyroxine completely stops at the time of dosing and that the endogenous concentration declines exponentially from the baseline level with a half-life of 7 days.

5.1.2 METHOD 2b

As applied above, Method 2 assumes that the C_{max} values for observed and baseline corrected levothyroxine data occur at the same time; T_{max} is the same for both measures. This is not necessarily the case.

Therefore, the data from Abbott Laboratories dosage form proportionality study (M01-323) and the tablet data from their relative bioavailability study (M01-324) were used to explore this question more fully. The baseline contribution to each observed concentration value, assuming exponential decay with a half-life of 7 days, was subtracted from the observed value to provide a baseline corrected value for each concentration. The maximum corrected concentration for each individual dosing and the corresponding sampling time were identified and recorded. This approach for obtaining a baseline corrected C_{max} value and the corresponding T_{max} value will be referred to as Method 2b. Only 12 out of the 135 C_{max} values obtained in this way occurred at a time different than the observed T_{max} values. Those 12 occurrences are listed below in Table 1.

Table 1: Individual Differences Between Method 2 and Method 2b for Corrected C_{max} and T_{max} Values

			C_{max} (mcg/dL)		T_{max} (hr)	
Subject	Study	Treatment	Method2	Method2b	Method2	Method2b
108	323	A	4.27	4.33	2.5	12
114	323	A	6.55	6.58	1.5	2.5
115	323	C	6.05	6.08	1.5	10
118	323	C	5.99	6.00	1.5	2.0
201	323	A	6.87	6.89	2.0	2.5
215	323	B	5.60	5.71	2.0	6.0
215	323	C	4.45	4.46	2.0	2.5
216	323	B	8.53	8.55	2.5	3.0
218	323	C	6.99	7.01	1.5	2.0
114	324	A	5.00	5.02	2.0	3.0
214	324	B	7.68	7.68	1.0	1.5
215	324	A	3.49	3.68	4.0	36

Study 323: Treatment A is 12 X 50 mcg tablets. Treatment B is 6 X 100 mcg tablets. Treatment C is 2 X 300 mcg tablets.
Study 324: Treatment A is 2 X 300 mcg tablets. Treatment B is 600 mcg in 15 mL solution.

5.1.3 JUSTIFICATION FOR USING METHOD 2 CORRECTION

The C_{max} values obtained using Method 2b were very similar in essentially all cases to the C_{max} values obtained by Method 2, which assumed that the baseline corrected C_{max} values occurred at the same time as those for the baseline uncorrected values. These results suggest that using Method 2 to obtain C_{max} estimates is quite reasonable and the additional complexity introduced by Method 2b is not necessary for the purposes of the these simulations.

5.2 Example Problem

In Abbott's biopharmaceutic study comparing 2 x 300 mcg Synthroid tablets to a 600 mcg Synthroid dose administered as a solution (M01-324), baseline uncorrected C_{max} and AUC_{0-48} values suggested that the solution and tablets were bioequivalent (SAS PROC MIXED), as shown in Table 2.

Table 2: Study M01-324, Baseline uncorrected parameters

	Ratio (%) of Central Values	
Formula	Point Estimate	90% CI
$100\% \times \frac{C_{max}\,\text{tablet}}{C_{max}\,\text{solution}}$	84.2	81.2 - 87.3
$100\% \times \frac{AUC_{(0-48)}\,\text{tablet}}{AUC_{(0-48)}\,\text{solution}}$	92.7	90.2 – 95.2

However, if one corrects for the baseline levothyroxine concentrations based on the baseline concentrations present when the 600 mcg doses are administered, a different conclusion is reached. The baseline corrected values using Method 1 correction, representing an estimate of the bioavailability of the exogenously administered levothyroxine, indicate that the tablet and solution are not bioequivalent (SAS PROC MIXED), as shown in Table 3.

Table 3: Study M01-324, Baseline corrected parameters (Method 1)

	Ratio (%) of Central Values	
Formula	Point Estimate	90% CI
$100\% \times \frac{C_{max}\,\text{tablet}}{C_{max}\,\text{solution}}$	71.7	67.2 – 76.4
$100\% \times \frac{AUC_{(0\text{-}48)}\,\text{tablet}}{AUC_{(0\text{-}48)}\,\text{solution}}$	79.6	73.7 – 86.0

The conclusion regarding the inequivalence of solution and tablet using baseline correction Method 2 (SAS PROC MIXED) is the same as that for values based on Method 1. The results using Method 2 are presented in Table 4.

Table 4: Study M01-324, Baseline corrected parameters (Method 2)

	Ratio (%) of Central Values	
Formula	Mean Ratio (%)	90% CI
$100\% \times \frac{C_{max}\,\text{tablet}}{C_{max}\,\text{solution}}$	72.2	67.9 – 76.8
$100\% \times \frac{AUC_{(0\text{-}48)}\,\text{tablet}}{AUC_{(0\text{-}48)}\,\text{solution}}$	82.6	77.6 - 87.9

The proposed simulations will further explore the behavior of the baseline uncorrected and baseline corrected parameters when used to assess the bioequivalence of levothyroxine products.

5.3 Parameter Values for Simulation of Bioequivalence Trials

To perform simulations using C_{max} and $AUC_{0\text{-}48}$ parameters that are baseline corrected by Methods 1 and 2, as well as uncorrected C_{max} and $AUC_{0\text{-}48}$ values, it is necessary to have estimates of between subject and within subject variances and covariances for these bioavailability parameters as well as for the baseline concentrations. For Method 2 it is necessary to have this information for T_{max} as well since this parameter is required to calculate the baseline uncorrected C_{max} using baseline corrected (exogenous) C_{max} and the baseline concentration (BL) corrected for exponential decay up to T_{max}. Estimates of mean parameters and between and within subject variance-covariance are ideally

obtained from studies with replicated treatments, but data from replicated single dose studies of levothyroxine are unavailable. Therefore, the results of the Synthroid dosage form proportionality study (Study M01-323) were used as a source for these estimates. In this study a total of 600 mcg of Synthroid as a single dose was administered as 12 x 50 mcg tablets, 6 x 100 mcg tablets and 2 x 300 mcg tablets on separate occasions to 36 healthy subjects (18F, 18M). This data set included values for 105 dosings for 36 subjects. Although not strictly a replicated design study, all three combinations of dosage forms were bioequivalent to each other based on baseline uncorrected and baseline corrected (Methods 1 and 2) data (Table 5) and were used to obtain estimates of bioavailability parameters and their between subject and within subject variance-covariance.

Table 5: Dosage Form Proportionality Study (M01-323)
Estimates of Relative Bioavailability

	Baseline Uncorrected	
	Geometric Mean	90% CI
C_{max}		
A vs B	101.3	98.3 – 104.4
A vs C	103.8	100.7 – 107.0
C vs B	97.6	94.7 – 100.6
AUC_{0-48}		
A vs B	101.0	98.8 – 103.3
A vs C	103.8	101.5 – 106.1
C vs B	97.4	95.3 – 99.5

	Baseline Corrected – Method 1	
	Geometric Mean	90% CI
C_{max}		
A vs B	102.6	96.4 – 109.2
A vs C	108.4	101.8 – 115.4
C vs B	94.7	89.0 – 100.7
AUC_{0-48}		
A vs B	102.8	95.0 – 111.3
A vs C	112.9	104.3 – 117.9
C vs B	91.1	84.2 – 98.5

	Baseline Corrected – Method 2	
	Geometric Mean	90% CI
C_{max}		
A vs B	102.1	96.2 – 108.3
A vs C	108.2	101.9 – 114.8
C vs B	94.3	88.9 – 100.1
AUC_{0-48}		
A vs B	102.6	96.4 – 109.2
A vs C	110.7	104.1 – 117.9
C vs B	92.6	87.1 – 98.6

SAS Proc Mixed
A – Twelve 50 mcg Synthroid Tablets
B – Six 100 mcg Synthroid Tablets
C – Two 300 mcg Synthroid Tablets

Since simulations using baseline corrections according to Method 2 require the T_{max} parameter and since this parameter is discrete rather than continuous (lower information content), it was decided to include both the M01-323 data and the 300 mcg tablet data (34 dosings for 34 subjects) from study M01-324 in the data set used as a basis for the simulation of Method 2. The inclusion of the M01-324 data provided additional information about the mean values and the between-subject variance-covariance but no additional information about within subject variance-covariance.

Linear mixed effect models (MEM) implemented in the NONMEM software (3) were used to estimate means and between- and within-subject variance-covariances for the natural logarithm transformed baseline uncorrected and baseline-corrected bioavailability parameters as well as log-transformed baseline concentrations and, for Method 2, the log-transformed T_{max} values. The FDA stipulates that C_{max} and AUC values be log-transformed for bioequivalence calculation. Although T_{max} values are discrete, their log-transformed values were modeled as continuous data in order to capture the potential correlation of T_{max} with other parameters. Although the distribution of log-transformed T_{max} values was improved relative to the distribution of untransformed values, the distribution was still heavy-tailed to the right. This was addressed in part with the algorithm used to convert the simulated, continuous T_{max} values to discrete T_{max} values (see section 5.4.4 below).

The NONMEM Control Files and Output are provided in Appendix II.

5.3.1 BASELINE UNCORRECTED DATA

The mixed-effect model used to analyze the log-transformed, baseline uncorrected C_{max} and $AUC_{0\text{-}48}$ values and log-transformed baseline concentrations from Study M01-323 is as follows:

LBL = THETA(1) + ETA(1) + EPS(1)

LCMAX = THETA(2) + ETA(2) + EPS(2)

LAUC = THETA(3) + ETA(3) + EPS(3)

where LBL, LCMAX and LAUC are the log-transformed observations for baseline concentration, C_{max} and $AUC_{0\text{-}48}$, respectively. The THETA's are the means to be estimated, the ETA's are random terms representing between subject variability, and EPS's are random terms representing within subject variability. The resulting mean and between and within subject variance parameter estimates are summarized in Table 6. The correlations between parameters are summarized in Table 7.

5.3.2 METHOD 1 CORRECTED DATA

The mixed-effect model used to analyze the log-transformed, baseline corrected C_{max} and $AUC_{0\text{-}48}$ values (Method 1) and log-transformed baseline concentrations

from Study M01-323 was the same as shown in 5.3.1 for uncorrected parameters. Results are provided in Tables 6 and 7.

5.3.3 METHOD 2 CORRECTED DATA

Since Method 2 required simulation of T_{max}, the mixed effect model describe in 5.3.1 was extended to include the log-transformed T_{max} values.

LBL = THETA(1) + ETA(1) + EPS(1)

LCMAX = THETA(2) + ETA(2) + EPS(2)

LAUC = THETA(3) + ETA(3) + EPS(3)

LTMAX = THETA(4) + ETA(4) + EPS(4)

where LTMAX is the log-transformed TMAX value and THETA(4), ETA(4) and EPS(4) are the estimated mean and random terms for LTMAX as describe in 5.3.1. Results are provided in Tables 6 and 7.

Table 6: Estimates of typical values and between and within subject variances for natural log transformed baseline concentrations and bioavailability parameters

Baseline Uncorrected (Data from Study M01-323)

Parameter	Typical Values		Between Subject		Within Subject	
	$\ln(P_{TV})$	P_{TV}	variance ω^2_{bs}	approximate CV(%)	variance σ^2_{ws}	approximate CV(%)
BL (mcg/dL)	1.92	6.82	0.0284	16.9	0.00360	6.00
C_{max} (mcg/dL)	2.57	13.1	0.0173	13.2	0.00572	7.56
AUC_{0-48} (mcg/dL)* hr	6.17	478	0.0213	14.6	0.00385	6.20

Baseline Corrected – Method 1 (Data from Study M01-323)

Parameter	Typical Values		Between Subject		Within Subject	
	$\ln(P_{TV})$	P_{TV}	variance ω^2_{bs}	approximate CV(%)	variance σ^2_{ws}	approximate CV(%)
BL (mcg/dL)	1.92	6.82	0.0284	16.9	0.00360	6.00
C_{max} (mcg/dL)	1.82	6.17	0.0241	15.5	0.0244	15.6
AUC_{0-48} (mcg/dL)* hr	5.01	149.9	0.0393	19.8	0.0436	20.9

Baseline Corrected – Method 2 (Data from Study M01-323 and Tablet Data from Study M01-324)

Parameter	Typical Values		Between Subject		Within Subject	
	$\ln(P_{TV})$	P_{TV}	variance ω^2_{bs}	approximate CV(%)	variance σ^2_{ws}	approximate CV(%)
BL (mcg/dL)	1.92	6.82	0.0251	15.8	0.00357	5.97
C_{max} (mcg/dL)	1.83	6.23	0.0246	15.7	0.0227	15.1
AUC_{0-48} (mcg/dL)* hr	5.20	181.3	0.0259	16.1	0.0271	16.5
T_{max} (hr)	0.992	2.70	0.0138	11.7	0.288	53.7

P_{TV} is the typical value of the parameter
ω^2_{bs} is the between-subject variance
σ^2_{ws} is the within-subject variance

Table 7: Correlation Coefficients for natural log transformed baseline concentrations and bioavailability parameters

Baseline Uncorrected (Data from Study M01-323)

Parameter	Between Subject	Within Subject
BL and C_{max}	0.8572	0.2644
BL and AUC_{0-48}	0.9270	0.2713
C_{max} and AUC_{0-48}	0.9689	0.5988

Baseline Corrected – Method 1 (Data from Study M01-323)

Parameter	Between Subject	Within Subject
BL and C_{max}	0.3524	-0.1750
BL and AUC_{0-48}	0.3742	-0.3815
C_{max} and AUC_{0-48}	0.9098	0.6009

Baseline Corrected – Method 2 (Data from Study M01-323 and Tablet Data from Study M01-324)

Parameter	Between Subject	Within Subject
BL and C_{max}	0.3260	-0.1900
BL and AUC_{0-48}	0.4746	-0.3345
BL and T_{max}	0.0472	-0.2832
C_{max} and AUC_{0-48}	0.8835	0.5927
C_{max} and T_{max}	-0.2692	-0.2511
AUC_{0-48} and T_{max}	-0.0505	0.0505

5.4 Simulation Conditions

The ability to detect a range (-0.5 to 0.5) of differences (Δ's) in extent of absorption between test and reference products is examined for the baseline uncorrected and baseline corrected C_{max} and AUC_{0-48} values. To simulate the effect of changes in the extent of absorption in the presence of significant endogenous concentrations, it is necessary to separate the contributions of the endogenous compound from the contributions of the exogenously administered compound. Only in this way is it possible to simulate changes in the extent of absorption of exogenous material in the presence of endogenous material. The simulations are constructed in the following manner.

5.4.1 SPECIFY TYPICAL VALUES.

Typical log transformed BL, log transformed baseline corrected (Method 1 or 2) C_{max}, and AUC_{0-48} values and, for Method 2, the log transformed T_{max} value are obtained from the MEM results provided in Table 6.

$LB_{TV} = \ln(BL_{TV})$

$LP_{R,TV} = \ln(P_{TV})$

$LT_{TV} = \ln(T_{max,\,TV})$ (Method 2 only)

where:

BL_{TV} is the typical value of the baseline

P_{TV} is the typical value of either C_{max} or AUC_{0-48}

$T_{max,\,TV}$ is the typical value of T_{max}

ln is the natural logarithm function

The subscript R indicates the reference product .

5.4.2 OBTAIN INDIVIDUAL PARAMETER VALUES.

Individual (subject specific) parameter values for the reference product are obtained from the typical values and a set of random terms drawn from the appropriate between subject multivariate normal distribution. Baseline corrected C_{max} and AUC_{0-48} values are adjusted for the assumed difference in extent of absorption for the test product relative to the reference product.

$LB_i = LB_{TV} + \eta_{B,i}$

$LP_{T,i} = LP_{R,TV} + \ln(1+\Delta) + \eta_{P,i}$

$LP_{R,i} = LP_{R,TV} + \eta_{P,i}$

$LT_i = LT_{TV} + \eta_{T,i}$ (Method 2 only)

where:

LB_i, $LP_{T,i}$, $LP_{R,i}$ and LT_i subject-specific values (i'th subject) for log transformed endogenous baseline values, (exogenous) bioavailability parameter values (C_{max} or AUC_{0-48}) and T_{max}, respectively. The subscript T indicates the test product.

η's are random variables that account for between subject variability, $\eta \sim MVN(0,\Omega_{bs})$. For each individual an η vector is generated. Information describing the between subject variance-covariance matrix,Ω_{bs}, is provided in Tables 6 and 7.

Δ is the fractional difference between Test and Reference products. Values of Δ equal to -0.5 to 0.5 at 0.05 increments were evaluated along with additional values of +/- 0.33.

5.4.3 OBTAIN DOSING PERIOD-SPECIFIC PARAMETER VALUES.

Dosing period-specific parameter values are obtained from the individual parameter values and a set of random terms drawn from the appropriate within subject multivariate normal distribution. The C_{max} and AUC_{0-48} values represent the contribution of exogenous levothyroxine and are the baseline corrected values expected to be calculated from the observed BL and the baseline uncorrected C_{max} and AUC_{0-48} values (see 5.4.4 and 5.4.5).

$LB_{i,a} = LB_i + \varepsilon_{B,i,a}$

$LB_{i,b} = LB_i + \varepsilon_{B,i,b}$

$LP_{T,i,a} = LP_{T,i} + \varepsilon_{P,i,a}$

$LP_{R,i,b} = LP_{R,i} + \varepsilon_{P,i,b}$

$LT_{i,a} = LT_i + \varepsilon_{T,i,a}$ (Method 2 only)

where:

$LB_{i,a}$ is the log transformed observed baseline concentration for dosing period "a".

$LB_{i,b}$ is the log transformed observed baseline concentration for dosing period "b".

$LP_{Ti,a}$ is the log transformed, baseline corrected parameter (C_{max} or AUC_{0-48}) for the test formulation (subject received test product in dosing period "a").

$LP_{Ri,b}$ is the log transformed, baseline corrected parameter (C_{max} or AUC_{0-48}) for the reference formulation (subject received reference product in dosing period "b").

$LT_{i,a}$ is the log transformed T_{max} for dosing period "a".

$LT_{i,b}$ is the log transformed T_{max} for dosing period "b".

ε's are random variables that account for within subject variability, $\varepsilon \sim MVN(0,\Sigma_{ws})$. The subscripts a and b indicate the different dosing periods. For each dosing period a separate vector of ε's is generated. Information describing the within subject variance-covariance matrix, Σ_{ws}, is provided in Tables 6 and 7.

These parameter values represent the contribution of exogenous levothyroxine and would be the baseline corrected values expected to be calculated from the observed BL, T_{max} and baseline uncorrected C_{max} and AUC_{0-48}values (see 5.4.4 and 5.4.5).

5.4.4 OBTAIN THE CONTRIBUTIONS OF ENDOGENOUS LEVOTHYROXINE TO BASELINE UNCORRECTED C_{MAX} AND AUC_{0-48} VALUES.

For Method 1, the contributions of endogenous levothyroxine to the total, observed C_{max} and AUC_{0-48} values, are BL and BL*48, respectively, where BL is obtained from 5.4.3.

For Method 2, the contribution of endogenous levothyroxine to the baseline uncorrected C_{max} value is based on BL and T_{max} from 5.4.3 assuming exponential decay up to T_{max} and a half-life of 7 days. The T_{max} used in this calculation is converted from a continuous variable to a discrete variable as described below. The endogenous contribution (Method 2) to the baseline uncorrected AUC_{0-48} value is based on BL from 5.4.3 and the log-linear trapezoid formula assuming exponential decay with a half-life of 7 days.

Let $BL_{i,a} = \exp(LB_{i,a})$ where $BL_{i,a}$ is the simulated baseline for individual i at dosing period a.

Method 1: Stable baseline

The contribution of endogenous levothyroxine to baseline uncorrected C_{max} is the observed baseline value, $BL_{i,a}$ or

$C_{max,\ endo,1,i,a} = BL_{i,a}$.

The contribution of endogenous levothyroxine to baseline uncorrected AUC_{0-48} is the observed baseline value, $BL_{i,a}$, multiplied by the AUC interval, 48 hours.

$AUC_{0-48,\ endo,1,i,a} = BL_{i,a} \times 48$.

Method 2: Exponential decline

Let $T_{max,\,i,a} = \exp(LT_{i,a})$ where $T_{max,\,i,a}$ is the simulated time to C_{max} for individual i at dosing period a. This is a continuous variable that is converted to a discrete variable according to the following table.

Continuous $T_{max,\,i,a}$ (hr)	Discrete $T_{max,\,i,a}$ (hr)	Simulated Percentage	Actual Percentage
<=.5	0.5	0.15	0
> 0.5 and <= 1	1	3.15	1.5
> 1 and <= 1.6	1.5	13.5	14
> 1.6 and <= 2.4	2	26.3	25.2
> 2.4 and <= 2.9	2.5	13.2	17
> 2.9 and <= 3.8	3	18.4	14
> 3.8 and <= 5	4	13.1	13
> 5 and <= 6.5	6	7.4	5.9
> 6.5 and <= 8.5	8	3.0	2.2
> 8.5 and <= 12	10	1.25	3.0
> 12 and <= 20	18	0.55	2.2
> 20 and <= 26	24	0	0
> 26	36	0	1.5

Comparison of the Simulated Percentage (n=2000) and the Actual Percentage (Study M01-323 and Tablet data from Study M01-324, n=135) indicate that this approach produces a reasonable representation of the observed T_{max} distribution. The C_{max} calculation will be relatively insensitive to modest differences in T_{max} because of the long, 7 day, half-life of levothyroxine.

The contribution of endogenous levothyroxine to baseline uncorrected C_{max} is the observed baseline value adjusted for exponential decay to time T_{max}.

$$C_{max,\,endo,2,i,a} = BL_{i,a} \times \exp(-0.693/(7 \times 24) \times \text{discrete } T_{max,\,i,a})$$

The contribution of endogenous levothyroxine to baseline uncorrected $AUC_{0\text{-}48}$ can be calculated using the log-linear trapezoid rule. The sampling interval is 48 hours

$$AUC_{0\text{-}48,endo,2,i,a} = (BL_{i,a} - BL_{i,a} \times \exp(-0.693/7 \times 2)) \times 48/\ln(BL_{i,a} / (BL_{i,a} \times \exp(-0.693/7 \times 2)))$$

5.4.5 OBTAIN BASELINE UNCORRECTED PARAMETERS.

The contributions of exogenous levothyroxine (baseline corrected C_{max} and $AUC_{0\text{-}48}$ from 5.4.3) are added to the contributions of endogenous levothyroxine (5.4.4) to obtain the

baseline uncorrected C_{max} and AUC_{0-48} values expected to be observed in a bioequivalence study.

$$P_{o,T,i} = P_{T,i,a} + P_{endo,i,a}$$

or

$$P_{o,R,i} = P_{R,i,a} + P_{endo,i,a}$$

where $P_{o,T,i}$ and $P_{o,R,i}$ =are the observed, baseline uncorrected parameters (C_{max} or AUC_{0-48}) for test and reference products, respectively.

5.4.6 BIOEQUIVALENCE STATISTICAL ANALYSIS

Bioequivalence studies were simulated as two-way crossover trials with no added period or sequence effects. Sample sizes of 24, 36 and 48 subjects were evaluated. An analysis of variance was used to obtain the residual error needed to calculate 90% confidence intervals for baseline uncorrected and baseline corrected C_{max} and AUC_{0-48} test/reference ratios expressed as a percentage. The ANOVA included treatment, period, sequence, and subject-within-sequence effects. The confidence limits for each parameter ratio were compared to a restricted acceptance range of 90 – 110% and the usual acceptance range of 80 - 125%. If the confidence interval extended beyond the acceptance range then the trial was recorded as a failure. For each assumed true difference in the extent of absorption and sample size, 1000 replicate trials were simulated and the percentage that passed (Pass Rate) was calculated.

The S-Plus simulation code is provided in Appendix III. In some cases it was necessary to subdivide the simulations so that they could be spread across multiple computers.

5.5 Simulation Results

5.5.1 ASSUMPTION 1 WITH BASELINE UNCORRECTED VERSUS METHOD 1 CORRECTED PARAMETERS

Power curves illustrating the probability of declaring bioequivalence as a function of the true percentage difference in extent of absorption, which compare the use of baseline corrected parameters (Method 1) to baseline uncorrected parameters, are provided in Figures 1-3 for samples sizes of 24, 36 and 48 subjects, respectively. If the usual acceptance range of 80 - 125% is applied to the baseline uncorrected C_{max} and AUC_{0-48} values, a test product that is 35% lower than the reference product in extent of absorption is likely to be declared bioequivalent to the reference. This is an unacceptable consumer risk. On the other hand, application of the usual acceptance criteria of 80 - 125% to the baseline corrected (Method 1) C_{max} and AUC_{0-48} values yields more appropriate consumer and producer risks. For a sample size of 24, there is some producer risk since even identical products have a small probability of being declared bioinequivalent in regard to AUC_{0-48}. However, the producer risk is improved for samples sizes of 36 and 48

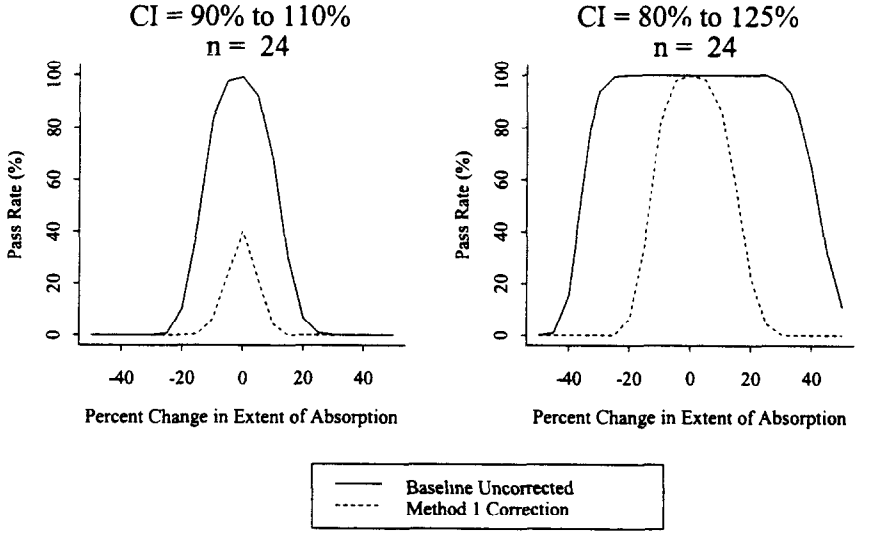
subjects. Consumer risk is maintained at an appropriate level; there is only a 5% probability that two products with a true difference at one extreme of the acceptance range would be declared bioequivalent. The baseline corrected (Method 1) C_{max} and AUC_{0-48} values exhibit Pass Rates of about 5%, the expected nominal rate, when the true difference in extent of absorption is – 20% or + 25%.

For sample sizes of 24 to 48 subjects, the use of a 90 – 110% acceptance range for the uncorrected C_{max} values provides similar power to using an 80 – 125% acceptance range for the corrected (Method 1) C_{max} values. For AUC_{0-48} and an acceptance range of 90-110%, the probability of declaring the two products bioequivalent is no greater than it is for declaring them equivalent with respect to each of Cmax and AUC_{0-48}. Therefore, the consumer risk is still low if the difference between the products is 20-25% for C_{max}. Nonetheless, the characteristics of uncorrected AUC with the narrower acceptance range is undesirable, especially if there were to be a test product similar to the reference for C_{max}, but rather different with respect to extent of absorption. On the other hand, the use of the 90 - 110% acceptance range and the baseline corrected (Method 1) values result in high producer risk. There is a high probability that even products with identical extents of absorption would not be declared bioequivalent. However, if a greater than 10% difference is clinically significant, this difficulty does not suffice to declare such a criterion inappropriate. If a greater than 10% difference is clinically significant, larger studies would be appropriate, assuming Method 1 correction itself is appropriate.

The baseline corrected (Method 1) C_{max} and AUC_{0-48} values exhibit Pass Rates of about 5%, the expected nominal rate, when the true difference is – 20% or + 25%. Simulation results are tabulated in Appendix IV as the number of failed trials out of 1000 replications.

Figure 1: Percent of Simulated Trials that Pass Bioequivalence Criteria vs. Percent Change in Extent of Absorption for N = 24.

Cmax



AUC0-48

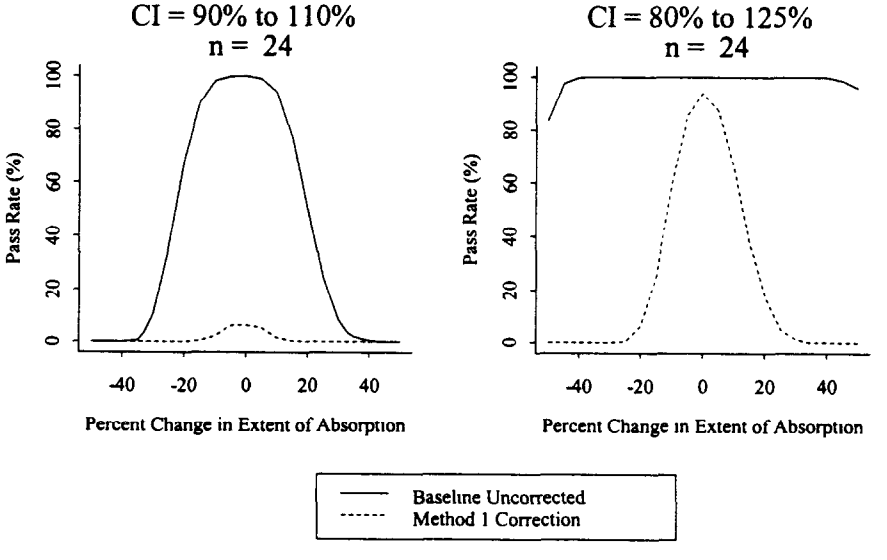
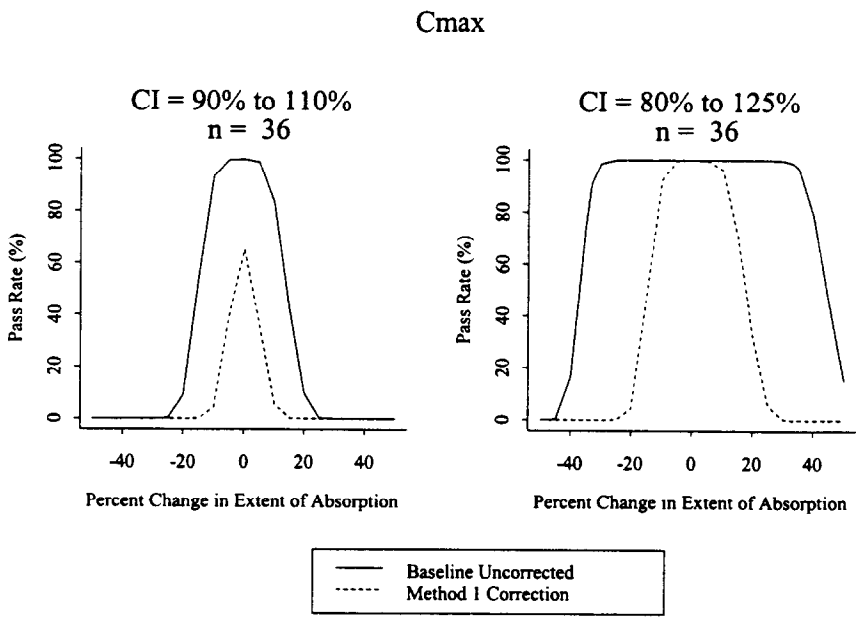


Figure 2: Percent of Simulated Trials that Pass Bioequivalence Criteria vs. Percent Change in Extent of Absorption for N = 36.



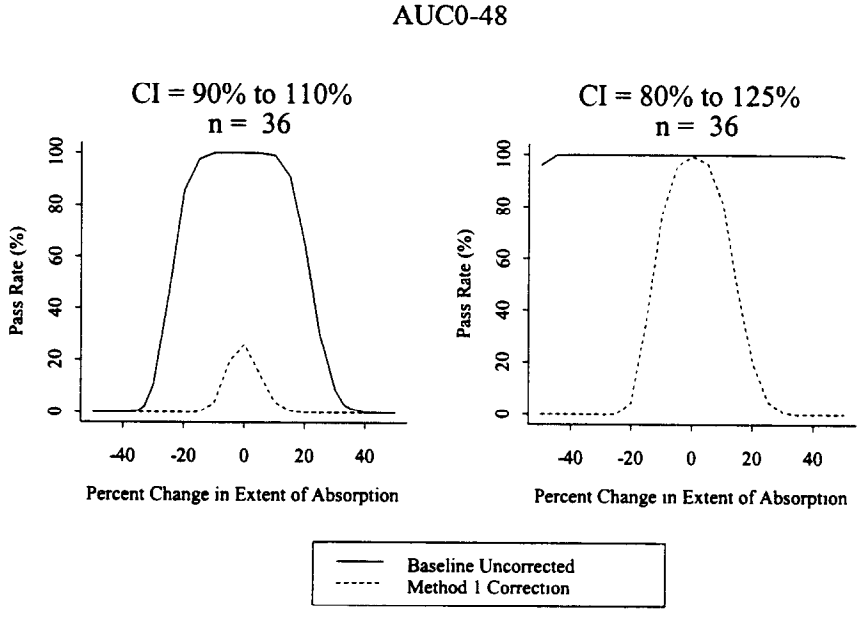
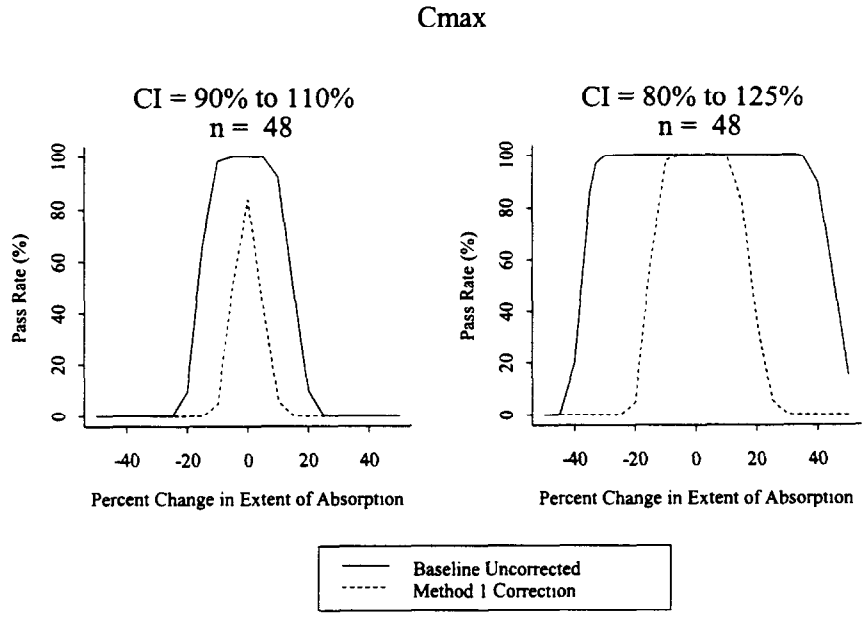
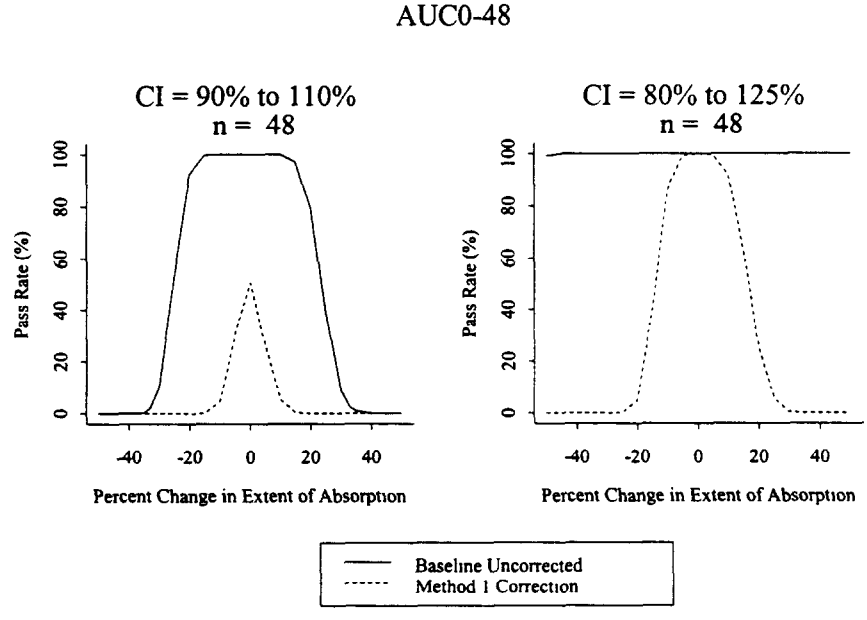


Figure 3: Percent of Simulated Trials that Pass Bioequivalence Criteria vs. Percent Change in Extent of Absorption for N = 48.





5.5.2 ASSUMPTION 2 WITH BASELINE UNCORRECTED VERSUS METHOD 2 CORRECTED PARAMETERS

The power curves comparing the use of baseline corrected parameters (Method 2) to baseline uncorrected parameters are provided in Figures 4-6 for sample sizes of 24, 36 and 48 subjects. They differ in only minor ways from Figures 1-3. Method 2 results in a somewhat larger fraction of the observed, uncorrected values being attributed to the exogenous dose and thereby makes the observed, baseline uncorrected values somewhat more sensitive to changes in the extent of absorption and reduces the probability of incorrectly concluding bioequivalence This effect, which can be seen by comparing Figures 1 and 4, 2 and 5, and 3 and 6, is very small and hardly noticeable for C_{max}, but larger and more obvious for AUC_{0-48}.

The baseline corrected (Method 2) C_{max} and AUC_{0-48} values exhibit Pass Rates of about 5%, the expected nominal rate, when the true difference is – 20% or + 25%. Simulation results are tabulated in Appendix IV as the number of failed trials out of 1000 replications.

Figure 4: Percent of Simulated Trials that Pass Bioequivalence Criteria vs. Percent Change in Extent of Absorption for N = 24.

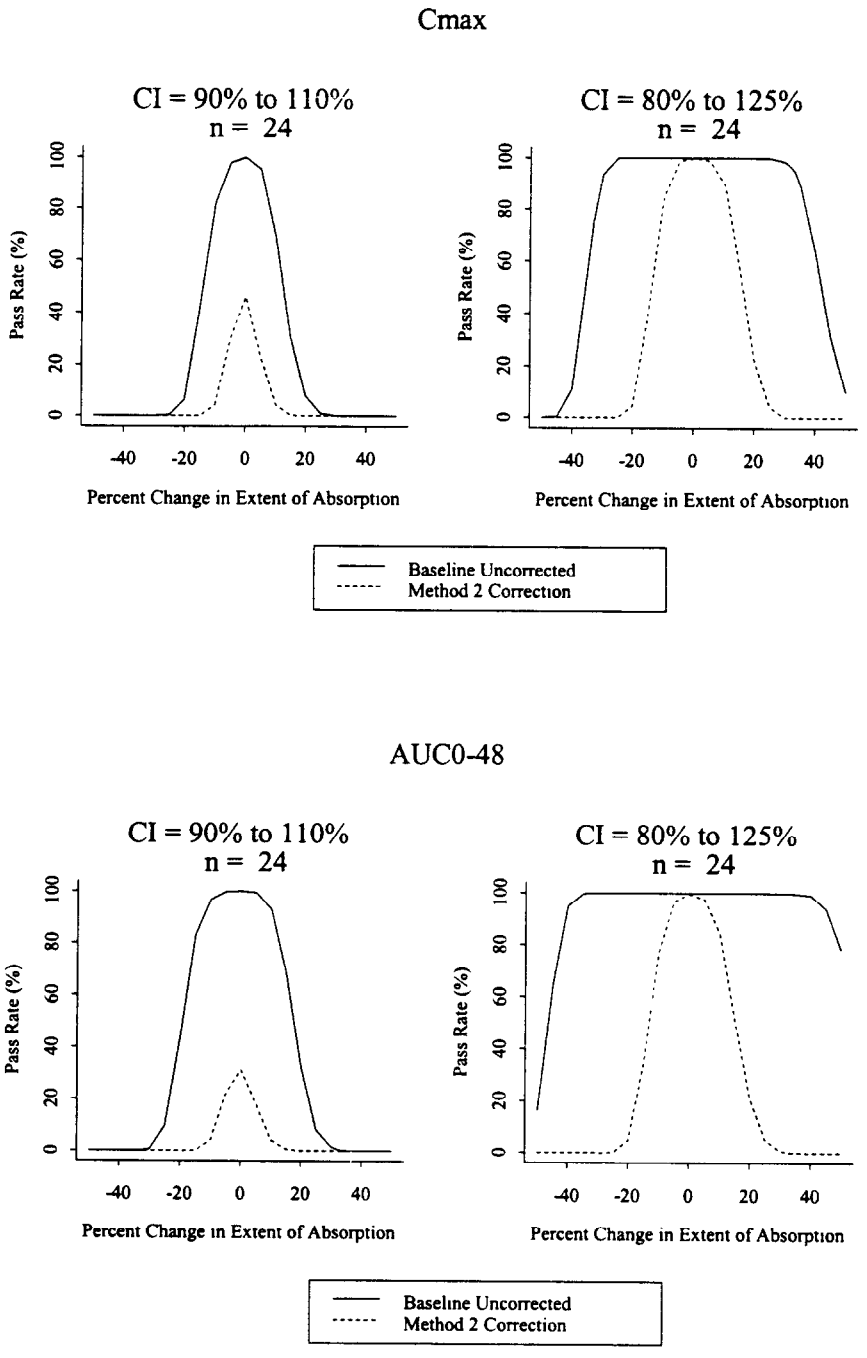
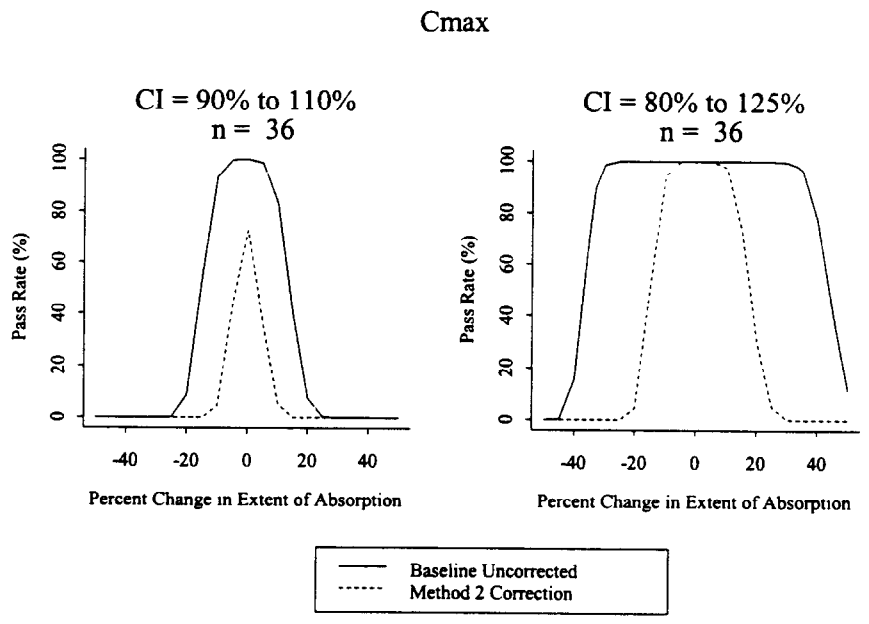


Figure 5: Percent of Simulated Trials that Pass Bioequivalence Criteria vs. Percent Change in Extent of Absorption for N = 36.



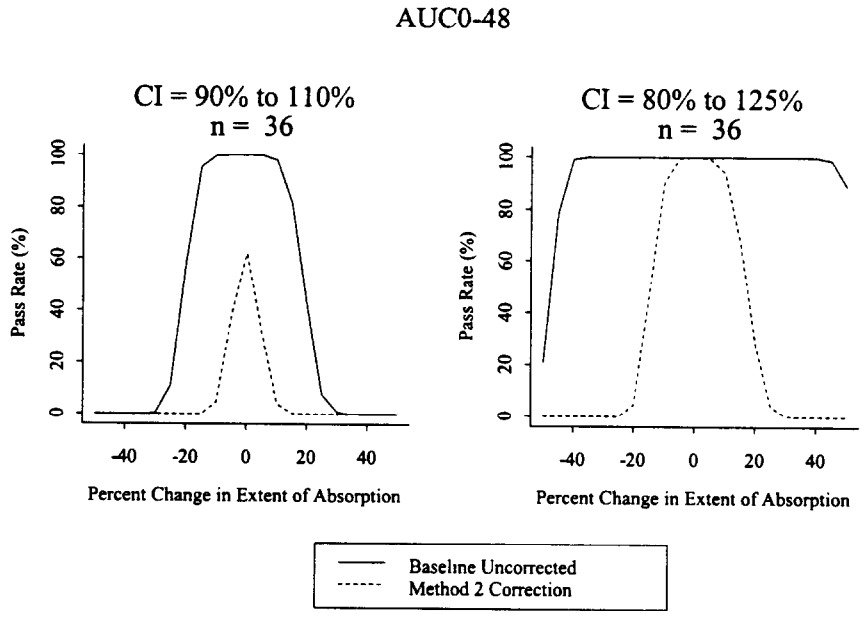
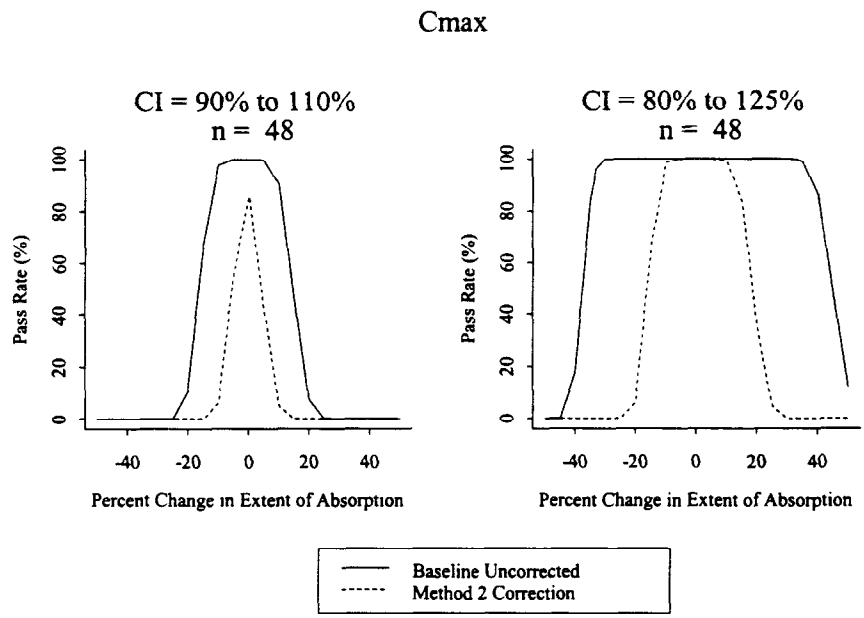
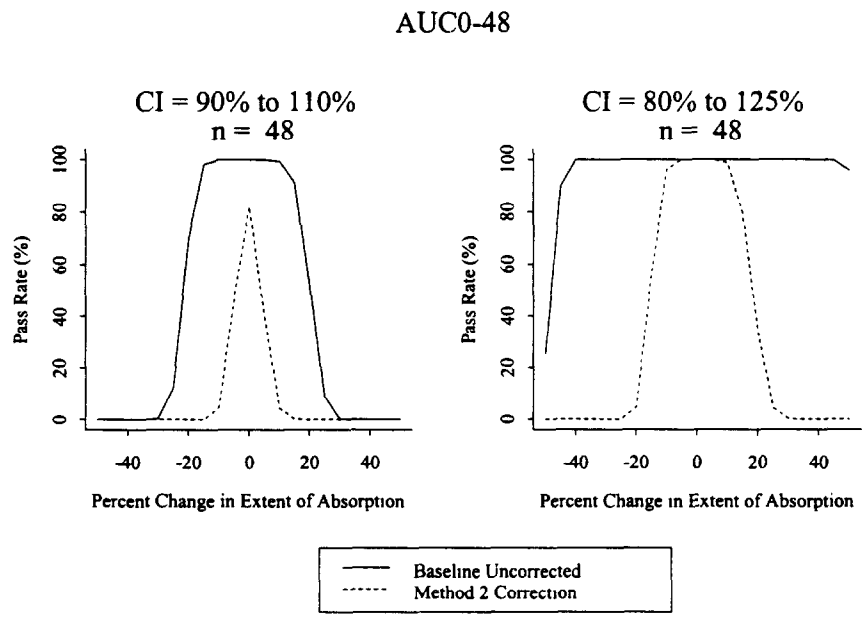


Figure 6: Percent of Simulated Trials that Pass Bioequivalence Criteria vs. Percent Change in Extent of Absorption for N = 48.





5.6 Conclusions

When a single dose of exogenous levothyroxine is given to healthy subjects, it is reasonable to expect that endogenous levothyroxine levels either remain approximately the same, if there is no suppression of endogenous production, or decline to some extent depending upon the degree of feedback suppression of levothyroxine production. If production is completely suppressed then the endogenous levothyroxine will decline at a maximum rate defined by its half-life, which is approximately 7 days. Thus, assuming a constant baseline of endogenous levothyroxine versus a baseline that decays exponentially with a 7 day half-life defines the limits for endogenous levothyroxine following a dose of exogenous levothyroxine. The simulation results provided in this report illustrate that given these very reasonable limiting assumptions about endogenous levothyroxine behavior in healthy subjects, the use of baseline uncorrected C_{max} and AUC_{0-48} values will result in an unacceptable probability of declaring two products bioequivalent when they actually differ by more than 20-25%. In fact, it is likely that products with differences in exogenous Cmax and AUC of 35% will be declared bioequivalent if bioequivalence is evaluated using baseline uncorrected parameters.

6 REFERENCES

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Appendix I

Simulation Analysis Plan

Simulation Plan for assessing alternative bioavailability calculations, study designs and acceptance criteria for determining the bioequivalence of levothyroxine sodium tablets.

Prepared February 12, 2002 by Thomas M. Ludden, Ph.D., GloboMax LLC

Objective: To explore via stochastic simulation 1) alternative approaches for the calculation of bioavailability parameters, 2) sample sizes and 3) acceptance criteria used to determine the bioequivalence of levothyroxine sodium tablets. These simulations will illustrate that baseline uncorrected levothyroxine concentration data from healthy volunteers is insensitive to changes in the extent of absorption of levothyroxine products. Products that exhibit a greater than 20% difference in extent of absorption will have a high probability of being deemed bioequivalent if the standard acceptance criteria of 90% confidence intervals falling within 80-125% is applied to baseline uncorrected data. The simulations will also provide information about the behavior of alternative choices for baseline correction of levothyroxine concentration data and the use of a narrower acceptance range, 90-110%.

Background: FDA guidance documents for levothyroxine sodium NDA's describe two biopharmaceutic studies in healthy subjects. One study (M01324) is a single dose, two way crossover study to compare the bioavailability of two x 300 mcg tablets to that of a 600 mcg dose administered as a solution. The second study (M01323) is a single dose, three way crossover study to assess the dosage form proportionality of 50, 100 and 300 mcg tablets. A total of 600 mcg as a single dose is administered as 12 x 50 mcg tablets, 6 x 100 mcg tablets and 2 x 300 mcg tablets on separate occasions. The suprapharmacologic dose of 600 mcg is administered in an effort to obtain levothyroxine concentrations above those normally present in healthy individuals. Data from studies performed as described in the FDA guidance, in spite of the large

dose, have yielded average maximum levothyroxine concentrations of about 14 mcg/dl, only twice the baseline, normal levothyroxine concentrations of 6 – 8 mcg/dl. The FDA instructed NDA applicants to use the baseline uncorrected data from these studies to estimate the bioavailability parameters, maximum observed concentration, Cmax, and area under the concentration-time curve 0 to 48 hours (AUC0_48). FDA is now recommending that this same procedure be applied to the assessment of bioequivalence. Given that a substantial portion of Cmax and AUC0_48 values calculated using baseline uncorrected data is related to normal, endogenous levothyroxine production, it is likely that these values will be much less sensitive to changes in bioavailability than is normally the case for bioequivalence assessment of nonendogenous compounds for which baseline levels are essentially zero. For example, in a recent study comparing 2 x 300 mcg Synthroid tablet to a 600 mcg Synthroid dose administered as a solution, baseline uncorrected Cmax and AUC0_48 values suggested that the solution and the tablets are bioequivalent as shown below.

Baseline uncorrected parameters

100%x Cmax_tablet/Cmax_solution = 84.2% (81.2 - 87.3, 90% CI)

100%x AUC0_48_tablet/AUC0_48_solution = 92.7% (90.2 – 95.2%)

However, if one corrects for the baseline levothyroxine concentrations based on the concentration present when the 600 mcg doses are administered, a different conclusion is reached. Levothyroxine concentrations were obtained at .5, .25 and 0 hr prior to dosing. If the average of these values is taken as representative of endogenous levothyroxine concentrations over the next 48 hours, then a baseline corrected Cmax can be calculated as the observed Cmax minus the baseline value and a baseline corrected AUC0_48 can be calculated as the baseline uncorrected AUC0_48 value minus the AUC0_48 due to endogenous levothyroxine, which is the baseline concentration x 48 hr. This way of correcting for endogenous levothyroxine is designated Method 1. These baseline corrected values, representing an estimate of the bioavailability of the exogenously administered levothyroxine, indicate that the tablet and solution are not bioequivalent.

Baseline corrected parameters (Method 1)

100% x Cmax_tablet/Cmax_solution = 71.7% (67.2 – 76.4%)

100% x AUC0_48_tablet/AUC0_48_solution = 79.6% (73.7 – 86.0%)

It is well known that levothyroxine concentrations are highly autoregulated in normal subjects. The levothyroxine concentrations resulting from the administration of the large, 600 mcg dose are likely to suppress to some extent endogenous levothyroxine production. However, even complete suppression of endogenous production would result in only a gradual decline in endogenous levothyroxine concentrations. This gradual decline is a result of the relatively long

half life for levothyroxine, about 7 days in normal subjects. This suggests a second approach to the correction for endogenous levothyroxine concentrations based on a half-life of 7 days, designated Method 2. A baseline corrected Cmax can be calculated as the observed Cmax minus the baseline value adjusted for exponential decay from time 0 to the time of maximum observed concentration, Tmax, assuming a half-life of 7 days.. A baseline corrected AUC0_48 can be calculated as the baseline uncorrected AUC0_48 value minus the AUC0_48 due to endogenous levothyroxine, which is calculated using the log-linear trapezoid rule assuming a half-life of 7days. The conclusion regarding the inequivalence of solution and tablet using this baseline correction (Method 2) is the same as that for values based on Method 1.

Baseline corrected parameters (Method 2)

100% x Cmax_tablet/Cmax_solution = 72.2 % (67.9 – 76.8%)

100% x AUC0_48_tablet/AUC0_48_solution = 82.6 (77.6 – 87.9%)

As applied above, Method 2 assumes that the Cmax values for observed and baseline corrected levothyroxine data occur at the same time; Tmax is the same for both measures. This is not necessarily the case. Therefore, the data from M01323 and the tablet data from M01324 were used to explore this question more fully. The baseline contribution to each observed concentration value, assuming exponential decay with a half-life of 7 days, was subtracted from the observed value to provide a baseline corrected value for each concentration. The maximum concentration for each individual and each dosing and its sampling time were identified and recorded. This approach for obtaining a baseline corrected Cmax value and the corresponding Tmax value will be referred to as Method 2b. Only 12 out of the135 Cmax values obtained in this way occurred at a time different from the Tmax for the observed Cmax. These 12 occurrences are summarized below. These Cmax values were very, very similar in essentially all cases to the Cmax values obtained by Method 2, which assumed that the baseline corrected Cmax values occurred at the time as those for the baseline uncorrected values.

			Cmax (mcg/dl)		Tmax (hr)	
Subject	Study	Treatment	Method2	Method2b	Method2	Method2b
108	323	A	4.27	4.33	2.5	12
114	323	A	6.55	6.58	1.5	2.5
115	323	C	6.05	6.08	1.5	10
118	323	C	5.99	6.00	1.5	2
201	323	A	6.87	6.89	2	2.5
215	323	B	5.60	5.71	2	6
215	323	C	4.45	4.46	2	2.5
216	323	B	8.53	8.55	2.5	3
218	323	C	6.99	7.01	1.5	2
114	324	A	5.00	5.02	2	3

214	324	B	7.68	7.68	1	1.5
215	324	A	3.49	3.68	4	36

These results suggest that using Method2 to obtain Cmax estimates is quite reasonable and the additional complexity introduced by Method 2b is not necessary for the purposes of the proposed simulations.

It should be noted that Methods 1 and 2 represent maximum and minimum baseline corrections, respectively. Method 1 represents no suppression of endogenous levothyroxine production and Method 2 represents complete suppression of endogenous levothyroxine production. Reality is probably somewhere in between.

The proposed simulations will further explore the behavior of the baseline uncorrected and baseline corrected parameters when used to assess bioequivalence.

Assumptions:

Simulations will be performed using Cmax and AUC0_48 parameters that are baseline corrected by Methods 1 and 2 as well as uncorrected Cmax and AUC0_48 values. To perform these simulations it is necessary to have estimates of between subject and within subject variability in these bioavailability parameters as well as the baseline concentrations. For Method 2 it is also necessary to include Tmax since this parameter is required to calculate the baseline uncorrected Cmax using baseline corrected Cmax and the BL corrected by exponential decay based up to Tmax. Estimates of between and within subject variability are ideally obtained from studies with replicated treatments, but data from replicated single dose studies of levothyroxine are unknown. Therefore, the results of the Synthroid dosage form proportionality study (MO1-323), which demonstrated bioequivalence across all three dosage forms based on baseline uncorrected and baseline corrected parameters (Method 1), were used as an alternative source for these variance estimates. Since simulations using baseline corrections according to Method 2 require the additional Tmax parameter and since this parameter is discrete rather than continuous (lower information content), it was decided to include the tablet data from study M01324 in the data set used as a bases for the simulation of Method 2. Since FDA guidance documents instruct the applicant to use the natural logarithm transformation of parameter values for assessing bioequivalence , a linear mixed effect model (MEM) was used to estimate between and within subject variability for each of the log transformed bioavailability parameters, baseline uncorrected and baseline corrected by Methods 1 and 2 as well as the log transformed baseline concentrations and, for Method 2, the log transformed Tmax value. Although the log transformed Tmax values have a skewed distribution, the transformation substantially decreased the asymmetry of the distribution. The results of these MEM analyses are provided in Table 2. Between and within subject correlations are provided in Table 3. These typical values (TV) and associated variance-covariance estimates will be used to simulate two way crossover bioequivalence trials assuming no period effect, no subject by formulation interaction and the same within subject variance-covariance for hypothetical test (T) and (R) products.

Simulations: The ability of the various uncorrected and corrected parameters to detect a range of differences (Δ's) between test and reference products will be examined. To simulate the effect of changes in the extent of absorption in the presence of significant endogenous concentrations, it is necessary to separate the contributions of the endogenous compound from the contributions of the exogenously administered compound. Only in this way is it possible to simulate changes in the extent of absorption of exogenous material in the presence of endogenous material. The simulations will be constructed in the following manner. [A mathematical description is provided in the Appendix.]

1. Specify typical values. Typical log transformed BL, log transformed baseline corrected (Method 1 or 2) Cmax and AUC0_48 values and, for Method 2, the log transformed Tmax value will be based on the MEM results provided in Table 2.

2. Obtain individual parameter values. Individual (subject specific) parameter values for the reference product will be obtained from the typical values and a set of random terms drawn from the appropriate between subject multivariate normal distribution. Adjust the baseline corrected Cmax and AUC0_48 values for the assumed difference in extent of absorption for the test product relative to the reference product.

3. Obtain dosing period-specific parameter values. Obtain dosing period-specific parameter values from the individual parameter values and a set of random terms drawn from the appropriate within subject multivariate normal distribution. The Cmax and AUC0_48 values represent the contribution of exogenous levothyroxine and would be the baseline corrected values expected to be calculated from the observed BL and the baseline uncorrected Cmax and AUC0_48 values (see Steps 4 and 5).

4. Obtain the contributions of endogenous levothyroxine to baseline uncorrected Cmax and AUC0_48 values. For Method 1, the contributions of endogenous levothyroxine to the total, observed Cmax and AUC0_48 values, are BL and BL*48, respectively, where BL is obtained from Step 3. For Method 2, the contribution of endogenous levothyroxine to the baseline uncorrected Cmax value is based on BL and discretized Tmax from Step 3 assuming exponential decay up to Tmax and a half-life of 7 days. The endogenous contribution (Method 2) to the baseline uncorrected AUC0_48 value is based on BL from Step 3 and the log-linear trapezoid formula assuming exponential decay with a half-life of 7 days. (see Appendix for details).

5. Obtain baseline uncorrected parameters. Add the contributions of exogenous levothyroxine (baseline corrected Cmax and AUC0_48 from Step 3) to the contributions of endogenous levothyroxine (Step 4) to obtain the baseline uncorrected Cmax and AUC0_48 values expected to be observed in a bioequivalence study.

In addition to the standard acceptance range for bioequivalence, 80-125%, a restricted range of 90-110% will be also be evaluated. The specific simulations to be performed are summarized in Table 4.

Evaluation: The primary result for each Case will be the percentage of 1000 replicates that meet the stipulated acceptance range (%pass). An informative way to present these results is by plotting %pass versus Δ for a given set of conditions [parameter set (uncorrected, method-1 or method-2), samples size and acceptance range].

Table 1
Dosage Form Proportionality Study
Estimates of Relative Bioavailability (%)

	Geomean	90% CI
Baseline Uncorrected		
Cmax		
A vs B	101.3	98.3 - 104.4
A vs C	103.8	100.7 - 107.0
C vs B	97.6	94.7 - 100.6
AUC0_48		
A vs B	101.0	98.8 - 103.3
A vs C	103.8	101.5 - 106.1
C vs B	97.4	95.3 - 99.5
Baseline Corrected - Method 1		
Cmax		
A vs B	102.6	96.4 - 109.2
A vs C	108.4	101.8 - 115.4
C vs B	94.7	89.0 - 100.7
AUC0_48		
A vs B	102.8	95.0 - 111.3
A vs C	112.9	104.3 - 117.9
C vs B	91.1	84.2 - 98.5
Baseline Corrected - Method 2		
Cmax		
A vs B	102.1	96.2 - 108.3
A vs C	108.2	101.9 - 114.8
C vs B	94.3	88.9 - 100.1
AUC0_48		
A vs B	102.6	96.4 - 109.2
A vs C	110.7	104.1 - 117.9
C vs B	92.6	87.1 - 98.6

A - Twelve 50 mcg Synthroid Tablets,
B - Six 100 mcg Synthroid Tablets,
C - Two 300 mcg Synthroid Tablets.

Table 2

Estimates of typical values and between and within subject variances for natural log transformed baseline concentrations and bioavailability parameters observed in study MO1-323

Parameter (P)	Typical Values		Between Subject		Within Subject	
	$\ln(P_{TV})$	$\exp(\ln(P_{TV}))$	variance ω^2_{bs}	approx. CV (%)	variance σ^2_{ws}	approx. CV(%)
Baseline Uncorrected (Study M01323)						
BL, mcg/dl	1.92	6.82	0.0284	16.9	0.00360	6.00
Cmax, mcg/dl	2.57	13.1	0.0173	13.2	0.00572	7.56
AUC0_48, (mcg/dl)*hr	6.17	478.2	0.0213	14.6	0.00385	6.20
Baseline Corrected – Method 1 (Study M01323)						
BL, mcg/dl	1.92	6.82	0.0284	16.9	0.00360	6.00
Cmax, mcg/d	1.82	6.17	0.0241	15.5	0.0244	15.6
AUC0_48, (mcg/dl)*hr	5.01	149.9	0.0393	19.8	0.0436	20.9
Baseline Corrected – Method 2 (Study M01323 and Tablet Data from Study M01324)						
BL, mcg/dl	1.92	6.82	0.0251	15.8	0.00357	5.97
Cmax, mcg/dl	1.83	6.23	0.0246	15.7	0.0227	15.1
AUC0_48, (mcg/dl)*hr	5.20	181.3	0.0259	16.1	0.0271	16.5
Tmax, hr	0.992	2.70	0.0138	11.7	0.288	53.7

Table 3

Correlation Coefficients

	Between Subject	**Within Subject**
Baseline Uncorrected		
BL and Cmax	0.8572	0.2644
BL and AUC0_48	0.9270	0.2713
Cmax and AUC0_48	0.9689	0.5988
Baseline Corrected – Method 1		
BL and Cmax	0.3524	-0.1750
BL and AUC0_48	0.3742	-0.3799
Cmax and AUC0_48	0.9098	0.6009
Baseline Corrected – Method 2		
BL and Cmax	0.3260	-0.1900
BL and AUC0_48	0.4746	-0.3345
BL and Tmax	0.0472	-0.2832
Cmax and AUC0_48	0.8746	0.5927
Cmax and Tmax	-0.2665	-0.2511
AUC0_48 and Tmax	-0.0513	0.0521

Table 4
Simulation Conditions

Case	Parameters	Δ's	Sample Size	Acceptance Range
IA1	Baseline Uncorrected	0 to .5 in steps of 0.05	24	80 – 125%
IA2				90 – 110%
IB1			36	80 – 125%
IB2				90 – 110%
IC1			48	80 – 125%
IC2				90 – 110%
IIA1	Baseline Corrected M1	0 to .5 in steps of 0.05	24	80 – 125%
IIA2				90 – 110%
IIB1			36	80 – 125%
IIB2				90 – 110%
IIC1			48	80 – 125%
IIC2				90 – 110%
IIIA1	Baseline Corrected M2	0 to .5 in steps of 0.05	24	80 – 125%
IIIA2				90 – 110%
IIIB1			36	80 – 125%
IIIB2				90 – 110%
IIIC1			48	80 – 125%
IIIC2				90 – 110%

Appendix to Simulation Analysis Plan

The simulation steps describe in the text are explicitly described below.

Step 1: Specify the typical value.

$LB_{TV} = \ln(BL_{TV})$

$LP_{R,TV} = \ln(P_{TV})$

$LT_{TV} = \ln(Tmax_{TV})$ (Method 2 only)

where:

BL_{TV} is the typical value of the baseline

P_{TV} is the typical value of either Cmax or AUC0_48 assuming a relative bioavailability of 1.

TmaxTV is the typical value of Tmax

ln is the natural logarithm function

The subscript R indicates the reference product .

Typical values for a specific method are those provided in Table 2.

Step 2: Obtain individual values.

$LB_i = LB_{TV} + \eta_{B,i}$

$LP_{T,i} = LP_{R,TV} + \ln(1-\Delta) + \eta_{P,i}$

$LP_{R,i} = LP_{R,TV} + \eta_{P,i}$

$LT_i = LT_{TV} + \eta_{T,i}$ (Method 2 only)

where:

LB_i, $LP_{T,i}$, $LP_{R,i}$ and LT_i subject-specific values (i'th subject) for log transformed endogenous baseline values, (exogenous) bioavailability parameter values (Cmax or AUC0_48) and Tmax, respectively. The subscript T indicates the test product.

η's are random variables that account for between subject variability, $\eta \sim MVN(0,\Omega_{bs})$. For each individual an η vector is generated. Information describing the between subject variance-covariance matrix, Ω_{bs}, is provided in Tables 2 and 3.

Δ is the fractional difference between Test and Reference products

Step 3: Obtain dosing period-specific values.

$LB_{i,a} = LB_i + \varepsilon_{B,i,a}$

$LB_{i,b} = LB_i + \varepsilon_{B,i,b}$

$LP_{T,i,a} = LP_{T,i} + \varepsilon_{P,i,a}$

$LP_{R,i,b} = LP_{R,i} + \varepsilon_{P,i,b}$

$LT_{i,a} = LT_i + \varepsilon_{T,i,a}$ (Method 2 only)

where:

$LB_{i,a}$ is the log transformed observed baseline concentration for dosing period "a".

$LB_{i,b}$ is the log transformed observed baseline concentration for dosing period "b".

$LP_{Ti,a}$ is the log transformed, baseline corrected parameter (Cmax or AUC0_48) for the test formulation (subject received test product in dosing period "a").

$LP_{Ri,b}$ is the log transformed, baseline corrected parameter (Cmax or AUC0_48) for the reference formulation (subject received reference product in dosing period "b").

$LT_{i,a}$ is the log transformed Tmax for dosing period "a".

$LT_{i,b}$ is the log transformed Tmax for dosing period "b".

ε's are random variables that account for within subject variability, $\varepsilon \sim MVN(0,\Sigma_{ws})$. The subscripts a and b indicate the different dosing periods. For each dosing period a separate vector of ε's is generated. Information describing the within subject variance-covariance matrix, Σ_{ws}, is provided in Tables 2 and 3.

These parameter values represent the contribution of exogenous levothyroxine and would be the baseline corrected values expected to be calculated from the observed BL, Tmax and baseline uncorrected Cmax and AUC0_48 values (see Steps 4 and 5).

Step 4: Obtain the contributions of endogenous levothyroxine.

Let $BL_{1,a} = \exp(LB_{1,a})$ where $BL_{1,a}$ is the simulated baseline for individual i at dosing period a.

Method 1: Stable baseline

The contribution of endogenous levothyroxine to baseline uncorrected Cmax is the observed baseline value, $BL_{1,a}$ or

$Cmax_{endo,1,i,a} = BL_{1,a}$.

The contribution of endogenous levothyroxine to baseline uncorrected AUC0_48 is the observed baseline value, $Bo_{i,a}$, multiplied by the AUC interval, 48 hours.

$AUC0_48_{endo,1,i,a} = BL_{1,a} \times 48$.

Method 2: Exponential decline

Let $Tmax_{i,a} = \exp(LT_{i,a})$ where $Tmax_{i,a}$ is the simulated baseline for individual i at dosing period a. This is a continuous variable that is converted to a discrete variable according to the following table. Comparison of the Simulated Percentage (n=4000) and the Actual Percentage (Study M01323 and Tablet data from Study M01324, n=135) indicate that this approach produces a reasonable representation of the observed Tmax distribution.

Continuous $Tmax_{1,a}$ (hr)	Discrete $Tmax_{i,a}$ (hr)	Simulated Percentage	Actual Percentage
<=.5	0.75	1.0	0
>.75 and <= 1.	1	3.6	1.5
> 1 and <= 1.6	1.5	13.6	14
>1.6 and <= 2.4	2	24.3	25.2
>2.4 and <= 2.95	2.5	14.5	17
>2.95 and <= 3.8	3	17.0	14
>3.8 and <= 5	4	13.3	13
> 5 and <= 6.5	6	8.2	5.9
> 6.5 and <= 8.5	8	3.4	2.2
> 8.5 and <= 12	10	1.6	3.0
> 12 and <= 20	18	0.4	2.2
> 20 and <= 26	24	<1	0
> 26	36	<1	1.5

The contribution of endogenous levothyroxine to baseline uncorrected Cmax is the observed baseline value adjusted for exponential decay to time Tmax.

$Cmax_{endo,2,1,a} = BL_{1,a} \times \exp(-0.693/(7 \times 24) \times \text{discrete } Tmax_{1,a})$

The contribution of endogenous levothyroxine to baseline uncorrected AUC0_48 can be calculated using the log-linear trapezoid rule. The sampling interval is 48 hours

$AUC0_48_{endo,2,1,a} =$
$(BL_{1,a} - BL_{1,a} \times \exp(-0.693/7 \times 2)) \times 48/\ln(BL_{1,a} / (BL_{1,a} \times \exp(-0.693/7 \times 2)))$

Step 5: Obtain baseline uncorrected parameters.

$P_{o,T,1} = P_{T,i,a} + P_{endo,1,a}$

or

$P_{o,R,1} = P_{R,1,a} + P_{endo,1,a}$

where $P_{o,T,1}$ and $P_{o,R,1}$ =are the observed, baseline uncorrected parameters (Cmax or AUC0_48) for test and reference products, respectively

Appendix IIa

Baseline Uncorrected NONMEM Code and Output: Synth_07

```
$PROBLEM Synthroid 323 Baseline Uncorrected Data get se's
$INPUT ID DV TYPE MDV L2 TRT PER TMAX CMAX AUC BL CMXH AUCH CMXE AUCE
$DATA m01323_03.dat IGNORE=C
$PRED
; LBL is lnBL
  LBL=THETA(1)+ETA(1)+EPS(1)
; LCX is lnCMAX
  LCX=THETA(2)+ETA(2)+EPS(2)
; LAX is lnAUC
  LAX=THETA(3)+ETA(3)+EPS(3)
  IYBL=0
  IYCX=0
  IYAX=0
  IF(TYPE.EQ.0)IYBL=1
  IF(TYPE.EQ.1)IYCX=1
  IF(TYPE.EQ.2)IYAX=1
  LPRD=IYBL*LBL+IYCX*LCX+IYAX*LAX
  Y=LPRD
  IPRD=EXP(LPRD)
$MSFI synth_04.msf
$COVR
```

```
$PROBLEM Synthroid 323 Baseline Uncorrected Data get se's
$INPUT ID DV TYPE MDV L2 TRT PER TMAX CMAX AUC BL CMXH AUCH CMXE AUCE
$DATA m01323_03.dat IGNORE=C
$PRED
; LBL is lnBL
  LBL=THETA(1)+ETA(1)+EPS(1)
; LCX is lnCMAX
  LCX=THETA(2)+ETA(2)+EPS(2)
; LAX is lnAUC
  LAX=THETA(3)+ETA(3)+EPS(3)
  IYBL=0
  IYCX=0
  IYAX=0
  IF(TYPE.EQ.0)IYBL=1
  IF(TYPE.EQ.1)IYCX=1
  IF(TYPE.EQ.2)IYAX=1
  LPRD=IYBL*LBL+IYCX*LCX+IYAX*LAX
  Y=LPRD
  IPRD=EXP(LPRD)
$MSFI synth_04.msf
$COVR
1NONLINEAR MIXED EFFECTS MODEL PROGRAM (NONMEM)    DOUBLE PRECISION NONMEM    VERSION V LEVEL 1.1
 DEVELOPED AND PROGRAMMED BY STUART BEAL AND LEWIS SHEINER

 PROBLEM NO.:         1
 Synthroid 323 Baseline Uncorrected Data get se's
0DATA CHECKOUT RUN:              NO
 DATA SET LOCATED ON UNIT NO.:    2
 THIS UNIT TO BE REWOUND:        NO
 NO. OF DATA RECS IN DATA SET:  735
 NO. OF DATA ITEMS IN DATA SET:  15
 ID DATA ITEM IS DATA ITEM NO.:   1
 L2 DATA ITEM IS DATA ITEM NO.:   5
 DEP VARIABLE IS DATA ITEM NO.:   2
 MDV DATA ITEM IS DATA ITEM NO.:  4
0LABELS FOR DATA ITEMS:
   ID      DV    TYPE     MDV      L2     TRT     PER    TMAX    CMAX
  AUC      BL    CMXH    AUCH    CMXE    AUCE
0FORMAT FOR DATA:
 (E4.0,E8.0,2E2.0,E4.0,2E2.0,E4.0,E5.0,E8.0,E5.0,E6.0,E7.0,2E7.0)

 TOT. NO. OF OBS RECS:     315
 TOT. NO. OF INDIVIDUALS:   36
0MODEL SPECIFICATION FILE INPUT
 EST. RESCALED:   NO
0COVARIANCE STEP OMITTED:    NO
 EIGENVLS. PRINTED:    NO
 SPECIAL COMPUTATION:  NO
```

```
 COMPRESSED FORMAT:     NO
1
```

0557

```
*************************************************************************************************************
********************                                                                      ********************
********************                 MINIMUM VALUE OF OBJECTIVE FUNCTION                  ********************
********************                                                                      ********************
*************************************************************************************************************
```

```
*************************************************      -1276.145      *************************************************
1
*************************************************************************************************************
********************                                                                      ********************
********************                    FINAL PARAMETER ESTIMATE                          ********************
********************                                                                      ********************
*************************************************************************************************************
```

```
THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

           TH 1      TH 2      TH 3
```

```
        1.92E+00  2.57E+00  6.17E+00


 OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

           ETA1      ETA2      ETA3

 ETA1
+        2.84E-02

 ETA2
+        1.90E-02  1.73E-02

 ETA3
+        2.28E-02  1.86E-02  2.13E-02


 SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

           EPS1      EPS2      EPS3

 EPS1
+        3.60E-03

 EPS2
+        1.20E-03  5.72E-03

 EPS3
+        1.01E-03  2.81E-03  3.85E-03

1
 ************************************************************************************************************************
 ********************                                                                                ********************
 ********************                          STANDARD ERROR OF ESTIMATE                            ********************
 ********************                                                                                ********************
 ************************************************************************************************************************


 THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

           TH 1      TH 2      TH 3
```

0558

```
         2.86E-02  2.31E-02  2.51E-02


 OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

            ETA1      ETA2      ETA3

 ETA1
+        1.03E-02

 ETA2
+        8.10E-03  6.39E-03

 ETA3
+        8.52E-03  6.78E-03  7.01E-03


 SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

            EPS1      EPS2      EPS3

 EPS1
+        5.75E-04

 EPS2
+        5.40E-04  9.45E-04

 EPS3
+        4.47E-04  7.00E-04  6.49E-04

1
 ************************************************************************************************************************
 *********************                                                                                  *********************
 *********************                          COVARIANCE MATRIX OF ESTIMATE                           *********************
 *********************                                                                                  *********************
 ************************************************************************************************************************

            TH 1      TH 2      TH 3      OM11      OM12      OM13      OM22      OM23      OM33      SG11      SG12      SG13
             SG22      SG23      SG33

 TH 1
+        8.20E-04
```

0559

```
 TH 2
+        5.36E-04  5.36E-04

 TH 3
+        6.43E-04  5.45E-04  6.29E-04

 OM11
+       -1.42E-04 -1.01E-04 -9.72E-05  1.06E-04

 OM12
+       -9.69E-05 -7.66E-05 -7.04E-05  8.13E-05  6.56E-05

 OM13
+       -1.02E-04 -7.80E-05 -7.26E-05  8.62E-05  6.86E-05  7.27E-05

 OM22
+       -7.25E-05 -6.40E-05 -5.81E-05  6.04E-05  5.02E-05  5.19E-05  4.09E-05

 OM23
+       -7.47E-05 -6.42E-05 -5.78E-05  6.54E-05  5.39E-05  5.63E-05  4.30E-05  4.60E-05

 OM33
+       -7.53E-05 -6.26E-05 -5.92E-05  6.79E-05  5.54E-05  5.87E-05  4.33E-05  4.69E-05  4.91E-05

 SG11
+       -2.17E-06 -1.44E-06 -1.98E-06 -8.13E-07 -7.12E-07 -7.33E-07 -3.95E-07 -5.21E-07 -6.03E-07  3.30E-07

 SG12
+       -4.65E-06 -1.26E-06 -2.48E-06 -7.18E-08 -3.82E-07 -3.48E-07 -8.59E-08 -1.67E-07 -2.91E-07  1.60E-07  2.92E-07

 SG13
+       -8.78E-07  3.33E-07 -3.49E-07 -1.94E-07 -2.34E-07 -2.32E-07 -2.06E-08 -1.27E-07 -1.73E-07  1.14E-07  1.52E-07  2.00E-07

 SG22
+       -6.84E-06 -1.41E-06 -2.71E-06 -1.81E-06 -1.88E-06 -1.83E-06 -1.46E-06 -1.52E-06 -1.58E-06  1.19E-07  2.14E-07  1.59E-07
          8.93E-07

 SG23
+       -2.11E-06 -1.31E-06 -8.41E-07 -9.64E-07 -7.62E-07 -7.90E-07 -4.68E-07 -6.43E-07 -7.75E 07  1.39E-07  1.35E-07  1.48E-07
          4.77E-07  4.90E-07

 SG33
+       -6.14E-06 -5.25E-06 -4.59E-06  1.51E-06  1.36E-06  1.34E-06  1.13E-06  1.04E-06  9.06E-07  9.12E-08  3.39E-08  9.72E-08
          2.45E-07  3.52E-07  4.21E-07

1
 ************************************************************************************************************************
 ********************                                                                                  ********************
```

0560

********** CORRELATION MATRIX OF ESTIMATE **********
********** **********
****

	TH 1	TH 2	TH 3	OM11	OM12	OM13	OM22	OM23	OM33	SG11	SG12	SG13
	SG22	SG23	SG33									
TH 1												
+	1.00E+00											
TH 2												
+	8.09E-01	1.00E+00										
TH 3												
+	8.96E-01	9.39E-01	1.00E+00									
OM11												
+	-4.81E-01	-4.23E-01	-3.76E-01	1.00E+00								
OM12												
+	-4.18E-01	-4.08E-01	-3.46E-01	9.74E-01	1.00E+00							
OM13												
+	-4.16E-01	-3.95E-01	-3.40E-01	9.80E-01	9.94E-01	1.00E+00						
OM22												
+	-3.96E-01	-4.32E-01	-3.63E-01	9.16E-01	9.70E-01	9.53E-01	1.00E+00					
OM23												
+	-3.84E-01	-4.09E-01	-3.40E-01	9.35E-01	9.81E-01	9.74E-01	9.92E-01	1.00E+00				
OM33												
+	-3.75E-01	-3.86E-01	-3.37E-01	9.40E-01	9.76E-01	9.83E-01	9.68E-01	9.88E-01	1.00E+00			
SG11												
+	-1.32E-01	-1.08E-01	-1.37E-01	-1.37E-01	-1.53E-01	-1.50E-01	-1.08E-01	-1.34E-01	-1.50E-01	1.00E+00		
SG12												
+	-3.01E-01	-1.01E-01	-1.83E-01	-1.29E-02	-8.72E-02	-7.55E-02	-2.49E-02	-4.55E-02	-7.69E-02	5.17E-01	1.00E+00	
SG13												
+	-6.86E-02	3.22E-02	-3.11E-02	-4.21E-02	-6.47E-02	-6.09E-02	-7.22E-03	-4.18E-02	-5.52E-02	4.45E-01	6.28E-01	1.00E+00
SG22												
+	-2.53E-01	-6.46E-02	-1.15E-01	-1.85E-01	-2.45E-01	-2.27E-01	-2.42E-01	-2.36E-01	-2.38E-01	2.18E-01	4.19E-01	3.77E-01
	1.00E+00											

```
 SG23
+       -1.05E-01 -8.08E-02 -4.79E-02 -1.33E-01 -1.34E-01 -1.32E-01 -1.05E-01 -1.35E-01 -1.58E-01  3.46E-01  3.58E-01  4.75E-01
          7.21E-01  1.00E+00

 SG33
+       -3.30E-01 -3.50E-01 -2.82E-01  2.26E-01  2.59E-01  2.42E-01  2.72E-01  2.35E-01  1.99E-01  2.45E-01  9.67E-02  3.35E-01
          4.00E-01  7.75E-01  1.00E+00

1
 ************************************************************************************************************************
 *********************                                                                          *********************
 *********************                  INVERSE COVARIANCE MATRIX OF ESTIMATE                   *********************
 *********************                                                                          *********************
 ************************************************************************************************************************


            TH 1      TH 2      TH 3      OM11      OM12      OM13      OM22      OM23      OM33      SG11      SG12      SG13
             SG22      SG23      SG33

 TH 1
+        1.77E+04

 TH 2
+        9.39E+03  3.55E+04

 TH 3
+       -2.33E+04 -4.07E+04  6.03E+04

 OM11
+       -4.70E+03  3.71E+03  1.20E+04  7.17E+05

 OM12
+       -4.13E+04 -1.84E+05  2.27E+05  2.17E+05  6.78E+06

 OM13
+        1.12E+05  1.95E+05 -3.18E+05 -1.72E+06 -6.85E+06  1.08E+07

 OM22
+       -1.31E+03 -1.07E+05  1.42E+05 -3.13E+05 -8.32E+05  1.71E+06  5.22E+06

 OM23
+        5.66E+04  3.87E+05 -4.88E+05  1.83E+05 -4.38E+06  3.47E+06 -6.88E+06  1.49E+07

 OM33
+       -1.24E+05 -2.95E+05  4.32E+05  9.14E+05  5.15E+06 -7.54E+06  1.33E+06 -7.63E+06  7.97E+06

 SG11
+       -1.32E+04  1.95E+04  4.82E+03  1.59E+05 -5.23E+04 -2.00E+05 -6.21E+04  3.92E+05 -1.50E+05  4.90E+06
```

```
 SG12
+        1.51E+05 -1.80E+05  1.08E+05 -6.17E+05  4.07E+06 -2.18E+06  1.59E+06 -6.83E+06  3.94E+06 -2.98E+06  1.62E+07

 SG13
+       -1.15E+05 -3.93E+04  6.01E+04 -1.37E+05 -1.08E+06  1.04E+06 -2.19E+06  4.29E+06 -2.09E+06 -5.42E+05 -7.99E+06  1.30E+07

 SG22
+        1.41E+05 -4.20E+04 -4.13E+04 -5.94E+05  1.17E+05  1.77E+06  2.51E+06 -3.43E+06 -2.31E+05  4.51E+05  2.31E+06 -1.90E+06
          5.88E+06

 SG23
+       -2.02E+05  1.42E+05 -6.42E+04  7.73E+05 -1.19E+04 -2.10E+06 -3.91E+06  6.51E+06 -1.18E+06  1.73E+05 -8.64E+06  3.24E+06
         -8.35E+06  2.30E+07

 SG33
+        1.49E+05 -7.48E+03 -1.09E+04  4.80E+05  5.04E+05 -1.66E+06  8.02E+05 -3.01E+06  2.67E+06 -1.24E+06  8.27E+06 -4.89E+06
          3.12E+06 -1.54E+07  1.75E+07
```

Appendix IIb

Baseline Correction Method 1 NONMEM Code and Output: Synth_05

```
$PROBLEM Synthroid 323 Baseline Correction Method 1 get se's
$INPUT ID DV TYPE MDV L2 TRT PER TMAX CMAX AUC BL CMXH AUCH CMXE AUCE
$DATA m01323_01.dat IGNORE=C
$PRED
; LBL is lnBL
  LBL=THETA(1)+ETA(1)+EPS(1)
; LCH is lnCMXH
  LCH=THETA(2)+ETA(2)+EPS(2)
; LAH is lnAUCH
  LAH=THETA(3)+ETA(3)+EPS(3)
  IYBL=0
  IYCH=0
  IYAH=0
  IF(TYPE.EQ.0)IYBL=1
  IF(TYPE.EQ.3)IYCH=1
  IF(TYPE.EQ.4)IYAH=1
  LPRD=IYBL*LBL+IYCH*LCH+IYAH*LAH
  Y=LPRD
  IPRD=EXP(LPRD)
$MSFI synth_01.msf
$COVR
```

```
NONLINEAR MIXED EFFECTS MODEL PROGRAM (NONMEM)    DOUBLE PRECISION NONMEM    VERSION V LEVEL 1.1
 DEVELOPED AND PROGRAMMED BY STUART BEAL AND LEWIS SHEINER

 PROBLEM NO.:         1
 Synthroid 323 Baseline Correction Method 1 get se's
0DATA CHECKOUT RUN:              NO
 DATA SET LOCATED ON UNIT NO.:    2
 THIS UNIT TO BE REWOUND:        NO
 NO. OF DATA RECS IN DATA SET:  735
 NO. OF DATA ITEMS IN DATA SET:  15
 ID DATA ITEM IS DATA ITEM NO.:   1
 L2 DATA ITEM IS DATA ITEM NO.:   5
 DEP VARIABLE IS DATA ITEM NO.:   2
 MDV DATA ITEM IS DATA ITEM NO.:  4
0LABELS FOR DATA ITEMS:
   ID      DV    TYPE     MDV      L2     TRT     PER    TMAX    CMAX
  AUC      BL    CMXH    AUCH    CMXE    AUCE
0FORMAT FOR DATA:
 (E4.0,E8.0,2E2.0,E4.0,2E2.0,E4.0,E5.0,E8.0,E5.0,E6.0,E7.0,2E7.0)

 TOT. NO. OF OBS RECS:     315
 TOT. NO. OF INDIVIDUALS:   36
0MODEL SPECIFICATION FILE INPUT
 EST. RESCALED:   NO
0COVARIANCE STEP OMITTED:    NO
 EIGENVLS. PRINTED:    NO
 SPECIAL COMPUTATION:  NO
 COMPRESSED FORMAT:    NO
```

```
*****************************************************************************************************************
********************                                                                      ********************
********************                    MINIMUM VALUE OF OBJECTIVE FUNCTION               ********************
********************                                                                      ********************
*****************************************************************************************************************


************************************************        -872.202        ************************************************
```

```
*********************************************************************************************************************
********************                                                                                  ********************
********************                                  FINAL PARAMETER ESTIMATE                        ********************
********************                                                                                  ********************
*********************************************************************************************************************


 THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

            TH 1      TH 2      TH 3

         1.92E+00  1.82E+00  5.01E+00


 OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

            ETA1      ETA2      ETA3

 ETA1
+        2.84E-02

 ETA2
+        9.22E-03  2.41E-02

 ETA3
+        1.25E-02  2.80E-02  3.93E-02


 SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

            EPS1      EPS2      EPS3

 EPS1
+        3.60E-03

 EPS2
+       -1.64E-03  2.44E-02

 EPS3
+       -4.78E-03  1.96E-02  4.36E-02
```

```
*****************************************************************************************************************
*******************                                                                          *******************
*******************                          STANDARD ERROR OF ESTIMATE                      *******************
*******************                                                                          *******************
*****************************************************************************************************************


THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

           TH 1      TH 2      TH 3

        2.86E-02  3.01E-02  3.90E-02


OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

           ETA1      ETA2      ETA3

 ETA1
+       1.01E-02

 ETA2
+       6.22E-03  6.56E-03

 ETA3
+       6.63E-03  9.09E-03  1.42E-02


SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

           EPS1      EPS2      EPS3

 EPS1
+       5.74E-04

 EPS2
+       9.76E-04  3.44E-03

 EPS3
+       1.49E-03  4.55E-03  7.70E-03
```

```
*********************************************************************************************************************
*********************                                                                                   *********************
*********************                          COVARIANCE MATRIX OF ESTIMATE                            *********************
*********************                                                                                   *********************
*********************************************************************************************************************
```

	TH 1	TH 2	TH 3	OM11	OM12	OM13	OM22	OM23	OM33	SG11	SG12	SG13
	SG22	SG23	SG33									
TH 1												
+	8.20E-04											
TH 2												
+	2.42E-04	9.07E-04										
TH 3												
+	3.08E-04	9.76E-04	1.52E-03									
OM11												
+	-1.48E-04	-5.49E-05	-2.40E-05	1.03E-04								
OM12												
+	-5.23E-05	-4.18E-05	-2.81E-05	5.10E-05	3.86E-05							
OM13												
+	-2.34E-05	-3.00E-05	-2.31E-05	4.38E-05	3.74E-05	4.39E-05						
OM22												
+	-4.07E-05	-7.26E-05	-1.00E-04	1.64E-05	1.16E-05	6.90E-06	4.30E-05					
OM23												
+	-3.13E-05	-8.75E-05	-1.33E-04	1.43E-05	7.86E-06	1.66E-06	5.25E-05	8.27E-05				
OM33												
+	-1.16E-05	-1.00E-04	-2.63E-04	3.55E-06	-3.36E-06	-5.80E-06	6.23E-05	1.11E-04	2.00E-04			
SG11												
+	-2.33E-06	-1.07E-06	-2.44E-06	-7.74E-07	-5.68E-07	-5.27E-07	4.53E-07	2.12E-07	-1.24E-07	3.29E-07		
SG12												
+	-9.38E-06	6.10E-06	6.24E-06	9.64E-07	-6.72E-07	-1.24E-06	1.66E-06	2.38E-06	2.01E-06	-5.03E-08	9.52E-07	
SG13												
+	-2.00E-06	1.16E-05	1.86E-05	2.11E-06	7.85E-07	1.05E-07	2.65E-07	-1.00E-06	-2.75E-06	-3.49E-07	7.70E-07	2.23E-06
SG22												

0569

```
+        1.48E-07 -1.36E-05 -1.11E-06 -8.85E-06 -4.57E-06 -3.25E-06 -5.19E-06 -7.12E-06 -9.87E-06  2.95E-07 -9.56E-07 -2.86E-07
          1.18E-05

 SG23
+        1.10E-05 -3.65E-05 -2.02E-05 -9.05E-06 -2.77E-06 -1.45E-06 -4.93E-06 -1.22E-05 -1.99E-05  7 88E-07 -2.11E-06 -2.36E-06
          1.00E-05  2.07E-05

 SG33
+       -2.66E-05 -1.14E-04 -1.20E-04 -1.59E-06  4.32E-06  4.70E-06  3.67E-06  2.56E-06 -3.68E-06  1.38E-06 -3.48E-06 -7.75E-06
          9.03E-06  2.73E-05  5.92E-05
```

****
****
****
****
****

CORRELATION MATRIX OF ESTIMATE

	TH 1	TH 2	TH 3	OM11	OM12	OM13	OM22	OM23	OM33	SG11	SG12	SG13
	SG22	SG23	SG33									
TH 1												
+	1.00E+00											
TH 2												
+	2.80E-01	1.00E+00										
TH 3												
+	2.76E-01	8.31E-01	1.00E+00									
OM11												
+	-5.08E-01	-1.80E-01	-6.08E-02	1.00E+00								
OM12												
+	-2.94E-01	-2.23E-01	-1.16E-01	8.10E-01	1.00E+00							
OM13												
+	-1.23E-01	-1.50E-01	-8.94E-02	6.52E-01	9.09E-01	1.00E+00						
OM22												
+	-2.17E-01	-3.68E-01	-3.92E-01	2.47E-01	2.84E-01	1.59E-01	1.00E+00					
OM23												
+	-1.20E-01	-3.19E-01	-3.75E-01	1.55E-01	1.39E-01	2.75E-02	8.81E-01	1.00E+00				
OM33												
+	-2.87E-02	-2.35E-01	-4.76E-01	2.48E-02	-3.82E-02	-6.18E-02	6.72E-01	8.61E-01	1.00E+00			
SG11												
+	-1.42E-01	-6.21E-02	-1.09E-01	-1.33E-01	-1.59E-01	-1.38E-01	1.20E-01	4.06E-02	-1.52E-02	1.00E+00		
SG12												
+	-3.36E-01	2.08E-01	1.64E-01	9.75E-02	-1.11E-01	-1.92E-01	2.59E-01	2.68E-01	1.45E-01	-8.98E-02	1.00E+00	
SG13												
+	-4.68E-02	2.58E-01	3.19E-01	1.39E-01	8.45E-02	1.06E-02	2.70E-02	-7.38E-02	-1.30E-01	-4.07E-01	5.28E-01	1.00E+00
SG22												

```
+        1.50E-03 -1.32E-01 -8.29E-03 -2.54E-01 -2.14E-01 -1.43E-01 -2.30E-01 -2.28E-01 -2.03E-01  1.50E-01 -2.85E-01 -5.56E-02
          1.00E+00

 SG23
+        8.44E-02 -2.66E-01 -1.14E-01 -1.96E-01 -9.79E-02 -4.79E-02 -1.65E-01 -2.94E-01 -3.09E-01  3.02E-01 -4.75E-01 -3.48E-01
          6.41E-01  1.00E+00

 SG33
+       -1.21E-01 -4.90E-01 -3.98E-01 -2.04E-02  9.02E-02  9.22E-02  7.28E-02  3.66E-02 -3.38E-02  3.12E-01 -4.64E-01 -6.74E-01
          3.41E-01  7.79E-01  1.00E+00
```

****
****
****
****
****

INVERSE COVARIANCE MATRIX OF ESTIMATE

	TH 1	TH 2	TH 3	OM11	OM12	OM13	OM22	OM23	OM33	SG11	SG12	SG13
	SG22	SG23	SG33									
TH 1												
+	3.82E+03											
TH 2												
+	-2.21E+02	8.65E+03										
TH 3												
+	-2.75E+02	-6.63E+03	6.60E+03									
OM11												
+	5.36E+03	6.88E+03	-6.68E+03	5.26E+04								
OM12												
+	7.10E+03	-1.61E+04	1.49E+04	-9.66E+04	4.97E+05							
OM13												
+	-9.27E+03	6.43E+03	-6.13E+03	2.63E+04	-2.85E+05	2.21E+05						
OM22												
+	8.85E+03	-1.05E+04	1.32E+04	7.19E+03	1.36E+04	-3.95E+04	2.35E+05					
OM23												
+	-1.32E+04	3.21E+04	-3.15E+04	2.27E+04	-1.62E+05	1.15E+05	-2.36E+05	3.93E+05				
OM33												
+	2.44E+03	-1.71E+04	1.70E+04	-2.10E+04	9.16E+04	-4.90E+04	5.39E+04	-1.46E+05	7.65E+04			
SG11												
+	3.18E+04	-4.75E+04	3.60E+04	-1.88E+04	2.24E+05	-1.06E+05	-1.06E+05	-2.20E+05	1.59E+05	5.25E+06		
SG12												
+	4.97E+04	-4.62E+04	2.76E+04	-6.65E+04	5.63E+05	-2.64E+05	1.38E+05	-4.64E+05	1.90E+05	1.61E+04	3.51E+06	
SG13												
+	2.21E+03	1.07E+04	-3.22E+03	3.92E+04	-2.83E+05	1.35E+05	-9.20E+04	6.38E+04	-2.46E+03	1.33E+06	-1.22E+06	2.09E+06
SG22												

```
+        1.44E+04 -1.35E+03  2.63E+03  1.62E+04  6.24E+04 -6.03E+04  1.06E+05 -1.26E+05  2.60E+04  6.24E+04  2.58E+05 -7.10E+04
         2.50E+05

 SG23
+       -2.11E+04  1.60E+04 -1.87E+04 -1.63E+04 -1.02E+04  5.47E+04 -1.91E+05  2.73E+05 -6.98E+04 -5.20E+05 -3.85E+04 -4.14E+05
         -2.42E+05  6.13E+05

 SG33
+        1.13E+04 -5.24E+03  9.88E+03  1.24E+04 -1.59E+04 -1.73E+04  8.21E+04 -1.36E+05  4.23E+04  2.97E+05  1.57E+04  4.01E+05
         8.82E+04 -3.15E+05  2.16E+05
```

Appendix IIc

Baseline Correction Method 2 NONMEM Code and Output: Synth_13

```
$PROBLEM Synthroid 323&4 tablet data Baseline Correction Method2 with Tmax
$INPUT ID STDY DV TYPE MDV L2 TRT PER TMAX CMAX AUC BL CMXH AUCH CMXE
    AUCE CME2 TMX2
$DATA method2_final.dat IGNORE=C
$PRED
; LBL is lnBL
  LBL=THETA(1)+ETA(1)+EPS(1)
; LCE is lnCMXE
  LCE=THETA(2)+ETA(2)+EPS(2)
; LAE is lnAUCE
  LAE=THETA(3)+ETA(3)+EPS(3)
; LT2 is lnTMAX
  LT2=THETA(4)+ETA(4)+EPS(4)
  IYBL=0
  IYCE=0
  IYAE=0
  IYT2=0
  IF(TYPE.EQ.0)IYBL=1
  IF(TYPE.EQ.5)IYCE-1
  IF(TYPE.EQ.6)IYAE=1
  IF(TYPE.EQ.9)IYT2=1
  LPRD=IYBL*LBL+IYCE*LCE+IYAE*LAE+IYT2*LT2
  Y=LPRD
  IPRD=EXP(LPRD)
$THETA
; lnBL
  1.5
; lnCE
  1.5
; lnAE
  5.5
; lnT2
  1.1
$OMEGA BLOCK (4)
 0.04
 0.001  0.04
 0.001 0.001 0.04
 0.001 0.001 0.001 0.04
$SIGMA BLOCK (4)
 0.04
 0.001 0.04
 0.001 0.001  0.04
```

```
 0.001 0.001  0.001  0.5
$EST MAXEVAL=9000 NSIG=4 METHOD=0 POSTHOC MSFO=synth_13.msf
$TABLE ID STDY TRT PER TYPE MDV LPRD NOPRINT ONEHEADER
     FILE=synth_13.tab
$COVR
```

```
NONLINEAR MIXED EFFECTS MODEL PROGRAM (NONMEM)    DOUBLE PRECISION NONMEM    VERSION V LEVEL 1.1
 DEVELOPED AND PROGRAMMED BY STUART BEAL AND LEWIS SHEINER

 PROBLEM NO.:          1
 Synthroid 323&4 tablet data Baseline Correction Method2 with Tmax
0DATA CHECKOUT RUN:                NO
 DATA SET LOCATED ON UNIT NO.:     2
 THIS UNIT TO BE REWOUND:         NO
 NO. OF DATA RECS IN DATA SET: 1660
 NO. OF DATA ITEMS IN DATA SET:  18
 ID DATA ITEM IS DATA ITEM NO.:   1
 L2 DATA ITEM IS DATA ITEM NO.:   6
 DEP VARIABLE IS DATA ITEM NO.:   3
 MDV DATA ITEM IS DATA ITEM NO.:  5
0LABELS FOR DATA ITEMS:
   ID     STDY      DV    TYPE     MDV      L2     TRT     PER    TMAX
 CMAX     AUC       BL    CMXH    AUCH    CMXE    AUCE    CME2    TMX2
0(NONBLANK) LABELS FOR PRED-DEFINED ITEMS:
 LPRD
0FORMAT FOR DATA:
 (2E3.0,E8.0,2E1.0,E3.0,2E1.0,E3.0,E4.0,E6.0,2E5.0,E6.0,4E6.0)

 TOT. NO. OF OBS RECS:      540
 TOT. NO. OF INDIVIDUALS:    97
0LENGTH OF THETA:  4
0OMEGA HAS BLOCK FORM:
  1
  1  1
  1  1  1
  1  1  1  1
0SIGMA HAS BLOCK FORM:
  1
  1  1
  1  1  1
  1  1  1  1
0INITIAL ESTIMATE OF THETA:
   0.1500E+01  0.1500E+01  0.5500E+01  0.1100E+01
0INITIAL ESTIMATE OF OMEGA:
 BLOCK SET NO.   BLOCK                                                          FIXED
        1                                                                         NO
                 0.4000E-01
                 0.1000E-02   0.4000E-01
                 0.1000E-02   0.1000E-02   0.4000E-01
                 0.1000E-02   0.1000E-02   0.1000E-02   0.4000E-01
0INITIAL ESTIMATE OF SIGMA:
 BLOCK SET NO.   BLOCK                                                          FIXED
        1                                                                         NO
                 0.4000E-01
```

0577

```
                 0.1000E-02   0.4000E-01
                 0.1000E-02   0.1000E-02   0.4000E-01
                 0.1000E-02   0.1000E-02   0.1000E-02   0.5000E+00
0ESTIMATION STEP OMITTED:           NO
 POP. ETAS OBTAINED POST HOC:      YES
 NO. OF FUNCT. EVALS. ALLOWED:    9000
 NO. OF SIG. FIGURES REQUIRED:       4
 INTERMEDIATE PRINTOUT:            YES
 ESTIMATE OUTPUT TO MSF:           YES
0COVARIANCE STEP OMITTED:    NO
 EIGENVLS. PRINTED:    NO
 SPECIAL COMPUTATION:  NO
 COMPRESSED FORMAT:    NO
0TABLES STEP OMITTED:    NO
 NO. OF TABLES:           1
0-- TABLE  1 --
 PRINTED:                NO
 HEADERS:               ONE
 FILE TO BE FORWARDED:   NO
0USER-CHOSEN ITEMS
 IN THE ORDER THEY WILL APPEAR IN THE TABLE:
   ID     STDY     TRT     PER    TYPE     MDV    LPRD
```

```
 MONITORING OF SEARCH:

0ITERATION NO.:     0     OBJECTIVE VALUE: -0.5038E+03     NO. OF FUNC. EVALS.:16
 CUMULATIVE NO. OF FUNC. EVALS.:   16
 PARAMETER:  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00
             0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00
             0.1000E+00  0.1000E+00  0.1000E+00  0.1000E+00
 GRADIENT:  -0.1309E+05 -0.1034E+05  0.3608E+05  0.6000E+03 -0.1926E+04 -0.4979E+02  0.4137E+02  0.4241E+01 -0.1134E+04  0.2455E+02
             0.4121E+01 -0.1049E+04 -0.2787E+01  0.7283E+02  0.2695E+03 -0.2433E+02  0.2522E+02  0.2570E+01  0.6147E+02 -0.1795E+00
             0.3417E+01 -0.5581E+02 -0.1627E+01  0.1041E+04
0ITERATION NO.:    48     OBJECTIVE VALUE: -0.1199E+04     NO. OF FUNC. EVALS.: 0
 CUMULATIVE NO. OF FUNC. EVALS.:  974
 PARAMETER:  0.1280E+00  0.1223E+00  0.9460E-01  0.9015E-01  0.7926E-01  0.1022E+01  0.1522E+01  0.1108E+00  0.7409E-01  0.2549E+01
            -0.7255E+00  0.3420E-01  0.9287E+00  0.5148E-01  0.2989E-01 -0.5725E+00 -0.1102E+01 -0.3037E+01  0.7406E-01  0.1818E+01
            -0.3412E+01  0.6361E-01  0.1792E+01  0.6780E-01
 GRADIENT:  -0.1464E-01 -0.2415E-02  0.4333E-01 -0.2855E-02  0.2036E-02  0.1188E-04 -0.1912E-04 -0.4865E-05 -0.8348E-03 -0.5556E-05
            -0.7161E-05  0.5809E-03  0.5338E-06  0.1413E-03  0.3020E-02 -0.6764E-04  0.4405E-04  0.6397E-06  0.7029E-03  0.1499E-04
            -0.1228E-05  0.3632E-02  0.1977E-05 -0.2108E-02
0MINIMIZATION SUCCESSFUL
 NO. OF FUNCTION EVALUATIONS USED:  974
 NO. OF SIG. DIGITS IN FINAL EST.:  4.6
```

0580

```
*****************************************************************************************************************
*******************                                                                      *********************
*******************                    MINIMUM VALUE OF OBJECTIVE FUNCTION               *********************
*******************                                                                      *********************
*****************************************************************************************************************


************************************************      -1198.599      ************************************************
```

```
*********************************************************************************************************************
********************                                                                                  ********************
********************                                  FINAL PARAMETER ESTIMATE                        ********************
********************                                                                                  ********************
*********************************************************************************************************************


THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

           TH 1      TH 2      TH 3      TH 4

        1.92E+00  1.83E+00  5.20E+00  9.92E-01


OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

           ETA1      ETA2      ETA3      ETA4

 ETA1
+       2.51E-02

 ETA2
+       8.10E-03  2.46E-02

 ETA3
+       1.21E-02  2.23E-02  2.59E-02

 ETA4
+       8.78E-04 -4.96E-03 -9.55E-04  1.38E-02


SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

           EPS1      EPS2      EPS3      EPS4

 EPS1
+       3.57E-03

 EPS2
+      -1.71E-03  2.27E-02
```

0581

```
 EPS3
+       -3.29E-03  1.47E-02  2.71E-02

 EPS4
+       -9.08E-03 -2.03E-02  4.46E-03  2.88E-01
```

```
*********************************************************************************************************************
********************                                                                                    ********************
********************                                STANDARD ERROR OF ESTIMATE                          ********************
********************                                                                                    ********************
*********************************************************************************************************************


THETA - VECTOR OF FIXED EFFECTS PARAMETERS   *********

           TH 1      TH 2      TH 3      TH 4

        2.03E-02  2.40E-02  2.49E-02  4.90E-02


OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS  ********

           ETA1      ETA2      ETA3      ETA4

ETA1
+       6.35E-03

ETA2
+       4.43E-03  6.12E-03

ETA3
+       4.70E-03  6.08E-03  7.02E-03

ETA4
+       7.10E-03  8.81E-03  6.64E-03  3.29E-02


SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS  ****

           EPS1      EPS2      EPS3      EPS4

EPS1
+       5.66E-04

EPS2
+       9.59E-04  3.24E-03
```

```
 EPS3
+        1.11E-03  3.35E-03  4.50E-03

 EPS4
+        4.10E-03  7.26E-03  9.35E-03  7.75E-02
```

0585

```
*****************************************************************************************************************
*******************                                                                                   *******************
*******************                          COVARIANCE MATRIX OF ESTIMATE                            *******************
*******************                                                                                   *******************
*****************************************************************************************************************
```

	TH 1 OM34	TH 2 OM44	TH 3 SG11	TH 4 SG12	OM11 SG13	OM12 SG14	OM13 SG22	OM14 SG23	OM22 SG24	OM23 SG33	OM24 SG34	OM33 SG44
TH 1												
+	4.11E-04											
TH 2												
+	1.08E-04	5.74E-04										
TH 3												
+	1.61E-04	4.78E-04	6.21E-04									
TH 4												
+	-5.61E-05	-2.54E-04	-1.81E-05	2.40E-03								
OM11												
+	-5.19E-05	-1.98E-05	-1.76E-05	5.63E-06	4.03E-05							
OM12												
+	-1.82E-05	-1.69E-05	-1.40E-05	1.27E-05	1.88E-05	1.96E-05						
OM13												
+	-1.67E-05	-1.50E-05	-1.17E-05	2.20E-05	2.01E-05	1.87E-05	2.21E-05					
OM14												
+	9.21E-06	1.09E-05	2.08E-05	1.99E-05	-1.59E-05	-1.33E-05	-1.01E-05	5.04E-05				
OM22												
+	-1.70E-05	-4.59E-05	-4.30E-05	6.09E-05	6.34E-06	4.04E-06	3.56E-06	-3.87E-06	3.74E-05			
OM23												
+	-1.53E-05	-3.56E-05	-3.70E-05	3.20E-05	8.81E-06	5.01E-06	4.77E-06	-5.09E-06	2.97E-05	3.69E-05		
OM24												
+	1.96E-06	6.16E-05	5.64E-05	-2.92E-05	-1.04E-05	-8.22E-06	-7.53E-06	7.40E-06	-1.04E-05	3.55E-06	7.76E-05	
OM33												
+	-7.42E-06	-2.37E-05	-5.63E-05	-3.85E-05	7.70E-06	3.54E-06	5.36E-06	-2.72E-06	2.45E-05	3.43E-05	2.36E-06	4.93E-05

```
 OM34
+        8.06E-06  2.62E-05 -1.83E-05 -7.93E-05 -1.14E-05 -4.42E-06 -2.83E-06  1.41E-05 -3.59E-06 -3.92E-06  2.77E-05  1.04E-05
          4.40E-05

 OM44
+        8.98E-05 -9.74E-05 -1.38E-04 -3.50E-04 -1.97E-05  1.07E-05  3.48E-06  8.24E-05  3.21E-06 -2.58E-05 -1.49E-04 -1.47E-05
          7.66E-06  1.09E-03

 SG11
+       -8.52E-07 -6.63E-07 -1.55E-06  3.64E-06 -5.58E-07 -2.49E-07 -2.31E-07  1.23E-06  4.66E-07  7.72E-08  7.96E-07 -1.78E-07
          9.42E-07 -2.34E-06  3.20E-07

 SG12
+       -5.10E-06  3.43E-06  2.04E-06 -1.07E-05  5.35E-07 -8.44E-07 -8.31E-07 -4.11E-07  9.54E-07  1.73E-06  1.95E-06  1.23E-06
          2.42E-07 -3.07E-06 -6.23E-08  9.19E-07

 SG13
+       -2.56E-06  4.05E-06  5.27E-06 -8.02E-06  1.13E-06  1.12E-07 -9.59E-08 -2.69E-06  6.08E-07  3.99E-07 -4.40E-07  2.92E-07
         -1.13E-07 -3.34E-06 -2.46E-07  5.09E-07  1.23E-06

 SG14
+        2.59E-09 -1.33E-05 -1.01E-05 -1.41E-05  1.99E-06  1.94E-06  1.47E-06 -4.64E-06 -8.37E-06 -6.60E-06 -7.71E-06 -4.86E-06
         -4.81E-06  1.85E-05 -1.07E-06 -1.37E-06  3.35E-07  1.68E-05

 SG22
+        1.67E-07 -8.76E-06 -3.27E-07  3.05E-05 -4.10E-06 -2.29E-06 -1.72E-06  4.75E-06 -5.29E-06 -4.51E-06  2.90E-06 -4.63E-06
          3.69E-06 -1.21E-05  1.00E-07 -8.08E-07 -2.69E-07  4.71E-07  1.05E-05
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	TH 1	TH 2	TH 3	TH 4	OM11	OM12	OM13	OM14	OM22	OM23	OM24	OM33
	OM34	OM44	SG11	SG12	SG13	SG14	SG22	SG23	SG24	SG33	SG34	SG44
SG23												
+	3.29E-06	-1.28E-05	-3.43E-06	5.56E-05	-3.88E-06	-3.09E-07	-1.01E-07	4.78E-06	-4.38E-06	-8.56E-06	-5.86E-06	-9.99E-06
	-2.32E-07	1.30E-05	4.70E-07	-1.61E-06	-1.23E-06	-7.62E-07	6.51E-06	1.12E-05				
SG24												
+	1.90E-05	1.20E-05	-6.07E-06	-7.84E-05	4.53E-06	5.59E-06	5.70E-06	-2.01E-06	-6.74E-06	-4.00E-06	-1.52E-05	2.83E-06
	-8.59E-06	8.24E-05	-9.75E-07	-9.91E-07	-2.07E-06	3.42E-06	-5.26E-06	5.69E-07	5.28E-05			
SG33												
+	-8.64E-06	-3.25E-05	-2.43E-05	9.14E-05	7.75E-07	3.70E-06	3.57E-06	2.49E-06	-2.29E-06	-4.26E-06	-5.77E-06	-9.70E-06
	-5.56E-06	1.37E-05	6.88E-07	-1.89E-06	-2.83E-06	-1.12E-06	5.02E-06	1.19E-05	3.33E-06	2.02E-05		
SG34												
+	2.36E-05	5.69E-05	3.54E-05	2.64E-05	-2.48E-06	-6.23E-06	-6.91E-06	-1.30E-05	-1.44E-05	-5.85E-06	2.59E-05	-5.46E-06
	-1.41E-05	-8.91E-05	-1.23E-06	-6.19E-07	-2.21E-06	4.05E-06	-4.05E-06	-5.89E-06	2.73E-05	2.89E-07	8.75E-05	
SG44												
+	-2.04E-04	1.57E-04	2.18E-04	1.75E-03	1.37E-05	-6.06E-05	-5.73E-05	-1.95E-04	2.09E-06	-9.56E-06	1.70E-04	-7.35E-05
	-9.50E-05	-1.85E-03	-2.11E-07	6.22E-07	9.85E-06	-2.37E-05	-1.60E-06	-1.61E-05	-2.02E-04	-6.54E-06	3.11E-04	6.01E-03

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CORRELATION MATRIX OF ESTIMATE

	TH 1 OM34	TH 2 OM44	TH 3 SG11	TH 4 SG12	OM11 SG13	OM12 SG14	OM13 SG22	OM14 SG23	OM22 SG24	OM23 SG33	OM24 SG34	OM33 SG44
TH 1												
+	1.00E+00											
TH 2												
+	2.23E-01	1.00E+00										
TH 3												
+	3.18E-01	8.00E-01	1.00E+00									
TH 4												
+	-5.65E-02	-2.16E-01	-1.48E-02	1.00E+00								
OM11												
+	-4.04E-01	-1.30E-01	-1.11E-01	1.81E-02	1.00E+00							
OM12												
+	-2.02E-01	-1.59E-01	-1.27E-01	5.83E-02	6.67E-01	1.00E+00						
OM13												
+	-1.76E-01	-1.34E-01	-9.98E-02	9.55E-02	6.74E-01	8.97E-01	1.00E+00					
OM14												
+	6.40E-02	6.40E-02	1.18E-01	5.72E-02	-3.52E-01	-4.21E-01	-3.02E-01	1.00E+00				
OM22												
+	-1.37E-01	-3.13E-01	-2.82E-01	2.03E-01	1.63E-01	1.49E-01	1.24E-01	-8.90E-02	1.00E+00			
OM23												
+	-1.24E-01	-2.44E-01	-2.44E-01	1.07E-01	2.29E-01	1.86E-01	1.67E-01	-1.18E-01	7.99E-01	1.00E+00		
OM24												
+	1.10E-02	2.92E-01	2.57E-01	-6.76E-02	-1.86E-01	-2.11E-01	-1.82E-01	1.18E-01	-1.92E-01	6.62E-02	1.00E+00	
OM33												
+	-5.21E-02	-1.41E-01	-3.22E-01	-1.12E-01	1.73E-01	1.14E-01	1.62E-01	-5.45E-02	5.71E-01	8.04E-01	3.82E-02	1.00E+00

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 OM34
+        5.99E-02  1.65E-01 -1.11E-01 -2.44E-01 -2.70E-01 -1.50E-01 -9.05E-02  2.98E-01 -8.83E-02 -9.71E-02  4.74E-01  2.22E-01
          1.00E+00

 OM44
+        1.34E-01 -1.23E-01 -1.69E-01 -2.17E-01 -9.41E-02  7.34E-02  2.25E-02  3.52E-01  1.59E-02 -1.29E-01 -5.12E-01 -6.36E-02
          3.50E-02  1.00E+00

 SG11
+       -7.43E-02 -4.89E-02 -1.10E-01  1.31E-01 -1.55E-01 -9.95E-02 -8.70E-02  3.07E-01  1.35E-01  2.25E-02  1.60E-01 -4.48E-02
          2.51E-01 -1.25E-01  1.00E+00

 SG12
+       -2.62E-01  1.49E-01  8.55E-02 -2.27E-01  8.80E-02 -1.99E-01 -1.84E-01 -6.04E-02  1.63E-01  2.97E-01  2.31E-01  1.83E-01
          3.81E-02 -9.71E-02 -1.15E-01  1.00E+00

 SG13
+       -1.14E-01  1.53E-01  1.91E-01 -1.48E-01  1.62E-01  2.27E-02 -1.84E-02 -3.42E-01  8.98E-02  5.92E-02 -4.51E-02  3.75E-02
         -1.54E-02 -9.16E-02 -3.93E-01  4.80E-01  1.00E+00

 SG14
+        3.12E-05 -1.36E-01 -9.90E-02 -7.03E-02  7.67E-02  1.07E-01  7.64E-02 -1.59E-01 -3.34E-01 -2.65E-01 -2.13E-01 -1.69E-01
         -1.77E-01  1.37E-01 -4.60E-01 -3.48E-01  7.39E-02  1.00E+00

 SG22
+        2.54E-03 -1.13E-01 -4.05E-03  1.92E-01 -1.99E-01 -1.59E-01 -1.13E-01  2.06E-01 -2.67E-01 -2.29E-01  1.02E-01 -2.03E-01
          1.72E-01 -1.13E-01  5.48E-02 -2.60E-01 -7.49E-02  3.55E-02  1.00E+00
```

	TH 1	TH 2	TH 3	TH 4	OM11	OM12	OM13	OM14	OM22	OM23	OM24	OM33
	OM34	OM44	SG11	SG12	SG13	SG14	SG22	SG23	SG24	SG33	SG34	SG44
SG23												
+	4.85E-02	-1.59E-01	-4.10E-02	3.39E-01	-1.82E-01	-2.08E-02	-6.44E-03	2.01E-01	-2.14E-01	-4.20E-01	-1.99E-01	-4.24E-01
	-1.04E-02	1.17E-01	2.48E-01	-5.02E-01	-3.31E-01	-5.55E-02	5.99E-01	1.00E+00				
SG24												
+	1.29E-01	6.91E-02	-3.35E-02	-2.20E-01	9.82E-02	1.74E-01	1.67E-01	-3.91E-02	-1.52E-01	-9.07E-02	-2.38E-01	5.56E-02
	-1.78E-01	3.45E-01	-2.37E-01	-1.42E-01	-2.57E-01	1.15E-01	-2.23E-01	2.34E-02	1.00E+00			
SG33												
+	-9.47E-02	-3.02E-01	-2.16E-01	4.15E-01	2.71E-02	1.86E-01	1.69E-01	7.79E-02	-8.32E-02	-1.56E-01	-1.45E-01	-3.07E-01
	-1.86E-01	9.27E-02	2.70E-01	-4.38E-01	-5.68E-01	-6.07E-02	3.44E-01	7.86E-01	1.02E-01	1.00E+00		
SG34												
+	1.25E-01	2.54E-01	1.52E-01	5.77E-02	-4.18E-02	-1.50E-01	-1.57E-01	-1.95E-01	-2.52E-01	-1.03E-01	3.14E-01	-8.31E-02
	-2.26E-01	-2.89E-01	-2.32E-01	-6.91E-02	-2.13E-01	1.06E-01	-1.33E-01	-1.88E-01	4.02E-01	6.86E-03	1.00E+00	
SG44												
+	-1.30E-01	8.43E-02	1.13E-01	4.62E-01	2.79E-02	-1.76E-01	-1.57E-01	-3.54E-01	4.42E-03	-2.03E-02	2.49E-01	-1.35E-01
	-1.85E-01	-7.25E-01	-4.81E-03	8.36E-03	1.15E-01	-7.47E-02	-6.35E-03	-6.20E-02	-3.59E-01	-1.88E-02	4.29E-01	1.00E+00

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****
********** INVERSE COVARIANCE MATRIX OF ESTIMATE **********
****
****

	TH 1	TH 2	TH 3	TH 4	OM11	OM12	OM13	OM14	OM22	OM23	OM24	OM33
	OM34	OM44	SG11	SG12	SG13	SG14	SG22	SG23	SG24	SG33	SG34	SG44
TH 1												
+	5.52E+03											
TH 2												
+	2.27E+03	1.13E+04										
TH 3												
+	-3.36E+03	-1.05E+04	1.33E+04									
TH 4												
+	-2.71E+02	5.73E+02	-5.18E+02	1.15E+03								
OM11												
+	3.35E+03	5.31E+02	-6.64E+02	1.44E+03	7.15E+04							
OM12												
+	1.44E+04	4.61E+03	-7.46E+03	-2.70E+03	-4.02E+04	4.77E+05						
OM13												
+	-6.26E+03	-7.79E+02	-2.46E+02	-2.16E+03	-3.34E+04	-2.84E+05	3.08E+05					
OM14												
+	7.27E+03	5.18E+03	-9.18E+03	-3.26E+03	-1.05E+04	8.94E+04	-2.67E+04	7.45E+04				
OM22												
+	1.20E+04	1.40E+04	-1.10E+04	-8.10E+02	-1.04E+04	7.56E+04	-3.08E+04	1.87E+04	2.40E+05			
OM23												
+	-2.07E+04	-1.79E+04	1.58E+04	-2.82E+03	2.80E+04	-1.78E+05	9.46E+04	-2.15E+04	-3.64E+05	8.06E+05		
OM24												
+	7.08E+03	1.43E+04	-1.64E+04	1.18E+03	7.28E+02	1.80E+04	-6.12E+03	-4.89E+03	1.00E+05	-2.10E+05	1.23E+05	
OM33												
+	8.21E+03	2.68E+03	1.16E+03	6.76E+02	-2.15E+04	1.03E+05	-5.62E+04	4.86E+03	1.46E+05	-4.05E+05	1.08E+05	2.67E+05

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 OM34
+        -1.10E+04 -2.40E+04  2.68E+04 -4.38E+02  1.81E+04 -5.91E+04  9.49E+03 -1.64E+04 -1.28E+05  2.97E+05 -1.35E+05 -1.60E+05
          2.10E+05

 OM44
+         8.19E+00  1.73E+01  5.08E+02 -1.69E+02  7.14E+02 -4.48E+03  5.60E+03 -6.34E+03  4.50E+03 -1.45E+04  1.33E+04  1.32E+04
         -1.22E+04  5.23E+03

 SG11
+         2.28E+04 -4.33E+04  6.12E+04 -7.63E+03 -3.60E+04  4.17E+04  1.13E+05 -8.61E+04  1.01E+05 -3.71E+05  9.92E+04  3.76E+05
         -1.83E+05  6.91E+04  7.89E+06

 SG12
+         5.48E+04 -9.54E+03  1.23E+04 -7.02E+03 -8.34E+04  5.52E+05 -1.01E+05  8.79E+04  2.76E+05 -4.87E+05  1.43E+04  2.91E+05
         -9.33E+04  9.99E+03  1.34E+06  4.28E+06

 SG13
+         1.36E+04  5.46E+03 -1.98E+04 -5.11E+03 -5.84E+04 -6.32E+04  5.62E+04  1.54E+05  7.81E+03 -1.14E+05  2 65E+04  8.39E+04
         -5.94E+04 -1.33E+04  1.06E+06 -1.22E+06  2.99E+06

 SG14
+         7.65E+03  2.56E+03  2.14E+03 -2.14E+03 -7.48E+03  4.68E+04 -4.24E+03  1.46E+04  6.09E+04 -5.82E+04  1.89E+04  4.02E+04
         -1.71E+04  3.26E+03  4.66E+05  4.60E+05 -6.65E+04  1.56E+05

 SG22
+         1.13E+04  1.30E+04 -8.03E+03 -1.01E+03 -1.03E+04  6.99E+04 -2.71E+04  2.02E+03  1.49E+05 -2.68E+05  7.61E+04  1.20E+05
         -1.16E+05  1.32E+04  4.05E+05  2.09E+05 -2.09E+03  3.15E+04  3.35E+05
```

```
            TH 1       TH 2       TH 3       TH 4       OM11       OM12       OM13       OM14       OM22       OM23       OM24       OM33
             OM34       OM44       SG11       SG12       SG13       SG14       SG22       SG23       SG24       SG33       SG34       SG44

 SG23
+        -1.30E+04 -1.04E+04  8.69E+02 -2.64E+03  3.24E+04 -1.45E+04  5.91E+04 -2.07E+04 -2.13E+05  4.90E+05 -8.43E+04 -1.95E+05
          1.25E+05 -1.69E+03 -2.28E+05  3.80E+05 -5.78E+05  3.77E+04 -3.61E+05  1.10E+06

 SG24
+         3.67E+03  3.48E+03 -3.17E+03 -1.36E+02 -2.97E+03  9.68E+03 -1.39E+04  7.39E+03  3.46E+04 -5.33E+04  3.25E+04  1.81E+04
         -2.70E+04  2.34E+03  1.19E+05  4.37E+03  6.97E+04  1.67E+04  6.16E+04 -1.04E+05  6.79E+04

 SG33
+         1.18E+04  3.12E+03  5.68E+03 -3.40E+03 -2.56E+04  1.82E+04 -3.73E+04  3.77E+04  1.29E+05 -2.90E+05  3.71E+04  1.46E+05
         -5.43E+04 -2.60E+03  1.28E+05 -5.74E+04  6.08E+05  1.79E+04  1.40E+05 -5.93E+05  4.91E+04  4.85E+05

 SG34
+        -6.10E+03 -1.20E+04  1.09E+04 -4.12E+02  7.61E+02 -1.26E+04  1.92E+04 -1.40E+03 -5.24E+04  1.18E+05 -6.48E+04 -5.56E+04
          7.53E+04 -5.62E+03  3.65E+04  1.63E+04  2.06E+04 -1.05E+04 -6.68E+04  1.32E+05 -4.81E+04 -6.23E+04  7.80E+04

 SG44
+         7.58E+02  1.38E+02 -7.87E+01 -5.79E+02 -1.26E+03  5.86E+03 -1.96E+02  2.53E+03  3.10E+03 -5.98E+03  3.82E+03  4.88E+03
         -4.25E+03  1.41E+03  2.22E+04  1.90E+04 -2.02E+03  4.17E+03  7.16E+03 -4.50E+03  4.42E+03  3.72E+03 -4.80E+03  1.22E+03
```

Appendix IIIa

Baseline Correction Method 1 Simulation Code

```
#     method1
# Modified 2/18/02 by TL j=3, i=8:23
# Documentation corrected 3/19/02 by TL
# replications
nreps <- 1000
# file name
filenm <- "D:\\Abbott\\Simulations\\"
# vector of samples sizes
nzz <- c(24,36,48)
#for (j in 1:length(nzz))
#{
j<-3
# select sample size
nzy <- nzz[j]
nsubs <- nzy
#vector of differences
# when zzz < 0 -> T < R, when zzz > 0 -> R < T
zzz <- c(-0.5,-0.45,-0.4,-0.35,-0.33,-0.3,-0.25,-0.2,-0.15,-0.1,-
0.05,0,0.05,0.1,0.15,0.2,0.25,0.3,0.33,0.35,
0.4,0.45,0.5)

#for (i in 1:length(zzz))
for (i in 8:23)
{
# run number
run <- i
#select difference
cdiff <- zzz[i]
# between subject var covr matrix for method 1 corrected data
bsvar <- matrix(nrow=3,ncol=3)
bsvar[,1] <- c(0.0284,0.00922,0.0125)
bsvar[,2] <- c(0.00922,0.0241,0.0280)
bsvar[,3] <- c(0.0125,0.0280,0.0393)
# within subject var covr matrix for method 1 corrected data
wsvar<-matrix(nrow=3,ncol=3)
```

```
wsvar[,1] <- c(0.00360,-0.00164,-0.00478)
wsvar[,2] <- c(-0.00164,0.0244,0.0196)
wsvar[,3] <- c(-0.00478,0.0196,0.0436)
# create typical value matrix for method 1 corrected data
# ln(BL), ln(CMAX), ln(AUC0_48)
typ <- rep(c(1.92,1.82,5.01),rep(nsubs,3))
dim(typ) <- c(nsubs,3)
# define p value
p <- .95
# create individual result matrix
lcmxo  <- matrix(nrow=nreps,ncol=9)
lcmxex <- matrix(nrow=nreps,ncol=9)
lauco  <- matrix(nrow=nreps,ncol=9)
laucex <- matrix(nrow=nreps,ncol=9)
# create summary matrix
xlist <-
c("run","cdiff","n2","lcmxon10","laucon10","lcmxon25","laucon25","lcmxen10","laucen10","lcmxen25","laucen25
")
xxx1  <- matrix(nrow=1,ncol=11)
# initialize counters
lcmxon10 <- 0
lcmxon25 <- 0
lcmxen10 <- 0
lcmxen25 <- 0
laucon10 <- 0
laucon25 <- 0
laucen10 <- 0
laucen25 <- 0
# set seed
set.seed(5389)
# set n2 equal to number of subjects
n2 <- nsubs
# calculate degrees of freedom
df <- n2-2
# set z = nreps and start replicates
z <- nreps
while(z){
# create data matrix
```

```
mlist <-
c("CID","TRT","SEQ","PD","BL","LBL","CMXEX","LCMXEX","CMXO","LCMXO","AUCEX","LAUCEX","AUCO","LAUCO")
y <- matrix(nrow=2*n2,ncol=14,dimnames=list(NULL,mlist))
# convert y to data frame and resave
yx <- as.data.frame(y)
y <- yx
# create ID, TRT, SEQ, and PD variables
y[,"CID"]  <- c(rep(seq(1,n2),2))
y[,"TRT"] <- c(rep(1,n2/2),rep(2,n2/2),rep(2,n2/2),rep(1,n2/2))
y[,"SEQ"] <- c(rep(1,n2/2),rep(2,n2/2),rep(1,n2/2),rep(2,n2/2))
y[,"PD"]  <- c(rep(1,n2),rep(2,n2))
# create individual deviations
xzbs <- rmvnorm(n2,mean=c(0,0,0),cov=bsvar)
# create individual's with treatment effect
xrws <- typ+xzbs
xtws <- typ+xzbs
# Adjust ln(Cmax) and ln(AUC) Test Product
xtws[,2:3] <- xtws[,2:3]+log(1+cdiff)
# calculate index values needed
n22 <- n2/2
n23 <- n2+n22+1
n24 <- 2*n2
n25 <- n22+1
n26 <- n2+1
n27 <- n2+n22
# create matrix for simulations
yhold <- matrix(nrow=n2*2,ncol=3)
#TRT=1 for test and TRT=2 for reference
yhold[1:n22,]   <- rmvnorm(n22,mean=xtws[1:n22,],cov=wsvar)
yhold[n23:n24,] <- rmvnorm(n22,mean=xtws[n25:n2,],cov=wsvar)
yhold[n25:n2,]  <- rmvnorm(n22,mean=xrws[n25:n2,],cov=wsvar)
yhold[n26:n27,] <- rmvnorm(n22,mean=xrws[1:n22,],cov=wsvar)
# create factors in matrix y
y[,"TRT"] <- factor(y[,"TRT"])
y[,"PD"]  <- factor(y[,"PD"])
y[,"SEQ"] <- factor(y[,"SEQ"])
y[,"CID"]  <- factor(y[,"CID"])
# simulated log transformed values for BL, AUCEX and CMXEX
```

0596

```
y[,"LBL"]    <- yhold[,1]
y[,"LCMXEX"] <- yhold[,2]
y[,"LAUCEX"] <- yhold[,3]
# untransformed values for BL, AUCEX and CMXEX
y[,"BL"]    <- exp(yhold[,1])
y[,"CMXEX"] <- exp(yhold[,2])
y[,"AUCEX"] <- exp(yhold[,3])
# calculation of observed auc and cmax values, method 1
y[,"AUCO"] <- y[,"BL"]*48 + y[,"AUCEX"]
y[,"CMXO"] <- y[,"BL"] + y[,"CMXEX"]
# log transformed values
y[,"LAUCO"] <- log(y[,"AUCO"])
y[,"LCMXO"] <- log(y[,"CMXO"])
# perform anova for log transformed observed Cmax, LCMXO
y1 <- aov(LCMXO~TRT+PD+SEQ+CID%in%SEQ,y)
y2 <- summary(y1)
# get error term
y3 <- y2[5,3]
# calculate standard error
y6 <- sqrt(as.numeric(y3)*2/n2)
# calculate means
trt <- y[,"TRT"]=="1"
y8  <- mean(y[trt,"LCMXO"])
y10 <- mean(y[!trt,"LCMXO"])
# calculate difference
y11 <- (y8-y10)
# get quantile
y12 <- qt(p,df)
# calculate lower 90% CL
ll <- exp(y11-y6*y12)
# calculate upper 90% CL
ul <- exp(y11+y6*y12)
# test for failure
nc10 <- 0
nc25 <- 0
if(ll<.9||ul>1.10){nc10 <- 1}
if(ll<.8||ul>1.25){nc25 <- 1}
# calculate index for lcmxo matrix
```

```
idx <- nreps-z+1
# save individual results in lcmxo
lcmxo[idx,] <- c(exp(y8),exp(y10),exp(y11),ll,ul,nc10,nc25,cdiff,nzy)
# count failures
lcmxon10 <- lcmxon10+nc10
lcmxon25 <- lcmxon25+nc25
# perform anova for log transformed, baseline corrected(M1) CMX, LCMXEX
y1 <- aov(LCMXEX~TRT+PD+SEQ+CID%in%SEQ,y)
y2 <- summary(y1)
# get error term
y3 <- y2[5,3]
# calculate standard error
y6 <- sqrt(as.numeric(y3)*2/n2)
# calculate means
trt <- y[,"TRT"]=="1"
y8  <- mean(y[trt,"LCMXEX"])
y10 <- mean(y[!trt,"LCMXEX"])
# calculate difference
y11 <- (y8-y10)
# get quantile
y12 <- qt(p,df)
# calculate lower 90% CL
ll <- exp(y11-y6*y12)
# calculate upper 90% CL
ul <- exp(y11+y6*y12)
# test for failure
nc10 <- 0
nc25 <- 0
if(ll<.9||ul>1.10){nc10 <- 1}
if(ll<.8||ul>1.25){nc25 <- 1}
# calculate index for lcmxex matrix
idx <- nreps-z+1
# save individual results in lcmxex
lcmxex[idx,] <- c(exp(y8),exp(y10),exp(y11),ll,ul,nc10,nc25,cdiff,nzy)
# count failures
lcmxen10 <- lcmxen10+nc10
lcmxen25 <- lcmxen25+nc25
# perform anova for log transformed observed AUC, LAUCO
```

```
y1 <- aov(LAUCO~TRT+PD+SEQ+CID%in%SEQ,y)
y2 <- summary(y1)
# get error term
y3 <- y2[5,3]
# calculate standard error
y6 <- sqrt(as.numeric(y3)*2/n2)
# calculate means
trt <- y[,"TRT"]=="1"
y8  <- mean(y[trt,"LAUCO"])
y10 <- mean(y[!trt,"LAUCO"])
# calculate difference
y11 <- (y8-y10)
# get quantile
y12 <- qt(p,df)
# calculate lower 90% CL
ll <- exp(y11-y6*y12)
# calculate upper 90% CL
ul <- exp(y11+y6*y12)
# test for failure
nc10 <- 0
nc25 <- 0
if(ll<.9||ul>1.10){nc10 <- 1}
if(ll<.8||ul>1.25){nc25 <- 1}
# calculate index for lauco matrix
idx <- nreps-z+1
# save individual results in lauco
lauco[idx,] <- c(exp(y8),exp(y10),exp(y11),ll,ul,nc10,nc25,cdiff,nzy)
# count failures
laucon10 <- laucon10+nc10
laucon25 <- laucon25+nc25
# perform anova for log transformed baseline corrected(M1) AUC, LAUCEX
y1 <- aov(LAUCEX~TRT+PD+SEQ+CID%in%SEQ,y)
y2 <- summary(y1)
# get error term
y3 <- y2[5,3]
# calculate standard error
y6 <- sqrt(as.numeric(y3)*2/n2)
# calculate means
```

```
trt <- y[,"TRT"]=="1"
y8  <- mean(y[trt,"LAUCEX"])
y10 <- mean(y[!trt,"LAUCEX"])
# calculate difference
y11 <- (y8-y10)
# get quantile
y12 <- qt(p,df)
# calculate lower 90% CL
ll <- exp(y11-y6*y12)
# calculate upper 90% CL
ul <- exp(y11+y6*y12)
# test for failure
nc10 <- 0
nc25 <- 0
if(ll<.9||ul>1.10){nc10 <- 1}
if(ll<.8||ul>1.25){nc25 <- 1}
# calculate index for laucex matrix
idx <- nreps-z+1
# save individual results in laucex
laucex[idx,] <- c(exp(y8),exp(y10),exp(y11),ll,ul,nc10,nc25,cdiff,nzy)
# count failures
laucen10 <- laucen10+nc10
laucen25 <- laucen25+nc25
# decrement loop counter
z <- z-1
}

# print data file for last replication
write.table(y,paste(filenm,"final.y.matrix.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames")

#
#     Make headers for each file on 1st iteration of j and i loops
#

if (j == 1 & i == 1)
{
#     LCMXO
header <- paste("Cmax.test","Cmax.ref","mean.diff","LCI90","UCI90","nc10","nc25","tx.diff","n",sep = ",")
```

0600

```
write.table(header,paste(filenm,"lcmxo.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = F)

#     LCMXEX
header <- paste("Cmax.test","Cmax.ref","mean.diff","LCI90","UCI90","nc10","nc25","tx.diff","n",sep = ",")
write.table(header,paste(filenm,"lcmxex.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = F)

#     LAUCO
header <- paste("AUC.test","AUC.ref","mean.diff","LCI90","UCI90","nc10","nc25","tx.diff","n",sep = ",")
write.table(header,paste(filenm,"lauco.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = F)

#     LAUCEX
header <- paste("AUC.test","AUC.ref","mean.diff","LCI90","UCI90","nc10","nc25","tx.diff","n",sep = ",")
write.table(header,paste(filenm,"laucex.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = F)

#     Summary file
header <- paste("Run","tx.diff","n","lcmxon10","laucon10","lcmxon25","laucon25","lcmxen10","laucen10",
"lcmxen25","laucen25",sep = ",")
write.table(header, paste(filenm,"sim.summary.m1.csv",sep = ""),sep = ",",dimnames.write =
"colnames",append = F)
}


#     Write files containing the results of each replicate
write.table(lcmxo,paste(filenm,"lcmxo.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = T)
write.table(lcmxex,paste(filenm,"lcmxex.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = T)
write.table(lauco,paste(filenm,"lauco.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = T)
write.table(laucex,paste(filenm,"laucex.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append = T)

#     Create summary file
xxx1[1,]<-c(run,cdiff,n2,lcmxon10,laucon10,lcmxon25,laucon25,lcmxen10,laucen10,lcmxen25,laucen25)
write.table(xxx1, paste(filenm,"sim.summary.m1.csv",sep = ""),sep = ",",dimnames.write = "colnames",append
= T)
} # end of i loop
#} # end of j loop
```

0601

Appendix IIIb

Baseline Correction Method 2 Simulation Code

```
# Method 2
# modified 2/18/02 j=1, i=16 to 23
#
#  NOTE:  Modifications made to this file allow simulations from zzz = 0.45 to -0.5
#         to be run and results added to existing files without rerunning previous
#         simulations.
#
#
# replications
nreps <- 1000
# file name
filenm <- "D:\\Abbott\\Simulations\\"
# vector of samples sizes
nzz <- c(24,36,48)
#for (j in 1:length(nzz))
#{
j<-1
# select sample size
nzy <- nzz[j]
nsubs <- nzy
#vector of differences
# when zzz < 0 -> T < R, when zzz > 0 -> R < T
zzz <- c(-0.5,-0.45,-0.4,-0.35,-0.33,-0.3,-0.25,-0.2,-0.15,-0.1,-0.05,0,0.05,0.1,0.15,0.2,0.25,0.3,0.33,0.35,
0.4,0.45,0.5)
#for (i in 1:length(zzz))
for (i in 16:23)
{
# run number
run <-  i
#select difference
cdiff <- zzz[i]
# between subject var covr matrix for method 2 corrected data
bsvar <- matrix(nrow=4,ncol=4)
bsvar[,1] <- c(0.0251,0.00810,0.0121,0.000878)
bsvar[,2] <- c(0.00810,0.0246,0.0223,-0.00496)
bsvar[,3] <- c(0.0121,0.0223,0.0259,-0.000955)
bsvar[,4] <- c(0.000878,-0.00496,-0.000955,0.0138)
```

0602

```
# within subject var covr matrix for method 2 corrected data
wsvar<-matrix(nrow=4,ncol=4)
wsvar[,1] <- c(0.00357,-0.00171,-0.00329,-0.00908)
wsvar[,2] <- c(-0.00171,0.0227,0.0147,-0.0203)
wsvar[,3] <- c(-0.00329,0.0147,0.0271,0.00446)
wsvar[,4] <- c(-0.00908,-0.0203,0.00446,0.288)
# create typical value matrix for method 2 corrected data
# ln(BL), ln(CMAX), ln(AUC0_48), ln(Tmax)
# means
typ <- rep(c(1.92,1.83,5.20,0.992),rep(nsubs,4))
dim(typ) <- c(nsubs,4)
# define p value
p <- .95
# create individual result matrix
lcmxo  <- matrix(nrow=nreps,ncol=9)
lcmxex <- matrix(nrow=nreps,ncol=9)
lauco  <- matrix(nrow=nreps,ncol=9)
laucex <- matrix(nrow=nreps,ncol=9)
# create summary matrix
xlist <- c("run","cdiff","n2","lcmxon10","laucon10","lcmxon25","laucon25","lcmxen10","laucen10","lcmxen25","laucen25")
xxx1  <- matrix(nrow=1,ncol=11)
# initialize counters
lcmxon10 <- 0
lcmxon25 <- 0
lcmxen10 <- 0
lcmxen25 <- 0
laucon10 <- 0
laucon25 <- 0
laucen10 <- 0
laucen25 <- 0
# set seed
set.seed(5389)
# set n2 equal to number of subjects
n2 <- nsubs
# calculate degrees of freedom
df <- n2-2
# set z = nreps and start replicates
z <- nreps
while(z){
# create data matrix
mlist <-
   c("CID","TRT","SEQ","PD","BL","LBL","CMXEX","LCMXEX","CMXO","LCMXO","AUCEX","LAUCEX","AUCO","LAUCO","TMAX","LTMAX")
y <- matrix(nrow=2*n2,ncol=16,dimnames=list(NULL,mlist))
```

```
# convert y to data frame and rename as y
yx <- as.data.frame(y)
y <- yx
# create CID, TRT, SEQ, and PD variables
y[,"CID"]  <- c(rep(seq(1,n2),2))
y[,"TRT"] <- c(rep(1,n2/2),rep(2,n2/2),rep(2,n2/2),rep(1,n2/2))
y[,"SEQ"] <- c(rep(1,n2/2),rep(2,n2/2),rep(1,n2/2),rep(2,n2/2))
y[,"PD"]  <- c(rep(1,n2),rep(2,n2))
# create individual deviations
xzbs <- rmvnorm(n2,mean=c(0,0,0,0),cov=bsvar)
# create individual's with treatment effect
xrws <- typ+xzbs
xtws <- typ+xzbs
#Adjust ln(Cmax) and ln(AUC) Test product
xtws[,2:3] <- xtws[,2:3]+log(1+cdiff)
# calculate index values needed
n22 <- n2/2
n23 <- n2+n22+1
n24 <- 2*n2
n25 <- n22+1
n26 <- n2+1
n27 <- n2+n22
# create matrix for simulations
yhold <- matrix(nrow=n2*2,ncol=4)
#TRT=1 for test and TRT=2 for reference
yhold[1:n22,]   <- rmvnorm(n22,mean = xtws[1:n22,] , cov=wsvar)
yhold[n23:n24,] <- rmvnorm(n22,mean = xtws[n25:n2,] , cov=wsvar)
yhold[n25:n2,]  <- rmvnorm(n22,mean = xrws[n25:n2,] , cov=wsvar)
yhold[n26:n27,] <- rmvnorm(n22,mean = xrws[1:n22,] , cov=wsvar)
# create factors in matrix y
y[,"TRT"] <- factor(y[,"TRT"])
y[,"PD"]  <- factor(y[,"PD"])
y[,"SEQ"] <- factor(y[,"SEQ"])
y[,"CID"]  <- factor(y[,"CID"])
# simulated log transformed values for BL, AUCEX and CMXEX
y[,"LBL"]    <- yhold[,1]
y[,"LCMXEX"] <- yhold[,2]
y[,"LAUCEX"] <- yhold[,3]
y[,"LTMAX"]  <- yhold[,4]
# untransformed values for BL, AUCEX and CMXEX
y[,"BL"]    <- exp(yhold[,1])
y[,"CMXEX"] <- exp(yhold[,2])
y[,"AUCEX"] <- exp(yhold[,3])
```

```
y[,"TMAX"]  <- exp(yhold[,4])
# make "TMAX" discrete
xtm <- y[,"TMAX"] <= .5
y[xtm,"TMAX"] <- .5
xtm <- y[,"TMAX"] > .5 & y[,"TMAX"] <= 1.
y[xtm,"TMAX"] <- 1
xtm<-y[,"TMAX"] > 1 & y[,"TMAX"] <= 1.60
y[xtm,"TMAX"] <- 1.5
xtm<-y[,"TMAX"] > 1.60 & y[,"TMAX"] <= 2.4
y[xtm,"TMAX"] <- 2
xtm<-y[,"TMAX"] > 2.4 & y[,"TMAX"] <= 2.9
y[xtm,"TMAX"] <- 2.5
xtm<-y[,"TMAX"] > 2.9 & y[,"TMAX"] <= 3.8
y[xtm,"TMAX"] <- 3
xtm<-y[,"TMAX"] > 3.8 & y[,"TMAX"] <=5
y[xtm,"TMAX"] <- 4
xtm<-y[,"TMAX"] > 5 & y[,"TMAX"] <= 6.5
y[xtm,"TMAX"] <- 6
xtm<-y[,"TMAX"] > 6.5 & y[,"TMAX"] <= 8.5
y[xtm,"TMAX"] <- 8
xtm<-y[,"TMAX"] > 8.5 & y[,"TMAX"] <= 12
y[xtm,"TMAX"] <- 10
xtm<-y[,"TMAX"] > 12 & y[,"TMAX"] <= 20
y[xtm,"TMAX"] <- 18
xtm<-y[,"TMAX"] > 20 & y[,"TMAX"] <= 26.
y[xtm,"TMAX"] <- 24
xtm<-y[,"TMAX"] > 26
y[xtm,"TMAX"] <- 36
# calculation of observed auc and cmax values, method 2
# calculate conc at 48 hr (2 days) post dose
c48 <- y[,"BL"]*exp(-.693/7*2)
# calculate contribution of endogenous level using log-linear trapezoid rule
y[,"AUCO"] <- (y[,"BL"]-c48)*48/log(y[,"BL"]/c48) + y[,"AUCEX"]
y[,"CMXO"] <- y[,"BL"]*exp(-.693/(7*24)*y[,"TMAX"]) + y[,"CMXEX"]
# log transformed values
y[,"LAUCO"] <- log(y[,"AUCO"])
y[,"LCMXO"] <- log(y[,"CMXO"])

# perform anova for log transformed observed Cmax, LCMXO
y1 <- aov(LCMXO~TRT+PD+SEQ+CID%in%SEQ,y)
y2 <- summary(y1)
# get error term
y3 <- y2[5,3]
```

```
# calculate standard error
y6 <- sqrt(as.numeric(y3)*2/n2)
# calculate means
trt <- y[,"TRT"]=="1"
y8 <- mean(y[trt,"LCMXO"])
y10 <- mean(y[!trt,"LCMXO"])
# calculate difference
y11 <- (y8-y10)
# get quantile
y12 <- qt(p,df)
# calculate lower 90% CL
ll <- exp(y11-y6*y12)
# calculate upper 90% CL
ul <- exp(y11+y6*y12)
# test for failure
nc10 <- 0
nc25 <- 0
if(ll<.9||ul>1.10){nc10<-1}
if(ll<.8||ul>1.25){nc25<-1}
# calculate index for lcmxo matrix
idx <- nreps-z+1
# save individual results in lcmxo
lcmxo[idx,] <- c(exp(y8),exp(y10),exp(y11),ll,ul,nc10,nc25,cdiff,nzy)
# count failures
lcmxon10 <- lcmxon10+nc10
lcmxon25 <- lcmxon25+nc25
# perform anova for log transformed, baseline corrected (M2) CMX, LCMXEX
y1 <- aov(LCMXEX~TRT+PD+SEQ+CID%in%SEQ,y)
y2 <- summary(y1)
# get error term
y3 <- y2[5,3]
# calculate standard error
y6 <- sqrt(as.numeric(y3)*2/n2)
# calculate means
trt <- y[,"TRT"]=="1"
y8 <- mean(y[trt,"LCMXEX"])
y10 <- mean(y[!trt,"LCMXEX"])
# calculate difference
y11 <- (y8-y10)
# get quantile
y12 <- qt(p,df)
# calculate lower 90% CL
ll <- exp(y11-y6*y12)
```