

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-181

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

**Office of Clinical Pharmacology and Biopharmaceutics
Pharmacometrics Review**

NDA/IND:	22-181
Compound:	Kuvan (sapropterin dihydrochloride, BH4, Phenoptin)
Submission Dates:	May 25, 2007
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Office of Clinical Pharmacology and Biopharmaceutics Pharmacometrics Review	1
1. Executive Summary	2
2. Introduction.....	3
2.1. Summary.....	3
2.2. Objective of the Analysis	4
3. Methods	4
3.1. Study Design	4
3.1.1. PKU-004 Substudy 02	4
3.2. Data	4
3.2.1. Pharmacokinetics	4
3.2.2. Data Checking	5
3.3. Pharmacokinetic Models.....	6
3.3.1. Structural Model.....	6
3.3.2. Covariate Model.....	6
3.3.3. Random Variance Models	7
3.4. Model Selection	7
3.4.1. Initial Model Selection.....	7
3.4.2. Final Model Selection	7
3.5. Software.....	8
4. Results and Discussion	8
4.1. Design Adequacy.....	8
4.2. Data Integrity	8
4.3. Base Model.....	10
4.3.1. Model description.....	10
4.3.2. Parameter estimation results	13
4.3.3. Goodness of fit.....	14
4.4. Model Selection	15
4.5. Final Model	18
4.5.1. Model description.....	18
4.5.2. Parameter estimation results	19
4.5.3. Goodness of fit.....	20
4.6. Model Qualification	21
5. Discussion.....	23
6. Labeling Recommendations.....	23
7. Appendice	23

1. EXECUTIVE SUMMARY

BioMarin has conducted a population pharmacokinetic (PK) study (PKU-004 substudy 002) to describe the PK characteristics of sapropterin dihydrochloride (Phenoptin or BH4) in patients with phenylketonuria (PKU) and to identify patient variables that may affect the PK variability. This was the only PK study conducted in the target patient population. The administered oral doses were 5, 10, and 20 mg/kg/day and BH4 concentrations were collected only at steady state. Seventy eight (78) of 80 PKU-004 subjects participated in the substudy and had their PK samples collected starting at Week 16, 20 and 22. Each subject had 4 samples collected that may be separated in 2 different occasions during Weeks 16 through 22. Two hundred and sixty five (265) evaluable plasma BH4 concentrations from 76 subjects were pooled and analyzed using population approach. The pharmacokinetic of BH4 was adequately described by a two compartment model with a short lag time (16.5 minutes) prior to first-order absorption, followed by biphasic elimination. A BASE term was included to account for the endogenous BH4. Total body weight was the only significant patient variable identified in this evaluation. Gender and age did not significantly affect the PK variability. The age effect was also determined by the empirical data evaluation. Estimates of total clearance and apparent volume of distribution of central compartment are 2100 L/h/70kg (53.9% BSV) and 8350 L/kg (55.7% BSV), respectively. The within-subject variability is 21.7% and the estimated mean terminal half-life is approximately 6.69 hours (range: 3.91 to 16.6 hours).

Reviewer's assessments:

- A BASE term was included in all final modeling steps to account for the endogenous BH4 level. However, this BASE term may not correctly reflect the baseline endogenous BH4 with the current PK sampling scheme. A more appropriate way to determine endogenous BH4 level and its variability is to have the several samples collected at various time points prior to drug administration on day 0 of the main PKU-004 study. Furthermore, the final estimate of BASE was 13.5 ng/mL. According to the sponsor's study report for Protocol 146-0402 (Determination of Tetrahydrobiopterin in Human Plasma by LC/MS/MS), the endogenous BH4 level was determined to be about 3.17 ng/mL. The endogenous BH4 estimate from population PK analysis was four times higher than that observed in study 146-0402. The BASE estimate seemed to be a scaling factor (i.e., approximately 13.5 ng/mL) that accounted for the remaining within-subject variability by shifting the individual predictions to be closer to the typical predictions. As a result, although the BASE term is significant in modeling aspects, the rationale for including this parameter and its interpretation are equivocal.
- The interpretation of the BASE term and determination of its appropriateness in the PK model are not completely resolved. Rather than endogenous BH4 levels, the BASE term seem to account for the dosing history uncertainty, compliance leading to higher BH4 accumulation than expected based on model that does not employ base model. Thus, the BASE term is an equivalent to a scaling factor.
- The effect of adding the BASE term on PK parameter estimates is significant. Further model exploration or optimization may be needed. The observed BH4 concentration-time profiles are, however, consistent with single-dose studies in that they appear to be close to zero at 24 hours. Therefore, the elimination half-life of Phenoptin in PKU patients is expected to be short. The data from PKU-005 and PKU-009 were used as supportive evidence. It is reasonable to assume that the pharmacokinetics of Phenoptin are similar between healthy subjects and patients.

2. RECOMMENDATIONS

Labeling claims made by the sponsor are acceptable.

- *The mean elimination half-life in PKU patients was approximately 6.7 hours (range 3.9 to 17 h), with values seen in healthy subjects.*

- A population pharmacokinetic analysis of sapropterin that included patients [REDACTED] showed no effect of age on sapropterin dihydrochloride pharmacokinetics.

3. INTRODUCTION

3.1. Summary

Sapropterin dihydrochloride (Phenoptin), a new molecular entity, is under investigation to reduce blood phenylalanine (Phe) levels [REDACTED] in patients with hyperphenylalaninemia (HPA) due to Phenylketonuria (PKU); [REDACTED]

Phenoptin is a synthetic preparation of the dihydrochloride salt of naturally occurring BH4, a cofactor for phenylalanine hydroxylase (PAH) enzyme that is responsible for converting Phe to tyrosine. The drug's primary pharmacologic action is to reduce blood Phe levels by stimulating the residual enzyme activity of the altered PAH in PKU patients and by supplementing BH4 level in patients with HPA due to defective BH4 biosynthesis. Phenoptin is formulated as an immediate-release tablet for oral administration. The PK and bioavailability of Phenoptin was explored in healthy volunteers in 2 Phase 1 studies (PKU-005 and PKU-009) using the same formulation and assay of BH4 as was used in the clinical studies. Both studies administered a single 10 mg/kg dose of Phenoptin to healthy volunteers, taken with water or orange juice as intact or dissolved tablets. Food effect was also investigated. In both studies, maximum concentration occurred at approximately 4 hours after single dose, t_{max} (range: 3-5 hours); elimination half-life ($t_{1/2}$) was also approximately 4 hours (range: 3-5 hours). Administration of Phenoptin as an intact tablet increased maximum concentration (C_{max}) and area-under-concentration-time curve (AUC) by approximately 20% compared to a dissolved tablet. Administration of Phenoptin as an oral solution with food increased C_{max} approximately 84% in water and 41% in orange juice.

BioMarin has conducted a population PK study (PKU-004 substudy 002). Population analysis was performed to describe the PK characteristics of Phenoptin in PKU patients and to identify patient factors that may affect the variability of Phenoptin PK. This was the only PK study conducted in the target patient population at doses of 5 mg, 10 mg, and 20 mg/kg/day with BH4 measurements only at steady state. The sponsor's analysis showed a short lag time prior to the appearance of measurable BH4 concentration, followed by rapid absorption, upon oral administration of Phenoptin. The PK of Phenoptin was adequately described by a two-compartment, first-order input model with first-order elimination. A BASE term was included to account for the endogenous levels of BH4. Total body weight was the only significant patient variable identified in this evaluation. Gender and age were not found to be significantly influential on Phenoptin clearance and/or volume of distribution. The final model had weight effect included in both clearance and volume of distribution. According to the final model, the mean $t_{1/2}$ was approximately 6.69 hours (range: 3.91 to 16.6 hours).

From this population PK analysis, the sponsor has included the following statements in the draft label (section 12.3 of draft labeling text):

- *The mean elimination half-life in PKU patients was approximately 6.7 hours (range 3.9 to 17 h), with values seen in healthy subjects.*
- *A population pharmacokinetic analysis of sapropterin that included patients [REDACTED] showed no effect of age on sapropterin dihydrochloride pharmacokinetics.*

3.2. Objective of the Analysis

The objectives of the population PK analysis were to characterize and identify factors that influence the PK and PK variability of Phenoptin in subjects with PKU.

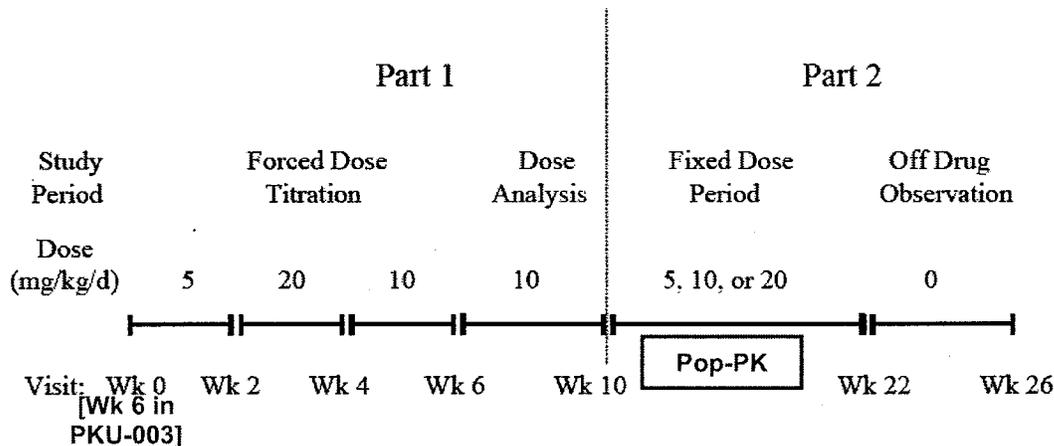
4. METHODS

4.1. Study Design

4.1.1. PKU-004 Substudy 02

This was an add-on population PK study of PKU-004, a Phase 3 open-label, 22-week, forced titration (5-20 mg/kg) trial in subjects with phenylketonuria (PKU) who had elevated phenylalanine levels. Study PKU-004 was an extension of PKU-003. Study PKU-003 was a randomized, double-blind, placebo-controlled pivotal trial to evaluate safety and efficacy of 10 mg/kg/day dose of Phenoptin in subjects with PKU for a duration of six weeks. Eighty (41 placebo, 39 drug) of 88 subjects in PKU-003 were enrolled in the main PKU-004 study. Week 0 for some PKU-004 subjects were their Week 6 in PKU-003. The schematic design for study PKU-004 is as shown in Figure 1. Population PK (substudy 02) was performed during the twelve-week fixed dose period (Weeks 10-22), during which subjects received a fixed dose (5, 10 or 20 mg/kg/day) according to their observed Phe levels at week 6. Collection of PK samples was performed during weeks 16-22. There were no specific exclusion or inclusion criteria for participation in the substudy.

Figure 1: Study Design (Source: CSR PKU-004)



4.2. Data

4.2.1. Pharmacokinetics

Seventy eight (78) out of 80 PKU-004 subjects agreed to participate in the substudy and had their PK samples collected starting at Week 16, 20 and 22. The substudy employed a sparse PK sampling scheme, which was based on optimal sampling design (D-optimal design). Each subject had 4 samples collected that could be separated in 2 different occasions during Weeks 16 through 22 (Table 1). Baseline (i.e., day 0, before first dose in PKU-004) BH4 levels were not collected. The PK database was consisted of 315 observations from 78 subjects, of which 265 plasma Phenoptin measurements from 76 subjects

were included in the population analysis. A total of 50 observations were omitted from the analysis for the following reasons:

- Below the limit of quantification (n=38, 12%)
- No dosing information (n=8, 2.5%)
- No samples recorded (n=4, 1.3%)

Covariate values were available for all 76 subjects; therefore, no imputation was needed.

Table 1: PK Collection Schedule (Source: CSR PKU-004 Substudy 02)

SUBJECT'S FIXED DOSE during weeks 10-22	PK COLLECTION TIME (during any of the weeks 16, 20, and/or 22)
5 mg/kg/day	SCHEDULE A
	Before dose
	0-6 minutes after dose
	1.2-3.7 hours after dose
	5.6-8.0 hours after dose
20 mg/kg/day	SCHEDULE B
	0-6 minutes after dose
	20-60 minutes after dose
	5-5.9 hours after dose
	7-8 hours after dose
10 mg/kg/day	May choose to follow Schedule A or Schedule B. * *Subject must adhere to a schedule once it has been chosen

4.2.2. Data Checking

Although it was not described in the study report, according to the data analysis plan, data validation checks were to be performed to identify potential errors in the data for the population PK analysis. Data checks for the potential errors would include, but not be limited to, missing data, inconsistencies between scheduled PK sample times and actual sample times, obvious errors in recording of dates and/or times (resulting in negative estimates of actual times, for example), physiologically unreasonable covariate values, and unit of measurement errors. Individual plasma concentration time profiles were examined for possible errors. In addition to validation checks, index plots of all population PK data variables were to be created and reviewed for possible outliers suggestive of errors in the dataset. The outliers were reviewed on an individual basis and either corrected, left as is, or excluded from the population analysis (based on availability of new data, or rules for handling data anomalies).

4.3. Pharmacokinetic Models

4.3.1. Structural Model

One, two and three compartment models with lag time and various input functions (i.e., first or zero order, or combination of both) were evaluated. The main PK parameters were clearance/fraction-of-bioavailability (CL/F) and volume of distribution of the central compartment/fraction-of-bioavailability (Vc/F). A nonlinear model with Michaelis-Menten kinetics was also tested.

4.3.2. Covariate Model

The demographic and laboratory covariates evaluated are listed in **Error! Reference source not found.** Creatinine clearance was computed using weight (WT) in the Cockcroft and Gault formula and added to the database during assembly. Body surface area was computed during model estimation step in NONMEM using the Dubois and Dubois formula. Race was not considered in covariate modeling because only 2 subjects were non-Caucasian.

Table 2: Summary of baseline demographics, N=78 (Source: CSR PKU-004 Substudy 02, sponsor's Table 11.1, page 41)

Demographic	Mean (standard deviation)	Median (range)	Number (percentage)
Age (years)	21.1 (9.64)	18 (9 – 50)	-
Weight (kg)	67.2 (21.8)	66.8 (28.2 – 144)	-
Body Surface Area (m ²)	1.72 (0.308)	1.73 (1.05 – 2.65)	-
Dose Group			
5 mg			6 (7.7)
10 mg	-	-	37 (47.4)
20 mg			34 (43.6)
Unknown			1 (1.3)
Race			
Caucasian	-	-	76 (97.4)
Non-Caucasian			2 (2.6)
Sex			
Males	-	-	45 (57.7)
Females			33 (42.3)

The only categorical variable, sex, was parameterized as,

$$TVP = \theta_{male} * SEX + \theta_{female} * (1 - SEX),$$

where TVP is the typical population parameter, θ_{male} and θ_{female} are parameter estimates for each gender (SEX=0 for male and 1 for female).

The continuous covariates were modeled in multiplicative power form:

$$TVP = \theta_{pop} * \prod_{i=1}^n \left[\frac{cov_{ij}}{mean(cov_i)} \right]^{\theta_i}$$

where θ_{pop} is the mean population value for TVP times the product of covariate effects for covariate i^{th} through n^{th} for the j^{th} subject; cov_{ij} represents the individual value for the i^{th} covariate normalized for the average value of that covariate; θ_i is the estimate of covariate effect.

4.3.3. Random Variance Models

The various residual error models evaluated were additive, proportional, and combined proportional additive. The between-subject variability (BSV) was assumed to be log-normally distributed and correlation between CL/F and Vc/F was allowed (i.e., full variance-covariance block). Covariance between other parameters such as absorption rate constant (Ka) and CL/F (and/or Vc/F) was also evaluated. Because some subjects had samples collected during both weeks 20 and 22, inter-occasion variability was also evaluated for CL/F. A baseline concentration (BASE) was assumed to reflect the endogenous Phenoptin level and accounted for in the residual error model.

4.4. Model Selection

4.4.1. Initial Model Selection

The first order conditional estimation method (FOCE) with interaction option of NONMEM was primarily used in modeling. Nested models were selected based on the likelihood ratio test (LRT) using NONMEM objective function value (OFV). Improvement in model goodness-of-fit (e.g., a full model versus a reduced one) was assessed primarily by a reduction in the OFV, which is approximately chi-squared distributed with the degree of freedom (df) equals to the difference in the number of parameters between reduced and full models. Other model assessment criteria were checking for visual improvements in the agreement between the observed and predicted plasma concentrations, reduction of the weighted residuals for the predicted concentrations, and reductions in BSV or residual variability.

The following criteria were used to evaluate the significance of a covariate effect:

- At least a 10-point decrease in the OFV from the base model ($p < 0.001$, $df=1$),
- Minimization of BSV and their imprecision,
- Reduction in the magnitude of the residual variability,
- Visual improvement in the prediction of the observed concentrations, and in the random scatter of weighted residuals around the line of identity in plots of weighted residuals versus predicted concentrations,
- Reduction of the asymptotic standard errors with respect to the parameter estimates

In addition, covariate factors had to have clinical or physiological relevance. If the magnitude of the change of the parameter due to a covariate influence was less than 20%, the covariate factor was not considered clinically relevant.

4.4.2. Final Model Selection

Model stability was evaluated using the condition number. The degree of collinearity of the parameter estimates was considered acceptable when the condition number is less than 20. Precision of parameter

estimates was determined by the 95% confidence intervals computed based on both the asymptotic standard errors of parameter estimates (produced by NONMEM covariance step), and the non-parametric bootstrapped samples. The predictive performance of the final model was evaluated by visual predictive check follows:

- 5,000 virtual subjects with weights simulated from the same distribution as in study PKU-004
- Steady-state concentrations were simulated for each of the 5,000 subjects over a 28.5 hour time period, all of whom received a 1000-mg dose.
- The distribution of simulated concentration profiles were then compared to the observed data through graphical visualization. All concentrations were dose-normalized.

4.5. Software

Nonlinear mixed effects modeling was implemented in NONMEM program (version V Level 1.1). Wings-for-NONMEM, a NONMEM programming interface, was used to invoke and automate bootstrap sampling in NONMEM. Diagnostic graphics, exploratory analyses, and post-processing of NONMEM output were performed using the S-Plus Professional Version 6.2 software.

5. RESULTS AND DISCUSSION

5.1. Design Adequacy

An informative PK sampling schedule is essential in population analysis of sparse data. A suboptimal sampling scheme can result in imprecise parameter estimates and model misspecification. A D-optimal design analysis was conducted using prior information (e.g., PK parameters and variability) to determine the optimal sampling times for PKU-004 substudy 02. Despite the usefulness of D-optimal design, the current sampling scheme was not 'optimal' due to some limitations:

- PK sampling times were designed to be only within the clinic visit time window (0-8 hours post dose). As thus, the PK sampling scheme did not cover the entire dosing interval. All PK data were collected within 8 hrs post dose at steady state, which may affect the estimates of the true terminal half lives and result in a wider range of estimate.
- The prior information was derived from non-compartmental analyses of legacy PK studies that utilized a different formulation and assay method.
- The samples were optimized using one-compartment models only. As thus, the parameters specific for two-compartment model (i.e., intercompartmental clearance, Q/F, and peripheral volume, V_p/F) could not be estimated with good precision.

5.2. Data Integrity

The quality of the analysis dataset appears adequate with a few minor errors.

- Although the report indicated that several subjects in the dose groups 5 and 10 mg/kg had PK samples collected at the end of dosing intervals, the reviewer's examination of the dataset showed that they were actually pre-dose concentrations (i.e., measurements prior to dosing on PK collection day). The sponsor treated these concentrations as those collected at the end of dosing interval in all of "time after dose" plots, which could be misleading because they assumed the exact dosing times for these concentrations were known and accurate; note that this had no impact on the PK analysis because clock-times (i.e., relative time to first dose) were used in the analysis, not the time-after-dose..

- Unlike the rest of the subjects, time 0 for IDs #15 and 57 started from beginning of week 12 or 16. This deviation would not affect the estimation of parameters because the first samples for these two subjects were collected when steady state had been reached, although the data points appeared to be earlier and misleading in the model diagnostic plots.

Table 3: Summary of data deviations (Source: CSR PKU-004 Substudy 02 dataset)

Subject ID	Remarks	Potential Impact on PK Analysis
15	No dose records prior to week 16; dose before first PK sample was approximately 26 days after week 16 visit	None since the first sample collected at steady-state
53	No actual dose time prior to 08/07 samples (week 22); date recording error is suspected because sampling times in clock hours seemed to follow the assigned Schedule A.	Minor since this was only one observation (out of 265) from 1 subject. If assuming the date/time of sampling is correct, then actual date/time of dose record is required; otherwise this observation should have been excluded.
57	No dose records prior to week 12; dose before first PK sample was in week 16	None since the first sample collected at steady-state

Figure 2: Plasma BH4 concentrations for all subjects (N=76)

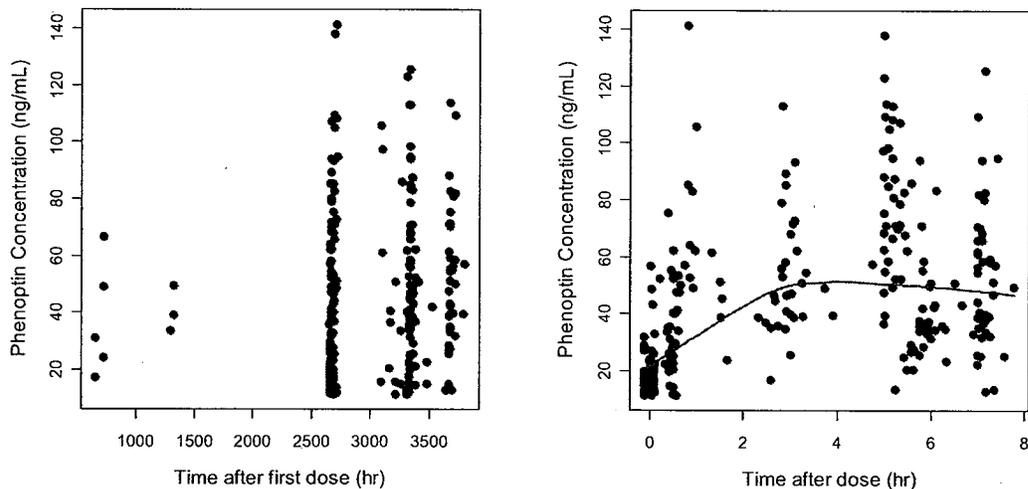
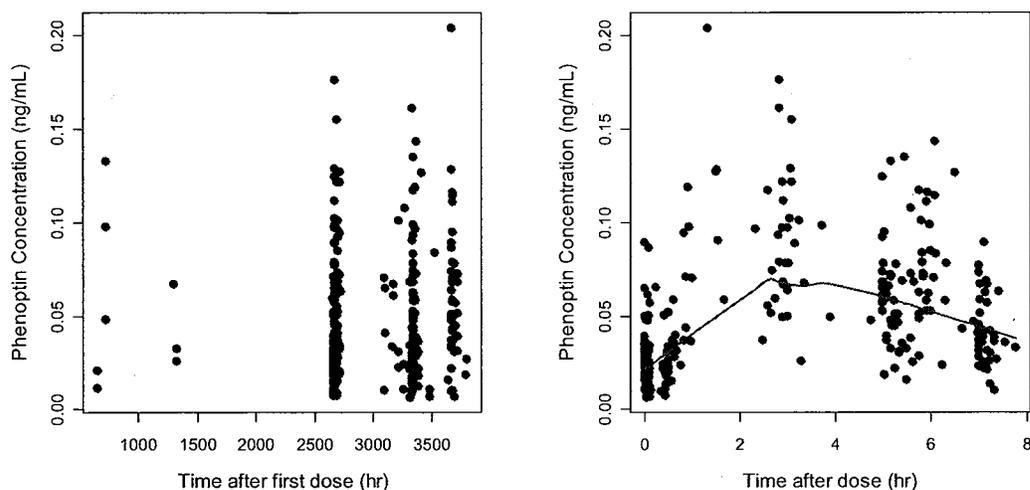


Figure 3: Dose-normalized plasma BH4 concentrations for all subjects (N=76)



According to plasma concentration-time profiles, some subjects appeared to have samples collected outside of the PK study period (i.e., 600-1400 hr or week 4-8 after first dose). These data points belonged to Subject IDs 15 and 57 and were actually determined during weeks 16-22 of study PKU-004; they appeared early in the plot because of the missing dose records prior to the PK study period as explained above. Concentrations were well above the lower limit of quantification (LLOQ, 5 ng/mL) at 8 hours post dose. There were 21 subjects with detectable pre-dose concentrations on PK collection days.

Although not clearly explained in the report, dosing times during the weeks prior to the dose on the PK collection day were not exact and assumed to be at 8 AM for all subjects. Where actual dosing times were not recorded, they were assumed to be at 8 AM. Dose compliance was assessed based on patients' diaries and counts of pills. Dosing times on the day of sample collection appear to be valid and at steady-state; therefore, the assumed times of previous doses are not expected to have an impact on the analysis.

5.3. Base Model

5.3.1. Model description

The Base Model selected was a two compartment linear model with first-order input following a lag time, a baseline endogenous concentration, and linear elimination. The BSV were included in CL/F and Vc/F and their correlation was accounted for. A proportional residual error model was determined to best describe the Base Model. The NONMEM model was parameterized as follows:

Lag time prior to absorption:	$ALAG = \theta_1$
First-order absorption rate constant:	$Ka = \theta_2$
CL/F with BSV:	$CL/F = \theta_3 \cdot \exp(\eta_1)$
Vc/F with BSV:	$CL/F = \theta_4 \cdot \exp(\eta_2)$
Volume of distribution of the peripheral compartment:	$Vp/F = \theta_5$
Inter-compartmental clearance:	$Q/F = \theta_6$

Baseline endogenous BH4 concentration:

BASE = 0₇

This reviewer disagreed with the Base Model selection for the following reasons:

- The BASE term may not correctly reflect the baseline endogenous Phenoptin with the current PK sampling scheme. The most appropriate way to determine endogenous Phenoptin level and its variability is to have the several samples collected at various time points prior to drug administration on day 0 of the main PKU-004 study. There were no such data available. Furthermore, the BASE term was modeled as a constant additive component to the individual predicted concentration without a variability component. The estimate of BASE was [REDACTED]; however, the report did not explain whether this estimate was in agreement with the literature value. According to sponsor's study report for Protocol 146-0402 (Determination of Tetrahydrobiopterin in Human Plasma by LC/MS/MS), the endogenous Phenoptin level was determined approximately 30% of the LLOQ of [REDACTED] L-biopterin in human plasma, which was translated to [REDACTED] of BH4. The endogenous level estimate from modeling was much higher than that from the analytical results. The BASE estimate seemed to act as a scaling factor (i.e., approximately [REDACTED]) that accounted for the residual variability by shifting the individual predictions closer to the typical predictions (PRED). The impact of this term on the PK parameter estimates is significant (e.g., Vp/F decreased by a magnitude of ten folds). The most obvious change was the random distribution of the residual variance, as shown in Figure 4 lower left panel (absolute IWRES versus IPRED). As a result, although the BASE term is significant in modeling aspects, the rationale for including this parameter and its interpretation are equivocal.
- The models referenced here are briefly summarized in Table 4. If BASE term is removed, the next best Base Model would be sponsor's model #44 (a two-compartment model). This model produced an extremely high BSV estimate for Vc/F (95.6%, Model #44) compared to a simple one-compartment model (36.6%, Model #10). In fact, all of the two-compartment models tested produced high BSV estimates when compared to one-compartment models. All in all, although the OFV improved significantly for two-compartment model (delta-OFV= -18 for Models #10 versus #36), the insufficient duration of PK sampling, the drastic increase in BSV, and the imprecision of parameter estimates for Q/F and Vp/F suggested over-parameterization and did not warrant the selection of a two-compartment Base Model. A simple one-compartment model (sponsor's Model #10) would have adequately described Phenoptin PK with relatively lower BSV and comparable residual variability.

Table 4: Summary of relevant models without the BASE term (Source: CSR PKU-004 Substudy 02, Appendix 16.1.9.3)

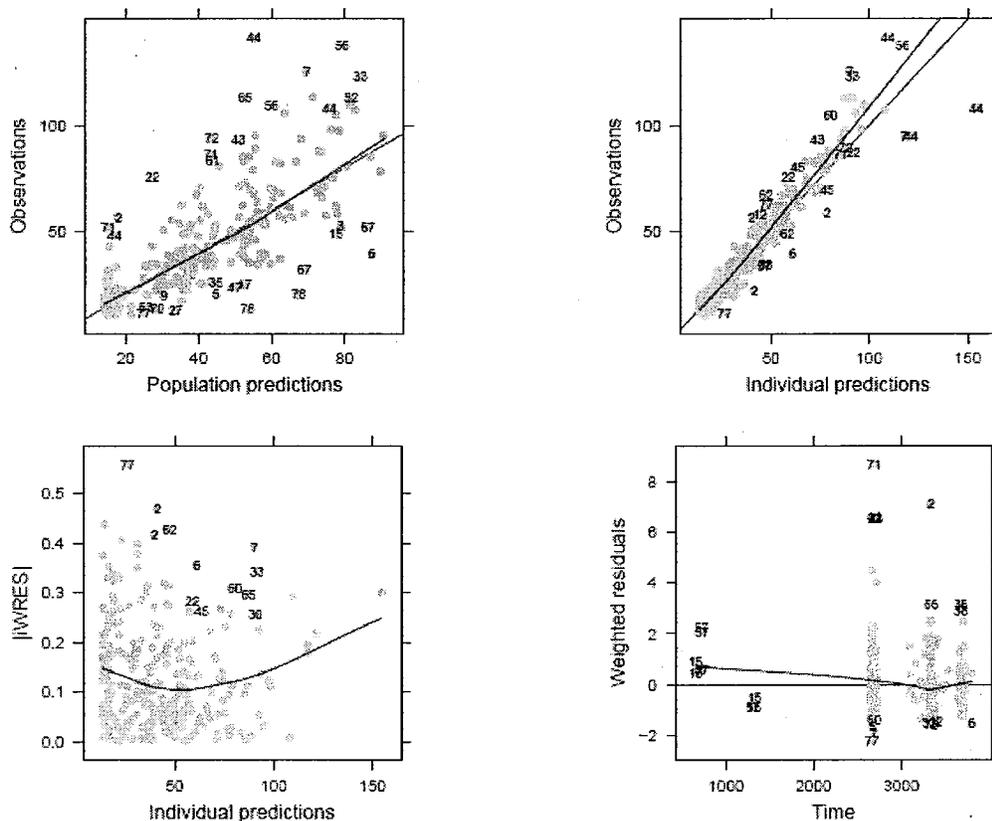
Model #	Descriptions	Estimates
10	Reviewer's choice of one-compartment, first order absorption with lag time; BSV in CL/F, and Vc/F, covariance block; proportional residual error	OFV = 1689.106 CL/F = 1000 L/h (BSV=43.6%) Vc/F = 17,200 L (BSV=36.6%) Residual error = 27%
12	Sponsor's best one-compartment, first order absorption with lag time; BSV in Ka, CL/F, and Vc/F, covariance block; proportional residual error	OFV = 1687.385 CL/F = 1010 L/h (BSV=44.3%) Vc/F = 17,300 L (BSV=41.5%) Residual error = 24%

Model #	Descriptions	Estimates
36	Two-compartment model , first order absorption with lag time; BSV in CL/F, and Vc/F, covariance block; proportional residual error	OFV = 1671.443 CL/F = 1110 L/h (BSV=41.6%) Vc/F = 11,900 L (BSV=59.2%) Q/F = 865 L/h Vp/F = 57,800 L Residual error = 24%
44	Sponsor's best two-compartment model , first order absorption with lag time; BSV in Ka, CL/F, and Vc/F, covariance block for all 3 parameters; proportional residual error	OFV = 1653.88 CL/F = 1130 L/h (BSV=43.1%) Vc/F = 8610 L (BSV=95.6%) Q/F = 1060 L/h Vp/F = 35,100 L Residual error = 22%

Note that the sponsor chose Model #12 as the best one-compartment model, however, this model was not significantly better than Model #10 (delta-OFV= -2). The reviewer deems Model #10 to be a more appropriate Base Model.

Figure 4: Comparison of goodness of fit diagnostic plots for models with and without BASE term.

Sponsor's Final Model with BASE



Parameter (Units)		Final Base Model Estimate		Bootstrap Model Estimates	
		Population Mean	95% CI	Median	95% CI
ALAG (h)	θ_1	0.306	0.245 – 0.367	0.313	0.157 – 0.385
Ka (h)	θ_2	0.552	0.309 – 0.795	0.564	0.334 – 1.13
CL/F (L/h)	θ_3	2030	1630 – 2430	2040	1620 – 2490
Vc/F (L)	θ_4	7730	4900 – 10600	7420	3567 – 11500
Vp/F (L)	θ_5	4000	1158 – 6842	4390	1600 – 25900
Q/F (L)	θ_6	937	171 – 1700	922	198 – 183000
BASE (ng/mL)	θ_7	13.9	11.8 – 16.0	13.8	11.1 – 15.8
R (CL,Vc)		0.436	-	0.443	0.155 – 0.735
IIV - CL/F (SD)		0.580	-	0.570	0.444 – 0.690
IIV - Vc/F (SD)		0.700	-	0.704	0.405 – 1.31
CCV (%CV)		21.4	-	21.1	18.2 – 23.9

ALAG = Lag Time

Ka = Absorption Rate Constant

CL/F = Apparent Clearance

Vc = Central Volume Compartment

Vp = Peripheral Volume Compartment

Q = Intercompartmental Clearance

BASE = Baseline endogenous concentration

R = Correlation between parameters

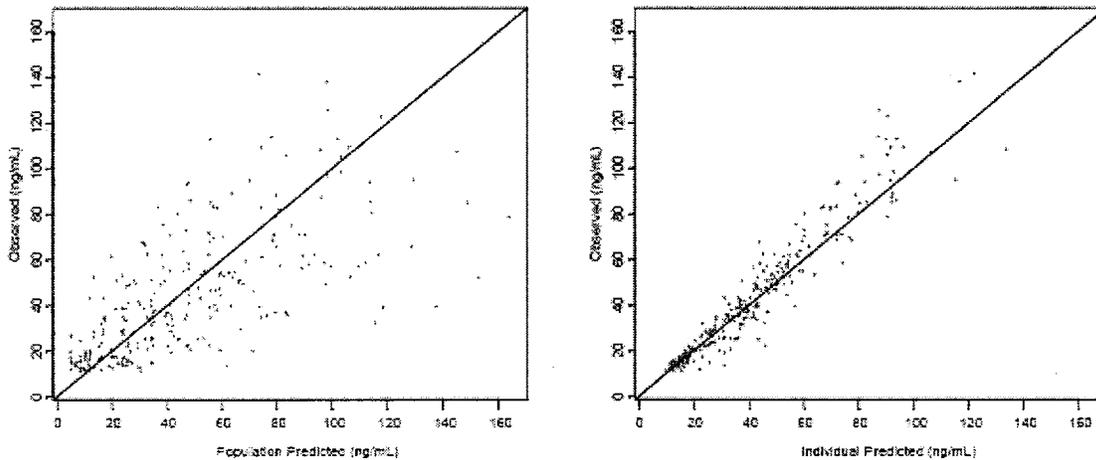
IIV = Inter-individual variability parameters

CCV = Constant coefficient of variation

5.3.3. Goodness of fit

The weighted residual (WRES) plots showed that the Base Model under-predicted at the lower concentrations and over-predicted at higher concentrations. The conditional weighted residuals (CWRES) are considered more reliable diagnostics in that they will always give better indication of structural model appropriateness. CWRES diagnostic plots in Figure 6 showed similar trend as seen in the standard WRES, which suggested that there was still room for improvement in the model.

Figure 5: Goodness of fit diagnostic plots for the sponsor's Base Model (Source: sponsor's figures 19 and 20, Appendix 16.1.9.3, page 98)



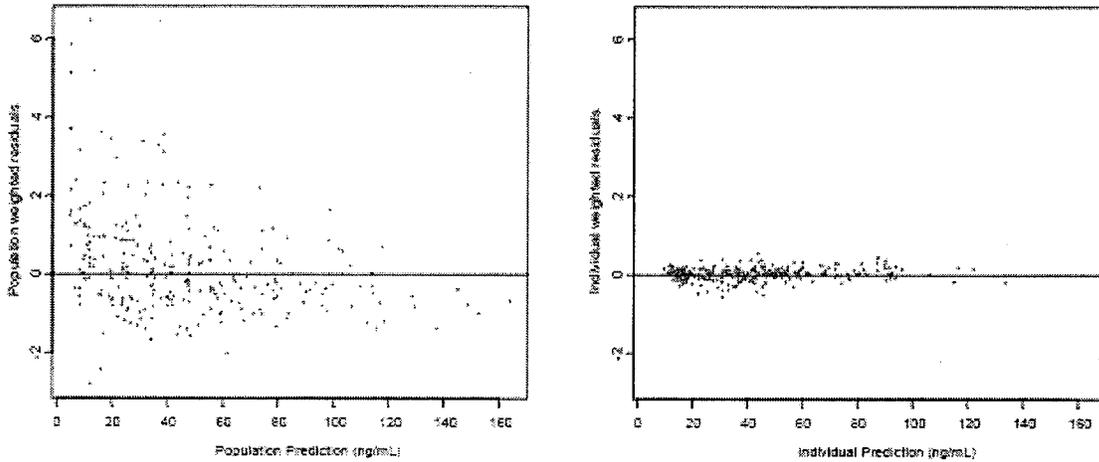
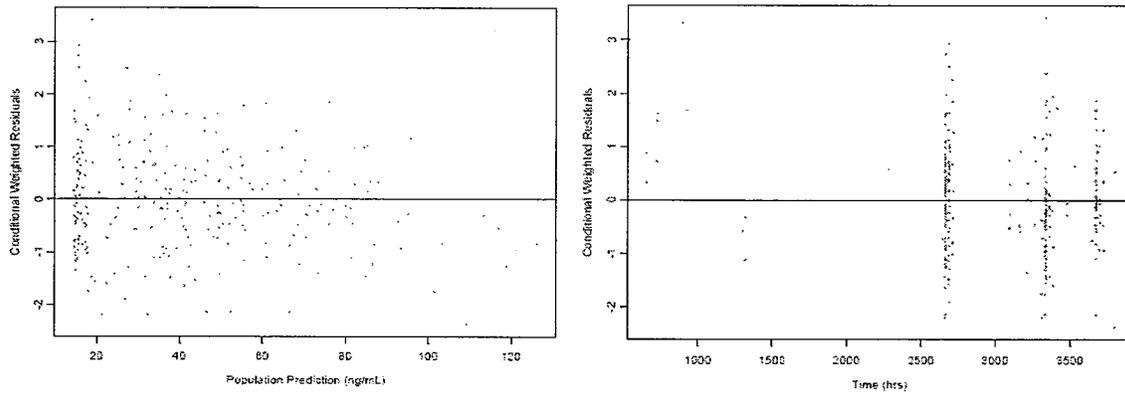


Figure 6: Conditional weighted residuals diagnostic plots for the sponsor's Base Model (Source: sponsor's figures 21 and 22, , Appendix 16.1.9.3, page 100)



5.4. Model Selection

The Base Model was selected based on criteria as described in Section 4.4.1. One, two, three compartment models were tried with lag time, first and zero order input, and various random variance models. Table 6 summarized the results of the relevant intermediate NONMEM runs.

Table 6: Summary of relevant NONMEM runs for selection of the Base Model (Source: PKU-004 Substudy 02 Report, Appendix 16.1.9.3)

*Model No.	*Model Name	Descriptions	OFV
10	Bio_1complin_alagka_clvbloc_prop	One-compartment, first order absorption with lag time; BSV in CL/F, and Vc/F, covariance block; proportional residual error	1689.106
12	Bio_1complin_alag_etaka_clvblock_prop	One-compartment, first order absorption with lag time; BSV in Ka, CL/F, and Vc/F, covariance block for all 3 parameters; proportional residual error	1687.385

*Model No.	* Model Name	Descriptions	OFV
24	Bio_1complin_dalag_etaclvb lock_prop	One-compartment, zero order absorption with lag time; BSV in CL/F, and Vc/F, covariance block; proportional residual error	1687.191
44	Bio_2complin_alag_etaclv2k ablock_prop	Two-compartment, first order absorption with lag time; BSV in Ka, CL/F, and Vc/F, covariance block for all 3 parameters; proportional residual error	1653.88
53	Bio_2complin_alagd_etaclv2 block_prop	Two-compartment, zero order absorption with lag time; BSV in CL/F, and Vc/F, covariance block; proportional residual error	1676.14
68	Bio_2complin_alag_etaclv2b lock_base_prop	Two-compartment, first order absorption with lag time and estimate of baseline endogenous BH4; BSV in Ka, CL/F, and Vc/F, covariance block; proportional residual error	1595.864

* Model number and name are as referenced in sponsor's study report

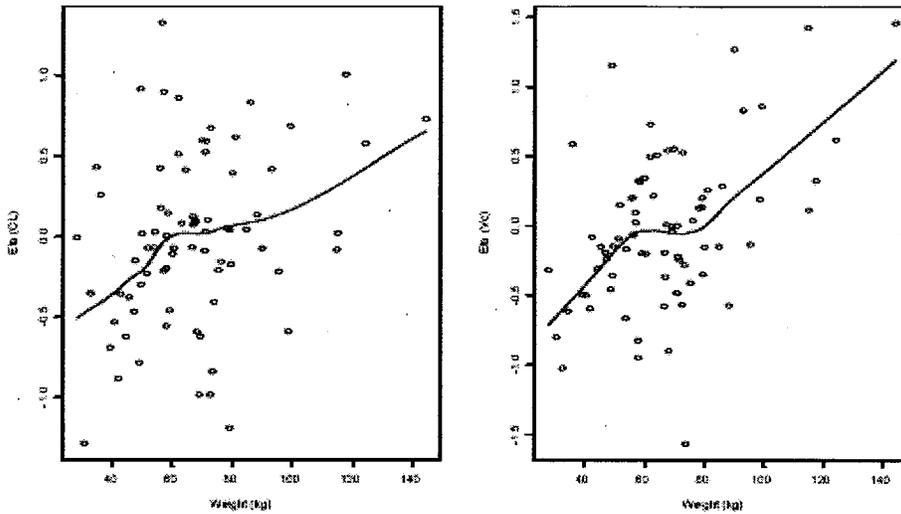
Model #68 was selected to be the best Base Model for forward testing of covariate effects. As mentioned above, the model estimate of the baseline endogenous term (BASE) is questionable. Furthermore, BSV in CL/F increased from 43% to 58% compared to model #44, the next best Base Model. Adding the BASE term seemed to decrease TVP drastically for Vp/F (from 35100 L to 4000 L).

Covariate models were evaluated based on the criteria set forth in Section 4.4.1. When each covariate was added separately to either CL/F or Vc/F in Base Model #68, only WT was found to have a significant effect on Vc/F (BSV reduced from 70% to 55.4%). WT effect on CL/F was not found to be significant even though the correlation between the conditional estimates of variance in CL/F and WT showed a positive linear relationship (Figure 7).

The model improved significantly when WT was added to both CL/F and Vc/F; a modest reduction for BSV in CL/F (58% to 53.9%), and a large relative reduction for BSV in Vc/F (70% to 55.7%). A model with combination of body surface area in CL/F and WT in Vc/F did not perform as well as WT effect in both parameters. Creatinine clearance did not significantly affect CL/F. No multiple combinations of covariates were attempted since no obvious correlations were found in the parameter variances after WT was already in the model.

Stepwise backward deletion was not performed to ensure no interactions among the population parameters.

Figure 7: Individual conditional estimates of variance in PK parameters from the Base Model versus Weight (Source: sponsor's figure 28, Appendix 16.1.9.3, page 106)



Age was not found to be a significant covariate. Examination of dose-normalized concentrations across quantiles of age showed no significant differences prior to t_{max} and immediately after t_{max} . Age may not be a significant covariate because it is confounded by WT, as seen in Figure 9, ~30% variability in WT can be explained by age.

Figure 8: Dose-normalized concentrations across Age quantiles

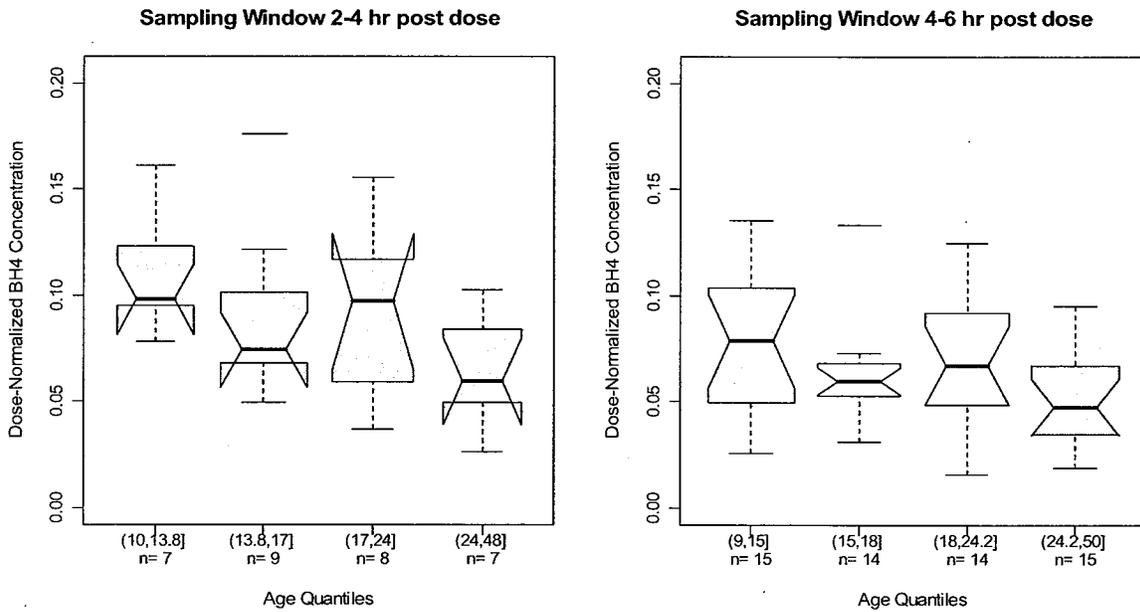
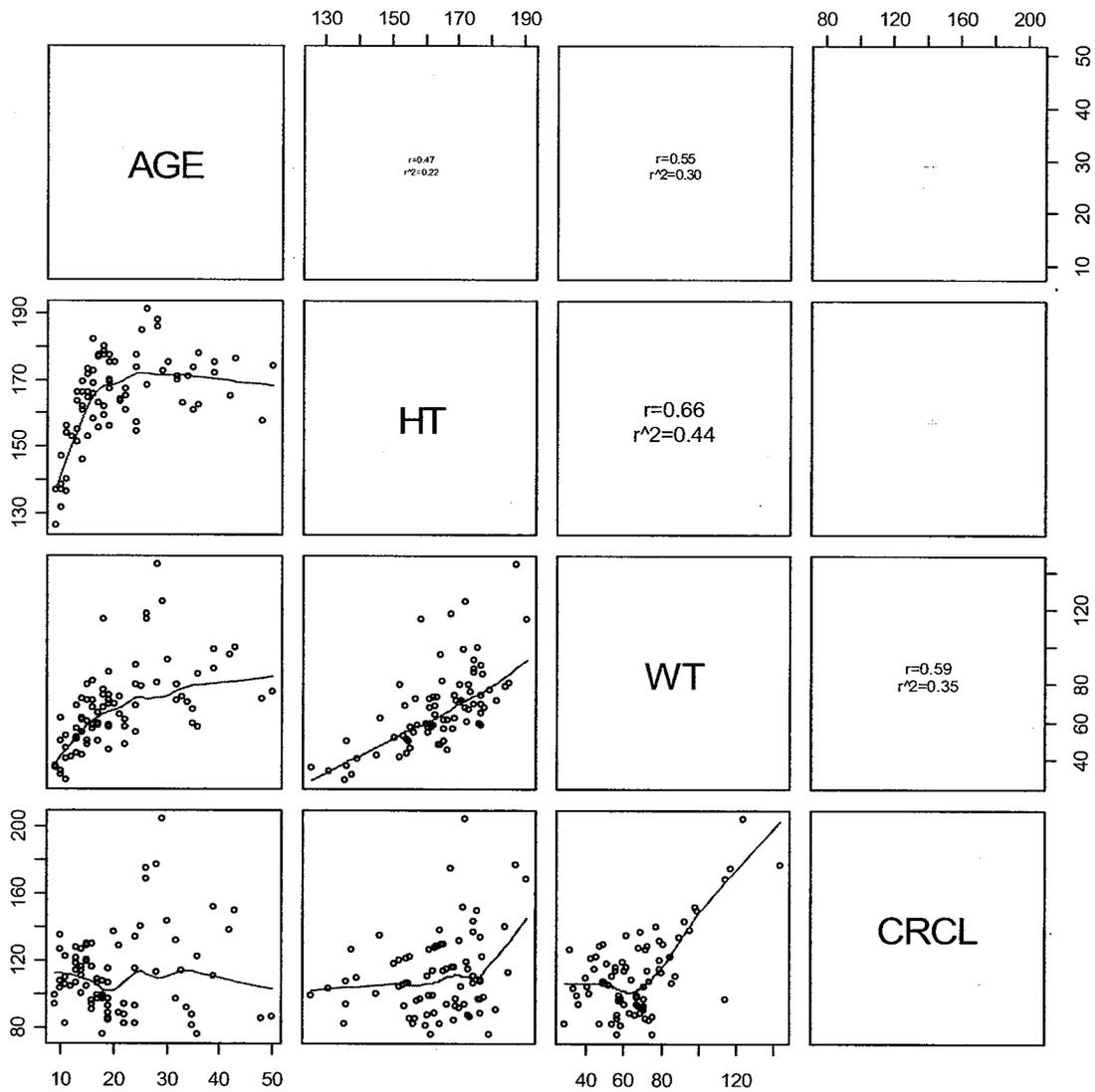


Figure 9: Pairwise correlations for continuous covariates



5.5. Final Model

5.5.1. Model description

The best Final Model for Phenoptin as identified in the sponsor's analysis was the Base Model with total body weight included on both CL/F and Vc/F (Model #116). The equations describing the Final Model are the following:

$$ALAG = \theta_1$$

$$Ka = \theta_2$$

$$\frac{CL}{F} = \theta_3 \cdot \left(\frac{Weight}{70} \right)^{\theta_8} \cdot \exp(\eta_1)$$

$$\frac{Vc}{F} = \theta_4 \cdot \left(\frac{Weight}{70} \right)^{\theta_8} \cdot \exp(\eta_2)$$

$$\frac{Vp}{F} = \theta_5$$

$$\frac{Q}{F} = \theta_6$$

$$BASE = \theta_7$$

WT was included in the Final Model for both CL/F and Vc/F even though it was not significant when added to CL/F alone. The sponsor's reason was that "there was a reduction in the inter-individual variability of clearance and the range in the typical value of clearance for the subjects recruited in the study was greater than 20%. As such addition of this parameter [WT] was considered to be clinically significant and was retained in the model." However, the relative reduction in BSV for CL/F was only 7.1% (from 58% in Base Model to 53.9% in the Final Model, absolute BSV reduction of 4%), which would not be considered clinically significant. Perhaps, a common reason to add WT in both parameters as *a priori* would be because of its biological ground and the well-known correlations with clearance and volume are supported by allometric studies.

5.5.2. Parameter estimation results

The following results are from CSR PKU-004 Substudy 002.

Table 7: Parameter estimates and standard errors for the Final PK Model (Source: sponsor's Table 17, Appendix 16.1.9.3, page 125)

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Parameter (Units)		Population Mean (SE [±])	Standard Deviation of Inter-Individual Variance (SE [±])
ALAG (h)	Θ_1	0.275 (13.7)	NA
Ka (1/h)	Θ_2	0.518 (24.1)	NA
CL/F (L/h/70kg)	Θ_3	2100 (9.9)	0.539 (25.3)
COV1	Θ_4	0.586 (34.0)	NA
Vc/F (L/70kg)	Θ_5	8350 (16.9)	0.557 (41.3)
COV2	Θ_6	1.13 (24.7)	NA
Vp/F (L)	Θ_7	4240 (42.5)	NA
Q/F (L/h)	Θ_8	862 (43.5)	NA
BASE (ng/mL)	Θ_9	13.5 (8.2)	NA
R (CL,Vc)		0.336	NA
CCV Residual Error (as %CV)			21.7 (13.3)

* - SE given as %CV; NA – Not Applicable

ALAG = Lag Time

Ka = Absorption Rate Constant

CL/F = Apparent Clearance

COV1 = Power function on CL/F

Vc/F = Central Volume Compartment

COV2 = Power function on Vc/F

Vp/F = Peripheral Volume Compartment

Q = Intercompartmental Clearance

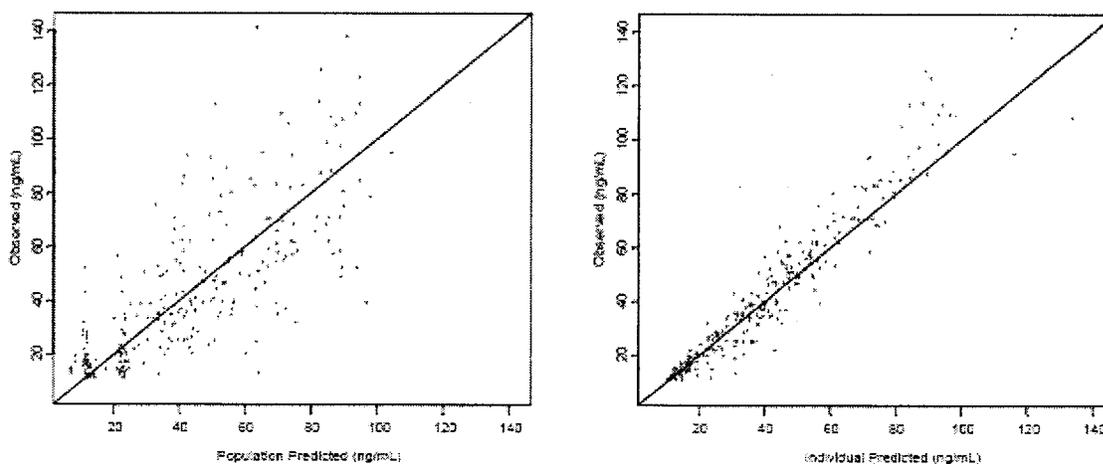
BASE = Endogenous Baseline Concentration

R = Correlation between parameters

5.5.3. Goodness of fit

According to the diagnostic plots for sponsor's Final Model (Figure 10), the model only improved slightly compared to the Base Model. The Final Model still under-predicted the lower and upper ends of observed concentration range. The CWRES also improved only slightly (Figure 11).

Figure 10: Goodness of fit diagnostic plots for the sponsor's Final Model (Source: sponsor's figures 63 and 64, Appendix 16.1.9.3, page 143)



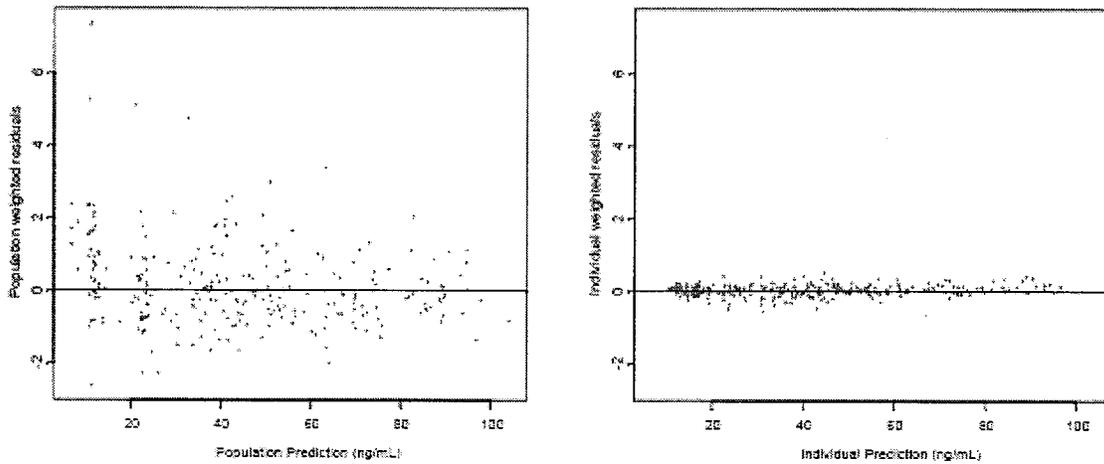
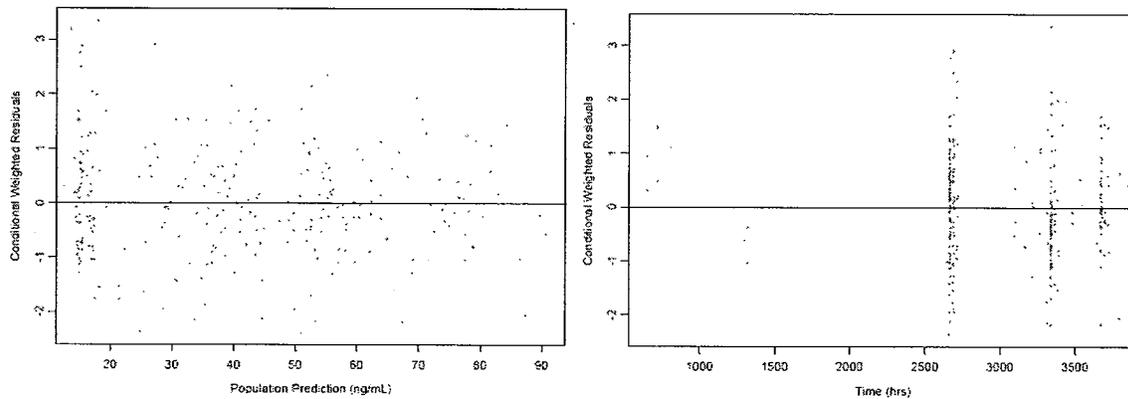


Figure 11: Conditional weighted residuals diagnostic plots for the Final Model (Source: sponsor's figures 65 and 66, Appendix 16.1.9.3, page 145)



5.6. Model Qualification

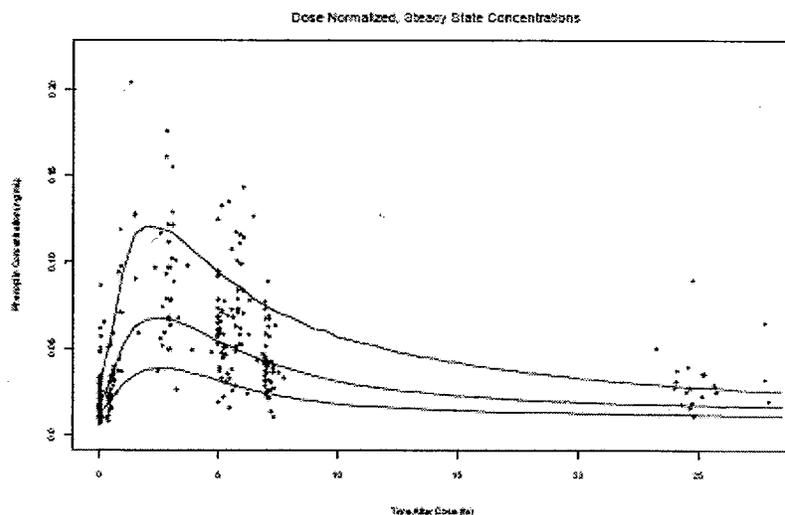
The 95% CIs estimated from the asymptotic standard errors obtained from the base model, and the non-parametric 95% CIs estimated from 1000 non-parametric bootstrap runs, are given in Table 8. Only runs that minimized successfully were used to compute the 95% CIs (919 runs). Visual predictive check (VPC) was used to check for of the model's ability to capture the variability in observed data. The sponsor's VPC plot (Figure 12) showed approximately 80% of the observed-BH4 concentrations fall between 10th and 90th percentile interval of simulated concentrations.

Table 8: 95% Confidence Intervals for the Final Covariate Model (Source: sponsor's Table 18, Appendix 16.1.9.3)

Parameter (Units)		Final Model Estimate		Bootstrap Model Estimates	
		Population Mean	95% CI	Median	95% CI
ALAG (h)	θ_1	0.275	0.201 – 0.349	0.288	0.165 – 0.365
Ka (h)	θ_2	0.518	0.273 – 0.763	0.551	0.333 – 1.22
CL/F (L/h/70kg)	θ_3	2100	1690 – 2500	2100	1680 – 2540
COV1	θ_8	0.586	0.196 – 0.977	0.576	0.176 – 0.955
Vc/F (L/70kg)	θ_4	8350	5590 – 11100	8000	2910 – 12600
COV2	θ_9	1.13	0.583 – 1.68	1.17	0.522 – 3.54
Vp/F (L)	θ_5	4240	712 – 7768	5020	1480 – 39000
Q/F (L/h)	θ_6	862	127 – 1597	821	187 – 390000
BASE (ng/mL)	θ_7	13.5	11.3 – 15.7	13.5	10.2 – 15.5
R (CL, Vc)		0.336	-	0.344	0.0119 – 0.701
IV - CL/F (SD)		0.539	-	0.526	0.383 – 0.662
IV - Vc/F (SD)		0.557	-	0.556	0.260 – 0.893
CCV (%CV)		21.7	-	21.3	18.3 – 24.2

ALAG = Lag Time
Ka = Absorption Rate Constant
CL/F = Apparent Clearance
COV1 = Power function on CL/F
Vc/F = Central Volume Compartment
COV2 = Power function on Vc/F
Vp/F = Peripheral Volume Compartment
Q = Inter-compartmental Clearance
BASE = Baseline endogenous concentration
R = Correlation between parameters
IV = Inter-individual variability parameters
CCV = Constant coefficient of variation

Figure 12: Visual predictive check for the Final Model (Source: sponsor's figure 69, Appendix 16.1.9.3, page 156)



VPC is typically shown for 90 or 95 percentile intervals. The sponsor's VPC plot only showed 80% intervals, which might look better than the 90 or 95 percentiles.

6. DISCUSSION

The pharmacokinetic of Phenoptin was adequately described by a two compartment model with a short lag time (16.5 minutes) prior to first-order absorption, followed by biphasic elimination. A BASE term was included to account for the endogenous BH4. Total body weight is the only significant patient variable that accounts for the BSV in CL/F and Vc/F, which is expected from a weight-based regimen. Gender and age did not significantly influence Phenoptin PK. As a result, the Final Model had only weight included in both CL/F and Vc/F. Estimates of CL/F and Vc/F were 2100 L/h/70kg (53.9% BSV) and 8350 L/kg (55.7% BSV), respectively. The within-subject (residual) variability was 21.7%. The analysis described the data well. However, due to the limitations in PK data collection and study design, the parameter estimates are associated with great uncertainty. The estimate of terminal half-life is 6.7 h (range: 3.9-17 h) in PKU subjects. The reported terminal half-life in previous single-dose PK studies (PKU-005 and PKU-009) in healthy volunteers was 4 h (range: 3-5 h). **Error! Reference source not found.** (Appendix: 8.1 Supportive data from PKU-005 and PKU-009) illustrates dose normalized plasma BH4 concentration-time profiles for all dose groups. In comparison with Figure 3, comparable time course between healthy subjects and patients was observed. Therefore, it is reasonable to assume that pharmacokinetics of healthy subjects and patients are similar.

7. LABELING RECOMMENDATIONS

Reviewer agreed with the following labeling claim:

The mean elimination half-life in PKU patients was approximately 6.7 hours (range 3.9 to 17 h), with values seen in healthy subjects.

A population pharmacokinetic analysis of sapropterin that included patients showed no effect of age on sapropterin dihydrochloride pharmacokinetics.

8. APPENDICE

8.1. Supportive data from PKU-005 and PKU-009

There were four cohorts in PKU-005

- a. prepared in water solution given in fasting conditions
- b. prepared in water solution given in fed conditions
- c. prepared in orange juice solution given in fasting conditions
- d. prepared in orange juice solution given in fed conditions

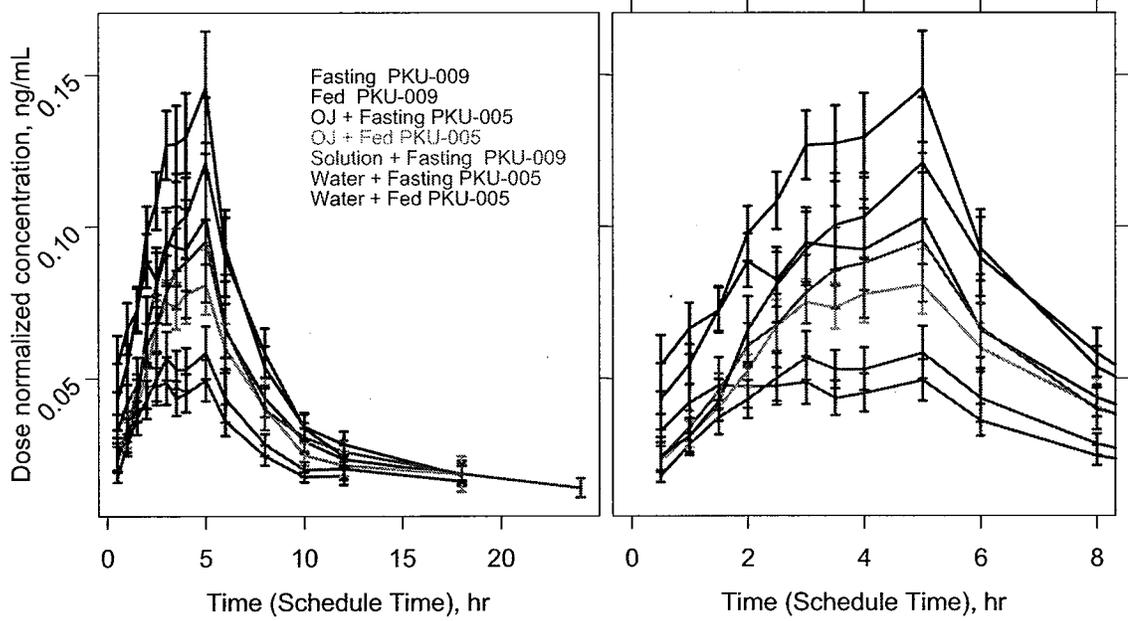
There were three cohorts in PKU-009

- a. administered after dissolution of tablet(s) in water given under fasting conditions
- b. administered as intact tablet(s) given under fasting conditions
- c. administered as intact tablet(s) given under fed conditions

where all dose groups received 10 mg/kg dose of Phenoptin.

Figure 13 illustrates dose normalized plasma BH4 concentration-time profiles for all dose groups.

Figure 13: Dose normalized plasma BH4 concentration (mean \pm 95% C.I.)-time profile after Phenoptin administration with different food status in studies PKU-005 and PKU-009. Left panel: all time points per collection schedule; Right Panel: Up to 8 hr post dose sampling.



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/s/

Pravin Jadhav

11/26/2007 05:43:39 PM

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The analyses was a part of clinical pharmacology review
by Dr. Hae-Young. This is a complete pharmacometrics
review.

Jogarao Gobburu

11/27/2007 11:03:00 AM

BIOPHARMACEUTICS



Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF CARDIOVASCULAR AND RENAL PRODUCTS

Date: November 7, 2007

From: Christine Garnett, Pharm.D.
Interdisciplinary Review Team for QT Studies
Division of Cardiovascular and Renal Products /CDER

Through: Norman Stockbridge, M.D., Ph.D.
Division Director
Division of Cardiovascular and Renal Products /CDER

To: Marlene Swider
Regulatory Project Manager
Division of Gastroenterology Products

Subject: QT-IRT Consult to NDA 22-181

This memo responds to your consult to us dated October 12, 2007 seeking our advice for the QT/cardiac safety evaluation of sapropterin under NDA 22-181 sponsored by BioMarin. The QT-IRT received and reviewed the following materials:

- Your consult and background package
- Clinical Pharmacology Table

Background

Sapropterin dihydrochloride is a new molecular entity currently undergoing priority NDA review as a novel treatment for phenylketonuria (PKU). Sapropterin has been designated as an Orphan Drug.

Sapropterin is an immediate-release tablet formulation developed to reduce blood phenylalanine (Phe) levels [REDACTED] in patients with hyperphenylalaninemia (HPA) due to PKU. Sapropterin increases the overall level of phenylalanine hydroxylase (PAH) activity by stimulating the residual enzyme activity of the altered PAH enzymes. [REDACTED]

[REDACTED]
[REDACTED] The dose may be adjusted within the range of 5 to 20 mg/kg/day according to the needs of the patient.

Sapropterin has been evaluated for safety and efficacy in approximately 580 patients with PKU, ages four years and older. Sapropterin was orally administered at doses of 5, 10, and 20 mg/kg/day once a day in clinical trials in support of the PKU indication.

According to the Division, study results showed that sapropterin was found to reduce blood Phe levels in BH4-responsive PKU patients (approximately 20% of patients treated), and was found to have a favorable safety profile. Most Adverse Events (AEs) noted in clinical trials were mild, and were most commonly headache, vomiting and diarrhea. No cardiac findings were noted; however, no routine ECG monitoring or evaluation was performed as part of the safety monitoring for the clinical studies conducted in PKU patients. The Division anticipates that sapropterin will likely receive approval for the treatment of PKU on or around November 28, 2007.

_____ the sponsor has conducted a phase 2 trial evaluating the effects of sapropterin 10 mg/kg/day for 8 weeks in patients with poorly controlled systemic hypertension. The sponsor reports that the mean values for ECG parameters, including QT interval, were similar between placebo and sapropterin-treated groups. Mean changes in ECGs from baseline were negligible in sapropterin-treated patients, and there were no adverse event reports that were suggestive of pro-arrhythmogenic effects of sapropterin.

Questions Posed by the Clinical Division

We have been asked by the Division to respond to the following questions:

1. Do you recommend that a thorough QT (TQT) or other cardiac safety study or evaluation be performed for sapropterin?

QT-IRT Response: Yes. ICH E14 recommends that new drugs having systemic bioavailability undergo clinical electrocardiographic evaluation during clinical development. Since sapropterin has been administered safely to healthy adult volunteers at doses up to 10 mg/kg, we recommend BioMarin perform a TQT study.

2. If yes, we anticipate that this study would be requested as a post-marketing commitment. Is the performance of a TQT (or other cardiac safety study or evaluation) as a post-marketing commitment reasonable or should a TQT or other evaluation be performed prior to approval?

QT-IRT Response: Whether to ask the sponsor to conduct a TQT study as a post-marketing commitment or prior to approval is not a decision for the QT-IRT. The review division has a better understanding of the factors which would go into such a decision (knowledge of the specific disease state and other treatment options; knowledge of the drug class; and a thorough understanding of the safety and efficacy profile of sapropterin) than does the QT-IRT.

3. If a TQT or other cardiac safety study/evaluation is recommended, please provide guidance as to the study design, appropriate study population (including age), length of study, appropriate positive and negative controls, and study endpoints. (Please see two attachments to this consult for more specifics and background information.)

QT-IRT Response: We recommend that the sponsor refer to ICH E14 when developing the protocol. In general, a single-dose, positive- and placebo-controlled crossover study in healthy volunteers may be sufficient to characterize the effects of administering sapropterin on the QT interval. The dose selected for the TQT study should give plasma concentrations to cover the expected high clinical exposure scenario in patients with PKU without compromising subject safety. It is not clear from the submitted data if the maximum

tolerated dose has been identified. We will provide specific comments on the study design once BioMarin submits a protocol for review.

Thank you for requesting our input into the development of this product under NDA. We welcome more discussion with you now and in the future.

Please feel free to contact us via email at cdcrdcrpqt@fda.hhs.gov

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/s/

Christine Garnett
11/7/2007 01:43:19 PM
BIOPHARMACEUTICS

Norman Stockbridge
11/7/2007 04:28:08 PM
MEDICAL OFFICER

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA	22-181
Submission Date	May 25, 2007
Brand Name	Kuvan
Generic Name	Sapropterin Dihydrochloride
Primary Reviewer	Hae-Young Ahn, Ph.D.
Team Leader	Sue Chih Lee, Ph.D.
PM Secondary Reviewer	Pravin Jadhav, Ph.D.
OCP Division	DCP3
OND Division	Division of Gastroenterology Products
Applicant	BioMarin Pharmaceuticals
Relevant IND(s)	69,708
Submission Type	NME: Priority for Therapeutic Gain
Formulation; Strength	Tablets; 100 mg
Proposed Indications	To reduce blood phenylalanine (Phe) levels _____ in patients with hyperphenylalaninemia (HPA) due to Phenylketonuria (PKU). _____ _____

Table of Contents

1. EXECUTIVE SUMMARY.....	2
1.1. RECOMMENDATION.....	2
1.2. PHASE 4 COMMITMENTS	2
1.3. COMMENTS.....	2
1.4. SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS	3
2. QUESTION BASED REVIEW.....	7
2.1. GENERAL ATTRIBUTES OF THE DRUG	7
2.1.1. <i>What pertinent regulatory background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?</i>	7
2.1.2. <i>What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?</i>	8
2.1.3. <i>What are the proposed mechanism(s) of action and therapeutic indication(s)?</i>	8
2.1.4. <i>What are the proposed dosage(s) and route(s) of administration?</i>	9
2.2. GENERAL CLINICAL PHARMACOLOGY	9
2.2.1. <i>What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?</i>	9
2.2.2. <i>What is the basis for selecting the response endpoints or biomarkers and how are they measured in clinical pharmacology and clinical studies?</i>	11
2.2.3. <i>Are the active moieties in the plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?</i>	15
2.2.4. <i>Exposure-response</i>	15
2.2.5. <i>What are the PK characteristics of the drug and its major metabolite?</i>	17
2.3. INTRINSIC FACTORS	20
2.4. EXTRINSIC FACTORS	20

2.5.	GENERAL BIOPHARMACEUTICS.....	21
2.5.2	<i>Are clinical formulations different from the to-be-marketed formulation? If so, are they comparable?</i>	21
2.5.3.	<i>In the pivotal clinical trials patients were given Kuvan dissolved in water, apple juice or orange juice. However, the sponsor proposes in the label that patients take Kuvan as dissolved in water or apple juice.....</i>	22
2.5.1.	<i>What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?</i>	27
2.6.	ANALYTICAL SECTION	28
2.6.1.	<i>How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?</i>	28
2.6.2.	<i>What bioanalytical methods are used to assess concentrations?</i>	29
3.	DETAILED LABELING RECOMMENDATIONS	30
4.	APPENDICES	32

1. Executive Summary

1.1. Recommendation

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 3 has reviewed the clinical pharmacology and biopharmaceutics information submitted to NDA 22-181 and finds it acceptable provided that a satisfactory agreement is reached between the sponsor and the Agency regarding the language to be included in the package insert

1.2. Phase 4 Commitments

A formal consult was sent to the QT-IRT team to inquire whether a thorough QT study is needed as a Phase 4 commitment.

1.3. Comments

1. Administration of Kuvan dissolved in orange juice has equivalent bioavailability to Kuvan dissolved in water administered under fasted conditions. Administration of Kuvan dissolved in orange juice after a high-fat, high-calorie meal is 25% less bioavailable than the tablets dissolved in water. Since orange juice contains a significant amount of Phe, the sponsor proposes that the drug be dissolved in water or apple juice. The bioavailability of the drug in apple juice has not been studied. The stability data on the drug dissolved in apple juice and water were comparable according to the reviewing chemist. Therefore, the sponsor's proposal of that the drug be dissolved in water or apple juice is acceptable.
2. Kuvan as an intact tablet was 20% more bioavailable than a dissolved tablet under fasted conditions. Kuvan tablets used in the study were manufactured by ~~_____~~

~~_____~~ This change is considered as a ~~_____~~ change which requires

Pharmacodynamics

The once daily dosing interval is based on the pharmacodynamic (PD) effect of sapropterin on Phe reduction rather than the known pharmacokinetics of sapropterin in humans. All of the PKU clinical studies with Kuvan were conducted using a once-daily (QD) dose.

The ability of Kuvan, when given as a single daily dose, to maintain stable blood Phe levels during a 24-hour period, was investigated in 2 substudies. Data from these substudies show that blood Phe levels remained relatively stable throughout a 24-hour observation period. In PKU-001 Substudy 01 eleven subjects received Kuvan 10 mg/kg/day for a total of 8 days. Mean blood Phe levels remained stable over the course of the 24 hours although inter-subject variability was large and there were many missing data points.

In PKU-004 Substudy 01, 12 subjects received Kuvan, 10 mg/kg/day from Weeks 6 to 10. Large variability in blood Phe levels between subjects was observed but the intra-subject variations in blood Phe levels were relatively small. Mean blood Phe levels were stable and there seemed to be no spikes of Phe levels in individual data.

Exposure-Response Relationships

The exposure-response relationships were studied in the Study PKU-004 which was a phase 3, open-label, forced dose-titration study. The mean blood Phe level observed at the end of each 2-week dosing period decreased as the dose of Kuvan increased, demonstrating an inverse relationship between the dose of Kuvan and mean blood Phe levels. The mean change in blood Phe level differed significantly between the dose groups; the differences between effect of 5 versus 10 mg/kg/day (and 5 vs 20 mg/kg/day) was statistically significant at $p < 0.0001$ and between 10 vs 20 mg/kg/day was statistically significant at $p = 0.0085$. The percentage of subjects with a $\geq 30\%$ reduction in blood Phe levels was 25%, 55%, 46% after dosing for 2 weeks with 5, 20, and 10 mg/kg/day, respectively.

In Study PKU-004, 85% of subjects experienced at least one AE. The sponsor reported that during the fixed-dose period, the percentage of subjects who had an AE was similar between the 5 mg/kg/day and the 20 mg/kg/day doses (50% and 57%), but slightly lower for the 10 mg/kg/day dose group (38%). There seemed to be no apparent relationship between the dose of Kuvan and the incidence of AE.

The sponsor has not conducted a thorough QT study. Neither were ECG assessments performed in the pivotal phase 3 studies, 003 and 006. Therefore, exposure-response (QT and QTc interval) relationship could not be determined.

Biopharmaceutics

The two Phase 1 PK studies, PKU-005 and PKU-009, were conducted using the same formulation of Kuvan as used in the clinical studies. T_{max} and $t_{1/2}$ were each approximately 4 hours regardless of mode of administration or delivery (swallowed intact

Kuvan™ (sapropterin dihydrochloride)

tablet vs. dissolved, water versus juice, fasted versus fed).

Two studies in healthy adult subjects evaluated the bioavailability of Kuvan at a dose of 10 mg/kg when administered dissolved in water versus orange juice (PKU-005) and when administered dissolved in water versus as an intact tablet (PKU-009); the effect of food on bioavailability was also evaluated in both studies.

Study PKU-005 showed that administration of Kuvan dissolved in orange juice has equivalent bioavailability to Kuvan dissolved in water administered under fasted conditions. Administration of Kuvan after a high-fat, high-calorie meal and dissolved in either vehicle resulted in a substantial increase in absorption compared to fasted conditions, approximately 84% in water and 41% in orange juice. Since orange juice contains a significant amount of Phe, the sponsor proposes that the drug be dissolved in water or apple juice. The bioavailability of the drug in apple juice has not been studied. The stability data on the drug dissolved in apple juice and water and submitted and they were comparable according to the reviewing chemist.

PKU-009 showed that administration of Kuvan under fasted conditions as an intact tablet resulted in an approximate 20% increase in the extent of absorption compared to a dissolved tablet. Kuvan tablets used in this study were manufactured by _____

_____ The change in the manufacturing process _____ change which requires a bioequivalence study. All the clinical trials used tablets dissolved in water, apple juice or orange juice. Therefore, it is recommended that Kuvan tablets be dissolved in water or apple juice although the sponsor proposes that Kuvan tablets may be _____ dissolved in water or apple juice.

The individual subject values for C_{max} and AUC_{0-t} across the different treatments in the 2 BioMarin studies are compared in Figures 1 and 2. With the exception of 1 or 2 spurious values, there was substantial overlap among the different ways of administering Kuvan (intact tablets, dissolved in water, dissolved in orange juice, fasted and fed). However, there was a clear trend toward an increase in exposure when Kuvan was administered with a high fat, high calorie meal.

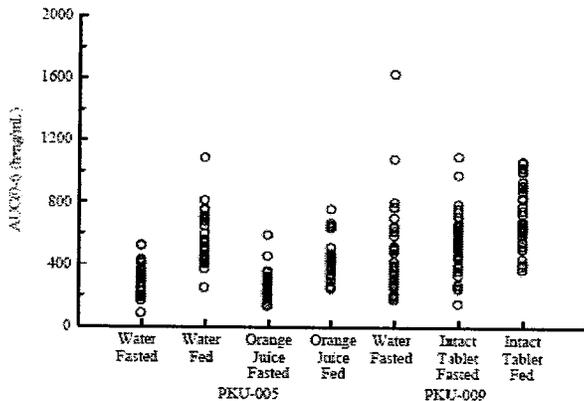


Figure 1: Comparison of individual subject AUC between studies PKU-005 and PKU-009 after oral administration of 10 mg/kg Kuvan dissolved in water, orange juice or as intact tablets under fasted and fed conditions

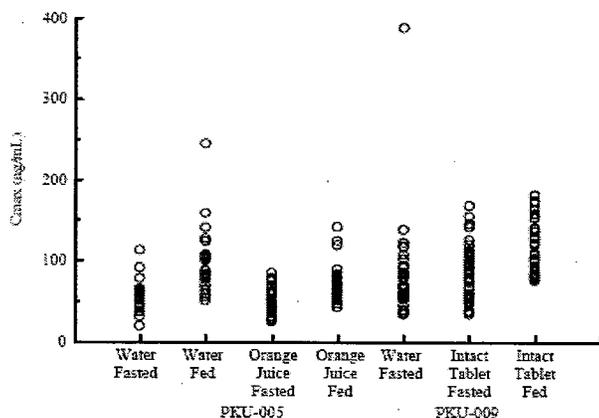


Figure 2: Comparison of individual subject Cmax between studies PKU-005 and PKU-009 after oral administration of 10 mg/kg Kuvan dissolved in water, orange juice or as intact tablets under fasted and fed conditions

2. Question Based Review

2.1. General Attributes of the Drug

2.1.1. What pertinent regulatory background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?

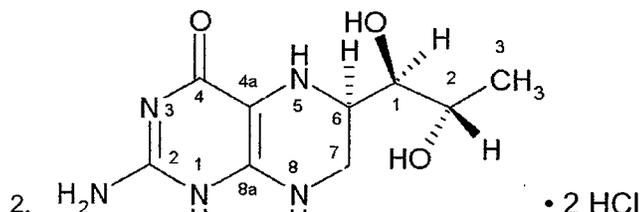
The regulatory history for Sapropterin is summarized as follows:

- 1992: Daiichi Suntory Pharm Co. received marketing approval in Japan under the name Biopten in a 2.5% granule formulation for use in the treatment of hyper-Phe due to primary BH4 deficiency. BioMarin had the rights to develop the product outside Japan.
- August 2004: IND 69,708 was opened to the Division of Metabolic and Endocrine Products (DMEP) for Kuvan for the treatment of BH4-responsive PKU.
- January 2004: Kuvan was granted orphan drug designation for the treatment of BH4-responsive PKU.
- January 2005: The sponsor submitted a fast track designation request but it was denied by the DMEP on the grounds that the development program was not assessing neurological effects and therefore not developing the drug as a treatment for a serious aspect of the disease.
- October 2005: IND 69,708 was transferred to Division of Gastroenterology Products (DGP).

Kuvan™ (sapropterin dihydrochloride)

- November 2005: The sponsor appealed the fast track designation denial, claiming that by reducing blood Phe levels, Kuvan treats a serious aspect of PKU. DGP accepted the sponsor's claim and Kuvan was granted fast track designation for the treatment of BH4-responsive PKU in January 2006.
- October 2006: A pre-NDA meeting was held between DGP and the sponsor.

2.1.2. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?



Sapropterin dihydrochloride is off-white to light yellow crystalline powder. It melts with decomposition at 239-241 °C. Sapropterin dihydrochloride is hygroscopic, and exhibits an approximately 2% increase in moisture content when stored at a relative humidity (RH) of 75%, with lesser increases in moisture content for lower relative humidity conditions.

At room temperature, sapropterin dihydrochloride is very soluble in water (> 1 g/mL). It is sparingly soluble in methanol (10 mg/mL) and ethanol (0.9 mg/mL), and practically insoluble (<0.1 mg/mL) in aprotic solvents such as diethyl ether.

The water/octanol partition coefficient: there is little or no distribution into octanol in the solutions at pH 1.2 – 4.0, indicating that the compound is hydrophilic.

2.1.3. What are the proposed mechanism(s) of action and therapeutic indication(s)?

Tetrahydrobiopterin (6R-BH4) is the naturally occurring pteridine, (6R)-2-amino-6-[(1R,2S)1,2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(1H)-pteridinone. 6R-BH4 is an endogenous cofactor for a variety of enzymes, including PAH. In some patients with PKU, 6R-BH4 can enhance the function of the mutated PAH enzyme, promoting oxidation of phenylalanine (Phe) to tyrosine, thus lowering blood Phe levels.

Kuvan (sapropterin dihydrochloride) is a synthetic formulation of 6R-BH4 that is proposed for marketing as an oral treatment for HPA in patients with PKU

Kuvan is proposed to increase the overall level of phenylalanine hydroxylase (PAH) activity by stimulating the residual enzyme activity of the altered PAH enzymes.

The proposed indication for Kuvan is to reduce blood Phe levels
in patients with HPA due to PKU.

2.1.4. What are the proposed dosage(s) and route(s) of administration?

The sponsor proposes the following:

- The starting dose of Kuvan for phenylketonuria is taken once daily.
- Kuvan may be taken with or without food. Tablets may be swallowed whole or dissolved in 4 to 8 oz. (120-240 mL) of water or apple juice. Dissolved preparations of Kuvan should be taken within 15 minutes.

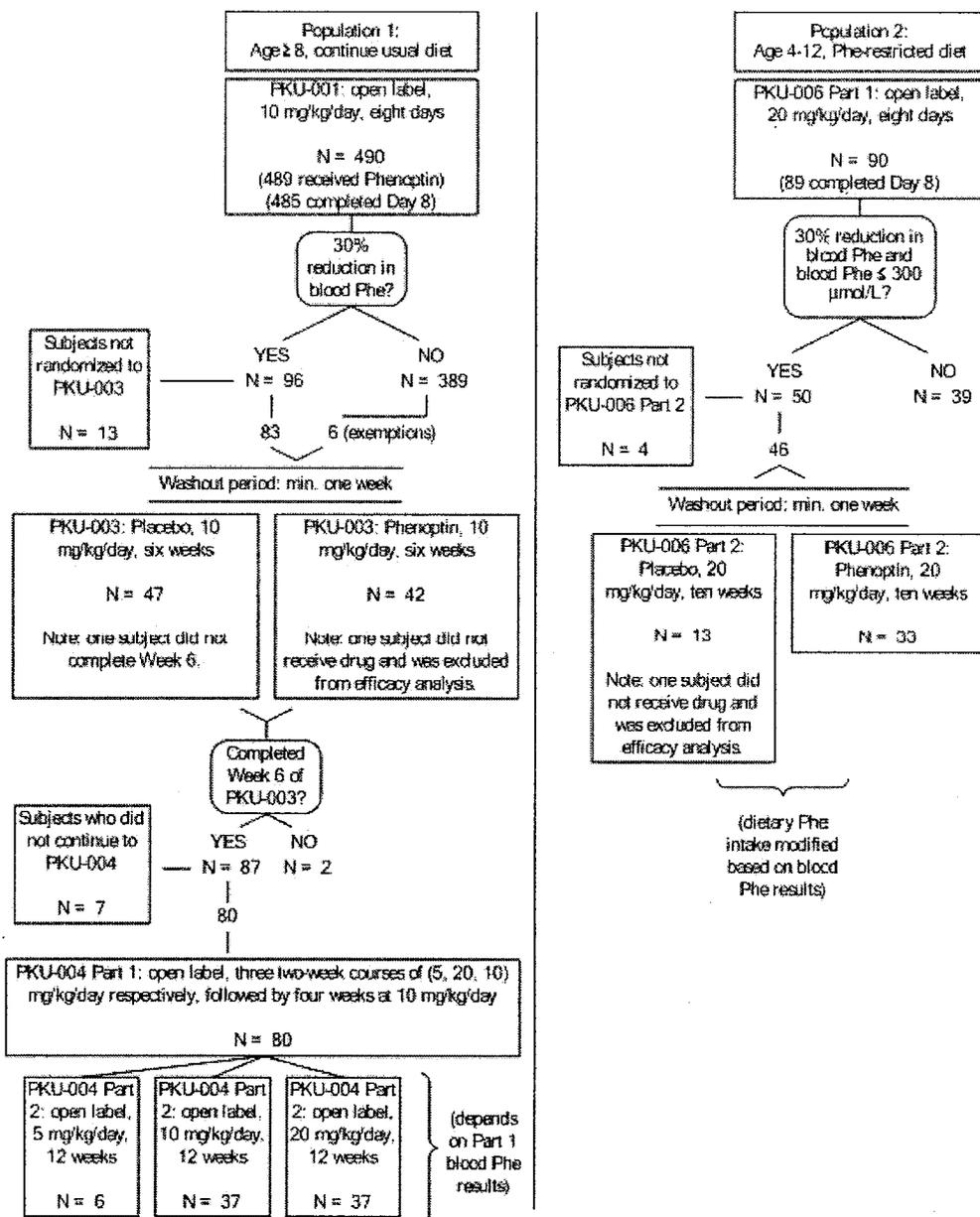
2.2. General Clinical Pharmacology

2.2.1. What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The clinical development program has focused on the pharmacodynamic response of Kuvan administration (i.e., the reduction in blood Phe), and consisted of 2 pivotal placebo-controlled studies (PKU-003 and PKU-006), 2 open-label studies (PKU-001 and PKU-004), 2 studies evaluating blood Phe levels over a 24-hour period (PKU-001 Substudy 01 and PKU-004 Substudy 01), and a population-based pharmacokinetic study (PKU-004 Substudy 02). Study PKU-004 included a study to investigate a dose-response relationship in the 5-20 mg/kg/day.

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Figure 3: Overview of clinical studies

The two controlled Phase 3 studies (Studies PKU-003 and PKU-006) assessed 2 situations in the clinical management of PKU: (1) lowering blood Phe levels into a safer range for subjects not following diet Phe restriction, mostly in teenagers and older subjects, and (2) increasing Phe tolerance in subjects following a restricted diet and blood Phe guidelines, most typical of pre-adolescents.

2.2.2. What is the basis for selecting the response endpoints or biomarkers and how are they measured in clinical pharmacology and clinical studies?

Phenylketonuria (PKU) is an autosomal recessive genetic metabolic disease caused by mutations in PAH gene that leads to abnormally elevated levels of Phe, which are toxic to the brain and result in a variety of neurophathologic conditions, including mental retardation, neurocognitive, and other neuropsychiatric disorders if left untreated. In order to establish quantitative relationship between Phe control and intelligence quotient (IQ) outcome, the sponsor commissioned a formal meta-analysis of the PKU literature and _____ performed the meta-analysis. The following was the outcome of the analysis:

- each 100 $\mu\text{mol/L}$ increase in blood Phe during the critical early childhood period predicts an average 1.3 to 3.1 point decrease in IQ, over a range of blood Phe from 423 to 750 $\mu\text{mol/L}$;
- each 100 $\mu\text{mol/L}$ increase in lifetime blood Phe (mean of blood Phe levels from birth through the time of testing) predicts an average 1.9 to 4.1 point decrease in IQ over a range of blood Phe from 394 to 666 $\mu\text{mol/L}$;
- each 100 $\mu\text{mol/L}$ increase in concurrent blood Phe predicts an average 0.5 to 1.4 point decrease in IQ, over a blood Phe range from 429 to 1664 $\mu\text{mol/L}$; and
- a statistically significant correlation between concurrent blood Phe and cerebral white matter abnormalities were also detected by MRI.

The analysis showed significant correlations between blood Phe and 2 independent neurologic outcomes. At the end of Phase 2 meeting, the Agency accepted blood Phe levels as an acceptable primary endpoint in the clinical studies with a plan for post-approval follow-up of treated patients to assess long term potential benefits of treatment (e.g., IQ and neuropsychological status).

The clinical development program has focused on the pharmacodynamic (PD) response of Kuvan administration (i.e., the reduction in blood Phe). The change in blood Phe levels was the primary endpoint for the Phase 3 PKU-003 study, and blood Phe was the metric against which increasing dietary Phe tolerance was assessed in PKU-006. A exposure-response (blood Phe levels) relationship was provided in the 5-20 mg/kg/day range in the Study PKU-004.

2.2.2.1. How was PD marker utilized in selecting dosing regimen in clinical trials?

The once daily dosing interval is based on the PD effect of sapropterin on Phe reduction rather than the known pharmacokinetics of sapropterin in humans (**note:** the drug has a plasma half-life of approximately 4-6 hours). All of the PKU clinical studies with Kuvan were conducted using a once-daily (QD) dose.

PKU-001 Substudy 01 and PKU-004 Substudy 01 were conducted to explore the ability of Kuvan to maintain reduced blood Phe levels during a 24 hour period, when given as a

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single daily dose, to sustain stable blood Phe levels during a 24-hour period in subjects with PKU who participated in Study 001 and Study 004. Data from these substudies have showed that blood Phe levels remain stable throughout a 24-hour observation period.

In PKU-001 Substudy 01 subjects received Kuvan 10 mg/kg/day for a total of 8 days according to the PKU-001 schedule of events. At each dose administration, subjects were to dissolve the drug tablets in 4-8 oz of water, orange juice or apple juice by mixing gently. The first dose was to be taken 3-5 hours after any meal; all other doses were to be taken 5-10 minutes before the morning meal. On the last day of treatment in (Day 8), additional blood Phe levels were obtained at 8:00 am prior to breakfast and prior to receiving the Kuvan dose, 12:00 noon prior to lunch, 4:00 pm, 6:00 pm prior to dinner, 10:00 pm, 12:00 midnight, and 8:00 am on the following morning prior to breakfast. Subjects were instructed to continue their usual diet without modification (ie, no change in daily Phe ingestion) during the 24-hour collection period. Subjects were prohibited from taking drugs known to inhibit folate synthesis (eg., methotrexate) during study participation.

Eleven subjects enrolled and all 11 subjects completed the 24 hour study. Of the 11 subjects enrolled, only 1 subject had a $\geq 30\%$ decrease in blood Phe level from baseline to Day 8 in PKU-001 and 10 were nonresponders (**note:** a responder was predefined as a $\geq 30\%$ decrease in blood Phe level from baseline). All blood Phe assays were performed using a tandem mass spectrometry.

The sponsor concluded that mean blood Phe levels remained stable over the course of the 24 hours and rose only slightly from a pre-dose baseline level of $747.4 \pm 152.6 \mu\text{mol/L}$ at 8:00 am to a peak level of $798.1 \pm 167.5 \mu\text{mol/L}$ at 10:00 pm, returning to starting levels of $745.5 \pm 188 \mu\text{mol/L}$ the following morning (Table 1). Change in blood Phe between the 8:00 am blood draw on Day 8 and subsequent time points showed mean changes ranging from $37.0 \pm 118.2 \mu\text{mol/L}$ at 4:00 pm to $-15.9 \pm 186.1 \mu\text{mol/L}$ at 12:00 midnight.

Table 1: Blood Phe levels over 24 hours

Blood Phe level ($\mu\text{mol/L}$)	(N = 11)
8:00 am	
n	10
Mean \pm SD	747.4 ± 152.6
12:00 pm	
n	11
Mean \pm SD	761.1 ± 168.5
4:00 pm	
n	9
Mean \pm SD	757.9 ± 168.2
6:00 pm	
n	8
Mean \pm SD	779.9 ± 268.1
10:00 pm	
n	9
Mean \pm SD	798.1 ± 167.5

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12:00 am	
n	9
Mean ± SD	705.0 ± 232.8
8:00 am (next day)	
n	8
Mean ± SD	745.5 ± 188.1

This reviewer does not agree with the sponsor’s conclusion regarding mean Phe values and its pattern. Mean values were calculated from many missing data. As shown in Table 2, only 6 subjects had entire data sets and 5 subjects had at least two missing data. There seems to be no pattern in terms of blood Phe levels after Kuvan administration. Individual data indicated large inter-subject variability but relatively small intra-subject variability.

Table 2: Blood Phe measurements during the 24-hr Substudy

Subject	8 am	Noon	4 pm	6 pm	10 pm	Midnight	8 am
0128-0013*						Not done	
0128-0011							
0128-0014							
0128-0015							
0128-0017							
0128-0018							
0128-0020							
0128-0021							
0128-0022							
0128-0023							
0128-0024							

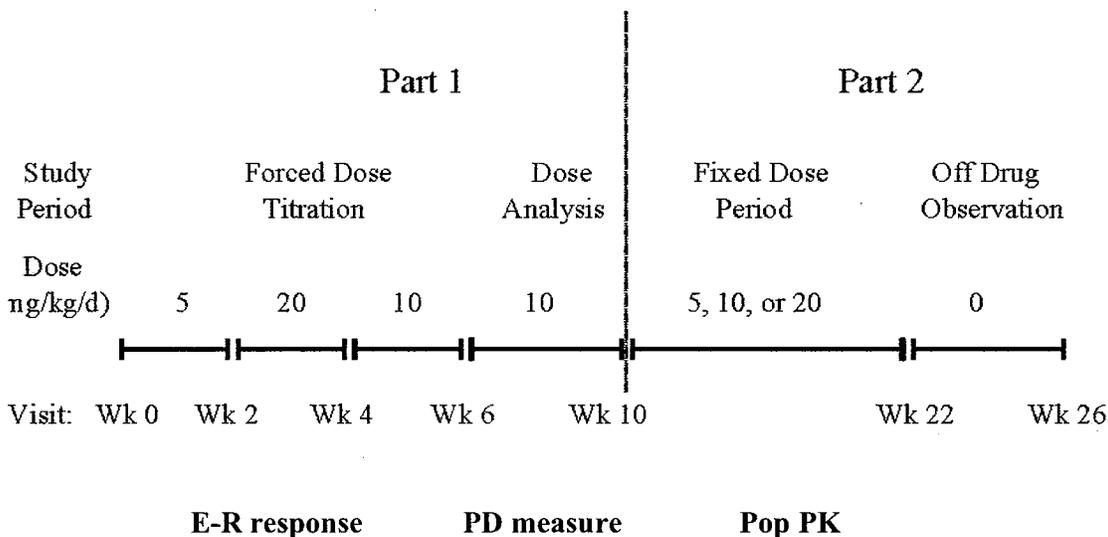
*responder

Overall, there seemed to be no apparent spike in the Phe levels during 24 hours.

In the second PD study (PKU-004 Substudy 01) 12 subjects received Kuvan, 10 mg/kg/day from Weeks 6 to 10 according to the PKU-004 schedule of events. While on treatment, subjects were admitted to the hospital for a 24-hour period and blood Phe levels were obtained at 8:00 am (prior to breakfast and receiving Kuvan), 12:00 noon (prior to lunch), 4:00 pm, 6:00 pm (prior to dinner), 10:00 pm, 12:00 midnight, and 8:00 am on the following morning (prior to breakfast and receiving the next Kuvan dose). All

blood Phe assays were performed using an ion exchange method.

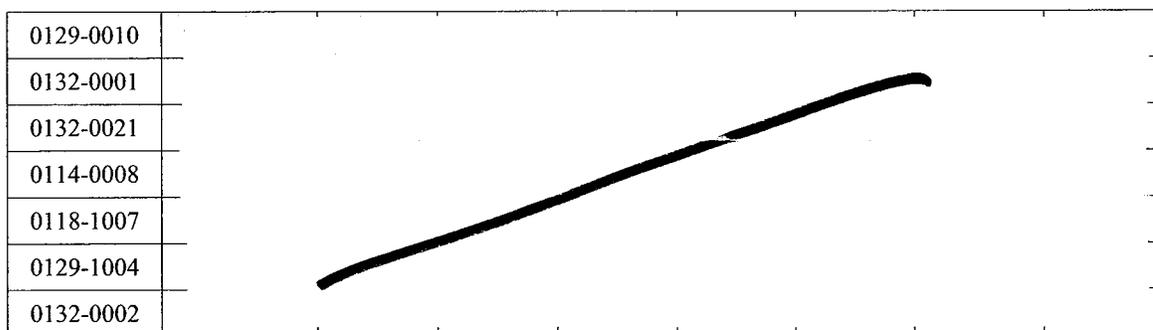
Figure 4: Schematic of the study design for Study PKU-004



The sponsor reported that among the 12 subjects evaluated (8 responders, > 30% reduction in blood Phe level from Week 0), mean blood Phe levels were stable, decreasing from an initial 8:00 am value of $661.1 \pm 432.8 \mu\text{mol/L}$ to a low value of $477.3 \pm 241.3 \mu\text{mol/L}$ at midnight (16 hours post Kuvan dose). However, the statement is not accurate. The low mean value of $477.3 \mu\text{mol/L}$ at midnight was derived from the data with one missing midnight measurement for the subject 0015-003, who happened to have the highest blood Phe values among patients. This reviewer, however, agreed with the sponsor that blood Phe levels were stable during 24 hours although inter-subject variability was large and dietary Phe intakes among patients varied as well. There seems to be no spike of Phe levels during 24 hours.

Table 3: Blood Phe measurements during 24 hour substudy

Subject	Dietary Phe intake, mg	Blood Phe level ($\mu\text{mol/L}$) at each timepoint						
		8 am	Noon	4 pm	6 pm	10 pm	Midnight	8 am
0015-0003	4903							
0018-0015	2639							
0018-0021	937							
0019-0001	5561							
0114-0011	364							



Overall conclusions:

Data from these substudies demonstrate that blood Phe levels remain stable throughout a 24-hour observation period. Therefore, once daily dosing can be justified.

2.2.3. Are the active moieties in the plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes, refer to the Analytical Section (2.7) for further details.

2.2.4. Exposure-response

2.2.4.1. What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

The exposure-response relationships were studied in the Study PKU-004, phase 3, open-label, forced dose-titration study. The study was an extension of Study PKU-003. Subjects received Kuvan in 3 consecutive 2-week courses of daily single oral doses of 5 mg/kg/day, followed by 20 mg/kg/day, and 10 mg/kg/day. Kuvan was administered dissolved in 4-8 oz of water, apple juice or orange juice. No specific instructions were given regarding the administration of Kuvan in relationship to meals. Eighty subjects were enrolled in the study.

The mean blood Phe level observed at the end of each 2-week dosing period decreased as the dose of Kuvan increased, demonstrating an inverse relationship between the dose of Kuvan and mean blood Phe levels. The mean change in blood Phe level differed significantly between the dose groups; the differences between effect of 5 versus 10 mg/kg/day (and 5 vs 20 mg/kg/day) was statistically significant at $p < 0.0001$ and between 10 vs 20 mg/kg/day was statistically significant at $p = 0.0085$. The percentage of subjects with a $\geq 30\%$ reduction in blood Phe levels was 25%, 55%, 46% after dosing for 2 weeks with 5, 20, and 10 mg/kg/day, respectively.

Table 4: Blood Phe levels

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	Mean Blood Phe levels (µmol/L) mean ± SD (N = 80)	Mean Change from Week 0 in blood Phe levels (µmol/L) mean ± SD (N = 80)	% of subjects with a ≥ 30% reduction in blood Phe level from Week 0 (N = 80)
<i>Dose-Titration Period</i>			
Week 0*	344.0 ± 398.0	--	--
End of 5 mg/kg/day dose (Week 2)	743.9 ± 384.4	-100.1 ± 295.2	25%
End of 20 mg/kg/day dose (Week 4)	580.8 ± 398.8	-263.3 ± 318.2	55%
End of 10 mg/kg/day dose (Week 6)	639.9 ± 381.8	-204.1 ± 303.0	46%

Table 5: Effect of Kuvan dose on blood Phe level

Estimated contrasts	Difference (µmol/L) mean ± SE	95% confidence interval	p-value
Change on 5 mg vs. change on 10 mg	104 ± 22.2	(60, 148)	<.0001
Change on 5 mg vs. change on 20 mg	163 ± 22.2	(119, 207)	<.0001
Change on 10 mg vs. change on 20 mg	59 ± 22.2	(15, 103)	0.0085

2.2.4.2. What are the characteristics of the exposure-response relationships for safety?

In Study PKU-004, 85% of subjects experienced at least one AE. Overall, 53% of subjects experienced an AE that was considered mild, 31% experienced an AE that was considered moderate, and only 1 subject (1%) experienced an AE that was considered severe (tooth abscess). Among the subjects who experienced an AE, 39% had an event that was judged by the investigator to be possibly or probably related to Kuvan. Three subjects each experienced a single serious adverse event (SAE); these were urinary tract infection, spinal cord injury, and a tibia fracture. None of the SAEs was judged to be related to Kuvan administration. No subject was withdrawn from the study because of an AE. The sponsor reported that during the fixed-dose period, the percentage of subjects who had an AE was similar between the 5 mg/kg/day and the 20 mg/kg/day doses (50% and 57%), but slightly lower for the 10 mg/kg/day dose group (38%). There seemed to have no apparent relationship between the dose of Kuvan and the incidence of AE.

2.2.4.3. Does this drug prolong the QT or QTc interval?

The sponsor has not conducted a thorough QT study. Neither were ECG assessments performed in the pivotal phase 3 studies, 003 and 006. ECGs were measured in the PK studies, Study PKU- 005 and -009. However, the assessments were conducted in screen and/or week1 or during follow-up phase but not at Tmax. Therefore, it is not feasible to investigate whether the drug prolongs the QT or QTc interval.

During a t-con held on May 30, 2006 with the DCRP, the division requested that ECG testing be performed at the time of “peak effect” of the drug in the HTN-001 protocol. However, the sponsor stated that the PK vs. PD effects of

sapropterin in the hypertension patient population were not yet well understood and therefore the time of peak effect was unknown at this point. Based on these factors, it was agreed that it would be appropriate to conduct the ECG testing. Testing was performed at screening, at Week 8 prior to the last dose of the study, and at the Week 12 visit, 4 weeks post final dose.

A question had been addressed to the QT-IRT group whether a formal thorough QT study should be requested as a phase 4 study, if the NDA is approved. The QT-IRT responded as follow: the Agency does not have a policy about orphan drugs, whether and when this drug should undergo a formal QT evaluation is a question for the review division.

_____ A formal consult was sent to the QT-IRT group inquiring whether a thorough QT study is needed as a phase 4 study.

2.2.4.4. Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The sponsor proposes that the starting dose of Kuvan for PKU _____ is _____ taken once daily. Doses of Kuvan may be adjusted in the range of 5-20mg/kg. However, this reviewer does not agree with the sponsor's proposal because of the following reasons:

1. Although there is clear dose-response relationship (i.e., the higher dose, the more efficacy), Study PKU-004 has demonstrated that the 10 mg/kg/day is efficacious: the end of dosing with the 10 mg/kg/day for 2 weeks 46% of patients had > 30% reduction in blood Phe level from Week 0, and mean change from Week 0 in blood Phe levels was -204 $\mu\text{mol/L}$.
2. The dose of 10 mg/kg/day was used in the pivotal, controlled, phase 3 study for PKU and proven to be efficacious.

It is suggested that the 10 mg/kg/day be the starting dose and the patients can be titrated up and down based on the response.

2.2.5. What are the PK characteristics of the drug and its major metabolite?

2.2.5.1. What are the single dose and multiple dose PK parameters?

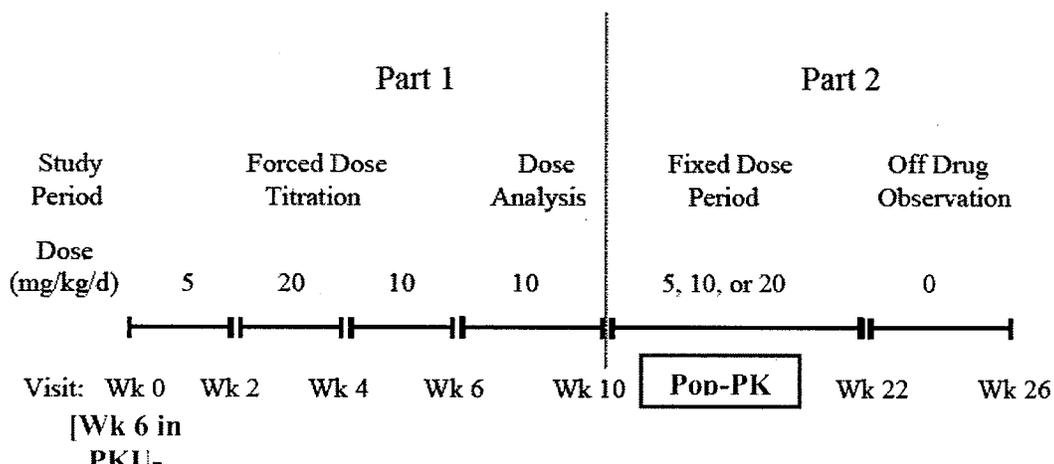
The single dose PK in healthy volunteers with the to-be-marketed formulation is described under the Section of 2.5 General Biopharmaceutics. Briefly, the two phase 1 PK studies demonstrated that T_{max} and $t_{1/2}$ were each approximately 4 hours regardless of mode of administration or delivery (swallowed as intact tablet vs dissolved, water versus orange juice, fasted vs fed). The multiple-dose PK studies were conducted with the 2.5% Sapropterin granule formulation over a 7-day period using a dosage of 300mg/day (100 mg TID) and a dosage of 600 mg/day (200 mg TID) and showed no accumulation. Since

those studies used different formulation and dosing regimen from the proposed formulation and dosing regimen, they would not be discussed in this document. The multiple dose PK with the to-be-marketed formulation was not conducted in healthy volunteers. However, the steady state PK study in patients was conducted using a population PK approach.

2.2.5.2. What is the PK in patients? How does the PK of the drug in healthy volunteers compare to that in patients?

Population analysis was performed to describe the PK characteristics of Kuvan in PKU patients and to identify patient factors that may affect the variability of Kuvan PK (PKU-004 substudy 002). This was the only PK study conducted in the target patient population at doses of 5 mg, 10 mg, and 20 mg/kg/day with BH4 measurements only at steady state. This was an add-on population PK study of PKU-004, a Phase 3 open-label, 22-week, forced titration (5-20 mg/kg) trial in subjects with PKU who had elevated phenylalanine levels. Study PKU-004 was an extension of PKU-003. Eighty subjects were enrolled in the main PKU-004 study and 78 subjects participated in the substudy. The schematic design for study PKU-004 is as shown in Figure 5. Population PK (substudy 02) was performed during the twelve-week fixed dose period (Weeks 10-22), during which subjects received a fixed dose (5, 10 or 20 mg/kg/day) according to their observed Phe levels at week 6. Collection of PK samples was performed during weeks 16-22. There were no specific exclusion or inclusion criteria for participation in the substudy.

Figure 5: Study Design



The pharmacokinetic of Kuvan was adequately described by a two compartment model with a short lag time (16.5 minutes) prior to first-order absorption, followed by biphasic elimination. A BASE term was included to account for the endogenous BH4. Total body weight is the only significant patient variable that accounts for the BSV in CL/F and Vc/F, which is expected from a weight-based regimen. Gender and age did not significantly influence Kuvan PK. As a result, the Final Model had only weight included in both CL/F

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and Vc/F. Estimates of CL/F and Vc/F were 2100 L/h/70kg (53.9% BSV) and 8350 L/70kg (55.7% BSV), respectively. The within-subject (residual) variability was 21.7%. The analysis described the data well. However, due to the limitations in PK data collection and study design, the parameter estimates are associated with great uncertainty. The estimate of terminal half-life is 6.7 h (range: 3.9-17 h) in PKU subjects. The reported terminal half-life in previous single-dose PK studies (PKU-005 and PKU-009) in healthy volunteers was 4 h (range: 3-5 h). The PM reviewer has concluded that it is reasonable to assume that pharmacokinetics of healthy subjects and patients are similar.

Figure 6: Plasma BH4 concentrations for all subjects (N=76)

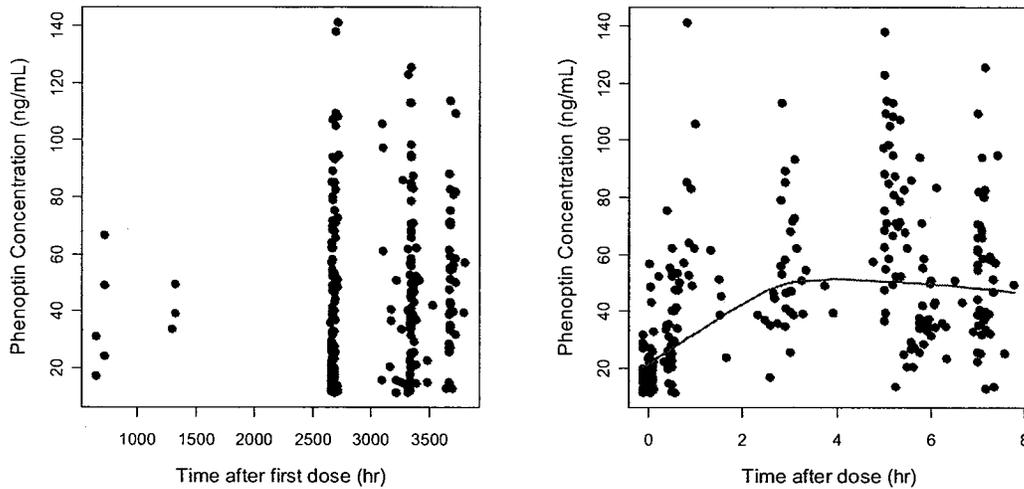


Table 6: Parameter estimates and standard errors for the Final PK Model

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Parameter (Units)		Population Mean (SE*)	Standard Deviation of Inter-Individual Variance (SE*)
ALAG (h)	θ_1	0.275 (13.7)	NA
Ka (1/h)	θ_2	0.518 (24.1)	NA
CL/F (L/h/70kg)	θ_3	2100 (9.9)	0.539 (25.3)
COV1	θ_5	0.586 (34.0)	NA
Vc/F (L/70kg)	θ_4	8350 (16.9)	0.557 (41.3)
COV2	θ_9	1.13 (24.7)	NA
Vp/F (L)	θ_7	4240 (42.5)	NA
Q/F (L/h)	θ_8	862 (43.5)	NA
BASE (ng/mL)	θ_7	13.5 (8.2)	NA
R (CL,Vc)		0.336	NA
CCV Residual Error (as %CV)			21.7 (13.3)

* - SE given as %CV; NA – Not Applicable

ALAG = Lag Time

Ka = Absorption Rate Constant

CL/F = Apparent Clearance

COV1 = Power function on CL/F

Vc/F = Central Volume Compartment

COV2 = Power function on Vc/F

Vp/F = Peripheral Volume Compartment

Q = Intercompartmental Clearance

BASE = Endogenous Baseline Concentration

R = Correlation between parameters

2.3. Intrinsic Factors

The findings of the population PK analysis in PKU patients indicated that gender and age did not significantly influence Kuvan PK but body weight could affect the Kuvan PK.

Other intrinsic factors such as renal insufficiency, hepatic insufficiency, and ethnicity have not been studied.

2.4. Extrinsic Factors

It has not been studied whether extrinsic factors such as drugs, herbal products, smoking and alcohol uses can influence dose-exposure and/or response of Kuvan. The sponsor states that there are no known clinically relevant PD interactions with other medicinal products or substances. Kuvan studies, however, excluded patients who required concomitant treatment with any drug known to inhibit folate synthesis (eg, methotrexate). BH4 is normally recycled and, in the last step of this process, q-dihydrobiopterin is reduced to active BH4 by the NADH-dependent dihydropteridine reductase (DHPR). Folate synthesis inhibitors, such as methotrexate, inhibit this activity of the enzyme both in vivo and in vitro. The Kuvan studies also excluded patients receiving levodopa based on one AE during DAP's development of Biopten. The sponsor reported that in one DAP study, a 13-year-old subject with prior history of convulsions and who was receiving levodopa was treated with sapropterin at a dose of 3 mg/kg/day and had an increase in the number of convulsions. The sponsor proposes to include the possible interactions between Kuvan and levodopa or folate synthesis inhibitors in the product labeling as a precaution.

2.5. General Biopharmaceutics

2.5.2 Are clinical formulations different from the to-be-marketed formulation? If so, are they comparable?

Table 7 is an overview of modifications in the manufacturing process, site of manufacture, debossment and the composition of the formulations during development.

Table 7: Overview of manufacturing modifications during development

	[REDACTED]	Lyne Laboratories ^{1,2}
Manufacturing Process	[REDACTED]	[REDACTED]
Debossment	[REDACTED]	"177"
Formulation	[REDACTED]	Modified
Sapropterin dihydrochloride	[REDACTED]	[REDACTED]
Dibasic calcium phosphate	[REDACTED]	[REDACTED]
Ascorbic acid	[REDACTED]	[REDACTED]
Croscopovidone	[REDACTED]	[REDACTED]
Sodium stearyl fumarate	[REDACTED]	[REDACTED]

¹Used in clinical studies:

- [REDACTED] - Study 001, 003, 004, 005, 006
- [REDACTED] - Study 004, and 006
- [REDACTED] - Study 004, 006
- [REDACTED] - Study 004, 006, 008, 009
- Lyne: - Study 008

²Proposed commercial formulation

³One of 6 lots was produced using [REDACTED]

⁴One of 5 lots was produced using [REDACTED]

In the modified formulation [REDACTED], the level of [REDACTED] was increased from [REDACTED]

Dissolution profiles were generated for tablets manufactured at the three manufacturing sites, [REDACTED]. All tablets were dissolved more than [REDACTED] in 30 minutes under the dissolution condition of USP paddle of 50 RPM with 0.1N HCl. Therefore, it is considered that the bioavailability of the products is not limited to the dissolution, and the formulation difference between the to-be-marketed and the clinical tablets would not affect the bioavailability of Kuvan.

The change in the manufacturing process from [REDACTED] considered as a [REDACTED] change which requires a bioequivalence study. The tablets manufactured by both processes were used in clinical studies. However, the drugs were

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dissolved in solution, which diminishes the difference between the manufacturing processes.

Therefore, it is concluded that the comparability between the clinical trial formulation and the to-be-marketed formulation has not been demonstrated.

2.5.3. In the pivotal clinical trials patients were given Kuvan dissolved in water, apple juice or orange juice. However, the sponsor [REDACTED] that patients take Kuvan as [REDACTED] dissolved in water or apple juice.

2.5.3.1. Is bioavailability different between intact tablets and dissolved tablets?

Administration of Kuvan as an intact tablet resulted in an approximate 20% increase in the extent of absorption compared to a dissolved tablet.

Study PKU-009 was an open-label, randomized, three-treatment, six-sequence, three-period crossover study in which 30 subjects were to complete 3 single-dose dosing periods and were randomized to one of six sequence groups where all dosing groups received Kuvan 10 mg/kg orally as follows: a: administered after dissolution of tablet(s) in water given under fasting conditions b: administered as intact tablet(s) given under fasting conditions c: administered as intact tablet(s) given 30 minutes after beginning to ingest a high-calorie, high-fat meal. Each subject received a single dose of 10mg/kg of Kuvan during each treatment period. The lot number used in this study was T140604B. Three single-dose treatment periods each separated by a minimum of 7 days.

Thirty (30) subjects were originally enrolled. The sponsor reported that six (6) subjects were withdrawn from the study after the first period because they tested positive for tobacco exposure and 1 subject withdrew after the second period for personal reasons. These subjects were replaced by 7 subjects who received the treatments the original subjects had not received. Seven (7) additional subjects were then enrolled and received all 3 treatments according to the sequences assigned to the original subjects as defined in the protocol. Total 44 subjects, ages 18 to 50 years, were enrolled in the study. Twenty (20) were female and 24 were male. Twenty five (25) were white, 11 were Hispanic or Latino, and 7 were black or African American and 1 was Asian/Asian Pacific Islander. Mean height was 172.3 cm (range: 157.5 to 191 cm) and mean weight was 70.8 kg (range: 51 to 99 kg). Although plasma concentrations of Kuvan were measured for the 7 discontinued subjects and the 7 incomplete replacements, all data from these 14 subjects was excluded from the statistical analyses.

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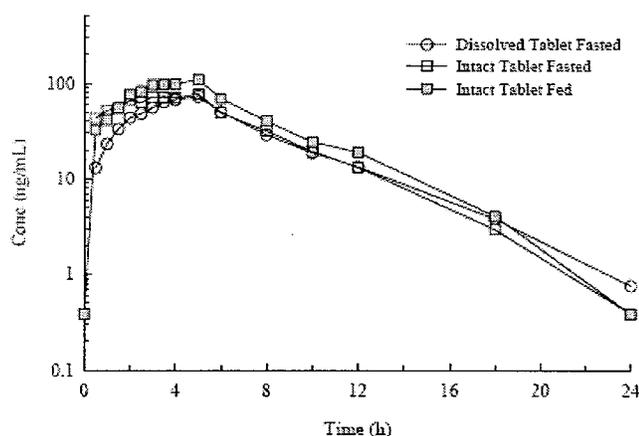


Figure 7: Mean plasma concentrations of BH₄ after oral administration of 10 mg/kg of Kuvan as dissolved and intact tablets under fasted and fed conditions

Mean plasma concentrations of BH₄ were lower when Kuvan was administered as a dissolved tablet compared to the intact tablet. Mean C_{max} was higher for the intact tablet as were mean values for AUC_(0-t) and AUC_(inf). The geometric mean ratios of intact-to dissolved tablet for all parameters measured, ranged from 118% to 121%. The median and range for T_{max} were essentially the same for the dissolved and intact tablets.

Table 8: Summary of Pharmacokinetic Parameters for BH₄ after Oral Administration of 10 mg/kg of Kuvan as Dissolved and Intact Tablets under Fasted Conditions

Parameter	Dissolved Tablet	Intact Tablet
C _{max} (ng/mL)	80.3 ± 63.3	91.2 ± 36.3
T _{max} (hr)	4.00	3.50
AUC _(0-t) (hr·ng/mL)	479 ± 292	550 ± 214
AUC _(0-∞) (hr·ng/mL)	597 ± 336	704 ± 202
t _{1/2} (hr)	5.31 ± 4.42	4.47 ± 3.37

Table 9: Statistical Comparison of Pharmacokinetic Parameters for BH₄ after Oral Administration of 10 mg/kg of Kuvan as Dissolved and Intact Tablets under Fasted Conditions

Parameter	Geometric mean ratio (%)	
	Estimate	90% CI
C _{max}	120.98	104.21 – 140.44
AUC _(0-t)	120.33	104.12 – 139.06
AUC _(0-∞)	118.04	98.16 – 141.96

Comments:

- One of 7 additional subjects (subject 0138-0306) had higher C_{max} (387.07 ng/mL) and larger AUC (1625.0 hr·ng/mL) compared to other subjects when the subject took Kuvan as dissolved tablets.
- The subjects who did not complete all treatment periods had comparable C_{max}s and AUCs.

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Administration of Kuvan as an intact tablet resulted in an approximate 20% increase in the extent of absorption compared to a dissolved tablet. Based on safety profile of the drug, 20% increase of bioavailability with an intact tablet would not impact on safety of the drug. Therefore, the sponsor proposes that the patients take [REDACTED] dissolved in water.

However, the drug products used in this study were manufactured [REDACTED] but the-to-be-marketed product will be manufactured by [REDACTED], and the change is considered as a [REDACTED] change. The tablets manufactured by both processes were used in clinical studies. However, the drugs were dissolved in solution, which diminishes the difference between the manufacturing processes. Therefore, it is recommended that Kuvan should be dissolved in solution.

2.5.3.1. What is the effect of vehicle (water, orange juice or apple juice) on Sapropterin absorption?

Administration of Kuvan in orange juice is equally bioavailable to Kuvan in water under fasted conditions. Administration of Kuvan dissolved in water and in orange juice resulted in comparable mean plasma concentrations and mean values for C_{max} and $AUC_{(0-\infty)}$. However, administration of Kuvan in orange juice is less bioavailable to Kuvan in water under fed conditions. A high fat, high calorie food increased the bioavailability of Kuvan. The smaller increase in mean plasma concentration was observed with orange juice as compared to water.

In the pivotal phase 3 study apple juice was used as a vehicle for drug administration but bioavailability of apple juice has not been investigated. (Note: In study 003, Kuvan tablets were administered orally once daily in the morning dissolved in 4-8 oz (120 – 240 mL) of water, apple juice or orange juice. The patients were instructed to ingest the entire solution within 15 minutes of dissolving tablets. In study 006, Kuvan tablets were administered dissolved in 4-8 oz of water or apple juice. Since the patients were in a Phe-restricted diet and orange juice contains a significant amount of Phe, orange juice was not used as a vehicle for drug administration.)

The vehicle effect was studied in Study PKU005. This study was an open label, randomized, 4-treatment, 4-sequence, 4-period crossover single oral dose study in which 28 subjects were randomized to 1 of 4 sequence groups where all dosing groups received Kuvan 10 mg/kg orally as follows: a: prepared in water solution given in fasting conditions, b: prepared in water solution given in fed conditions, c: prepared in orange juice solution given in fasting conditions, d: prepared in orange juice solution given in fed conditions. A washout period of at least 7 days separated each dose administration. Blood samples for pharmacokinetic analysis were drawn at selected time points during each treatment period: within 30 minutes prior to dose, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 18.0, and 24.0 hours post dose. The lot number used throughout the study was Lot T140402-A.

All 28 subjects were randomized and were dosed in Periods 1, 2, and 3. Subject 0138-0002 withdrew from the study because of an AE (streptococcal pharyngitis) prior to Period 4 in which Kuvan in water under fasted conditions was to be administered. This subject's data for the first 3 treatment periods were analyzed and used in the appropriate

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statistical comparisons by the sponsor and its approach was accepted by this reviewer. Thus, the analysis population included all 28 subjects for Treatment Periods 1, 2, and 3 and 27 subjects for Treatment Period 4.

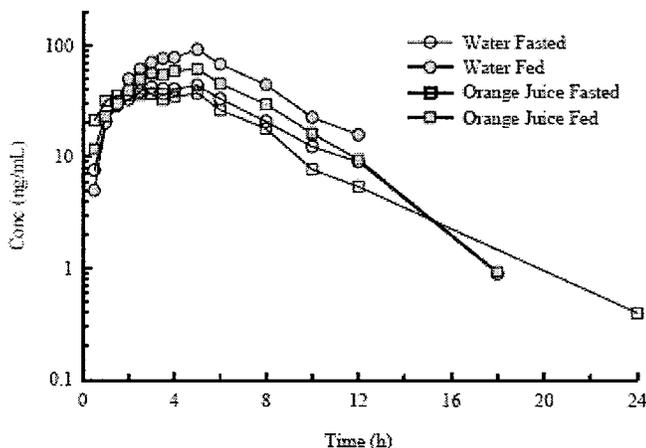


Figure 8: Mean plasma concentration of BH₄ after oral administration of 10 mg/kg of Kuvan in water and orange juice under fed and fasted conditions

Administration of Kuvan in water and orange juice under fasted conditions resulted in comparable mean plasma concentrations and mean values for C_{max} and $AUC_{(0-t)}$. The geometric mean ratios, orange juice-to-water, for C_{max} and $AUC_{(0-t)}$ were 95.4% and 90.2%, respectively. However, administration of Kuvan under fed conditions (high fat, high calorie meal) resulted in an increase in the mean plasma concentrations and mean values for C_{max} and $AUC_{(0-t)}$ when Kuvan was administered in water as opposed to orange juice. The geometric mean ratios, orange juice-to-water, for the 2 parameters were 73.4% and 74.6%, respectively.

Table 10: Summary of Pharmacokinetic Parameters for BH₄ After Oral Administration of Kuvan in Water And Orange Juice Under Fed And Fasted Conditions

Parameter ¹	Water Fasted	Water Fed	Orange Juice Fasted	Orange Juice Fed
C_{max} (ng/mL)	54.0 ± 18.6 [27] (51.1)	99.4 ± 38.8 [28] (93.6)	51.6 ± 17.5 [28] (48.6)	71.8 ± 23.4 [28] (68.7)
T_{max} (h)	4.00 [27] (2 - 6)	5.00 [28] (3 - 6)	3.00 [28] (2 - 8)	4.00 [28] (3 - 6)
$AUC_{(0-t)}$ (h·ng/mL)	309 ± 104 [27] (289)	557 ± 169 [28] (534)	273 ± 98.5 [28] (258)	415 ± 127 [28] (398)
$AUC_{(inf)}$ (h·ng/mL)	386 ± 110 [8] (371)	634 ± 179 [20] (611)	333 ± 79.9 [11] (324)	521 ± 155 [20] (499)
λ_z (h ⁻¹)	0.1985 ± 0.0868 [8]	0.2484 ± 0.0609 [20]	0.2512 ± 0.1272 [11]	0.2038 ± 0.1097 [20]
$t_{1/2}$ (h)	4.68 ± 3.36 [8]	2.97 ± 0.84 [20]	3.77 ± 2.83 [11]	4.34 ± 2.20 [20]

Table 11: Geometric Mean Ratios for C_{max} , $AUC_{(0-t)}$ and $AUC_{(inf)}$ under Fasted and Fed Conditions After Oral Administration Of Kuvan In Water And Orange Juice

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Parameter	Geometric Mean Ratio (%) ¹		
	Estimate	90% Confidence Interval	
Water Fed vs. Water Fasted			
C _{max}	183.62	164.57	→ 204.88
AUC(0-t)	186.64	170.07	→ 204.81
AUC(inf)	159.11	132.36	→ 191.27
Orange Juice Fed vs. Orange Juice Fasted			
C _{max}	141.23	126.75	→ 157.36
AUC(0-t)	154.25	140.73	→ 169.08
AUC(inf)	155.77	133.54	→ 181.69
Orange Juice Fasted vs. Water Fasted			
C _{max}	95.42	85.52	→ 106.47
AUC(0-t)	90.23	82.22	→ 99.02
AUC(inf)	83.56	68.39	→ 102.09
Orange Juice Fed vs. Water Fed			
C _{max}	73.39	65.87	→ 81.78
AUC(0-t)	74.57	68.03	→ 81.74
AUC(inf)	81.80	72.68	→ 92.07

Comments:

- Administration of Kuvan in water and in orange juice under fasted conditions resulted in comparable mean plasma concentrations and mean values for C_{max} and AUC(0-t).
- Administration of Kuvan in water and orange juice after a high fat, high calorie meal resulted in substantial increases in mean values for C_{max} and AUC(0-t). The smaller increase in mean plasma concentration was observed with orange juice as compared to water.
- Kuvan tablets were administered orally once daily in the morning after being dissolved in 4-8 oz (120 – 240 mL) of water, apple juice or orange juice in Study PKU-003. It is not feasible to detect the difference on efficacy or safety between vehicles because of no records stating which patients used what vehicle. In study 006, Kuvan tablets were administered dissolved in 4-8 oz of water or apple juice. Although relative bioavailability of the drug dissolved in apple juice to either water or orange juice has not been studied, the two pivotal phase 3 studies showed that the drug is efficacious and safe when the drug is administered dissolved in water, orange juice or apple juice.
- In Study PKU-001 and 004, Kuvan tablets were administered in water, apple juice and orange juice. No information was in datasets on who used which liquid.
- This reviewer has learned from the reviewing chemist that the sponsor had submitted the stability data on the drug in apple juice and the data indicated that the drug in apple juice and water are comparable.
- Therefore, it is concluded that the sponsor's proposal of dissolving tablets in water and apple juice is acceptable.

2.5.1. What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The effect of food on the bioavailability of Kuvan was studied in PKU-009 and PKU-005. The two studies indicated that food (high fat, high calorie meal) increased the bioavailability of the drug. Administration of Kuvan as an intact tablet with a high-calorie, high-fat meal resulted in an approximate 30% increase in the extent of absorption. Administration of Kuvan with food in either water or orange juice results in a substantial increase in absorption – approximately 84% in water and 41% in orange juice.

In Study PKU-009 administration of the intact tablet with a standard high-fat high-calorie meal resulted in a substantial increase in the mean plasma BH₄ concentrations and mean values for C_{max} and AUC_(0-t). The geometric mean ratios of fed-to-fasted for all parameters measured ranged from 126% to 139%. The T_{max} values were essentially the same under fed and fasted conditions, suggesting that the increase seen with food was in the extent but may not the rate of absorption.

Table 12: Pharmacokinetic Parameters after Oral Administration of 10 mg/kg of Kuvan as Intact Tablets under Fasted Conditions and under Fed Conditions to Healthy volunteers

Parameter	Fasted	Fed
C _{max} (ng/mL)	91.2 ± 36.3	121 ± 33.6
T _{max} (hr)	3.50	4.00
AUC _(0-t) (hr·ng/mL)	550 ± 214	709 ± 221
AUC _(0-∞) (hr·ng/mL)	704 ± 202	825 ± 256
t _{1/2} (hr)	4.47 ± 3.37	4.28 ± 2.79

Table 13: Statistical Comparison of Pharmacokinetic Parameters after Oral Administration of 10 mg/kg of Kuvan as Intact Tablets under Fasted Conditions and Fed Conditions

Parameter	Geometric mean ratio (%)	
	Estimate	90% CI
C _{max}	138.63	119.42 – 160.93
AUC _(0-t)	133.69	115.68 – 154.50
AUC _(0-∞)	125.61	104.29 – 151.30

Study PKU-005 showed whether dissolved in water or in orange juice, administration of Kuvan after a high fat, high calorie meal resulted in a substantial increase in mean plasma concentrations and mean values for C_{max} and AUC_(0-t). Administration of an aqueous solution with food resulted in geometric mean ratios, fed-to-fasted, of 184% for C_{max} and 187% for AUC_(0-t). The magnitude of the increase with orange juice was less — geometric mean ratios of 141% and 154% for C_{max} and AUC_(0-t), respectively.

Table 14: Geometric Mean Ratios for Cmax, AUC(0-t) and AUC(0-∞) under Fasted and Fed Conditions after Oral Administration of Kuvan in Water and Orange Juice

Parameter	Geometric Mean Ratio (%)	
	Estimate	90% CI
Water Fed vs Water Fasted		
Cmax	183.62	164.57 – 204.88
AUC(0-t)	186.64	170.07 – 204.81
AUC(0-∞)	159.11	132.36 – 191.27
Orange Juice Fed vs. Orange Juice Fasted		
Cmax	141.23	126.75 – 157.36
AUC(0-t)	154.25	140.73 – 169.08
AUC(0-∞)	155.77	133.54 – 181.69

Comments:

- High fat meals increased the bioavailability of Kuvan especially when the drug was administered as solution dissolved in water, 87% increase compared to fasting condition with the 90% CI of 205%.
- Pivotal clinical studies were conducted regardless to meals or no instruction was made whether patients could take the drug under fasting or fed conditions. It may not be feasible to triage the food effect on efficacy or safety.
- In Study PKU-001 and 004, Kuvan tablets were taken in the morning but no specific instructions were given regarding the administration of Kuvan in relationship to meals. In study 001, the first dose was to be taken 3-5 hours after any meal and all other doses were to be taken 5-10 minutes before the morning meal.
- ~~_____~~
However, this reviewer recommends that the drug be taken with food in order to optimize the bioavailability of the drug.

2.6. Analytical Section

2.6.1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

The BH₄ conversion ratio was calculated as the molar ratio:

$$\%BH_4 \text{ Conversion Ratio} = \frac{\text{Mean of } \text{Found}}{\text{Nominal BH}_4 \text{ Concentration}} \times \frac{MW_{BH_4}}{MW} \times 100$$

where MW_{BH₄} = 241.2 and MW = 237.2

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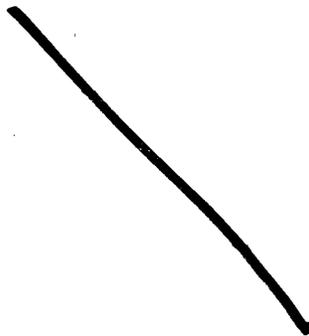
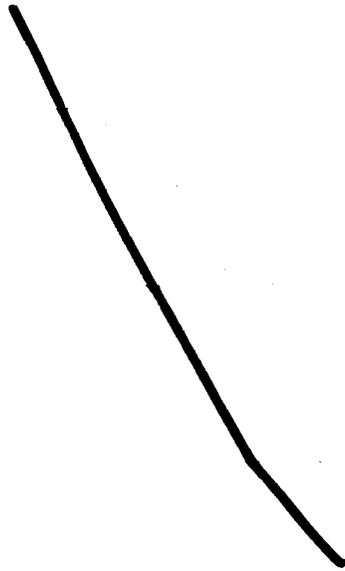
10.6% for the blood control

Linearity:

Linear up to a dilution factor of 128 and down to the Phe level of 12.86 $\mu\text{mol/L}$

Many methods were used to analyze samples in PKU-006 at different clinical sites. Detailed methods were not described but Phe testing equivalency report was included in the submission. In support of PKU-006, — clinical trial services collected basic testing information from local labs. The report demonstrated comparable results.

3. Detailed Labeling Recommendations



21 Page(s) Withheld

 Trade Secret / Confidential

✓ Draft Labeling

 Deliberative Process

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/s/

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