PROJECT 230108
PROTOCOL 2023-5439

A PILOT, PHASE 1, RANDOMIZED, OPEN-LABEL, SINGLE-DOSE, FOUR-WAY CROSSOVER STUDY TO COMPARE THE PHARMACOKINETICS OF SAPROPTERIN DIHYDROCHLORIDE 100 mg/mL ORAL SUSPENSION (PRODUCT CODE: RLF-OD032) (TEST) WITH KUVAN® (SAPROPTERIN DIHYDROCHLORIDE) 100 mg POWDER FOR ORAL SOLUTION (REFERENCE) AND TO EVALUATE THE EFFECT OF FOOD AND THE EFFECT OF WATER ON THE BIOAVAILABILITY OF SAPROPTERIN DIHYDROCHLORIDE 100 mg/mL ORAL SUSPENSION IN HEALTHY SUBJECTS

Sponsor:	APR Applied Pharma Research s.a. Via Giuseppe Corti 5 6828 Balerna Switzerland
Contract Research Organization:	[redacted]
Protocol Version:	Final
Date:	10-MAY-2024

CONFIDENTIAL

Protocol Historical File

Version	Brief description/summary of changes	Date
Final	Version submitted to the Independent Ethics Committee (IEC).	10-MAY-2024

Sponsor Signature Page

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Investigator Signature Page

I have carefully read this study protocol and agree that it contains all necessary information required to conduct this study. I agree to conduct the study according to this protocol (including any amendments) and in accordance with the clinical site's Standard Operating Procedures (SOPs), ICH Good Clinical Practice (GCP), all other applicable regulations, and the recommendations laid down in the most recent version of the Declaration of Helsinki.

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List of Abbreviations

AE	adverse event
ALT (SGPT)	alanine aminotransferase
ANOVA	analysis of variance
ANCOVA	analysis of co-variance
AST (SGOT)	aspartate aminotransferase
$AUC_{0\text{-}inf}$	area under the concentration-time curve from time zero to infinity (extrapolated)
$AUC_{0\text{-}t}$	area under the concentration-time curve from time zero until the last observed concentration
BH4	tetrahydrobiopterin
β-HCG	beta-human chorionic gonadotropin
BMI	body mass index
BUN	blood urea nitrogen
CBC	complete blood count
CDER	Center for Drug Evaluation and Research
CI	confidence interval
Cl/F	apparent clearance
C_{max}	maximal observed concentration
CTA	Clinical Trial Application
CV	coefficient of variation
DMP	data management plan
ECG	electrocardiogram
EOS	end of study
ERB/IRB	Ethics/Institutional Review Board
ET	early termination
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma glutamyl transpeptidase
GMP	Good Manufacturing Practice
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HEENT	head, eyes, ears, nose, and throat
HIV	human immunodeficiency virus

ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
K_{el}	terminal elimination rate constant
LD	lactate dehydrogenase
LH	luteinizing hormone
MedDRA	Medical Dictionary for Regulatory Activities
MRHD	maximum recommended human dose
NOL	No Objection Letter
OTC	over-the-counter
PAH	phenylalanine hydroxylase
PCF	Privacy Consent Form
PCP	phencyclidine
Phe	phenylalanine
PK	pharmacokinetic(s)
R^2	coefficient of determination obtained from regression analysis
RBC	red blood cell
SAE	serious adverse event
SAS	statistical analysis system
SOP	standard operation procedure
SUSAR	suspected, unexpected, serious adverse reaction
$T_{½\ el}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
T_{lag}	time of observation prior to the first observation with a measurable (non-zero) concentration
T_{max}	time when the maximal concentration is observed
VCF	Volunteer Consent Form
V_z/F	apparent volume of distribution
WBC	white blood cell

Synopsis of Protocol

Project Number:	230108
Protocol number	2023-5439
Title of the Study:	A Pilot, Phase 1, Randomized, Open-Label, Single-Dose, Four-Way Crossover Study to Compare the Pharmacokinetics of Sapropterin Dihydrochloride 100 mg/mL Oral Suspension (Product Code: RLF-OD032) (Test) with Kuvan® (Sapropterin Dihydrochloride) 100 mg Powder for Oral Solution (Reference) and to Evaluate the Effect of Food and the Effect of Water on the Bioavailability of Sapropterin Dihydrochloride 100 mg/mL Oral Suspension in Healthy Subjects
Clinical Research Facility:	[redacted]
Biomedical Laboratory Facility:	[redacted]
Investigational Products:	Test: Sapropterin dihydrochloride 100 mg/mL oral suspension (product code: RLF-OD032) (APR Applied Pharma Research s.a., Switzerland) Reference: Kuvan (sapropterin dihydrochloride) 100 mg powder for oral solution [redacted]
Study Phase and Type:	Phase 1 – Comparative Pharmacokinetic (PK), Food-Effect, and Water-Effect
Objectives:	Primary objectives: • [redacted] Secondary objective: • [redacted]
Endpoints:	Primary endpoints: • PK o Uncorrected and baseline-corrected sapropterin AUC_{0-t}, AUC_{0-inf}, and C_{max}

	Secondary endpoints: [redacted]
Study Design:	This is a single center, Phase 1, randomized, open-label, single-dose, 4-treatment, 4-period, 4-sequence, crossover study designed to compare the PK of sapropterin from the Test and Reference products, and to evaluate the effect of food and the effect of water administration on the bioavailability of sapropterin from the Test product in healthy subjects. In each period, subjects will receive a single 10 mg/kg dose of sapropterin dihydrochloride on Day 1, under fasting or fed conditions, and with or without water, followed by 24 hours of PK sampling. The study will include a screening visit from Day -30 to Day -1. In each period, eligible subjects will be admitted to the clinical site on Day -1 and will be confined until completion of the assessments on Day 2. There will be a washout period of at least 7 days between doses.
Study Population:	It is planned to enroll 16 healthy adult males and females for participation in this study. All attempts will be made to have an equal number of males and females.
Inclusion/Exclusion Criteria:	Inclusion Criteria Subjects must meet all of the following criteria to be included in the study: 1. Male or female, light smoker (no more than 10 cigarettes daily) or non-smoker, ≥18 and ≤50 years of age, with body mass index (BMI) ≥18.5 and ≤30.0 kg/m^2 and body weight ≥50.0 kg for males and ≥45.0 kg for females. 2. Healthy as defined by: a. the absence of clinically significant illness and surgery within 30 days prior to dosing. b. the absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, hematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease. 3. Female subjects of non-childbearing potential must be: a. post-menopausal (no menstrual period at least 12 consecutive months without any other medical cause and FSH and LH values consistent with being menopausal); or b. surgically sterile (bilateral oophorectomy, bilateral salpingectomy, hysterectomy or tubal ligation) at least 3 months prior to dosing.

	4. Sexually active female subjects of childbearing potential must be willing to use an acceptable contraceptive method throughout the study as detailed in section 7.1. 5. Willing to take off dentures or mouth piercing at the time of dosing. 6. Able to understand the study procedures and provide signed informed consent to participate in the study. Exclusion Criteria Subjects to whom any of the following applies will be excluded from the study: 1. Any clinically significant abnormal finding at physical examination. 2. Clinically significant abnormal laboratory test results (may be repeated up to two times) or positive serology test results for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, or human immunodeficiency virus (HIV)-1 and HIV-2 antibodies at screening. 3. Positive pregnancy test or lactating female subject. 4. Positive urine drug screen. 5. Known allergic reactions to sapropterin dihydrochloride or other related drugs, or to any excipient in the formulation. 6. Clinically significant ECG abnormalities or vital signs abnormalities (systolic blood pressure lower than 90 or over 150 mmHg, diastolic blood pressure lower than 50 or over 90 mmHg, or pulse rate less than 50 or over 100 bpm) at screening. ECG and vitals signs may be repeated up to two times, to determine if the values are significantly abnormal. 7. Recreational use of soft drugs (such as marijuana) within 1 month or hard drugs (such as cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within 3 months prior to screening. 8. History of alcohol addiction requiring treatment. 9. History of abuse of medicinal product or drugs within the last 3 years. 10. History or presence of alcoholism within the last 3 years. (>40 g ethanol/day or more than 10 units per week [1 unit =150 mL of wine, or 360 mL of beer, or 45 mL of 45% alcohol]). 11. Use of medications within the timeframes specified in section 7.2. 12. Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to dosing, administration of a biological product in the context of a clinical research study within 90 days prior to dosing, or concomitant participation in an investigational study involving no drug or device administration. 13. Known predisposition to seizures. 14. Donation of plasma within 7 days prior to dosing or donation or loss of 500 mL or more of whole blood within 8 weeks prior to dosing.

	15. Presence of orthodontic braces or orthodontic retention wires, or any physical findings in the mouth or tongue that would be likely to interfere with successful completion of the dosing procedure. 16. Females who: a. Have discontinued or changed the use of implanted, intrauterine, intravaginal, or injected hormonal contraceptives within 6 months prior to study treatment administration. b. Have discontinued or changed the use of oral or patch hormonal contraceptives within 1 month prior to study treatment administration. 17. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.
Study Treatments:	In each period, subjects will receive one of the following treatments, according to the randomization scheme: Treatment A: 1 x 10 mg/kg dose* of sapropterin dihydrochloride oral suspension 100 mg/mL administered without water under fasting conditions. Treatment B: 1 x 10 mg/kg dose* of sapropterin dihydrochloride oral suspension 100 mg/mL administered without water under fed conditions. Treatment C: 1 x 10 mg/kg dose* of sapropterin dihydrochloride oral suspension 100 mg/mL administered with water under fed conditions. Treatment D: 1 x 10 mg/kg dose* of Kuvan (sapropterin dihydrochloride) 100 mg powder for oral solution dissolved in water and administered under fed conditions. [redacted] For Treatment C, the administration of the suspension will be followed by a glass of water so that the total volume of suspension + water is 240 mL (± 5 mL). For Treatment D, the powder for oral solution will be dissolved in 120 mL (± 3 mL) of water within 15 minutes before administration and the solution will be swallowed at the time of study treatment administration. To ensure that the entire dose is consumed, the dosing cup will be rinsed twice with an additional 60 mL (± 1 mL) of water each time (total volume of 120 mL (± 2 mL)) and the content will be swallowed.
Study Procedures:	Blood samples for PK analysis will be collected in each period at -1, -0.5, pre-dose (0-hour), and 0.333, 0.667, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12, 16, and 24 hours post-dose. Blood pressure, pulse rate, respiration rate, and temperature will be measured at screening and end-of-study (EOS). Temperature will be measured daily during confinement. Blood pressure and pulse rate will be measured before dosing (0-hour) and at 4 and 6 hours post-dose of each period. 12-lead ECG will be measured at screening, before dosing (0-hour), at 4 and 6 hours post-dose of each period, and at EOS.

	Other safety procedures will be performed at pre-defined times throughout the study as specified in section 8.3, 8.4, 8.5, and 8.6. Subjects will be monitored throughout the study by the clinical staff for AEs and concomitant medication use.
Statistical Analyses:	The primary objective of this study is to evaluate the relative bioavailability of a new oral suspension formulation. Since this study is exploratory, no formal hypothesis testing is defined. PK analysis: For sapropterin, using the PROC MIXED procedure in SAS, analysis of variance (ANOVA) will be performed on log-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max} parameters at the alpha level of 0.05 on uncorreted data, ANCOVA (Analysis of Co-Variance) will be applied for baseline corrected analysis, using the actual total dose included in the ANOVA statistical model as a covariate. The ratio of geometric means (A/D, B/D, C/D, B/C, A/C, and B/A) and 90% confidence interval (CI) for the ratio of geometric means, based on least-squares means from the ANOVA of the log-transformed data, will be calculated for AUC_{0-t}, AUC_{0-inf}, and C_{max}. Uncorrected and baseline-corrected data will be presented. Safety and tolerability analysis: Safety and tolerability of sapropterin dihydrochloride will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study treatment, outcome, duration, and management), vital signs, 12-lead ECGs, clinical laboratory tests, and physical examinations. Treatment-emergent adverse events (TEAEs) will be tabulated by treatment. Safety and tolerability data will be reported using descriptive statistics.

Table 1. Schedule of Events

Study Stage	Screening	Admission (check-in-of Period 1)	Admission of Period 2,3,4	Treatment Periods[12]	
Day	**-30 to -1 (of Period 1)**	**-1 of Period 1 or prior to Period 1 dosing**	**-1 of Period 2, 3, 4**	**1**	**2 EOS/ET[13]**
Screening consent[1]	X				
Informed consent[2]		X			
Inclusion/exclusion criteria	X[3]	X[4]			
Demographic data	X[3]				
Medical and medication history	X[3]	X	X		
Confinement[5]		X	X	X	
Discharge					X
Study treatment administration					
Sapropterin dihydrochloride oral suspension				X[6]	
Kuvan powder for oral solution				X[6]	
Pharmacokinetics					
Blood samples for PK analysis[7]				X	X
Safety					
Physical examination[8]		X			X
Body measurements (height, weight, BMI)	X[3]	X[9]			
Vital signs[10]	X[3]	X	X	X	X
12-lead ECG[11]	X[3]			X	X
Serology tests	X[3]				
Biochemistry, hematology, and urinalysis tests	X[3]				X
FSH/LH (for post-menopausal females)	X[3]				
Serum pregnancy tests	X[3]	X	X		X
Urine pregnancy test		X	X		
Urine drugs of abuse	X[3]	X	X		
Breath alcohol		X	X		
Monitoring and recording of AEs and prior/concomitant medication use		X	X	X	X

Abbreviations: AE = adverse event; BMI = body mass index; ECG = electrocardiogram; EOS = end of study; ET = early termination; FSH = follicle-stimulating hormone; LH = luteinizing hormone; PK = pharmacokinetic.

1 Includes privacy and volunteer consent. During screening, subjects will consent to and sign privacy and volunteer consent forms.

2 An informed consent form (ICF) specific to this study will be signed by all subjects at Period 1 check-in (Day -1), and before any study specific procedure.

3 Screening procedures can be done either at the screening visit or at Period 1 check-in (or repeated at both screening visit and Period 1 check-in if deemed necessary by the Investigator).

4 Complete outstanding procedures for inclusion/exclusion criteria confirmation.

5 Confinement period is at least 10.5 hours pre-dose until 24 hours post-dose.

6 On Day 1 of each period, subjects will receive one of the four treatments, according to the randomization scheme.

7 A total of 20 blood samples will be collected for PK analysis in each period: -1, -0.5, pre-dose (0-hour), and 0.333, 0.667, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12, 16, and 24 hours post-dose.

8 A complete physical examination will be performed at Period 1 check-in. A brief physical examination will be performed at EOS. Refer to sections 8.1 and 8.4 for more details.

9 Weight only at check-in of Period 1.

10 Blood pressure, pulse rate, respiration rate, and temperature will be measured at screening and EOS. Temperature will be measured daily during confinement. Blood pressure and pulse rate will be measured before dosing (0-hour) and at 4 and 6 hours post-dose of each period.

11 12-lead ECG will be measured at screening, before dosing (0-hour), at 4 and 6 hours post-dose of each period, and at EOS.

12 There are 4 treatment periods. There will be a washout period of at least 7 days between doses.

13 EOS evaluations will be performed prior to study discharge of the last period. In case of ET, EOS procedures will be performed as soon as possible.

1. Introduction

1.1 Background Information

Sapropterin dihydrochloride is a synthetic preparation of the dihydrochloride salt of naturally occurring tetrahydrobiopterin (BH4), the cofactor for the enzyme phenylalanine hydroxylase (PAH). Sapropterin dihydrochloride is indicated to reduce blood phenylalanine (Phe) levels in adult and pediatric patients one month of age and older with hyperphenylalaninemia due to BH4-responsive phenylketonuria.[1,2] In patients 7 years and older, the recommended starting dose of sapropterin dihydrochloride is 10 to 20 mg/kg taken once daily. AEs associated with sapropterin dihydrochloride are listed in the Prescribing Information.[1]

PAH hydroxylates Phe through an oxidative reaction to form tyrosine. In patients with phenylketonuria, PAH activity is absent or deficient. Treatment with BH4 can activate residual PAH enzyme activity, improve the normal oxidative metabolism of Phe, and decrease Phe levels in some patients.[1,2]

When sapropterin dihydrochloride was administered in healthy subjects as intact tablets under fasting conditions, the calculated median T_{max} for intact tablets was shorter than that for dissolved tablets, although the ranges were comparable. Plasma sapropterin concentrations were comparable at 2 hours after administration of intact tablets under fed and fasting conditions. However, sapropterin concentrations continued to increase under fed conditions, which was not the case under fasting conditions.[3] Detailed pharmacokinetic parameters of sapropterin are shown in the table below.

Table 2. Pharmacokinetic Parameters of Sapropterin Dihydrochloride 10 mg/kg Administered as Intact or Dissolved Tablets in the Fasted State, and as Intact Tablets in the Fed or Fasting States.

	Dosing Regimen		
Parameter	Intact/Fasting (n = 30)	Dissolved/Fasting (n = 30)	Intact/Fed (n = 30)
C_{max}, ng/mL	84.1 (42.2)	63.0 (28.5)	105 (32.1)
T_{max}, median (range), h	2.77 (1–5)	5.00 (1–6)	4.50 (2–5)
AUC_{0-t}, ng · h/mL	474 (235)	337 (186)	635 (246)
$AUC_{0-\infty}$, ng · h/mL*	559 (220)	511 (237)	752 (307)
λ_z, h^{-1}*	0.21 (0.08)	0.18 (0.09)	0.27 (0.08)
$t_{1/2}$, h*	3.67 (1.16)	4.65 (2.03)	2.80 (1.05)

*Because λ_z could not be determined for some subjects due to insufficient concentration data in the terminal phase of the plasma curve, it was not possible to calculate $t_{1/2}$ and $AUC_{0-\infty}$ for these subjects. Thus, the number of subjects for the intact/fasting, dissolved/fasting, and intact/fed regimens in this comparison were 14, 17, and 18, respectively.

Based on the clinical pharmacology and biopharmaceutics review of Kuvan[4], the pharmacokinetic parameters of sapropterin dihydrochloride 100 mg powder for oral solution are expected to be comparable to the 100 mg tablets based on their composition, solubility, and osmolarity. The powder formulation contains the same active ingredient in the same concentration, exhibits rapid dissolution using the dissolution method for sapropterin dihydrochloride tablets, has similar osmolarity in water, and its inactive ingredients are not expected to affect drug absorption and bioavailability.[4] Absolute bioavailability or bioavailability for humans after oral administration is not known. In human plasma (*in vitro*), the protein-binding rate remained constant (22%–34%) within the concentration range of endogenous levels (approximately 3-10 ng/mL).[2]

Sapropterin is a synthetic form of BH4 and is expected to be metabolized and recycled by the same endogenous enzymes. *In vivo* endogenous BH4 is converted to quinoid dihydrobiopterin and is metabolized to dihydrobiopterin and biopterin. The enzymes dihydrofolate reductase and dihydropteridine reductase are responsible for the metabolism and recycling of BH4.[1,2]

1.2 Study Rationale

1.2.1 Rationale for the Dose

According to FDA Draft Guidance on Sapropterin Dihydrochloride[6], a dose of 10 mg/kg will be administered to subjects. To account for the fact that the Reference drug is supplied as 100 mg powder for oral solution, the dose for each subject will be calculated by multiplying the subject's weight measured on Day -1 of Period 1 by 10 mg/kg and then rounding up to the next 100 mg dose. This dose can be safely administered to healthy volunteers and should allow a good characterization of the pharmacokinetic profile of sapropterin.

1.2.2 Rationale for Baseline Correction

Sapropterin is an endogenous compound and should therefore be analyzed both with and without baseline correction. Therefore, for baseline correction, for each subject and treatment period, the baseline value will be defined as the mean of the -1 hour, -0.5 hour, and pre-dose (0) samples obtained before dosing, for that same subject and period.

1.2.3 Rationale for the Study Population

A healthy volunteer population has been selected for the study because healthy subjects with no concomitant diseases and using no concomitant medications represent a homogenous population allowing for proper evaluation of the PK, safety, and tolerability of a drug without confounding factors.

Available pregnancy registry data have not reported an association with sapropterin dihydrochloride and major birth defects, miscarriage, or adverse maternal or fetal outcomes when sapropterin dihydrochloride was used during pregnancy.[1] An embryo-fetal development study with sapropterin dihydrochloride in rats using oral doses up to 3 times the maximum recommended human dose (MRHD) given during the period of organogenesis showed no effects. In a rabbit study using oral administration of sapropterin dihydrochloride during the period of organogenesis, a rare defect, holoprosencephaly, was noted at 10 times the MRHD.[1,2] Since there have been no studies with pregnant women, it is uncertain whether there is human fetal risk associated with the use of sapropterin dihydrochloride. Therefore, only male and non-pregnant, non-lactating female subjects will be included in the study.

1.3 Benefit/Risk Assessment

Risks to subjects in this study are related to common procedures performed (e.g., venipuncture) and the documented AEs listed in the current reference product information. Subjects are also at risk of exposure to infectious diseases, such as flu (influenza), cold (typically caused by a rhinovirus), or COVID-19, by being in the presence of people who may have a virus but do not have any symptoms. These risks are communicated to the subjects in the consent forms. In case of the re-emergence of COVID-19, or any other infectious disease, safety precautions such as masking, distancing, testing, etc. may be reinstated as deemed necessary by the study doctor and/or according to local public health guidelines.

The inclusion and exclusion criteria have been chosen to select subjects who are known to be free from any significant illness, history of autoimmune diseases, and from any condition that could impact their safety or interfere with meeting the study objectives. The proposed safety screening and monitoring assessments are deemed to be sufficient to monitor potential risks of sapropterin dihydrochloride administration. There is no anticipated therapeutic benefit for the healthy subjects in this study.

In other clinical trials, the following AEs (greater than 4%, and higher than placebo) have been reported by phenylketonuria patients after taking sapropterin: [1]

- Headache (15%)
- Rhinorrhea (11%)
- Pharyngolaryngeal pain (10%)
- Diarrhea (8%)
- Vomiting (8%)
- Cough (7%)
- Nasal congestion (4%)

2. Objectives

Primary objectives:

- [redacted]

Secondary objective:

- [redacted]

3. Endpoints

Primary endpoints:

- PK
    - Uncorrected and baseline-corrected sapropterin AUC_{0-t}, AUC_{0-inf}, and C_{max}

Secondary endpoints:

[redacted]

4. Study Design

This is a single center, Phase 1, randomized, open-label, single-dose, 4-treatment, 4-period, 4-sequence, crossover study designed to compare the PK of sapropterin from the Test and Reference products, and to evaluate the effect of food and the effect of water administration on the bioavailability of sapropterin from the Test product in healthy subjects.

In each period, subjects will receive a single 10 mg/kg dose of sapropterin dihydrochloride on Day 1, under fasting or fed conditions, and with or without water, followed by 24 hours of PK sampling.

This study will employ general screening. The study will start on the day the first subject signs the study's Ethics/Institutional Review Board (ERB/IRB) approved ICF. The end date of the clinical trial will be the last visit of the last subject. The anticipated end of study date for most subjects will be their last scheduled study procedure. Unscheduled visits and AE follow-up may occur after the end of study procedures. The expected duration of subject participation in this study (from voluntary signing of the ICF to the last scheduled study procedure) will be approximately 24 days.

The washout between drug administrations for each subject will be at least 7 days (±3 hours). The washout may be extended. The duration of subject participation will be extended by adding on the extra washout days.

5. Study Population

5.1 Number of Subjects

It is planned to enroll 16 subjects for participation in this study. [redacted]

5.2 Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

1. Male or female, light smoker (no more than 10 cigarettes daily) or non-smoker, ≥18 and ≤50 years of age, with body mass index (BMI) ≥18.5 and ≤30.0 kg/m^2 and body weight ≥50.0 kg for males and ≥45.0 kg for females.
2. Healthy as defined by:
   a. the absence of clinically significant illness and surgery within 30 days prior to dosing.
   b. the absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, hematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease.
3. Female subjects of non-childbearing potential must be:
   a. post-menopausal (no menstrual period at least 12 consecutive months without any other medical cause and FSH and LH values consistent with being menopausal); or
   b. surgically sterile (bilateral oophorectomy, bilateral salpingectomy, hysterectomy or tubal ligation) at least 3 months prior to dosing.
4. Sexually active female subjects of childbearing potential must be willing to use an acceptable contraceptive method throughout the study as detailed in section 7.1.
5. Willing to take off dentures or mouth piercing at the time of dosing.

6. Able to understand the study procedures and provide signed informed consent to participate in the study.

5.3 Exclusion Criteria

Subjects to whom any of the following applies will be excluded from the study:

1. Any clinically significant abnormal finding at physical examination.
2. Clinically significant abnormal laboratory test results (may be repeated up to two times) or positive serology test results for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, or human immunodeficiency virus (HIV)-1 and HIV-2 antibodies at screening.
3. Positive pregnancy test or lactating female subject.
4. Positive urine drug screen.
5. Known allergic reactions to sapropterin dihydrochloride or other related drugs, or to any excipient in the formulation.
6. Clinically significant ECG abnormalities or vital signs abnormalities (systolic blood pressure lower than 90 or over 150 mmHg, diastolic blood pressure lower than 50 or over 90 mmHg, or pulse rate less than 50 or over 100 bpm) at screening. ECG and vitals signs may be repeated up to two times, to determine if the values are significantly abnormal.
7. Recreational use of soft drugs (such as marijuana) within 1 month or hard drugs (such as cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within 3 months prior to screening.
8. History of alcohol addiction requiring treatment.
9. History of abuse of medicinal product or drugs within the last 3 years.
10. History or presence of alcoholism within the last 3 years. (>40 g ethanol/day or more than 10 units per week [1 unit =150 mL of wine, or 360 mL of beer, or 45 mL of 45% alcohol]).
11. Use of medications within the timeframes specified in section 7.2.
12. Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to dosing, administration of a biological product in the context of a clinical research study within 90 days prior to dosing, or concomitant participation in an investigational study involving no drug or device administration.
13. Known predisposition to seizures.
14. Donation of plasma within 7 days prior to dosing or donation or loss of 500 mL or more of whole blood within 8 weeks prior to dosing.
15. Presence of orthodontic braces or orthodontic retention wires, or any physical findings in the mouth or tongue that would be likely to interfere with successful completion of the dosing procedure.

16. Females who:
    a. Have discontinued or changed the use of implanted, intrauterine, intravaginal, or injected hormonal contraceptives within 6 months prior to study treatment administration.
    b. Have discontinued or changed the use of oral or patch hormonal contraceptives within 1 month prior to study treatment administration.
17. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.

5.4 Subject Withdrawal and Replacement

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator or designee may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with the clinical site's SOP:

- Safety reason;
- Non-compliance with protocol requirements;
- Significant protocol deviation;
- Positive pregnancy test, drug screen, or alcohol breath test.

Subjects who experience vomiting within 10 hours after study treatment administration will be removed from that period of the study. Their continued participation in the study will be evaluated by the Investigator and PK Scientists prior to the next study treatment administration.

Subjects who experience vomiting after this time will be assessed for their continued participation in the study by the Investigator.

Subjects excluded from dosing in one period as per criteria listed above, may be invited to participate in subsequent periods of the study if deemed appropriate by the Investigator and appropriate from a statistical standpoint (i.e., would permit adequate statistical comparison). However, subjects with positive pregnancy test, drug screen, or alcohol breath test will be definitively withdrawn from the study.

Subjects who withdraw prior to the first dosing may be replaced with a standby subject. Subjects who withdraw or are withdrawn from the study after the first dosing will not be replaced.

Subjects who withdraw or are withdrawn will be asked to remain at the clinic until the Investigator or designee agrees that the subject is fine and can be discharged. As soon as subject withdrawal is confirmed, blood sampling will be stopped. A PK blood sample may be collected at the time of withdrawal if deemed required by the Investigator. EOS/early termination (ET) procedures will be performed at the time of withdrawal from the study or as soon as possible thereafter.

5.4.1 Use of Data for Discontinued Subjects

Data that was obtained prior to a subject's discontinuation in this study will be maintained, used, and included in subsequent analyses, as applicable. If a subject withdraws from the study, the subject may request the destruction of samples.

No analyses will be performed on the samples outside of the protocol requirements without the subject's consent.

6. Study Treatments

6.1 Study Treatment Supplies and Accountability

It is the responsibility of the Sponsor to ensure that study treatments provided for this study are manufactured under Good Manufacturing Practice (GMP) and are suitable for human use. The reference product will be obtained from [redacted]. The Sponsor is responsible to ship a sufficient amount of dosage units to allow the clinical site to maintain an appropriate sampling for the study.

The study treatments will be stored at the clinical site pharmacy as per applicable requirements in a locked, environmentally-controlled medication room with restricted access. Container(s) will be labeled according to applicable regulations.

Individual doses for each subject and period will be dispensed at the clinical site pharmacy, as per appropriate SOP, according to the randomization scheme, and according to the subject's weight measured at Period 1 check-in, in appropriate syringes/containers indicated with at least the project number, the period number and the subject number/spare number. Please refer to the pharmacy and dosing plans for detailed procedures on dispensing.

All study treatments received at the site will be inventoried and accounted for throughout the study and the result recorded in the study treatment accountability record according to the clinical site appropriate SOP.

At the completion of the study, unused study treatment(s) will be retained at the clinical site, returned to the Sponsor, or destroyed, as requested by the Sponsor. No study treatment(s) will be destroyed without written permission from the Sponsor.

6.2 Investigational Products

Test: Sapropterin dihydrochloride 100 mg/mL oral suspension (product code: RLF-OD032) (APR Applied Pharma Research s.a., Switzerland)

Reference: Kuvan (sapropterin dihydrochloride) 100 mg powder for oral solution [redacted]

6.3 Identification of Treatments

Treatment A: 1 x 10 mg/kg dose* of sapropterin dihydrochloride oral suspension 100 mg/mL administered without water under fasting conditions.

Treatment B: 1 x 10 mg/kg dose* of sapropterin dihydrochloride oral suspension 100 mg/mL administered without water under fed conditions.

Treatment C: 1 x 10 mg/kg dose* of sapropterin dihydrochloride oral suspension 100 mg/mL administered with water under fed conditions.

Treatment D 1 x 10 mg/kg dose* of Kuvan (sapropterin dihydrochloride) 100 mg powder for oral solution dissolved in water and administered under fed conditions.

6.4 Randomization and Blinding

This study will be an open-label study due to the objective nature of the data. Subjects will be administered each treatment according to the block randomization scheme. A 4-sequence Williams design will be used (e.g.: ADBC, BACD, CBDA and DCAB):

Table 3. Treatment Sequence

	Treatment			
Sequence	**Period 1**	**Period 2**	**Period 3**	**Period 4**
DCAB	D	C	A	B
ADBC	A	D	B	C
BACD	B	A	C	D
CBDA	C	B	D	A

Subjects will be assigned consecutive subject numbers in an ascending order. Each number will identify a subject and determine the sequence of study treatment administration according to the randomization scheme.

The randomization code will not be available to the bioanalytical facility until the clinical and analytical phases of the study have been completed.

6.5 Study Treatment Administration

Subjects will be asked to take off dentures or mouth piercing at the time of dosing. A mouth check will be performed to ensure study treatment and fluid (when applicable) consumption. The time of dosing will be set as the time the oral suspension or solution begins to be swallowed. For

[redacted]

Treatments A and B: t[redacted]

[redacted]

Treatment C: t[redacted]

[redacted]

For Treatment D, the powder for oral solution will be dissolved in 120 mL (± 3 mL) of water within 15 minutes before administration and the solution will be swallowed at the time of study treatment administration. The powder should be placed in water and stirred until dissolved. The powder should dissolve completely.[1,2] To ensure that the entire dose is consumed, the dosing cup will be rinsed twice with an additional 60 mL (± 1 mL) of water each time (total volume of 120 mL (± 2 mL)) and the contents will be swallowed. No additional water will be given to the subjects.

6.6 Administration of Food and Fluid

For Treatment A:

Subjects will fast from at least 10 hours prior to study treatment administration until at least 4 hours post-dose.

For Treatment B, C, and D:

After an overnight fast of at least 10 hours, subjects will consume a high-fat, high-calorie breakfast starting 30 minutes prior to study treatment administration.

Table 4. High-Fat, High-Calorie Breakfast

	Calories (kcal)	**Total Fat (g)**	**Carbohydrates (g)**	**Protein (g)**
2 eggs	140	10	0	12
10 g of butter for cooking eggs	70	8	0	0
2 slices of whole wheat toast (approximately 64 g)	160	2	31	7
10 g of butter for toast	70	8	0	0

3 triangle pieces of hash brown potatoes (approximately 130 g)	218	14	23	2
250 mL of whole milk	160	8	12	8
3 strips of chicken bacon (approximately 63 g)	105	7	0	9
Total weight (g)		57	66	38
Total calories (kcal)	923	514	262	152
Relative caloric content		56%	28%	16%

***Note**: Due to supply chain issues, some ingredients may not be available therefore, the content of the high-fat, high-calorie breakfast is subject to change. Any change will be notified to the Sponsor and ensured that the total caloric content and breakdown between fat, carbohydrate, and protein is maintained according to FDA requirements: high-fat (approximately 50 percent of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories). The meal should derive approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.*[7]

Subjects who do not consume the entire meal in the required time will be dismissed from that period of the study prior to study treatment administration. Their continued participation in the remainder of the study will be determined by the Investigator or designate.

Subjects will fast for at least 4 hours post-dose.

For all Treatments:

Standardized meals will be provided throughout confinement (e.g., at 4 hours for lunch, 9 hours for dinner and 13.5 hours for snacks). The meals will be identical for all periods.

Water will be restricted from one hour prior to study treatment administration until one hour post-dose (except the water administered with the study treatment in Treatment C and D). Access to water will otherwise be freely available to subjects.

7. Study Restrictions

Adherence to the restrictions will be confirmed and recorded at each check-in. Subjects who do not comply with these restrictions will be assessed by the Investigator/PK Scientists regarding their continued participation in the study.

7.1 Contraception

Subjects participating in this study must use an acceptable single- or double-method of contraception as defined below:

- Females: from 21 days prior to first study treatment administration until 28 days after the last PK blood sample in the study

- Males: from the day of first study treatment administration until 28 days after the last PK blood sample in the study

Table 5. Acceptable Effective Methods of Contraception

Single-Method	Double-Method (must use at least two)	
Surgically sterile	Diaphragm with Spermicide	Non-hormonal IUD
Postmenopausal	Cervical cap with Spermicide	Vasectomy
Remain abstinent	Condom with Spermicide	Hormonal contraception
Hormonal contraception includes: Oral contraceptives, intra-vaginal devices, hormonal IUD, injections, transdermals, and implants		

Male subjects will be instructed not to donate sperm from the day of first study treatment administration until at least 28 days after the last PK blood sample in the study.

7.2 Concomitant Medications

Subjects will be required to avoid using medications for the timeframes specified below and throughout the study:

- Depot injection or implant of any drug for 3 months prior to dosing;
- Prescription medications for 14 days prior to dosing;
- Any vaccine, including COVID-19 vaccine, for 14 days prior to dosing;
- Over-the-counter (OTC) medications and natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) for 7 days prior to dosing (with the exception of the occasional use of acetaminophen up to 2 g daily).

No concomitant medications will be allowed during the study, with the exception of hormonal contraceptives, medications required for the medical management of an AE, and medications exempted by the Investigator on a case-by-case basis that are judged unlikely to affect the PK profile of the study treatment or subject safety (e.g., topical drug products without significant systemic absorption).

Medications taken by subjects before the first dose will be documented as prior medications and medications taken by subjects after the first dose through to the last study day will be documented as concomitant medications. Any prior or concomitant medication use, other than the allowed medications stated above, will be reviewed and evaluated on a case-by-case basis by the Investigator and PK Scientists to determine if they affect a subject's eligibility or continued participation in the study, or for potential impact on the study results. In accordance with the prescribing information for Kuvan, subjects taking medications known to inhibit folate metabolism, or levodopa, after being administered sapropterin, will be monitored. Subjects taking medications known to affect nitric oxide-mediated vasorelaxation after being administered sapropterin will be monitored for hypotension.

7.3 Drugs, Nicotine and Alcohol

Subjects will be required to abstain from:

- Soft or hard drugs from screening and throughout the study;
- Any nicotine product (including cigarettes) during confinement. No more than 10 cigarettes per day will be allowed during the washout period. No nicotine products (other than cigarettes) will be allowed during the washout period;
- Alcohol-based products from 48 hours prior to admission until after the last PK blood sample collection of each period.

7.4 Diet

Subjects will be required to abstain from:

- Food or beverages containing grapefruit, Seville orange, starfruit, pomegranate, pineapple, or pomelo from 7 days prior to dosing and throughout the study;
- Food or beverages containing xanthine derivatives or xanthine-related compounds (coffee, black/green tea, chocolate) or energy drinks from 48 hours prior to dosing until after the last PK blood sample collection of each period;
- Food containing poppy seeds from 24 hours prior to admission of each period.
- Chewing gum will be prohibited at all times during the confirment.

7.5 Posture and Physical Activity

Subjects will be required to remain seated and avoid lying down or sleeping for 6 hours after study treatment administration.

Failure of subjects to comply with these requirements does not constitute a deviation from the protocol if it is medically necessary, required for procedures, or to go to the bathroom. When appropriate, subjects will be accompanied by a staff member while walking. Vigorous activity will be prohibited at all times during the confinement.

8. Study Procedures

Subjects must provide written informed consent (privacy, volunteer, and informed consent) prior to initiation of any study procedures.

Unless otherwise specified, study procedures will be conducted in accordance with clinical site SOPs.

Every effort will be made to schedule and perform the procedures as close to the nominal time as possible, giving considerations to appropriate posture conditions, practical restrictions, and other procedures to be performed at the same time point.

PK blood sample collection will be performed closest to the nominal time. When vital signs measurement or ECG recording coincide with a blood collection, they should preferably be performed before the blood collection, whenever possible.

Table 6. Scheduled Windows of Allowance for Vital signs and ECG

Procedure	Timepoint	Acceptable Window from Scheduled time
Vitals	Pre-dose (0-hour)	Within 180 minutes prior to dosing
	>0 hours	±20 minutes
ECG	Pre-dose (0-hour)	From check-in to dosing
	>0 hours	±30 minutes

8.1 Screening Procedures (-30 to -1)

Recruitment into this study will initially involve [redacted] recruitment procedures and general screening, during which volunteers will voluntarily consent to and sign an ERB/IRB approved Volunteer Consent Form (VCF) and Privacy Consent Form (PCF).

An ICF specific to this study will be signed by all subjects prior to any study specific procedures occurring.

Screening will include the following procedures either at the screening visit or at Period 1 check-in:

- Screening evaluations (Screening consent: Inclusion/Exclusion Criteria);
- Medical and medication history;
- Demographic data collection and height and weight measurements, as well as BMI calculation;
- Vital signs (blood pressure, pulse rate, respiration rate, and temperature) will be measured after the subjects have been resting for at least 3 minutes in a sitting position;
- 12-lead ECG will be recorded after the subjects have been resting in a semi-recumbent or supine position;
- Blood and urine sample collection.

Table 7. Blood and Urine Tests Performed at Screening

Biochemistry	Hematology	Urinalysis
Random glucose Total bilirubin BUN Creatinine Alkaline phosphatase Calcium LD AST (SGOT) ALT (SGPT) Serum electrolytes (sodium, potassium, and chloride) GGT FSH LH ***Note:*** *FSH and LH will be tested only in female subjects with a menstrual history consistent with being postmenopausal.*	CBC with differential: • WBC count with differential • Hemoglobin • Hematocrit • RBC count and indices Platelet count	Specific gravity pH Leukocytes Nitrites Protein Glucose Ketones Blood Microscopic examination ***Note:*** *microscopic examination will only be performed when any of the following urinalysis tests are reported abnormal (i.e., outside of normal ranges): leukocyte, nitrite, protein, and/or blood. The microscopic examination will be conducted on the same urine sample as the abnormal urinalysis test.*
Serology	**Drugs of Abuse**	**Immunohematology**
HIV-1 and HIV-2 antibodies HCV antibody HBsAg	Amphetamines Barbiturates Benzodiazepines Cannabinoids Cocaine Methadone Opiates Phencyclidine Tricyclic antidepressants	Serum β-HCG (females only)

Abbreviations: ALT (SGPT) = alanine aminotransferase; AST (SGOT) = aspartate aminotransferase; β-HCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CBC = complete blood count; FSH = follicle-stimulating hormone; GGT = gamma glutamyl transpeptidase; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; LD = Lactate dehydrogenase; LH = luteinizing hormone; RBC = red blood cell; WBC = white blood cell.

8.2 Test/ Procedures at check-in

Check-in of Period 1

- ICF signature for the study (if not done before);
- Medical and medication history;
- Any outstanding screening procedure that is not completed, or needs to be repeated;
- Complete physical examination including assessments of the following: head, eyes, ears, nose, throat (HEENT), neck, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination;
- Temperature measurement;

- Weight;
- Urine drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, phencyclidine, and tricyclic antidepressants);
- Urine β-HCG and serum β-HCG (females only);
- Breath alcohol;
- Confirm inclusion/exclusion criteria.

Check-in of Period 2, 3, 4

- Temperature measurement;
- Urine drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, phencyclidine, and tricyclic antidepressants);
- Urine β-HCG and serum β-HCG (females only);
- Breath alcohol;
- Medical and medication history.

Clinical study personnel reserve the right to conduct additional testing (e.g., breath alcohol test, diagnostic imaging) at any time throughout the study.

8.3 On-study procedures:

- Temperature measurement daily;
- Vital signs (blood pressure and pulse rate) will be measured after the subjects have been resting for at least 3 minutes in a sitting position: before dosing (0-hour), and at 4 and 6 hours post-dose;
- 12-lead ECG will be recorded after the subjects have been resting in a semi-recumbent or supine position: before dosing (0-hour), at 4 and 6 hours post-dose;
- Study treatment administration; and
- Pharmacokinetic Blood Sample Collection: -1, -0.5, pre-dose (0), and 0.333, 0.667, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12, 16, and 24 hours post-dose.

Table 8. Time Windows for PK Blood Samples

Day	Time point	Time window
1	-1 hour, and -0.5 hour	±5 minutes
	Pre-dose (0-hour)	Within 5 minutes
	0.333, 0.667, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6 hours	±1 minute
	8, 12, and 16 hours	±3 minutes

2	24 hours	±3 minutes

Samples collected outside the acceptable window will not be considered as protocol deviations, but rather the actual clock time of each sample collection will be recorded.

8.4 End of study procedures

End-of-study for each subject is defined as the last study specific procedure in the last period of the study or the last study specific procedure prior to the discontinuation of a subject from the study.

End-of-study will include the following procedures:

- A brief physical examination including assessments of the following: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject;
- Vital signs (blood pressure, pulse rate, respiration rate, and temperature) will be measured after the subjects have been resting for at least 3 minutes in a sitting position;
- 12-lead ECG will be recorded after the subjects have been resting in a semi-recumbent or supine position; and
- Blood and urine sample collection.

Table 9. Blood and Urine Tests Performed at End of Study

Biochemistry	**Hematology**	**Urinalysis**
Random glucose Total bilirubin BUN Creatinine Alkaline phosphatase Calcium LD AST (SGOT) ALT (SGPT) GGT Serum electrolytes (sodium, potassium, and chloride)	CBC with differential: • WBC count with differential • Hemoglobin • Hematocrit • RBC count and indices Platelet count	Specific gravity pH Leukocytes Nitrites Protein Glucose Ketones Blood Microscopic examination ***Note:*** *microscopic examination will only be performed when any of the following urinalysis tests are reported abnormal (i.e., outside of normal ranges): leukocyte, nitrite, protein, and/or blood. The microscopic examination will be conducted on the same urine sample as the abnormal urinalysis test.*
Immunohematology		
Serum β-HCG (females only)		

Abbreviations: ALT (SGPT) = alanine aminotransferase; AST (SGOT) = aspartate aminotransferase; β-HCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CBC = complete blood count; GGT = gamma glutamyl transpeptidase; LD = Lactate dehydrogenase; RBC = red blood cell; WBC = white blood cell.

For subjects who are removed or withdrawn from the study, end-of-study procedures will still be conducted. Subjects who do not return for end-of-study procedures will be considered lost-to-follow-up.

If a subject is not able to repeat any end-of-study tests at the clinic, they may repeat the test with their local healthcare provider/laboratory. All external results will be reviewed by the Investigator.

8.5 Pharmacokinetic Assessments

8.5.1 Pharmacokinetic Blood Sample Collection and Processing

In each period, blood samples for PK analysis will be collected via an intravenous catheter or by direct venipuncture.

The PK Scientists will be contacted to evaluate the subject's continued participation in the study if any of the following incidents occur in any period:

- The 24-hour scheduled blood draw is missed; or
- At least two consecutively scheduled blood draws are missed.

The impact of these incidents on the estimation of PK parameters will be made as close to the time of occurrence as possible.

The planned volume of blood to be collected for this study, including that collected for eligibility and safety purposes, should not exceed 500 mL. Additional tests or blood draws could be performed, if deemed required by the Investigator or study staff.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in a laboratory manual.

Plasma concentrations of the study treatment will be determined using a validated analytical method. Details of the analytical method will be provided in a separate document.

8.6 Safety and Tolerability Assessments

Subjects will be monitored throughout the study by the clinical staff for AEs. Adequate medical surveillance will be assured during the confinement period. The Investigator will be on-site until 4 hours after the last subject is dosed and on call throughout the study. If necessary, the Investigator or designee at the clinical site or a healthcare professional in a nearby hospital will administer treatment for any AE(s). A crash cart or emergency bag containing the necessary rescue material and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency.

Safety parameters will be assessed by the Investigator or designee, using the clinical site acceptance ranges as suggested guidelines in making the medical assessment.

For eligibility purposes, abnormal vital signs measurements or clinical laboratory test results may be repeated up to two times if an abnormal result is observed at the initial reading. Moreover, abnormalities found in the ECG may need to be confirmed by repeated measurements. In the event that the participation of a subject in the study is delayed and some screening procedures had been performed outside of the prescribed screening window, outdated screening procedures can be repeated.

Safety assessments scheduled during the study will be repeated according to the clinical site SOPs or upon request from the Investigator or designee. Any abnormal repeated measurement will be evaluated and repeated, if judged necessary. Further action may be taken upon the Investigator or designee's request.

8.7 Adverse Events

8.7.1 Definition of Adverse Event

An AE is defined as any untoward medical occurrence in a patient or clinical trial subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

8.7.2 Recording of Adverse Events

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study treatment. AEs will be collected and documented from the time of signing the ICF and throughout the study. AEs will be followed-up until complete resolution, or until the Investigator or designee judges safe to discontinue follow-up. The severity of AEs and relationship to the study treatment will be classified according to the sections below.

AEs will be classified according to the Medical Dictionary for Regulatory Activities (MedDRA) and reported with respect to severity, duration, relationship to study treatment(s) and action taken.

8.7.3 Assessment of Severity

The severity of AEs will be described and documented using the following definitions:

Table 10. Severity of Adverse Events

Severity	Description
Mild	Awareness of signs and symptoms, but are easily tolerated; are of minor irritant type; causing no limitations of usual activities. Signs or symptoms may require minor action.
Moderate	Discomfort severe enough to cause some limitations of usual activities and may require action.
Severe	Incapacitating with inability to carry out usual activities or significantly affects clinical status, and requires specific action and/or medical attention.

8.7.4 Assessment of Relationship to Study Treatment

Each AE must be classified based on medical judgment and according to the following relationship categories: probable, possible, remote/unlikely, and unrelated. The definitions for the relationship categories are as follows:

Table 11. Adverse Event Relationship Categories

Category	Description
[Related] **Probable** (must have first three points)	This category applies to AEs that are considered, with a high degree of certainty, to be related to the investigational product. An AE may be considered probable, if: 1. It follows a reasonable temporal sequence from the administration of the study treatment. 2. It cannot be reasonably explained by the known characteristics of the participant's clinical state, environmental or toxic factors or other modes of therapy administered to the participant. 3. It disappears or decreases on cessation or reduction in dose (there are important exceptions when an AE does not disappear upon discontinuation of the study treatment, yet relatedness clearly exists; e.g. (1) bone marrow depression, (2) tardive dyskinesias). 4. It follows a known pattern of response to the suspected study treatment. 5. It reappears upon re-challenge.
[Related] **Possible** (must have first two points)	This category applies to AEs in which the connection with the investigational product administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when: 1. It follows a reasonable temporal sequence from the administration of the study treatment. 2. It may have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. 3. It follows a known pattern of response to the suspected study treatment.

Remote/Unlikely (must have first two points)	In general, this category is applicable to an AE that meets the following criteria: 1. It does not follow a reasonable temporal sequence from the administration of the study treatment. 2. It may readily have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. 3. It does not follow a known pattern of response to the suspected study treatment. 4. It does not reappear or worsen when the study treatment is re-administered.
Unrelated	This category is applicable to AEs that are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.), and do not meet the criteria for medication relationship listed under remote/unlikely, possible, or probable.

8.7.5 Definition of Serious Adverse Event

A serious adverse event (SAE) is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life-threatening (i.e., its occurrence places the subject at immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Is otherwise considered to be an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Medical and scientific judgement should be exercised in deciding whether an event should be considered as an Important Medical Event. Examples of such medical events include:
  - Allergic bronchospasm requiring intensive treatment in an emergency room or at home;
  - Blood dyscrasias or convulsions that do not result in hospitalization;
  - Development of drug dependency or drug abuse.

8.7.6 Reporting of Adverse Events

8.7.6.1 Serious Adverse Event Reporting to the Sponsor

Any SAE will be reported to the Sponsor (or representative) within 24 hours of learning of the event. The notification must be directed to:

SAE contact information:

[redacted]

8.7.6.2 Serious Adverse Event Reporting to the IEC and Regulatory Agency(ies)

It is the responsibility of the clinical site to report suspected, unexpected, serious adverse reactions (SUSARs) to the IEC responsible for the study per their policies. Report of fatal or life-threatening SUSARs must be made as soon as possible, but no later than 7 calendar days after first knowledge of the event. Report of SUSARs that are neither fatal nor life-threatening must be made as soon as possible, but no later than 15 calendar days after first knowledge of the event.

The Sponsor (or representative) is responsible for notifying the regulatory agency(ies) of SUSARs observed during the study conduct per their regulations, as soon as possible but no later than 7 calendar days after becoming aware of the information when fatal or life-threatening, or 15 calendar days when neither fatal nor life-threatening. The Sponsor (or representative) is responsible to comply with any other applicable regulatory requirement(s) related to the reporting of SAE to other regulatory authority(ies).

8.7.7 Pregnancy

In the event a dosed female participant or the female partner of a dosed male participant (i.e., has received at least one dose of the investigational product) becomes pregnant during or shortly after participation in the study, this pregnancy will be reported to the Sponsor (or representative) within 24 hours of first knowledge of the event. Any subject who becomes pregnant during the study will be immediately withdrawn. Follow-up information regarding the course and outcome of the pregnancy will be documented (after obtaining the consent of the female partner) as per site's SOP. If the outcome of the pregnancy meets the criteria of reportable event, reporting of the event to the IEC responsible for the study and/or to applicable regulatory agency(ies) will be performed as per site's SOP.

8.8 Premature Termination of the Study

The study may be prematurely terminated by the Investigator following consultation with the Sponsor, by the Sponsor or by the regulatory authorities. Following a decision to discontinue the trial, the Investigator will promptly inform the active study subjects and the IEC responsible for this trial, stating the reasons for discontinuation of the study. It is the responsibility of the Sponsor (or representative) to report the premature termination of the study to the regulatory authority(ies), when required by the applicable regulatory requirement(s).

9. Statistical Analyses

For all PK analysis, all concentrations below the limit of quantification will be set to 0.

Samples with invalid concentration (due to bioanalytical or clinical issue) will be replaced by "0.00" when it occurs prior to dosing for PK parameters calculation. Otherwise, they will be set to missing for tabulation, graphical representation, and calculation purposes if it occurs after dosing.

The primary objective of this study is to evaluate the relative bioavailability of a new oral suspension formulation. Since this study is exploratory, no formal hypothesis testing is defined.

9.1 Analysis Populations

9.1.1 Safety Population

The safety population is defined as all subjects who receive at least one dose of the study treatment.

9.1.2 Pharmacokinetic Population

The PK population will include all subjects data from the safety population who complete at least two periods, including Treatment B, and for whom the PK profile can be adequately characterized.

9.1.3 Pharmacokinetic Statistical Population

The PK statistical population will include all subject's data from the PK population who complete at least two periods, where a statistical comparison can be made for at least one PK parameter and who have not experienced any protocol deviations or other circumstances to exclude the subject from the PK statistical analysis.

Individual period data will be excluded if a baseline-corrected pre-dose concentration (at time 0-hour) greater than 5% of the corresponding C_{max} is observed for a subject.

9.1.4 Samples to be Assayed

Samples from subjects included in the PK dataset and who experience vomiting within the criteria for removal, as defined in the section 5.4 will be assayed.

9.2 Pharmacokinetic Parameters

The actual post-dose sample collection times will be used in the PK analysis.

The following PK parameters and observations will be calculated based on data from the PK population [redacted] later) for ba

- AUC_{0-t}: Area under the concentration-time curve from time zero until the last observed concentration, as calculated by the linear up/log down variant of the trapezoidal method.

- $AUC_{0\text{-}inf}$: Area under the concentration-time curve from time zero to infinity (extrapolated)
- Residual area: Percentage of $AUC_{0\text{-}inf}$ due to extrapolation from the time of the last observed concentration to infinity, calculated as $[1 – (AUC_{0\text{-}t}/AUC_{0\text{-}inf})] \times 100$
- C_{max}: Maximal observed concentration
- T_{max}: Time when the maximal concentration is observed
- T_{lag}: Time of observation prior to the first observation with a measurable (non-zero) concentration.
- $T_{½\ el}$: Terminal elimination half-life
- K_{el}: Terminal elimination rate constant
- Cl/F: Apparent clearance
- V_z/F: Apparent volume of distribution
- R^2: Coefficient of determination obtained from regression analysis

Additional PK parameters may be calculated. K_{el}, $T_{½\ el}$, and $AUC_{0\text{-}inf}$ parameters will not be estimated for concentration-time profiles where the terminal linear phase is not clearly defined.

Sapropterin is an endogenous compound and therefore, should be analyzed both with and without baseline correction. Pharmacokinetic parameters will be calculated based on uncorrected plasma concentrations and baseline-corrected plasma concentrations, as appropriate. Baseline corrected for sapropterin will be performed by calculating the mean plasma pre-dose levels for each subject within each period of the study using the measured concentrations at the -1, -0.5, and the 0-hour time points. The mean of these pre-dose values will be the *baseline* for that period for that subject. The baseline value will be subtracted from all subsequent measured concentrations (including 0-hour) for the same subject within the same period. Negative baseline adjusted levels will be set to zero before the PK analysis. No adjustment of the pre-dose or post-dose concentrations will be performed for the baseline uncorrected data.

9.3 Pharmacokinetic Statistical Analysis

Statistical analysis described in this section will be based on the PK statistical population.

Individual and mean plasma concentration versus time curves will be presented for both linear and semi-log scales.

Descriptive statistics for the PK parameters of baseline-corrected and uncorrected sapropterin will be calculated. Descriptive statistics will include number of observations, arithmetic mean, standard deviation, geometric mean (where applicable), coefficient of variation (CV), median, minimum, and maximum.

Statistical analysis will be performed on quality assured data, with unbalanced sequences if necessary, from subjects in the statistical dataset. The PROC MIXED procedure from SAS® (version 9.4 or later) will be used.

ANOVA will be performed on log-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max} parameters at the alpha level of 0.05 on uncorrelated data, the statistical model will include sequence, treatment, period, and subject(sequence) as a random effect.

ANCOVA (Analysis of Co-Variance) on log-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max} parameters at the alpha level of 0.05 will be applied for baseline corrected analysis, using the actual total dose included in the ANOVA statistical model as a covariate, the statistical model will include sequence, treatment, period, and actual total dose as fixed effects and subject(sequence) as a random effect.

In case the study is dosed in groups, considering the study conduct will occur at the same clinical site, assignment to groups is random, and the dosing of the groups will occur within a relatively short timeframe, it is not anticipated that dosing in groups will impact the study results. As such, the group effect will not be considered in the statistical model.

Using the same statistical model, the least-squares-means, the differences between the treatments least-squares-means, and the corresponding standard errors of these differences will be estimated for log-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max} parameters. Based on these statistics, the ratios of the geometric means for treatments and the corresponding 90% confidence interval will be calculated for the following comparisons:

- Treatment A versus Treatment D
- Treatment B versus Treatment D
- Treatment C versus Treatment D
- Treatment B versus Treatment C
- Treatment A versus Treatment C
- Treatment B versus Treatment A

These contrasts will be estimated separately using only the data from the treatments involved in the comparison.

Treatment differences in T_{max} will be evaluated by a nonparametric approach (Wilcoxon signed rank test) on the untransformed values.

These statistics will be used to evaluate the effect of food on the test formulation and the performance of the test formulation with and without water.

Additional PK statistical analysis may be performed. For example, an analysis with potency corrected data (to adjust the ratio and 90% CI for the comparison between Test and Reference B/D), in the event that the measured study treatment content of the lots of the Test and Reference products would differ by more than 5%.

9.4 Safety and Tolerability Analysis

Demographic parameters will be summarized descriptively.

Safety and tolerability analysis will be performed for all subjects in the safety population. No inferential statistical analysis of safety data is planned.

Safety and tolerability of sapropterin dihydrochloride will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study treatment, outcome, duration, and management), vital signs, 12-lead ECGs, clinical laboratory tests, and physical examinations. AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA).

TEAEs will be tabulated by treatment. Safety and tolerability data will be reported using descriptive statistics.

10. Data Collection

The source data will be collected on paper as per site SOPs. Source data will reflect the ALCOA+ principles, then they should be attributable, legible, contemporaneous, original, accurate, and complete. Source documents will be maintained in order to maintain data integrity. The Investigator and/or the clinical staff have the responsibility of ensuring the accuracy, completeness, legibility, and timeliness of the source data.

Details on the data management process will be described in a data management plan (DMP).

11. Regulatory Considerations and Quality Assurance

11.1 IEC Approval of Protocol and Other Study Documents

The Investigator(s) agree to provide the IEC with all appropriate documents, including a copy of the protocol/amendments, ICFs, advertising text (if any), and any other written information provided to study subjects. The trial will not begin until the Investigators have obtained the IEC favourable written approvals for the above-mentioned study documents. A properly executed written ICF shall be read, signed, and dated by each subject prior to entering the trial or prior to performing any study procedure. Adequate information will be provided to subjects in order for the subject to make an informed decision regarding his/her participation. Prior to signing any consent form, subjects will be allowed adequate time to consider the potential risks associated with their participation in the study. The original signed and dated ICF will be kept at the clinical site and a copy will be given to the subject.

In the event that the protocol is amended, the revised protocol must be approved by the IEC prior to its implementation, unless the changes involve only logistical or administrative aspects of the trial. If a revised ICF is introduced during the study, each subject's further consent must be obtained. The new version of the ICF must be approved by the IEC, prior to subsequently obtaining each subject's consent.

The Investigator and the Sponsor's representative must sign the protocol and its amendments (if any) before initiating the study.

It is the Sponsor's responsibility to submit the protocol and its amendments (if any), and the ICFs to regulatory authorities when necessary.

11.2 Compliance

This study will be conducted in compliance with the protocol, GCP, all applicable regulations, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (title 21), and any IEC requirements relative to clinical studies. The study will also be conducted in compliance with the recommendations laid down in the most recent version of the Declaration of Helsinki, with the exception that registration of such Phase 1 trials in a publicly accessible database is not mandatory. As required by the Canadian regulatory agency, a Clinical Trial Application (CTA) will be submitted before the beginning of the study and a No Objection Letter (NOL) must be received prior to enrollment.

11.3 Quality Assurance and Monitoring

The Sponsor or its designee will perform the quality assurance and quality control activities of this study; however, responsibility for the accuracy, completeness, security, and reliability of the study data presented to the Sponsor lies with the Investigator generating the data. When applicable, quality assurance procedures will be performed according to the site SOPs.

The study will be monitored internally according to the site monitoring plan and SOP to ensure that it is conducted and documented properly according to the protocol, GCP, and all applicable regulatory requirements. The Sponsor is entitled to access information about the status of the study and to review the original documents of the study. In addition, the Sponsor may decide to engage an external consultant for monitoring activities.

The Sponsor may arrange audits as part of the implementation of quality assurance to ensure that the study is being conducted in compliance with the protocol, SOPs, GCP, and all applicable regulatory requirements. In such case, audits will be independent of and separate from the routine monitoring and quality control functions.

11.4 Financial Disclosure and Obligations

11.5 Insurance

[redacted]

11.6 Confidentiality and Retention of Study Records

This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties. Persons to whom this study protocol is disclosed must be informed that all the information herein is confidential and may not be further divulged. These restrictions will apply as well to all future communications if deemed privileged or confidential. Publication of the study results may only be allowed with written permission from the Sponsor.

All information on a subject obtained during the conduct of the study will be kept confidential. Subjects will be identified by an anonymized identifier on all samples and study records provided to the Sponsor or designee. In compliance with ICH GCP, the Sponsor's authorized representatives, monitor(s), auditor(s), IEC, and regulatory authority(ies) will be granted direct access to the subject's original trial-related records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations. Consent from the subject for disclosure of such information will be obtained in writing in the ICF. In addition, should a subject require medical care or hospitalization during the course of the study, the clinical site may contact the treating physician with the subject's consent, except that consent may not be requested if there is an emergency situation. If the results of the study are published, the subject's identity will remain confidential.

The clinical site will maintain adequate study records according to local and agency applicable regulatory requirements and data integrity principles. The Sponsor will be notified prior to the destruction of study records or their moving from the site.

12. References

1 Kuvan, Prescribing Information. Version revised on 03/2020. Drug Databases, FDA. Available online at: www.accessdata.fda.gov/scripts/cder/daf/

2 Kuvan, Product Monograph. Version revised on JUL 05, 2022. Drug Product Database, Health Canada. Available online at: health-products.canada.ca/dpd-bdpp/index-eng.jsp

3 Musson D.G., *et al*. Relative Bioavailability of Sapropterin From Intact and Dissolved Sapropterin Dihydrochloride Tablets and the Effects of Food: A Randomized, Open-Label, Crossover Study in Healthy Adults. Clinical Therapeutics. Volume 32, Number 2, 2010.

4 Kuvan, Clinical Pharmacology and Biopharmaceutics Review(s). Version submitted on FEB 09, 2013. Drug Databases, Available online at: www.accessdata.fda.gov/drugsatfda_docs/nda/2013/205065Orig1s000ClinPharmR.pdf

5 Center for Drug Evaluation and Research (CDER), FDA. Guidance for Industry: Bioavailability Studies Submitted in NDAs or INDs – General Considerations. April 2022.

6 FDA Draft Guidance on Sapropterin Dihydrochloride Tablets. Recommended Sep 2008; Revised Sep 2012.

7 Center for Drug Evaluation and Research (CDER), FDA. Guidance for Industry: Assessing the Effects of Food on Drugs in INDs and NDAs — Clinical Pharmacology Considerations.