European Medicines Agency
*Evaluation of Medicines for Human Use*

Doc.Ref.: EMEA/604757/2008

# CHMP ASSESSMENT REPORT

# FOR

# Kuvan

International Nonproprietary Name: **sapropterin**

**Procedure No. EMEA/H/C/000943**

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

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# 1. BACKGROUND INFORMATION ON THE PROCEDURE

## 1.1 Submission of the dossier

The applicant Merck KGaA submitted on 30 October 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Kuvan, which was designated as an orphan medicinal product EU/3/04/199 on 8 June 2004. Kuvan was designated as an orphan medicinal product in the following indication: treatment of hyperphenylalaninemia (HPA). The calculated prevalence of this condition was 17 per 100,000 EU population.

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

The application submitted is a complete dossier composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

The applicant applied for the following indication treatment of hyperphenylalaninemia (HPA) in adult and paediatric patients with phenylketonuria (PKU) or tetrahydrobiopterin (BH4) deficiency.

**Protocol Assistance:**
The applicant received Protocol Assistance from the CHMP on 15 June 2005. The Protocol Assistance pertained to non-clinical and clinical aspects of the dossier.

**Licensing status:**
The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP* and the evaluation teams were:

Rapporteur: Patrick Salmon Co-Rapporteur: Pieter Neels

**During the meeting on 21-25 March 2008, the CHMP agreed on a switch of Rapporteurship, being from then onwards Dr. Salmon the Rapporteur and Dr. Neels the Co-Rapporteur.*

## 1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 30 October 2007.
- The procedure started on 23 November 2007.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 29 February. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 13 February 2008.
- During the meeting on 17-19 March 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 19 March 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 May 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 4 July 2008.
- During the CHMP meeting on 21-24 July 2008, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant .
- The applicant submitted the responses to the CHMP consolidated List of Questions on 20 August 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 9 September 2008.

- During the meeting on 22-25 September 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Kuvan on 25 September 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 22 September 2008.
- The CHMP opinions were forwarded in all official languages of the European Union, to the European Commission, which adopted the corresponding Decision on 2 December 2008.

## 2 SCIENTIFIC DISCUSSION

### 2.1 Introduction

Hyperphenylalaninemia (HPA) is defined as a chronic abnormal elevation in blood phenylalanine levels. Phenylalanine (Phe) is one of 8 essential amino acids that cannot be synthesised de novo in the human body. Physiologic requirements for Phe are met exclusively by dietary protein intake. In addition, Phe is a necessary precursor for synthesis of tyrosine, which is considered a conditionally essential amino acid due to its dependence on Phe metabolism. Tyrosine, in turn, serves as a precursor for neurotransmitter and thyroid hormone syntheses. Usual dietary intake of protein provides excess amounts of Phe and blood Phe levels are maintained within non-toxic levels via utilisation, metabolism and excretion.
Since the conversion of Phe to tyrosine by phenylalanine hydroxylase (PAH) normally accounts for approximately 75% of dietary Phe disposal, disorders of Phe metabolism can cause abnormal elevations in blood Phe levels, or HPA. Although such conditions are rare, severe HPA has serious clinical consequences for affected individuals, including severe neurocognitive delay and mental retardation, neuromotor disability and adverse pregnancy outcomes for affected women. In the application for Orphan Medicinal Product designation, the population prevalence for HPA in European countries was estimated to be 1.7 in 10,000 (EMEA/5516/045). In recognition of the serious and debilitating nature of HPA and its rare occurrence, this condition has been granted orphan drug status in the EU, US, Australia and Switzerland.

Two genetic conditions, PKU and BH4-deficiency, account for the majority of cases of clinically significant HPA. Both of these conditions are detectable in newborn screening programs. HPA in both conditions can be controlled by dietary restriction of whole protein, with concomitant administration of commercial Phe-free protein supplements to provide adequate nutritional intake of protein. Although this dietary therapy has proven to be beneficial in lowering blood Phe levels and preventing the severe neurological consequences of HPA, most affected individuals are unable to maintain adequate control of blood Phe levels with diet therapy since the severe restriction of dietary protein is impractical in daily life. In addition, the Phe-free protein supplements, which are necessary to maintain protein nutrition, are widely acknowledged to be unpalatable.

Sapropterin dihydrochloride, a synthetic version of 6R-BH4, the naturally occurring cofactor, is proposed as an oral treatment for hyperphenylalaninaemia (HPA) in patients with phenylketonuria (PKU) or BH4 deficiency. In patients with PKU, the role of sapropterin dihydrochloride is to enable endogenous PAH activity and to partially restore oxidative metabolism of phenylalanine, resulting in decreased blood phenylalanine levels in PKU patients. In patients with BH4 deficiency, sapropterin dihydrochloride is proposed to restore endogenous PAH activity by providing an exogenous source of the missing cofactor.

### 2.2 Quality aspects

**Composition**

Kuvan contains sapropterin dihydrochloride as the active substance. It is presented as soluble tablets, which are dissolved in water prior to administration. Each soluble tablet contains 100 mg of sapropterin dihydrochloride, which is equivalent to 76.8 mg of sapropterin. The dose is dependent on body weight and is adjusted for maximum therapeutic effect, but will usually be in the range 5-20 mg/kg/day.

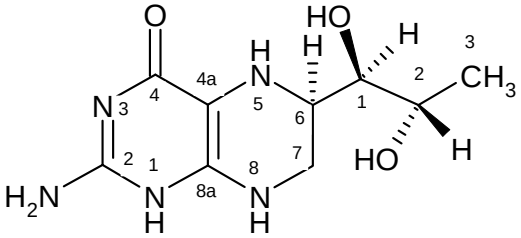
Other ingredients include ascorbic acid, crospovidone, anhydrous calcium hydrogen phosphate, mannitol, riboflavin and sodium stearyl fumarate.

The tablets are packaged into HDPE bottles. A silica gel desiccant canister is placed into each bottle together with a pharmaceutical-grade polyester coil, which is placed in each bottle to fill excess space and prevent damage. The bottle is induction sealed with an inner cap liner and the closure is a child-resistant cap.

**Active Substance**

Sapropterin is a synthetic formulation of the naturally-occurring 6R-diastereomer of tetrahydrobiopterin ('BH4'), an endogenous compound which is a co-factor of phenylalanine hydroxylase, the enzyme responsible for phenylalanine metabolism.

Sapropterin is the recommended INN and it refers to the 6R diastereomer of tetrahydrobiopterin. The active substance is the dihydrochloride salt. Its chemical name is (6R)-2-amino-6-[(1R,2S)-1,2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(1H)-pteridinone dihydrochloride and its structure is shown below:




Sapropterin dihydrochloride is a crystalline powder. It is hygroscopic and very soluble in water (> 1 g/ml).

There are three chiral centers, the absolute configurations of which are given in the chemical name. Two of the chiral centers are found in the dihydroxypropyl side chain. The third chiral center is found at the juncture of the dihydroxylpropyl side chain with the pteridone ring at C-6. The "R" absolute configuration at this center is necessary for pharmacological effect, while the 6S form may cause inactivation of phenylalanine hydroxylase, thus inhibiting the effects of the 6R form.

Sapropterin dihydrochloride exhibits polymorphism and many crystalline forms were identified during the course of crystallisation studies. Form B is a thermodynamically stable crystalline anhydrate and it can be specifically identified by X-ray powder diffraction. Given that the finished product is formulated as soluble tablets that will be dissolved prior to administration and in view of the high aqueous solubility of the active substance, the existence of polymorphism is not a source of concern.

The chemical structure of sapropterin dihydrochloride has been confirmed using elemental analysis, mass spectroscopy, infrared spectroscopy, $^{13}C$ and $^{1}H$ NMR, ultraviolet spectroscopy, optical rotation of the active substance.

- **Manufacture**

The synthesis of sapropterin dihydrochloride involves a number of synthetic and purification steps and uses commercially available starting materials. The critical steps in the synthesis have been identified and have been appropriately validated. The manufacturing process has been shown to consistently produce the desired 6R isomer with an optical purity of ≥ 99%. X-ray diffraction studies show that the polymorph manufactured with the proposed synthetic process is the stable anhydrous crystal Form B.

Related substances arising either from the route of synthesis or from degradation have been identified and some of them are specified impurities. The specification limits for those impurities above the limit of max.0.15% have been toxicologically qualified.

The solvents used in the synthesis have been shown to be efficiently removed during the purification and drying operations and appropriate specifications have been set.

- **Specification**

The active substance specification includes tests for appearance, identification (IR, UV, optical rotation), assay (HPLC), related substances (HPLC), residual solvents (GC), pH, loss on drying (Ph. Eur), and heavy metals (Ph. Eur).

Batch analysis results have been provided for 23 pilot and 6 commercial-scale batches; commercial batches were manufactured by the proposed manufacturer. All the batches were within the proposed specifications.

- **Stability**

Stability studies were carried out on pilot scale and production scale batches according to the ICH requirements. Samples were stored at 25°C/60 % RH for up to 12 months and at 40°C/75 % RH for 6 months.

The parameters tested were appearance, assay, related substances and loss on drying using the analytical methods intended for release testing. The samples were also tested in selected timepoints for polymorphic purity using X-ray powder diffraction.

The long-term and accelerated stability data show no significant changes in the appearance, or in X-ray diffraction results demonstrating that there is no polymorphic transition state. The observed changes in assay values are within the method intermediate precision limits and no downward trend is observed. In addition the related substance impurity levels have not increased significantly over time.

The results presented support the proposed re-test period when the active substance is stored at the recommended conditions (controlled room temperature, protected from moisture).

**Medicinal Product**

- **Pharmaceutical Development**

Kuvan has been developed as an immediate release solid dosage form intended to be administered orally as dissolved tablets (administration: dissolution in water, the solution should be administered within 15 to 20 minutes). The proposed formulation evolved from a granule formulation (Biopten®) approved in Japan in 1992. The clinical development program as well as the non-clinical studies had been initiated using the granule formulation. Later on, there was a re-development of the granule formulation to an immediate release tablet due to manufacturing considerations and in order to improve patient compliance.

In the tablet formulation the same excipients were used as in the already marketed granules with minor modifications. Mannitol was retained as a sweetener, ascorbic acid as an antioxidant and riboflavine as a colorant, while calcium hydrogen phosphate dihydrate was replaced with the anhydrate. In addition a disintegrant (crospovidone) and a lubricant (sodium stearyl fumarate) were used in the tablet formulation. The compatibility of the active substance with the excipients used has been demonstrated by stability data.

Since the tablets are intended to be dissolved in water the short-term chemical stability of sapropterin dihydrochloride in the recommended medium has been investigated. Stability data have been provided for up to 180 minutes. The data show that the levels of individual and total related substances remained within specification for 60 minutes after being dissolved in water. Furthermore, it has been shown that two tablets can be dissolved in as little as 5 ml of water, which facilitates administration to infants.

For the manufacturing process development a standard direct compression process has been used. The minor formulation and process modifications that have occurred during the development have no impact of the final product profile and the equivalency of the sapropterin dihydrochloride tablets used in clinical studies and the proposed commercial formulation and manufacturing process has been demonstrated with dissolution and stability studies.

- **Manufacture of the Product**

The manufacturing process is a standard direct compression process. All critical process parameters have been identified and controlled by appropriate in-process controls. The validation report for three consecutive commercial-scale batches has been provided. All three batches met the validation acceptance criteria and complied with the product specification.

- **Product Specification**

The specification for the finished product at release and shelf life includes tests for appearance, identification (HPLC, UV), assay, (HPLC), disintegration, (Ph. Eur), loss on drying, ascorbic acid content, (HPLC), content uniformity (Ph.Eur), dissolution (Ph. Eur), related substances (HPLC) and microbiological attributes.

The proposed disintegration test limit in the specification does not meet the requirements of the Ph. Eur. monograph for soluble tablets (testing is at 37 °C instead of 15-25 °C) at either the time of release or during the shelf-life. Nonetheless, it has been shown that the tablets dissolve readily, when they are stirred in water at room temperature. This derogation from the Ph.Eur. requirement does not raise concerns about the clinical performance of the product, because, as directed in the SPC and Package Leaflet, prior to administration the tablets should be stirred by the patient until dissolved. The fact that the active substance is freely soluble in water (> 1 g/ml) as well as in acidic and basic aqueous solutions provides additional assurance that a longer disintegration time would not affect bioavailability, as complete dissolution would be reached before the drug entered the upper intestine. Finally the inclusion of a dissolution test in the specification ($Q \geq 85\%$ at 15 minutes), is another safeguard that bioavailability will not be adversely affected by the potentially longer disintegration time of the tablets.

Batch analysis data from 6 pilot scale batches have been presented. All batches met the test limits as defined in the release specification and test methodology valid at the time of batch release. Process validation data on three commercial scale batches have been provided.

- **Stability of the Product**

Stability studies were conducted under long-term (25 °C/60% RH) and accelerated (40 °C /75% RH) conditions in accordance with ICH Q1A, 'Stability Testing of New Drug Substances and Products'. The parameters tested were appearance, disintegration, loss on drying, ascorbic acid content, dissolution, dissolution profile, assay, related substances, total aerobic count using the analytical procedures employed for release.

Data have been provided for six batches of the proposed market formulation packaged in 100 $cm^3$ HDPE bottles for 12 months under long-term and 6 months under accelerated conditions.

Data have been provided for six batches of the proposed market formulation packaged in 100 $cm^3$ HDPE bottles for 12 months under long-term and 6 months under accelerated conditions.

In addition to the above, supportive data have been provided for five batches of a development formulation, which differs from the proposed market formulation only in the content of lubricant. Two of these batches were packaged in aluminium/aluminium blisters, and the other three batches were packaged in HDPE bottles. The studies were conducted under ICH conditions and results are available for 24 months under long-term conditions and for 6 months under accelerated conditions.

The results from all the above studies indicate that the stability of the tablets is excellent The only noticeable chemical change is an increase in the content of dihydrobiopterin; as this is a metabolite of the drug substance, the increase is not a cause for concern in itself. There has also been a slight increase in disintegration time for those samples stored at accelerated conditions, but not at long term conditions; an appropriate condition has therefore been included in the SPC, i.e. 'store below 25 °C.

One batch of tablets was subjected to photostability testing in accordance with the ICH note for guidance on 'Photostability testing of new drug substances and products' (ICH Q1B). No evidence of degradation was detected following exposure to UV/visible light.

One batch of tablets was subjected to stability testing under simulated in-use conditions for 8 weeks. The results indicate that the tablets remained stable under long-term conditions (25 °C/60% RH) for the full period of the study. A warning to store the tablets in the original pack for protection from moisture, and a direction to close the bottles tightly, have been included in the SPC and label/leaflet texts and are justified by the results of the in-use stability study.

**Discussion on chemical, pharmaceutical and biological aspects.**

The quality of Kuvan is adequately established. In general, satisfactory chemical and pharmaceutical documentation has been submitted for marketing authorization. There are no major deviations from EU and ICH requirements.

The active substance is well characterised and documented. It exhibits optical activity and has several polymorphic forms; however the route of synthesis has been demonstrated to consistently produce the desired isomer and polymorphic form. In addition appropriate specifications have been established to ensure its quality. The excipients are commonly used in these types of formulations and comply with Ph. Eur. requirements. The packaging material is commonly used and well documented. The manufacturing process of the finished product is a standard process that has been adequately described. Stability tests indicate that the product under ICH guidelines conditions is chemically stable for the proposed shelf life. At the time of the Opinion there were a few unresolved issues that did not affect the benefit/risk of the product and will be addressed as post-authorisation commitments.

## 2.3 Non-clinical aspects

### Pharmacology

- Primary pharmacodynamics

Non-clinical primary pharmacodynamic studies were conducted in normal rodents where HPA was induced by administering the BH4 biosynthesis inhibitor, DAHP (2,4-diamino-6-hydroxypyrimidine). Such studies demonstrated that 6R-BH4 supplementation by systemic route can reverse the effects of inhibitors of the BH4 biosysnthesis pathway. Indeed, sapropterin reversed DAHP-induced increases in plasma/liver Phe levels at doses levels from 5 mg/kg p.o. in rats and at 20 mg/kg p.o. in guinea pigs. Moreover, in the guinea pig pregnant model, lowering in maternal and foetus plasma/liver Phe levels was observed and appeared primarily due to a reduction of overall blood Phe in the mother, resulting in a lowering of transplacental Phe transfer and foetal uptake. However, a transplacental transfer of sapropterin to the foetus could not be excluded. While the DAPH-induced BH4 deficiency animal model is considered acceptable as a model of HPA due to BH4 deficiency. There is no appropriate animal models for HPA due to PKU, due to the extreme heterogeneous genotypic variation in the PKU population. However, from the clinical development program, sapropterin dihydrochloride increased the overall levels of PAH activity, partially restored normal physiologic phenylalanine oxidative capacity, and reduced blood phenylalanine concentrations in PKU patients responsive to 6R-BH4.

Furthermore, sapropterin reversed intracerebral monoamine depletion induced by α-methyltyrosine, a TPH inhibitor, as well as the associated decreases in motor activity. The single oral dosing of sapropterin to 3-week old mice, resulted in some activation of the intracerebral monoamine metabolism; such effects were pronounced at doses as high as 1000 mg/kg but minimal at 300 mg/kg.

Increases in the brain neurotransmitter levels (DA, 5-HT, NE and metabolites) were more evident when sapropterin was administered through striatal dialysis perfusion or intracisternally. These were marginally affected following single i.p. administration to juvenile or adult rats with no dose correlation and not consistent signal throughout various rat ages. Following repeat i.p dosing at doses ≥ 10 mg/kg starting when rats were 7-day old the degree of increase in total intracerebral biopterin content gradually decreased with age. Brain DA, 5-HT and NE and their metabolites increased in 7-day old rats, but not in 14- or 21-day old, suggesting a higher CNS penetration in very young rats.

- Secondary pharmacodynamics

A review of the literature concerning secondary pharmacodynamic effects of sapropterin dihydrochloride in multiple animal models of cardiovascular diseases with underlying endothelial dysfunction (associated with BH4 deficiency), including hypertension, congestive heart failure and diabetes not only indicated the absence of adverse secondary pharmacodynamic effects, but also that the pharmacological intervention with sapropterin dihydrochloride reduced the oxidative stress and resulted in a restoration of endothelial function in these models.

- Safety pharmacology programme

The majority of the *in vivo* safety pharmacology studies were not performed in accordance with GLP principles. Taking into account that the studies were performed up to 1989, thus, before the ICHS7A guideline was published, and taking into account the clinical experience with the sapropterin

dihydrochloride granule formulation, this was considered acceptable. However, as no individual animal data were provided, the evaluation of the studies is hampered.

*Central nervous system*

The battery of tests aimed at assessing the effects of orally administered sapropterin dihydrochloride on the central nervous system (motor activity and coordination, barbiturate-induced sleep prolongation, convulsions, pain and body temperature, showed no major findings up to 300 mg/kg, except for a significant prolongation of thiopental sodium-induced sleeping time and a slight increase in muscular tone in male Wistar rats and a slight increase in motor activity in male ddY mice. At doses ranging between 100 and 600 mg/kg p.o. sapropterin dihydrochloride showed antidepressive, anxiolytic and ameliorative effects on memory and learning disorder behavioural tests in rodents, suggesting that brain CNS penetration may occur at these dosages.

*Cardiovascular and respiratory system*

Cardiovascular safety pharmacology study findings were minimal. Since the $IC_{50}$ for the effect of sapropterin on hERG potassium current was > 690 µM which far exceeds the maximal therapeutic plasma concentration at the highest anticipated clinical dose of 20 mg/kg, it can be concluded that sapropterin does not block Ikr channels. This is indicative of negligible risk for delayed ventricular repolarisation (QT interval prolongation) and *torsades de pointes*.

No effects on the cardiovascular system (dogs by telemetry) or respiratory function parameters (rats, by plethysmography) were noted in two GLP-compliant studies after single oral sapropterin administration at 100 mg/kg (dog) and 225 mg/kg (rat). It was only after a high intravenous dose (30 mg/kg) in anesthetized dogs that a significant decrease in respiration and heart rates (but with no changes in mean arterial blood pressure or ECG morphology) was observed.

*Other systems*

Other findings from the general (safety) pharmacology experiments consisted of an increased emesis in dogs at sapropterin dihydrochloride doses ≥ 10 mg/kg in fasting conditions in a single study. However, emesis was not observed in subsequent dog safety pharmacology studies at oral doses of up to 100 mg/kg but in fed conditions.

No other effects of sapropterin dihydrochloride have been observed in pharmacology experiments aimed at evaluating the autonomic nervous, gastrointestinal and genitor-urinary systems.

- Pharmacodynamic drug interactions

BH4 acts to enhance nitric oxide synthetase (NOS) activity and could have a synergistic effect with phosphodiesterase type 5 (PDE-5) inhibitors, such as sildenafil, on vasorelaxation. Animal studies do not, however, indicate interaction between sildenafil and sapropterin.

**Pharmacokinetics**

Similarities exist in the pharmacokinetic profile of sapropterin dihydrochloride across the species studied (mouse, rat, rabbit and monkey) and with the limited clinical pharmacokinetic studies. Sapropterin dihydrochloride and its metabolites were measured in all species either by HPLC and/or mass spectrometry (LC/MS/MS) assay.

Based on PK studies and allometric scaling in rats and cynomolgus monkeys, pharmacokinetic parameters can be summarised as below.

**Table: Exposures after a single oral dose in the human, rat & monkey:**

| | Study | BW[1] (kg) | Dose (mg) | Dose/BW (mg/kg) | tmax (h) | Cmax (ng/mL) | AUC (ng/mL/h) | Cmax/Dose (ng/mL/mg/kg) | AUC/Dose (ng/mL/h/mg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| **Rat** | PHN-104-PK-SR | 0.25 | 2.5 | 10 | 2.0 | 75 | 265 | 7.5 | 26.5 |
| **Cynomolgus Monkey** | PHN-110-PK-SR | 4 | 40 | 10 | 2.9 | 344 | 1300 | 34.4 | 130.0 |
| **Human[2]** | P1501 | 70 | 100 | 1.43 | 3.1 | 6.8 | 59.8 | 4.8 | 41.9 |
| | | | 200 | 2.86 | 2.6 | 12.5 | 92.0 | 4.4 | 32.2 |

[1] BW: body weight

[2] Phase I study of sapropterin hydrochloride – single dose and multiple dose studies

The rat model is considered to be appropriate for the toxicology studies.
However, there are no pharmacokinetic data, including metabolism and tissue distribution data, to support the use of marmoset monkeys in the toxicology studies and as such safety margins cannot be calculated in this species.

*Absorption*
Absorption studies were performed in rats, cynomolgus monkeys, mice and pregnant rabbits by both i.v. and p.o. routes. Tmax values ranging between 1-3 hours were comparable to that found in human studies. The half-lives were 1.2, 1.1 and 1.4 h in mice, rats and monkeys, respectively which were slightly lower than those obtained in human fasting and non-fasting studies (t½ ~ 3-4.8 h).
In the rat, sapropterin dihydrochloride was mainly absorbed through the small intestine and unlike human data, food had no effect on its pharmacokinetic profile.
AUC in the majority of studies, increased with dose but in a slightly less than dose-proportional manner.
The absolute bioavailability was 7-12 % in 6 week old rats and ~9 % in cynomolgus monkeys but is unknown for humans. Comparable results were observed between single and repeat oral dosing, indicating that there was no accumulation or persistence.
Total biopterin (BP) levels were higher in 2 week vs. 6 week old rats thus highlighting the potential higher degree of absorption of sapropterin dihydrochloride from the gastrointestinal tract of younger animals.

*Distribution*
Biodistribution studies revealed that in rats, once absorbed, sapropterin dihydrochloride is distributed mainly into the liver, adrenals (consistent with the sites where tetrahydrobiopterin acts as a coenzyme for aromatic amino acid hydroxylases) and kidneys (the main excretory organ).

Murine studies demonstrate that infused [$^{14}$C](6R)-BH4 localises primarily to liver and kidney in postnatal animals, with minimal uptake into brain, adrenal medulla or bone marrow. This distribution was hypothesised to reflect the tissue distribution of PAH, whereas tissues containing tyrosine and tryptophan hydroxylases and significant endogenous BH4 synthesis (*e.g.* adrenal) had relatively little uptake of exogenously administered BH4. In these same studies, the oxidised product, biopterin, did not accumulate in any tissue and was rapidly excreted. The tissue distribution was apparently age-dependent, with no tissue uptake into liver or the inner cortex of the kidney in newborn (5-7 day old) mice, paralleling the maturation of PAH expression in these tissues. In postnatal mice, [$^{14}$C]BH4 apparently did not significantly cross the blood brain barrier. However, BH4 was found to cross the placenta and distribute throughout foetal tissues, including foetal brain and adrenal in mice.

Binding of BP to plasma proteins is low in rat and human plasma, indicating a very low potential for sapropterin dihydrochloride to displace other agents from plasma proteins. Red blood cell partitioning experiments in rats and monkeys showed that at concentrations exceeding the erythrocyte binding capacity, the concentration of total BP in plasma increase linearly.

Total BP concentration was increased in fetus and in milk after i.v. but not p.o. administration of sapropterin dihydrochloride at 10 mg/kg in pregnant rats.

*Metabolism*
Although no formal metabolism studies were performed in the clinic, the rat and human catabolic pathways of tetrahydrobiopterin are thought to be very similar based on scientific literature. Tetrahydrobiopterin (6R-BH4 [presumed unchanged drug]), dihydrobiopterin (DHBP), biopterin (BP), pterin (PT), and 6-hydroxylumazine (6-OH-Lu) were the main urinary metabolites detected in rats which were comparable to those found in humans except for lumazine metabolites (the human liver is not reported to express the enzyme involved in the formation of these metabolites). Repeated administration of sapropterin dihydrochloride did not up-regulate CYP-dependent drug-metabolising enzymes in the liver microsomes.

*Excretion*
Rat excretion studies showed that 72 h after a single oral administration about 75% of the radioactive dose administered (3H-sapropterin dihydrochloride) was excreted in faeces and 7% in urine. In

Cynomolgus monkeys 24 h after a single oral administration of sapropterin dihydrochloride 2.3% of the dose was excreted in urine as total biopterins. An additional study in rats with the radiolabelled compound indicated that the biliary route plays a minor role in elimination. It is considered that the use of a tritium label to evaluate the quantitative excretion profile of BP in this instance cannot be considered a quantitative or indeed a reliable method given that it does not discriminate between $^{3}H_2O$ or $^{3}$H-sapropterin dihydrochloride. However, taking into account the results of a dedicated i.v. study in rats with the unlabelled compound (the dose was almost completely excreted in urine either as unchanged drug or metabolites within 6 h of dosing) it can be concluded that the majority of orally administered sapropterin dihydrochloride will be excreted in faeces due to limited absorption from the gastro-intestinal tract. The fraction absorbed is mostly excreted with the urine.

**Toxicology**

The toxicology of sapropterin dihydrochloride was characterised in a series of acute (single–dose), repeat–dose, genotoxicity, carcinogenicity, reproductive and developmental, juvenile, antigenicity, and metabolite toxicity studies. These studies were conducted in five different species (mouse, rat, rabbit, marmoset, and guinea pig), via four routes of administration (oral [p.o.], subcutaneous [s.c.], intravenous [i.v.], and intraperitoneal [i.p.]). All pivotal toxicology studies were conducted in accordance with the principles of Good Laboratory Practice (GLP).

- Single dose toxicity

Single-dose toxicity studies of sapropterin dihydrochloride via p.o, i.v., and s.c administrations were performed in adult mice, rats and marmosets. In rats, following p.o. administration of sapropterin dihydrochloride, histopathological findings included atrophy of the glandular and forestomach (≥2500 mg/kg), findings that could not be seen following i.v. or s.c. administration. There were no kidney changes. In marmoset monkeys, degenerative changes in renal tubules were observed at doses ≥ 1’000 mg/kg p.o. and ≥ 150 mg/kg s.c.

- Repeat dose toxicity (with toxicokinetics)

The effects resulting from the long-term oral administration of sapropterin dihydrochloride were evaluated through two 13- and 52-week repeated-dose toxicity studies performed in rats and marmoset monkeys.

The systemic safety profile of sapropterin dihydrochloride was assessed in the rat and the marmoset monkey. The rat was chosen based on the early pharmacological studies demonstrating the ability of oral sapropterin dihydrochloride to revert the induced HPA to control values. The choice of the marmoset monkeys was based on the finding of an increased total BP concentration in the CSF upon oral sapropterin dihydrochloride administration at doses not associated to systemic toxic effects. This species allowed neurological examination to be conducted at various timepoints within the toxicology studies.
The 52-week rat repeat dose toxicity study demonstrated slight renal changes of unknown mechanism (basophilic change in collecting tubules) at the highest dose level (400 mg/kg). Consequently the NOAEL is set at 40 mg/kg/day.

In the 2-year rat carcinogenicity study, treatment-related non-neoplastic lesions identified also the kidney as target organ of toxicity. Lesions included distended renal papillary tubules found in 7/60 females and 4/60 males that received 250 mg/kg/day and in 1/32 males that received 25 mg/kg/day. None of the control animals was affected; the incidence was statistically significant in females treated at 250 mg/kg/day. Additional non-neoplastic lesions occurring only in the high dose group (250 mg/kg/day) include: acute ascending pyelonephritis (1/60 M and 3/60 F); basophilic cortical tubules (1/60 F), dilated cortical tubules (4/60 F) and pigment deposition in cortical tubular cells (1/60 M). The predominance of findings in females is not in favour of an exacerbation of the age-related chronic progressive nephropathy. These lesions most probably correspond to an exacerbation of the kidney changes that are observed in the rat repeat dose toxicity studies (slight basophilic changes).

In the repeat dose studies in marmosets, all findings were not considered to be toxicologically significant.

- Genotoxicity

Although Sapropterin dihydrochloride produced a positive result in bacterial gene mutation assays and in the chromosomal aberration test in CHL and CHO cells, it did not prove to be mutagenic when assessed in human peripheral blood lymphocytes. Additionally, the potential of orally (both single and repeat dose) administered sapropterin dihydrochloride to induce micronuclei formation in bone marrow progenitor cells in mice also proved negative. The *in vitro* positive results are possibly related to the auto-oxydation of sapropterin, generating reactive oxygen species such as hydrogen peroxide (as seen with other anti-oxydants) which is not the case in vivo or to more efficient protective mechanisms and high levels of catalase in human peripheral blood lymphocytes cultures.
Overall, with the plasma exposure in these *in vivo* tests after repeat dosing being up to 45 times higher than human exposure, the biological significance of these positive *in vitro* findings is doubtful.

- Carcinogenicity

Oral carcinogenicity studies conducted in mice and rats indicated no potential of sapropterin dihydrochloride to induce the formation of either neoplastic or hyperplastic lesions. The higher incidence of benign phaeochromocytomas of the adrenal medulla in the rat carcinogenicity study in the 250 mg/kg/day and 80 mg/kg/day dose groups compared with the control group was not considered to represent evidence of an oncogenic potential since it was observed in one sex only, the incidence of malignant phaeochromocytoma was unaffected and the incidence is well within the historical control range of the testing facility while it was unusually low in the vehicle-treated male animals (1/60, 1.7 %). No increased incidence of adrenal hyperplasia was observed in any of the rodent carcinogenicity studies, or in the chronic studies in rats and marmosets.

- Reproduction Toxicity

Reproductive toxicity assessment was performed in the both rats and rabbits via oral administration, the full set of reproductive and developmental studies were performed in the rat with an additional embryo-fetal developmental study performed in the rabbit.

Following administration of Sapropterin dihydrochloride no effects were observed in any of the parameters examined in the mothers or offspring (foetuses or neonates) at dose levels up to 400 mg/kg/day in the rat, with the exception of a slight but significant reduction in the number of viable pups at the high dose. This was not confirmed in the rabbit embryofoetal development study. The NOAEL in the rat is considered to be 40 mg/kg/day. In the rabbit at the highest dose tested (600 mg/kg/day)there was an observed increase in the number of external malformations (holoprosencephaly) skeletal abnormalities but these increases were not found to be significant and were found in control animals albeit at a lower incidence. The NOAEL in the rabbit was considered to be 60 mg/kg/day for the mothers and embryofoetal development.

None of the reproductive and developmental toxicity studies included toxicokinetic evaluations. A bridging pharmacokinetic study has been performed only in pregnant NZW rabbits and safety margins for embryofoetal development are more than 20-fold. Due to the lack of experimental toxicokinetic analysis in the pregnant rat no assessment can be provided with respect to safety margins at the observed NOAEL levels. The pharmacokinetic data were obtained in the rat following dosing at 10 and 100 mg/kg and as such exposure levels at 400 mg/kg/day can not be commented upon.

Pharmacokinetic studies in late-stage pregnant rat (GD 18-20) and during lactation confirm that 2 – 2.5 hr post oral administration of 10 mg/kg of sapropterin dihydrochloride, plasma exposures were comparable to that of male rats given the same dose (PHN-104-PK-SR). The absence of gender differences in absorption were also demonstrated by using the tritium-labelled compound. Foetal absorption was only seen following i.v. administration of 10 mg/kg.dose. Administration of an i.v. dose of 10 mg/kg, 7 days after delivery, demonstrated about a 3-fold increase in milk exposure. The absence of milk or foetal exposure following p.o. dosing of 10 mg/kg sapropterin dihydrochloride is considered to be related to the low maternal exposure levels. The i.v. exposure levels are more comparable to the exposures achieved at higher p.o. dose levels.

- Toxicokinetic data

No toxicokinetic assessment was carried out with any of the repeat dose studies in rat and marmoset as per the ICH S3A guideline.
Pharmacokinetic data performed in the rat, namely 10 mg/kg and 100 mg/kg, do not compare with doses used in the toxicity studies and in the marmoset, toxicokinetic data are lacking. Thus no dose relationship has been ascribed to the systemic exposure achieved in the animals to the doses used in the toxicity studies.

However, a safety margin of 2.6 was estimated using the extrapolated (rat and human) AUCs, the same safety margin can be estimated based on the chronic NOAEL (320 mg/kg) in marmosets corrected by allometric scaling and the highest human therapeutic dose. This could represent a conservative approach since exposure in marmosets at NOAEL is estimated to be higher than the exposure in rats at the NOAEL.

- Other toxicity studies

*Juvenile toxicity*
A single acute dose study in mice and 2 single and repeat dose studies in the rat were performed as part of the juvenile oral toxicity package. Acute studies were performed in 7-21-day old rats and mice. The LD50 in 7-day old rats was determined to be 1108 and 1445 mg/kg in males and females respectively. At necropsy the only finding of note was haemorrhage throughout the glandular stomach at doses above 1680 mg/kg, this findings was considered to be likely due to the large dose volume and acidity (pH 0.5- 1.0) of the solution rather than any pharmacological action. In rats 21-days old the LD50 were consistently higher, 2278 and 2287 mg/kg for male and female mice and 2930 and 3222 mg/kg for male and female rats. It was considered that the difference in LD50 values was due to higher absorption of sapropterin dihydrochloride in younger animals, as observed in the pharmacokinetic studies.
Following 2-week repeat dosing in pre-weaned rats a significant decrease in body weight gain was observed at the high dose (320 mg/kg/day) together with an increased frequency and degree (from very slight to slight) of basophilic changes, dilation of the kidney tubules and thickening of the Bowmans capsule. The NOAEL was considered to be 80 mg/kg/day. In the 4-week repeat dose study in 21-day old rats no significant changes were observed in any of the measured parameters and the NOAEL was considered to be 500 mg/kg/day. Although such data may suggest sapropterin dihydrochloride as being potentially more toxic in early postnatal age, this was most likely due to the higher absorption rate of sapropterin dihydrochloride through the gastrointestinal tract of animals of two-weeks of age and younger compared to 6-week old rats; target organs of toxicity were consistent with those already identified in the studies in adult animals.

*Antigenicity-allergenicity*
Sapropterin dihydrochloride did not demonstrate allergenic or antigenic potential in mouse and guinea pig models designed to detect immunoglobulin E and anaphylactoid-type responses.

*Metabolites*
Regarding the metabolites of sapropterin dihydrochloride, dedicated safety experiments (including safety pharmacology and acute toxicity) have been conducted on both dihydrobiopterin and pterin. Dihydrobiopterin and pterin were examined for pharmacologic effects in mice (thiopental-induced sleep, p.o.), rats (anxiolytic effect, p.o.) and dogs (cardiovascular system and respiratory rate, i.v.) models. Each substance showed no effect at doses of 100 or 300 mg/kg (p.o.) or at a dose of 30 mg/kg (i.v). In single dose toxicity studies conducted in mice no acute toxic effect (including death) occurred when dihydrobiopterin and pterin were administered orally at doses at least 2.5-fold higher than the oral LD50 of sapropterin dihydrochloride.

*Impurities*
For the drug substance batches used in the non-clinical studies, the purity levels of Biopterin, BH2, R-THBL, S-BH4 and THP were not reported. Therefore, all release specifications will be reviewed periodically and revised to below the ICH qualification threshold limit of >0.15% where possible and if justified by the accumulated data. The applicant committed to perform the above as a follow-up measure.

*Excipients*
No excipients were identified in the proposed drug product formulation that could represent a safety risk to humans. All of them (mannitol, crospovidone, dibasic calcium phosphate, ascorbic acid, sodium stearylfumarate, riboflavin) comply with the EU pharmacopoeia and none represents a novelty in the pharmaceutical field or may pose any undue risk for the intended patient population.

**Ecotoxicity/environmental risk assessment**
A $PEC_{surface\ water}$ of 0.0022 and 0.0060 □g/L was calculated using a refined $F_{pen}$ of 0.00056% & 0.00157% and a $DOSE_{ai}$ value of 768 mg. Therefore the $PEC_{surface\ water}$ is below the 0.01 □g/L threshold concentration. Thus sapropterin is unlikely to pose a risk to the environment.

**Discussion on the non-clinical aspects**

Sapropterin dihydrochloride is a synthetic version of 6R-BH4, the naturally occurring cofactor of hydroxylases for phenylalanine, tyrosine and tryptophan. In patients with PKU, the role of sapropterin dihydrochloride is to enable endogenous PAH activity and to partially restore oxidative metabolism of phenylalanine, resulting in decreased blood phenylalanine levels in PKU patients. In patients with BH4 deficiency, sapropterin dihydrochloride is proposed to restore endogenous PAH activity by providing an exogenous source of the missing cofactor.
As there are no appropriate animal models for HPA due to PKU, non-clinical primary pharmacodynamic studies were conduced in normal rodents where HPA was induced by administering a BH4 biosynthesis inhibitor. Such studies demonstrated that 6R-BH4 supplementation by systemic route can reverse the effects of inhibitors of the BH4 biosysnthesis pathway.

Similarities exist in the pharmacokinetic profile of sapropterin dihydrochloride across the species studied (mouse, rat, rabbit and monkey) and with the limited clinical pharmacokinetic studies.
The absolute bioavailability was 7-12 % in 6 week old rats and ~9 % in cynomolgus monkeys but is unknown for humans. Although no formal metabolism studies were performed in the clinic, the rat and human catabolic pathways of tetrahydrobiopterin are thought to be very similar based on scientific literature. Repeated administration of sapropterin dihydrochloride did not up-regulate CYP-dependent drug-metabolising enzymes in the liver microsomes. The majority of orally administered sapropterin dihydrochloride is excreted in faeces due to limited absorption from the gastro-intestinal tract. The fraction absorbed is mostly excreted with the urine.

The kidney was identified as a target organ of toxicity in the rat in single-dose and repeat-dose studies (slight tubular basophilia) at doses resulting in very low safety margins compared to the human exposure. The Applicant has agreed to monitor potential reports on kidney findings in the post-approval commitments as well as in individual case safety reports in context of routine pharmacovigilance activities outlinedin the risk management plan.
Juvenile animal data suggest that sapropterin dihydrochloride may be potentially more toxic in early postnatal age, but this was most likely due to the higher absorption of sapropterin dihydrochloride in younger animals.
There was no developmental or embryofoetal toxicity in rats and rabbits.
Oral carcinogenicity studies conducted in mice and rats indicated no potential of sapropterin dihydrochloride to induce the formation of either neoplastic or hyperplastic lesions.
Sapropterin is not expected to pose a risk to the environment.

## 2.4 Clinical aspects

**Introduction**

**GCP**

The Clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.
The clinical pharmacology studies were designed to demonstrate both the pharmacodynamic effect of a single oral dose of sapropterin dihydrochloride on blood phenylalanine levels over 24 hours in PKU patients (PKU-001 Sub-study 01, PKU-004 Sub-study 01), and the PK of blood sapropterin dihydrochloride levels following the oral administration of sapropterin dihydrochloride at three different daily doses (PKU-004 Sub-study 02). The dose effect of sapropterin dihydrochloride at 5 mg/kg, 20 mg/kg, and then 10 mg/kg on the change in blood phenylalanine levels from baseline at the end of consecutive 2-week dosing periods was also assessed. All of the PKU clinical studies with sapropterin dihydrochloride immediate release tablets were conducted using a once-daily dose.

The main clinical trials with sapropterin dihydrochloride included two pivotal, randomised, placebo-controlled, Phase 3 studies: PKU-003 and PKU-006 (Part 2).

The overall development plan is depicted in the diagram below:

**Pharmacokinetics**
The pharmacokinetics (PK) of sapropterin dihydrochloride was evaluated in healthy subjects in four Phase I studies (three studies with sapropterin dihydrochloride granules, and one Phase I study with sapropterin dihydrochloride tablets).

The pharmacokinetics of sapropterin dihydrochloride have also been independently reported in two publications using a non-pharmaceutical grade preparation.

The initial Phase I pharmacokinetic study of the 2.5% and 10% granule formulations (study number P1501), was conducted by Suntory in 1985, according to the acceptable standards appropriate at that time. All other sponsored studies with granule formulations (FB1602, FB1701) were conducted in accordance with the Japanese good clinical practice regulations in effect at that time, and the more recent study with sapropterin dihydrochloride tablets (PKU-005) in accordance with the ICH E6 guideline on Good Clinical Practice (GCP).

A summary of the clinical pharmacokinetic studies is shown in the following 2 tables:

**Phase I Studies Evaluating Sapropterin Dihydrochloride Granules in Healthy Volunteers**

| Study No./ Period | Type of Study | Summary of Objectives | Sapropterin Dosage and Regimen | Study Subjects |
|---|---|---|---|---|
| P1501<br>Nov–Dec 1985 | Phase I<br>Open-label | Assess tolerability and pharmacokinetics of single and multiple doses | 2.5% *granules*<br>Single-dose:<br>100 mg and 200 mg p.o.<br>Multiple-dose: 100 mg p.o. TID x 7 days | Healthy adult male volunteers<br>sapropterin:<br>N=6 (single-dose)<br>N=6 (multiple dose) |
| FB1602<br>Aug–Sept 1996 | Phase I<br>Single-blind | Assess safety and pharmacokinetics of a 7-day multiple-dose regimen | 2.5% *granules*<br>200 mg p.o.<br>TID x 7 days | Healthy adult male volunteers<br>sapropterin: N=6<br>Placebo: N=2 |
| FB1701<br>March 1997 | Phase I<br>Open-label | Assess safety of a 7-day multiple-dose regimen with 10% granules<br>Confirm plasma biopterin concentrations on Day 1 of drug administration | 10% *granules*<br>200 mg p.o.<br>TID x 7 days | Healthy adult male volunteers<br>sapropterin: N=6 |

**Phase I Studies Evaluating Sapropterin Dihydrochloride Tablets in Healthy Volunteers**

| Study No./ Period | Type of Study | Summary of Objectives | Sapropterin Dosage and Regimen | Study Subjects |
|---|---|---|---|---|
| PKU-005<br>Apr–May 2005 | Phase I<br>Open-label | Relative bioavailability of sapropterin dihydrochloride administered p.o. in orange juice or water, and effect of high fat, high calorie meal on bioavailability<br>Safety and tolerability | Single 10 mg/kg p.o. dose in each of<br>– Water/fed<br>– Water/fasted<br>– Orange juice/fed<br>– Orange juice/fasted<br>Order of treatments varied in 4 groups, treatments separated by 7 days | 28 healthy adult male and female volunteers enrolled<br>27 evaluable |

- Absorption

The pharmacokinetics of sapropterin dihydrochloride in patients with PKU were examined in PKU-004 Sub-study 02.The results showed that sapropterin dihydrochloride is rapidly absorbed after a short initial time lag, with a bi-exponential decline following attainment of peak levels, with pharmacokinetics following a 2-compartment, first-order input model with first-order elimination.

Bioavailability of sapropterin is documented in an open-label, randomised, 4-treatment, 4-sequence, 4-period, crossover single dose study (PKU-005) in healthy volunteers in a fasted and fed state. Absorption under fasted conditions was equivalent when dissolved in either water or orange juice. After a high fat high calorie meal, absorption was increased to a greater degree after dissolution in water, compared to dissolution in orange juice. In the clinical trials, sapropterin was dissolved in water, apple or orange juice in PKU-003 or in PKU-006, in 120-240ml of water or apple juice (no orange juice). Therefore, it is mentioned in the SPC (section 4.2) that sapropterin should be administered "*with a meal as single daily dose, at the same time each day, preferably in the morning*."

The absolute bioavailability of sapropterin was not studied in humans but pre-clinical studies suggest that the absolute bioavailability in humans may be similar to the rat and the monkey, i.e. ranging from 7 to 10%.
The mean terminal half-life was 6.69 hours (range 3.91 to 16.6 hours).

- Distribution

Evidence of distribution is based on murine studies (See also non-clinical section). These studies demonstrate that intravenous BH4 distributes to tissues almost completely within minutes, with negligible urinary excretion of intact BH4.

- Metabolism

No special metabolic studies were performed.
Sapropterin dihydrochloride is a synthetic version of the naturally occurring cofactor, 6R-BH4, and the metabolic fate is assumed to be as for the naturally occurring cofactor.
Presumably, a large proportion of the BH4 serving as a cofactor for aromatic amino acid hydroxylases enters the BH4 regeneration pathway. Consistent with this assumption, following oral administration of BH4 in healthy individuals, <10% is recovered as pterin or lumazine metabolites in urine and faeces, implying substantial regeneration and/or tissue uptake and metabolism. From the bloodstream, exogenously administered BH4 must then transit the cell membrane to function as a co-factor for the intracellular aromatic amino acid hydroxylases.

- Excretion

Evidence of excretion is based only on murine studies which showed that the oxidised product, biopterin, did not accumulate in any tissue and was rapidly excreted.

- Dose proportionality and time dependencies

Assuming 4 half-lives for clearance, coverage is estimated to be 26.8 hours, which is considered to support once-daily dosing. Results also showed that there was little accumulation with daily doses even at the highest dose regimen.

- Special populations

No studies have been performed in patients with either renal or hepatic insufficiency. This is reflected in the SPC.

*Paediatric patients*

Based on the results of the animal studies, age-related variability in absorption could result in increased sensitivity to sapropterin in younger children which could result in a greater degree of dose-related reduction in blood phenylalanine levels. Therefore, it is recommended in the SPC that blood phenylalanine and tyrosine levels should be tested, particularly in children, one to two weeks after each dose adjustment and monitored frequently thereafter, under the direction of the treating physician.
Based on population PK analyses (PKU-004 Substudy 02) and published studies, the sapropterin dose can be adjusted in children of all ages within the range of 5 to 20 mg/kg per day to achieve blood Phe control.
Many of the patients in the studies were children but the use of, or effects of, sapropterin in those under the age of 4 years is not documented. Therefore, the use in children under the age of 4 is not recommended as reflected in the SPC. Further data will be collected in the post-marketing phase as a follow-up measure.

- Pharmacokinetic interaction studies

Drug interaction studies were not performed in part due to the rarity of HPA.
However, in pre-clinical repeat dose studies in rats, no induction of hepatic CYP450 enzymes was observed.
Folic acid and vitamin B12 may increase BH4 levels; although the mechanisms are not completely defined. Ascorbic acid (*e.g.* Vitamin C) has been shown to prevent the auto-oxidation of BH4 to BH2. Inhibitors of dihydrofolate reductase (DHFR) such as methotrexate, aminopterin or trimethoprim, may also inhibit the activity of DHPR and theoretically prevent salvage of BH4.

**Pharmacodynamics**

- Mechanism of action

Sapropterin is a synthetic version of the naturally occurring 6R-BH4, which is a cofactor of the hydroxylases for phenylalanine, tyrosine and tryptophan.

Sapropterin acts by increasing the overall level of PAH activity, partially restoring normal physiologic phenylalanine oxidative capacity, and reducing blood phenylalanine concentrations in PKU patients responsive to 6R-BH4. It has been proposed that 6R-BH4 may help stabilise PAH proteins, compensate for the decreased affinity of mutant PAH for 6R-BH4, act as a chaperone to facilitate folding and preservation of functional PAH Tetramers or stimulate an increase in catalytic activity by increasing the BH4 concentration.

- Primary and Secondary pharmacology

Sapropterin dihydrochloride has a plasma half-life of approximately 3-5 hours, however the pharmacodynamics activity over 24 hours, as assessed as blood phenylalanine decrease after once daily administration of sapropterin dihydrochloride has been observed.

Demonstration of a specific response to sapropterin was a requirement to enter the pivotal studies. Pharmacodynamic response to sapropterin dihydrochloride was examined over a 24-hour period in PKU-004 Sub-study 01, conducted between the 6 and 10 week visits of PKU-004, when all subjects were maintained on a daily sapropterin dihydrochloride dose of 10 mg/kg/day. Of the 12 subjects 8 had a blood phenylalanine level at the first sub-study time point that was ≥30% lower than their mean Week 0 value of 718.3±168.3 µmol/L. This degree of percent decrease in blood phenylalanine level was selected as a criterion for pharmacodynamic response. For these 8 subjects, the blood phenylalanine level at the first sub-study time point was 446.6±108.8 µmol/L, declining to 378.8±139.8 µmol/L at 4 pm and returning to 438.8±133.1 µmol/L at 8 am, 24 hours after the previous dose of sapropterin dihydrochloride. These data suggest a stable reduction of blood phenylalanine levels over a 24 hour dose interval, and provide support for once-daily dosing of sapropterin dihydrochloride in patients with HPA due to PKU.

*Pharmacodynamic interactions*

BH4 acts to enhance nitric oxide synthetase (NOS) activity and could have a synergistic effect with pharmacodynamicsE5 inhibitors, such as sildenafil, on vasorelaxation. Animal studies do not, however, indicate interaction between sildenafil and sapropterin.

Synergism may also theoretically occur with antihypertensive agents, such as minoxidil, that act as a nitric oxide agonist as part of their mechanism of action. Caution about these possible interactions is mentioned in the SPC (section 4.5).

**Clinical efficacy**

The clinical development program for sapropterin dihydrochloride included two pivotal, randomised, placebo-controlled, Phase 3 studies: PKU-003 and PKU-006 (Part 2). For these trials, reduction in blood phenylalanine levels as compared to placebo control was used as a clinical surrogate efficacy endpoint for prevention of HPA-related neurotoxicity, and this was accepted in the CHMP SAWG protocol assistance for the clinical development program (EMEA/174572/2205).

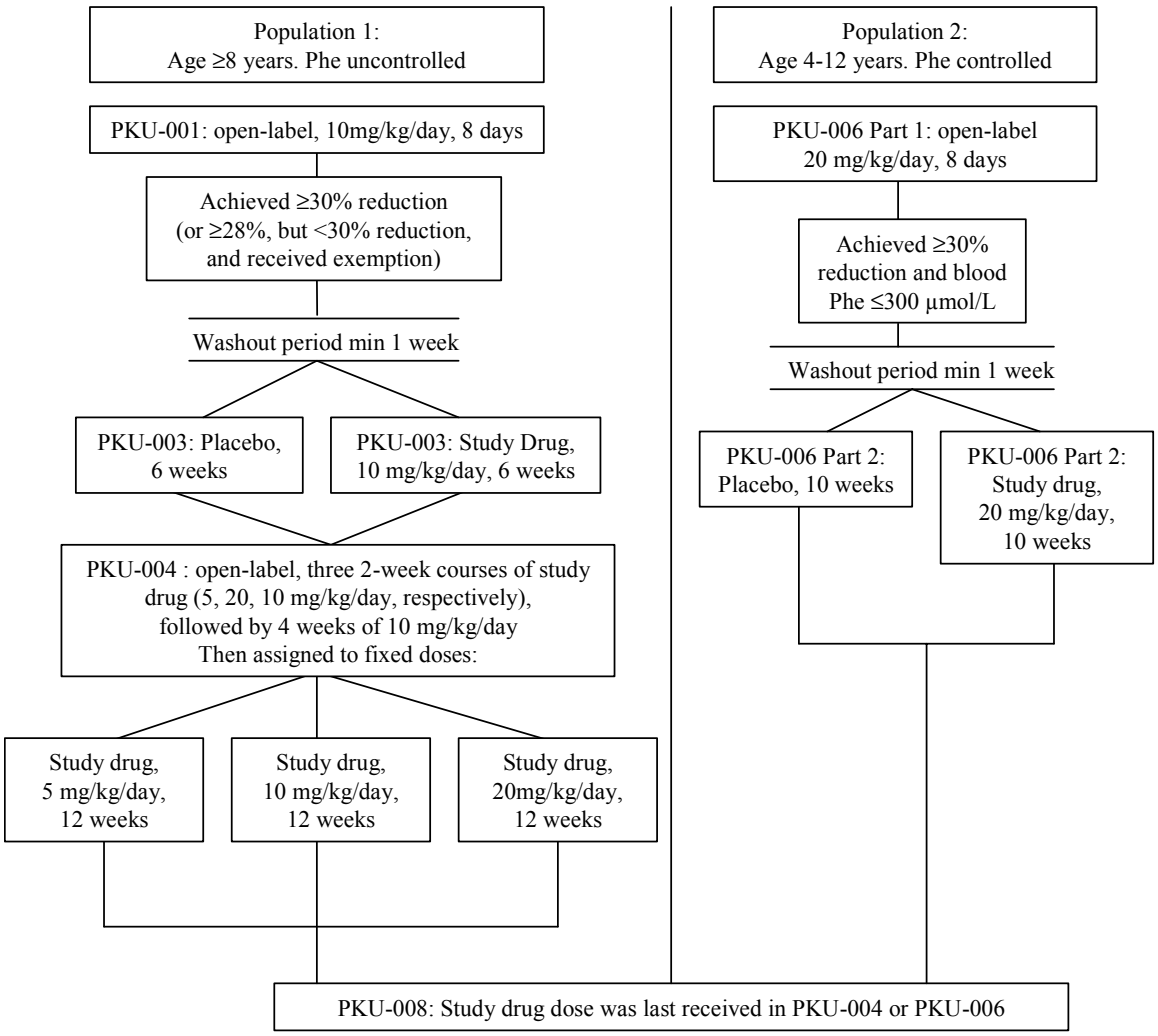
Both pivotal studies were preceded by short-term - PKU-001 and PKU-006 (Part 1) - open-label treatment response studies to identify responders to sapropterin dihydrochloride treatment, thereby reducing the likelihood of not detecting a true response due to the rarity of the condition.

In addition, PKU-004 was a long term open label study looking at safety and tolerability of 3 doses of 5, 10 or 20 mg/kg/day of sapropterin.

## Summary table of Clinical trials

| | | | | | No. of Subjects Exposed: | |
|---|---|---|---|---|---|---|
| **Study** | **Title** | **Study Population** | **Study Design** | **Sapropterin dihydrochloride tablets –Dose and Regimen** | **Sapropterin dihydrochloride Placebo** | **Placebo** |
| **Placebo-Controlled Clinical Studies in PKU** | | | | | | |
| PKU-003 | A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Phenoptin™ (sapropterin dihydrochloride) 1 in Subjects with Phenylketonuria | PKU | Phase 3Double-blind | 10 mg/kg/day PO x 6 weeks | 41 | 47 |
| PKU-006 Part 2 | A Phase 3, Multicenter, Randomized, Double-blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Phenoptin™ (sapropterin dihydrochloride) 1 20 mg/kg/day to Increase Phenylalanine Tolerance in Phenylketonuric Children on a Phenylalanine-restricted Diet | PKU | Phase 3 Double-blind | 20 mg/kg/day PO x 10 weeks | 33 | 12 |
| **Open-Label Clinical Studies in PKU** | | | | | | |
| PKU-001 | A Phase 2, Multicenter, Open-Label Study to Evaluate the Response to and Safety of an 8-Day Course of Phenoptin™ (sapropterin dihydrochloride)1 Treatment in Subjects with Phenylketonuria Who Have Elevated Phenylalanine Levels | PKU | Phase 2 Openlabel | 10 mg/kg/day PO x 8 days | 489 | NA |
| PKU-004 | A Phase 3, Multicenter, Open-Label Extension Study of Phenoptin™ (sapropterin dihydrochloride) 1 in Subjects with Phenylketonuria Who Have Elevated Phenylalanine Levels | PKU | Phase 3 Openlabel | 5-20 mg/kg/day PO x 22 weeks | 80 | NA |
| PKU-006 Part 1 | A Phase 3, Multicenter, Randomized, Double-blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Phenoptin™ (sapropterin dihydrochloride)1 20 mg/kg/day to Increase Phenylalanine Tolerance in Phenylketonuric Children on a Phenylalanine-restricted Diet | PKU | Phase 3 Openlabel | 20 mg/kg/day PO x8 days | 90 | NA |

A diagram of these clinical trials is depicted below:




- Dose response studies

*Methods*
Two short-term open-label treatment response studies (**PKU-001** and **PKU-006 -Part 1**) were performed to identify responders to sapropterin dihydrochloride treatment. In addition, **PKU-004** was a long term open label study looking at safety and tolerability of 3 doses of 5, 10 or 20 mg/kg/day of sapropterin.

The 10mg/kg/day dose in PKU-001 was selected on the basis that publications had documented the fact that doses of between 7.5 and 20mg/kg had been administered as a loading doses to more than 1900 subjects; 10 published studies evaluating a total of 28 subjects with PKU had shown that 10mg/kg/day was the most frequently used 6R-BH4 dosage that produced sustained reduction in blood phenylalanine levels.
The 20mg/kg/day dose in PKU-006 was selected on the basis that publications had documented the fact that doses of between 7.5 and 20mg/kg had been administered as a loading doses to more than 1900 subjects; studies have provided evidence for adequate long term safety of the dose.

**PKU-004** was a long term open label study looking at safety and tolerability of three two week courses of 5, 20 and then 10 mg/kg/day followed by 4 weeks of 10 mg/kg/day and then assigning of patients (on the basis of week 2 and week 6 phenylalanine levels and using a protocol algorithm) to fixed doses of 5, 10 or 20 mg/kg/day of sapropterin from week 10 to week 22.
Primary endpoints were to assess safety and tolerability but secondary endpoints included assessment of safety and tolerability of 3 different doses
Of the eighty (80) subjects enrolled, 39 subjects had received sapropterin dihydrochloride tablets and 41 subjects had received placebo in PKU-003. 18% of patients had no treatment gap (PKU-003) and

82% had treatment gaps of 63.2 days for the active in PKU-003 and 70 days for the placebo in PKU-003. Subjects from the placebo arm had higher week 0 blood phenylalanine levels than patients treated with active but at the end of the 5mg/kg/day dosing period, mean blood levels were similar.
All enrolled subjects received at least one dose of sapropterin dihydrochloride and completed the study through the Week 10 visit, and 79 subjects completed the study through the Week 22 visit. One subject was withdrawn at Week 16 due to non-compliance with study procedures. All subjects who enrolled in the study were included in the efficacy analyses.

*Results*

**PKU-001**

Of the 485 subjects in PKU-001 who received at least one dose of study drug and who had blood phenylalanine level measurements at both Day 1 and Day 8, 96 (19.8%) were responders, as defined for the enrichment design of the program (*i.e.* ≥30% reduction in blood Phenylalanine level from Day 1 to Day 8). The mean ± SD change and mean ± SD percent change in blood phenylalanine levels from Day 1 to Day 8 for responders were −391.8 ± 185.3 µmol/L and −50.0 ± 16.0%, respectively. When changes in blood phenylalanine levels from Day 1 to Day 8 for protocol-defined responders were examined by baseline blood phenylalanine level (<600 µmol/L, n=31; ≥600 µmol/L; n=65), the mean (± SD) changes were −286.3 ± 84.0 µmol/L for subjects with baseline levels <600 µmol/L and −442.0 ± 199.2 µmol/L for subject with baseline levels ≥600 µmol/L, while the mean ± SD percent changes were −55.3 ± 16.7% and −47.5 ± 15.2%, respectively.

**PKU-006** Part 1

In this study, 90 subjects were enrolled and received at least one dose of sapropterin dihydrochloride. With the identification of 50 subjects who met eligibility criteria for PKU-006 Part 2, 46 were randomised 3:1 to sapropterin dihydrochloride or placebo treatment, and 45 received at least one dose of study drug (33 subjects received sapropterin dihydrochloride tablets, 12 subjects received placebo). Forty one subjects (32 sapropterin dihydrochloride, 9 placebo) completed Part 2 of the study by attending the Week 10 visit.

Data for 89 of the 90 subjects (99%) enrolled in Part 1 of the study were used in the efficacy analyses. One subject was excluded from the analyses due to lack of a Day 8 blood phenylalanine measurement.

**PKU-004**

The comparison of 3 Daily Dose Levels (5, 10 and 20 mg/kg/day) on Reduction of Blood Phenylalanine was performed during the forced dose titration period in PKU-004 in support of the intended dose range.
The Persistence of Effect of Sapropterin Dihydrochloride to reduce Blood Phenylalanine Levels was performed over the 22-week treatment period in PKU-004. A supportive analysis, compared to placebo, was performed for PKU-003.

*Primary Efficacy Results*

In the final fixed dose part of the study, of the 80 subjects, 6 (8%) received 5 mg/kg/day of sapropterin dihydrochloride, 37 (46%) received 10 mg/kg/day, and 37 (46%) received 20 mg/kg/day during the fixed dose period of PKU-004. The mean ± SD blood phenylalanine levels at the Weeks 12 - 22 visits ranged between 619.8 ± 371.0 and 652.2 ± 382.5 µmol/L. On average, subjects maintained a stable reduction in phenylalanine levels.

Subjects who received a fixed sapropterin dihydrochloride dose of 10 or 20 mg/kg/day had comparable mean blood phenylalanine levels at Weeks 12, 16, 20, and 22 as they had previously had on the same dose in the forced dose-titration period. Although subjects who received the fixed 5 mg/kg/day dose of sapropterin dihydrochloride had the lowest phenylalanine levels they manifested greater variability (possibly related to the small numbers of patients in this group) and did not have as low a mean blood phenylalanine level as observed for the 5 mg/kg/day dose during the forced-dose titration period.

*Secondary Efficacy Results*
During the forced-dose titration period (Week 0 visit until Week 6 visit), the mean blood phenylalanine level observed at the end of each 2 week dosing period was inversely related to the dose of sapropterin dihydrochloride. At baseline (Week 0), the mean ± SD blood Phenylalanine level was 844.0 ± 398.0 µmol/L. After sapropterin dihydrochloride dosing at 5 mg/kg/day (Week 2), 10 mg/kg/day (Week 6), and 20 mg/kg/day (Week 4), mean ± SD blood Phenylalanine levels were 743.9 ± 384.4 µmol/L, 639.9 ± 381.8 µmol/L, and 580.8 ± 398.8 µmol/L, respectively. For each pair-wise comparison of the dose levels, the mean change in blood phenylalanine levels differed significantly ($p<0.01$).
From the Week 6 visit to the Week 10 visit, during which all subjects received 10 mg/kg/day of sapropterin dihydrochloride, subjects maintained blood phenylalanine levels similar to those that occurred on the same dose during the dose titration period (Weeks 4 to 6), with a mean ± SD blood phenylalanine level at Week 10 of 645.2 ± 393.4 µmol/L.

Overall, the changes in phenylalanine levels were directly related to the dose of phenylalanine and the differences in effect were statistically significant when compared between dose levels. For the 5mg dose 25%, for the 20mg 55% and for the 10mg dose 46% of patients achieved more than 30% reduction in phenylalanine levels at the weeks 2, 4, 6 and 10 visits. The difference between the 5 and 10mg/kg/day was statistically significant at $p<0.0001$ and between 10 and 20mg/kg/day at $p=0.0085$. Twice as many subjects achieved more than 30% reduction in blood levels after receiving 10mg compared to 5mg/kg/day.
The results suggest that most patients with phenylalanine will achieve blood phenylalanine reductions with doses of between 5 and 10mg/kg/day.

Stable reduction of blood phenylalanine levels following a daily sapropterin dihydrochloride, 10 mg/kg/day was demonstrated in PKU-004 Sub-study 01. Daily dosing of sapropterin dihydrochloride in patients with HPA due to PKU is further supported by results of the population PK analysis in PKU-004 Sub-study 02. The stability of blood phenylalanine reduction observed over the 24 hour dose interval period in PKU-004 Sub-study 01 contrasts with the wide fluctuation in blood phenylalanine levels that occur during the day in patients with PKU who are treated only with dietary phenylalanine restriction.

*Overall*, the results of PKU-004 study confirmed that the changes in Phe blood levels were directly related to the dose of Sapropterin in subjects with PKU. The difference in effects was statistically significant.
In mPKU, information from the literature using a non-pharmaceutical preparation indicate that normalization of blood Phe levels may occur at BH4 doses less than 10 mg/kg/day.

Persistent reduction in mean blood Phenylalanine levels and acceptable safety were demonstrated at this dose level for 37 subjects with HPA due to PKU.

- Main studies

The clinical development program for sapropterin dihydrochloride included two pivotal, randomised, placebo-controlled, Phase 3 studies: **PKU-003 and PKU-006 (Part 2).**

METHODS

*Study Participants*

For **PKU-003**, responders were identified in PKU-001, using a ≥30% reduction in blood Phenylalanine from baseline after an 8-day course of sapropterin dihydrochloride 10 mg/kg/day. Responders were then eligible for randomisation in PKU-003. Subjects completing PKU-003 were then eligible for continuation into PKU-004, a Phase 3 open-label, 22-week extension study that included an initial forced-dose titration study.

Subjects had PKU, were at least 8 years of age with elevated blood phenylalanine levels at screening, were not following a strict diet and had responded to sapropterin in PKU-001. Age 8 was selected as a 6 week period on placebo without dietary control could result in elevated phenylalanine levels and a risk of neurological sequelae.

Sampling for genotype analysis was performed for subjects participating in PKU-001 and genetic diversity was demonstrated in PKU-001 and PKU-003, as requested, by consideration of the types of mutation and comparison to an international PAH mutation database.

For **PKU-006**, responders were identified in Part 1, which involved an 8-day course of sapropterin dihydrochloride 20 mg/kg/day, using the criteria of a ≥30% reduction in blood phenylalanine and blood phenylalanine ≤300 µmol/L at day 8. Responders were then eligible for randomisation into PKU-006, Part 2, with a primary efficacy endpoint of increase in phenylalanine tolerance during treatment with sapropterin dihydrochloride 20 mg/kg/day, and a secondary efficacy endpoint of reduction in blood phenylalanine in the 3 weeks prior to adjustment of phenylalanine intake.

Subjects had PKU with a screening blood phenylalanine level less than or equal to 480µmol/L, were between the ages of 4 and 12 years, were responsive to sapropterin (as documented in part 1 of the study involving 8 days sapropterin 20mg/kg/day and day 8 phenylalanine less than or equal to 300 µmol/L) and were under dietary control with a phenylalanine restricted diet as shown by an estimated phenylalanine tolerance< 1000mg/day and at least 6 months blood phenylalanine control prior to screening (phenylalanine level less than or equal to 480µmol/L).

*Treatments*

Based on the CHMP scientific advice, the final clinical development program included investigations of a 10 mg/kg/day dose in PKU-003, 20 mg/kg/day dose in PKU-006 and doses of 5, 10, and 20 mg/kg/day in PKU-004. Thus, the dose ranges used for the Phase 3 program support the range of weight-based doses proposed for the product.

In **PKU-003**, during the 6 week double blind treatment period, subjects received once daily doses of 100mg of sapropterin dihydrochloride or corresponding placebo dissolved in 120-240ml of water apple or orange juice. Doses were to be taken in the morning as the number of tablets equivalent to 10/mg/kg body weight.

In **PKU-006**, patients received 20mg/kg/day sapropterin phenylalaninenoptin or placebo dissolved in 120-240ml of water or apple juice (no orange juice). Subjects were instructed to maintain a stable phenylalanine diet and were to keep a 3 day diet record and a record of all foods and beverages. At weeks 3, 5, 7 and 9, subjects phenylalanine intake was increased or decreased based on blood phenylalanine at weeks 2, 4, 6, and 8 respectively.

The 6-week treatment period for PKU-003 was not extended to 3 months because of ethical concerns regarding maintenance of placebo treatment for an extended period, particularly since this study involved children. Instead, additional safety data were collected during the 22-week treatment period of the Phase 3, open-label, extension study, PKU-004, and also are being collected in PKU-008, an ongoing Phase 3b safety trial in which sapropterin dihydrochloride tablets are provided in acknowledgement of the absence of alternative drug product. PKU-004 was further re-designed to include exposure across the intended treatment dose range of 5 to 20 mg/kg/day, and included an initial forced-dose titration study. Therefore, it was not feasible to also include a randomised withdrawal design within this protocol.

*Objectives*

The final design of PKU-006 was intended to demonstrate the potential benefit of sapropterin treatment to increase dietary Phenylalanine tolerance and thereby allow a less restrictive and more palatable diet for patients with PKU, as agreed by the CHMP scientific advice.

In **PKU-003**
The primary objective was to evaluate the efficacy of sapropterin in reducing phenylalanine levels in subjects with PKU after 6 weeks of treatment.
Secondary objectives were to compare sapropterin to placebo with respect to weekly changes in phenylalanine levels during 6 weeks of treatment and the proportion of subjects who had levels less than 600umol/L at week 6.
In **PKU-006**
The primary objective was to evaluate the efficacy of sapropterin to increase phenylalanine tolerance in children with PKU who are following a phenylalanine restricted diet.
Secondary objectives were to evaluate the ability of sapropterin to reduce phenylalanine levels, in the 3 weeks prior to adjustment of phenylalanine intake. to compare sapropterin.

*Outcomes/endpoints*

**Reduction in Blood Phenylalanine Levels**
Reduction in blood phenylalanine compared to placebo was accepted as the surrogate clinical efficacy endpoint for the pivotal trials within the clinical development program (EMEA/174572/2005) and was incorporated in the design of both studies PKU-003 and PKU-006 Part 2. Reduction in blood phenylalanine levels was also an efficacy endpoint for each of the 3 open label trials, PKU-001, PKU-004, and PKU-006 Part 1.
The use of reduction in blood phenylalanine as a surrogate clinical efficacy endpoint was based on studies, including a meta-analysis, showing correlations between blood phenylalanine levels and neurological outcomes in patients with HPA due to PKU. Furthermore, blood phenylalanine levels are universally used in the diagnosis and clinical management of patients with HPA due to PKU and BH4 deficiency.
The normal level of blood phenylalanine in individuals without PKU is approximately 60 µmol/L (1 mg/dL) and varies inversely with age. Reference ranges listed by Mayo Medical Laboratories, a primary reference laboratory for the clinical program, as determined by ion exchange chromatography, are listed below.

**Blood Phenylalanine Reference Range by Mayo Medical Laboratories***

| | Age | | | | |
|---|---|---|---|---|---|
| | **Premature** | **0 to 1 month** | **1 to 24 months** | **2 to 18 years** | **Adult** |
| Blood Phenylalanine (µmol/L) | 98-213 | 38-137 | 31-75 | 26-91 | 35-85 |

*Phenylalanine and Tyrosine, Plasma 2007

In individuals with PKU, blood phenylalanine levels may be 20-fold or more above the normal range with normal dietary phenylalanine intake, although the phenotype varies considerably, with some affected individuals having levels that are only modestly elevated.
Current management guidelines for HPA due to PKU do not aim at normalisation of blood phenylalanine levels. Instead, the goal is reduction of blood phenylalanine levels into selected therapeutic ranges. In HPA due to BH4 deficiency, normalisation of blood phenylalanine levels is often observed with BH4 therapy.

**Increase in Dietary Phenylalanine Tolerance**
The second primary efficacy objective for the clinical development program was to demonstrate the ability of sapropterin dihydrochloride tablets to increase the dietary phenylalanine tolerance in subjects with HPA due to PKU, controlled on a phenylalanine-restricted diet. This endpoint was evaluated in PKU-006 Part 2.
Current management of HPA is based on restriction of dietary protein to reduce phenylalanine intake to levels that will allow maintenance of target phenylalanine levels and the level of dietary phenylalanine that allows achievement and maintenance of target phenylalanine levels varies between patients and is referred to as the dietary phenylalanine tolerance. In addition, current management includes ingestion of phenylalanine-free protein supplements to maintain daily protein requirements. Patients with HPA due to PKU may tolerate only a small fraction of the usual daily intake of protein and must meet >80% of daily protein requirements using phenylalanine-free protein supplements. Maintenance of this degree of protein restriction is impractical in daily life, also considering that the

phenylalanine-free protein supplement is known to be unpalatable. Poor compliance with diet management is a well-known occurrence in children and adults with HPA due to PKU and may begin in infancy.

In addition to the two primary efficacy objectives, three additional efficacy objectives were considered within the clinical development program:

**Reduction in Blood Phenylalanine to <600 µmol/L**
Demonstrating the ability of sapropterin dihydrochloride tablets to decrease the blood phenylalanine level to <600 µmol/L at the end of study in patients with a blood henylalanine level ≥600 µmol/L prior to treatment was an additional efficacy objective evaluated in PKU-003. A secondary analysis for this efficacy objective was also performed for the open-label, short-term study, PKU-001. The boundary blood phenylalanine level of 600 µmol/L was selected as being consistent with commonly used therapeutic guidelines for control of blood phenylalanine in individuals with HPA due to PKU. Internationally agreed consensus guidelines for target blood phenylalanine levels for treatment of HPA are not available. Infants with HPA are typically identified in national newborn screening programs. For Germany, the level of HPA required for initiation of treatment of infants involves persistent levels of blood phenylalanine >600 µmol/L on normal intake. The UK consensus recommendation is to treat infants with confirmed blood phenylalanine >400 to 600 µmol/L, as measured for several days on normal protein intake.
Treatment guidelines for HPA due to PKU after the newborn period are displayed below for the 4 national guidelines (UK, US, Germany, France) and representative published guidelines for other countries.

**Therapeutic Targets for Blood Phenylalanine Levels (µmol/L)**

| Country/Year (reference) | Age Category | | |
|---|---|---|---|
| | **Infant** | **Child** | **Adult** |
| **National Guidelines** | | | |
| UK/1993 | 120-360 | 120-480 | 120-700 |
| Germany/1997 | 0-10 years old: 40-240<br>10-15 years old: ≤900 | >15 years old: ≤1'500 | |
| USA/2000<br>(NIH 2001[i]) | 0-12 years old : 120-360 | >12 years old : 120-900<br>(ideal is 120-600) | |
| France/2005<br>(Abadie 2005[ii]) | 0-10 years old: 120-300<br>10 years old-adult: <900 | <1200-1'500 | |
| **Other Published Guidelines** (Schweitzer-Krantz and Burgard 2000[iii]) | | | |
| Ireland/1987 | No age specified: 200-400 | | |
| Denmark/1995 | 0-8 years old: 180-400<br>>8-10 years old: <600<br>>10-12 years old: <700<br>>12-18 years old: <900 | | <1'500 |
| East Europe/1998 | 0-6 years old: <360<br>>6-10 years old: <480<br>>10-15 years old: <600 | | <900 |

Upon review of the various national guidelines and consultation with experts, achievement of a blood level of <600 µmol/L in subjects with a pre-treatment blood phenylalanine level of ≥600 µmol/L was chosen as representative of a typical clinical therapeutic goal.

*Sample size*

In **PKU-003**, sample size was calculated for the primary efficacy criterion and was based on a pilot study. The sample size calculation assumed a mean difference between placebo and active of 159umol/L and a 2-sided type 1 error rate of 0.05. Under these conditions, a sample size of 80 randomised subjects (40 in each group) would provide 95% power to detect a difference in mean blood phenylalanine level between placebo and active.

In **PKU-006**, assuming a mean phenylalanine supplement of 0mg/kg/day and a mean phenylalanine supplement of 17.5mg/kg/day at week 10 in the sapropterin group, a standard deviation of 16mg/kg/day and a two sided type 1 error rate equal to 0.05, then a sample size of 30 subjects receiving sapropterin gave 99% power to detect the specified increase in total daily phenylalanine supplement tolerated.

*Randomisation*
In **PKU-003**, subjects were randomised in a 1:1 ratio to receive either 10mg/kg/day to sapropterin dihydrochloride or placebo. Randomisation was stratified by study site and screening visit blood phenylalanine level. 96 subjects were considered as responders in PKU-001 and so, eligible for PKU-003. Nineteen (19) enrolled on PKU-003 under protocol amendment 1 and 70 were screened under protocol amendment 2. I subject withdrew from the study prior to receiving any study drug because he was unable to comply with the study schedule. One subject randomised to placebo withdrew at week 4 because of non compliance with dosing.

With the identification of 50 subjects who met eligibility criteria for **PKU-006 Part 2**, 46 were randomised 3:1 to sapropterin dihydrochloride or placebo treatment, and 45 received at least one dose of study drug (33 subjects received sapropterin dihydrochloride tablets, 12 subjects received placebo). Forty one subjects (32 sapropterin dihydrochloride, 9 placebo) completed Part 2 of the study by attending the Week 10 visit. Randomisation to treatment group was stratified by the average blood phenylalanine level in the 6 months prior to screening in part 1.

*Blinding (masking)*
There were no specific issues with blinding. Placebo and active were similar.

*Statistical methods*
In **PKU-003**, descriptive statistics were used to summarise data in the CRFs. Continuous variables were summarised by the number of subjects, mean, median, standard deviations, minimum and maximum values and where appropriate, other percentiles. Primary and secondary endpoints were tested at a 2-sided type 1 error rate of 0.05. Unless stated to be otherwise, all p-values were 2-sided.

In **PKU-006**, categorical variables using frequencies and percentages and continuous variables using means and standard deviations, as well as means, ranges and appropriate percentiles were used.

Before locking CRF databases, a detailed statistical analysis plan was developed. The primary endpoint was the amount of phenylalanine tolerated after 10 weeks treatment while maintaining adequate phenylalanine levels. The mean supplement tolerated was compared to zero using a one sample t test. The supplement was calculated using changes in the amount of supplement prescribed and was defined as the cumulative increase or decrease in phenylalanine supplement prescribed while the subject blood phenylalanine was <360µmol/L.

RESULTS

*Participant flow*
In **PKU-003**, a total of 89 subjects from 27 study centres, were randomised and 88 subjects received at least one dose of either sapropterin dihydrochloride tablets (41 subjects) or placebo tablets (47 subjects).
Nineteen (19) subjects had blood phenylalanine level ≥600 µmol/L and the remaining 70 subjects were entered after Protocol Amendment 2 and had a screening blood phenylalanine level ≥450 µmol/L. The majority of the 88 treated subjects had a screening blood phenylalanine level ≥600 µmol/L. This level was chosen because tests of executive and higher cognitive function had shown consistent improvement when blood phenylalanine levels were less than 600.
6 subjects were non responders (28%) reduction and 3 subjects had historically high blood levels,

Nine(9) subjects in the placebo group and 7 subjects in the sapropterin dihydrochloride group were entered under Protocol Amendment 2 and had a screening blood Phenylalanine level ≥450 µmol/L, but <600 µmol/L. Forty seven (47) patients were randomised to placebo and 42 to active.

1 subject was withdrawn because of non compliance and 87 patients completed the 6 week study

In **PKU-006**, 90 subjects were enrolled in part 1 at 15 sites. Fifty subjects were classified as responders and 39 as non responders (one subject withdrew before day 8 for non compliance). Forty six (46) subjects were randomised at 11 sites. Percentages of subjects with mean blood phenylalanine<300µmol/L in the 6 months prior to part 1 screening were 42% and 50% in the placebo and sapropterin groups respectively while the percentage with mean blood phenylalanine≥300 µmol/L in the 6 months prior to Part 1 screening was 58%in the placebo and 50% in the sapropterin groups

*Recruitment*

Study **PKU-003**
Patients entering this study were responders in PKU-001 Subjects were enrolled at 15 sites and 2 satellite sites in North America and 12 sites in Europe.

Study **PKU-006**
Patients entering this study were responders in part 1 of the study in which 90 subjects between 4 and 12 years received sapropterin 20mg/kg/day for 8 days and to be a responder was required to have a reduction in blood phenylalanine of greater than or equal to 30%, and who had blood levels of less than 300µmol/L at day 8

*Baseline data*
In **PKU-003**, baseline blood phenylalanine levels for the sapropterin dihydrochloride group and the placebo group were similar, 842.7 ± 299.6 and 888.3 ± 323.1 µmol/L, respectively.

In **PKU-006**, percentages of subjects with mean blood phenylalanine<300µmol/L in the 6 months prior to part 1 screening were 42% and 50% in the placebo and sapropterin groups respectively while the percentage with mean blood phenylalanine≥300 µmol/L in the 6 months prior to Part 1 screening was 58%in the placebo and 50% in the sapropterin groups

*Numbers analysed*
In **PKU-003**, a total of 89 responders out of 489 patients were randomised and 88 subjects received at least one dose of either sapropterin dihydrochloride tablets (41 subjects) or placebo tablets (47 subjects). The majority of the 88 treated subjects had a screening blood phenylalanine level ≥600 µmol/L. Nine subjects in the placebo group and 7 subjects in the sapropterin dihydrochloride group were entered under Protocol Amendment 2 and had a screening blood phenylalanine level ≥450 µmol/L, but <600 µmol/L. The 88 subjects who received at least one dose of study drug were included in the efficacy analyses.

For **PKU-006** Part 2, all 45 subjects who received at least one dose of study drug were included in the efficacy analyses.

*Outcomes and estimation*

There was high inter-subject variability in the response to sapropterin. In **PKU-003**, over the 6-week study, there was a significant ($p<0.001$) mean decrease in blood phenylalanine levels for the sapropterin dihydrochloride treatment group compared to the placebo group, with a mean ± SE between-group difference of 245 ± 52.5 µmol/L. The mean ± SD baseline blood phenylalanine levels for the sapropterin dihydrochloride group and the placebo group were similar, 842.7 ± 299.6 and 888.3 ± 323.1 µmol/L, respectively. The mean ± SD change in blood phenylalanine levels from baseline to Week 6 was −235.9 ± 257.0 µmol/L for the sapropterin dihydrochloride group and 2.9 ± 239.5 µmol/L for the placebo group.
It was noted that reductions in phenylalanine were seen across the spectrum of baseline levels and a baseline blood level cannot be used as a predictor of response to sapropterin.

In **PKU-006** Part 2, the phenylalanine supplement at the Week 0 visit of PKU-006 Part 2 was 0 mg/kg/day; phenylalanine supplement was not started until the Week 3 visit. The amount of phenylalanine supplement tolerated (*i.e.* phenylalanine supplement prescribed while maintaining adequate blood phenylalanine control) was defined as the cumulative increase/decrease in phenylalanine supplement prescribed at the visit prior to the last visit when the subject's blood phenylalanine level was <360 µmol/L.
Over the 10 week study period, the mean ± SD phenylalanine supplement tolerated by subjects in the sapropterin dihydrochloride group, 20.9 ± 15.4 mg/kg/day, was significantly different from zero ($p<0.001$).
Of the 33 subjects who received sapropterin dihydrochloride, 11 (33.3%) tolerated a phenylalanine supplement of 31 to 50 mg/kg/day (50 mg/kg/day was the maximum amount of Phenylalanine supplementation allowed by the protocol), 10 (30.3%) tolerated 11 to 30 mg/kg/day, and 7 (21%) tolerated 1 to 10 mg/kg/day; 5 subjects (15%) were unable to tolerate any phenylalanine supplementation. Of the 12 subjects in the placebo group, none were able to tolerate phenylalanine supplementation above 10 mg/kg/day.

**Secondary Efficacy Results**
In **PKU-003**, the mean ± SD blood phenylalanine levels for the sapropterin dihydrochloride group decreased from 842.7 ± 299.6 µmol/L at baseline to 619.9 ± 354.7 µmol/L at Week 1 and remained below this level for the duration of treatment. For the placebo group, blood phenylalanine levels fluctuated only slightly from the mean ± SD baseline level of 888.3 ± 323.1 µmol/L, reaching a Week 1 mean ± SD level of 862.2 ± 345.6 µmol/L and remaining above this level for the duration of the study. The mean ± SD change in blood phenylalanine levels from baseline to Week 1 was − 222.9 ± 192.4 µmol/L for the sapropterin dihydrochloride group and − 25.7 ± 232.3 µmol/L for the placebo group.
The effect of sapropterin dihydrochloride relative to placebo was sustained and unchanged throughout the treatment period. The estimated difference in mean ± SE blood phenylalanine level between the two treatment groups (sapropterin dihydrochloride − placebo) was −230 ± 43.4 µmol/L at Week 6. Fifty four percent (54%) of subjects in the sapropterin dihydrochloride group and 23% of subjects in the placebo group had Week 6 blood phenylalanine levels <600 µmol/L ($p=0.004$). In the subgroup of subjects whose baseline blood Phenylalanine levels had been ≥600 µmol/L, 42% of those in the sapropterin dihydrochloride group and 13% of those in the placebo group had Week 6 blood phenylalanine levels <600 µmol/L ($p=0.012$).

A post hoc analysis of the proportion of subjects who had blood phenylalanine levels <360 µmol/L at Week 6 (end-of-study) was performed. No subject had a screening blood phenylalanine level <360 µmol/L. At Week 6, 13 of 41 subjects (32%) in the sapropterin dihydrochloride group and 1 of 47 subjects (2%) in the placebo group had blood phenylalanine levels <360 µmol/L ($p<0.001$). For subjects with screening blood phenylalanine levels ≥600 µmol/L, 26% of subjects in the sapropterin dihydrochloride group and 3% of subjects in the placebo group had Week 6 blood phenylalanine levels <360 µmol/L.

In **PKU-006** Part 2, for subjects treated with sapropterin dihydrochloride, the mean ± SD change in blood phenylalanine from the Week 0 visit to the Week 3 visit was 148.5 ± 134.2 µmol/L, which was a significant decrease from the Week 0 visit ($p<0.001$).
Another secondary efficacy endpoint, comparison between treatment groups of the amount of phenylalanine supplement tolerated, was analysed using a two way analysis of variance (ANOVA) model with effects for blood phenylalanine level stratum and treatment group. The adjusted mean ± SE phenylalanine supplement tolerated was 21.0 ± 2.3 mg/kg/day for subjects in the sapropterin dihydrochloride group and 3.3 ± 3.9 mg/kg/day for subjects in the placebo group. The difference between the two treatment groups was statistically significant ($p<0.001$).

*Ancillary analyses*

**Genotype Analysis**

Subjects enrolled in **PKU-001** were invited to participate in a study involving analysis of PAH genotype. Of the 485 subjects who completed PKU-001, 400/485 (82.5%) had full PAH genotype analysis. An additional 85 subjects had indeterminate PAH genotypes, probably due to technical limitations rather than misdiagnosis. Among the 400 fully genotyped subjects, 57/400 (14.3%) were homozygous for a single PAH mutation and 343/400 (85.7%) were compound heterozygotes. A total of 118 different PAH gene mutations were identified. A total of 72/88 (82%) of subjects in **PKU-003** had full PAH genotype analysis. The PAH genotype patterns were similar between the placebo and sapropterin dihydrochloride treatment groups but detailed analysis was not possible. due to the large number of observed mutations and the fact that the majority of subjects had compound heterozygosity for PAH mutations.
Comparison of PAH genotypes to the response of blood phenylalanine to sapropterin dihydrochloride treatment in PKU-001 did not reveal any consistent patterns.

- Analysis performed across trials (pooled analyses and meta-analysis)

No analyses were performed across trials for efficacy

- Clinical studies in special populations

No studies were performed in special populations.

- Supportive studies

Due to the particularly rare condition of BH4 deficiency, no specific studies were performed but evidences from published studies were provided in support of this application.
Although the current presentation of sapropterin was not used in any of the studies, these publications suggest a significant effect on the Phe levels in this subgroup of patients with lower doses of sapropterin (2 to 5 mg/kg/day) and an effect seen in studies for up to 133 months.

**Biopten Study** (D272) was used to support Biopten (sapropterin dihydrochloride 2.5% granules) registration in Japan, enrolled and treated 16 subjects with BH4 deficiency. Sapropterin dihydrochloride treatment began at a dose of 1.5 to 10 mg/kg/day, and the dose was increased or decreased based on clinical symptoms and examination results. Daily dosing ranged from 0.8 to 37 mg/kg/day, with maintenance dosing ranging from 2 to 5 mg/kg/day. Sapropterin dihydrochloride was administered for a mean of 15.5 months; 15 subjects were treated for 10 to 20 months and 1 subject was treated for 4 months.
Assessment of clinical effectiveness was based on the degree of improvement in blood phenylalanine levels, a meaningful increase in dihydrobiopterin (BH2; indicating oxidation of 6R-BH4) for patients with DHPR deficiency, and decrease of the urine neopterin to biopterin ratio, (indicating 6R-BH4 utilisation) for patients with PTPS deficiency. Based on this global improvement rating, there was marked improvement in 87.5% (14/16) of patients and marked or moderate improvement in all 16 patients. When overall improvement, general safety, and other clinical assessments were taken into account, 87.5% (14/16) of patients had a score of very useful, and all 16 patients had a score of very useful or useful.
For all 16 subjects, blood phenylalanine levels were lower after treatment with sapropterin dihydrochloride and were maintained within the normal range (≤3 mg/dL [≤181.6 μmol/L]) during the treatment period.

Other literature studies reported by Wang *et al.,* and Chien *et al.* in HPA patients with BH4 deficiency were also in favour of an improvement under a treatment including a non-pharmaceutical preparation of BH4.

- Discussion on clinical efficacy

Two short-term open-label treatment response studies (PKU-001 and PKU-006 -Part 1) were performed to identify responders to sapropterin dihydrochloride treatment.
Responders were defined by the criterion of a ≥30% decrease in blood phenylalanine from baseline after 8 days of daily sapropterin dihydrochloride at the same dose used in the consequent, respective pivotal trial. The 30% cut off represents a significant reduction in blood phenylalanine based on limited published observations and expert opinion, and is not directly relevant to proposed clinical therapeutic targets per se.

The results of PKU-004 have documented a dose response. Overall, the changes in phenylalanine levels were directly related to the dose of sapropterin and the differences in effect were statistically significant when compared between dose levels. For the 5mg dose 25%, for the 20mg 55% and for the 10mg dose 46% of patients achieved more than 30% reduction in phenylalanine levels at the weeks 2, 4, 6 and 10 visits. The difference between the 5 and 10mg/kg/day was statistically significant at $p<0.0001$ and between 10 and 20mg/kg/day at $p=0.0085$. Twice as many subjects achieved more than 30% reduction in blood levels after receiving 10mg compared to 5mg/kg/day.

For BH4 deficiency, the starting dose for Sapropterin, 2 to 5 mg/kg/day, is based on clinical data for sapropterin dihydrochloride 2.5% granules (Biopten), which contain the same drug substance. Information from the Biopten registration trials and post-marketing experience, as well as information from the published literature using a non-pharmaceutical preparation indicate that reduction or normalisation of blood phenylalanine levels may occur at sapropterin dihydrochloride doses less than 10 mg/kg/day, particularly in patients with BH4 deficiency due to PTPS deficiency. On the other hand, some individuals with BH4 deficiency due to DHPR deficiency may require doses of sapropterin dihydrochloride up to 20 mg/kg/day, and the daily dose may need to be divided. In addition, the total daily dose may need to be split into 2 or 3 doses a day. It has been postulated that this difference in dosing regimen reflects the facts that DHPR deficiency prevents endogenous regeneration of 6R-BH4. Therefore, the proposed dose range for BH4 deficiency is specified as 5 to 20 mg/kg/day, which may need to be split into 2 to 3 doses per day to optimise treatment

In the two phase III studies provided PKU-003 and PKU-006, the applicant has documented a statistically significant dose related effect which was maintained for the duration of the studies.

In **PKU-003**, there were statistically significant differences between the sapropterin and placebo treated patients' phenylalanine levels at the end of the study. The mean ± SD baseline blood phenylalanine levels for the sapropterin dihydrochloride group and the placebo group were similar, 842.7 ± 299.6 and 888.3 ± 323.1 μmol/L, respectively. The estimated difference in mean ± SE blood phenylalanine level between the two treatment groups (sapropterin dihydrochloride − placebo) was −230 ± 43.4 μmol/L at Week 6.

In **PKU-006**, 11 (33.3%) of the 33 subjects who received sapropterin dihydrochloride tolerated a phenylalanine supplement of 31 to 50 mg/kg/day, 10 (30.3%) tolerated 11 to 30 mg/kg/day, and 7 (21%) tolerated 1 to 10 mg/kg/day; 5 subjects (15%) were unable to tolerate any phenylalanine supplementation. Of the 12 subjects in the placebo group, none were able to tolerate phenylalanine supplementation above 10 mg/kg/day. In subjects treated with sapropterin dihydrochloride, the mean ± SD change in blood phenylalanine from the Week 0 visit to the Week 3 visit was 148.5 ± 134.2 μmol/L, which was a significant decrease from the Week 0 visit ($p<0.001$).

The results suggest that most patients with hyperphenylalaninaemia will achieve blood phenylalanine reductions with doses of between 5 and 10mg/kg/day. These patients must continue a restricted phenylalanine diet and undergo regular clinical assessment (such as monitoring of blood phenylalanine and tyrosine levels, nutrient intake, and psycho-motor development).

**Clinical safety**

- Patient exposure

In total, 647 subjects have been treated with one or more doses of sapropterin dihydrochloride, in either a tablet or granule formulation, in clinical studies of HPA due to PKU or BH4 deficiency or in healthy volunteers.

In PKU-001, PKU-003, PKU4 and PKU-006, a **total of 579 individuals with PKU** received at least one dose of sapropterin dihydrochloride tablets including 125 children (≥4 to <12 years old), 157 adolescents (≥12 to <18 years old) and 297 adults (≥18 years old). Forty four point three percent of subjects (44.3% [255/575]) were aged ≥ 18 years and received sapropterin dihydrochloride for < 10 days as part of a screening study to identify responders.

- Adverse events

In the combined, placebo-controlled trials, PKU-003 and PKU-006 Part 2, the treatment-emergent adverse events which were reported by ≥ 5% of subjects treated with sapropterin dihydrochloride tablets and at a rate which was in excess over placebo (i.e. where the proportion of sapropterin dihydrochloride-treated subjects reporting the event exceeding the proportion of placebo subjects reporting the same event by at least 3 percentage points), included: rhinorrhoea, pharyngolaryngeal pain, diarrhoea, and contusion. Other adverse events which were reported by at least 3 sapropterin dihydrochloride-treated subjects and at a rate which was in excess over placebo in either one of the individual studies included: headache, nasal congestion, vomiting and cough. Except for headache, no other neuropsychological adverse events were most common in the pivotal trials

**Treatment-Emergent Adverse Events in the Combined Placebo-Controlled Trials, PKU-003 and PKU-006 Part 2, Occurring in ≥ 5% of Sapropterin Dihydrochloride-Treated Subjects and in Excess Over Placebo**

| | PKU-003 and PKU-006 Part 2 Combined | | | |
|---|---|---|---|---|
| | Placebo Subjects (n=59) Events (n=120) | | Sapropterin dihydrochloride 10-20 mg/kg/day Subjects (n=74) Events (n=153) | |
| Preferred Term | Subjects n (%) | Events n (%) | Subjects n (%) | Events n (%) |
| Diarrhoea | 3 ( 5.1) | 3 ( 2.5) | 6 ( 8.1) | 7 ( 4.6) |
| Pharyngolaryngeal pain | 1 ( 1.7) | 1 ( 0.8) | 7 ( 9.5) | 9 ( 5.9) |
| Rhinorrhoea | 0 ( 0.0) | 0 ( 0.0) | 8 ( 10.8) | 8 ( 5.2) |
| Contusion | 1 ( 1.7) | 1 ( 0.8) | 4 ( 5.4) | 4 ( 2.6) |

(a) Occurring in at least 5% of Sapropterin treated subjects and this is at least 3% more than the % of Placebo treated subjects with AEs in the same Preferred Term.

**Withdrawal**

The potential effects of drug withdrawal were evaluated in the context of the placebo-controlled studies, PKU-003 and PKU-006 Part 2, by examining the AEs that occurred following treatment discontinuation ("post-treatment period").

From the results of this analysis, there did not appear to be any pattern to the events that emerged following discontinuation of sapropterin dihydrochloride tablets and no safety concerns relevant with withdrawal effects were identified.

**Rebound**

Rebound was examined in the context of study PKU-006 and the break in treatment that occurred between Parts 1 and 2.

The results of this analysis suggest not surprisingly that continued treatment with sapropterin dihydrochloride in necessary to maintain effect as, even in subjects maintained on a phenylalanine-

restricted diet, phenylalanine levels may rebound upon cessation of treatment. However, in subjects experiencing rebound, resumption of treatment with sapropterin dihydrochloride is effective in returning phenylalanine levels to the desired therapeutic level.

- Serious adverse events and deaths

No deaths were reported in among the studies of sapropterin dihydrochloride tablets in PKU. No deaths were reported in the clinical study D272 of sapropterin dihydrochloride granules in BH4 deficiency.

It is unlikely that any of the six serious adverse events (hospitalisation for strep throat, two cases of appendicitis, back pain, hospitalisation for urinary tract infection and fractured tibia) reported in the dossier were related to sapropterin.

- Laboratory findings

**Hypophenylalaninenylalaninemia**
Since phenylalanine is an essential amino acid, persistence of blood phenylalanine levels below the normal range is undesirable. For the analyses of safety data in the pivotal trials, PKU-003 and PKU-006 Part 2, hypophenylalaninaemia was defined as the occurrence of a **blood phenylalanine level ≤26 µmol/L**.
5 of the 41 subjects in the sapropterin dihydrochloride treatment group for **PKU-003** experienced at least one occurrence of blood phenylalanine levels ≤120 µmol/L; there were no low levels reported for the placebo group and no subjects in either treatment group with levels below 40 or 26 µmol/L.
In **PKU-006 Part 2**, 27/33 (81.8%) of subjects randomised to treatment with sapropterin dihydrochloride tablets had at least one occurrence of a blood Phenylalanine level ≤120 µmol/L, as compared to 6/12 (50%) of placebo subjects. Of the 27 sapropterin dihydrochloride-treated subjects with a blood phenylalanine level ≤120 µmol/L during PKU-006 Part 2, 12 had at least one blood phenylalanine level ≤40 µmol/L and 9 had at least one level ≤26 µmol/L (36.3% and 27.3%, respectively, of all the sapropterin dihydrochloride treated subjects); comparable numbers for the placebo treatment group were 1/12 (8.3%) and 1/12 (8.3%), respectively.
In study **PKU-006 Part 1**, 40 (44.9%) subjects had one or more phenylalanine level measurement ≤ 120 µmol/L. Of these, 35.0% (14/40) of the subjects had a measurement ≤ 40 µmol/L and 20.0% (8/40) had a measurement ≤ 26 µmol/L.

In comparison, 6 subjects (1.2%) participating in study PKU-001 had a phenylalanine level measurement ≤ 120 µmol/L. Of these, 1 subject had a measurement ≤ 26 µmol/L.
In PKU-004, 6 (7.5%) subjects had phenylalanine levels ≤ 120 µmol/L. This incidence was higher than that seen in PKU-001 (1.2%), but lower than in the sapropterin dihydrochloride-treated subjects in PKU-003 (12.2%). No subject had a result ≤ 40 or ≤ 26 µmol/L during PKU-004.

In studies PKU-003, PKU-001 and PKU-004, the proportion of subjects having one or more low phenylalanine value was higher among the paediatric subjects (≥ 4 to < 18 years) than in the adults (≥ 18 years). Because all subjects enrolled in studies PKU-006 Parts 1 and 2 were aged 4 to 12 years, no comparison of low phenylalanine values between paediatric subjects and adults is possible in these studies.

The higher occurrence of low blood phenylalanine levels during sapropterin dihydrochloride treatment in PKU-006 Part 2 as compared to PKU-003 was not unexpected since subjects in PKU-006 were pre-selected to have blood phenylalanine levels controlled on a phenylalanine-restricted diet, whereas subjects in PKU-003 had uncontrolled blood phenylalanine levels. In addition, the dose of sapropterin dihydrochloride tablets was higher in PKU-006 (20 mg/kg/day) than in PKU-003 (10 mg/kg/day).

Low phenylalanine levels were not reported in the published Study D272 or in the post-marketing surveillance study of sapropterin dihydrochloride granules in BH4-deficiency, although such events may have occurred.

In these studies and in the literature, downward adjustment of the BH4 dose even after diet liberalisation was evident for some subjects, suggesting that blood phenylalanine levels may have been below the desired therapeutic or normal range.

- Safety in special populations

Studies in patients with renal or liver insufficiency were not included in the clinical development. Therefore, no data are available for sapropterin dihydrochloride use in these subgroups. These subgroups have been included as important missing information in the Risk Management Plan and will be addressed in routine pharmacovigilance.

In the pre-clinical program, nephrotoxicity was identified as a possible safety concern, based on studies in which very high doses of sapropterin were administered to animals. There were no reports of renal laboratory abnormalities and no treatment-related renal adverse events observed in the clinical development program.

There were no hepatic concerns raised in the pre-clinical studies, and there were no reports of hepatic abnormalities or treatment-related hepatic adverse events observed in the clinical development program. Hepatic laboratory shift tables, which address the advice given during the CHMP Protocol Assistance, did not reveal any abnormalities

Subjects who were pregnant or lactating were excluded from all studies. In one published case report of a pregnant patient with mild hyperphenylalaninaemia due to PKU who was treated with tetrahydrobiopterin (6R-BH4), no adverse effects on the pregnancy or newborn were noted.

- Safety related to drug-drug interactions and other interactions

No formal study of interactions between sapropterin dihydrochloride and other drugs has been carried out.
In the combined placebo-controlled trials, PKU-003 and PKU-006 Part 2, a similar proportion of subjects in the sapropterin dihydrochloride treatment group used one or more concomitant drugs as compared to the placebo group. Among the sapropterin dihydrochloride-treated subjects, 48 (64.9%) used one or more concomitant medication. While in the placebo group, 45 (76.3%) used one or more concomitant medication. The most frequently reported concomitant medications (≥ 10% of subjects in either treatment group) included propionic acid derivatives (ibuprofen), anilides (paracetamol) and multivitamins. Similar proportions of sapropterin dihydrochloride and placebo subjects used these drugs. All other concomitant drugs were reported by fewer than 10% of subjects in either treatment group.
No drug interaction data are available from study D272 of sapropterin dihydrochloride granules in subjects with BH4 deficiency.

- Discontinuation due to adverse events

There were no discontinuations due to drug-related adverse events

- Post marketing experience

Sapropterin dihydrochloride tablets has not been approved for marketing in the treatment of HPA due to BH4 deficiency.
Sapropterin dihydrochloride granules (Biopten 2.5% Granules) has been approved for the treatment of BH4 deficiency in Japan. Results of a pre-approval and post-marketing surveillance study conducted in Japan are summarised.
A pre-approval and 10-year post-marketing safety surveillance program was conducted by DSP in patients with atypical hyperphenylalaninaemia due to BH4 deficiency. Safety results were classified as adverse drug reactions (ADRs), and the terms used in this summary and in the full report have not been coded using standard MedDRA SOCs or PTs.
The only ADRs reported in the pre-approval surveillance program were 2 cases of diarrhoea reported for 1 of the 19 patients (5.3%).
Thirty-two ADRs were reported for 11 of 30 patients (36.7%) in the post-marketing surveillance program. The major ADRs were convulsion and exacerbation of convulsion in 3 of the 30 patients (10.0%), and GGT increased in 2 of the 30 patients (6.7%). The annual number of ADRs reported was

0 to 8 ADRs reported per year for 0 to 3 patients per year (0.0% to 10.0% of patients in the surveillance program). Two patients' deaths considered unrelated to sapropterin dihydrochloride granules treatment were reported during or immediately following the post-marketing surveillance period. DSP concluded that no clinical problems related to treatment with sapropterin dihydrochloride granules were evident, based on this surveillance program.
In the post-marketing surveillance program, the frequency and incidence of ADRs categorised by body system was: 16 events of central and peripheral nervous system disorders in 5 patients (16.7%), 1 event of an autonomic nervous system disorder in 1 patient (3.3%), 6 events of vision disorders in 2 patients (6.7%), 3 events of psychiatric disorders in 2 patients (6.7%), 1 event of a gastrointestinal system disorder in 1 patient (6.7%), and 5 events of liver and biliary system disorders in 4 patients (13.3%). None of the ADRs were considered serious. Twenty-one of the ADRs in 7 subjects were considered unexpected events. All ADRs were transient, and subjects recovered or improved without discontinuing sapropterin dihydrochloride granules treatment, except for 1 subject in whom stammering, involuntary movement of lips, and ocular displacement were continuously reported from the sixth year of the program onward while sapropterin dihydrochloride granules treatment was continued.
Since 16 ADRs in the central and peripheral nervous system disorders category were reported for 5 subjects (16.7%) in the post-marketing surveillance program, neurological symptoms underwent intensive review. All subjects who developed these ADRs, except for 1 subject with convulsion, had developed neurological symptoms before the start of sapropterin dihydrochloride granules treatment. In most subjects, it was difficult to distinguish a treatment-emergent ADR from the subjects' pre-existing neurological symptoms. The subject for whom convulsion was reported did not have neurological symptoms before the start of treatment, but developed clonic convulsion of the upper limbs and abnormal eye movement (staring) for several seconds about 3 months after the start of sapropterin dihydrochloride granules treatment at 1 month of age. The severity of the ADR was mild and the subject recovered following treatment with an anticonvulsant. Overdose of concomitantly administered levodopa may have been related to these symptoms.
ADRs in the gastrointestinal disorders category were limited to 1 report of loose stool and 2 reports of diarrhoea in 2 subjects (6.7%).
ADRs in the liver and biliary system disorders category were reported for 4 subjects (13.3%): 2 reports of GGT increased and 1 report each of hepatic function disorder, AST increased and ALT increased.
Two (2) deaths, both considered to be unrelated to sapropterin dihydrochloride granules treatment, were reported as part of the DSP post-marketing surveillance program. One death was reported during the 10-year surveillance period and 1 was reported 5 days after completion of the surveillance period. One patient, a male, suffered an airway obstruction secondary to a large amount of sputum, developed severe complications, and died from sepsis at age 4 years and 5 months. The second patient, also a male, was found dead in his apartment 5 days after the surveillance period had ended, at age 24 years and 10 months, having apparently died "several days" earlier of complications related to his underlying BH4 deficiency with severe muscle stiffness. Autopsies were not performed in either case. No other serious AEs were identified in the study report for the surveillance study.
Gender, diagnosis, medical history, concomitant levodopa and/or anticonvulsant drug use, and concomitant therapies were evaluated as potential factors affecting ADR incidence. A statistically significant difference was observed for medical history, with the frequency of ADRs higher in subjects with complications than in those without complications. There was no association between type of complication (neurological or non-neurological) and the ADRs developed. The reasons for the higher ADR frequency in patients with complications could not be determined. No significant difference in ADR frequency was apparent in comparisons by gender, diagnosis, concomitant levodopa and/or anticonvulsant drug use, or concomitant therapies.

The conclusion from this surveillance study was that long-term treatment with Sapropterin dihydrochloride granules did not pose any significant overall safety problems, or specific concerns in patients with pre-existing neurological disorders.

- Discussion on clinical safety

Sapropterin was well tolerated.
Hypophenylalaninaemia, as defined by the occurrence of a blood phenylalanine level ≤26 µmol/L, was observed to occur more frequently with sapropterin-dihydrochloride treatment as compared to placebo. This is an expected consequence of the effect of sapropterin to decrease blood phenylalanine levels and may indicate a need to increase dietary phenylalanine intake or adjust the dose of sapropterin dihydrochloride tablets.

Common adverse events among the sapropterin dihydrochloride-treated subjects that were reported in excess over placebo (*i.e.* by a difference of at least 3 percentage points greater than the proportion of placebo subjects reporting the same event) in either the combined or one of the individual placebo-controlled trials, include: headache, rhinorrhoea, pharyngolaryngeal pain, vomiting, diarrhoea, nasal congestion, cough and contusion.
The observed adverse events within this dose range were mostly mild to moderate and transient in nature. There were no deaths and serious adverse reactions were not related to sapropterin.

The recommended starting dose of 2 to 5 mg/kg/day for Sapropterin treatment of BH4 deficiency is consistent with the safety data from the Study D272 and the post-marketing surveillance study for sapropterin dihydrochloride 2.5% granules (Biopten). No safety concerns were evident within this dose range.
Patients treated with Kuvan must continue a restricted phenylalanine diet and undergo regular clinical assessment (such as monitoring of blood phenylalanine and tyrosine levels, nutrient intake, and psycho-motor development).

Special attention was given to analysis of data regarding hepatic laboratory test results, clinical adverse reactions, and neurological and psychological/behavioural events.
In addition to the provided Risk Management Plan, further safety data will be collected in the post-marketing phase as follow-up measures:

- a post-marketing drug registry for patients >4 years old. The registry will include data for elderly patients (without an upper age limit), maternal pregnancy, and patients with renal or hepatic insufficiency and children, including childhood growth and neurocognitive outcomes.
- a study to evaluate neurocognitive function, long-term safety and effects on growth parameters in children up to the age of 8 years.
- a study in paediatric patients with PKU, 0 to 4 years old, to evaluate population pharmacokinetics, efficacy and safety.

## 2.5 Pharmacovigilance

**Detailed description of the Pharmacovigilance system**

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

It provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

**Risk Management Plan**

The MAA submitted a risk management plan.

**Summary Table of the Risk Management Plan**

| Safety concern | Proposed pharmacovigilance activities (routine and additional) | Proposed risk minimisation activities (routine and additional) |
|---|---|---|
| **Important identified risks** | | |
| **Gastrointestinal disorders**<br>• Vomit<br>• Diarrhoea<br>• Abdominal pain | • Routine pharmacovigilance activities (ICSRs, aggregate data analyses in accordance with applicable laws)<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, section 4.2 and 4.8<br>Additional<br>Not applicable |
| **Hypophenylalaminemia** | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, sections 4.2, 4.4 and 4.8<br>Additional<br>Not applicable |
| **Rebound** | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, sections 4.2<br>Additional<br>Not applicable |
| **Drug interactions**<br>• Dihydrofolate reductase inhibitors<br>• Vasodilators using the NO metabolic pathway<br>• Levodopa | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, sections 4.2 and 4.5<br>Additional<br>Not applicable |
| **Potential risks** | | |
| **Nephrotoxicity** | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Section 5.3 of the SPC describes the kidney findings in animals.<br>Additional<br>Not applicable |
| **Limited / missing information** | | |
| **Size of safety database** | • Routine pharmacovigilance activities<br>• Clinical studies PKU-007, PKU-008<br>• Post-authorisation studies | Routine<br>Not applicable<br>Additional<br>Not applicable |
| **Limited long-term exposure data** | • Routine pharmacovigilance activities<br>• Clinical study PKU-008<br>• Post-authorisation studies | Routine<br>Not applicable<br>Additional<br>Not applicable |
| **Limited BH4 deficiency subgroup data** | • Routine pharmacovigilance activities<br>• Clinical study PKU-007 | Routine<br>Not applicable<br>Additional<br>Not applicable |

| | | |
|---|---|---|
| | • Post-authorisation studies | |
| **Pregnant women** | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, section4.6.<br>Additional<br>Not applicable |
| **Children ≤ 4 years of age** | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, sections 4.1, 4.2 and 4.4<br>Additional<br>Not applicable |
| **Elderly**<br>**Patients with renal or hepatic insufficiency** | • Routine pharmacovigilance activities<br>• Post-authorisation studies | Routine<br>Adequately described in the SPC, sections 4.2 and 4.4<br>Additional<br>Not applicable |

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information

## 2.6 Overall conclusions, risk/benefit assessment and recommendation

**Quality**

The quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. There are no unresolved quality issues, which have a negative impact on the Benefit Risk balance of the product.

**Non-clinical pharmacology and toxicology**
As there are no appropriate animal models for HPA due to PKU, non-clinical primary pharmacodynamic studies were conduced in normal rodents where HPA was induced by administering a BH4 biosynthesis inhibitor. Such studies demonstrated that 6R-BH4 supplementation by systemic route can reverse the effects of inhibitors of the BH4 biosysnthesis pathway.

Similarities exist in the pharmacokinetic profile of sapropterin dihydrochloride across the species studied (mouse, rat, rabbit and monkey) and with the limited clinical pharmacokinetic studies.
The majority of orally administered sapropterin dihydrochloride is excreted in faeces due to limited absorption from the gastro-intestinal tract. The fraction absorbed is mostly excreted with the urine.

The kidney was identified as a target organ of toxicity in the rat in single-dose and repeat-dose studies (slight tubular basophilia) at doses resulting in very low safety margins compared to the human exposure. Therefore, the occurrence of possible kidney changes will be monitored during clinical use as mentioned in the risk management plan.
Juvenile animal data suggest that sapropterin dihydrochloride may be potentially more toxic in early postnatal age, but this was most likely due to the higher absorption of sapropterin dihydrochloride in younger animals.
There was no developmental or embryofoetal toxicity in rats and rabbits.
Oral carcinogenicity studies conducted in mice and rats indicated no potential of sapropterin dihydrochloride to induce the formation of either neoplastic or hyperplastic lesions.
Sapropterin is not expected to pose a risk to the environment.

**Efficacy**
Sapropterin is intended to provide the necessary co-factor for phenylalanine hydroxylase (PAH), allowing hydroxylation of phenylalanine to tyrosine in patients with phenylketonuria (PKU) by acting as a co-factor to increase the residual activity of the mutated PAH enzyme, and to replace the missing 6R-BH4 in patients who lack BH4.

In the two phase III studies provided PKU-003 and PKU-006, the applicant has documented a statistically significant dose related effect which was maintained for the duration of the studies.

In **PKU-003**, the estimated difference in mean ± SE blood phenylalanine level between the two treatment groups (sapropterin dihydrochloride − placebo) was −230 ± 43.4 μmol/L at Week 6.

In **PKU-006**, 11 (33.3%) of the 33 subjects who received sapropterin dihydrochloride tolerated a phenylalanine supplement of 31 to 50 mg/kg/day, 10 (30.3%) tolerated 11 to 30 mg/kg/day, and 7 (21%) tolerated 1 to 10 mg/kg/day; 5 subjects (15%) were unable to tolerate any phenylalanine supplementation. Of the 12 subjects in the placebo group, none were able to tolerate phenylalanine supplementation above 10 mg/kg/day. In subjects treated with sapropterin dihydrochloride, the mean ± SD change in blood phenylalanine from the Week 0 visit to the Week 3 visit was 148.5 ± 134.2 μmol/L, which was a significant decrease from the Week 0 visit ($p<0.001$).

**Safety**

Sapropterin cannot reduce blood levels in all patients with raised phenylalanine levels but does appear to reduce levels in subjects who have been shown to respond to BH4. In prescribing this product, it is therefore important to stress that the product does not work in all patients with PKU or BH4 deficiency but only in those who have shown a definite effect.
In addition, it is important to ensure that patients treated are monitored to ensure an adequate response and an absence of excess effect in phenylalanine levels.
As mentioned in the SPC (section 4.2 and 4.4), "Patients treated with Kuvan must continue a restricted phenylalanine diet and undergo regular clinical assessment (such as monitoring of blood phenylalanine and tyrosine levels, nutrient intake, and psycho-motor development)."

Sapropterin was well tolerated with most of the adverse events mild or moderate. Common adverse events among the sapropterin dihydrochloride-treated subjects that were reported in excess over placebo (*i.e.* by a difference of at least 3 percentage points greater than the proportion of placebo subjects reporting the same event) in either the combined or one of the individual placebo-controlled trials, include: headache, rhinorrhoea, pharyngolaryngeal pain, vomiting, diarrhoea, nasal congestion, cough and contusion.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.
Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

The results of user testing demonstrated that at least 90% of the participants were able to find each point of information. It also showed that at least 90% of those participants were able to express the information in their own words. The leaflet therefore fulfils the EU requirements for user testing.

**Risk-benefit assessment**

In the two phase III studies provided PKU-003 and PKU-006, the applicant has documented a statistically significant dose related effect which was maintained for the duration of the studies.
The results suggest that most patients with hyperphenylalaninaemia will achieve blood phenylalanine reductions with doses of between 5 and 10mg/kg/day.
In prescribing this product, it is important to stress that the product does not work in all patients with PKU or BH4 deficiency but only in those who have shown a definite effect.

In addition, it is important to ensure that patients treated are monitored to ensure an adequate response and an absence of excess effect in phenylalanine levels.

Sapropterin was well tolerated with most of the adverse events mild or moderate.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that routine pharmacovigilance was adequate to monitor the safety of the product.
The CHMP, having considered the data submitted, was of the opinion that:

- Pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.
- No additional risk minimisation activities were required beyond those included in the product information.

**Similarity with authorised orphan medicinal products**
The CHMP is of the opinion that Kuvan is not similar to any authorised orphan medicinal product within the meaning of Article 3 of Commission Regulation (EC) No. 847/200.

**Recommendation**

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Kuvan in the following indication:

> "Kuvan is indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients of 4 years of age and over with phenylketonuria (PKU) who have been shown to be responsive to such treatment.
>
> Kuvan is also indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients with tetrahydrobiopterin (BH4) deficiency, who have been shown to be responsive to such treatment."

was favourable and therefore recommended the granting of the marketing authorisation.

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