

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-181

PHARMACOLOGY REVIEW(S)

Comments on N22181 sapropterin dihydrochloride
From A. Jacobs 12/5/07

There do not appear to be any pharm-tox related approvability issues.

I. The Labeling

A. I concur with the pregnancy category C

B. Section 13.1 carcinogenesis, mutagenesis, impairment of fertility

Even so, the results in the Ames test were equivocal at best. The statement in the labeling could be softened to reflect this.

C. Section 8.3 Nursing mothers

The nonclinical toxicology studies and carcinogenicity findings of adrenal pheochromocytoma do warrant the severity of warning against nursing. There was no evidence of excretion of the drug in milk after oral administration, and no statistically significant results in the peripostnatal studies, in which the rats nursed. These peripostnatal studies should be mentioned in the nonclinical toxicology section of labeling.

It would seem that human mothers should not nurse until it is known that the infants do not have the genetic disease of the mother.

11. The review

The labeling recommendations should be in the executive summary.

An addendum to the review should comment/concur with the final labeling for nonclinical toxicology sections.

I have discussed these comments with the reviewer and/or TL.

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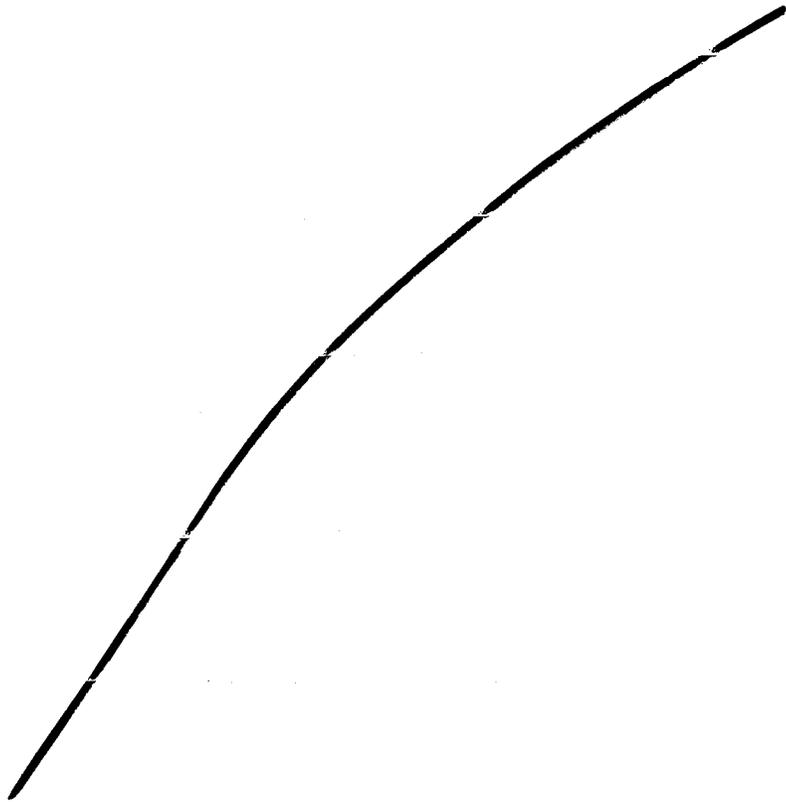
/s/

Abby Jacobs
12/4/2007 04:16:14 PM
PHARMACOLOGIST



Previously Proposed Version:

Carcinogenesis, Mutagenesis, Impairment of Fertility



Revised Version:

A 2-year carcinogenicity study was conducted in F-344 rats, and a 78-week carcinogenicity study was conducted in CD-1 mice. In the 104-week oral carcinogenicity study in rats, sapropterin doses of 25, 80, and 250 mg/kg/day (0.2, 0.7, and 2 times the maximum recommended human dose of 20 mg/kg/day, respectively, based on body surface area) were used. In the 78-week oral carcinogenicity study in mice, sapropterin doses of 25, 80, and 250 mg/kg/day (0.1, 0.3, and 2 times the recommended human dose, respectively, based on body surface area) were used. In the 2-year rat carcinogenicity study, there was a statistically significant increase in the incidence of benign adrenal pheochromocytoma in male rats treated with the 250 mg/kg/day (about 2 times the maximum recommended human dose, based on body surface area) dose, as compared to vehicle-treated rats. The mouse carcinogenicity study showed no evidence of a carcinogenic effect, but the study was not ideal due to its duration of 78 instead of 104 weeks.

Fang Cai, Ph.D.
Pharmacologist, HDF-180

Date

Comments:

Sushanta Chakder, Ph.D.

Date

Acting Supervisory Pharmacologist, HDF-180

IND
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HFD-181/CSO.
HFD-180/Dr. Chakder
HFD-180/Dr. Cai
R/D Init. : Dr. Chakder, December 4, 2007

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/s/

Fang Cai
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PHARMACOLOGIST

Sushanta Chakder
12/4/2007 03:10:30 PM
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22-181
SERIAL NUMBER: 0000
DATE RECEIVED BY CENTER: 05/25/2007
PRODUCT: Kuvan (sapropterin duhydrochloride) tablets
INTENDED CLINICAL POPULATION: Patients with hyperphenylalaninemia due
to phenylketonuria

SPONSOR: BioMarin Pharmaceutical, Inc.
DOCUMENTS REVIEWED: Electronic submission of the NDA
REVIEW DIVISION: Division of Gastroenterology Products (HFD-180)

PHARM/TOX REVIEWER: Fang Cai, Ph.D
PHARM/TOX SUPERVISOR (Acting): Sushanta Chakder, Ph.D
DIVISION DIRECTOR (Acting): Daniel A. Shames, M.D
PROJECT MANAGER: Cristi L. Stark, M.S

Date of review submission to Division File System (DFS): November 26, 2007

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: The sponsor conducted adequate preclinical studies with sapropterin HCl to determine the safety of the drug. Thus, from a preclinical standpoint, the NDA application is approvable. However, the relevant findings of preclinical studies should be included in the labeling as recommended.
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling: Included in the labeling section of the review.

II. Summary of nonclinical findings

A. Pharmacologic activity

Sapropterin dihydrochloride is the hydrochloride salt of R-tetrahydrobiopterin (6R-BH₄), which is a coenzyme of phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), and contributes to the biosynthesis of monoamine neurotransmitters [e.g. dopamine (DA), norepinephrine (NE), serotonin (5-HT)].

Nonclinical pharmacology studies of sapropterin HCl have shown an increase in release of endogenous 6R-L-erythro-5,6,7,8,-tetrahydrobiopterin (6R-THBP) without affecting monoamine levels from normal rat striatum and activation of both the dopaminergic and serotonergic systems, as detected by a decrease in [¹¹C]N-methylspiperon ([¹¹C]-NMSP) signal using CT (PET), in normal rhesus monkey striatum. In animal models of intracerebral monoamine depletion, sapropterin HCl was shown to reverse DAHP (2,4-diamino-6-hydroxypyrimidine) and α -methyltyrosine (α -MT) induced depletions in monoamines. Sapropterin reversed increased phenylalanine (phe) level in blood and liver caused by DAHP in rats. Increased maternal and fetal serum Phe and liver Phe caused by DAHP was also reversed by administration of sapropterin to mother guinea pigs. Additionally, sapropterin HCl administration attenuated α -MT induced decreases in motor activity. However, sapropterin HCl showed no effect on p-chlorophylalanine (p-CPA)-induced decreases in brain monoamine levels. Sapropterin reversed decreased endogenous production of 6R-BH₄ and restored catecholamine production at pharmacologic levels.

In mice, thiopental-induced sleep prolongation was observed with sapropterin HCl at a dose of 300 mg/kg (p.o.). Anesthetized dogs dosed with sapropterin HCl at 30 mg/kg (i.v.) exhibited a decrease in respiration rate and pulse rate as well as a tendency toward inhibition of the maximum elevation speed of pressure within the left ventricle (LV dP/dt max). Sapropterin up to 100 mg/kg (p.o) had no cardiovascular effects in conscious dogs. Sapropterin caused tremor (at 100 mg/kg, p.o.) and shortened immobility time (at 60, 100 and 600 mg/kg, p.o.) in male mice. It does not block IKr channels in HEK293 cells that stably express the human hERG channels.

B. Brief overview of nonclinical findings

ADME:

Sapropterin was rapidly absorbed in male mice, rats and monkeys following oral administration and the total concentration in plasma reached its peak (T_{max}) approximately at 1 hr, 2 hrs and 3 hrs, respectively. Following oral dosing, Sapropterin was eliminated with half-lives (t_{1/2}) of 1.3 hrs in mice, 1.1 hrs in rats and 1.4 hrs in monkeys, respectively. The bioavailability was 7%-12% in rats, and approximately 9% in monkeys. Plasma and organ vs time concentrations of total did not change following repeated oral administration at doses up to 100 mg/kg in rats. The same non-accumulation was observed in presumed pregnant rabbits at 6 and 60 mg/kg/day p.o, however, when dosed at 600 mg/kg/day for 13 days accumulation was observed following the final dose. Sapropterin was distributed mainly to the kidney and liver of rats. After 10mg/kg, i.v. dose of sapropterin HCl to lactating rats, the concentration of in milk increased to about 3X the endogenous level at 2.5 hr post-dosing. Sapropterin does not activate or inhibit the cytochrome P450 metabolic pathway. The drug was excreted mainly in rat urine after i.v. administration. 7,8-Dihydrobiopterin [DHBP], pterin, 7,8-Dihydropterin (DHPT) and 6-hydroxylumazine [6-OH-Lu] were identified as metabolites in rat urine following i.v. administration of sapropterin HCl. Sapropterin does not interact with plasma protein or erythrocytes and thus does not bioaccumulate or persist unless dosed above 100 mg/kg/day p.o.

Toxicology:

Acute (single-dose) toxicity studies of sapropterin via p.o., i.v. and s.c. administrations were performed in mice, rats and marmosets. The minimal lethal doses (MLDs) for mice were 256 mg/kg for males and 320 mg/kg for females (i.v.). The MLDs for both male and female mice were 204 mg/kg (s.c) and 1000 mg/kg (p.o.). The MLDs for both male and female rats were 320 mg/kg (i.v.). The MLDs were 1250 mg/kg for male rats and 2500 mg/kg for female rats (p.o.). The MLDs were 1125 mg/kg for male rats and 1687.5 mg/kg for female rats (s.c.). The MLD for monkeys was not determined after i.v. administration and was 2000 mg/kg for p.o. administration. The MLD was 300 mg/kg for male monkey and was not determine for female monkey (s.c.). The MLD for 7-day old male and female rats was 1200 mg/kg. The MLDs were 1717 mg/kg for juvenile male mice (p.o) and 2330 mg/kg for juvenile female mice (p.o.). After p.o. administration to juvenile rats, the MLDs were 2330 mg/kg for males and 3162 mg/kg for females. Transient tremor, ataxia, tachypnea, and dreaseced mortor activity were observed in adult rats and mice after i.v administration. In marmoset monkeys, salivation, emesis, yellow staining around the mouth and genitalia were noted. In addition, the female monkeys were subdued with marked tremor, ataxia and sluggish movements before death. Hunched posture, decreased motor activity, respiratory difficulties, ataxia and piloerection were seen in juvenile mice and rats after p.o.

Repeat dose oral toxicity studies were conducted in adult rats and marmosets. Hypertrophy of parafollicular cells of the thyroid was noted in both rat and monkey toxicity studies. Increased incidence of pituitary gland hyperplasia occurred at doses \geq 40 mg/kg/d in 52-week rat study. In the 13-Week monkey study, enteritis was observed in 320 mg/kg/d females. Toxicity of the kidney (dilated distal tubules, dilated Bowman's capsule, thickened glomerular membrane), liver (centriacinal lymphoid follicle, mineralized fibrous focus) and the ovary (regressing corpora lutea, luteinizing artretic

follicles) was observed at doses of 80 and 320 mg/kg/d. In a 52-Week monkey study, increased incidences in cerebellar basophilic concretions in the brain, chronic ileitis and myocardial fibrosis was observed with no dose-related increase in incidence or severity. Increased incidences in transitional cell hyperplasia (kidney, male of 80 mg/kg/d) and lymphoid hyperplasia (stomach, 320 mg/kg/d group) were observed. Glandular hyperplasia and adenomyosis (uterus) was observed in females dosed 20 and 320 mg/kg/d. In repeated oral dose toxicity study in juvenile rats (2 and 4-week), basophilia of proximal tubules, dilatation of renal tubules and pelvis, and thickened Bowman's capsule occurred at 320 mg/kg/d.

Sapropterin tested positive in the bacterial reverse mutation and chromosome aberration tests but was not mutagenic when assessed in the *in vivo* mouse micronucleus test.

The carcinogenic potential of sapropterin was assessed in a 104-week oral carcinogenicity study in rats and a 78-week oral carcinogenicity study in mice. There was a dose-related increase in incidence of benign adrenal pheochromocytoma in male rats. The incidence of benign adrenal pheochromocytoma in high dose (250 mg/kg/day) male rats was significantly increased relative to the control group. In female mice, a slight but non-significant increase in incidence of pulmonary adenoma was observed at the mid dose (80 mg/kg/day) and high dose (250 mg/kg/day). However, the mouse study was not ideal due to its duration of only 78 weeks.

In the fertility and early embryonic development study in rats, sapropterin at a oral dose up to 400 mg/kg/d did not produce any effects on fertility and reproductive functions of male and female rats. Teratogenicity studies with sapropterin have been conducted in rats at oral doses up to 400 mg/kg/day and in rabbits at oral doses up to 600 mg/kg/day. Sapropterin was not teratogenic in rats. However, there was a significant reduction in the number of live fetuses and a significant reduction in the body weights of live fetuses from F1 dams treated with the 400 mg/kg/day dose. In the rabbit teratogenicity study, there was a significant increase in the incidence of holoprosencephaly at the 600 mg/kg/day compared to controls. In the post-natal and peri-natal developmental rat study with sapropterin, the average number of still births was increased in 40 and 400 mg/kg/d dams, and birth rate was also decreased in 400 mg/kg/d dams. An increased incidence of newborn pups (F2) with thymic cervical residue was noted in all treated groups.

Sapropterin HCl had no antigenic potential under the conditions of the studies in guinea pigs and mice.

C. Nonclinical safety issues relevant to clinical use:

Sapropterin dihydrochloride is excreted in the milk of intravenously, but not orally treated lactating rats. It is not known whether sapropterin HCl is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from sapropterin HCl and because of the potential for tumorigenicity shown for sapropterin HCl in the rat carcinogenicity, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-181

Review number: 01

Sequence number/date/type of submission: 000/May 25, 2007/Original; 001/July 25, 2005; 002/July 30, 2007; 003/August 29, 2007; 004/August 30, 2007

Information to sponsor: Yes () No (x)

Sponsor and/or agent: BioMarin Pharmaceutical Inc. 105 Digital Drive, Novato, CA 94949.

Manufacturer for drug substance: _____

Reviewer name: Fang Cai, Ph.D

Division name: Division of Gastroenterology Products

HFD #: 180

Review completion date:

Drug:

Trade name: Kuvan™

Generic name: sapropterin dihydrochloride, Tetrahydrobiopterin (6R-BH4 or BH4)

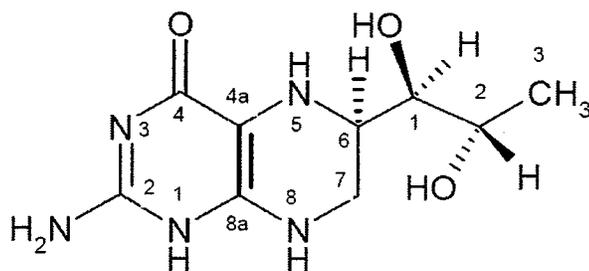
Code name: SUN 0588

Chemical name: (6R)-2-amino-6-[(1R,2S)-1,2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(1H)-pteridinone dihydrochloride

CAS registry number: 69056-38-8

Molecular formula/molecular weight: C₉H₁₅N₅O₃·2HCl/314.17

Structure:



• 2 HCl

Drug class: Phenoptin™ is an enzyme cofactor related to tetrahydrobiopterin. Tetrahydrobiopterin (BH4 or 6R-BH4) formulations have been available for more than a decade, and they are primarily used in a loading test to exclude BH4 deficiency in newborn infants with high blood phenylalanine (Phe) levels. Prior to 1999, the BH4

formulation used in Europe (Schircks Laboratories, Jona, Switzerland) contained a mixture of two isomers of the cofactor: 6S-BH4 and the active isomer 6R-BH4. In 1999, a purer formulation, containing primarily 6R-BH4, became available. 6R-BH4 may normalize the physiology of Phe control, possibly through the activation, stabilization, or enhanced folding of phenylalanine hydroxylase (PAH) enzyme.

Intended clinical population: patients with hyperphenylalaninemia (HPA) due to phenylketonuria (PKU)

Clinical formulation:

Quantitative Composition Per Tablet

Ingredient	100 mg Proposed Commercial Tablet Formulation "B"		100 mg Clinical Study Tablet Formulation "A"	
	Percentage (%)	Amount per Tablet (mg)	Percentage (%)	Amount per tablet (mg)
Sapropterin dihydrochloride	100%	100.00	100%	100.00
<hr/>				
Dibasic calcium phosphate				
Crospovidone				
Ascorbic acid				
Sodium stearyl fumarate				
TOTAL	100%	300 mg	100%	300 mg

Route of administration: Oral

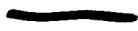
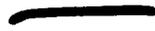
Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

STUDY	STUDY #	LOT #	TESTING LAB	PAGE # OF REVIEW
Pharmacology				13
<u>Absorption</u>				
Dose Ranging Pharmacokinetics of 6R-BH4 after Single Oral Dose Administration in Male C57BL/6 Mice *	0166-06-033	---		50
Pharmacokinetics of Sapropterin Hydrochloride in the Rat (I) (Single Dose Administration)	PHN-104-PK-SR	8886202 8885Y05	Suntory Institute for Biomedical Research, Osaka, Japan	50/69
Pharmacokinetics of Sapropterin Hydrochloride in the Monkey	PHN-110-PK-SR	8886202 8885Y05	Suntory Institute for Biomedical Research, Osaka, Japan	50/69/73
Introcoporeal Kinetics of Apropterin-Hydrochloride through a Variety of Administering Pathways in Rats and Mice	PHN-112-PK-SR	8890101	Suntory Ltd. Medicine Center in Gunma, Japan,	50
Repeat Oral Dose Toxicokinetics of Phenoptin™ (6R-BH4) in CD-1 Mice*	0162-06-012	05JM-090		56
Pharmacokinetics of Sapropterin Hydrochloride in the Rat (II) (Repeated Dose Administration)	PHN-105-PK-SR	8886202	Suntory Ltd. Biopharma Tech Center, Gunma, Japan.	50
Oral (Gavage) Toxicokinetic Study of Phenoptin™ (Tetrahydrobiopterin; 6RBH4) in Presumed Pregnant NZW Rabbits*	0162-05-018	05JM-090		57
<u>Distribution</u>				
Plasma Protein Binding and Distribution into Blood Cells of Sapropterin Hydrochloride	PHN-106-PK-SR	8886202	Bio-Pharm Tech Center, Suntory Ltd. Ohra-gun, Gunma Prefecture, Japan	73
Fetal Migration and Milk Migration of Sapropterin Hydrochloride	PHN-107-PK-SR	8886202	Bio-Pharm Tech Center, Suntory Ltd. Oura-gun, Gunma Prefecture, Japan	58
<u>Metabolism</u>				
A Study on Sapropterin Hydrochloride Metabolites in Rat Urine	PHN-109-PK-SR	8886202	Suntory Biomedical Research Center in Osaka, Japan	64
A Study on Liver Microsomal Drug-Metabolizing Enzyme Induction by Sapropterin Hydrochloride	PHN-108-PK-SR	8886202	Suntory Ltd. Biopharma Tech Center in Gunma, Japan	64
Dosage Setting Tests of Pterin (Oral) in Mice	PHN-85-AT-SR	300603R	Medicine Center, Suntory, Ltd. Yuraki-gun, Gunma, Japan	128
An Acute Toxicity Test of Pterin (oral) in Mice	PHN-86-AT-SR	300603R	Medicine Center, Suntory, Ltd. Yuraki-gun, Gunma, Japan	128

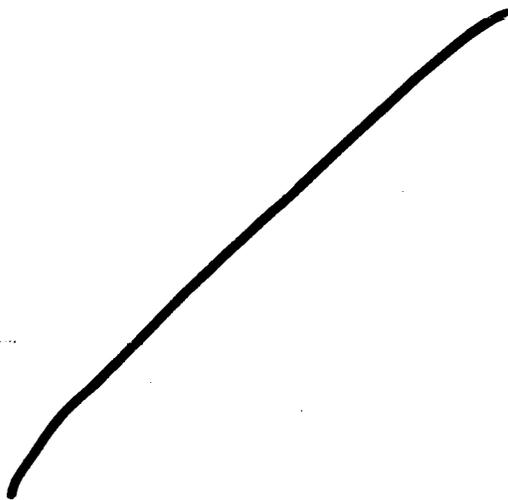
A Dosage Setting Test of Dihydrobiopterin (oral and Intravenous) in Mice	PHN-87-AT-SR	300604R	Medicine Center, Suntory, Ltd. Yuraki-gun, Gunma	128	
An Acute Toxicity Test of Dihydrobiopterin (oral and Intravenous) in Mice	PHN-88-AT-SR	300604R	Medicine Center, Suntory, Ltd. Yuraki-gun, Gunma, Japan	128	
<u>Other Pharmacokinetic Studies</u>					
Endogenous  Levels in Various Species*	PHN-103-PK-SR	---	Suntory Institute for Biomedical Research, Osaka, Japan.	77	
Toxicology					
<u>Single Dose Toxicity</u>					
<u>Mouse:</u>					
Acute Toxicity Study (oral)	PHN-57-AT-SR	8885Y05		83	
Acute Toxicity Study (i.v)	PHN-58-AT-SR	8885Y05		83	
Acute Toxicity Study (Subcutaneous Administration)	PHN-59-AT-SR	8885Y05		84	
<u>Rat:</u>					
Acute Toxicity Study (oral)	PHN-60-AT-SR	8885Y05		83	
Acute Toxicity Study (i.v)	PHN-61-AT-SR	8885Y05		83	
Acute Toxicity Study (Subcutaneous Administration)	PHN-62-AT-SR	8885Y05		84	
<u>Marmoset monkey:</u>					
Acute Toxicity Study (oral)	HN-63-AT-SR	8886506		84	
Acute Toxicity Study (i.v.)	PHN-64-AT-SR	8886506	84		
Acute Toxicity Study (Subcutaneous Administration)	PHN-65-AT-SR	8886506	85		
<u>Repeat Dose Toxicity</u>					
<u>Rat:</u>					
2-Week Toxicity Study	PHN-66-AT-SR	8885Y05	86		
13-Week Toxicity Study with a 5-Week Recovery Period	PHN-67-AT-SR	88886506	88		
52-Week Toxicity Study (oral)	PHN-70-AT-SR	8887402 8887301 8887Z05	91		
4-Week Preliminary Toxicity Study (oral)	PHN-115-AT-SR	8887904	97		

<u>Marmoset Monkey:</u>					
4-Week Preliminary Toxicity Study (oral)	PHN-68-AT-SR	8886506		100	
13-Week Toxicity Study with a 4-Week Recovery Period	PHN-69-AT-SR	8886506		104	
52-Week Toxicity Study with a 4-Week Recovery Period	PHN-71-AT-SR	8886506		108	
		8886607			
		8886708			
<u>Mouse:</u>					
13-Week Preliminary Toxicity Study (oral)	PHN-117-AT-SR	8887904			114
<u>Genetic toxicology Study</u>					
Reverse mutation test in bacteria	PHN-84-AT-SR	8885Y05		131	
Chromosome aberration test with Chinese hamster lung cells	PHN-84-AT-SR	8885Y05		134	
Micronucleus Test of SUN 0588 Using Mice (single dose)	PHN-84-AT-SR	8885Y05		137	
Micronucleus Test of SUN 0588 Using Mice (repeated dose)*	PHN-89-AT-SR	8889403	Medicine Center, Suntory, Ltd. Yuraku-gun, Gunma, Japan	139	
<u>Carcinogenicity Studies:</u>					
104-Week Oncogenicity Study (oral) in Rats	PHN-116-AT	8887904 8888301 8888302 8888503 8888908 8889101 8889302 8889403		143	
78-Week Oncogenicity Study (oral) in Mice	PHN-118-AT-SR	8888302 8888907		172	
<u>Reproductive and Developmental Toxicology:</u>					
Segment I (oral) fertility and reproductive performance study in rats	PHN-77-AT-SR	8886506		194	
Segment II (oral) teratogenicity study in rats	PHN-78-AT-SR	8885Z06		201	
Segment II (oral) teratogenicity study in rats (Supplemental Study)	PHN-79-AT-SR	8886405		217	

Dose-finding study for segment II in rabbits	PHN-80-AT-SR	8886101 8885Z06 8886405		223
Segment II (oral) teratogenicity study in rabbits	PHN-81-AT-SR	8886101 8886607		222
Segment III (oral) pre- and post-natal development in rats (segment III)	PHN-82-AT-SR	K7F98T K8B78T		229
<u>Other toxicity studies</u>				
Acute Toxicity Study:				
<u>Mouse:</u>				
Acute Oral Toxicity Study in the Three Week Old Mouse	PHN-72-AT-SR	8888302		118
<u>Rats:</u>				
Acute Toxicity Testing (oral) in 7-day old Rats	PHN-75-AT-SR	8888301	Suntory, Ltd. Pharmaceutical Center, Oura-gun, Gunma, Japan	118
Acute Oral Toxicity Study in the Three Week Old Rat	PHN-73-AT-SR	8888302		119
Repeat Toxicity Study:				
<u>Rats:</u>				
2-week Toxicity Study (oral) in 7 Days Old Rats	PHN-76-AT-SR	8888706	Medicine Center, Suntory, Ltd. Yuraku-gun, Gunma, Japan	120
4-Week Toxicity Study (oral) in 21 Days Old Rats	PHN-74-AT-SR	8888302		123
Special toxicology studies				
<u>Antigenicity</u>				
SUN 0588 Antigenicity Test	PHN-83-AT-SR	8887Z05		243
<u>Local Stimulation</u>				
Local Stimulation Tests via Intraperitoneal Administration*	PHN-100-AT-SR	---	Suntory Biomedical Research Institute, Japan	242

*: New studies submitted and were reviewed under NDA 22-181. Most of the studies were reviewed under IND 69,708, and were incorporated in the DNA review.

Studies not reviewed within this submission:



2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Sapropterin dihydrochloride is the hydrochloride salt of R-tetrahydrobiopterin (6R-BH₄), which is a coenzyme of phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TPH), and contributes to the biosynthesis of monoamine neurotransmitters [e.g. dopamine (DA), norepinephrine (NE), serotonin (5-HT)]. 6R-BH₄ is the naturally occurring pteridine, 6R-L-erythro-5,6,7,8 tetrahydrobiopterin (6R-THBP) and is only biochemically active in the enantiomeric R form.

Nonclinical pharmacology studies conducted to characterize sapropterin HCl have shown an increase in release of endogenous 6R-THBP without affecting monoamine levels from normal rat striatum and activation of both the dopaminergic and serotonergic systems, as detected by a decrease in [¹¹C]-NMSP signal using CT (PET), in normal rhesus monkey striatum. In animal models of intracerebral monoamine depletion, sapropterin HCl was shown to reverse DAHP (2,4-diamino-6-hydroxypyrimidine) and α -methyltyrosine (α -MT) induced depletions in monoamines. Sapropterin reversed increased phenylalanine (phe) in blood and liver caused by DAHP in rats. Increased maternal and fetal serum Phe and liver Phe caused by DAHP was also reversed by administration of sapropterin to mother guinea pigs. Additionally, sapropterin HCl administration also attenuated α -MT induced decreases in motor activity. However, sapropterin HCl showed no effect on p-chlorophylalanine (p-CPA)-induced decreases in brain monoamine levels. Sapropterin reversed decreased endogenous production of 6R-BH₄ and at pharmacologic levels restored catecholamine production.

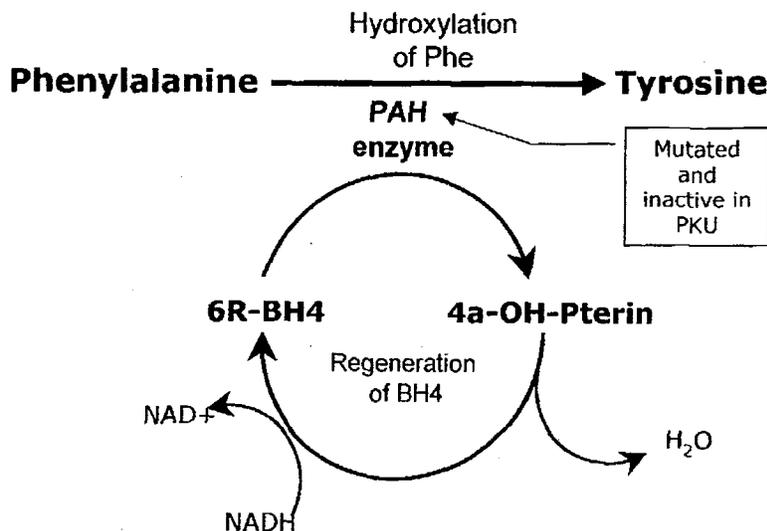
2.6.2.2 **Primary pharmacodynamics:** Sapropterin HCl has been demonstrated to reduce plasma phenylalanine level in patients with PKU/Hyperphenylalaninemia.

Mechanism of action:

The mechanism by which exogenous 6R-BH4 lowers Phe levels in some hyperphenylalaninemia (HPA)/PKU patients not having a 6R-BH4 deficiency and thus having normal or even elevated levels of endogenous 6R-BH4 has not been well established. It is proposed that mutations in the Phenylalanine hydroxylase (PAH) gene reduce or eliminate the activity of the enzyme phenylalanine hydroxylase (PAH), whose primary role is to hydroxylate phenylalanine (Phe) to tyrosine. PAH is primarily found in the liver, where it utilizes 6R-BH4 as its natural cofactor in Phe hydroxylation. PAH-mediated hydroxylation of Phe is the primary output in Phe homeostasis, and is the requisite first step in total oxidation of Phe to CO₂ and H₂O (Figure 1), (Scriver CR, 2001, McGraw-Hill). 6R-BH4 is a natural cofactor that binds to PAH and then reacts with oxygen to form an active intermediate that hydroxylates Phe, turning it into tyrosine (Kaufmsan, S. 1986 J. Pediatr.).

Three mechanisms of pharmacological action of 6R-BH4 that result in an increase in PAH activity include: 1) the “Km mutant theory” which postulates that there is reduced affinity between 6R-BH4 and the mutant PAHs that is reflected in a higher Michaelis-Menten constant, Km; a higher 6R-BH4 concentration shifts the equilibrium in favor of binding, 2) optimized regulation of PAH gene expression, and 3) activation or stabilization of the mutant PAH dimer form of the normally tetrameric PAH enzyme. Therefore, 6R-BH4 may normalize the physiology of Phe control, possibly through the activation, stabilization, or enhanced folding of the PAH enzyme.

Figure 1: Biochemistry of Phenylalanine Metabolism



Drug activity related to proposed indication: Sapropterin hydrochloride has been shown to lower blood phenylalanine levels clinically. Nonclinical pharmacology studies

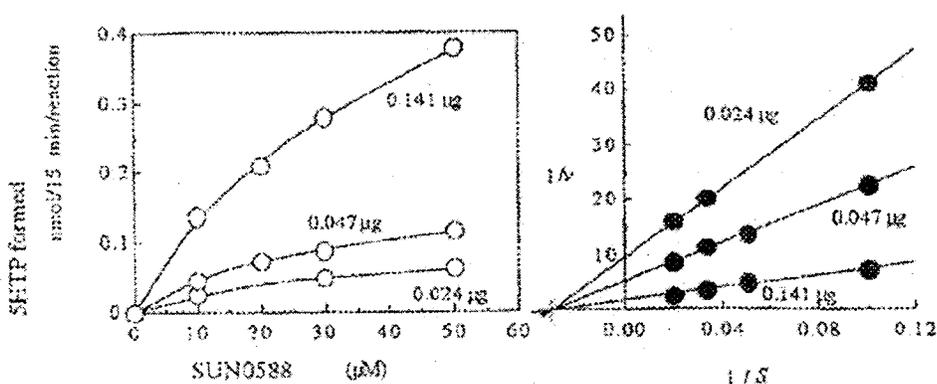
conducted to characterize sapropterin HCl have shown an increase in release of endogenous 6R-THBP without affecting monoamine levels from normal rat striatum and activation of both the dopaminergic and serotonergic systems, as detected by a decrease in [^{11}C]-NMSP signal using CT (PET), in normal rhesus monkey striatum. In animal models of intracerebral monoamine depletion, sapropterin HCl was shown to reverse DAHP (2,4-diamino-6-hydroxypyrimidine) and α -methyltyrosine (α -MT) induced depletions in monoamines. Sapropterin reversed increased phenylalanine (phe) in blood and liver caused by DAHP in rats. Increased maternal and fetal serum Phe and liver Phe caused by DAHP was also reversed by administration of sapropterin to mother guinea pigs.

Primary pharmacodynamics: (* New Studies submitted and were reviewed under NDA 22-181)

Role of SUN 0588 (sapropterin) as a Coenzyme in the Enzymatic Hydroxylation Reactions of Tryptophan and Phenylalanine (Study #: PHN-90-PC-SR)*

In this non-GLP study, tryptophan hydroxylase (TPH), obtained from mouse mastocytoma cells, was activated by anaerobic incubation with dithiothreitol (DTT). The activated enzyme was then incubated with catalase, sapropterin (4.5 -75.5 nmol), NADH, dihydropteridine reductase (DPR), and the substrate, tryptophan. HPLC was used to measure the reaction product, 5-HTP. As shown in Figure 1, the amount of 5-hydroxy-L-tryptophan (L-5HTP) formed indicates the typical Michaelis-Menten-type SUN 0588 dependence, and the K_m value computed from the Lineweaver-Burk plot was $32.6 \pm 1.7 \mu\text{M}$.

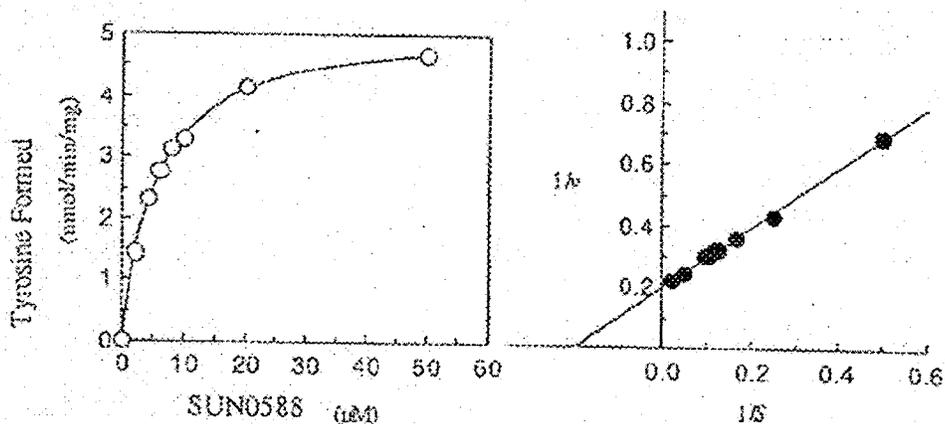
Figure 1: Dependence of mouse mastocytoma tryptophan hydroxylase activity on SUN 0588 concentration : Computation of Michaelis constant



The concentration of tryptophan, the amino acid substrate, was fixed at 200 μM .

Rat phenylalanine hydroxylase (PAH) was incubated with NADH, DPR, sapropterin (0-50 μM), and its substrate, L-phenylalanine. HPLC was used to measure the reaction product of PAH, tyrosine. The K_m for SUN 0588 was 5.17 μM (Figure 2).

Figure 2: Dependence of rat liver phenylalanine hydroxylase activity on SUN 0588 concentration : Computation of Michaelis constant



The concentration of phenylalanine, the amino acid substrate, was fixed at 200 μ M.
The amount of alanine hydroxylase was assumed to be 200 μ g/assay.

The sponsor stated that the K_m values derived from the above-mentioned reactions matched the expected values from the literature, and chemically synthesized sapropterin exhibited bioactivity equivalent to the endogenous cofactor, (6R)-5,6,7,8-tetrahydrobiopterin.

Effects of Sapropterin on 6R-BH4 and Release of Monoamines from Rat Striatum (Study #: 0162-06-011)*

Under diethyl ether anesthesia, a microdialysis probe was stereotactically inserted into the striatum of male Wistar rats weighing 250-300 grams in this non-GLP study. The probe was continuously perfused with Ringer's solution (9.5 μ L/min). Every 20 min, the effluent was collected in tubes containing 20 μ L perchloric acid. 6R-BH4 and dopamine (DA) levels were quantified using an electrochemical HPLC method.

When the striatum was dialyzed with Ringer's solution containing varying concentrations of 6R-BH4 (0.25, 0.5 and 1.0 mM), recovery of DA through the dialysis membrane increased in a concentration-dependent manner. Maximal DA levels were observed at 2-hrs post-infusion and persisted to 4-hrs post-infusion. The 6R-BH4-induced increase in DA levels in dialysates was not a result of inhibition of DA uptake because nomifensine (20-200 mg/kg), which is a specific inhibitor of DA uptake did not block 6R-BH4-induced DA release. The 6R-BH4-induced increase in DA levels in dialysates was abolished after pretreatment with tetrodotoxin (50 μ M) added to the perfusion fluid. These results suggest that sapropterin has transient DA releasing activity that is independent of the biosynthesis of DA.

Effect of SUN 0588 on the Total Intracerebral Content of [REDACTED] and Intracerebral Monoamine Metabolism in Juvenile and Mature Rats (Study #: PHN-92-PC-SR)*

In this non-GLP study, male and female Wistar rats at 3 days, 1, 2, 3 and 9 weeks, and 2 years of age were administered a single i.p. dose of sapropterin (2, 10 or 50 mg/kg) and anterior cerebellum tissue was homogenized 1-hr post-dose to measure total [REDACTED] and catecholamine content by HPLC. In a second part of the study, 3-day old rats were administered multiple i.p. doses (5, 12 or 19 total doses, once daily) of 2, 10 or 50 mg/kg sapropterin. Anterior cerebellum tissue was treated in the same manner with total [REDACTED] and catecholamine content measured. In a third phase of the study, 7-day and 9-week old rats were administered a single i.p. dose of sapropterin at 2, 10 or 50 mg/kg and anterior cerebellum tissue was sampled at 15 min, 1, 2, 4 or 8 hrs post-dose to measure total [REDACTED] and monoamine content. The following monoamine: noradrenaline (NA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), serotonin (5-HT), and 5 hydroxyindoleacetic acid (5-HIAA) were determined.

Total [REDACTED] content in brain homogenate dose-dependently increased in all rats. However, no dose-dependent relationship or correlation between sapropterin dose and neurotransmitter levels (DA, 5-HT, NA) and their metabolites (DOPAC, HVA and 3-MT) in the anterior cerebellum were observed in any animal (see Tables 1, 2, 3 and 4 below).

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Table 1: Effect of SUN 0588 on the contents of dopamine and its metabolites in the rat brain (anterior cerebellum)

Anterior cerebellum	Aged 3 days	Aged 1 week	Aged 2 weeks	Aged 3 weeks	Aged 9 weeks	Aged 2 years
DA						
saline	98.1±2.2	157.5±5.0	182.1±1.2	213.7±5.0	582.2±15.6	589.5±25.6
vehicle	94.4±3.7	154.4±1.0	188.5±2.9	217.2±8.1	578.8±16.4	654.0±27.7
SUN 0588 2 mg/kg	90.8±2.9	157.7±4.1	182.3±2.1	200.9±1.3	582.2±22.4	581.0±42.8
10 mg/kg	92.7±3.5	182.0±5.8	175.4±3.7 *	218.6±9.3	574.6±19.1	556.4±12.4 **
50 mg/kg	85.0±11.4	182.0±4.7	181.1±1.5	231.2±5.8	565.7±19.0	613.2±37.7
DOPAC						
saline	15.5±0.4	34.9±0.9	38.4±0.6	42.2±1.0	80.6±4.4	73.9±4.1
vehicle	15.4±0.8	35.6±0.8	43.4±0.9 **	42.8±2.0	90.3±5.1	93.8±4.3 **
SUN 0588 2 mg/kg	14.9±0.4	33.5±1.2	47.4±1.8	48.8±1.9 *	114.7±1.8 **	99.1±7.7
10 mg/kg	14.3±0.8	33.3±0.9	45.9±3.1	48.4±2.5	94.7±2.9	95.9±2.9
50 mg/kg	15.6±1.9	33.2±0.7 *	47.0±0.6 *	51.5±2.0 *	95.0±3.9	106.7±9.5
HVA						
saline	34.9±1.0	42.3±1.5	45.7±1.0	32.0±0.7	28.1±1.3	31.0±2.9
vehicle	37.8±1.0	41.9±1.4	49.1±0.9 *	34.0±2.2	32.3±2.0	37.2±2.5
SUN 0588 2 mg/kg	35.1±1.5	43.9±1.7	46.2±0.5 *	30.8±1.1	37.1±1.7	32.6±2.8
10 mg/kg	35.8±2.0	44.1±1.2	45.8±0.5 *	33.2±1.6	35.4±2.3	32.4±1.3
50 mg/kg	32.3±4.5	47.2±2.3	45.8±1.2	35.4±1.1	38.4±1.4	41.4±4.6
(DOPAC+HVA)/DA						
saline	0.515±0.019	0.491±0.010	0.487±0.004	0.353±0.008	0.183±0.004	0.178±0.007
vehicle	0.584±0.010 *	0.510±0.013	0.487±0.010 *	0.353±0.012	0.212±0.011	0.201±0.009
SUN 0588 2 mg/kg	0.553±0.012	0.492±0.009	0.497±0.009	0.395±0.004 *	0.257±0.006 **	0.228±0.005 *
10 mg/kg	0.544±0.028	0.489±0.009	0.503±0.005	0.378±0.004	0.227±0.008	0.230±0.009 *
50 mg/kg	0.571±0.033	0.504±0.017	0.487±0.008	0.378±0.008	0.234±0.007	0.240±0.012 *

The test drug was intraperitoneally given as a single dose.

Each figure indicates the mean±S.E. of 5-6 rats.

Units: ng/g tissue

Significantly different from the vehicle group: *, p < 0.05; **, p < 0.01

Significantly different between the saline and vehicle groups: #, p < 0.05; ##, p < 0.01

Table 2: Effect of SUN 0588 on the contents of noradrenaline and serotonin and their metabolites in the rat brain (anterior cerebellum)

Anterior cerebellum	Aged 3 days	Aged 1 week	Aged 2 weeks	Aged 3 weeks	Aged 9 weeks	Aged 2 years
NA						
saline	43.8±1.2	50.6±2.1	48.6±0.8	53.8±1.5	130.8±2.4	163.1± 7.2
vehicle	42.7±2.6	48.8±1.0	49.1±2.0	54.0±1.5	140.4±3.1 *	180.9± 9.1
SUN 0588 2 mg/kg	43.9±1.3	50.1±1.5	52.3±2.0	54.1±0.7	138.0±8.0	187.2±12.8
10 mg/kg	43.8±1.3	51.2±1.6 *	48.7±1.5	56.0±1.9	135.2±5.3	189.1± 2.7
50 mg/kg	33.4±3.5	51.8±1.5 *	52.1±1.9	82.8±0.8 ***	134.0±5.7	171.5±10.9
5-HT						
saline	82.4±2.5	118.5± 8.0	105.5±1.4	118.0±7.3	308.8± 1.9	283.7±12.3
vehicle	71.8±3.8	107.1± 1.1	104.3±1.4	103.8±5.1	322.2± 8.3	297.7±15.2
SUN 0588 2 mg/kg	59.4±1.5 *	112.8± 4.1	106.8±3.5	99.8±2.5	275.9± 8.2**	255.2±13.8
10 mg/kg	83.8±2.8	111.2± 4.7	102.9±4.8	99.5±8.9	280.8± 5.2**	254.3± 1.8*
50 mg/kg	80.8±4.3	118.1±10.3	105.5±2.9	107.0±4.8	305.1±12.2	270.7±18.2
5-HIAA						
saline	49.1±2.6	129.1± 9.8	159.2±3.0	163.6± 7.3	281.1±12.9	248.9±12.5
vehicle	62.8±3.9 *	120.8± 5.8	181.4±8.1	155.8± 9.8	288.9±11.8	281.4±14.5
SUN 0588 2 mg/kg	40.7±0.8 **	121.3± 4.0	189.4±4.8	145.0± 4.8	324.3± 7.5**	281.8±15.8
10 mg/kg	52.0±3.0	118.0± 5.3	165.9±8.8	138.1±18.8	295.7± 5.2*	248.5± 2.5
50 mg/kg	46.8±3.8 **	133.3±14.8	153.4±6.5	154.2± 5.5	313.1±12.0*	286.4±23.7
5-HIAA/5-HT						
saline	0.786±0.022	1.081±0.033	1.510±0.034	1.482±0.038	0.817±0.038	0.946±0.036
vehicle	0.873±0.025 *	1.149±0.045	1.549±0.062	1.486±0.041	0.828±0.027	0.946±0.024
SUN 0588 2 mg/kg	0.687±0.018 **	1.134±0.033	1.588±0.021	1.388±0.060	1.178±0.024 ***	1.026±0.018 *
10 mg/kg	0.849±0.043	1.047±0.043	1.808±0.031	1.481±0.055	1.058±0.036 ***	0.978±0.013
50 mg/kg	0.767±0.020 **	1.109±0.048	1.455±0.048	1.448±0.047	1.061±0.031 ***	1.131±0.020 ***

The test drug was intraperitoneally given as a single dose.

Each figure indicates the mean±S.E. of 5-6 rats.

Units: ng/g tissue

Significantly different from the vehicle group: *, p < 0.05; **, p < 0.01; ***, p < 0.001

Significantly different between the saline and vehicle groups: #, p < 0.05

Table 3: Effect of SUN 0588 on the contents of dopamine and its metabolites in the rat brain (anterior cerebellum)

Anterior cerebellum	Age in days		
	7	14	21
DA			
saline	99.5±2.4	119.0±5.8	167.3±6.8
vehicle	100.7±2.1	123.8±5.5	186.5±5.5
SUN 0588 2 mg/kg	104.0±2.4	135.1±5.7	187.4±12.4
10 mg/kg	109.2±2.7 *	122.5±5.0	171.5±12.3
50 mg/kg	106.2±2.1	130.6±4.6	182.5±2.5
DOPAC			
saline	17.9±0.4	18.4±0.8	23.5±0.9
vehicle	17.3±0.5	19.7±0.9	27.4±0.6 **
SUN 0588 2 mg/kg	18.5±0.4	21.4±0.9	28.1±2.6
10 mg/kg	20.5±0.7 **	19.9±1.1	25.6±2.0
50 mg/kg	20.5±0.4 ***	21.5±0.7	26.6±0.6
HVA			
saline	38.9±0.9	38.5±2.2	34.5±1.0
vehicle	40.6±1.1	43.0±1.5	38.6±1.5 *
SUN 0588 2 mg/kg	42.1±0.6	47.4±2.4	36.7±2.5
10 mg/kg	46.1±1.2 **	42.0±1.4	36.6±2.5
50 mg/kg	46.0±1.0 **	46.4±1.6	41.3±1.6
(DOPAC+HVA)/DA			
saline	0.572±0.011	0.477±0.012	0.348±0.008
vehicle	0.575±0.011	0.480±0.023	0.355±0.014
SUN 0588 2 mg/kg	0.584±0.011	0.509±0.008	0.345±0.008
10 mg/kg	0.610±0.007 *	0.506±0.011	0.364±0.009
50 mg/kg	0.627±0.016 *	0.521±0.011	0.373±0.007

The test drug was intraperitoneally given once daily at the age of 3 days and later.

Each figure indicates the mean±S.E. of 5-6 rats.

Units: ng/g tissue

Significantly different from the vehicle group: *, p < 0.05; **, p < 0.01; ***, p < 0.001

Significantly different from the saline group: #, p < 0.05; ##, p < 0.01

Table 4: Effect of SUN 0588 on the contents of adrenalin and serotonin and their metabolites in the rat brain (anterior cerebellum)

Anterior cerebellum	Age in days		
	7	14	21
NA			
saline	42.5±1.1	40.9±2.2	54.0±2.3
vehicle	40.1±1.3	40.1±1.6	59.1±2.0
SUN 0588 2 mg/kg	44.9±1.0 *	45.1±1.7	65.2±1.9
10 mg/kg	45.6±1.9 *	43.4±1.0	56.6±2.6
50 mg/kg	44.3±1.2 *	42.8±1.1	58.9±1.1
5-HT			
saline	68.7±4.3	60.1±3.5	79.4±3.9
vehicle	59.6±3.7	56.7±2.8	83.0±5.0
SUN 0588 2 mg/kg	76.0±4.6 *	63.1±2.9	95.5±4.6
10 mg/kg	68.0±2.8	59.1±2.1	80.6±3.6
50 mg/kg	75.2±5.0 *	62.4±2.9	85.8±2.8
5-HIAA			
saline	58.1±4.6	67.1±3.9	85.5±4.8
vehicle	47.6±3.0	64.0±2.7	103.7±2.6 **
SUN 0588 2 mg/kg	68.0±6.1 *	73.5±3.4	103.2±4.9
10 mg/kg	54.8±2.1	66.6±2.3	100.7±4.2
50 mg/kg	68.2±6.7 *	69.7±3.6	98.9±2.6
5-HIAA / 5-HT			
saline	0.843±0.027	1.119±0.025	1.076±0.024
vehicle	0.801±0.027	1.134±0.029	1.267±0.057 *
SUN 0588 2 mg/kg	0.838±0.031	1.165±0.021	1.086±0.043 *
10 mg/kg	0.807±0.017	1.128±0.011	1.253±0.040
50 mg/kg	0.898±0.038	1.117±0.025	1.156±0.027

The test drug was intraperitoneally given once daily at the age of 3 days and later.

Each figure indicates the mean±S.E. of 5-6 rats.

Units: ng/g tissue

Significantly different from the vehicle group: *, p < 0.05

Significantly different from the saline group: #, p < 0.05; ##, p < 0.01

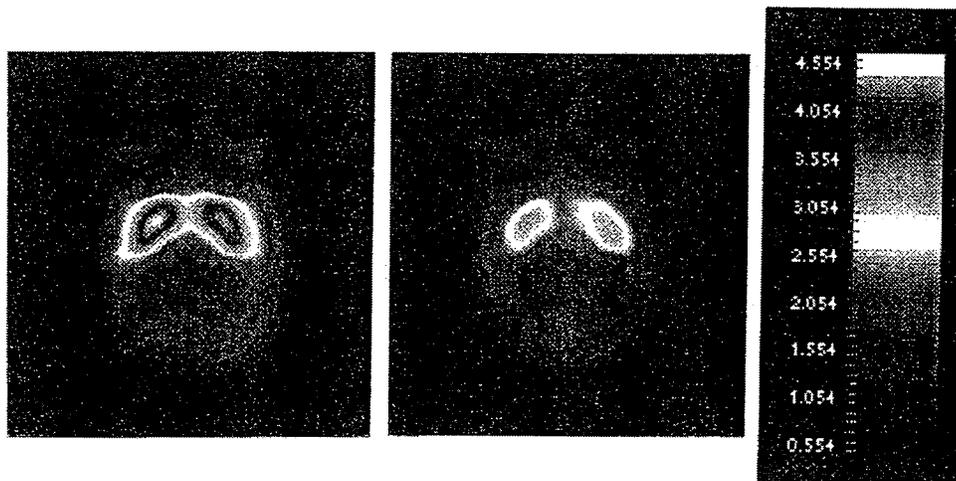
In juvenile rats aged 14 days and under, single or repeated intraperitoneal administration at a low dose level (≤ 10 mg/kg) caused the total intracerebral NA level to increase significantly without any difference between ages. However, the dose administered was 5 times higher in animals aged 9 weeks than in animals aged 7 days. The peak total intracerebral NA level in the former was slightly lower (approximately 4 times higher than the endogenous level) than in the latter (approximately 5-6 times higher than the endogenous level). Thus, the increase in the total intracerebral NA content differed greatly between animals aged 7 days and 9 weeks.

Effects of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin on the Dopaminergic and Cholinergic Receptors as Evaluated by Positron Emission Tomography in the Rhesus Monkey (Study #: PHN-119-PC-SR)

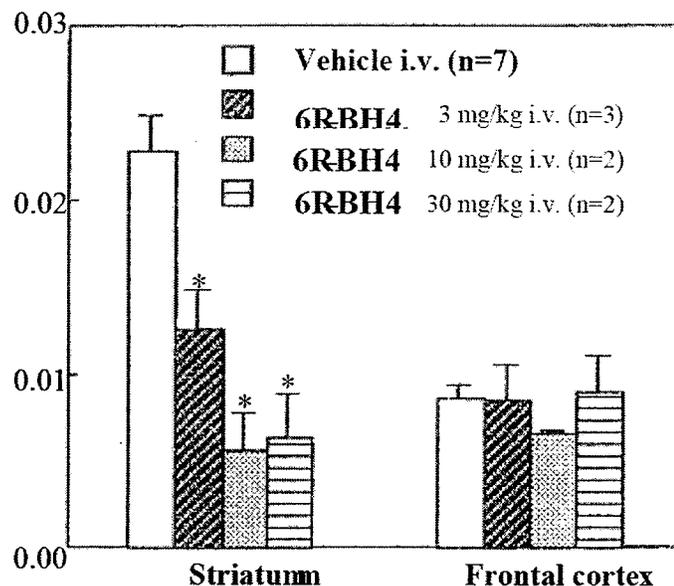
A positron CT (PET) [scanner] was used to study the effects of sapropterin HCl on the dopaminergic nervous system within the brain of the rhesus monkey. [^{11}C]N methylspiperone ([^{11}C]NMSP) was used as the radioligand to measure binding with the DAD_2 receptor. Sapropterin HCl (3, 10, 30 mg/kg i.v.) was administered 1 hr before the administration of the radioligand.

The administration of sapropterin HCl markedly decreased the uptake of [^{11}C]NMSP into the striatum. The uptake of [^{11}C]NMSP was highest in the striatum, moderate in the cerebral cortex, and low in the cerebellum (Figure 1). Administration of sapropterin HCl at 3 mg/kg (i.v.) markedly decreased the uptake of [^{11}C]NMSP into the striatum. Furthermore, the association rate constant, k_3 , for the [^{11}C]NMSP and the striatum dopamine D_2 receptor, which is determined from the gradient of the striatum/cerebellum radioactivity ratio, decreased dose-dependently as the result of the administration of 3, 10, and 30mg/kg (i.v.) of sapropterin HCl (Figure 2). The sponsor postulated that this result was attributable to sapropterin HCl promotion of striatum DA release suggesting that sapropterin HCl activates the dopaminergic and serotonergic systems of the striatum.

Figure 1: Effects on [^{11}C]N-methylspiperone Binding (cumulative images 25-60 min. after ligand administration)



Horizontal cross-sections including striatum: (left, vehicle (iv.) administration; right, (iv.) administration of 3 mg/kg of sapropterin HCl).

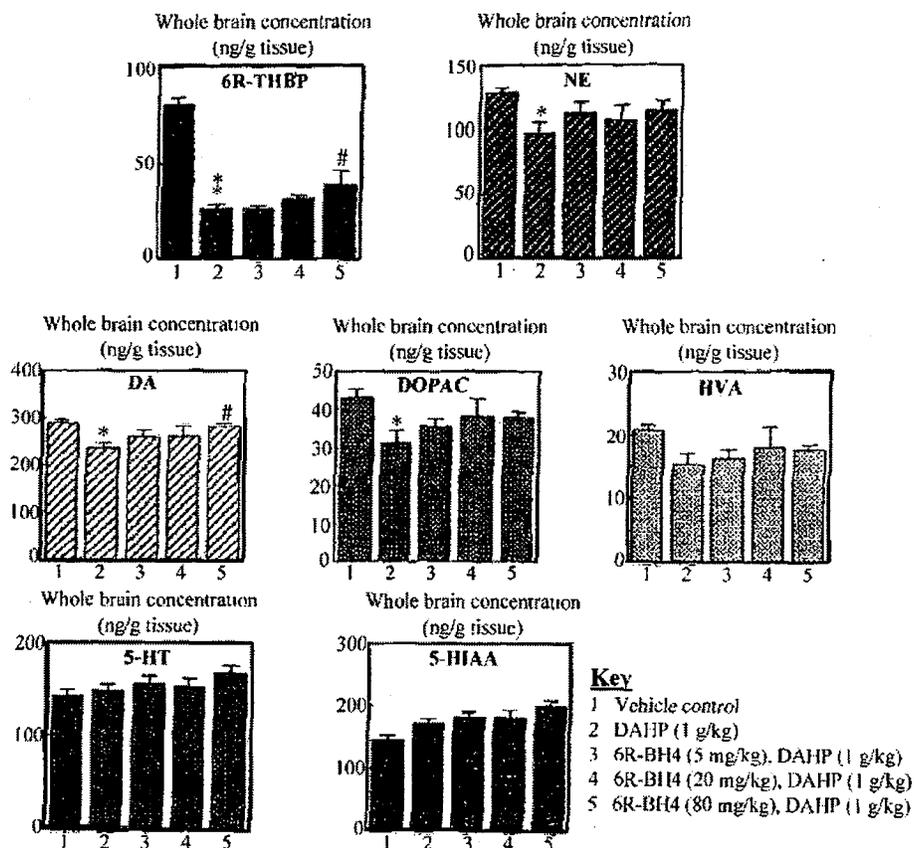
Figure 2: Effects of Sapropterin HCl [¹¹C]NMSP on Association Rate Constant k₃

Each column shows the mean \pm standard deviation for 2-7 animals. A significant difference exists between vehicle administration groups at * $p < 0.05$.

Effects of Sapropterin on 2,4-Diamino-6-Hydroxypyrimidine (DAHP)- Induced Decreases in the Intracerebral 6R-BH4 and Monoamines Levels

One g/kg of DAHP (2,4-diamino-6-hydroxypyrimidine), an inhibitor of the rate-limiting enzyme (GTP Cyclohydrolase) for 6R-THBP biosynthesis, was administered to rats i.p. four times; every four hrs over a 12 hr period (e.g. at 18:00, 22:00, 2:00, 6:00). Sapropterin (5, 20, 80 mg/kg; p.o.) was administered 1 hr after (e.g., at 7:00) the final dose of DAHP. Three hours later (e.g., at 10:00), the brains were extracted under ether anesthesia. Brain monoamine and 6R-THBP levels were quantified using HPLC methodology. As a result of DAHP administration, the 6R-THBP content of the brains decreased to about 30% of that of the control group, and the contents of NE, DA, and DOPAC also decreased significantly. DAHP-induced reductions in 6R-THBP and DA contents were restored significantly by 80 mg/kg of sapropterin HCl. DAHP-induced NE, DA, DOPAC and homovannilic acid (HVA) reductions also tended to be reversed. Conversely, 5-HT and 5-hydroxy-3-indoleacetic acid (5-HIAA), failed to decrease with DAHP administration and little change was observed with sapropterin HCl administration) (Figure below).

Figure: Effects of Oral Administration of Sapropterin HCl on Intracerebral 6R-BH4 and Monoamine Contents in DAHP-Treated Rats



Each column shows the mean ± standard deviation for 5-6 animals. *p<0.05, **p<0.01. #p<0.05 (Duncan's new multiple range test).

Action of R-THBP and Creation of Model of Atypical Hyperphenylalaninemia Caused by Tetrahydrobiopterin Deficiency (Study #: PHN-91-PC-SR)*

In this non-GLP study, Hartley guinea pigs, in late stage pregnancy (identified by the start of the separation of the pubic bone), were administered DAHP (2,4-diamino-6-hydroxypyrimidine) (1g/kg, p.o.) or saline (control, p.o.) every 12 hrs for 3 days. Six hrs after the final administration, animals were euthanized and fetal and maternal blood and livers were sampled; six hrs after the final administration of DAHP, a subset of animals received 20 mg/kg R-THBP (p.o.) followed 3 hrs later by tissue sampling. Maternal and fetal serum phenylalanine (Phe), liver Phe and total **————** were measured by HPLC.

DAHP significantly decreased total **————** and increased Phe levels in fetal and maternal blood and livers compared to controls (Table 1). Treatment with R-THBP

reversed this effect by decreasing Phe and increasing total α -MSA levels to or above control levels in both dams and fetuses (Table 1). This study demonstrated that R-THBP, when administered p.o. to dams, is absorbed via the gastrointestinal tract and increases the maternal blood concentration of total α -MSA which subsequently increases the total α -MSA levels in the fetal liver and blood.

Table 1: Action of R-THBP with Respect to Blood Phenylalanine, Blood Total α -MSA, and Liver Total α -MSA in the Atypical Hyperphenylalaninemia Mode

		In Blood		In Liver
		Phenylalanine (mg/dl)	Total α -MSA (pmol/mL)	Total α -MSA (μ g/g tissue)
Mothers	Control group	0.49 \pm 0.08 (5) **	36.7 \pm 4.0 (10) ***	0.67 \pm 0.04 (3) ***
	DAHP	2.61 \pm 0.42 (7)	8.4 \pm 2.2 (7)	0.09 \pm 0.02 (7)
	DAHP + R-THBP	1.04 \pm 0.07 (2)	15947 \pm 5349 (2)	8.00 \pm 2.51 (3)
Fetuses	Control group	0.81 \pm 0.03 (3) *	23.7 \pm 2.6 (7) **	0.42 \pm 0.05 (7) ***
	DAHP	5.27 \pm 1.16 (4)	12.1 \pm 0.3 (5)	0.03 \pm 0.003
	DAHP + R-THBP	1.98 \pm 0.24 (3) *	5366 \pm 1277 (3) *	(26)
				0.95 \pm 0.31 (5) *

The numbers represent the mean \pm standard deviation. Numbers within parentheses are the number of animals.

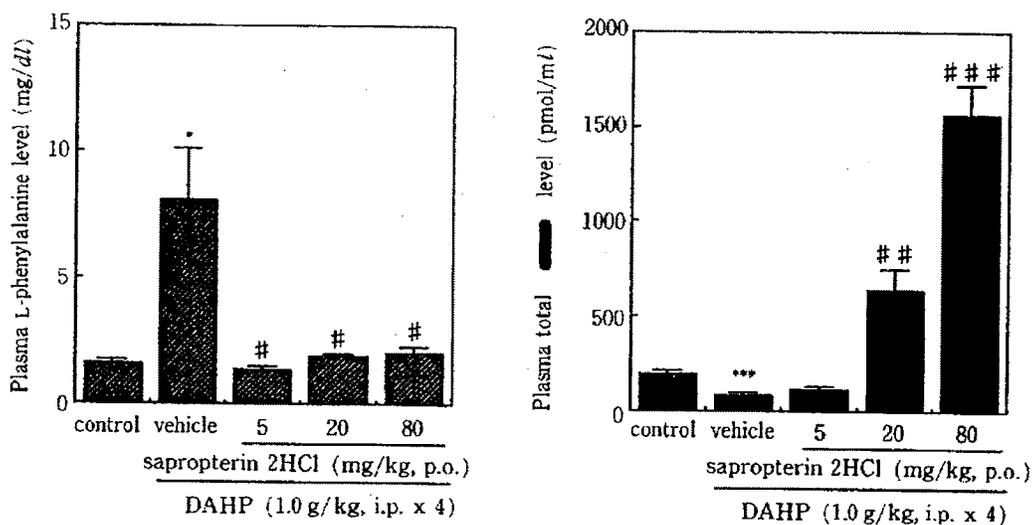
* p < 0.05, ** p < 0.01, *** p < 0.001 (Significant difference exists between DAHP group and control group or DAHP + R-THBP group. Student's t-test.)

The Effect of Sapropterin in Rats with Hyperphenylalaninemia Due to Defective Synthesis of Tetrahydrobiopterin (Study #: PHN-99-PC-SR)*

Female Wistar rats (age 4 weeks, 5-6/group) were assigned to five treatment groups: (1) control group (0.1 M PBS solution, pH 7.4, i.p.), (2) DAHP (i.p.) vehicle (p.o.) group, (3) DAHP (i.p.) and sapropterin (5 mg/kg, p.o.) group, (4) DAHP (i.p.) and sapropterin (20 mg/kg, p.o.) group, and (5) DAHP (i.p.) and sapropterin (80 mg/kg, p.o.) in this non-GLP study. A dose of 1.0 g/kg DAHP was administered i.p. four times at 4 hr intervals, and sapropterin was administered once (p.o.), 1 hr after the final administration of DAHP. Three hrs later, animals, under light ether anesthesia, were incised abdominally and blood was collected while the liver was removed. Total α -MSA and Phenylalanine (Phe) levels were measured in blood and liver by HPLC with a fluorescence detector.

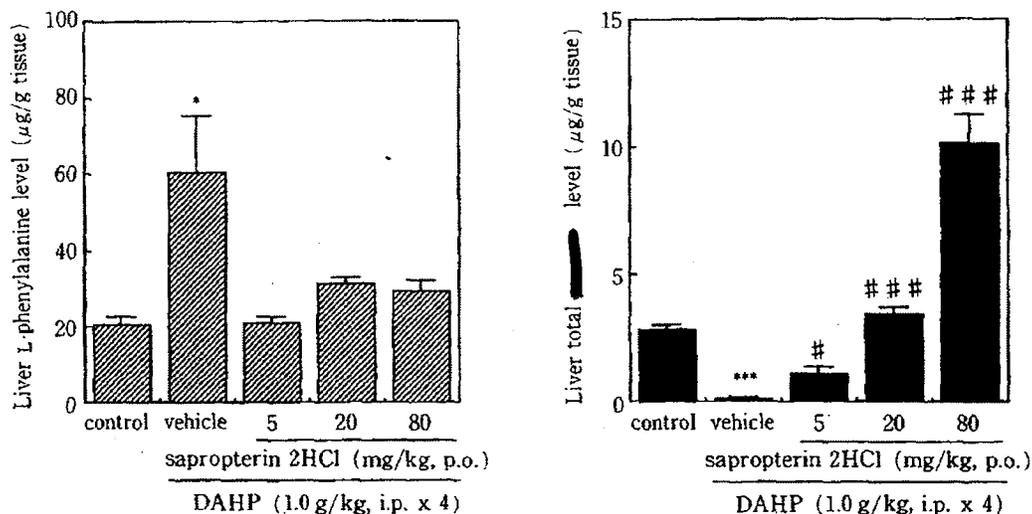
DAHP (1.0 g/kg, i.p.) reduced total **tryptophan** content in plasma to 43% of the control group, and total **tryptophan** quantity in the liver to 4% compared of the control group (Figures 1 and 2). Conversely, plasma Phe levels increased to approximately 5-fold greater than the control group levels, and liver Phe levels increased approximately 3-fold to that of control group (Figures 1 and 2). Total **tryptophan** levels, reduced markedly by i.p. DAHP administration, were increased dose-dependently in both plasma and liver after administration of sapropterin (5, 20, and 80 mg/kg, p.o.) (Figures 1 and 2). Additionally, Phe was decreased to the control group levels at all sapropterin dose levels (Figures 1 and 2).

Figure 1: Effects of Sapropterin on Hyperphenylalaninemia and Lowered Plasma Total **tryptophan Level Induced by 2, 4-diamino-6 hydroxypyrimidine (DAHP) in Rats**



Each column represents the mean \pm SEM of 5-6 rats. Ssignificantly different from the control at *: P < 0.05 and ***: P < 0.001, respectively. Significantly different from the DAHP-treated control at #: P < 0.05, ##: P < 0.01 and ###: P < 0.001, respectively.

Figure 2: Effects of sapropterin dihydrochloride on elevated L-phenylalanine level and lowered total α -MT level in the liver of DAHP-treated rats.

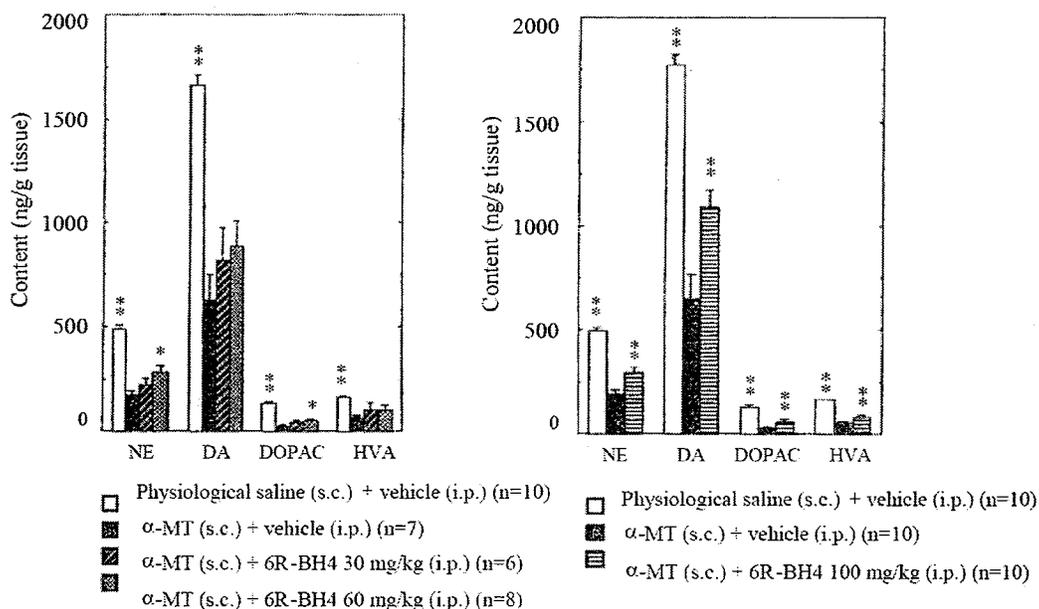


Each column represents the mean \pm SEM of 5-6 rats. Significantly different from the control at *: $P < 0.05$ and ***: $P < 0.001$, respectively. Significantly different from the DAHP-treated control at #: $P < 0.05$ and ###: $P < 0.001$, respectively.

Effects of Sapropterin on α -MT-Induced Decreases in the Intracerebral Catecholamine Content (Study #: PHN-93-PC-SR & 0162-06-006)

In this non-GLP study, 8 hrs after the subcutaneous (s.c.) administration of 250 mg/kg tyrosine hydroxylase inhibitor, α -methyltyrosine (α -MT), to mice (male ddY, aged 3 weeks), significant decreases in the mouse brain contents of NE, DA, and DOPAC and HVA were observed. Administration of sapropterin HCl at dose of 30, 60, and 100 mg/kg (i.p.), immediately after administration of α -MT, caused no effect at 30 mg/kg, but significant improvements in NE and DOPAC were observed in the 60 mg/kg group. Moreover, significant improvements were observed in the sapropterin HCl 100 mg/kg group for NE, DA, DOPAC, and HVA levels (Figure below).

Figure: Effects of Sapropterin HCl (i.p.) on Catecholamines Levels in the Brains (whole brain excluding cerebellum) of Mice Treated with α -methyltyrosine (α -MT) (s.c.)



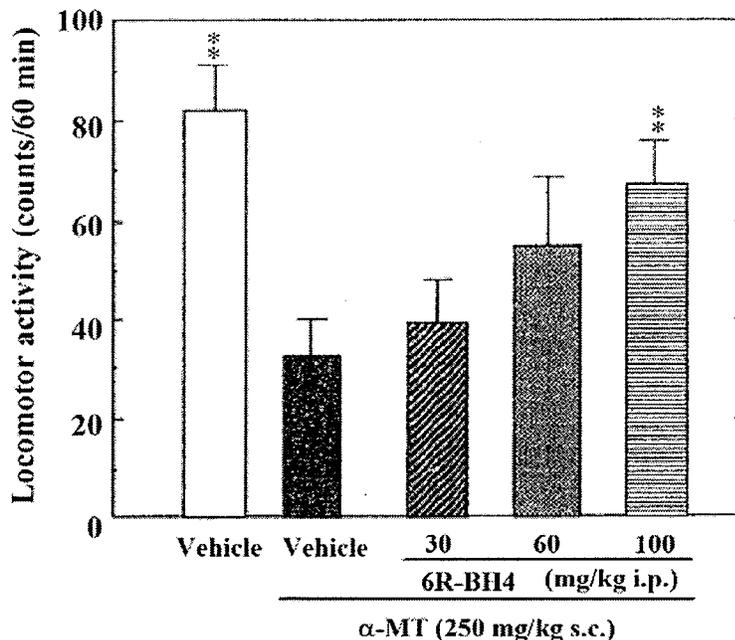
Each column shows the mean \pm standard deviation. A significant difference exists vs. the α -MT administration group at * p <0.05 and ** p <0.01 (Dunnett's two-tailed test).

Effects of Sapropterin on p-CPA-induced Decreases in Intracerebral Indolamine Content (Study #: PHN-93-PC-SR)

A tryptophane hydroxylase inhibitor, pCPA (200 mg/kg) followed by sapropterin HCl (30 or 100 mg/kg) were given i.p. to mice (male ddY; 3 weeks of age) once daily for 2 days. 8 hours after dosing on day 2, the mice were decapitated and the whole brain was removed quickly. The cerebellum and pineal body were cut off. The intracerebral contents of monoamines and their metabolites were determined by HPLC.

Administration of the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (p-CPA), decreased brain contents of 5-HT and 5-HIAA in mice at 8 hr after the final administration of p-CPA (Figure below). These decreases were unaffected by the sapropterin HCl dosed immediately after p-CPA administration.

Figure: Effects of Sapropterin on Locomotor Activity Reduction in Mice Treated with α -methyltyrosine (α -MT)



Each column shows the mean for 6 to 28 animals, and the vertical bars show the standard deviation. A significant difference exists vs. the α -MT administration group at $^{**}p < 0.01$ (Mann-Whitney U-test).

2.6.2.3 Secondary pharmacodynamics

The sponsor stated that this secondary pharmacology for 6R-BH4 was established from the scientific literature and was not directly investigated by the sponsor.

6R-BH4 is a cofactor for nitric oxide synthase (NOS) generating NO that activates the guanylate cyclase producing cGMP, which induces vascular smooth muscle relaxation. Pharmacodynamic studies in animal models demonstrate that some cardiovascular diseases with underlying endothelial dysfunction, including hypertension, congestive heart failure (CHF), atherosclerosis, pulmonary hypertension, and diabetes are associated with BH4 deficiency, and that replacement of BH4 restores endothelial function and reduces oxidative stress.

Replacement of 6R- BH4 through the administration of sepiapterin, a synthetic precursor of 6R-BH4, and a direct metabolic intermediate in BH4 biosynthesis, restored vascular function (Mitchell et al., 2002, Biol. Res. Nurs., 2003, Hypertension, and 2005, Current Hypertension Reviews). In addition, nephrectomy in rats (5/6th kidney removal to mimic renal failure), induces hypertension and increases blood pressure, that is normalized with i.p. 6R-BH4 treatment (Podjarny et al., 2004, Nephrol. Dial. Transplant.). In the angiotensin II infusion induced hypertensive rat model, 6R-BH4 treatment attenuated the

increased inducible NOS expression and increased oxidative stress (nitro-tyrosine and superoxide anion) (Kase et al., 2005, J. Hypertens.)

Decreased 6R-BH4 levels in diabetic animal models are due to suppression of GTPCH (GTP cyclohydrolase, a rate-limiting enzyme) in the 6R-BH4 biosynthetic pathway (Shinozaki et al., 1999, Diabetes; Meininger et al., 2000, Biochem. J.; Meininger CJ, 2003, SPS Publications). Insulin resistance in fructose-fed rats had reduced 6R-BH4 levels and impaired endothelial NOS (eNOS) activity (Shinozaki et al., 1999, Diabetes). Restoration of 6R-BH4 levels with administration of 6R-BH4 or sepiapterin restored dilation in aortic rings and NO production in the endothelial cells in diabetic rats (Shinozaki et al., 2000, Circ. Res., Meininger CJ, 2003, SPS Publications). These data indicate that 6R-BH4 administration improved endothelial function presumably through improved eNOS activity. Overexpression GTPCH in transgenic mouse that was induced diabetes by streptozotocin had greatly reduced production of reactive oxygen species (ROS) compared to wild-type streptozotocin-induced diabetic mice.

Transgenic overexpression of GTPCH prevented hypoxia-induced pulmonary hypertension in hyperphenylalaninemic mutant mouse (hph-1) that has a defect in GTPCH and subsequently reduced BH4 level. The degree of replacement with BH4, as measured by level of expression of GTPCH in individual animals, determined the degree of reversal of hypoxia-induced pulmonary hypertension and secondary effects, such as right ventricular hypertrophy (Khoo et al., 2004, Mol. Genet. Metab; Nandi et al., 2005, Circulation).

BH4 treatment reverses transaortic clamping (TAC)-induced CHF in the male eNOS-null mice (eNOS -/-) and male normal mice ((Takimoto et al., 2005, Nat. Med.).

2.6.2.4 Safety pharmacology

(* New Studies submitted and were reviewed under NDA 22-181)

Effects of 6R-BH4 on Cloned hERG Potassium Channels Expressed in Mammalian Cells (Study #: 0162-06-005)*

This is a GLP study. HEK293 cells (n = 2-3 cells per concentration) stably transfected with hERG cDNA were incubated alone (control, saline) or in the presence of 6R-BH4 (85 and 690 μ M; targeted concentrations of 100 and 1000 μ M, respectively), or 60 nM terfenadine (positive control). Peak hERG tail current amplitude was measured prior to and after exposure to the above compounds.

Sapropterin negatively inhibited hERG potassium current by $-9.6 \pm 2.0\%$ at 85 μ M and $-23.0 \pm 3.3\%$ at 690 μ M (mean \pm SEM). Because no inhibition was observed following 6R-BH4 application to the cells, but rather negative inhibition, the 50% inhibitory concentration (IC₅₀) for the effect of 6R-BH4 on hERG potassium current was estimated

to be > 690 μ M. Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by $81.2 \pm 3.0\%$ (n = 2). In effect, the dose of sapropterin that blocks the Ikr channel was not determined.

General Pharmacological Effects of SUN 0588 (Part 2) (Study #: PHN-98-PC-SR) and CNS and General Pharmacological Effects of a Metabolite and a Degradation Product of SUN 0588 (Study #: PHN-96-PC-SR)

In this non-GLP study (PHN-98-PC-SR), effects of Sapropterin on the central nervous system, cardiovascular and respiratory Systems, autonomic nervous system, gastrointestinal system and genitourinary system were studied in mouse, rats and dogs. In addition, local anesthetic actions (infiltration anesthesia and topical anesthesia) and blood coagulation system (prothrombin and activated partial thromboplastin) were also tested in guinea pigs or rabbits.

Dihydrobiopterin (DHP) and pterin, the principal metabolite and catabolite, respectively, of sapropterin were examined for anti-conflict effect and general pharmacologic effects in the non-GLP study (PHN-96-PC-SR) utilizing mice, rats and dogs. Each substance showed no remarkable effects at doses of 100 or 300 mg/kg (p.o.) or at a dose of 30 mg/kg (i.v.).

The general pharmacological test results with sapropterin or its mebatolites are summarized in the Table below.

Table: summary of general pharmacological test results with sapropterin HCl or its metabolites

Test Parameter (Test Method)		Species (No. Animals/ Dosage Group)	Administration route	Dose (mg/kg)	Summary of Results
General symptoms		Mouse (6)	p.o.	100 300	Increased grooming at 300 mg/kg (4/6 animals)
		Rat (5)	p.o.	100 300	Increased trunk muscle tone at 300 mg/kg (3/5 animals)
		Dog (3)	p.o.	30 100 300	Emesis in 1/3 cases at 100 mg/kg, and emesis in all 3 cases at 300 mg/kg
Central nervous system	Motor activity	Mouse (4-8)	p.o.	100 300	No effect
	Hours of sleep Thiopental-induced	Mouse (5-6)	p.o.	30 100 300	Significant prolongation increase during 1 hr pretreatment at 300 mg/kg
	Convulsions Max. electroconvulsion	Mouse (7)	p.o.	100, 300	No effect
	Pentetrazol convulsion	Mouse (7)	p.o.	100, 300	No effect
	Pain Acetic acid writhing Tail-pinch	Mouse (6) Mouse (7)	p.o. p.o.	100, 300 100, 300	No effect No effect

Test Parameter (Test Method)		Species (No. Animals/ Dosage Group)	Administration route	Dose (mg/kg)	Summary of Results
Central nervous system	Body temperature <i>Normal body temp.</i>	Rat (6)	p.o.	100, 300	No effect
	Yeast-induced hyperthermia	Rat (6)	p.o.	100, 300	No effect
	Muscle tone (suspension method)	Mouse (5)	p.o.	100, 300	No effect
	Coordinated movement (rotarod)	Mouse (5)	p.o.	100, 300	No effect
	Brain waves Spontaneous brain waves (chronic)	Unanesthetized rat (4-5)	p.o.	300	No effect
Respiratory and circulatory systems	Respiratory rate	Anesthetized dog (3-4)	i.v.	10, 30	Decrease at 30 mg/kg
	Blood pressure				No effect
	Pulse rate				Decrease at 30 mg/kg
	Maximum elevation speed of pressure in left ventricle				Inhibitory tendency at 30 mg/kg
	Electrocardiogram				No effect
	Blood flow volume Common carotid artery blood flow volume				No effect
	Femoral artery blood flow volume				No effect

Test Parameter (Test Method)		Species (No. Animals/ Dosage Group)	Administration route	Dose (mg/kg)	Summary of Results
Autonomic nervous system	Change in blood pressure and heart rate resulting from intravenous administration of norepinephrine and acetylcholine, bilateral common carotid artery occlusion or vagus nerve stimulation	Anesthetized dog (5)	i.v.	10, 30	No effect
Gastrointestinal system	Gastric evacuation ability Intestinal transportation ability	Mouse (7-8)	p.o.	30, 100, 300	No effect
	Amount of gastric acid secretion	Rat with ligated gastric outlet (5)	p.o.	100, 300	No effect
	Gastric mucosa	Rat (5)	p.o.	100, 300	No effect
	Emetic action	Dog (4)	p.o.	10, 30, 100	Emesis in 1/4 cases at both 10 mg/kg (pH 2 solution) and 30 mg/kg (pH 1.4), and emesis also in 2/4 cases at 100 mg/kg (pH 0.8)
Genitourinary system	Effects on uterine motility Non-pregnant uterus	Anesthetized rat (6)	i.v.	30	No effect
	Pregnant uterus	Anesthetized rat (4)	i.v.	30	No effect
	Effects on urine volume and urine electrolyte excretion	Rat (5)	p.o.	100, 300	No effect

Test Parameter (Test Method)		Species (No. Animals/ Dosage Group)	Administration route	Dose (mg/kg)	Summary of Results
Other	Local anesthetic action Infiltration anesthesia	Guinea pig (6)	s.c.	0.3% and 1% solutions per 250 μ L	42.1% inhibition at 1%
	Topical anesthesia	Guinea pig (6)	Eye drops	3% solution / 3 drops	No effect
	Blood coagulation system Prothrombin time (PT) Activated partial thromboplastin time (aPTT)	Rabbit (3)	p.o.	100, 300	No effects on PT and aPTT
General pharmacology of metabolites and catabolites	General symptoms	Mouse (6)	p.o.	300 (DHBp) 300 (pterin)	Neither pterin nor dihydrobiopterin had an effect.
		Rat (5)	p.o.	300	Neither pterin nor dihydrobiopterin had an effect.
	Thiopental-induced sleep	Mouse (5-6)	p.o.	300	Neither pterin nor dihydrobiopterin had an effect.
	Respiratory rate, blood pressure, pulse rate, left-ventricular pressure dP/dt max., common carotid artery and femoral artery blood flow volumes and ECG	Anesthetized dog (6)	i.v.	30	Dihydrobiopterin had no effect.
	Emetic action	Unanesthetized dog (5)	p.o.	100	Neither pterin nor dihydrobiopterin had an effect.

General Pharmacological Effects of SUN 0588 (Part 1) (Study #: PHN-97-PC-SR)*

In this non-GLP study, effects of Sapropterin on the central nervous system, cardiovascular and respiratory Systems, gastrointestinal system and urogenital system were studied in mouse, rats and dogs. In addition, local anesthetic actions (infiltration anesthesia) were also tested in guinea pigs. The general pharmacological test results with sapropterin are summarized in the Table below.

Table: summary of general pharmacological test results with sapropterin HCl

Test Parameters (Test Methods)		Species (No. animals/Dosage Group)	Administration route	Dose (mg/kg)	Summary of Results
Central nervous system	motor activity	Male mouse/ ddY (9)	p.o.	1, 3	No effects
	hexobarbital sodium-Induced sleep duration	Male mouse/ ddY (6)	p.o.	1, 3	No effects
	electroshock-induced convulsions	Male mouse/ ddY (6)	p.o.	1, 3	No effects
	Acetic acid writhing (Pain)	Male mouse/ ddY (6)	p.o.	1, 3	No effects
	Normothermia	Male Wistar rats (6)	p.o.	1, 3	No effects
	Muscle tone	Male mouse/ ddY (6)	p.o.	1, 3	No effects

	Locomotor Activity	Male mouse/ ddY (6)	p.o.	1, 3	No effects
	spontaneous electroencephalogram	Male Rat/ Wistar (2)	i.v	3	No effects
Respiratory and Cardiovascular system	blood pressure, heart rate, left ventricular pressure (LVP), and its dP/dt max	Conscious Dog/ Mongrel (male and female, 4)	p.o and i.v	1, 3	No effects
	blood pressure, heart rate, cardiac output, vertebral arterial blood flow, and coronary arterial blood flow	Anesthetized thoracotomized Dog/ Mongrel (male and female, 5)	i.v	1, 3	No effects
	respiration, blood pressure, heart rate, common carotid arterial blood flow, portal vein blood flow, femoral blood flow, renal blood flow, and carbon dioxide concentration in expired gas	Anesthetized Dog/ Mongrel. (male and female, 4)	i.v	1, 3	No effects
Digestive System	gastric evacuation and intestinal transit ability	Male mouse/ ddY (number not reported)	p.o.	1, 3	No effects
	gastric juice volume	Male Rat/ Sprague Dawley (number not reported)	Direct pyloric administration	1, 3	No effects
Urogenital System	urine volume and urinary electrolyte secretion	Male Rat/ Wistar (5)	p.o.	1, 3	No effects
Other	Local Anesthetic Effects	Male Guinea Pig/ Hartley (6)	s.c	0.1%	No effect

A Cardiovascular Safety Pharmacology Study in Telemetry Implanted Beagle Dogs after Orally (gavage) Administered 6R-BH4 ((6R)-Tetrahydrobiopterin; Sapropterin dihydrochloride) (Study #: 0166-06-015)*

Methods: This was a GLP study. Eight telemeterized male Beagle dogs (6-10 months old; 7.5-11.4 kg) were divided into three sapropterin dose groups (20, 50, and 100 mg/kg) and a vehicle group. The dogs were administered sapropterin or vehicle (reverse osmosis water) orally. The study was a Latin Square crossover design, meaning that each dog received each dose of sapropterin or vehicle with a washout between administrations of at

least three days (See Table below). Sapropterin was administered by oral gavage once daily on Days 1, 4, 8, and 11 of the dosing phase, at a dose volume of 5 mL/kg.

Study Design

Crossover-Dose Pattern

Animal No.	Dose Level/Dose Period ^a			
	Dose Period 1	Dose Period 2	Dose Period 3	Dose Period 4
	Day 1	Day 4	Day 8	Day 11
H04420	L	H	C	M
H04421	H	M	L	C
H04422	C	L	M	H
H04423	M	C	H	L
H04424	C	H	M	L
H04425	H	L	C	M
H04426	M	C	L	H
H04427	L	M	H	C

a Treatment associated with the C, L, M, and H designations is shown in the following table.

Dose	Dose Level (mg/kg)	Dose Volume ^a (mL/kg)	Dose Concentration ^a (mg/mL)
C - Control	0	5.0	0
L - Low	20	5.0	4
M - Mid	50	5.0	10
H - High	100	5.0	20

Note: C - Control animals received RO water only.

a Animals were dosed by oral gavage at a dose volume of 5 mL/kg/dose.

Telemetry was used to measure heart rate, systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), pulse pressure, body temperature, and LV dP/dt max. The ECG waveform (Lead II position), blood pressure, left ventricular pressure, and abdominal body temperature measurements were recorded predose, and 2, 4, 8, 12 and 24 hours postdose on all dosing days. Blood samples (approximately 1.5 to 2 mL) were taken predose (one day prior to each day of dosing) and approximately 1 hour postdose on Days 1, 4, 8, and 11 of the dosing phase.

Results: The dominant clinical sign observed was occasional abnormal feces (liquid or mucoid feces). The abnormal feces were present during the predose phase and did not appear to worsen after dosing. The incidence of abnormal feces that did occur during the dosing phase did not appear dose-related. No other noteworthy clinical signs were observed.

Plasma levels of calculated BH4 for the 50 and 100 mg/kg-dose levels at 1 hr post dose were approximately 4551 ± 538 and 8663 ± 1717 ng/mL, respectively.

Animal No. H04425 on Day 1 of the dosing phase at 2 and 4 hours postdose had one ventricular premature complex following receipt of the 100 mg/kg sopropterin, which can be considered a normal variant. Normal sinus arrhythmia and a fluctuation in heart rate occurred throughout the study in all animals and in all phases. No drug-related effects were observed in intervals of RR, QT and QTc (Fridericia method).

There were no drug-related effects on systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), pulse pressure (systolic-diastolic pressure), body temperature, LV dP/dt max. Some of the cardiovascular parameters are summarized in the Tables below.

Table 1
Mean Systolic Arterial Pressure Data

(mm Hg)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
0	Mean	148	129	141	131	138	142
	SD	16.0	8.4	6.1	7.6	6.6	8.6
	N	7	7	7	7	7	7
20	Mean	144	128	145	131	143	141
	SD	13.3	6.9	11.9	10.3	6.9	9.3
	N	7	7	7	7	7	7
50	Mean	142	129	145	132	140	143
	SD	10.4	7.8	15.4	6.3	10.8	14.4
	N	7	7	7	7	7	7
100	Mean	147	133	142	134	137	139
	SD	15.4	10.1	5.2	7.0	8.5	7.2
	N	7	7	7	7	7	7

**Appears This Way
On Original**

Table 2
Mean Diastolic Arterial Pressure Data

(mm Hg)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
0	Mean	83	69	79	69	76	76
	SD	8.8	5.8	5.4	4.0	7.2	8.5
	N	7	7	7	7	7	7
20	Mean	79	68	82	66	77	79
	SD	8.6	4.4	8.4	7.5	9.0	5.9
	N	7	7	7	7	7	7
50	Mean	79	69	82	71	77	80
	SD	6.2	4.3	6.2	3.9	6.7	7.9
	N	7	7	7	7	7	7
100	Mean	82	72	80	72	75	76
	SD	7.7	6.1	6.1	5.6	8.1	4.6
	N	7	7	7	7	7	7

Table 3
Mean Arterial Pressure Data

(mm Hg)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
0	Mean	108	90	102	91	98	101
	SD	11.3	5.4	6.1	5.4	5.7	9.2
	N	7	7	7	7	7	7
20	Mean	104	89	106	89	100	102
	SD	10.0	4.3	8.8	7.4	8.2	6.7
	N	7	7	7	7	7	7
50	Mean	103	91	106	93	99	104
	SD	7.8	4.1	9.6	4.3	7.1	10.0
	N	7	7	7	7	7	7
100	Mean	107	94	103	94	97	98
	SD	10.1	7.6	6.1	6.0	7.5	4.3
	N	7	7	7	7	7	7

Table 4
Mean Arterial Pulse Pressure Data

(mm Hg)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
0	Mean	65	60	62	61	61	65
	SD	8.7	5.3	4.2	5.5	5.6	3.5
	N	7	7	7	7	7	7
20	Mean	65	60	63	65	65	62
	SD	7.3	3.8	8.2	7.6	7.5	6.7
	N	7	7	7	7	7	7
50	Mean	63	61	63	61	63	63
	SD	5.6	5.9	10.4	4.6	7.1	8.9
	N	7	7	7	7	7	7
100	Mean	65	60	62	62	62	63
	SD	9.4	7.1	6.1	5.6	8.3	6.2
	N	7	7	7	7	7	7

Table 7
Mean +dP/dt_{max} Data

(mm Hg/second)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
0	Mean	4224	3322	3980	3420	3414	4452
	SD	709.0	358.1	729.9	463.5	482.0	879.7
	N	8	8	8	8	8	8
20	Mean	4271	3326	4222	3553	3569	4071
	SD	697.2	327.7	601.4	627.5	878.0	834.3
	N	8	8	8	8	8	8
50	Mean	4209	3608	4270	3559	3378	4145
	SD	616.4	499.0	576.5	557.2	742.8	641.1
	N	8	8	8	8	8	8
100	Mean	4525	3479	4348	3561	3381	3776
	SD	1061.0	606.5	659.3	471.7	632.8	674.7
	N	8	8	8	8	8	8

Ethopharmacological Assessment of SUN 0588 (Study #: PHN-95-PC-SR)*

This was a non-GLP study. The effects of administration of sapropterin to rodents at doses up 100 mg/kg (i.p), 600 mg/kg (p.o.) or 300 µg/mouse (intracisternally) on a forced swim test, conflict or aggressive behavior, memory and learning, or motor activity are summarized in the Table below based on sponsor's information.

Table: Summary of Ethopharmacological Assessment of SUN 0588

Test Parameters (Test Methods)		Species (No. animals/Dosage Group)	Administration route	Dose (mg/kg)	Summary of Results
Central nervous system	Behavior using forced swim test	Male Mouse/ ICR (4-8)	i.p.	30, 60, 100	1/8 deaths, tremor in 3/8 and absent righting reflex in 2/8 at 100 mg/kg; Shorter immobility time at 60 and 100 mg/kg at 24 hrs postdose
		Male Mouse/ ICR (6-7)	p.o	300, 600	Shorter immobility time at 600 mg/kg
	Conflict Behavior	Male Rat/ Wistar (6-9)	p.o	10, 30, 100, 300	At 300 mg/kg, however, the frequency of drinking for 2% saline solution was significantly increased; no effects on drinking tap water
	Scopolamine-induced amnesia (Memory)	Male Mouse/ ddY (9-20)	i.p	3, 10, 30, 100	No Anti-amnesiac effect at 30 and 100 mg/kg 1 or 5 hrs after the test session; Anti-amnesiac effect at 10 mg/kg immediately after learning
		Male Mouse/ ddY (7-23)	i.p	3, 10, 30, 100	Anti-amnesiac effect at 3 and 10 mg/kg 24 hrs prior to scopolamine administration; or at 30 mg/kg 5 hrs prior to scopolamine administration
		Male Mouse/ ddY (8-10)	Intracisternal	0.1, 0.3	No effects

		Male Mouse/ ddY (9-23)	p.o	1, 3	Anti-amnesia effect at 1 or 3 mg/kg when SUN0588 was given q.d for 4 days
	Learning disorder (Cerebral infarction induced by microsphere injection)	Male Rat/ Wistar (8)	i.p	10	Ameliorated learning acquisition disorder with repeat dose
	T-maze learning	Male Rat/ Wistar (8)	i.p before learning test	10	Ameliorated learning acquisition disorder
	increased motor activity with kainic acid (KA)– induced unilateral cortex damage	Rat/ Fischer 344 (Juveniles- <i>gender not specified</i> , 6-8)	i.p	10	No effects
	hyperactivity induced by intracisternal administration of 6 hydroxydopamine (6- OHDA)	Rat/ Fischer 344 (Juveniles- <i>gender not specified</i> , 5-6)	p.o	600	Exercise capacity increased on treatment day 1 after a repeated oral dose of 600 mg/kg
		Male Rat/ Fischer 344 (7)	i.p	10 for 6 & 7 days	No effects
	Aggressive behavior (muricide) in rats induced by removal of the olfactory bulbs	Male Rat/ Wistar king (4-8)	i.p	10, 30, 60	No effects
	increased motor activity caused in Mongolian gerbils by bilateral complete common carotid artery obstruction followed by reperfusion	Male & female Gerbil/ Mongolian (9-10)	i.p.	3	No effects

**Respiratory Safety Pharmacology Study Using the Head-Out Body
Plethysmography Model of Orally Administered 6R-BH4 in Rats (Study #: 0166-06-
021)***

Methods: This was a GLP study. Male Sprague Dawley rats (6-8 weeks old) were assigned to four dose groups and administered as a single oral dose of sapropterin at 0, 25, 75 or 225 mg/kg. Animals were placed in a head-out plethysmograph to assess the

effects of sapropterin on respiratory parameters (tidal volume, rate (breaths per minute) and minute volume).

Baseline data were collected for approximately 1 hour on the day prior to dosing. Following dosing, data were collected continuously for at least 6 hours and analyzed approximately 0.5, 1, 1.5, 2, 3, 4, 6, and 24 hours postdose. For the 24 hour time point, all animals were loaded into the chamber at least 30 minutes prior to the start of data collection, and data were collected for at least 30 minutes for all animals (based on the last animal dosed). Blood was collected from all animals predose and approximately 1 and 4 hours postdose on Day 1 of the dosing phase.

Results: Plasma levels of BH4 at 1 hour post dose for the 25, 75 and 225 mg/kg dose levels were approximately BQL, 356 ± 64 and 601 ± 28 ng/mL, respectively. No treatment effect was observed for tidal volume, mean minute volume and respiratory rate at any measured time point. A single dose of up to 225 mg/kg sapropterin administered by oral gavage to male Sprague Dawley rats did not cause any significant changes in respiratory parameters. Based on these results, the no-observable-adverse-effect level (NOAEL) on the respiratory system for 6R-BH4 administered orally, as a single dose to male rats, was 225 mg/kg.

2.6.2.5 Pharmacodynamic drug interactions

(* New Studies submitted and were reviewed under NDA 22-181)

Sapropterin couples eNOS and restores physiological NO production. NO binds to the heme group of guanylate cyclase, increases the production of cyclic guanosine monophosphate (cGMP) and thus induces calcium related vasorelaxation. Pharmacodynamically, sildenafil citrate also increases vasorelaxation through phosphodiesterase 5 (PDE5) inhibition, impeding the catabolism of cGMP to GMP and thus extending the pharmacodynamic vasorelaxation.

Respiratory Safety Pharmacology Study Using the Head-Out Body Plethysmography Model of Orally Administered 6R-BH4 in Combination with Sildenafil Citrate in Rats (Study #: 0166-06-022)*

Methods: Male Sprague Dawley rats (6 rats/group, 8.5 to 9.5 week old) were assigned to three dose groups and administered a single oral dose of sapropterin at 225 mg/kg (group 2), sildenafil citrate at 50 mg/kg (group 3), or a combination of 225 mg/kg sapropterin and 50 mg/kg sildenafil citrate (group 4). A fourth group was included as a vehicle-treated group (group 1). The dose volume was 5 ml/kg. Animals were placed in a head-out plethysmograph to assess the effects of sapropterin on respiratory parameters (tidal volume, rate (breaths per minute) and minute volume). Other Four groups of animals (3 rats/group) received the same treatments as described above used for toxicokinetic study. Blood was collected into K2-EDTA tubes from all toxicokinetic animals predose and approximately 1 and 4 hours postdose on Day 1 of the dosing phase.

Baseline data were collected for at least 1 hour on the day prior to dosing. Following dosing, data were collected continuously for at least 6 hours and analyzed approximately 0.5, 1, 1.5, 2, 3, 4, 6, and 24 hours postdose. For the 24 hour time point, all animals were loaded into the chamber at least 30 minutes prior to the start of data collection and data were collected for at least 30 minutes for all animals.

Results: The plasma concentrations of 6R-BH4 were 900.14 ± 437.99 ng/ml and 623.47 ± 178.99 ng/ml at 1 hr postdose; 1109.59 ± 332.79 ng/ml and 966.35 ± 208.86 ng/ml at 4 hrs postdose for group 2 and group 4 animals, respectively.

A single oral gavage dose of 6R-BH4 (225 mg/kg) or sildenafil citrate (50 mg/kg) alone or in combination administered to male Sprague Dawley rats did not cause any significant changes in respiratory function compared to those of control rats. In addition, no significant changes in respiratory functions were observed in rats treated with a combination of 6R-BH4 (225 mg/kg) and sildenafil citrate (50 mg/kg) versus treatment with sildenafil citrate alone.

A Cardiovascular Safety Pharmacology Study in Telemetry implanted Beagle Dogs after Orally (gavage) Administered 6R-BH4 ((6R)-Tetrahydrobiopterin; Sapropterin dihydrochloride) in Combination with Sildenafil Citrate (Study #: 0166-06-020)*

Methods: Eight telemeterized male Beagle dogs (7- 10 months old; 7.8-12.0 kg) were divided into three dose groups (100 mg/kg sapropterin, 30 mg/kg sildenafil citrate, and a combination of 100 mg/kg sapropterin with 30 mg/kg sildenafil citrate) and a vehicle (reverse osmosis water) group. The dogs were administered all doses orally on days 1, 4, 8 and 11. The study was a Latin Square crossover design, meaning that each dog received each dose with a washout between administrations of at least three days (See Table below). Telemetry was used to measure heart rate, systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), pulse pressure, body temperature, LV dP/dt max and the ECG waveform (Lead II position).

For each dosing day, ECG and pressure measurements were recorded for at least 90 minutes prior to dosing, continuously for at least 8 hours after dosing, and then for one period of at least 15 minutes in duration each hour through at least 24 hours postdose. ECG intervals for evaluation were predose and 2, 4, 8, 12, and 24 hours postdose. At least 1 minute segments of contiguous most artifact-free ECG complexes for each animal within 10 minutes of the time points up to 8 hours and then within 1.5 hours after 8 hours were reviewed by a board-certified veterinary consultant for interpretation. Quantitative evaluation of ECG measurements, including RR interval, QT, and rate-corrected QT (QTc, using Fridericia's method) were done. For each dosing day, blood pressure assessments were taken predose and approximately 2, 4, 8, 12, and 24 hours postdose. Positive inotropic effects (+dP/dtmax) and heart rate were calculated from a left

ventricular pressure signal predose and approximately 2, 4, 8, 12, and 24 hours postdose. Intraabdominal temperature measurements were taken for each dosing day predose and approximately 2, 4, 8, 12, and 24 hours postdose.

Blood samples were taken from the jugular vein for hematology, coagulation, and clinical chemistry after the last telemetry collection was completed.

Table: Study Design

Animal ID	Dose Period 1	Dose Period 2	Dose Period 3	Dose Period 4
	Day 1	Day 4	Day 8	Day 11
No. 1 (H04782)	6R-BH4 ^a	6R-BH4 + Sildenafil ^b	Control ^c	Sildenafil ^d
No. 2 (H04783)	6R-BH4 + Sildenafil	Sildenafil	6R-BH4	Control
No. 3 (H04784)	Control	6R-BH4	Sildenafil	6R-BH4 + Sildenafil
No. 4 (H04785)	Sildenafil	Control	6R-BH4 + Sildenafil	6R-BH4
No. 5 (H04786)	Control	6R-BH4 + Sildenafil	Sildenafil	6R-BH4
No. 6 (H04787)	6R-BH4 + Sildenafil	6R-BH4	Control	Sildenafil
No. 7 (H04788)	Sildenafil	Control	6R-BH4	6R-BH4 + Sildenafil
No. 8 (H04789)	6R-BH4	Sildenafil	6R-BH4 + Sildenafil	Control

a Animals given 6R-BH4 received 100 mg/kg at a dose volume of 5 mL/kg.

b Animals given the combination treatment received 6R-BH4 (100 mg/kg; dose volume of 5 mL/kg) followed by a dose of sildenafil citrate (30 mg/kg; dose volume of 5 mL/kg) for a total volume of 10 mL/kg.

c Animals in the control group received reverse osmosis water at a dose volume of 10 mL/kg.

d Animals given sildenafil citrate received 30 mg/kg at a dose volume of 5 mL/kg.

Results:

No test article-related effects on clinical signs, abdominal body temperature, food consumption, or body weights were noted during the study.

QT interval was decreased at 2 and 4 hours postdose in animals given sildenafil citrate alone or in combination with 6R-BH4 (2hr postdose: ↓11.35% and ↓10.9% for sildenafil or sildenafil + 6R-BH4, respectively; 4 hrs postdose: ↓14.5% and ↓9.5% for sildenafil or sildenafil + 6R-BH4, respectively) compared to the controls. The QT interval was not different between sildenafil citrate alone and the combination with 6R-BH4. No QT changes from controls were seen in animals given 6R-BH4 alone. Therefore, the QT changes are attributable to sildenafil citrate administration and addition of 6R-BH4 did not alter the effects of sildenafil citrate on QT interval. No test article-related changes were seen in heart rate-corrected QT intervals (QTc). Thus, administration of 6R-BH4 with sildenafil citrate did not alter the effect of sildenafil citrate on QT interval. The data of intervals of RR, QT, QTc is summarized in the table 4 below.

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Table 4
Mean Electrocardiographic Data

Dose Level (mg/kg)		Predose			2 Hours Postdose			4 Hours Postdose			8 Hours Postdose		
		RR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QT Int (msec)	QTc Int (msec)
Control (0)	Mean	623	232	274	674	229	265	710	228	261	711	239	259
	Stdev	110.1	11.1	12.5	288.9	20.0	11.5	315.4	21.2	11.7	188.1	15.7	7.9
	N	8	8	8	8	8	8	8	8	8	8	8	8
6R-BH4 (100)	Mean	572	226	274	659	224	260	604	223	265	607	225	266
	Stdev	136.7	21.0	15.1	174.9	19.1	13.7	125.1	15.3	12.2	78.5	14.2	10.7
	N	8	8	8	8	8	8	8	8	8	8	8	8
Sildenafil (30)	Mean	545	232	272	484	203	260	470	195	253	589	230	256
	Stdev	39.1	11.9	12.3	83.0	16.0	8.5	132.4	15.8	8.3	150.6	18.4	12.0
	N	8	8	8	8	8	8	8	8	8	8	8	8
6R-BH4 + Sildenafil (100/30)	Mean	600	226	270	548	204	250	505	206	262	525	209	259
	Stdev	112.1	17.8	14.7	116.3	16.5	11.7	150.7	17.7	11.7	82.5	18.4	16.9
	N	8	8	8	8	8	8	8	8	8	8	8	8

Table 4
Mean Electrocardiographic Data

Dose Level (mg/kg)		12 Hours Postdose			24 Hours Postdose		
		RR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QT Int (msec)	QTc Int (msec)
Control (0)	Mean	771	242	265	758	237	263
	Stdev	172.7	14.1	11.9	217.1	21.1	9.5
	N	8	8	8	8	8	8
6R-BH4 (100)	Mean	725	239	268	729	237	265
	Stdev	175.1	18.6	11.4	154.7	17.4	12.3
	N	8	8	8	8	8	8
Sildenafil (30)	Mean	654	226	261	666	237	273
	Stdev	109.8	15.4	10.6	133.8	16.5	16.4
	N	8	8	8	8	8	8
6R-BH4 + Sildenafil (100/30)	Mean	677	226	260	730	238	265
	Stdev	166.1	17.6	13.3	110.9	14.0	12.6
	N	8	8	8	8	8	8

Arrhythmias were observed in several animals. Two animals had paroxysmal ventricular tachycardia with frequent ventricular premature complexes. When sapropterin (100 mg/kg) in combination with sildenafil (30 mg/kg) was given to dogs orally, paroxysmal ventricular tachycardia (PVT) and frequent ventricular premature complex (VPC) were noted in one (animal No H04783) out of 8 dogs 4 and 8 hrs postdose on dose period 1. Another dog (H04787) had VPC after receiving 6R-BH4, sildenafil or combination of these two drugs. PVT with frequent VPC was observed in animal H04786 on dose period 4 before receiving 6R-BH4 and 2 hr postdose. Animal H04788 had VPC 24 hrs after receiving sildenafil on dose period 1. The arrhythmias are summarized in sponsor's table below.

Table: Arrhythmias in animals treated with 6R-BH4 and Sildenafil

Animal No.	Treatment	Predose (2 Points)	2 Hours Postdose	4 Hours Postdose	8 Hours Postdose	12 Hours Postdose	24 Hours Postdose
H04788	Sildenafil						1 VPC
H04787	Control	1 VPC					1VPC
	6R-BH4	1 to 2 VPC	1 to 2 VPC		1 to 2 VPC		1 to 2 VPC
	Sildenafil			1 VPC		1 VPC	3 VPC
	6R-BH4 + Sildenafil			1 VPC			
H04786	6R-BH4	PVT and frequent VPC (both points)	Frequent VPC				
H04783	Control						1 VEC
	6R-BH4 + Sildenafil			PVT and frequent VPC	PVT and frequent VPC		

VPC = ventricular premature complex.
 VEC = ventricular escape complex.
 PVT = paroxysmal ventricular tachycardia.

The sponsor stated that the study design included the use of a left ventricular pressure catheter could, at least in part, contribute to intermittent ventricular tachycardia or a high frequency of ventricular premature complexes due to the local irritation of the myocardium. In addition, based on the study design and ECG test results, the sponsor stated that these left ventricular arrhythmias may not be a direct result of test article administration and these events are sporadic and may be precipitated by the presence of a catheter in the left ventricle of the heart. However, under the study design and the presence of a catheter in the left ventricle of the heart, an association between the combination test article administration and these arrhythmias (VPC, VEC and PVT) could not be entirely ruled out.

No treatment-related changes were observed in systolic blood pressure, mean arterial pressure, or inotropic state (+dP/dtmax).

When compared to the controls, sildenafil citrate and sildenafil citrate in combination with sopropterin resulted in decreased diastolic pressure in dogs 2 and 4 hrs postdose (2hr postdose: ↓7.9% and ↓9.2% for sildenafil or sildenafil + 6R-BH4, respectively; 4 hrs postdose: ↓12.7% and ↓8.8% for sildenafil or sildenafil + 6R-BH4, respectively) (See Table 6 below). This decrease in pressure was accompanied by an increase in heart rate (2 to 12 hrs postdose: ↑15.2% -31.9% for sildenafil; ↑20.3%-31.9% for sildenafil +6R-BH4; see Table 10 below) and an increase in arterial pulse pressure (2 to 8 hrs postdose: ↑9.8% -20.7% for sildenafil; ↑3.4%-10.3% for sildenafil +6R-BH4; see Table 8 below). A decrease in blood pressure and an increase in heart rate are documented effects of sildenafil citrate in dogs (NDA #20-895). 6R-BH4 did not augment or attenuate the decreased diastolic pressure and increased heart rate caused by sildenafil. Animals given the combination treatment had lower arterial pulse pressure than the sildenafil citrate alone at 2 and 8 hours postdose (see Table 10 below); this finding suggests that 6R-BH4 has an attenuating effect on the pulse pressure increase after administration of sildenafil

citrate. No effect of 6R-BH4 alone was noted on any of the cardiovascular parameters measured.

The addition of 100 mg/kg sapropterin to 30 mg/kg sildenafil citrate did not potentiate the cardiovascular effects of sildenafil citrate in these dogs. However, an association between ventricular arrhythmias (PVT and PVC) and administration of combination of 6R-BH4 with sildenafil cannot be fully ruled out.

Table 6
Mean Diastolic Blood Pressure Data

Dose Level (mg/kg)		(mm Hg)					
		Pre-dose	2 hours	4 hours	8 hours	12 hours	24 hours
Control (0)	Mean	80	76	79	68	70	78
	SD	8.7	10.5	4.4	5.8	7.5	6.8
	N	8	8	8	8	8	8
6R-BH4 (100)	Mean	82	78	76	66	71	79
	SD	5.8	8.1	8.4	5.8	8.1	9.2
	N	8	8	8	8	8	8
Sildenafil (30)	Mean	82	70	69	67	68	75
	SD	5.5	5.4	4.7	9.7	5.2	8.4
	N	8	8	8	8	8	8
6R-BH4 + Sildenafil (100/30)	Mean	84	69	72	68	67	77
	SD	8.2	7.0	6.9	8.3	10.1	11.3
	N	8	8	8	8	8	8

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Table 8
Mean Arterial Pulse Pressure Data

(mm Hg)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
Control (0)		Mean	62	58	61	59	63
		SD	7.7	6.4	5.2	4.2	7.2
		N	8	8	8	8	8
6R-BH4 (100)		Mean	60	58	58	57	63
		SD	8.1	7.6	5.8	6.7	9.1
		N	8	8	8	8	8
Sildenafil (30)		Mean	63	70	67	71	68
		SD	7.6	7.6	6.3	6.5	5.9
		N	8	8	8	8	8
6R-BH4 + Sildenafil (100/30)		Mean	61	64	67	61	67
		SD	5.6	8.4	7.2	8.0	8.7
		N	8	8	8	8	8

Table 10
Mean Heart Rate Data

(Beats/minute)

Dose Level (mg/kg)		Predose	2 hours	4 hours	8 hours	12 hours	24 hours
Control (0)		Mean	106	102	111	94	79
		SD	9.1	20.2	14.4	16.7	15.0
		N	8	8	8	8	8
6R-BH4 (100)		Mean	115	107	110	97	78
		SD	18.2	22.1	14.3	11.8	12.9
		N	8	8	8	8	8
Sildenafil (30)		Mean	114	132	139	124	91
		SD	8.9	19.1	11.5	28.3	12.4
		N	8	8	8	8	8
6R-BH4 + Sildenafil (100/30)		Mean	112	127	137	124	95
		SD	16.3	12.0	17.3	21.6	19.7
		N	8	8	8	8	8

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

(* New Studies submitted and were reviewed under NDA 22-181.)

2.6.4.1 Brief summary

Following oral administration, sapropterin HCl is absorbed mainly in the small intestine. The concentration of total [redacted] in plasma (the sum of reduced [redacted] and oxidized [redacted]) is maximal by 1 hr after p.o administration in mice, 2 hr after p.o administration in rats, and by 3 hr. after p.o administration in monkeys. The bioavailability was 7%-12% in rats, and approximately 9% in monkeys. When presumed pregnant rabbits were treated with 6, 60 and 600 mg/kg of sapropterin on gestation day (GD) 6 to GD 18, the increase with dosage was less than dose-proportional at day 6 of gestation, and the exposure at the lower dosage may have decreased with continued dosage, whereas the exposure increased at the higher dosage. There was no evidence of accumulation or decrease in C_{max} after four daily doses of 125, 250 and 500 mg/kg to mice by comparison with results following the first dose. Although exposure did increase with increased dosage, AUC values normalized to dose decreased slightly with increasing dose levels. Total plasma [redacted] concentration after repeated sapropterin HCl administrations (once daily for 14 days) to rats at 100 mg/kg p.o. was almost identical to that observed after a single-dose, indicating no accumulation or persistence. Sapropterin HCl is metabolized in the liver and primarily distributed to the kidneys and liver in rats. The drug is excreted mainly in urine after i.v administration. 7,8-Dihydrobiopterin [DHBp], [redacted], pterin, 7,8-Dihydropterin [DHBT], and 6-hydroxylumazine [6-OH-Lu] were identified as metabolites in rat urine following i.v. administration. Large amounts of THBP and DHBp and DHXaPT (7,8-dihydro-xanthopterin) as well as a small amount of [redacted] appeared in the urine of healthy humans administered (R,S)-THBP intravenously. Since human liver is devoid of pterin deaminase, no lumazine metabolites (metabolites with oxidative elimination of the amino group at the 2-position) in human urine have been documented. On the other hand, 6-OH-Lu is reportedly excreted in feces. In addition, biolumazine, resulting from [redacted] oxidation have formed through metabolic degradation by bacterial flora in the gastrointestinal tract.

2.6.4.2 Methods of Analysis

The dihydrochloride of tetrahydrobiopterin (6R-BH₄) and its metabolites (quinoid dihydrobiopterin (R-q-DHBp), dihydrobiopterin (DHBp) and [redacted] are called total [redacted]. The total [redacted] concentration is measured by means of acidic oxidation of the biological sample, and only the oxidized [redacted] (DHBp + [redacted]) concentration by means of alkaline oxidation. The reduced [redacted] (6R-BH₄ + R-q-DHBp) concentration is computed as the difference between the total [redacted] and the oxidized [redacted] concentrations. [redacted] were measured using HPLC with fluorometric detection in non-GLP study. In GLP study, [redacted] were measured using an HPLC tandem mass spectrometry separation and detection system.

In whole-body autoradiography distribution studies using ³H-Sapropterin, ³H was introduced at the 6-position of a pterin ring. Even after this isomer is converted to R-q-

DHBP, the ^3H is maintained. However, when it is oxidized to DHBP, the ^3H detaches, thereby forming $^3\text{H}_2\text{O}$. Thus, active 6R-BH4 and R-q- DHBP remain radioactive as cofactors, but DHBP do not remain radioactive. The $^3\text{H}_2\text{O}$ evaporates in the process that dries the prepared whole-body section, so it is assumed that the darkening on the autoradiogram mainly represents the distribution of reduced (6R-BH4 \pm R-q-DHBP). Alternatively, it could be postulated that the radioactivity detected in the quantitative tissue distribution test contains both reduced, labeled with ^3H as well as $^3\text{H}_2\text{O}$ -derived radioactivity.

2.6.4.3 Absorption

Dose Ranging Pharmacokinetics of Tetrahydrobiopterin after Single Oral Dose Administration in Male C57BL/6 Mice (Study #: 0166-06-033)*

Methods: Single doses of sapropterin (36, 200 and 400 mg/kg) were administered orally to male C57BL/6 mice (24/group). Terminal blood samples were collected at 1, 2, 6 and 24 hours post dose for plasma BH4 concentration analysis.

Results: Results are summarized in the table below. Sapropterin appeared to be rapidly absorbed after p.o. doses of 36, 200 or 400 mg/kg. The observed T_{max} was 1 hr postdose for each group. Plasma BH4 concentrations were below the level of quantitation at 24 hr post-dose in each group (100 ng/mL equivalent to 237 ng/mL BH4). AUC increased approximately in proportion to the increase in dose from 36 to 400 mg/kg.

Table: Pharmacokinetics Parameters if Sapropterin Administered Orally in C57/BL6 Mice

Dose (mg/kg)	Administration Route	C _{max} (ng/ml)	T _{max} (hr)	AUC _{inf} (ng.hr/ml)	T _{1/2} (h)	AUC/dose
400	p.o.	17332	1	46878	1.2	117
200	p.o.	9100	1	22894	1.3	114
36	p.o.	2058	1	5787	1.2	161

Pharmacokinetics of Sapropterin Hydrochloride in the Rat (I) (Single Dose Administration) (Study #: PHN-104-PK-SR); Pharmacokinetics of Sapropterin Hydrochloride in the Monkey (Study #: PHN-110-PK-SR); Pharmacokinetics of sapropterin hydrochloride in the rat (II) (repeated dose administration) (Study #: PHN-105-PK-SR); Intracorporeal kinetics of Apropterin hydrochloride through a variety of administering pathways in rats and mice (Bosster test) (Study #: PHN-112-PK-SR).

The absorption of sapropterin was also investigated in rats, mice and monkeys in these studies. The results are summarized the Tables below.

Pharmacokinetics of sapropterin HCl (SUN 0588) in rats was investigated using SUN 0588 or sapropterin HCl tritiated at the 6-position of its pteridine ring (³H-SUN 0588). Sponsor stated that doses were chosen upon reference to plasma endogenous concentration in humans and anticipated clinical dosage (1-3 mg/kg), since there is a marked difference in plasma concentration of endogenous between humans and rats. That is, a low dose level of 10 mg/kg corresponding to approximately 5 to 10 times the anticipated clinical dosage and a high dose level of 100 mg/kg being 10 times the low dose were adopted, because the plasma endogenous concentration in rats (40-50 ng/ml) was about 10-fold higher than that in humans (4-5 ng/mL).

SINGLE DOSE PK DATA IN RATS

Rat: Sites of gastrointestinal absorption of SUN 0588

	Animal No.	Endogenous level	Intragastric administration	Intraduodenal administration	Intrajejunal administration
Concentration in plasma (ng/mL)	1	44.42	44.05	4792.49	2155.74
	2	34.79	37.41	4785.09	3744.30
	3	34.39	39.31	2204.86	4206.98
	4	32.85	43.55	4707.18	1301.73
	5	31.31	48.11	4800.10	2691.00
	Mean ±S.E.	35.55 ± 2.30	42.49 ± 1.89	4257.80 ± 513.58	2071.09 ± 526.38
Concentration in whole blood (ng/mL)	1	382.59	346.39	10802.10	5634.72
	2	294.21	360.73	10892.73	6584.57
	3	318.90	393.37	5550.85	9824.51
	4	434.42	329.54	10819.95	3879.15
	5	318.42	376.80	10789.99	7031.14
	Mean ±S.E.	349.68 ± 25.73	361.37 ± 11.18	9791.12 ± 1060.28	5270.30 ± 972.18

100 mg/kg

Difference in absorption by age in rats (10 mg/kg; p.o.)

Age	4 days ¹⁾	1 week ²⁾	2 weeks ¹⁾	3 weeks ¹⁾	4 weeks ¹⁾	6 weeks ³⁾	8 weeks ¹⁾
Conc. in plasma (ng/mL)							
Endogenous level	51.8 ±3.93	49.4 ±3.33	40.6 ±1.01	44.7 ±3.23	38.3 ±1.43	36.4 ±0.76	33.5 ±0.80
C _{max} (2 hr)	706.1 ±92.6	908.3 ±24.2	906.2 ±21.3	179.9 ±8.96	177.8 ±17.5	113.1 ±14.7	135.9 ±15.5
Conc. in whole blood (ng/mL)							
Endogenous level	483.8 ±42.3	451.6 ±10.3	308.7 ±27.7	348.0 ±34.4	209.8 ±4.48	251.2 ±6.95	149.5 ±3.51
C _{max} (2 hr)	1109.1 ±80.5	1097.5 ±58.9	930.9 ±27.2	492.2 ±35.1	397.3 ±36.2	303.0 ±7.32	233.9 ±16.6

1) n=5, 2) n=6, 3) n=4, mean±S.E.

Single Dose PK Parameters showing estimation of GI absorption rate of SUN 0588 in rats

6-week-old rats: Estimation from ΔAUC

Dose mg/kg	Route of administration	C _{endo} ^{a)} ng/ml	C _{max} ng/ml	ΔC _{max} ^{b)} ng/ml	T _{max} hr	ΔAUC ng+hr/ml	T _{1/2} hr	Absorption rate ^{c)} (%)
100	p.o.	38.2	1227	1189	1.0	4571	1.1 (2- 6hr)	11.8
10	p.o.	33.5	108	75	2.0	285	1.1 (2- 6hr)	6.8
10	i.v.	33.5	—	—	—	3881	0.8(0.5-3hr)	—
1	i.v.	33.5	—	—	—	529	0.3(0.5-3hr)	—

a) C_{endo}: Endogenous total concentration

b) C_{max} - C_{endo}

c) Absorption rate based on AUC comparison

2-week-old rats: Estimation from ΔAUC

Dose mg/kg	Route of administration	C _{endo} ^{a)} ng/ml	C _{max} ng/ml	ΔC _{max} ^{b)} ng/ml	T _{max} hr	ΔAUC ng+hr/ml	T _{1/2} hr	Absorption rate ^{c)} %
10	p.o.	49.2	1150	1101	2.0	6593	2.6 (2-6hr)	52.3
1	p.o.	49.2	174	125	2.0	824	3.4 (2-6hr)	65.3
1	i.p.	49.2	—	—	—	1281	0.8(0.5-3hr)	—

a) C_{endo}: Endogenous total concentration

b) C_{max} - C_{endo}

c) Absorption rate, as percent of absorption rate after a 1-mg/kg i.p. dose, based on comparison of AUCs

Rat: Effect of food ingestion on absorption of SUN 0588 (I)

Tissue	Tissue Concentration of (ng/g ml)								
	0hr	0.5 hr	1hr	2hr	3hr	6hr	9hr	24hr	
100 mg/kg p.o. 15-hr fasted	Plasma	38±1 (88.7)	914±11 (879) (90.1)	1227±98 (1189) (89.8)	1217±139 (1179) (89.9)	713±132 (675) (88.6)	149±22 (111) (88.4)	82±5 (24) (83.8)	37±4 (---) (88.3)
	Blood	181±12 (89.7)	818±19 (457) (89.2)	852±106 (891) (91.0)	902±170 (741) (91.8)	804±90 (843) (91.7)	329±18 (168) (87.0)	199±9 (38) (91.8)	124±4 (---) (89.7)
100 mg/kg p.o. Non-fasted	Plasma	50±1 (90.1)	702±155 (625) (89.5)	1023±289 (973) (87.0)	698±108 (846) (90.6)	682±54 (632) (82.0)	334±46 (284) (91.9)	113±2 (83) (91.0)	42±1 (---) (51.2)
	Blood	290±12 (97.0)	881±19 (391) (94.8)	1303±201 (1013) (83.8)	918±56 (829) (96.1)	845±32 (655) (98.2)	892±53 (402) (98.6)	382±33 (72) (98.8)	263±8 (---) (96.0)

(): Increase in concentration due to dosing, []: Reduced/total ratio (%), (---): Below pre-dosing level
Mean±S.E. (n=2-5)

Rat: Effect of food ingestion on absorption of SUN 0588 (II)

		Tmax (hr)	ΔCmax (ng/ml)	ΔAUC (ng*hr/ml)
Plasma	Fasted	1	1189±99	4571
	Non-fasted	1	973±270	4803
Blood	Fasted	2	741±182	3868
	Non-fasted	1	1013±303	5356

Rats: Bioavailability of SUN 0855 in rats

Age (weeks)	Dose mg/kg	i.v.		p.o.	
		Plasma ΔAUC ¹⁾	Brain ΔAUC ¹⁾	Plasma ΔAUC ¹⁾	Brain ΔAUC ¹⁾
6	100	---	---	4571	377.8
	10	3881	307.2	285	7.2 ²⁾
	1	529	15.9 ²⁾	---	---
2	10	---	---	6593	2017
	1	---	---	824	701

1) ΔAUC: ng.h/ml - Not determined ; 2) The increase rate is not more than 1% of endogenous level; the value is not very reliable.

Rat: Tissue total concentrations after single and repeated dosing with SUN 0588

In 6-week-old Sprague-Dawley rats receiving repeated oral administration of SUN 0588 at 100 mg/kg/day for 14 days, whole blood, plasma and tissue total concentrations over time after the last dose were assessed in comparison with tissue total

concentrations over time following a single dose. The results showed no conspicuous difference in tissue total concentrations over time between the single dosing and the 14-day oral repeated dosing with SUN 0588. It is concluded therefore that absorption, distribution and excretion of SUN 0588 are barely affected by repeated dosing.

Single Dosing

	0 hour	2 hour	6 hour	24 hour	48 hour
Plasma (ng/ml)	31.0± 4.2	617.4± 69.5	104.5± 2.0	34.2± 3.4	36.5± 1.1
Blood (ng/ml)	84.9± 4.3	542.6± 50.3	189.2± 6.8	79.6± 5.9	87.4± 7.3
Liver (ng/g)	1607.0± 99.5	9839.9± 786.3	2468.2± 166.5	1752.7± 72.3	1758.7± 101.8
Brain (ng/g)	84.2± 2.3	93.8± 8.0	87.1± 0.9	84.2± 0.3	77.5± 2.3
Adrenal (ng/g)	1953.6± 590.6	2080.4± 405.8	1893.5± 432.7	1611.2± 365.9	1201.7± 218.1
Kidney (ng/g)	152.2± 11.0	2609.5± 262.5	381.9± 15.1	159.0± 1.7	151.2± 11.2
Spleen (ng/g)	338.2± 33.7	689.0± 43.6	521.0± 35.9	412.9± 51.7	466.8± 39.6

(N=3, Mean±S. E.)

Repeat Dosing

	0 hour	2 hour	6 hour	24 hour	48 hour
Plasma (ng/ml)	31.7± 1.0	656.7± 114.9	85.4± 1.7	32.3± 1.9	30.3± 0.9
Blood (ng/ml)	87.6± 4.7	612.8± 69.3	165.9± 7.2	91.4± 2.6	81.4± 13.0
Liver (ng/g)	1584.7± 162.5	10287.8± 1620.5	2497.2± 48.9	1334.1± 239.6	1694.9± 44.6
Brain (ng/g)	90.4± 1.0	97.3± 9.0	93.5± 4.2	79.1± 2.5	80.6± 3.1
Adrenal (ng/g)	1513.9± 88.1	1777.8± 350.7	2807.1± 251.5	1529.3± 54.5	1022.1± 28.4
Kidney (ng/g)	153.0± 11.8	2500.1± 15.4	292.0± 35.6	134.9± 8.4	144.2± 7.8
Spleen (ng/g)	418.0± 37.7	683.2± 67.7	510.1± 15.4	382.3± 24.1	435.5± 60.4

(N=3, Mean±S. E.)

Concentration fluctuation of total in blood plasma after i.v. and i.p. administration of SUN 0588 in rats

	Concentration of total in blood plasma (µg/ml.)			
	0hr	0.5hr	1hr	2hr
10 mg/kg i.v.	0.030±0.001	3.31±0.07	1.45±0.05	0.565±0.087
10 mg/kg i.p.	0.030±0.001	3.90±0.09	1.90±0.19	0.694±0.023

Concentration fluctuation of total [redacted] in blood plasma after intravenous administration and subcutaneous administration of SUN 0588 in rats

	Concentration of total [redacted] in blood plasma (µg/mL)				
	0hr	0.5hr	2hr	6hr	24hr
200 mg/kg i.v.	0.046 ±0.002	92.1 ± 3.9	17.0 ± 2.8	1.41 ±0.36	0.107 ±0.042
300 mg/kg i.v.	0.046 ±0.002	166.6 ±12.7	61.2 ± 25.6	22.5 ± 3.8	
500 mg/kg i.v.	0.046 ±0.002	313.6 ±13.5	151.7 ± 2.8	43.8 ± 5.1	
200 mg/kg s.c.	0.046 ±0.002	101.7 ± 8.3	31.7 ± 1.8	2.26 ±0.16	0.075 ± 0.007
500 mg/kg s.c.	0.046 ±0.002	157.5 ± 15.8	98.2 (n=1)	33.3 ± 7.2	
1000 mg/kg s.c.	0.046 ±0.002	180.5 ± 21.7	260.6 ± 21.9	151.9 ± 17.4	
2000 mg/kg s.c.	0.046 ±0.002	121.8 ± 20.9	375.7 ±20.2	427.2 ± 40.6	

Mean ± S.E. (n=3)

Mouse: Concentration fluctuation of total [redacted] in blood plasma after i.v. and s.c. administration of SUN 0588 in mice

	Concentration of total [redacted] in blood plasma (µg/mL)				
	0hr	0.5hr	2hr	6hr	24hr
200 mg/kg i.v.	0.078 ±0.004	131.9 ± 5.5	73.4 ± 8.1	21.78 ± 5.26	0.646 ± 0.131
200 mg/kg s.c.	0.078 ±0.004	150.4 ± 15.3	55.8 ± 2.3	21.90 ± 1.92	0.369 ± 0.093

Mean ± S.E. (n=3-9)

Monkey: Endogenous [redacted] in blood of cynomolgus monkeys

Concentration (ng/ml)	Total [redacted]	Oxidized [redacted]	Reduced [redacted]	Reduced/Total Ratio
Concentration in plasma	17.2	3.7	13.6	78.8%
Concentration in whole blood	49.8	6.7	43.1	86.7%
Concentration in erythrocytes	89.5	10.3	79.2	88.8%

Note) Animal No. 8412 was bled/analyzed for [redacted] on two separate occasions one month apart; therefore, data from this monkey were treated as data from two separate animals.

Pharmacokinetic Parameters of Total [redacted] in Plasma after Single-Dose Administration of Sapropterin to Female Monkeys

Dose (mg/kg)	Administration Route	Cendo 1 (ng/ml)	Cmax (ng/ml)	ΔCmax2 2 (ng/ml)	Tmax (hr)	ΔAUC3 (ng.hr/ml)	T1/2 (hr)	F4 (%)
10	p.o.	17.4±1.3	344±149	344±148.5	2.9±0.2	1301±144	1.42±0.17	9.0
1	i.v.	17.1±2.1				1449±68.4	0.82±0.14	

1 Endogenous total [redacted] concentration

² $C_{max} - C_{endo}$

³ Computed based on trapezoid rule, by using the value (ΔC) obtained by subtracting C_{endo} from the actually measured value (C) of plasma concentration.

⁴ Bioavailability (F) was computed by using ΔAUC at the time of 1-mg/kg intravenous injection.

$$F = [\Delta AUC_{po}] / [DOSE_{po}] / [\Delta AUC_{iv}] / 1 \times 100 (\%)$$

(mean value \pm standard error, n = 3)

Repeat Oral Dose Toxicokinetics of Phenoptin (6R-BH4) in CD-1 Mice (Study #: 0162-06-012)*

Methods: Six-week-old male CD-1 mice (45/group) were administered 125, 250, and 500 mg/kg/day of Phenoptin (sapropterin) by oral gavage at a dose volume of 5 mL/kg each day for four days. Blood was collected for toxicokinetic analysis pre-dosing, and at 0.5, 1, 2, 3, 4, and 6 or 7 hrs post-dosing on days 1 and 4.

Results: Phenoptin administration resulted in a dose-dependent increase in plasma concentrations in total in CD-1 mice. T_{max} values ranged from 0.5 to 2 hours post-dosing with no apparent correlation to dose level or dosing day. C_{max} increased with increasing dose levels but was slightly less than dose linear. There was no evidence of accumulation or decrease in C_{max} after four daily doses by comparison with results following the first dose. Although exposure did increase with increased dosage, AUC values normalized to dose decreased slightly with increasing dose levels. There was no change in exposure after four daily doses at any of the three dose levels. The TK parameters are summarized in the Table below.

Table: Toxicokinetic Parameters in Male CD-1 Mouse Plasma Following 4-Day Repeated Oral Dosing of Phenoptin

Dose (mg/kg)	Day	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-6} (hr*ng/mL)	AUC_{0-6}/D ((hr*ng/mL)/(mg/kg))	AUC_{last} (hr*ng/mL)	AUC_{last}/D ((hr*ng/mL)/(mg/kg))
125	1	1.0	3824.7	10131	81.050	10131	81.050
	4	0.5	4628.2	10997	87.979	11375	91.002
250	1	1.0	6920.5	17732	70.929	17732	70.929
	4	2.0	6981.2	20586	82.345	21422	85.686
500	1	1.0	11098	29475	58.949	29475	58.949
	4	0.5	10888	28537	57.073	30023	60.045

Oral (Stomach Tube) Toxicokinetic Study of Phenoptin (6R-BH4) in Pregnant New Zealand White Rabbits (Study #: 0162-05-018)*

Methods: The timed-mated rabbits [Hra:(NZW)SPF] were assigned to four dose groups (n=6). Sapropterin or the vehicle alone (sterile water for injection) were administered orally via stomach tube to female rabbits once daily on days 6 through 18 of presumed gestation at doses of 0 (Vehicle), 6, 60 or 600 mg/kg/day. The rabbits were also examined for clinical observations, abortions, premature deliveries and deaths daily. Body weight and feed consumption were recorded daily. Blood samples were collected pre-dose and at approximately 30 min, 1, 2, 3, 4 and 6 hours post-dose on gestation days 6 and 18. Plasma samples were analyzed for L-biopterin and the plasma concentrations of sapropterin were estimated from those L-biopterin levels.

On day 18 of presumed gestation, each female rabbit was sacrificed via an intravenous injection of a euthanasia solution after the last blood sample collection, Caesarean-sectioned and examined for pregnancy status (number and viability of conceptuses were recorded). Live conceptuses were sacrificed via an intraperitoneal injection of the euthanasia solution. Carcasses were discarded without further evaluation.

Results:**Mortality and clinical signs:**

All rabbits survived to schedule sacrifice. Observations of scant feces and/or no feces in the cage pan for two does (5647 and 5648) at 600 mg/kg/day at approximately day 14 or 17 of gestation were consistent with diminished feed consumption and body weight losses in these rabbits at the same time.

Body weights and body weight changes:

Does at 600 mg/kg/day had a 3% reduction in body weight as compared to the controls on day 18 of gestation. During the entire dosage period, this group gained an average of only 0.01 kg, which was significantly reduced ($p \leq 0.05$) as compared to the control group (0.20 kg). Average changes in body weight gain for intervals within the dosage period were generally reduced at 600 mg/kg/day, and the loss of 0.01 kg for days 12 to 15 of gestation was also significantly reduced ($p \leq 0.05$) as compared with the control group (gain of 0.07 kg).

Feed consumption:

Consistent with the changes in body weights, both absolute and relative feed consumption values were lower at 600 mg/kg/day with significantly reduced values ($p \leq 0.05$) for the interval between days 12 to 15 of gestation, as compared with the control group (60% of the control's absolute and relative value). Feed consumption values at 6 and 60 mg/kg/day were comparable with the control values.

Sectioning and Litter Observations:

Caesarean-sectioning observations were based on 6, 6, 6 and 5 pregnant does in the

0, 6, 60 and 600 mg/kg/day dosage groups, respectively. No Caesarean-sectioning or litter parameters (implantations and viable embryos) were affected by dosages of the test article as high as 600 mg/kg/day.

TK data:

The average plasma concentrations of sapropterin in the pregnant rabbits increased in a dose-related manner after the first dosage and the last dosage. The ratios of Cmax (ng/mL) values for 60 and 600 mg/kg/day doses were 1554:5896 (1:3.8) on GD 6 and 992:10374 (1:10.5) on GD 18. These ratios of Cmax (ng/mL) values suggested: 1) that the increase with dosage was less than dose-proportional at day 6 of gestation, and 2) that exposure at the lower dosage may have decreased with continued dosage, whereas the exposure increased at the higher dosage. The t_{1/2} was at least four hours. Dose related increases in plasma BH4 concentrations were observed for doses of 60 mg/kg/day and higher. The TK parameters are summarized in the Table below.

Table: Toxicokinetic Parameters of Sapropterin Administration Orally in Presumed Pregnant Rabbits

Parameters ¹	6 mg/kg/day		60 mg/kg/day		600 mg/kg/day	
	GD ² 6	GD ² 18	GD ² 6	GD ² 18	GD ² 6	GD ² 18
Cmax (ng/mL)	378(1)	– ³	1,544 ± 734 (6)	992 ± 195 (6)	5,896 ± 1,618 (5)	10,374 ± 7,241 (5)
Tmax (hr)	0.98 (1)	– ³	0.92 (6)	0.68 (6)	1.20 (5)	1.41 (5)
AUC(0-t) (h·ng/mL)	465 (1)	– ³	3,671 ± 1,679 (6)	1,589 ± 599 (6)	21,023 ± 3,218 (5)	32,896 ± 28,772 (5)

¹ Mean and standard deviation (N) (N≥2) except for Tmax for which the median (N) is reported. If N = 1, then the individual value is shown.

² GD Gestation Day

³ There were no BH4 plasma concentrations ≥ LOQ (100 ng/mL) on GD 18.

2.6.4.4 Distribution

The distribution of sapropterin was investigated in the following studies: Pharmacokinetics of Sapropterin Hydrochloride in the Rat (I) (Single Dose Administration) (Study #: PHN-104-PK-SR) and Fetal Migration and Milk Migration of sapropterin hydrochloride (Study #: PHN-107-PK-SR). The results are summarized as the followings.

Rat: Difference in endogenous level by age

Tissue	6-week-old	2-week-old
Plasma	33±3 (94.8%)	49±2 (87.8%)
Blood	214±14 (93.5%)	325±24 (95.6%)
Brain	80±5 (—)	128±3 (—)
Liver	1423±32 (98.5%)	—
Kidney	140±9 (91.3%)	—
Adrenal	3031±595 (93.8%)	—
Spleen	662±30 (78.5%)	—

Mean±S.E. (n=3-10), (): Reduced/total ratio, —: Not assayed

Rat: Tissue distribution of in 6-week-old rats following p.o. administration of SUN 0588 (Mean ± SE, n=2-5)

Best Possible Copy

Dose (administration route)	Oral Administration		(mean ± standard deviation, n = 2-5)						
	Organ name	Before dose	Hr 0.5 post-dosing	Hr 1 post-dosing	Hr 2 post-dosing	Hr 3 post-dosing	Hr 6 post-dosing	Hr 9 post-dosing	Hr 24 post-dosing
10 mg/kg (p.o.)	Plasma	33±3 [98.4%]	88±4 [93.5%]	103±8 [85.9%]	108±11 [81.3%]	78±7 [73.0%]	40.2±2 [73.0%]	34±3 [81.3%]	30±1 [95.8%]
	Whole blood	214±14 [93.5%]	237±7 [91.8%]	241±9 [88.7%]	255±13 [94.8%]	254±11 [92.2%]	211±4 [97.0%]	161±5 [97.4%]	170±2 [97.3%]
	Brain	80±5 [91.3%]	79±2 [95.8%]	82±4 [95.7%]	86±2 [95.9%]	79±7 [95.2%]	80±2 [93.9%]	78±2 [93.3%]	74±2 [95.6%]
	Liver	1423±32 [96.5%]	1376±77 [96.4%]	1601±74 [95.7%]	1512±93 [95.7%]	2072±108 [96.6%]	1353±24 [96.3%]	1372±162 [95.4%]	771±124 [96.5%]
	Kidneys	140±9 [91.3%]	413±28 [95.8%]	415±40 [95.7%]	474±24 [95.9%]	435±39 [95.2%]	131±3 [93.9%]	124±0 [93.3%]	156±11 [95.6%]
	Adrenal glands	3031±595 [93.8%]	3289±90 [87.5%]	3119±682 [89.4%]	416±71 [93.4%]	428±32 [91.3%]	3692±258 [94.5%]	3916±459 [90.8%]	1967±116 [95.3%]
	Spleen	662±30 [78.5%]	691±142 [60.9%]	806±78 [77.4%]	761±49 [74.3%]	710±36 [65.5%]	589±44 [63.0%]	707±41 [51.4%]	502±15 [68.3%]

Dose (administration route)	Oral Administration		(mean ± standard deviation, n = 2-5)						
	Organ name	Before dose	Hr 0.5 post-dosing	Hr 1 post-dosing	Hr 2 post-dosing	Hr 3 post-dosing	Hr 6 post-dosing	Hr 9 post-dosing	Hr 24 post-dosing
100 mg/kg (p.o.)	Plasma	38±1 [88.7%]	91.4±11 [90.1%]	1227±98 [89.8%]	1217±139 [90.9%]	713±132 [88.6%]	149±22 [86.4%]	62.5±5 [83.8%]	37±4 [86.3%]
	Whole blood	161±12 [89.7%]	618±13 [89.2%]	852±106 [91.0%]	902±170 [91.8%]	804±90 [91.7%]	329±16 [187.0%]	199±9 [38.8%]	124±4 [89.7%]
	Brain	106±5 [91.8%]	122±3 [91.3%]	156±9 [93.4%]	135±11 [91.7%]	143±3 [93.4%]	128±7 [93.3%]	121±11 [94.2%]	96±2 [92.6%]

Parentheses contain the amount of increase from pre-administration value. Brackets contain the reduced-form ratio.

(--): Below pre-dose level

Rat: Tissue distribution of [redacted] in 6-week-old rats following i.v. administration of SUN 0588 (Mean ± SE, n=3)

Intravenous Administration		(mean ± standard deviation, n = 3)							
Dose (administration route)	Organ name	concentration in organs (ng/g or ml)							
		Before dose	Hr 0.5 post-dosing	Hr 1 post-dosing	Hr 2 post-dosing	Hr 3 post-dosing	Hr 6 post-dosing	Hr 9 post-dosing	Hr 24 post-dosing
10 mg/kg (i.v.)	Plasma	33±3 [94.8%]	2678±34 (2645) [92.0%]	1385±63 (1350) [92.8%]	481±38 (448) [90.1%]	201±18 (168) [87.4%]	56±4 (23) [92.3%]	36±3 (3) [87.4%]	31±2 (-) [92.5%]
	Whole blood	214±14 [93.5%]	2629±85 (2415) [94.7%]	1489±57 (1275) [95.0%]	984±55 (770) [95.8%]	591±30 (377) [94.5%]	287±8 (73) [97.0%]	221±14 (7) [96.0%]	148±1 (-) [98.6%]
	Brain	80±5 (53)	135±5 (53)	134±6 (54)	124±9 (44)	136±32 (56)	93±7 (13)	86±7 (6)	98±3(18)
	Liver	1423±32 [96.5%]	18155±2165 (16732) [83.6%]	10612±1462 (9189) [83.4%]	5727±369 (4304) [83.3%]	1841±170 (418) [77.9%]	873±43 (-) [87.7%]	873±38 (-) [91.5%]	877±108 (-) [93.4%]
	Kidneys	140±9 [91.3%]	16608±1589 (16468) [92.7%]	5451±361 (5311) [95.4%]	2031±287 (1891) [95.3%]	1041±267 (901) [94.6%]	203±14 (63) [93.7%]	155±14 (15) [91.3%]	162±3 (22) [89.4%]
	Adrenal glands	3031±595 [93.8%]	6765±273 (3734) [66.1%]	5608±250 (2577) [63.3%]	4508±303 (1477) [72.7%]	4479±578 (1448) [81.7%]	4037±260 (1006) [80.1%]	3501±197 (470) [84.9%]	2419±136 (-) [89.2%]
	Spleen	662±30 [78.5%]	2394±77 (1732) [57.9%]	1834±146 (1172) [59.1%]	1494±179 (832) [55.5%]	1503±37 (84) [63.2%]	873±71 (211) [59.6%]	886±32 (224) [64.6%]	536±50 (-) [67.6%]

Intravenous Administration		(mean ± standard deviation, n = 3)							
Dose (administration route)	Organ name	concentration in organs (ng/g or ml)							
		Before dose	Hr 0.5 post-dosing	Hr 1 post-dosing	Hr 2 post-dosing	Hr 3 post-dosing	Hr 6 post-dosing	Hr 9 post-dosing	Hr 24 post-dosing
1 mg/kg (i.v.)	Plasma	33±3 [94.8%]	312±7 (279) [88.8%]	162±5 (129) [87.0%]	69±5 (36) [92.0%]	35±2 (2) [83.2%]	31±1 (-) [90.8%]	29±2 (-) [89.9%]	
	Whole blood	214±14 [93.5%]	406±8 (192) [92.3%]	321±3 (107) [94.3%]	225±16 (11) [93.5%]	236±14 (22) [94.1%]	198±13 (-) [97.2%]	185±13 (-) [96.3%]	
	Brain	80±5 (11)	91±2 (11)	94±1 (14)	55±3 (-)	78±4 (-)	81±6 (1)	83±5 (3)	
	Liver	1423±32 [96.5%]	3178±403 (1755) [91.2%]	2461±246 (1038) [91.8%]	1403±94 (-) [95.0%]	1171±101 (-) [92.1%]	1030±41 (-) [95.2%]	995±19 (-) [96.0%]	
	Kidneys	140±9 [91.3%]	1278±134 (1138) [96.5%]	781±112 (641) [96.7%]	287±42 (147) [95.7%]	186±36 (46) [95.5%]	108±18 (-) [95.0%]	144±10 (4) [96.7%]	
	Adrenal glands	3031±595 [93.8%]	2640±301 (-) [81.4%]	3554±586 (532) [88.4%]	2635±577 (-) [80.4%]	3567±368 (536) [87.4%]	3946±465 (915) [90.4%]	3270±593 (239) [85.3%]	
	Spleen	662±30 [78.5%]	784±63 (118) [61.0%]	714±19 (52) [43.2%]	776±66 (114) [47.2%]	778±87 (116) [56.2%]	824±77 (162) [65.0%]	728±184 (66) [53.3%]	

Parenteses contain the amount of increase from pre-administration value. Brackets contain the reduced-form ratio.

(--): Below pre-dose level

Rat: Tissue distribution of  in 2-week-old rats following administration of SUN 0588

		Tissue Concentration of (ng/g ml)							
Tissue		0hr	0.5 hr	1hr	2hr	3hr	6hr	9hr	24hr
1mg/kg i. p.	Plasma	49±2 (87.8)	340±21 (791) (94.9)	417±5 (368) (94.7)	186±16 (147) (93.9)	128±12 (79) (93.7)	93±9 (44) (93.8)	66±2 (17) (93.5)	50±1 (1) (92.3)
	Blood	325±24 (95.8)	789±45 (444) (94.6)	605±38 (280) (95.1)	393±51 (147) (94.8)	340±22 (79) (94.9)	322±30 (44) (94.5)	317±12 (17) (94.7)	243±14 (1) (94.8)
1mg/kg p. o.	Plasma	49±2 (87.8)	74±1 (25) (91.4)	131±12 (82) (93.4)	174±28 (125) (92.9)	130±12 (81) (92.4)	101±8 (52) (92.2)	82±10 (33) (92.1)	53±2 (4) (92.3)
	Blood	325±24 (95.8)	426±22 (101) (96.8)	409±28 (84) (96.3)	458±45 (131) (96.2)	435±59 (110) (96.2)	426±49 (101) (96.2)	380±60 (35) (95.8)	305±12 (---) (95.6)
	Brain	128±3	—	157±4 (29) (85.9)	158±2 (30) (84.8)	184±7 (36) (86.0)	164±4 (36) (87.0)	158±3 (30) (84.3)	155±3 (27) (86.7)
10mg/kg p. o.	Plasma	49±2 (87.8)	293 ¹⁾ (244) (82.7)	548±79 (500) (93.5)	1150±56 (1101) (93.3)	932±155 (883) (92.8)	428±85 (378) (89.9)	282±26 (238) (90.2)	49±2 (0) (88.2)
	Blood	325±24 (95.8)	498 ¹⁾ (173) (95.1)	619±72 (294) (93.2)	1158±57 (833) (94.1)	1288±40 (961) (94.8)	661±112 (338) (93.3)	490±88 (165) (91.7)	281±32 (---) (88.3)
	Brain	128±3	170±9 (42) (85.0)	181±6 (33) (82.5)	209±18 (81) (80.5)	213±9 (85) (84.1)	231±14 (103) (82.0)	248±8 (120) (80.2)	173±8 (45) (79.5)

(): Increase in concentration due to dosing. [] : Reduced/total ratio (%), (---): Below pre-dosing level
 Mean±S.E. (n=2-10), 1) N=1

Rat: Tissue radioactivity concentrations after oral administration of ³H-SUN 0588 (100 mg/10 MBq/kg) ---6 weeks old

Tissue	Radioactivity density (µg eq. Of  /g or ml)		Tissue	Radioactivity density (µg eq. Of  ; or ml)	
	Hr 2 post-dosing	Hr 6 post-dosing		Hr 2 post-dosing	Hr 6 post-dosing
Plasma	3.44±0.23	8.27±0.79	Pancreas	2.61±0.19	6.77±0.45
Whole blood	2.87±0.21	7.53±0.91	Testes	1.40±0.15	6.28±0.58
Brain	2.02±0.08	4.24±1.20	Epididymis	2.07±0.12	5.97±0.74
Pituitary gland	4.69±1.78	4.76±0.88	Prostate gland	1.78±0.11	6.15±0.58
Eyes	2.23±0.10	7.05 ± 0.58	Seminal	2.20±0.21	6.74±0.59

			vesicles		
Harderian glands	2.14±0.20	5.21±0.63	Skin	2.32±0.25	5.61±0.47
Submaxillary glands	1.66±0.03	5.71±0.64	Muscles	3.07±1.39	6.58±0.44
Thyroid gland	1.65±0.54	4.09±0.46	Bone marrow	1.73±0.51	4.75±0.30
Thymus	2.19±0.10	6.24±0.55	White fat	0.20±0.07	2.61±1.31
Heart	2.17±0.08	5.57±0.72	Brown fat	2.17±0.99	2.61±1.31
Lungs	2.57±0.10	5.62±0.40	Mesenteric lymph nodes	14.9±8.4	6.71±0.69
Liver	13.4±1.6	8.75±0.85	Stomach	25.7±3.7	8.79±1.55
Kidneys	6.95±0.23	7.83±0.72	Small intestine	405±193	33.8±27.8
Adrenal glands	1.50±0.16	5.15±0.70	Large intestine	5.33±1.31	352±49
Spleen	2.71±0.09	5.08±1.54	Cecum	14.4±8.9	7.34±2.88

(mean ± standard deviation, n = 3)

Rat: Tissue radioactivity concentrations after oral of administration of ³H-SUN 0588 (Corrected values) --6 weeks old

Tissue	Corrected concentration (µg. eq. of \blacktriangleright /g or mL)	
	2hr	6hr
Plasma	1.19	2.61
Whole blood	0.62	1.87
Liver	11.11	3.09
Kidney	4.70	2.17

**Rat: Tissue radioactivity concentrations after oral of administration
of ³H-SUN 0588 – 2 weeks old**

Tissue	Radioactivity concentration ($\mu\text{g. eq. of } \text{---} \text{g or mL}$)	Corrected value ($\mu\text{g. eq. of } \text{---} \text{/mL}$)
Plasma	3.44 ± 0.23	1.42
Whole blood	2.87 ± 0.21	0.85
Brain	2.02 ± 0.08	0
Liver	4.69 ± 1.78	2.67
Kidney	2.23 ± 0.10	0.21
Adrenal	2.14 ± 0.20	----
Spleen	1.66 ± 0.03	----

**Total Concentration in Maternal Animals and Fetuses after p.o.
Administration of Sapropterin (10 mg/kg) to Pregnant Rats**

Sample		Total concentration (ng/ml)		
		Before dose	2 hr	6 hr
Dam	Whole blood	78.7±6.6	145±7*	85.2±7.8
	Plasma	24.4 (n=2)	110±10*	30.0±0.3*
	Liver	853±62	1739±50**	1037±16*
	Placenta	614±27	427±67	361±56*
Fetus		321±29	257±7	322±28

* p<0.05; ** p<0.01 Significant difference relative to pre-administration value (Student's t-test)
(mean ± standard deviation, n = 3)

The fetal migration of SUN 0588 was studied by means of whole-body autoradiography and the HPLC based measurement of the tissue concentration of

Whole-body autoradiography: Sponsor stated that radioactivity distribution comparable to that in dam blood was observed in the placenta and uterine membrane, 0.5 hr after the i.v. administration of 10 mg/kg of ³H-SUN 0588. The concentration of radioactivity in the fetus was lower than that in the dam's blood and was distributed uniformly throughout the body. The distribution of radioactivity in the dam was almost identical to that in the male rat. The radioactivity distribution after the oral administration of 10 mg/kg of ³H-SUN 0588 was mainly in the GI tract. As for the other tissues, only a low level was observed in the liver, and migration of radioactivity to the fetus was not observed. (Only autoradiographs were provided. No numerical data).

The total endogenous concentration of SAPROPTERIN in the fetus when no SUN 0588 was administered was approximately 300 ng/g. This concentration was about 10x the dam plasma concentration. Even after the oral administration of 10 mg/kg of SUN 0588, the concentration was almost unchanged.

Milk migration: The milk migration of SUN 0588 was studied by measuring the concentration of SAPROPTERIN in milk. The results are summarized in the Table below.

The total SAPROPTERIN concentration in milk prior to SUN 0588 administration was almost equivalent to that in plasma (i.e., approximately 70 ng/ml). The total SAPROPTERIN concentration in mammary glands was about 20X that in plasma (i.e., approximately 1000 ng/ml).

When 10 mg/kg of SUN 0588 was administered intravenously, the mammary gland concentration of SAPROPTERIN increased to about 6X the pre-administration level at 2.5 hr after administration, and almost the same concentration was maintained even 6.5 hr after administration. The total SAPROPTERIN concentration in milk rose gradually after administration, and 6.5 hr after administration it reached a level at least 5X that before administration.

After 10 mg/kg of SUN 0588 was administered orally, the total SAPROPTERIN concentration in the mammary glands showed a tendency to rise slightly above the level before administration. However, the SAPROPTERIN concentration in milk was nearly constant before and after administration, so no effect attributable to administration was observed.

Rat: Milk Migration of SAPROPTERIN after oral and i.v. Administration of SUN 0588

		Total SAPROPTERIN concentration (ng/g or ml)				
		0 hr	1.5 hr	2.5 hr	3.5 hr	6.5 hr
IV	Plasma	51.9 ± 4.3		365.8 ± 44.6		75.0 ± 7.1
10mg/kg	Milk	69.9 ± 11.4	246.8 ± 44.6	243.2 ± 30.9	307.7 ± 59.9	445.4 ± 103.7
	Mammary g.	987.0 ± 101.1		6456.7 ± 546.4		5507.2 ± 880.0
PO	Plasma	51.9 ± 4.3		95.0 ± 24.8		42.7 ± 5.4
10mg/kg	Milk	69.9 ± 11.4	67.9 ± 27.2	51.5 ± 3.2	63.1 ± 3.4	61.6 ± 9.9
	Mammary g.	987.0 ± 101.1		1968.3 ± 485.6		1645.5 ± 557.1

2.6.4.5 Metabolism

The metabolism of Sapropterin was investigated in the following studies: A study on Sapropterin Hydrochloride metabolites in rat urine (Study #: PHN-109-PK-SR) and A

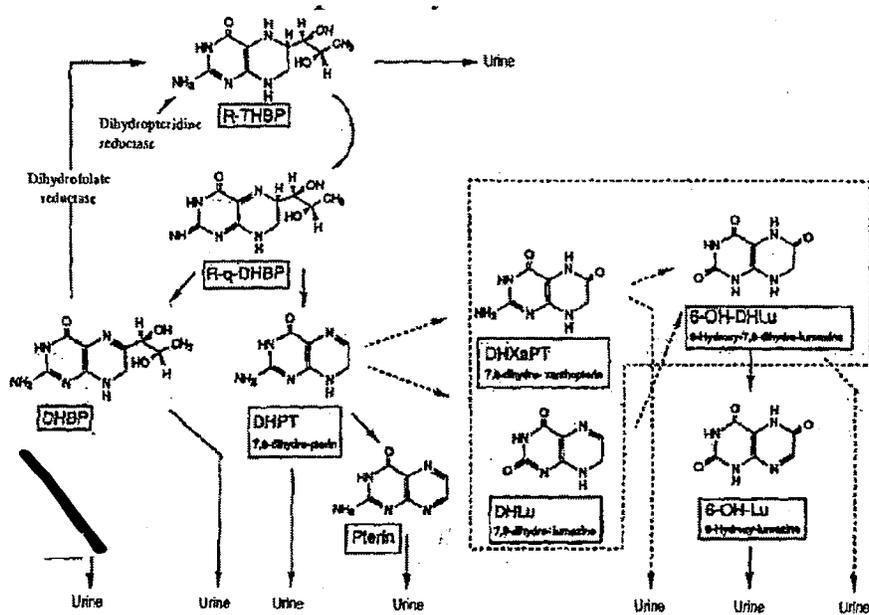
study on liver microsomal drug-metabolizing enzyme induction by sapropterin hydrochloride (Study #: PHN-108-PK-SR).

7,8-Dihydrobiopterin [DHBP], [redacted] pterin and 6-hydroxylumazine [6-OH-Lu] were identified as metabolites in rat urine by HPLC co-chromatography following administration of sapropterin hydrochloride [SUN 0588: (-)-(1'R, 2'S, 6R)-2-amino-6-(1', 2'-dihydroxypropyl)-5,6,7,8-tetrahydro- 4(3H)Pteridinone dihydrochloride]. In the rat urine excreted during 6 hours after a 10 mg/kg intravenous dose of SUN 0588, 30.8% of the dose given was excreted as unchanged compound R-THBP, 19.0% as 7,8-Dihydrobiopterin [DHBP], 0.9% as [redacted], 0.9% as pterin and 1.2% as 6-hydroxylumazine [6-OH-Lu]. As the pterin excretion in urine increased substantially after acidic iodine oxidation of the urine sample, possible excretion of 7,8-dihydropterin [DHPT] in urine was estimated. Furthermore, there was a marked increase in urinary recovery of 6-OH-Lu (to 41.5% of the dose) following urine sample oxidation with dilute iodine, suggesting possible metabolism to 7,8-dihydro-xanthopterin [DHXApt] and 7,8-dihydro-6-hydroxy-lumazine [6-OH-DHLu] and excretion in urine. The sum of the amounts of these metabolites excreted corresponded to 99-101% of the dose given; it is thus evident that SUN 0588 administered was rapidly excreted in urine. 7,8-Dihydroxylumazine [DHLu] or 7,8-dihydro-xanthopterin [DHXApt] was considered likely to occur as an intermediate metabolite in the metabolic pathway from DHPT to 6-OH-DHLu, but neither lumazine [Lu] nor xanthopterin [XApt] was detected in iodine oxidized urine solution.

SUN 0588 is excreted as an intact compound in urine. But, if utilized in tissues as the coenzyme R-THBP, it is converted to R-q-DHBP, which in turn is reduced to R-THBP by dihydropterin reductase or oxidized to DHBP or DHPT. The DHBP is either excreted as it is in urine, or reduced in part to R-THBP by dihydrofolate reductase or oxidized so far as to [redacted] and excreted in urine. The DHPT is further oxidized to be excreted as pterin in urine or even excreted as is in urine. By further oxidation, it is excreted as 6-OHLu in urine. It could not be ascertained, however, whether the metabolite detected as 6-OH-Lu following oxidation with dilute iodine was derived from 6-OH-DHLu or DHXApt.

Sponsor stated that, it has been reported that large amounts of THBP and DHBP and DHXApt as well as a small amount of [redacted] appeared in the urine of healthy humans administered (R,S)-THBP intravenously. It has also been described that the human liver is devoid of pterin deaminase and no lumazine metabolites (metabolites with oxidative elimination of the amino group at the 2-position) in human urine have been documented. In feces, on the other hand, not only 6-OH-Lu are reportedly excreted, but also biolumazine resulting from [redacted] oxidation after deamination at the 2-position, sepiapterin and 2'-deoxy-sepiapterin forming via conversion of the side chain at the 6-position, and sepialumazine and 2'-deoxysepialumazine resulting from their oxidation after deamination at the 2-position. These are all considered to have formed through metabolic degradation by bacterial flora in the gastrointestinal tract.

Estimated metabolic pathways of SUN 0588 in rats



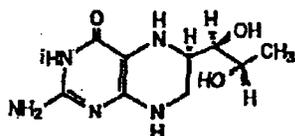
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Chemical Structures of SUN 0588 and Its Metabolites:

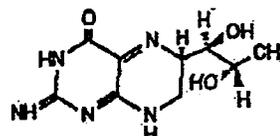
SUN 0588(R-TBHP)

(R)-Tetrahydrobiopterin



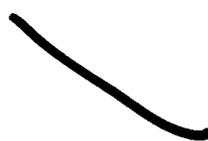
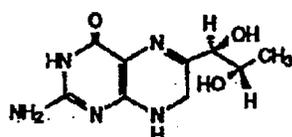
R-q-DHBP

(R)-quinonoid-dihydrobiopterin



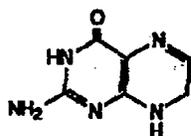
DHBP

7,8-dihydro-biopterin

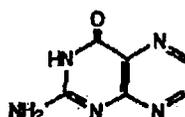


DHPT

7,8-dihydro-pterin

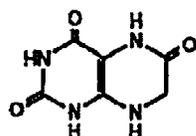


pterin



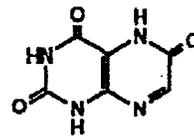
6-OH-DHLu

7,8-dihydro-6-hydroxy-lumazine



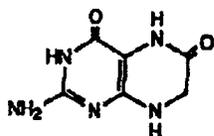
6-OH-Lu

6-hydroxy-lumazine



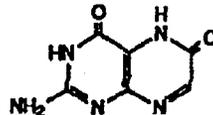
DHXaPT

7,8-dihydro-xanthopterin



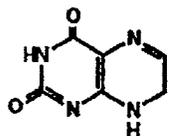
XaPT

xanthopterin

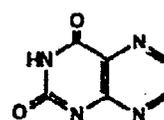


DHLu

7,8-dihydro-lumazine



lumazine(Lu)



To determine whether drug metabolizing activity might be induced by long term treatment with SUN 0588, the liver weight and liver microsomal cytochrome b₅ and cytochrome P-450 relative contents and drug-metabolizing activity were determined in male SD rats following treatment with 100 mg/kg/day SUN 0588 orally for 7 days, and assessed in comparison with a control group. Phenobarbital (PB) and β-naphthoflavone (BN) – (drug metabolizing enzyme inducers) were used as positive controls.

In response to treatment with PB as well as to that with BN, rats exhibited significant increases in liver weight and cytochrome P-450 content but no change in cytochrome b₅ content. Aminopyrine N-demethylation activity significantly increased in the rats treated with PB, and a significant increase in aniline p-hydroxylation activity occurred in the rats treated with BN. These results agree well with the known facts regarding induction of hepatic drug-metabolizing enzyme activity by PB and BN. The SUN 0588 treated group, in contrast, showed no difference from the control group with respect to cytochrome b₅ content, cytochrome P-450 content, aminopyrine N-demethylation activity, or aniline p-hydroxylation activity even after repeated dosing for 7 days. Body weight changes during treatment and post-treatment macroscopic appearance of the liver in the SUN 0588 treated group practically did not differ from those in the control group. SUN 0588 is thus not considered to induce hepatic drug-metabolizing enzyme activity.

Effect of treatment with SUN 0588 on the activities of rat liver microsomal cytochrome b₅ content, cytochrome P-450 content, amino pyrine N-demethylation and aniline p-hydroxylation activity

(mean ± standard deviation, n = 5)						
Treatment	Body weight after sacrifice (g)	Liver weight (g)	Enzyme amount (nmol/mg prot.)		Metabolic activation (nmol/mg prot.)	
			Cytochrome b ₅	Cytochrome P-450	Aminopyrine N-demethylation	Aniline p-hydroxylation
Control (saline) 0.4 ml/kg/day × 7 days, p.o.	184±6 (100%)	5.7±0.3 (100%)	0.569±0.038 (100%)	1.129±0.062 (100%)	10.37±0.62 (100%)	0.943±0.044 (100%)
Sapropterin 100 mg/kg/day × 7 days, p.o.	188±2 (102%)	5.6±0.1 (98%)	0.582±0.029 (103%)	1.073±0.087 (95%)	10.55±0.44 (102%)	0.989±0.055 (105%)
Phenobarbital 80 mg/kg/day × 3 days, intraperitoneally	194±2 (105%)	8.4±0.2 (147%)	0.525±0.028 (92%)	2.148±0.219* (190%)	13.80±1.12* (133%)	1.102±0.080 (117%)
β-naphthoflavone 40 mg/kg/day × 3 days, intraperitoneally	216±4 (117%)	9.0±0.3 (158%)	0.611±0.020 (107%)	1.328±0.065* (118%)	9.75±0.73 (94%)	1.305±0.094** (138%)

Parentheses contain percentage of control.

* p<0.05

** p<0.01 Significant difference with respect to control value (Student's t-test)

2.6.4.6 Excretion

The excretion of sapropterin was investigated in rats and monkeys in the following studies: Pharmacokinetics of Sapropterin Hydrochloride in the Rat (I) (Single Dose Administration) (Study #: PHN-104-PK-SR) and Pharmacokinetics of Sapropterin hydrochloride in the Monkey (Study #: PHN-110-PK-SR). The results are summarized as the followings.

Rat: Excretion in Urine, Feces, and Bile after Administration of 3H-Sapropterin to Rat

N	Sex	Dose (mg/kg)	Administration Route	Time (hr)	Excretion rate in urine (% of dose)	Excretion rate in feces (% of dose)	Excretion rate in bile (% of dose)	Total excretion rate (% of dose)
4	Male	100	p.o.	0-72	6.9 ± 0.8	75.2±3.5	–	82.0 ± 2.7
3	Male	10	i.v.	0-72	78.1 ± 0.8	1.5±0.3	–	79.6 ± 0.5
2*	Male	10	i.v.	0-72	64.6 (mean)	0.8 (mean)	8.1 (mean)	73.5 (mean)

--: Not measured

*Separate study in which excretion rate in bile was measured (mean ± standard deviation)

6-week-old rats: Estimation from urinary excretion rate

Route of administration	Dose (mg/kg)	72-hour cumulative excretion rate of radioactivity (% of dose)	Absorption rate (% of dose)
p.o.	100	6.9 ± 0.78	8.8
i.v.	10	78.1 ± 0.77	----

Absorption rate (%) = Urine (p.o.) / Urine (i.v.) × 100

Rat: Excretion of endogenous  in urine, feces and bile per day in rats

	Excretion of Total ($\mu\text{g/day}$)	Reduced / Total (%)
Urine ¹⁾	$69.8 \pm 5.66^{3)}$	75.6 ± 4.3
Feces ¹⁾	$0.4 \pm 0.16^{3)}$	— ³⁾
Bile ²⁾	$3.5 \pm 0.39^{4)}$	— ⁵⁾

1) From the same rats, 2) Only bile was collected, 3) n=3, 4) n=4, 5) Not done

Rat:  excretion in urine and feces after intravenous administration of SUN 0588

Time (hr)	Urine		Feces
	Excretion of Total (% of Dose)	Reduced Total (%)	Excretion of Total (% of Dose)
0~6	44.8 ± 3.3	76.5 ± 2.7	0.10 ± 0.15
6~24	0.9 ± 0.7	51.6 ± 5.5	0.04 ± 0.02
0~24	45.7 ± 4.0	74.3 ± 2.5	0.06 ± 0.17

Rat:  excretion in urine and feces after oral administration of SUN 0588

Time (hr)	Urine		Feces	
	Excretion of Total (% of Dose)	Red Tot (%)	Excretion of Total (% of Dose)	Red Tot (%)
0~24	2.4 ± 0.9	70.4 ± 5.7	50.2 ± 3.0	(89.0 ± 3.4)
24~48	0.1 ± 0.1	60.5 ± 14.9	0.5 ± 0.4	(73.6 ± 9.1)
0~48	2.5 ± 0.8	70.8 ± 3.7	50.7 ± 2.6	(88.9 ± 3.4)

Rat: Cumulative excretion rates of radioactivity in urine and feces after i.v. or oral administration of $^3\text{H-SUN 0588}$

Time (hr)	i.v. (10 mg/kg) Excretion of radioactivity (% of dose)			p.o. (100 mg/kg) Excretion of radioactivity (% of dose)		
	Urine	Feces	Total	Urine	Feces	Total
0~3	58.0±3.4	0.1±0.0	58.0±3.4	1.9±0.6	9.2±3.1	11.1±3.5
0~6	59.4±3.2	0.1±0.0	59.5±3.2	3.7±0.4	32.1±7.7	35.7±7.5
0~12	70.5±1.2	0.7±0.2	71.2±1.1	4.8±0.4	70.0±4.2	74.8±3.8
0~24	73.3±1.4	1.2±0.2	74.4±1.2	5.2±0.5	74.9±3.5	80.1±3.0
0~48	76.0±1.0	1.4±0.3	77.4±0.8	6.1±0.6	75.1±3.5	81.2±2.9
0~72	78.1±0.8	1.5±0.3	79.6±0.5	6.9±0.8	75.2±3.5	82.0±2.7

Mean±S.E.

Rat: Cumulative excretion rates of radioactivity in urine, feces and bile after i.v. or oral administration of $^3\text{H-SUN 0588}$

Time (hr)	Excretion of radioactivity (% of dose)			
	Urine	Feces	Bile	Total
0~3	51.1±10.1	0.1±0.02	1.9±0.37	53.0±9.7
0~6	59.0±7.2	0.1±0.00	2.4±0.46	61.5±6.8
0~12	61.4±6.3	0.1±0.02	3.3±0.58	64.7±5.8
0~24	63.0±6.0	0.7±0.38	5.1±0.46	68.7±5.2
0~48	64.6±5.5	0.8±0.38	7.8±0.74	73.2±4.4
0~72	64.6±5.5	0.8±0.38	8.1±0.83	73.5±4.3

Mean±S.E. (n=2)

Monkey: Total [redacted] in urine after administration of SUN 0588 to Cynomolgus monkey

	Animal No.	Dose of SUN 0588 (mg)	Urinary excretion of total [redacted]						
			Endogenous 24 hr mean	µg/kg b.w.	0-24 hr	0-24 hr ΔExcretion	0-24 hr Δ%Excretion	24-48 hr	48-72 hr
i. v. 1mg/kg	8407	2.84 (2.14)	122						
	8408	2.89 (2.02)	143						
	8410	3.20 (2.42)	150 ¹⁾						
	8412	5.50 (4.15)	167 ¹⁾						
	Mean S. E.		148 ± 9.3	— —	929 ± 336	784 ± 328	26.3% ± 5.77 %	150 ± 13.1	—
p. o. 10 mg/kg	8407	28.0 (21.1)	159 ²⁾						
	8408	25.0 (18.9)	70 ²⁾						
	8412	54.9 (41.4)	162						
	Mean S. E.		130 ± 30.2	— —	842 ± 392	711 ± 381	2.3% ± 0.76 %	152 ± 4.0	127 ± 8.0
Endogenous total [redacted] excretion (Mean of values from / monkeys)			139 ± 12.8	41.2 ± 4.54					

- 1) Mean of data on 3 pre-dosing days (#8410: 150±13.5, #8412: 167±53.3)
- 2) Mean of data on 2 pre-dosing days (#8407: 159±31.5, #8408: 70)

Rat: Urinary excretion of metabolites after intravenous administration of SUN 0588 (Ascorbic acid/cysteine -added urine collection) Dose: 10mg/kg of SUN 0588 (equivalent to 2.152 mg of [redacted])

Metabolite	Blank urine (6 hr)	Urinary excretion [µg, free form, [redacted] equivalent in parentheses]			ΔExcretion	Excretion rate (%)
		Urine post-dosing				
		0~3hr	3~6hr	0~6hr		
R-THBP (SUN 0588)	6.29 (4.75)	825.33 (823.18)	58.25 (44.74)	884.58 (867.92)	878 (883)	30.8
DNBP	30.01 (29.78)	408.70 (403.27)	35.84 (35.54)	442.54 (438.81)	413 (409)	19.0
[redacted]	0.19	17.43	1.57	18.00	18	0.9
[redacted]	18.85	864.30	75.20	1039.50	1021	47.4
pteria	0.79 (1.11)	12.68 (18.41)	1.24 (1.80)	13.90 (20.21)	13 (19)	0.9
Total pterin ²⁾	2.11 (3.07)	123.27 (179.25)	10.29 (14.88)	133.56 (194.21)	131 (181)	8.9