Rat: Urinary excretion of metabolites after intravenous administration of SUN 0588 (Non-added urine collection) Dose: 10 mg/kg of SUN 0588 (equivalent to 2.265mg of [redacted])

Metabolite	Urinary excretion [μg, free form, [redacted] equivalent in parentheses]					Excretion rate (%)
	Blank urine (3 hr)	Urine post-dosing			ΔExcretion	
		0~3hr	3~6hr	0~6hr		
R-THBP (SUN 0588)	1.59 (1.20)	44.65 (33.26)	16.81 (12.69)	60.86 (45.95)	58 (44)	1.9
DHBP	6.59 (6.53)	1004.14 (995.66)	17.24 (17.09)	1021.38 (1012.75)	1008 (1000)	44.2
[redacted]	0.11	51.31	0.96	52.27	52	2.3
pterin	0.02 (0.03)	19.43 (28.25)	0.62 (0.90)	20.05 (29.15)	20 (29)	1.3
6-OH-Lu	1.17 (1.54)	21.20 (27.82)	1.65 (2.17)	22.85 (30.09)	20 (27)	1.2
Metabolites assayed as 6-OH-Lu after oxidation with dilute iodine	18.03 (21.11)	682.11 (898.35)	63.16 (83.18)	745.27 (981.53)	713 (939)	41.5

Protein Binding:

The protein binding of sapropterin was investigated in the following studies: Plasma Protein Binding and Distribution into Blood Cells of Sapropterin Hydrochloride (Study #: PHN-106-PK-SR) and Pharmacokinetics of Sapropterin hydrochloride in the Monkey (Study #: PHN-110-PK-SR).

Plasma protein binding of [redacted] in rats (10 mg/kg i.v.)

	Total [redacted]		Reduced [redacted]	
	Conc. (ng/ml)	Binding ratio (%)	Conc. (ng/ml)	Binding ratio (%)
Endogenous level	42.2±7.8	3.1±2.2	41.4±8.4	12.1±6.9
30 min. after dose	2893±189	6.2±0.4	2758±186	5.5±0.7

(mean ± standard deviation, n = 3)

Plasma protein binding of [redacted] in humans

	Total [redacted]		Reduced [redacted]	
	Conc. (ng/ml)	Binding rate (%)	Conc. (ng/ml)	Binding rate (%)
Endogenous level	3.1±0.2	22.2±3.6	–	–
Sapropterin addition	5.1±0.3	22.7±2.7	–	–
	5.5±0.3	33.7±7.0	–	–
	9.0±0.3	25.4±7.3	5.8±0.3	31.3±6.9
	51.8±1.0	6.9±3.0	47.3±0.5	7.8±1.7
	468±43	8.5±3.0	437±10	6.8±1.4

(mean value ± standard deviation, n = 2–4)
– Not Detected

Total [redacted] concentration in blood cells (Rats: 100 mg/kg; p.o.)

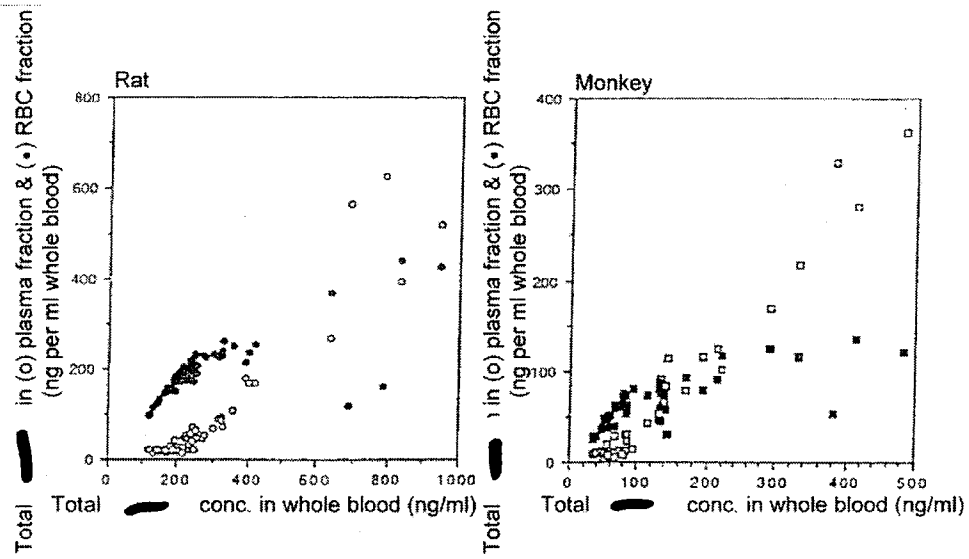
Animal No.	Plasma and whole blood total [redacted] concentration: Conc. in plasma ng/mL	Conc. in whole blood ng/mL	Conc. in plasma fraction[1), 2)]	Conc. in blood cell fraction[1), 3)]	Total [redacted] concentration in fractions on Sepracell-MN: Erythrocyte Fr. Conc.[1)]	Erythrocyte Fr. Prop.[4)]	Lymphocyte Fr. Conc.[1)]	Lymphocyte Fr. Prop.[4)]	Plasma Fr. Conc.[1)]	Plasma Fr. Prop.[4)]	Total conc.[1)]
Endogenous											
1											
2											
3											
4											
5											
6											
After a 100 mg/kg p.o. dose of SUN 0588											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											

1) Concentration per ml of whole blood (ng/ml of blood); 2) Total [redacted] concentration in plasma x 0.55; 3) (Total [redacted] in whole blood) - (Concentration in plasma fraction); 4) Proportion as percent of total concentration in erythrocyteflymphocyteJplasma fractions

Total [redacted] concentration in plasma - Comparison of PPP (platelet poor plasma) and PRP (platelet rich plasma) – Rat: 100 mg/kg; p.o.

Animal No.	Total [redacted] concentration in plasma (ng/mL) PPP (Platelet poor plasma)	PRP (Platelet rich plasma)	PRP/PPP ratio
Endogenous level			
1	[redacted]		0.763
2			0.949
3			0.995
After a 100 mg/kg p.o. dose of SUN 0588			
4	[redacted]		0.910
5			0.953
6			0.896
7			0.872

Blood Cell Distribution of [redacted] in Rat and Monkey



PK/TK summary:

The absorption, distribution, metabolism and excretion of sapropterin HCl was characterized in eleven studies, in five species; mouse, rat, dog, rabbit and monkey, and via two routes of administration; i.v. and p.o. The intended clinical route of

administration is p.o. Sapropterin HCl is absorbed mainly in the small intestine, including the duodenum. 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 half-lives were 1.1 hr and 1.4 hr in rats and monkeys, respectively. The half-lives were 1.2-1.3 hr in mice treated with single dose of 36, 200 and 400 mg/kg p.o. The areas under the plasma concentration-time curve (ΔAUCs) in rats treated with 10 and 100 mg/kg p.o. were 265 and 4571 ng.h/ml, respectively. The AUCs in mice treated with 36, 200 and 400 mg/kg p.o. were 5787, 22894 and 46878 ng.h/ml, respectively. After 10mg/kg p.o. administration to monkeys the ΔAUC was 1301 ng.h/ml. The bioavailability was 7%-12% in rats, and approximately 9% in monkeys. There was no evidence of accumulation or decrease in Cmax following repeated daily doses in rats or mice. When rats were treated with a single-dose of sapropterin HCl, it was primarily distributed to the kidneys, adrenal gland, and liver. The total organ concentration of [redacted] after a single-dose (10 mg/kg, p.o.) was increased in the kidney to about 3X the endogenous level and increased in the adrenal glands and liver to about 1.4X of the endogenous level. However, at this dose level little was distributed to the brain. In contrast, after a single-dose of 100 mg/kg p.o., the brain [redacted] level rose to about 1.5X the endogenous level.

When ^{3}H-sapropterin HCl was administered i.v. to rats on day 18 of gestation, the radioactivity distributed to the placenta and uterine wall at almost the same level as in the blood of a maternal animal. However, little radioactivity was distributed to the fetus. After 10 mg/kg, i.v. dose of sapropterin HCl to lactating rats, the concentration of [redacted] in milk increased to about 3X the endogenous level at 2.5 hr post-dosing.

The protein binding of [redacted] in plasma was relatively low (3%-12% in rats; 22%-34% in humans). When sapropterin HCl was administered to rats and monkeys, nonlinearity (saturability of distribution to red blood cell fraction) was also observed in the red blood cell distribution of [redacted]

The following were detected as urinary metabolites after administration of sapropterin HCl, 10 mg/kg, i.v., to rats: tetrahydrobiopterin (6R-BH4 [presumed unchanged drug]), dihydrobiopterin, [redacted] pterin, and 6-hydroxylumazine. Therefore, it was assumed that sapropterin HCl passes through the metabolic pathway for endogenous 6R-BH4.

In rats, 6R-BH4 and dihydrobiopterin were mainly excreted in urine after the single-dose of sapropterin HCl, 10 mg/kg, i.v.. The sum of the 6R-BH4 and the metabolites detected in urine up to 6 hr post-dosing reached about 100% of the administered dose, indicating that sapropterin HCl and its metabolites were excreted rapidly in urine.

2.6.4.7 Pharmacokinetic drug interactions

Sapropterin is not metabolized through the Cyp-P450 drug metabolizing enzyme system and does not induce Cyp-P450 enzymatic activity. The rat and human catabolic pathways are similar and therefore it is assumed that sapropterin passes through the

catabolic pathway for endogenous 6R-BH4. Additionally, the protein-binding rate of sapropterin in plasma was relatively low. Thus, sapropterin is not expected to have any potential for pharmacokinetic drug interaction via these pathways.

2.6.4.8 Other Pharmacokinetic Studies

Endogenous [redacted] Levels in Various Species (Study #: PHN-103-PK-SR)*

Methods: Endogenous plasma [redacted] concentrations were measured in humans (healthy adult males, 25 to 40 years old), monkeys (cynomolgus, female, age unknown), dogs (beagle, males, 8-9-month-old), rats (Sprague Dawley [SD] strain, males, 6-week-old and 4-day-old; 4-week-old male SPF Wistar rats), and mice (ICR strain, males, 7-week-old; ddY starin, males, 4-week-old).

The effects of circadian rhythms on endogenous [redacted] levels were explored in mice (ICR strain, males, 7-week-old). The effects of fasting on [redacted] levels were explored in rats (Sprague Dawley [SD] strain, males, 8-week-old). The [redacted] levels of 4-day-old and 8-week-old rats (Sprague Dawley [SD] strain, males) were compared.

Blood samples were collected from the heart of anesthetized rats and mice. Blood was collected via a foreleg vein of the dog or via a leg vein of the monkey. Blood of healthy volunteers was collected via a forearm vein. All the blood samples were immediately treated with 1 mL of an acidic oxidizing solution or an alkaline oxidizing solution.

Animals used to measure [redacted] concentration in the brain were thoroughly exsanguinated, the brain was excised, a suitable volume of 0.1 N phosphoric acid solution was added, the tissue was homogenized, and a predetermined amount of the homogenate was oxidized in the same manner as the blood.

Results: The total concentration of [redacted] in plasma and whole blood varied with the animal species and was highest in mice, followed by rats > dogs > monkeys > humans. Although the whole-blood level was comparable to the plasma level in humans, the whole-blood level was higher than the plasma level by approximately 3-fold in monkeys, 4-fold in dogs, 6- to 9-fold in rats, and 2- to 5-fold in mice. In addition, the ratio of reduced [redacted] to total [redacted] varied across animal species. The proportion of [redacted] accounted for by reduced [redacted] was approximately 65% in humans and higher in the other species. Species differences in endogenous [redacted] levels are summarized in the Table 1 below.

Table 1: Endogenous [redacted] Concentration in Plasma and whole blood of Each Animal Species

Animal	n	Plasma (ng/ml) Total [redacted]	Reduced [redacted]	Red./Tot(%)	Blood (ng/ml) Total [redacted]	Reduced [redacted]	Red./Tot(%)
Men	8	4.14± 0.19	2.73 ± 0.20	(65.8± 3.6)	4.12± 0.16	2.74 ± 0.12	(67.2± 3.7)
Monkey	8	18.98± 2.24	14.61 ± 1.62	(77.5± 2.4)	51.27± 9.72	46.13 ± 4.35	(89.9± 0.8)
Dog	8	18.52± 1.40	13.20 ± 1.17	(70.7± 2.2)	72.89± 5.78	65.57 ± 3.71	(89.8± 0.5)
Rat(Wistar)	3	41.63± 2.43	36.54 ± 2.73	(87.6± 1.4)	353.64±10.11	335.70 ±10.87	(94.9± 0.4)
Rat(S.D.)	4	49.91± 1.98	44.99 ± 1.84	(90.1± 0.6)	291.94±12.61	282.88 ±10.82	(97.0± 0.7)
Mouse(ddY)	2	76.43±12.67	60.10 ±18.80	(76.7±11.8)	407.61±40.26	381.70 ±39.21	(93.6± 0.4)
Mouse(ICR)	12	126.95± 2.80	108.59 ± 2.71	(85.5± 0.7)	230.14±12.98	218.80 ±13.42	(94.7± 0.8)

Mean ± S.E.

When the circadian rhythms of the total plasma concentrations of [redacted], were studied in mice over a 12–hr period from the 9th hr to the 21st hr, the levels remained virtually unchanged. Thus, circadian rhythms have no effect on endogenous [redacted] levels in the plasma (see Table 2 below)

Table 2: Diurnal Variation in Endogenous [redacted] Levels in Rats

		[redacted] concentration (ng/ml) 9hr	12hr	15hr	18hr	21hr
Blood	Total [redacted]	189.58±46.36	247.09±38.98	243.77±11.92	224.57±38.46	230.41±10.99
	Reduced [redacted]	176.54±48.13	231.07±38.54	284.99±12.66	213.83±42.63	223.32±11.39
Plasma	Total [redacted]	116.81±15.12	123.39± 6.23	130.11± 0.93	122.20± 4.06	122.67± 5.59
	Reduced [redacted]	91.50± 6.34	104.17± 5.45	114.26± 1.09	103.59± 3.67	105.92± 6.44

Mean ± S.E. (n=2~3)

In rats, whole blood and plasma [redacted] levels decreased to approximately 55% and 75%, respectively, of their initial levels with fasting for 16 hr, but no significant decrease was seen in the [redacted] level in the brain. With fasting for 6 hr, only the whole-blood level decreased, to approximately 70% of the initial level (See Table 3 below).

Table 3: Effect of Food consumption on Endogenous [redacted] Levels in Rats

	Total [redacted] Concentration (ng/ml or g) not fasted	6hr fasted	15hr fasted
Plasma	50.32 ± 1.31	51.28 ± 1.35	38.16 ± 1.36**
Blood	290.04 ± 12.48	207.19 ± 13.60**	161.12 ± 11.60**
Brain	120.23 ± 3.14		106.10 ± 4.96

Mean ± S.E. (n= 3~5)
** Significant different from corresponding value of not fasted (p< 0.01).

The whole-blood [redacted] level in 4-day-old rats was approximately 480 ng/mL and decreased approximately 70% by 8 weeks of age. The plasma [redacted] concentration in 4-day-old rats was approximately 50 ng/mL, and decreased approximately 35% by 8 weeks of age, a smaller proportional decrease than was seen in whole blood. Age difference in endogenous [redacted] levels in rats are summarized in the Table 4 below.

Table 4: Age difference in endogenous [redacted] levels in rats

	Total [redacted] concentration (ng/ml)						
Age	4 days[1)]	1 week[2)]	2 weeks[1)]	3 weeks[1)]	4 weeks[1)]	6 weeks[3)]	8 weeks[1)]
Plasma	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
Blood	483.6 ±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

mean±S.E.
1) n=5, 2) n=6, 3) n=4

2.6.4.9 Discussion and Conclusions

Sapropterin was rapidly absorbed in male mice, rats and monkeys following oral administration and the total [redacted] concentration in plasma reached its peak (Tmax) at 1 hr, 2 hrs and 3 hrs, respectively. Following oral dosing, Sapropterin was eliminated with half-lives (t1/2) of 1.3 hrs in mice, 1.1 hrs in rats and 1.4 hrs in monkey, respectively. The bioavailability was 7%-12% in rats, and approximately 9% in monkeys. Plasma and organ vs time concentrations of total [redacted] did not change following repeated oral administration 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 distributes mainly to the kidney and liver. Pregnant rats showed no significant increase in sapropterin concentration in the placenta and fetus as compared to endogenous levels found in this species. After 10 mg/kg, i.v. dose of sapropterin HCl to lactating rats, the concentration of [redacted] in milk increased to about 3X the endogenous level at 2.5 hr post-dosing. Sapropterin does not activate the cytochrome P450 metabolic pathway. The drug is excreted mainly in urine after i.v. administration. 7,8-Dihydrobiopterin [DHBP], [redacted] pterin and 6-hydroxylumazine [6-OH-Lu] were identified as metabolites in rat urine following i.v. administration of sapropterin HCl.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General 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 LD_{50} of sapropterin via the p.o. route of administration was 1592 and 1393 mg/kg for male and female mice, respectively, and 2272 and 2268 mg/kg for male and female rats, respectively. The p.o. LD_{50} in marmosets was estimated to be between 1000 and 2000 mg/kg for both sexes. No clinical signs were observed when sapropterin was administered to mice and rats via p.o. In marmoset monkeys, single oral dose of 2000 mg/kg caused salivation, emesis, yellow staining around the mouth and genitalia and orange staining on the perch. In addition, the females were subdued with marked tremor, ataxia and sluggish movements before death.

Repeat dose (2, 13 and 52-week) toxicity studies were conducted in rats. In each of these studies rats were dosed once daily orally (gavage) with 4, 40 and 400 mg/kg of SUN0588. In the 13-week rat study, minimal neutrophilic infiltration of the kidney was observed at HD. At the end of the recovery period, minimal to slight microscopic lesions were observed in the kidney (neutrophilic infiltration of the papilla), liver (focal necrosis) and testes (atrophy) of HD rats. In the 52 week rat study, increased incidence of pituitary gland hyperplasia (pars distalis) occurred at doses $\geq$ 40 mg/kg/d. Basophilic change was noted in the collecting tubule of the kidneys at 400 mg/kg/d while hypertrophy of parafollicular cells of the thyroid was noted in all treated groups (except MD females). The target organs of toxicity were pituitary gland, kidney, liver, testes and thyroid.

Repaet dose (4, 13 and 52-week) toxicity studies were also conducted in marmosets. The marmosets were dosed orally with 20, 80 and 320 mg/kg/d of SUN0588. In the 13-Week study, ovarian weight increased dose-dependently in treated females. Enteritis was observed in HD females. Changes in 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 in MD and HD groups. Increased incidence of lymphoid follicles and parafollicular cell hypertrophy of the thyroid was noted in HD monkeys. The kidney, liver and ovarian pathology showed partial recovery. In a 52-Week study, lymphocytic foci of adrenal gland were noted in the HD group. At dose $\geq$ 20 mg/kg/d, myocardial fibrosis (with no dose-related increase in incidence or severity) and increased incidence of periportal inflammatory cell foci (liver) were observed. Increased incidence of transitional cell hyperplasia (kidney, MD group), increased mitotic index in papillary duct (kidney, HD group) and increased incidence of lymphoid hyperplasia (stomach, HD group) were observed in males. Lymphocytic infiltration and parafollicular cell hyperplasia (thyroid) was observed in most treated groups with no dose-related increase in incidence or severity. Glandular hyperplasia and adenomyosis (uterus) was observed in females dosed 20 and 320 mg/kg/d. These lesions were minimal to slight in severity with occasional moderate findings and showed partial recovery. The target organs of

toxicity were adrenal, brain, heart, GI tract, kidney, liver, ovary, uterus, stomach and thyroid.

In the acute (single–dose) p.o. toxicity study with sapropterin in weanling animals (7–day old rats), the LD_{50} of sapropterin was 1108 and 1445 mg/kg for male and female rats, respectively. In the acute (single– dose) p.o. toxicity studies with sapropterin in juvenile animals (3–week old mice and rats), the LD_{50s} of sapropterin were 2278 and 2287 mg/kg for male and female 3–week old mice, respectively, and 2930 and 3222 mg/kg for male and female 3–week old rats, respectively.

The 2-week repeated-dose oral toxicity study in juvenile rats (7-days old) was associated with blood around the anus of rats dosed at 320 mg/kg/d. Renal toxicity (basophilia of proximal tubules, dilatation of renal tubules and pelvis, and thickened Bowman's capsule) occurred at 320 mg/kg/d. The 4-week oral toxicity study in 21-days old juvenile rats, there were toxicities in the adrenal cortex (hypertrophy), kidney (tubular dilatation and basophilia), liver (foci of leucocytes), uterus (dilatation), mandibular lymph node (follicular hyperplasia) and thymus (hemorrhage), heart (fibrosis) and harderian gland (inflammation).

Genetic toxicology:

Sapropterin tested positive in the bacterial reverse mutation and the chromosome aberration tests but was not mutagenic when assessed in the mouse micronucleus test. Thus, Sapropterin has mutagenic and/or clastogenic potential.

Carcinogenicity:

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 statistically significant 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).

Reproductive toxicology:

In the fertility and early embryonic development study in rats, sapropterin at a dose of 400 mg/kg/d (7 x MRHD, BSA) decreased insemination index and fertility index. NOAEL on fertility (males and females) is 40 mg/kg/d (0.7x MRHD) and NOEAL for early embryonic development is 400 mg/kg/d (7X MRHD).

In the rat embryofetal development study with sapropterin, an increased incidence in poorly ossified supraoccipital bone, left umbilical artery and a 2-fold increase in ventricular septal defect was noted in the F1 fetuses at $\geq$ 40 mg/kg/d (0.7x MRHD). Except for the dose-related increase in incidence of resorptions in F2 fetuses from F1

offsprings whose dam received sapropterin treatment, there was no abnormality in the development of F2 fetuses. The NOAEL for fetuses is 40 mg/kg/d (0.7X MRHD) based on the 13.3% decrease in number of live births at the 400 mg/kg/d dose level. NOAEL for dams is 400 mg/kg/d (7X MRHD).

In the peri-natal and posti-natal developmental rat study with sapropterin in rats, 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. Increased incidence of prolonged diestrus was observed in female pups (F1) from all treated dams. There was no effect of treatment on the reproductive ability of F1 pups from treated F0 dams. There was an increase in number of sternebrae in F2 pups from 400 mg/kg/d F1 dams. A slight increase in incidence of ventricular septal defect was observed in F2 pups from F1dams that were exposed to sapropterin. An increased incidence of newborn pups (F2) with thymic cervical residue was noted in all treated groups. There were no growth suppressive effects or teratogenic effects in any groups. NOAEL for reproductive ability (dams and F1 offspring) and effects on peri- and post-natal development is 40 mg/kg/d (0.7X MRHD) based on the decreased birth rate in HD dams and the skeletal abnormalities observed in a HD fetus.

In the rabbit teratology study with sapropterin, a dose of 600 mg/kg/d (20X MRHD, BSA) caused an increased incidence in early resorbed fetuses and holoprosencephaly. The incidence of holoprosencephaly (2/13 litters or 4/96 fetuses in 600 mg/kg/d group) was about ten times higher than the incidence reported in the studies of rabbits (email communication with the sponsor; historic data from [redacted] 1-2/50 litters or 1-2/500 fetuses). A dose-related increase (6, 60 and 600 mg/kg/d represent 0.1x, 2x and 20x MRHD) in incidence of fetuses with malformations and 13th ribs was noted. Overall an increased incidence of skeletal abnormalities was observed in the 600 mg/kg/day. NOAEL for fetal and maternal effects is 60 mg/kg/d (2X MRHD) based on fetal malformations and skeletal abnormalities observed at the HD and early fetal resorption in 600 mg/kg/d dams.

Special toxicology:

Sapropterin HCl had no antigenic potential under the conditions of these studies (in two *in vivo* test systems, the guinea pig and two strains of mice).

2.6.6.2 Single-dose toxicity

Tolerability of 6R-BH4 Administered to Male Beagle Dogs Via Oral Gavage (Study #: 0166-06-023)*

This is a non-GLP study. Two male beagle dogs (9.5-11.4 kg) were orally dosed (gavage) with 50 mg/kg of sapropterin on day 1 and the same dogs were redosed with 100 mg/kg sapropterin on day 3. Animals were observed for clinical signs of toxicity and blood samples were obtained prior to dose and at 1 hour post dose to confirm sapropterin exposure. 6R-BH4 was below the level of quantification in all pre-dose samples and was measurable in all 1 hour post-dose samples. Both the 50 and 100 mg/kg doses were well tolerated by the study animals with no adverse events noted.

Table: Plasma Analysis of Total L-Biopterin and Calculated BH4

	sapropterin dose (mg/kg)			
	50		100	
Animal Number	Pre-dose L-Biopterin (ng/mL)	1 hour post-dose L-Biopterin (ng/mL)	Pre-dose L-Biopterin (ng/mL)	1 hour post-dose L-Biopterin (ng/mL)
IYL-2	[redacted]			
CVE-AKV				

BQL = below level of quantitation, <50 ng/mL L-Biopterin

Acute Toxicology Studies: Animals were given a single dose of the drug and observed for 14 days.

SPECIES	DOSE (mg/kg)	ADVERSE EFFECTS
Mouse	204, 256, 320, 400, 500 – i.v. 5/sex/group	• Mortality: Deaths occurred in 2/5 (M) dosed 256 mg/kg; 5/5 (M) and 4/5 (F) dosed 320 mg/kg; and 5/5 (M) and 5/5 (F) dosed 400 and 500 mg/kg on days 1 and 2. Cause of death was not established. • Clinical signs: transient tremor, ataxia, jumping, lateral recumbency, clonic convulsion, tachypnea & sedation in the 400 & 500 mg/kg groups. • Necropsy: no abnormalities were observed. • LD50: Sponsor proposed 264 (220-314) mg/kg for males and 301 (251-361) mg/kg for females.
Mouse	250, 500, 1000, 2000, 4000 – p.o.; 5/sex/group	• Mortality: Deaths occurred in 1/5 (M) and 1/5 (F) dosed 1000 mg/kg; 3/5 (M) and 4/5 (F) dosed 2000 mg/kg and 5/5 (M) and 5/5 (F) dosed 4000 mg/kg on Days 1 and 2. Cause of death was not established. • Clinical signs: None noted. • Necropsy: Red discoloration of the gastric mucosa was noted in all the dead animals at necropsy. No abnormalities were observed in the surviving animals.

		• Histopathology: The gastric changes were noted to be erosion or hemorrhage of the glandular stomach or atrophy of the forestomach. In the surviving animals of the 250 and 500 mg/kg groups, which showed no abnormal changes at necropsy, erosion of the glandular stomach was also noted microscopically after the completion of the observation period. Sponsor stated that the gastric disorders seem to suggest that HCl liberated from SUN 0588 (dihydrochloride) might have acted to cause the histopathologic changes of the gastric mucosa, however, no primary cause of death could be found. • LD50: Sponsor proposed 1592 (924 to 2841) mg/kg for males and 1393 (815 to 2396) mg/kg for females.
Mouse	163, 204, 256, 320, and 400 mg/kg – s.c. 5/sex/group.	• Mortality: Deaths occurred in 2/5 (M) and 2/5 (F) in the 204 mg/kg group, 2/5 (M) in the 256 mg/kg • group, 5/5 (M) and 4/5 (F) in the 320 mg/kg group and all males and females in the 400 mg/kg group on Day 1. Cause of death was not established. Clinical signs: The clinical signs included a fragile change (the opening with ulcer) at the dorsal injection site in all animals from 3 hours after dosing. • Necropsy: A fragile change (the opening with ulcer) was observed in all animals at the injection site. • Histopathology: The fragile changes included ulcer, atrophy, granuloma, hemorrhage, fat degeneration/necrosis and degeneration of smooth muscle. These changes were severe in the 400 mg/kg group. Since the severity of these findings was dose-dependent, the sponsor stated that HCl liberated from SUN 0588 (a dihydrochloride) in the injection site might have caused such local toxic changes. • LD50: Sponsor proposed 239 (196 to 287) mg/kg for males and 270 (220 to 339) mg/kg for females.
Rat	204, 256, 320, 400, 500 mg/kg – i.v. 5/sex/group.	• Mortality: Deaths occurred in 2/5 (M) and 1/5 (F) in the 320 mg/kg group, 4/5 (M) and 3/5 (F) in the 400 mg/kg group, and 5/5 (M) and 5/5 (F) in the 500 mg/kg group on Days 2 and 3. Cause of death was not established. • Clinical Signs: Jumping, tachypnea and decreased locomotor activity was observed in 2 males and a female in the HD (500 mg/kg) group just after dosing, lasting for about 30 seconds. • LD50: Sponsor proposed 342 (287 to 407) mg/kg for males and 372 (312 to 448) mg/kg for females.
Rat	313, 625, 1250, 2500, 5000 mg/kg – p.o. 5/sex/group.	• Mortality: Deaths occurred in 1/5 (M) in the 1250 mg/kg group, 2/5 (M) and 3/5 (F) in the 2500 mg/kg • group and 5/5 (M) and 5/5 (F) in the 5000 mg/kg group on Days 1 and 2. Clinical signs: None • observed. Cause of death was not established. Necropsy: Red discoloration of the gastric mucosa was observed only in the dead animals. • Histopathology: These gastric changes were noted to be erosion and atrophy of the glandular/forestomach. Sponsor stated that HCl liberated from SUN 0588, (a dihydrochloride) might have acted as a strong acid to cause the erosion. • LD50: Sponsor proposed 2272 (1306 to 4378) mg/kg for male rats and 2268 (1320 to 3963) mg/kg for female rats.
Rat	500, 750, 1125, 1687.5, 2531.3 mg/kg – s.c. 5/sex/group.	• Mortality: Deaths occurred in 3/5 (M) in the 1125 mg/kg group, 5/5 (M) and 2/5 (F) in the 1687.5 • mg/kg group, and 5/5 (M) and 5/5 (F) in the 2531.3 mg/kg group on Days 1 and 2. Cause of death was not established. Clinical signs: Included hardness at the injection site observed 2 hours post-dose in all

		animals. This finding was observed continuously until the death among the dead animals and until Day 1 among the surviving animals. All surviving animals showed the fragile change (the opening with ulcer) at the injection site from Day 2 to the completion of observation period. • Necropsy: The dead animals showed hardness and subcutaneous change at the injection site, and the surviving animals showed the fragile change (the opening with ulcer) at the injection site. • Histopathology: The fragile changes correlated with ulcer, inflammation and degeneration in skin, subcutaneous granuloma, disturbance of hair formation and degeneration of smooth muscle. Sponsor stated that HCl liberated from SUN 0588 (a dihydrochloride) might have caused such changes in the skin and subcutaneous tissues. No primary cause of death could be found under the conditions of this study. • LD50: Sponsor proposed 1084 (789 to 1492) mg/kg for males and 1720 (1250 to 2430) mg/kg for females.
Monkey	150, 300	• Mortality: Both marmosets that received 300 mg/kg were killed prematurely, the male on

(marmoset)	mg/kg – i.v. 1/sex/group	Day 5 and the female on Day 6. • Clinical signs: Signs observed in HD animals included yellow staining of the cage tray paper, cage perches and genitalia, diarrhea with yellow mucoid perianal staining, subdued behavior or drowsiness, partially closed eyes, reduced heart rate and muscle tone, whole body tremor and pallor. Signs in marmosets treated at 150 mg/kg comprised yellow staining of cage tray paper and, in the female only, diarrhea with perianal soiling, isolated incidents of partially closed eyes, subdued mood and pallor and transient orange/red swelling at the injection site with more persistent bruising and subsequent scab formation. • Food consumption: Food intake after the administration of SUN 0588 was lower in animals that received 300 mg/kg than that recorded before treatment. In animals treated at 150 mg/kg the female showed a slight reduction in the overall consumption of fruit. • Body weight: All animals lost body weight following treatment. Both animals treated at 150 mg/kg showed normal weight changes during the second half of the observation period. • Necropsy: Ante mortem veterinary examination of animals treated at 300 mg/kg revealed reduced heart rate and muscle tone, photophobia, drowsiness and subdued mood. In addition the extremities of the male were cool and there was swelling of the left mandible. In the female the postural reflexes were markedly depressed or absent. Photophobia was also seen on Day 13 of the observation period in the female treated at 150 mg/kg together with matted hair in the sacral area. • Hematology: Slight reductions in PCV, hemoglobin concentration, RBC and MCHC and an increase in MCV were observed in the 300 mg/kg group relative to pretreatment values. No hematology changes were seen in marmosets dosed 150 mg/kg. • Clinical chemistry: 300 mg/kg -↑ in alkaline phosphatase, ALT and AST activites, high phosphorus (2x), urea, creatinine (20x) and bilirubin concentrations and low plasma protein concentrations with a low albumin to globulin ratio in the female. 150 mg/kg -↑ in alkaline phosphatase activity (3-4x), urea (8x – male), creatinine (37x – male) and phosphorus (4x – male). • Organ weight: The liver, kidney and adrenal weights were higher in animals that received 300 mg/kg than in the lower dosage group. • Necropsy: Macroscopic examination of the female treated at 150 mg/kg revealed perianal soiling. Pale or dark foci were seen on the kidneys of both monkeys treated at 150 mg/kg and the female that received 300 mg/kg. • Histopathology: 300 mg/kg - nephrosis in the proximal convoluted tubules and proteinaceous fluid in the distal tubules, with a focal pre-terminal change of subcapsular necrosis in the female. Distal tubule nephrosis with cast formation was found in the low dose animals with tubular mineralization in the female only. Severity ranged from minimal to moderate with no dose-dependency. Kidney is the target organ. • LD50: was between 150 and 300 mg/kg.
Monkey (marmoset)	1000, 2000 mg/kg – p.o. 1/sex/group.	• Mortality: Both marmosets that received 2000 mg/kg died or were killed in extremis ~ three days after dosing. • Clinical signs: 2000 mg/kg -salivation for ~ 1 hr after dosing, emesis shortly after dosing, yellow staining around the mouth and genitalia and orange staining on the perch from ~ 1 hr after dosing onwards. In addition, the female was subdued with marked tremor, ataxia and sluggish movements before death. 1000 mg/kg - No signs of reaction to treatment were observed. • Food consumption: A reduction in food intake was observed in both animals receiving

		2000 mg/kg; food intake of animals treated at 1000 mg/kg was unaffected. • Body weight: The female given 2000 mg/kg lost weight progressively; the bodyweights of the other animals were unaffected. • Hematology: 2000 mg/kg (F) -↓ in PCV, hemoglobin concentration and RBC; ↑ MCV in the male dosed 1000 mg/kg and the female dosed 2000 mg/kg. • Clinical chemistry: No treatment-related effects. • Urinalysis: No treatment-related effects. • Organ weight: Increased liver and kidney weights in were noted in both marmosets treated at 2000 mg/kg. The organ weights of animals treated at 100 mg/kg were not affected by treatment. • Necropsy: Pale kidneys with occasional dark foci in the HD female. No abnormality

		attributable to treatment was found in LD animals. • Histopathology: 2000 mg/kg - nephrosis in the proximal and distal convoluted tubules. The effects were much less marked or absent in the animals that received 1000 mg/kg/day. • LD50: was between 1000 and 2000 mg/kg.
Monkey (marmoset)	150, 300 mg/kg – s.c. 1/sex/group	• Mortality: The HD male died 4 days post-dose and the female was sacrificed in extremis six days post-dose. The LD male was killed seven days post-dose. • Clinical signs: Yellow or orange staining of the cage tray paper, perigential staining, liquid faeces, subdued mood, partially closed eyes, weakness, tremor, pallor and discolouration of the injection sites. • Food consumption: food intake ↓ progressively in HD animals after dosing and became lower than pre-treatment values; a reduction in food intake was observed in the LD group as well. • Body weight: Body weight ↓ progressively after treatment and was apparent in both HD animals and in the LD male. A similar, but less marked, trend was also evident in the LD female. • Sponsor stated that Veterinary examination before death of the HD animals revealed prostration in the male with no apparent voluntary control over limbs, tail or bladder. The female was lying on the cage floor with greatly reduced muscle tone and postural reflexes. Ante mortem examination of the LD male revealed reluctance to move with subdued mood, unsteadiness, shivering, photophobia, labored respiration, reduced muscle tone and a reduced heart rate. The LD female, before scheduled termination, had a slight reduction in limb muscle tone and a red ring enclosing a pale area around the treatment site. • Hematology: No treatment related effects. • Clinical chemistry: Increases in alkaline phosphatase (2X), ALT (3-24X) and AST (2X) activities in the majority of animals and high urea (2-10X), creatinine (18-29X), glucose (1-3X), bilirubin (2X - HD female only); ↑ K and P concentrations and ↓ Na and Cl concentration in the animals that died prematurely. Low albumin/globulin ratios were also evident before death in the HD male and slightly low protein concentration was observed in the HD female. • Urinalysis: No treatment-related abnormalities. • Organ weight: No treatment related effects. • Necropsy: Macroscopic examination showed encrustation of the injection site in both HD animals; in the HD male this was associated with thickening of the subcutis with occasional dark areas. Pale and dark areas were seen at the injection sites of LD animals, bordered, in the female by pale punctate encrustations. • Histopathology: Microscopic examination of both males and the high dosage female revealed nephrosis in the proximal and distal convoluted tubules, casts and tubular proteinaceous fluid; the HD female also showed slight proliferation of the papillary epithelium. The surviving LD female had nephrosis of the distal tubules, cast and tubular proteinaceous fluid. The LD male also showed occasionally slight mineralization of tubules. • LD50: was 150 mg/kg.

2.6.6.3 Repeat-dose toxicity

Study title: Two-Week Oral Repeated-Dose Toxicity Study in Rats

Key study findings:

- SUN0588 was well tolerated up to doses of 400 mg/kg/d for 2 weeks.
- There were no treatment-related changes in clinical signs, body weight, food consumption, hematology, blood biochemistry and organ weights.
- Based on these results, a HD level of 400 mg/kg was chosen for the 13-week repeated oral dose toxicity study in rats.
- NOAEL was not established since histopathology was not conducted.

Study no: PHN-66-AT-SR
Conducting laboratory and location: [redacted]
Date of study initiation: May 22, 1986.
GLP compliance: Yes (Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8885Y05, purity was not provided.
Formulation/vehicle: SUN0588 dissolved in distilled water.

Methods (unique aspects):
Dosing:
Species/strain: Rat/Wistar.
#/sex/group or time point (main study): 6/sex/group.
Satellite groups used for toxicokinetics or recovery: None.
Age: 5 weeks old.
Weight: 138-152 (M); 118-127 (F).
Doses in administered units: 4, 40 and 400 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Twice weekly.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Blood samples were drawn prior to terminal sacrifice for hematology evaluation.
Animals were fasted for 16 hours before terminal sacrifice.
Clinical chemistry: Blood samples were drawn prior to terminal sacrifice for clinical chemistry evaluation. Animals were fasted for 16 hours before terminal sacrifice.
Urinalysis: Not conducted.
Gross pathology: Organs/tissues examined are indicated in the histopathology table.
Organs weighed: Liver, kidneys, spleen and adrenal glands.
Histopathology: Not conducted.
Toxicokinetics: Not conducted.

Results:
Mortality: None.
Clinical signs: No treatment-related clinical signs.
Body weights: No treatment-related changes in body weight.
Food consumption: No treatment-related changes in food consumption.
Ophthalmoscopy: No data.
Electrocardiography: No data.
Hematology: No treatment-related changes in hematology parameters.
Clinical chemistry: No treatment-related clinical chemistry changes.

Urinalysis: No data.
Organ weights:

Dose (mg/kg/d)	0	4	40	400
Spleen (mg)	520	457*	524	482
Spleen (mg%)	335	292	327	308

Gross pathology: No treatment-related gross findings.
Histopathology: No data.
Toxicokinetics: No data.

Summary of individual study findings: Proposed clinical dose = 10 mg/kg – 370 mg/m^2. Effects of SUN0588 were investigated in Wistar rats treated orally once daily at dose levels of 4, 40 and 400 mg/kg daily for 2 weeks. These doses represent ~ 0.1X, 1X and 7X MRHD (maximum recommended human dose) based on mg/m^2. There were no treatment-related changes in clinical signs, body weight, food consumption, hematology, blood biochemistry and organ weights. Based on these results, a HD level of 400 mg/kg was chosen for the 13- week repeated oral dose toxicity study in rats. NOAEL could not be established since histopathology was not conducted.

Study title: 13-Week Repeated Oral Toxicity Study in Rats With 5-Week Recovery Period.

Key study findings:

- There were no treatment-related deaths or effects on body weight or food consumption.
- Monocytes were significantly decreased in females dosed 400 mg/kg/d at the end of the recovery period.
- Urinalysis revealed increases in specific gravity (females dosed 400 mg/kg/d), Na, K and Cl in females dosed 4 and 400 mg/kg/d at the end of the treatment period. At the end of the recovery period Na and Cl levels in urine were decreased.
- Relative thyroid weight was decreased in females dosed 4 and 400 mg/kg/d at the end of the treatment period with no correlative histopathology (reversed at end of recovery). At the end of the recovery period, male rats dosed 40 and 400 mg/kg/d had decreased weights of pituitary (no correlative histopathology) and testes (atrophy in 1/6 males).
- The target organs include the kidney (neutrophilic infiltration of the papilla), liver (focal necrosis) and testes (atrophy).
- NOAEL is 400 mg/kg/d.

Study no: PHN-67-AT-SR
Conducting laboratory and location: [redacted]

Date of study initiation: November 14, 1986.
GLP compliance: Yes (Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 88886506, [redacted]

Formulation/vehicle: SUN0588 dissolved in distilled water.

Methods (unique aspects):
Dosing:
Species/strain: Rat/Wistar.
#/sex/group or time point (main study): 8/sex/group for control, MD and HD groups; 5/sex/group for the LD group.
Satellite groups used for toxicokinetics or recovery: 6/sex/group from the control, MD and HD groups were used for recovery.
Age: 5 weeks old.
Weight: 139-161 g (M), 121-147 (F).
Doses in administered units: 4, 40 and 400 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Weekly.
Ophthalmoscopy: Conducted on all rats pre-treatment and week 13, and on all control and HD rats at recovery week 5.
EKG: Not conducted.
Hematology: Blood samples for hematology evaluation were collected from fasted (16 hr) animals prior to necropsy.
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected from fasted (16 hr) animals prior to necropsy.
Urinalysis: Performed on all rats at week 13 and recovery week 5.
Gross pathology: Organs/tissues collected for gross examination are indicated in the histopathology table.
Organs weighed: Organs/tissues collected for gross examination are indicated in the histopathology table.
Histopathology: Organs and tissues from the control and HD groups were processed for microscopic examination. Kidneys from the MD group were also processed for examination since slight neutrophilic infiltration in the renal papilla, dilatation and epithelial degeneration of the papillary ducts were noted in a HD male.
Toxicokinetics: No data.

Results:
Mortality: None.
Clinical signs: Alopecia was noted in a female (No. 11) of the 40 mg/kg group on Days 66 to 73 of treatment and in a male (No. 22) and a female (No. 46) of the 400 mg/kg group on Days 58 to 66 of treatment and from Day 41 of treatment to Day 35 of recovery, respectively. No other abnormalities in clinical signs were observed throughout the treatment and recovery periods.
Body weights: No treatment-related effects on body weight.
Food consumption: No treatment-related effects on food consumption.

Ophthalmoscopy: No treatment-related ophthalmic effects.
Electrocardiography: No data.
Hematology: There were no treatment related effects at the end of the treatment period.
End of Recovery Data: Blood samples from control, MD and HD groups were examined.

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Monocyte (%)		6.5				4.2		2.0** (69%↓)

** p<0.01

Clinical chemistry: No treatment-related effects on clinical chemistry.
Urinalysis: Week 13 Data

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Sp. Gravity		1.030		1.040		1.037		1.045*(2%↑)
Na (mg/Rat/16 hr)		6.2		15.4**(148%↑)		12.7		15.9**(157%↑)
K (mg/Rat/16 hr)		31.6		66.0*(108%↑)		55.8		61.4*(94%↑)
Cl (mg/Rat/16 hr)		13.6		33.4*(146%↑)		29.4		41.6**(206%↑)

* p<0.05; ** p<0.01

Urinalysis: End of recovery data – only samples from control, MD and HD groups were examined.

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Na (mg/Rat/16 hr)		13.4				7.2** (46%↓)		15.4
Cl (mg/Rat/16 hr)		33.6				19.4*(42%↓)		35.3

* p<0.05; ** p<0.01

Organ weights: Week 13 Data

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Thyroid (mg %)		4.7		3.7*(21%↓)		4.2		3.8*(19%↓)

* p<0.05

Organ weights: End of recovery data – only samples from control, MD and HD groups were examined.

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Pituitary gland (mg)	15				12*(20%↓)		11*(27%↓)	
Testes (g)	3.9				3.4**(13%↓)		3.6*(8%↓)	

* p<0.05; ** p<0.01

Gross pathology: No treatment-related gross pathology findings.
Histopathology: Organs and tissues from the control and HD groups were processed for microscopic examination. Kidneys from the MD group were also processed for

examination since slight neutrophilic infiltration in the renal papilla, dilatation and epithelial degeneration of the papillary ducts were noted in a HD male.
Week 13 Data: n = 10/sex/group - **1 = minimal; 2 = slight; 3 = moderate; 4 = marked**

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Kidney: neutrophils infiltration of papilla	0				0		1(2)	

End of Recovery Data: n = 6/sex/group – Only tissues from control and HD groups were examined.

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Pituitary: cyst	0						2(2)	
Testes: atrophy	0						1(2)	
Liver: focal necrosis								1(2)

Toxicokinetics: TK was not conducted in this study. The rat TK data provided for 1 mg/kg (265 ng.h/ml) and 100 mg/kg (4571 ng.h/ml) in the pharmacokinetics section is not linear. Therefore it was not possible to estimate what systemic exposure would be for a 400 mg/kg dose. The multiples derived are therefore based on BSA (mg/m^2).

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m^2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In the 13 week study, rats were dosed once daily orally (gavage) with 4, 40 and 400 mg/kg of SUN0588. These doses represent 0.1X, 1X and 7X the proposed clinical dose based on mg/m^2. There were no treatment-related deaths or effects on body weight or food consumption. Monocytes were significantly decreased in females dosed 400 mg/kg/d (7X) at the end of the recovery period. Urinalysis revealed increases in specific gravity (females dosed 400 mg/kg/d), Na, K and Cl in females dosed 4 (0.1X) and 400 mg/kg/d (7X) at the end of the treatment period. At the end of the recovery period Na and Cl levels in urine were decreased. Relative thyroid weight was decreased in females dosed 4 (0.1X) and 400 mg/kg/d (7X) at the end of the treatment period with no correlative histopathology (reversed at end of recovery). At the end of the recovery period, male rats dosed 40 and 400 mg/kg/d had decreased weights of pituitary (no correlative histopathology) and testes (atrophy in 1/6 males). The target organs include the kidney (neutrophilic infiltration of the papilla), liver (focal necrosis) and testes (atrophy). NOAEL is 400 mg/kg/d (7X) based on the histopathology.

Study title: 52-Week Oral Toxicity Study of SUN0588 in Rats

Key study findings:

- Mortality occurred at all dose levels: 1/40 (control), 3/40 (LD), 1/40(MD) and 4/40 (HD). The control male rat (# 76), the LD group (#s 12, 209, 298) and the HD group (# 9) died of gavage error. One HD male (# 50) died of a tumor (fibrosarcoma). Two HD rats (#s 64 and 39) were sacrificed in extremis. Animal #s 7 and 39 had meningitis. Sponsor stated that the cause of demise of the remaining animals could not be determined but was not considered treatment related.
- There were no treatment-related effects on body weight and food consumption.
- Urinary chloride levels were increased by 133% in HD males.
- Uterine weight was decreased in MD females by 22% relative to control.
- The target organs include the pituitary gland (hyperplasia of pars distalis), kidney (basophilic change in collecting tubule) and thyroid (hypertrophy of parafollicular cells).
- NOAEL is 4 mg/kg/d based on histopathology.

Study no: PHN-70-AT-SR
Conducting laboratory and location: [redacted]
Date of study initiation: May 18, 1987.
GLP compliance: Yes (Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8887402, [redacted] Lot # 8887301 [redacted] Lot # 8887Z05, [redacted]
Formulation/vehicle: SUN0588 dissolved in distilled water.

Methods (unique aspects):
Dosing:
Species/strain: Rat/Wistar.
#/sex/group or time point (main study): 20/sex/group.
Satellite groups used for toxicokinetics or recovery: None.
Age: 5 weeks old.
Weight: 131-151 (M); 130-147 (F).
Doses in administered units: 4, 40 and 400 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weekly for the first 13 weeks and monthly thereafter.
Food consumption: Weekly for the first 13 weeks and monthly thereafter.
Ophthalmoscopy: Conducted predose, during Weeks 26 and 51 in 10 control and HD animals.
EKG: Not conducted.
Hematology: Blood samples for hematology evaluation were collected prior to terminal necropsy.
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected prior to terminal necropsy.

Urinalysis: Conducted on Weeks 26-27 and during Week 51.
Gross pathology: Organs/tissues isolated for gross examination are indicated in the histopathology table.
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Only tissues from control and HD groups were processed for evaluation. In addition, organs judged to be macroscopically abnormal and the pituitary gland, thyroid, kidney and testes were also processed for evaluation.
Toxicokinetics: Not conducted.

Results:
Mortality: N = 20/sex/group

Dose (mg/kg/d)	0	4	40	400
Males	1/20 # 76 – Week 25	1/20 # 12 – Week 40	0/20	3/20 # 9 – Week 17 # 64* – Week 38 # 50 – Week 46
Females	0/20	2/20 # 7 – Week 30 # 48 – Week 43	1/20 # 52 – Week 39	1/20 # 39* – Week 34

*** Animal numbers 64 (M) and 39 (F) were sacrificed moribund**

Clinical signs: Incidence of clinical signs. Empty cells = no clinical signs.

Dose (mg/kg/d)	0		4		40		400	
Sex	M	F	M	F	M	F	M	F
Thickening of R. ear	2		1	1	1		3	
Epileptic convulsion	1	1	2		1		1	2
Alopecia	1	3		1	1			3
Mass – axillary region			1					
Swelling – L. hind limb					1			
Mass – R. flank							1	
↓ Locomotor activity		1					1	1
Piloerection		1					2	
Leanness							1	
Ataxia							1	
Moribund, gasping							1	1
Mass on chest		1						
Periurethral discharge								1

Body weights: No treatment-related effects on body weight.
Food consumption: No treatment-related effects on food consumption.
Ophthalmoscopy: No treatment-related ophthalmic findings.
Electrocardiography: No data.
Hematology: No treatment-related effects in males.

Dose (mg/kg/d)	0	4	40	400

MCHC (%)	32.7	32.0**	32.2**	32.4

** **p<0.01**

Clinical chemistry: No treatment-related effects on clinical chemistry parameters.
Urinalysis: No treatment-related effects in females.

Dose (mg/kg/d)	**0**	**4**	**40**	**400**
Cl (mg/rat/16 hr)	10.4	9.4	8.1	24.2**

** **p<0.01**

Organ weights: No treatment-related effects in males.

Dose (mg/kg/d)	**0**	**4**	**40**	**400**
Uterus (mg)	1120	1135	869*	951

* **p<0.05**

Gross pathology: Moribund and early decedent animals: * **sacrificed moribund**

MALES				
Dose (mg/kg/d)	**Animal #**	**Day of death**	**Organ**	**Findings**
0	76	171	Lung	Dark red in color, foamy discharge from trachea.
4	12	278	Thoracic cavity Dodenum	Cloudy hydrothorax, suppurative pleuritis. Mass.
400	9	117	Lung	Dark red in color.
	50	321	R. axillary region	Subcutaneous mass.
	64*	263	Neck R. axillary region Glandular stomach Kidney	Retention of green material. Subcutaneous mass. Dark red point. Hypertrophy.
FEMALES				
4	7	209	Neck R. axillary region Lung Adrenal	Subcutaneous mass. Subcutaneous mass. Dark red in color. Hypertropgy.
	48	298	Lung	Dark red in color.
40	52	270	Neck	Subcutaneous mass.
400	39*	236	Stomach Adrenal	Dark red point. Hypertrophy.

Gross pathology: Terminal sacrifice – empty cells indicate zero incidence.

Dose (mg/kg/d)	**0**		**4**		**40**		**400**	
Sex	**M(19)**	**F(20)**	**M(19)**	**F(18)**	**M(20)**	**F(19)**	**M(17)**	**F(19)**
Glandular stomach Red point	1		1					
Testes (R) Atrophy	1				1			
Atrophy (bilateral)					1			
Epididymis (R)	1				1			

Atrophy								
Atrophy (bilateral)					1			
R. Ear Thickening	2		1					
Nodule					1		3	
Liver Reddish brown spot	1	2				4	1	
White spot			1					
Nodule		1						
Reddish brown mass				1				
Adhesion to diaphragm								1
Lung Grayish white spot	1		2		1	1	2	3
Pituitary Reddish lesion		2	1		1			
L. axillary region Cutaneous mass			1					
Skin Focal loss of hair		1		1	1			2
Right hind leg Cutaneous mass					1			
Vagina Mass		1		1				
Chest Mass				1				
Uterus Mass				1				
Adrenal Hypertrophy						1		
Ovary (unilateral) Mass						1		2
Cyst (unilateral)								1

Histopathology: Moribund and early decedent animals

MALES				
Dose (mg/kg/d)	**Animal #**	**Day of death**	**Organ**	**Findings**
0	76	171	Lung Adrenal Kidney	Hemorrhage(2); Edema (1) Angiectasis (2) Chronic nephropathy (1)
4	12	278	Heart Stomach	Cell infiltration (1) Adenocarcinoma
400	9	117	Thymus	Hemorrhage (2)
			Lung	Edema (3)
	50	321	Thymus Adrenal Skin (axillary area)	Atrophy (3) Cortical hyperplasia (1) Fibrosarcoma

	64*	263	Thymus Thyroid Kidney Stomach Skin Esophagus	Hemorrhage (2); Atrophy (2); Inflammation (3) Inflammation (3) Basophilic change-collecting tubule (2); necrosis of tubular epithelium (1) Necrosis (1) Inflammation (3) Inflammation (3)
FEMALES				
4	7	209	Cerebrum Cerebellum Thymus Thyroid Esophagus Lung Heart Liver Spleen Adrenal Pancreas Trachea Skin (axillary area)	Meningitis (1) Meningitis (1) Atrophy (3); Inflammation (3) Inflammation (2) Inflammation (2) Pleuritis (1) Pericarditis (1) Vacuolation (1) Atrophy of white pulp (2) Angiectasis (1) Inflammation (2) Inflammation (2) Inflammation (3)
	48	298	Lung Heart Spleen	Congestion (3) Pericarditis (1) Atrophy of white pulp (1)
40	52	270	Thymus Liver Skin (neck)	Atrophy (1) Vacuolation (1) Abscess (3)
400	39*	236	Cerebrum Cerebellum Spinal cord Thymus Liver Adrenal Kidney Stomach	Meningitis (2) Meningitis (2) Meningitis (1) Atrophy (2) Eosinophilic change (2) Angiectasis (1) Chronic nephropathy (1) Necrosis (1)

*** sacrificed moribund; 1 = slight; 2 = moderate; 3 = marked**

Histopathology: Terminal sacrifice - Only tissues from control and HD groups were processed for evaluation. In addition, organs judged to be macroscopically abnormal and the pituitary gland, thyroid, kidney and testes were also processed for evaluation.

Dose (mg/kg/d)	**0**		**4**		**40**		**400**	
Sex	**M(19)**	**F(20)**	**M(19)**	**F(18)**	**M(20)**	**F(19)**	**M(17)**	**F(19)**
Pituitary: Pars distalis Hyperplasia	0	1(1)	2(1)	2(1)	1(1)	4(1)	3(1)	6(1)
Adenoma: pars distalis	0	1(1)	1(2)	1(1)	3(2)	0	0	0
Cyst	0	0	2(1)	0	0	0	4(1)	1(1)
Kidney:collecting tubule Basophilic change	2(1)	0	1(1)	0	2(1)	0	6(1)	3(1)
Chronic nephropathy	15 10(1) 5(2)	7(1)	12 10(1) 2(2)	5(1)	12 8(1) 4(2)	9(1)	15 9(1) 3(2) 3(3)	10 8(1) 2(2)
Pyelitis	1(1)	0	1(1)	0	0	0	0	1(1)
Thyroid: Hypertrophy of parafollicular cell	0	0	2(1)	2(1)	1(1)	0	2(1)	2(1)

Lung: Foam cell in subpleural alveoli	4(1)	2(1)	2(1)	0	1(2)	1(1)	2(2)	4 3(1) 1(2)
Thickening-alveolar wall	0	0	0	0	0	0	1(1)	0

1 = slight; 2 = moderate; 3 = marked

Toxicokinetics: TK was not conducted in this study. The rat TK data provided for 1 mg/kg (265 ng.h/ml) and 100 mg/kg (4571 ng.h/ml) in the pharmacokinetics section is not linear. Therefore it was not possible to estimate what systemic exposure would be for a 400 mg/kg dose. The multiples derived are therefore based on BSA (mg/m2).

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In a 52 week oral (gavage) toxicity study, rats were dosed with 4, 40 and 400 mg/kg/d of SUN0588. These doses represent 0.1X, 1X and 7X the MRHD based on mg/m^2. Mortality occurred at all dose levels: 1/40 (control), 3/40 (LD), 1/40(MD) and 4/40 (HD). The control male rat (# 76), the LD group (#s 12, 209, 298) and the HD group (# 9) died of gavage error. One HD male (# 50) died of a tumor (fibrosarcoma). Two HD rats (#s 64 and 39) were sacrificed in extremis. Animal #s 7 and 39 had meningitis. Sponsor stated that the cause of demise of the remaining animals could not be determined but was not considered treatment related. Urinary chloride level was increased by 133% in HD males. Uterine weight was decreased in MD females by 22% relative to control. The target organs include the pituitary gland (hyperplasia of pars distalis), kidney (basophilic change in collecting tubule) and thyroid (hypertrophy of parafollicular cells). NOAEL is 4 mg/kg/d (0.1X MRHD) based on histopathology.

Study title: Oral Dose-Ranging Repeated Dose Toxicity Study of Sapropterin HCl in Rats for 4 Weeks.

Key study findings:

- The study was not adequately designed. Hematology, clinical chemistry, urinalysis, histopathology and toxicokinetics were not conducted.
- There were no treatment-related clinical signs or effects on body weight, food consumption and gross pathology.
- Slight decrease in liver weight occurred in LD rats and MD females. Relative kidney weight was slightly increased in HD females. Testicular weight was slightly increased in all treated males relative to control.
- NOAEL could not be established since histopathology was not performed.
- MTD was not established in this study since the HD used did not induce any toxicity and/or mortality.

Study no: PHN-115-AT-SR.

Conducting laboratory and location: [redacted]

Date of study initiation: November 24, 1987.
GLP compliance: Yes (Europe, Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8887904, [redacted]
Formulation/vehicle: SUN0588 was dissolved in distilled water.

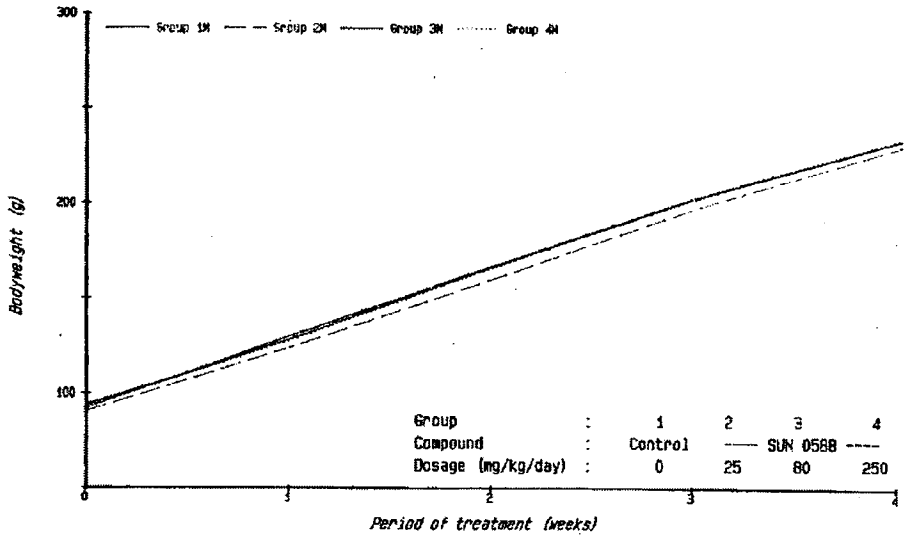
Methods (unique aspects):
Dosing:
Species/strain: Rat/ F-344
#/sex/group or time point (main study): 10/sex/group.
Satellite groups used for toxicokinetics or recovery: None.
Age: 5-6 weeks at study initiation.
Weight: 45-62 g (M), 48-67 g (F).
Doses in administered units: 25, 80 and 250 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weighed on Days 3 and 7 and weekly thereafter.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Not conducted.
Clinical chemistry: Not conducted.
Urinalysis: Not conducted.
Gross pathology: Organs/tissues collected for gross examination are indicated in the histopathology table.
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Not conducted.
Toxicokinetics: Not conducted.

Results:
Mortality: None.
Clinical signs: No treatment-related clinical signs. Bright yellow staining of cage tray papers was observed in rats dosed 250 mg/kg/d during weeks 3 and 4.
Body weights: (g) - No treatment-related effects on body weight.

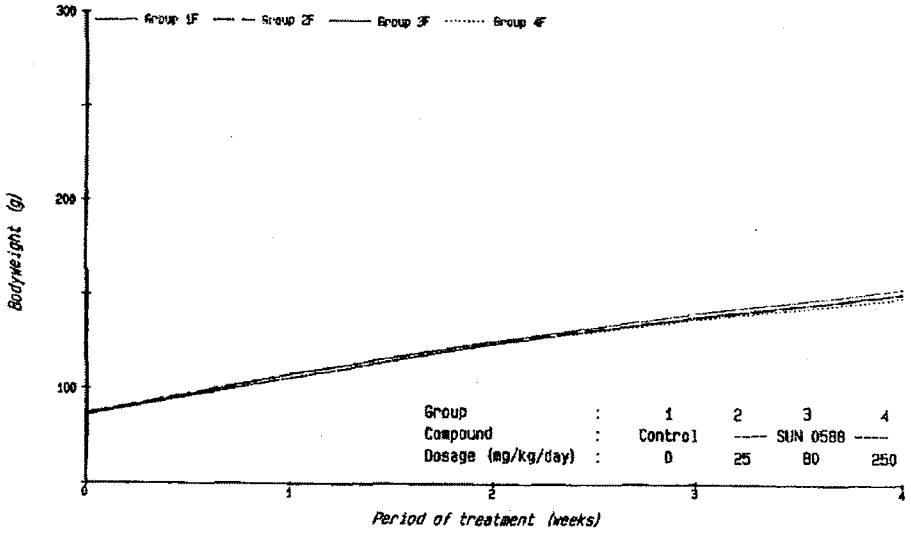
Dose (mg/kg/d)	0		25		80		250	
Sex	M	F	M	F	M	F	M	F
Day 0	92	87	90	87	94	86	93	87
Day 20	234	154	231	151	234	151	235	149
Change in wt.	142	67	141	64	140	65	142	62

Males:



Best Possible Copy

Females:



Food consumption: No treatment-related effects on food consumption.
Ophthalmoscopy: No data.
Electrocardiography: No data.
Hematology: No data.
Clinical chemistry: No data
Urinalysis: No data.
Organ weights:

Dose (mg/kg/d)	0		25		80		250	
Sex	M	F	M	F	M	F	M	F
Liver (g)	12.7	6.8	11.5** (9%↓)	6.2*(9%↓)	12.2	6.1**(10%↓)	12.6	6.5
Liver (%)	5.51	4.46	5.15**(7%↓)	4.22	5.36	4.16*(7%↓)	5.51	4.46
Kidneys (%)	0.89	0.88	0.87	0.89	0.90	0.91	0.92	0.93*(6%↑)
Testes (%)	1.12		1.16*(4%↑)		1.17		1.16*(4%↑)	

Gross pathology: No treatment-related gross findings.
Histopathology: No data.
Toxicokinetics: TK was not conducted in this study. The rat TK data provided for 1 mg/kg (265 ng.h/ml) and 100 mg/kg (4571 ng.h/ml) in the pharmacokinetics section is not linear. Therefore it was not possible to estimate what systemic exposure for the doses used in this study. The multiples derived are therefore based on BSA (mg/m^2).

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m^2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In a 4 week toxicity study, rats were dosed orally, once daily with 25, 80 and 250 mg/kg/d SUN0588. These doses represent 0.4X, 1X and 4X MRHD based on mg/m^2. There were no treatment-related clinical signs or effects on body weight, food consumption and gross pathology. Slight decrease in liver weight occurred in LD rats and MD females. Relative kidney weight was slightly increased in HD females. Testicular weight was slightly increased in all treated males relative to control. NOAEL could not be established since histopathology was not performed. MTD was not established in this study since the HD used did not induce any toxicity and/or mortality.

Study title: 4-Week Oral Toxicity Study of SUN 0588 in Marmosets

Key study findings:

- The LD female died under anesthesia following cerebrospinal fluid sampling on Day 27 of treatment.
- Though not statistically significant, decreases in erythrocyte parameters were noted. Dose dependent decreases in PCV, RBC and Hg were observed in treated males. Only RBC decreased dose dependently in treated females. WBC was slightly decreased in MD females but increased in HD females.
- The administration of SUN 0588 did not reveal any overt toxicity. Histopathology was not conducted hence NOAEL could not be established.
- Based on findings of this study, doses of 20, 80 and 320 mg/kg/d were chosen for the 13 week study on marmosets.

Study no: PHN-68-AT-SR.
Conducting laboratory and location: [redacted]
Date of study initiation: November 14, 1986.
GLP compliance: Yes (USA, Europe, Japan).

QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: Lot # 8886506,
Formulation/vehicle: SUN 0588 dissolved in distilled water.

Methods (unique aspects):
Dosing:
Species/strain: Monkey/Marmoset.
#/sex/group or time point (main study):1/sex/goup.
Satellite groups used for toxicokinetics or recovery: None.
Age: 50-58 weeks at study initiation.
Weight: 243-278 g (M); 210-279 g (F).
Doses in administered units: 20, 80 and 320 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 4 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Daily.
Ophthalmoscopy: Conducted before study initiation and before termination of treatment.
EKG: Not conducted.
Hematology: Blood samples for hematology evaluation were collected before treatment and before termination of study from fasted monkeys.
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected before treatment and before termination of study from fasted monkeys.
Urinalysis: Overnight urine samples were collected before treatment and before termination of treatment from monkeys deprived of food and water.
Gross pathology: Organs/Tissues collected for gross pathology examination are indicated in the histopathology table.
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Not conducted.
Toxicokinetics: Blood samples were collected from each animal before treatment and before termination of treatment to demonstrate proof of absorption.
Cerebrospinal fluid sampling: Cerebrospinal fluid samples were collected from each monkey 2 hr after the last administration of SUN 0588.

Results:
Mortality: There were no treatment-related deaths. The female receiving 20 mg/kg/d died under general anesthesia following cerebrospinal fluid sampling on Day 27 of treatment.

Clinical signs: Diarrhea (loose or liquid feces) was more frequently observed during the treatment period than before the study started. The incidence was not dosage-related and was clearly less during the last week of treatment than earlier in the study. Isolated incidents of emesis or salivation occurred in a few marmosets. These incidents were most frequent in the male receiving 320 mg/kg/day. All surviving animals were moderately to severely depressed following cerebrospinal fluid sampling and all except

the male treated at 20 mg/kg/day had marked hind limb ataxia. These signs were considered to be a consequence of intracranial hemorrhage caused by sampling and unrelated to treatment with SUN 0588.

Body weights: No treatment-related effects on body weight.
Food consumption: No treatment-related effects.
Ophthalmoscopy: No treatment-related ophthalmic findings.
Electrocardiography: No data.
Hematology: Week 3 data. There were no control animals in this study.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
PCV (%)			53	57	47 (11%↓)	43(25%↓)	44(17%↓)	44(23%↓)
Hg (g%)			16.8	18.5	14.1(16%↓)	13.7(26%↓)	13.3(21%↓)	13.8(25%↓)
RBC (106/mm3)			7.6	7.6	7.2(5%↓)	5.9(22%↓)	6.0(21%↓)	5.3(30%↓)
WBC (103/mm3)				8.4		8.3*(1%↓)		9.2*(10%↑)

* **p<0.05**

Clinical chemistry: No treatment-related effects on clinical chemistry.
Urinalysis: No treatment-related effects on urinalysis parameters.
Organ weights: No treatment-related effects on organ weights.
Gross pathology: n = 1/sex/group – empty cells indicate no gross pathology findings.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Brain: Dark red fluid in cranial cavity		1*						
Liver: Distended bile duct		1*						
Lungs: pale and dark areas		1*						
Cervical L.N.: Large				1*				
Ovaries: Small				1*				

* **Same animal**

Histopathology: No data.

Toxicokinetics: Analysis of plasma levels of [redacted], a product of SUN 0588 metabolism, demonstrated that SUN 0588 was absorbed and was present in all animals 2 hours after the last dose. Plasma concentrations of [redacted] clearly increased with dose, although levels found in animals dosed with 80 or 320 mg/kg/day were not directly proportional to those in monkeys dosed 20 mg/kg/day.

Plasma and cerebrospinal fluid concentration of [redacted] in Marmosets

		PLASMA (ng/ml)			Cerebro-spinal fluid (ng/ml)
Animal No.		Total [redacted]	Oxidized	Reduced (R/T %) [redacted]	Total [redacted]
1 M♂ No.401 (20mg/kg)	pre				
	post				
	Δ	35.84	9.13	26.71 (74.5%)	----
2 M♂ No.403 (80mg/kg)	pre				
	post				
	Δ	400.14	208.87	191.27 (47.8%)	----
3 M♂ No.405 (320mg/kg)	pre				
	post				
	Δ	1418.25	653.94	764.31 (53.9%)	----
Mean♂	pre	41.11	36.79	4.31 (10.5%)	----
1 F♀ No.400 (20mg/kg)	pre				
	post				
	Δ	238.06	72.05	166.01 (69.7%)	----
2 F♀ No.402 (80mg/kg)	pre				
	post				
	Δ	614.13	553.57	60.56 (9.9%)	----
3 F♀ No.404 (320mg/kg)	pre				
	post				
	Δ	1873.98	1231.84	642.14 (34.3%)	----
Mean♀	pre	59.10	51.72	7.38 (12.5%)	----

pre : sampling at 10 Nov. 1986
post : sampling at 11 Dec. 1986
Δ : post - pre

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m^2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In a 4-Week oral toxicity study, marmosets were dosed orally with 20, 80 and 320 mg/kg/d of SUN0588. The doses used represent 0.3X, 1X and 5X MRHD based on mg/m^2. There were no treatment-related deaths. The LD

female died under anesthesia following cerebrospinal fluid sampling on Day 27 of treatment. Though not statistically significant, decreases (11-30%) in erythrocyte parameters were noted. Dose-dependent decreases in PCV, RBC and Hg were observed in treated males. Only RBC decreased dose dependently in treated females. WBC was slightly decreased in MD females but increased in HD females. The administration of SUN 0588 did not reveal any overt toxicity. Histopathology was not conducted hence NOAEL could not be established. Based on findings of this study, doses of 20, 80 and 320 mg/kg/d were chosen for the 13 week study on marmosets.

Study title: 13-Week Oral Toxicity Study in Marmosets Followed By a 4 WeeK Recovery Period

Key study findings:

- Two control males and one LD female were sacrificed in extremis and two control females died during the treatment period. Sponsor stated that these deaths were considered to be incidental and that of the LD female cannot be attributed to treatment with SUN 0588 since there were no deaths in the higher dose groups.
- A slight decrease in MCV (10%) occurred in LD females. This was considered an incidental finding.
- Triglycerides were decreased in LD (43%) and MD (46%) males but not in HD males. T. cholesterol was increased by 75% and 70% in MD and HD males respectively, relative to control. Phospholipids were increased by 54% in MD females.
- Urine volume was decreased by 57% in HD males. Urine SG was slightly increased in MD males.
- Ovarian weight increased dose-dependently in treated females whiles weights of the uterus plus cervix were slightly increased in MD and HD females relative to control. These were not reversed at the end of the recovery period.
- The target organs of toxicity included the GI tract (enteritis), kidney (dilated distal tubules, dilated Bowman's capsule, thickened glomerular membrane), liver (centriacinal lymphoid follicle, mineralized fibrous focus), thyroid (lymphoid follicles, parafollicular cell hypertrophy) and the ovary (corpora lutea, regressing corpora lutea, luteinizing artretic follicles). The kidney, liver and ovarian pathology showed partial recovery.
- NOAEL is 80 mg/kg/d.

Study no: PHN-69-AT-SR.
Conducting laboratory and location: [redacted]
Date of study initiation: March 18, 1987
GLP compliance: Yes (Europe, Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8886506, [redacted]
Formulation/vehicle: A solution of SUN0588 in distilled water.

Methods (unique aspects):

Dosing:
Species/strain: Monkey/Marmoset.
#/sex/group or time point (main study): 5/sex/control and HD groups, 3/sex/LD and MD groups.
Satellite groups used for toxicokinetics or recovery: 2/sex/ control and HD groups for recovery.
Age: 49-59 weeks old.
Weight: 215-301 g.
Doses in administered units: 20, 80 and 320 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 4 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Daily.
Ophthalmoscopy: Prior to study initiation and at week 13.
EKG: Not conducted.
Hematology: Blood samples for hematology evaluation were collected prior to treatment, on weeks 4 and 13 from overnight fasted animals. Bone marrow smears were obtained from all animals at necropsy.
Clinical chemistry: Blood samples for clinical chemistry evaluation were collected prior to treatment, on weeks 4 and 13 from overnight fasted animals.
Urinalysis: Prior to treatment and during week 13.
Gross pathology: Organs/tissues collected for gross examination are indicated in the histopathology table.
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Tissues from all dose groups were processed for examination. The left kidney (cortex and medulla) and liver were processed for electron microscopy.
Toxicokinetics: Not conducted.

Results:
Mortality:

Dose (mg/kg/d)	0	20	80	320
Incidence	2/5 (M); 2/5 (F)	1/3 (F)	0	0

Two control males and one female receiving 20 mg/kg/day were sacrificed in extremis and two control females died during the treatment period. Of these animals, the control males were sacrificed during weeks 2 and 11 respectively and the LD female during Week 7; the two control females died during Weeks 6 and 14 respectively. Sponsor stated that these deaths were considered to be incidental and that of the LD female cannot be attributed to treatment with SUN 0588.
Clinical signs: Sponsor stated that the clinical observations on these animals before death are considered to be consistent with Marmoset Wasting Syndrome. These observations included hunched posture, subdued mood, poor muscle tone, tremor, ataxia, collapse, bradycardia, bloated appearance, slow and deep respiration, liquid feces and dehydration.

Other signs observed included yellow staining of cage tray paper, salivation and emesis mainly in the HD group.

Body weights: No treatment-related effects on body weight.
Food consumption: No treatment-related effects on food consumption.
Ophthalmoscopy: No treatment-related ophthalmic findings. Photophobia was observed in 1/5 HD female after week 12.
Electrocardiography: No data.
Hematology: Week 13 data - Empty cells indicate no significant difference was noted relative to control. No significant changes at the end of the recovery period.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
MCV (cμ)	72	73	69	66*	74	72	71	71

* p<0.05

Clinical chemistry: Week 12 data - Empty cells indicate no significant difference was noted relative to control. No significant changes at the end of the recovery period.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Triglycerides (mg%)	143		82*(43%↓)		77*(46%↓)		122	
T. Cholesterol (mg%)	118		146		207*(75%↑)		200*(70%↑)	
Cl (mEq/l)	94		99*(5%↑)		96		95	
Phospholipids (mg%)		217		255		334*(54%↑)		244

* p<0.05

Urinalysis: Week 12 data - No significant changes at the end of the recovery period.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Volume (ml)	8.2		7.2		5.3		3.5*(57%↓)	
SG	1.010		1.007		1.025*(2%↑)		1.021	

* p<0.05

Organ weights: Week 13 data - Empty cells indicate no significant difference was noted relative to control.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Ovary (g)		0.09		0.15		0.17		0.22
Ovary (%)		0.03		0.05		0.06		0.07
Uterus + cervix (g)		0.19		0.18		0.28		0.26
Uterus + cervix (%)		0.07		0.06		0.10		0.09

Organ weight: Week 4 Recovery Data – Only HD groups were examined.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Ovary (g)								0.22
Ovary (%)								0.07
Uterus + cervix (g)								0.31
Uterus + cervix (%)								0.10

Gross pathology: No treatment-related abnormalities.
Histopathology: Week 13 Data – Tissues from all dose groups were examined. Empty cells indicate zero incidence. N = 3/sex/group.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Ileum Enteritis								1/3'(3)
Colon Enteritis								1/3'(2)
Jejunum Enteritis								1/3'(2)
Rectum Enteritis								1/3'(2)
Kidney Dilated bowman's cap		1/3(1)				1/3(1)	2/3 (1)	
Glomerular basement membrane - thickened		1/3(2)				2/3(1)		1/3(3)
Dilated distal tubules	2/3(1)	2/3 1/3(1) 1/3(2)	2/3 1/3(1) 1/3(2)	0/3	1/3(1)	3/3(1)	3/3 1/3(1) 1/3(2) 1/3(3)	2/3(2)
Liver: Centriacinar lymphoid follicle					1/3(2)		1/3(1)	
Mineralized fibrous Focus							1/3(2)	
Thyroid Lymphoid follicles		1/3(2)				1/3(1)		2/3(1)
Parafollicular cell hypertrophy								1/3(2)
Ovaries: Corpora lutea		0/3		0/3		2/3x		1/3x
Regressing corpora lutea		0/3		1/3x		2/3x		1/3x
Luteinizing artretic follicles		0/3		0/3		2/3x		1/3x

1 = minimal, 2 = slight, 3 = moderate, x = present, ' = same animal

Histopathology: Week 4 Recovery Data. Only tissues from HD groups were examined. Empty cells indicate zero incidence.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Kidney Dilated distal tubules							1/3(1)	
Interstitial nephritis							1/3(1)	
Liver: Centriacinar lymphoid follicle							1/3(1)	1/3(1)
Ovaries: Regressing corpora lutea								2/3(1)

Electron microscopic examination of the liver and left kidney from all treated groups was similar to those of the control group.

Toxicokinetics: TK was not conducted in this study. The monkey TK data provided for 10 mg/kg (1301 ng.h/ml) is for Cynomolgus monkey. Since the present study used marmosets, it was not possible to estimate systemic exposure from this data. The multiples derived are therefore based on BSA (mg/m^2).

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In a 13-Week oral toxicity study, marmosets were dosed orally with 20, 80 and 320 mg/kg/d of SUN0588 followed by a 4 week recovery period. The doses used represent 0.3X, 1X and 5X MRHD based on mg/m^2. Two control males and one LD female were sacrificed in extremis and two control females died during the treatment period. These deaths were considered to be incidental since there were no deaths in the higher dose groups. Urine volume was decreased by 57% in HD males. Ovarian weight increased dose-dependently in treated females whiles weights of the uterus plus cervix were slightly increased in MD and HD females relative to control. These were not reversed at the end of the recovery period. The increased ovarian weights correlated with the presence of corpora lutea and luteinizing artretic follicles. The target organs of toxicity included the GI tract (enteritis), kidney (dilated distal tubules, dilated Bowman's capsule, thickened glomerular membrane), liver (centriacinal lymphoid follicle, mineralized fibrous focus), thyroid (lymphoid follicles, parafollicular cell hypertrophy) and the ovary (corpora lutea, regressing corpora lutea, luteinizing artretic follicles). The kidney, liver and ovarian pathology showed partial recovery. NOAEL is 80 mg/kg/d (1X) based on histopathology.

Study title: 52-Week Oral Toxicity Study In Marmosets Followed by a 4-Week Recovery Period

Key study findings:

- Slight decrease in body weight was observed in HD animals at the end of the recovery period.

- Food consumption was decreased by 10% in HD females at the end of the recovery period.
- Increases in platelets (27%) and PT (8%) were observed in HD females at the end of the treatment period. At the end of the recovery period, platelets were still increased by 16% in HD females.
- A slight increase in creatinine (17%) occurred in HD males. AST was decreased by 35% and 34% in LD males and females respectively, and by 18% in HD females at the end of the treatment period. At the end of the recovery period, Cl level was slightly increased in HD males.
- The target organs of toxicity include the adrenal (lymphocytic foci), brain (cerebellar basophilic concretions), heart (myocardial fibrosis), ileum (chronic ileitis), kidney (interstitial nephritis, pyelitis, transitional cell hyperplasia, increased mitotic index in papillary duct), liver (periportal inflammatory cell foci), stomach (lymphoid hyperplasia), thyroid (lymphocytic infiltration), uterus (glandular hyperplasia, adenomyosis).
- At the end of the recovery period, lesions were observed in the epididymis and thyroid (lymphocytic infiltration), gall bladder (leukocytic foci), perobronchial, cervical and mesenteric lymph nodes (paracortical hyperplasia) of HD animals. In addition, lesions in the thyroid (dilated follicles with secretion), urinary bladder (transitional cell hyperplasia) and mesenteric lymph node (sinus histiocytosis) were observed in HD females.
- NOAEL is 80 mg/kg/d based on histopathology.

Study no: PHN-71-AT-SR.
Conducting laboratory and location: [redacted]
Date of study initiation: November 3, 1987.
GLP compliance: Yes (Europe, Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot #s 8886506, [redacted] 8886607, [redacted] and 8886708, [redacted]
Formulation/vehicle: SUN0588 dissolved in distilled water.

Methods (unique aspects):
Dosing:
Species/strain: Monkey/Marmoset.
#/sex/group or time point (main study): 6/sex/group for control and HD groups; 4/sex/group for LD and MD groups.
Satellite groups used for toxicokinetics or recovery: 2/sex/group for control and HD groups were used for recovery.
Age: Between 47 and 109 weeks.
Weight: 214-418 g.
Doses in administered units: 20, 80 and 320 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 4 ml/kg.

Observations and times:

Clinical signs: Daily.
Body weights: Weekly.
Food consumption: Weekly.
Ophthalmoscopy: Conducted prior to treatment, at Weeks 26 and 51 of treatment and on Day 24 of the recovery period.
EKG: Not conducted.
Hematology: Blood samples were obtained from non-fasted animals prior to treatment, at Weeks 24 and 50 of treatment and on Day 28 of the recovery period for hematology evaluation.
Clinical chemistry: Blood samples were obtained from non-fasted animals prior to treatment, at Weeks 25 and 51 of treatment and on Day 31 of the recovery period for clinical chemistry evaluation.
Urinalysis: Urine samples were collected overnight from animals deprived of food and water prior to treatment, at Weeks 23 and 49 of treatment and on Day 23 of the recovery period.
Gross pathology: Organs/tissues collected for gross examination are indicated in the histopathology table (at the end of the section of toxicology).
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Tissues from all dose groups were processed for evaluation. In addition, the left kidney (cortex and medulla) and liver samples were processed for electron microscopy.
Toxicokinetics: Not conducted.

Results:
Mortality: None.
Clinical signs: Bright yellow staining of the cage tray paper was occasionally observed in HD females during the first 39 weeks of treatment; # 704 was the most frequently affected. One animal was again affected in Week 52. A few Incidents were occasionally noted in the HD males during the first 40 weeks of treatment and two isolated incidents occurred in MD males. Salivation was observed in a majority of HD animals during the treatment period, with isolated incidents in lower dose groups, particularly the MD group. Emesis was occasionally observed in the MD and HD groups. None of these signs was observed during the four week recovery period.
Body weights:

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Week 1	140	140	140	140	140	140	150	130
Week 52	150	150	140	150	140	140	150	130 (13%↓)
Recovery Week 1	140	160					150	130 (19%↓)
Recovery Week 4	140	130					130 (7%↓)	120 (7%↓)

Only control and HD groups were examined during the recovery period.

Food consumption: (g)

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Day -1	343	331	352	325	346	326	344	331
Day 364	392	398	399	390	381	391	373	378

							(5%↓)	(5%↓)
Recovery Day -1	390	460					392	373
Recovery Day 28	382	418					384	376 (10%↓)

Only control and HD groups were examined during the recovery period.

Ophthalmoscopy: Week 51 data

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Photophobia	1/2*	1/2	0/2	2/2	1/2	0/2	0/2	0/2
Recovery Week 3								
Photophobia	1/2*	0/2					0/2	1/2

Only control and HD groups were examined during the recovery period; * same animal

Electrocardiography: No data.

Hematology: Week 50 data.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Platelets (10^3/mm3)	224	235	231	271	283	247	273	323*(27%↑)
PT (sec)	6.8	6.5	6.8	6.7	6.8	6.8	6.9	7.0**(8%↑)
Recovery Week 3								
Platelets (10^3/mm3)	253	282					285	327*(16%↑)

Only control and HD groups were examined during the recovery period, * p<0.05, **p<0.01

Clinical chemistry: Week 51 data.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Creatinine (mg%)	0.6		0.6		0.6		0.7*(17%↑)	
AST (IU/l)	134	107	87*(35%↓)	71***(34%↓)	126	99	99	88*(18%↓)
Recovery Week 4								
Chloride (mEq/l)	103		NOT EXAMINED				109*(6%↑)	

Only control and HD groups were examined during the recovery period, * p<0.05, ** p<0.01. *** p<0.001

Urinalysis: No treatment-related effects on urinalysis parameters.

Organ weights: No treatment-related effects on organ weight.

Gross pathology: Week 52 data. N = 6/sex/group (control and HD); N = 2/sex/group (LD, MD & recovery).

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Cervical L.N. Large (unilateral)			1/2	1/2	1/2			
Lungs Pale/Dark areas			1/2				1/2	
Incomplete collapse							2/2	
Peribronchial L.N. Dark					1/2			
Spleen Pale					1/2			

Large		2/2						
Abnormal shape						1/2		
Adrenal glands Dark					1/2			
Liver Firm mass (es)							1/2	
Thyroid gland Large (unilateral)							1/2	
Ovaries Dark		1/2						
Mass(es) on ovaries		1/2		2/2				
Large ovaries				1/2				
Recovery Week 4								
Cervical L.N.: Large	1/2	1/2						
Mesenteric L.N. Dark	1/2	1/2	**NOT EXAMINED**				1/2	
Oraries: Large								1/2

Only control and HD groups were examined during the recovery period. Empty cells indicate zero incidence.

Histopathology: Week 52 data. N = 6/sex/group (control and HD); N = 2/sex/group (LD, MD & recovery). Tissues from all dose groups were examined. Empty cells indicate zero incidence.

Dose (mg/kg/d)	**0**		**20**		**80**		**320**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Adrenal gland Lymphocytic foci							2/4 1/4(1) 1/4(2)	
Brain: Cerebellar basophilic concretions						1/4(1)		1/4(1)
Femur Marrow degeneration								1/4(1)
Heart (Ventricle) Myocardial fibrosis			1/4(1)	1/4(1)		1/4(1)		1/4(1)
Ileum: Chronic ileitis						1/4(1)		1/4(1)
Kidney Interstitial nephritis		4/4(1)	2/4 1/4(1) 1/4(2)	2/4 1/4(1) 1/4(2)	1/4(1)	1/4(1)	3/4 2/4(1) 1/4(2)	3/4(1)
Pyelitis	1/4(1)	1/4(1)			1/4(2)			2/4(1)
Transitional cell hyperplasia	2/4(1)				3/4 2/4(1) 1/4(2)		2/4(1)	1/4(1)
↑ mitotic index in papillary duct cells							1/4(2)	
Liver: Periportal inflammatory cell foci		1/4(1)	1/4(1)	2/4(1)		4/4(1)	2/4(1)	3/4(1)
Stomach Lymphoid hyperplasia		1/4(2)			1/4(2)		2/4 1/4(2) 1/4(3)	1/4(2)
Thyroid: Lymphocytic infiltration	1/4(1)	1/4(1)	2/4 1/4(2)			1/4(1)	2/4 1/4(1)	3/4 1/4(1)

			1/4(3)	2/4(2)	1/4(2)		1/4(2)	2/4(2)
Parafollicular cell hyperplasia	1/4(2)		1/4(2)		2/4 1/4(2) 1/4(3)	2/4(2)	1/4(2)	
Uterus Glandular hyperplasia				1/4(3)				1/4(3)
Adenomyosis								1/4(2)
	0 mg/kg/d		**Recovery Week 4**				**320 mg/kg/d**	
Epididymides: Lymph -ocytic infiltration							1/2(1)	
Gall bladder Leukocytic foci			**NOT EXAMINED**				1/2(1)	
Ileum: chronic ileitis								1/2(1)
Kidney: Inflammatory Cell foci	1/2(1)	1/2(1)					2/2(1)	1/2(1)
Glomerulosclerosis								1/2(1)
Cervical L.N: Paracort -ical hyperplasia	2/2 1/2(1) 1/2(2)						1/2(1)	2/2 1/2(1) 1/2(2)
Mesenteric L.N: Parac -ortical hyperplasia							1/2(2)	1/2(2)
Sinus histiocytosis								1/2
Peribronchial L.N. Paracortical hyperplasia							1/2(1)	1/2(1)
Liver: Inflammatory Cell foci	1/2(1)	1/2(1)					2/2 1/2(1) 1/2(2)	1/2(1)
Stomach: Lymphocyti -c infiltration	2/2(1)	1/2(1)					1/2(1)	2/2(1)
Thyroid: Lymphocyti -c infiltration		2/2 1/2(1) 1/2(2)					2/2(3)	
Dilated follicles with secretion								1/2(2)
Urinary bladder: Tra -nsitional cell hyperpla -sia		1/2(1)						1/2(2)

Only control and HD groups were examined during the recovery period. Empty cells indicate zero incidence.

Toxicokinetics: No data.

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In a 52-Week oral toxicity study, marmosets were dosed orally, once daily with 20, 80 and 320 mg/kg/d of SUN0588 followed by a 4 week recovery period. The doses used represent 0.3X, 1X and 5X MRHD based on mg/m^2. Slight decreases in body weight (HD animals) and food consumption (HD females) were observed at the end of the recovery period. Slight increases in platelets and PT were observed in HD females at the end of the treatment period. The platelets showed partial recovery. A slight increase in creatinine (HD males) and decrease in AST (LD group and HD females) was seen at the end of the treatment period. At the end of the recovery period, Cl level was slightly increased in HD males. The target organs of toxicity include

the adrenal (lymphocytic foci), brain (cerebellar basophilic concretions), heart (myocardial fibrosis), ileum (chronic ileitis), kidney (interstitial nephritis, pyelitis, transitional cell hyperplasia, increased mitotic index in papillary duct), liver (periportal inflammatory cell foci), stomach (lymphoid hyperplasia), thyroid (lymphocytic infiltration), uterus (glandular hyperplasia, adenomyosis). At the end of the recovery period, lesions were observed in the epididymis and thyroid (lymphocytic infiltration), gall bladder (leukocytic foci), perobronchial, cervical and mesenteric lymph nodes (paracortical hyperplasia) of HD animals. In addition, lesions in the thyroid (dilated follicles with secretion), urinary bladder (transitional cell hyperplasia) and mesenteric lymph node (sinus histiocytosis) were observed in HD females. NOAEL is 80 mg/kg/d (1X) based on histopathology.

Study title: Oral Dose-Ranging Repeated Dose Toxicity Study of Sapropterin HCl in Mice for 13 Weeks.

Key study findings:

- The study was not adequately designed. Hematology, clinical chemistry, urinalysis, histopathology and toxicokinetics were not conducted.
- Mortality (not dose related) occurred in 1/13 control males, 1/13 LD (M &F) and 1/13 MD (F). Cause of death could not be determined since histopathology was not performed.
- Food consumption was decreased (not SS) by 11% in HD males.
- Gross examination revealed large adrenal gland (1 HD, F), dark harderian gland (2 LD, 1 MD and 2HD females), large cervical and mesenteric lymph nodes (1HD, F each), lung mass (1HD, M), large preputial gland (1 MD and 1 HD, M), swollen spleen (1 HD, F) and distended uterus (2 MD and 2 HD females).
- NOAEL could not be established since histopathology was not performed.
- For males, 250 mg/kg/d may be MTD based on 11% decrease in food consumption. MTD was not established for females.

Study no: PHN-117-AT-SR.
Conducting laboratory and location: [redacted]
Date of study initiation: February 15, 1988.
GLP compliance: Yes (Europe, Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8887904; [redacted]
Formulation/vehicle: UN0588 dissolved in distilled water.

Methods (unique aspects)**:**
Dosing:
Species/strain: Mouse/CD-1.
#/sex/group or time point (main study): 12/sex/group.
Satellite groups used for toxicokinetics or recovery: None.
Age: 4-5 weeks at study initiation.
Weight: 16-21 g (M), 15-20 g (F).

Doses in administered units: 25, 80, 250 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Weighed on Days 3 and 7, and thereafter weekly.
Food consumption: Weekly.
Ophthalmoscopy: Not conducted.
EKG: Not conducted.
Hematology: Not conducted.
Clinical chemistry: Not conducted.
Urinalysis: Not conducted.
Gross pathology: Organs/tissues isolated for gross examination are indicated in the histopathology table (at the end of the section of toxicology).
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Not conducted.
Toxicokinetics: Not conducted.

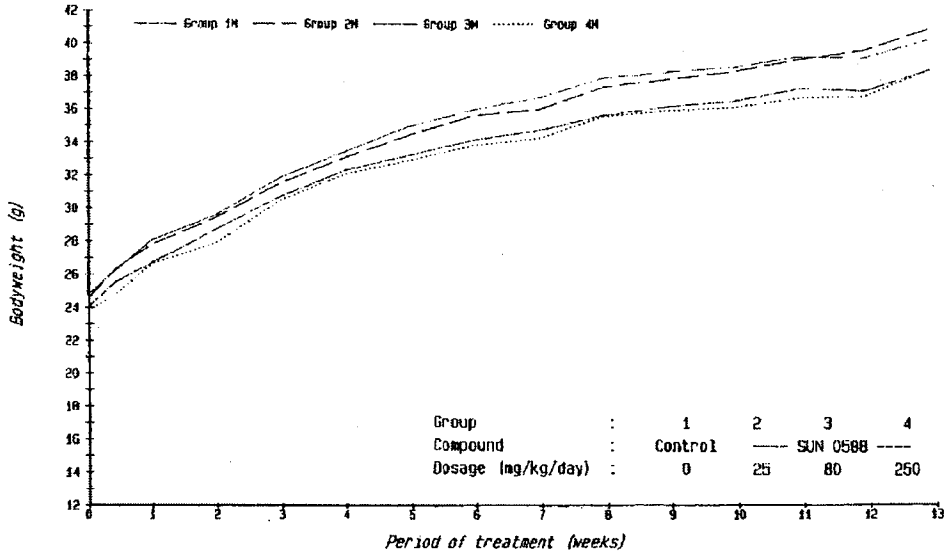
Results:
Mortality: n = 12/sex/group

Dose (mg/kg/d)	0		25		80		250	
Sex	M	F	M	F	M	F	M	F
No. of Deaths	1	0	1	1	0	1	0	0
# 002 M (0 mg/kg/d)	Died on Day 4 of treatment. Gross examination revealed slight emaciation, dark liver, firm pale/dark masses on all liver lobes and a pale spleen. Cause of death not known.							
# 022 M (25 mg/kg/d)	Died on Day 2 of treatment. Gross examination revealed yellow viscous fluid in the duodenum, jejunum. Cause of death not known.							
# 063 F (25 mg/kg/d)	Died on Day 3 of treatment. Gross examination revealed distended uterus with fluid. Cause of death not known.							
# 081 F (80 mg/kg/d)	Died on Day 3 of treatment. Gross examination did not reveal any abnormalities. Cause of death not known.							

Clinical signs: No treatment-related clinical signs.
Body weights: (g) - No treatment-related effects on body weight.

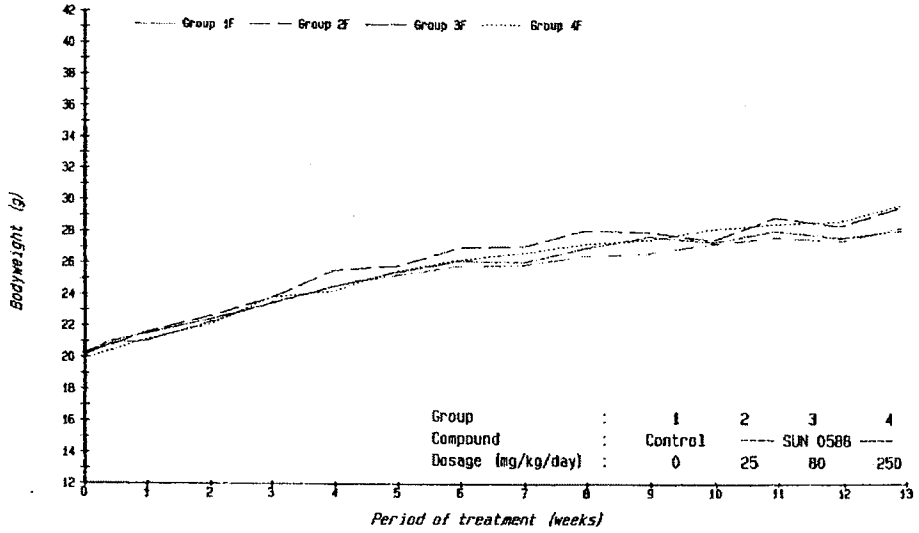
Dose (mg/kg/d)	0		25		80		250	
Sex	M	F	M	F	M	F	M	F
Day 0	24.8	20.2	24.6	20.3	24.1	20.3	23.8	19.9
Day 91	40.2	28.3	40.8	29.5	38.3	28.1	38.3	29.7
Body weight Gain	15.4	8.1	16.2	9.2	14.2	7.8	14.5	9.8

Males:



Females:

Best Possible Copy



Food consumption: (g/mouse/week): Though not statistically significant, food consumption in HD males was decreased by 11% relative to control.

Dose (mg/kg/d)	0		25		80		250	
Sex	M	F	M	F	M	F	M	F
Week 1	35	28	34	28	33	26	32	26
Week 13	38	33	40	37	38	34	34	33
% change from control	-	-	5↑	12↑	-	3↑	11↓	-

Ophthalmoscopy: No data.
Electrocardiography: No data.
Hematology: No data.

Clinical chemistry: No data.
Urinalysis: No data.
Organ weights: No treatment-related effects on organ weight.
Gross pathology: Week 13 data. Organs/Tissues from all dose groups were examined.

Dose (mg/kg/d)	0		25		80		250	
Sex	M(11)	F(12)	M(11)	F(11)	M(12)	F(11)	M(12)	F(12)
Adrenal gland Large								1
Harderian gland Dark				2		1		2
Cervival L.N. Large								1
Mesenteric L.N. Large								1
Lung Mass							1	
Preputial gland Large					1		1	
Spleen Swollen								2
Uterus Distended						2		2

Empty cells indicate zero incidence

Histopathology: No data.
Toxicokinetics: No data.

Summary of individual study findings: *Clinical dose = 10 mg/kg = 370 mg/m^2. Multiple dose (7 days) human PK for the 200 mg TID (10% formulation) = 600 mg/d = 366 ng.h/ml. This dose is equivalent to the 10 mg/kg (600 mg/d for a 60 kg human) and may have similar systemic exposure.* In a 13 week oral toxicity study, mice were dosed orally, once daily with 25, 80 and 250 mg/kg/d SUN0588. These doses represent 0.2X, 0.7X and 2X MRHD based on mg/m^2. There were no treatment related effects on body weight or clinical signs. Mortality (not dose related) occurred in 1/13 control males, 1/13 LD (M &F) and 1/13 MD (F). Cause of death could not be determined since histopathology was not performed. Food consumption was decreased (not SS) by 11% in HD males. Gross examination revealed large adrenal gland (1 HD, F), dark harderian gland (2 LD, 1 MD and 2HD females), large cervical and mesenteric lymph nodes (1HD, F each), lung mass (1HD, M), large preputial gland (1 MD and 1 HD, M), swollen spleen (1 HD, F) and distended uterus (2 MD and 2 HD females). NOAEL could not be established since histopathology was not performed. For males, 250 mg/kg/d may be MTD based on 11% decrease in food consumption. MTD was not established for females.

Other toxicity studies:
SINGLE DOSE TOXICITY STUDIES IN JUVENILE ANIMALS

Single-Dose Toxicity Studies of Sapropterin HCl in Juvenile Mice (21-days old) (Study #: PHN-72-AT-SR)

Sapropterin HCl was administered orally once to 3-week old [redacted]:CD-1 mice at dose levels of 932, 1265, 1717, 2330 and 3162 mg/kg (5 animals/sex/group). Animals were observed for 14 days for mortality and signs of toxicity. Deaths occurred in 3 males in the 1717 mg/kg group, 3 females in the 2330 mg/kg group and all animals in the 3162 mg/kg group. Deaths occurred between study days 2 and 3. Therefore, the minimum lethal dose of sapropterin HCl was 1717 mg/kg for 3-week old male mice and 2330 mg/kg for 3-week old female mice. Sponsor stated that the LD_{50} values were 2278 mg/kg for males and 2287 mg/kg for females. Clinical signs of decedents consisted of lethargy, hunched posture, decreased motor activity and piloerection. Necropsy findings for the same animals were confined to yellow staining of the fur and altered gastrointestinal contents.

Study Design and Results of a Single-Dose Toxicity Study of Sapropterin in Juvenile Mice (21-days old)

Species/Strain/Age	Route of Administration	Dose (mg/kg)	Sex	No. of animals/group	Results	
					LD50 *	Findings
Mouse/CD-1/3 weeks old	p.o.	932, 1265, 1717, 2330, 3162	M F	5 5	M: 2278 mg/kg F: 2287 mg/kg	Clinical Signs in Decedents: hunched posture, ataxia, decreased motor activity, respiratory difficulties and piloerection.

Observation period: 14 days * Calculated by Probit Method
Sapropterin Lot #: 8888302
Study Location: [redacted]
Study Dates: September 20, 1988 – October 6, 1988

Single-Dose Toxicity Studies of Sapropterin HCl in Juvenile SD Rats (7-days old) (PHN-75-AT-SR)

Sapropterin HCl was administered orally once to 1-week old [redacted] CD-1 SD rats at dose levels of 1200, 1680, 2350, 3800 and 4620 mg/kg (6 animals/sex/group). Animals were observed for 14 days for mortality and signs of toxicity. Deaths occurred in 4 males and 2 females in the 1200 mg/kg dose group, 4 males and 4 females in the 1680 mg/kg dose group, all males and 5 females in the 2350 mg/kg group and all animals in the 3300 and 4620 mg/kg dose groups. Deaths occurred between study days 1 and 8. Therefore, the minimum lethal dose of sapropterin HCl was 1200 mg/kg for 7-day old male and female mice. Sponsor stated that the LD_{50} values were 1108 mg/kg for males and 1445 mg/kg for females. Clinical signs of decedents consisted of decrease of voluntary movements,

prone resting, bradypnea and cyanosis. Upon necropsy, hyperemia or hemorrhage was evident throughout the glandular stomach of animals dosed orally with ≥ 1680 mg/kg sapropterin HCl.

Study Design and Results of a Single-Dose Oral Toxicity Study of Sapropterin in Weanling Rats (7-days old)

Species/ Strain/Age	Route of Administration	Dose (mg/kg)	Sex	No. of Animals/Group	Results	
					LD50 *	Findings
Rat/ 7 Days Old/ ● .CD (SD)	p.o.	1200, 1680, 2350, 3800, 4620	M F	6 6	M: 1108 mg/kg F: 1445 mg/kg	Voluntary movements ↓ Prone resting: Bradypnea: Necropsy: hyperemia, hemorrhage throughout glandular stomach at doses ≥ 1680mg/kg p.o..

Observation period: 14 days * Calculated by Probit Method
Sapropterin Lot #: 8888301
Study Location: Suntory, Ltd. Medicines Center
Study Dates: April 17, 1989 – November 27, 1989

Single-Dose Toxicity Study of Sapropterin HCl in Juvenile Wistar Rats (21-days old) (Study #: PHN-73-AT-SR)

Sapropterin HCl was administered orally once to 3-week old ● :Wistar rats at dose levels of 1265, 1717, 2330, 3162 and 4291mg/kg (5 animals/sex/group). Animals were observed for 14 days for mortality and signs of toxicity. Deaths occurred in 1 male in the 2330 mg/kg group, 4 males and 2 females in the 3162 mg/kg group, and 4 males and all females in the 4291 mg/kg group. Deaths occurred between study days 1 and 2. Therefore, the minimum lethal dose of sapropterin HCl was 2330 mg/kg for 3-week old male rats and 3162 mg/kg for 3-week old female rats. Sponsor stated that the LD_{50} values were 2930 mg/kg for males and 3222 mg/kg for females. Clinical signs of decedents consisted of hunched posture, ataxia, animals were confined to yellow or brown staining of the fur and altered gastrointestinal contents.

Study Design and Results of a Single-Dose Toxicity Study of Sapropterin in Juvenile Rats (21-days old)

Species/Strain/Age	Route of Administration	Dose (mg/kg)	Sex	No. of animals/group	Results	
					LD50 *	Findings
Rat/ ● :CD/3 weeks old	p.o.	1265, 1717, 2330, 3162, 4291	M F	5 5	M: 2930 mg/kg F: 3222 mg/kg	Clinical Signs in Decedents: hunched posture, ataxia, decreased motor activity, respiratory

						difficulties and piloerection. Necropsy of decedents: yellow or brown staining of the fur, altered gastrointestinal contents

Observation period: 14 days * Calculated by Probit Method
Sapropterin Lot #: 8888302
Study Location: [redacted]
Study Dates: September 20, 1988 – October 6, 1988

REPEATED-DOSE TOXICITY STUDIES IN JUVENILE ANIMALS

Study title: 2-Week Oral Toxicity Study of SUN 0588 in Juvenile Rats (7days old)

Key study findings:

- 1/12 males in the control group was sacrificed moribund on Day 4 of treatment. One out of 12 MD females was found dead on Day 8. Sponsor attributed the demise of this rat to gavage error.
- Blood was observed around the anus of 1/12 males and 5/12 females in the HD group (Days 2-7).
- Body weight gain was decreased (not SS) by 10% and 7% in HD males and females respectively.
- RBC and PCV were slightly increased in HD males.
- A/G ratio was slightly increased while Ca was slightly decreased in HD males. LDH was increased by 27% in HD females.
- Slight increases in relative weights of the liver, submaxillary salivary gland, lung and brain occurred in HD males. Absolute weight of the thymus was decreased by 23% in HD males.
- The kidney is the target organ (basophilia of proximal tubules, dilatation of renal tubules and pelvis, and thickened Bowman's capsule).
- NOAEL is 80 mg/kg/d (1X MRHD, BSA) based on the increased incidence and severity of histopathology findings at HD.

Study no: PHN-76-AT-SR.
Conducting laboratory and location: Medicine Center, Suntory Ltd., 2716-1 Oaza-Aka-Iwaaza- Kurakake, Chiyoda-cho, Yuraku-gun, Gunma, Japan.
Date of study initiation: May 11, 1989.
GLP compliance: Yes (Japan).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8888706, [redacted]
Formulation/vehicle: A solution of SUN 0588 in water.

Methods (unique aspects):

Dosing:
Species/strain: Rat/SD.
#/sex/group or time point (main study): 12/sex/group.
Satellite groups used for toxicokinetics or recovery: None.
Age: 7 days at study initiation.
Weight: 14.0-19.6 g (M); 15.0-18.7 g (F).
Doses in administered units: 20, 80 and 320 mg/kg/d for 2 weeks.
Route, form, volume, and infusion rate: Oral (gavage); 5 ml/kg/d.

Observations and times:
Clinical signs: Daily.
Body weights: Determined prior to dosing, once every 2 days during the first week, and once every 3 days during the second week.
Food consumption: No information on how this was measured.
Ophthalmoscopy: Conducted on the final day of dosing.
Auditory test: Auricular reflex and systemic reaction to a stimulus of 12,000 Hz sounds with a Galton whistle were examined on the final day of dosing.
EKG: Not conducted.
Hematology: One day after the final day of dosing, blood samples were collected for routine hematology evaluation.
Clinical chemistry: One day after the final day of dosing, blood samples were collected for routine clinical chemistry evaluation.
Urinalysis: Urine samples were collected once after the final day of dosing for evaluation.
Gross pathology: Organs/tissues isolated for gross examination are indicated in the histopathology table showed at the end of the section of toxicology.
Organs weighed: Organs weighed are indicated in the histopathology table.
Histopathology: Tissues from control and HD groups were processed and examined except for the kidney where tissues from LD and MD groups were examined as well.
Toxicokinetics: Not conducted.

Results:

Mortality:

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Deaths	1/12	0/12	0/12	0/12	0/12	1/12	0/12	0/12

One male in the control group was sacrificed moribund on Day 4 due to deterioration of general condition. One MD female was found dead on Day 8. Sponsor attributed the demise of this animal to gavage error (no supporting gross pathological evidence was provided).
Clinical signs:

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Hypoactivity	1/12 (D4)	0/12	0/12	0/12	0/12	0/12	0/12	0/12

Blood around anus	0/12	0/12	0/12	0/12	0/12	0/12	1/12 (D5,6)	5/12 (D2-7)

D = Treatment day when clinical sign was observed

Body weights:

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Day 1	17.2	16.7	17.0	16.6	17.4	16.6	17.3	16.5
Day 16 (M); Day 17 (F)	61.9	66.3	63.4	66.9	64.1	65.7	57.6	62.7
Gain	44.7	49.6	46.4	50.3	46.7	49.1	40.3	46.2
Decrement	-	-	-	-	-	-	4.4	3.4
% Decrement	-	-	-	-	-	-	10	7

Food consumption: No data.
Ophthalmoscopy: No treatment related findings.
Auditory test: No treatment related findings.
Electrocardiography: No data.
Hematology: No treatment related changes in females.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
RBC (10^6/mm3)	5.7		5.7		5.7		6.2*(9%↑)	
PCV (%)	35.2		35.7		36.3		38.5*(9%↑)	

*** p < 0.05**

Clinical chemistry:

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
A/G Ratio	1.97		2.03		2.02		2.13**(8%↑)	
Ca (mg/dl)	9.91		9.93		9.74		9.54*(4%↓)	
LDH (mU/ml)		152		173		157		193**(27%↑)

*** p < 0.05; ** p < 0.01**

Urinalysis: No treatment related effects on urinalysis parameters.
Organ weights: No treatment related organ weight changes in females.

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Liver (g %)	3.7		3.8		3.9		4.0**(8%↑)	
Submax. Gland (g %)	0.34		0.34		0.35		0.38*(12%↑)	
Thymus (g)	0.26		0.25		0.25		0.20**(23%↓)	
Lung (g %)	0.79		0.80		0.80		0.85*(8%↑)	
Brain (g %)	2.94		3.00		2.96		3.27*(11%↑)	

*** p < 0.05; ** p < 0.01**

Gross pathology: No treatment related gross findings except for a black focus on the lung of 1/12 HD females.
Histopathology:

Dose (mg/kg/d)	0		20		80		320	
Sex	M	F	M	F	M	F	M	F
Kidney Basophilia; Proximal tubules	2/11(1)	5/12 4/12(1) 1/12(2)	3/12(1)	5/12(1)	3/12(1)	2/12(1)	6/12(2)	2/12(2)
Dilatation: renal tubules	0/11	5/12(1)	2/12(1)	0/12	1/12(1)	0/12	6/12(2)	2/12(2)
Bowman's capsule: thickened	1/11(1)	3/12(2)	2/12(2)	0/12	0/12	0/12	5/12(2)	3/12(2)
Pelvic dilatation	2/11(2)	0/12	1/11(2)	1/12(1)	0/12	1/12(1)	1/12(2)	1/12(1)

1 = minimal; 2 = slight

Toxicokinetics: TK was not conducted in this study. The rat TK data provided for 10 mg/kg (265 ng.h/ml) and 100 mg/kg (4571 ng.h/ml) in the pharmacokinetics section is not linear. Therefore it was not possible to estimate what systemic exposure would be for a 320 mg/kg dose. The multiples derived are therefore based on BSA (mg/m^2).

Summary of individual study findings: *MRHD = 10 mg/kg = 250 mg/m^2 (based body surface area for a child).*
In an oral toxicity study, juvenile rats (7days old) were dosed with SUN 0588 at 20, 80 and 320 mg/kg/d once daily for 2 weeks. These doses represent 0.5X, 2X and 8X MRHD, based on BSA respectively (please note that these multiples were derived using the adult rat factor of 6 to convert mg/kg to mg/m^2). There were no drug-related deaths. Blood was observed around the anus of 1/12 males and 5/12 females in the HD group (Days 2-7). Slight decrease in body weight gain was observed in the HD group. Slight increases in PCV, RBC, A/G ratio and a decrease in Ca occurred in HD males. LDH was slightly increased in HD females with no correlative histopathology. Weights of the liver, submaxillary salivary gland, lung and brain were slightly increased while weight of the thymus was decreased in HD males. The kidney is the target organ (basophilia of proximal tubules, dilatation of renal tubules and pelvis, and thickened Bowman's capsule). NOAEL is 80 mg/kg/d (2X MRHD, BSA) based on the increased incidence and severity of histopathology findings at HD.

Study title: 4-Week Oral Toxicity Study of SUN 0588 in Juvenile Rats (21 days old)

Key study findings:

- Slight increase in prothrombin time was noted in treated males. Slight decreases in erythrocyte parameters (PCV, HB, RBC) occurred in MD females.
- Decreases in triglycerides (26%), phospholipids (12%) and T. cholesterol (16%) were observed in MD males. A slight decrease in phosphorus occurred in HD males. Slight decreases in AST (HD females) and ALT (LD & HD females) was observed. Albumin increased slightly in LD and HD females while A/G ratio increased slightly in HD females.
- Minimal to slight decreases in urine volume, pH, SG, Na, K and Cl were observed in treated rats with no dose-related pattern.

- Relative weight of the spleen was increased with no correlative histopathology.
- The target organs of toxicity include the adrenal cortex (hypertrophy), kidney (tubular dilatation and basophilia), liver (foci of leucocytes), uterus (dilatation), mandibular lymph node (follicular hyperplasia), thymus (hemorrhage) and harderian gland (inflammation).
- NOAEL is 500 mg/kg/d (8X MRHD) based on the low incidence and minimal to slight severity of histopathology findings in the 500 mg/kg/d group. Incidence and severity of histopathology findings in controls are similar to those of the 500 mg/kg/d group.

Study no: PHN-74-AT-SR.
Conducting laboratory and location: [redacted]

Date of study initiation: August 24, 1988.
GLP compliance: Yes (U. K.).
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8888302, [redacted]
Formulation/vehicle: A solution of SUN 0588 in distilled water.

Methods (unique aspects):
Dosing:
Species/strain: Rat/Wistar.
#/sex/group or time point (main study): 10/sex/group.
Satellite groups used for toxicokinetics or recovery: None.
Age: 21 days old at study initiation.
Weight: 30-57 g (M); 25-57 g (F).
Doses in administered units: 5, 50, 500 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), 5 ml/kg.

Observations and times:
Clinical signs: Daily.
Body weights: Daily.
Food consumption: Weekly.
Ophthalmoscopy: Conducted prior to treatment and before study termination.
EKG: Not conducted.
Hematology: Blood samples were collected from all animals after 3 weeks of treatment for hematology evaluation.
Clinical chemistry: Blood samples were collected from all animals after 3 weeks of treatment for clinical chemistry evaluation.
Urinalysis: Urine samples were collected overnight from all animals (fasted and deprived of water) after 3 weeks of treatment for urinalysis evaluation.
Gross pathology: Tissues/organs collected for gross examination are indicated in the histopathology table.
Organs weighed: Organs weighed are indicated in the histopathology table (at the end of the section of toxicology).

Histopathology: Tissues from control and HD groups were processed and examined microscopically. In addition, all tissues exhibiting gross abnormalities were examined microscopically.
Toxicokinetics: Not conducted.

Results:

Mortality: None.
Clinical signs: Except for transient salivation observed in the HD group, there were no other treatment related clinical signs.
Body weights: No treatment related effect on body weight.
Food consumption: No treatment related effect on food consumption.
Ophthalmoscopy: No treatment related ophthalmic findings.
Electrocardiography: No data.
Hematology:

DOSE (mg/kg/d)	0		5		50		500	
Sex	M	F	M	F	M	F	M	F
Prothrombin time (s)	14.8		15.3*(3%↑)		15.5**(5%↑)		15.5**(5%↑)	
PCV (%)		41		41		40**(2%↓)		41
HB (g %)		14.0		14.0		13.5*(4%↓)		14.1
RBC (106/mm3)		6.6		6.4		6.3*(5%↓)		6.6

*** p<0.05; ** p<0.01**

Clinical chemistry:

DOSE (mg/kg/d)	0		5		50		500	
Sex	M	F	M	F	M	F	M	F
Triglycerides (mg %)	139		122		103**(26%↓)		132	
Phospholipids (mg %)	138		127		121*(12%↓)		135	
T. Cholesterol	70		64		59*(16%↓)		71	
P (mEq/l)	3.3		3.3		3.2		3.1**(6%↓)	
AST (IU/l)		149		162		142		124*(17%↓)
ALT (IU/l)		35		29*(17%↓)		31		26**(26%↓)
Albumin (umin)		3.5		3.7*(6%↑)		3.5		3.8***(9%↑)
A/G Ratio		1.3		1.4		1.3		1.6***(23%↑)

*** p<0.05; ** p<0.01' *** p<0.001**

Urinalysis:

DOSE (mg/kg/d)	0		5		50		500	
Sex	M	F	M	F	M	F	M	F
Volume (ml)	6.0		4.5**(25%↓)		4.0*** (33%↓)		5.0**(17%↓)	
pH	6.5	6.1	6.3**(3%↓)	6.0	6.3**(3%↓)	5.9*(2%↓)	6.4	6.0
SG	1037	1051	1044*(1%↑)	1061*(1%↑)	1049*** (1%↑)	1056	1041	1060

Na (mEq)	0.63		0.57		0.45*(29%↓)		0.55	
K (mEq)	1.17	0.61	1.01*(14%↓)	0.65	1.03*(12%↓)	0.74*(37%↓)	1.07	0.74* (37%↓)
Cl (mEq)	0.78		0.70		0.62*(21%↓)		0.66	

*** p<0.05; ** p<0.01' *** p<0.001**

Organ weights:

DOSE (mg/kg/d)	**0**		**5**		**50**		**500**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen (%)	0.27		0.28		0.29		0.30*(11%↑)	

*** p<0.05**

Gross pathology:

DOSE (mg/kg/d)	**0**		**5**		**50**		**500**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Uterus								
Fluid Distension		0/10		1/10		3/10		1/10

Histopathology: Tissues from control and HD groups were processed and examined. microscopically. In addition, all tissues exhibiting gross abnormalities were examined microscopically.

DOSE (mg/kg/d)	**0**		**5**		**50**		**500**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
R. Adrenal cortex Focal cortical hypertrophy	0/10	0/10					1/10(3)	0/10
L. Kidney Medullary tubular dilatation	0/10	0/10					0/10	1/10(2)
Heart, ventricle Focal fibrosis	0/10	0/10					0/10	1/10(2)
R. Kidney Basophilia, cortical tubules	2/10(2)	1/10(2)					2/10(2)	1/10(2)
Liver, sinusoids Foci of leucocytes	0/10	1/10(2)					1/10(1)	3/10(2)
Uterus Dilatation		1/10(2)		1/10(2)		3/10(2)		2/10(2)
Mandibular LN Parafollicular hyperplasia	2/10 1/10(2) 1/10(3)	7/10(2)	0/10	1/10(3)	0/10	0/10	4/10(2)	5/10(2)
Mandibular LN Follicular hyperplasia	1/10(2)	1/10(2)	0/10	0/10	0/10	2/10 1/10(2) 1/10(3)	0/10	2/10(2)
Thymus Hemorrhage	1/10(2)						2/10(2)	
Harderian gland Inflammation	0/10	0/10	0/10	1/10(4)	1/10(2)	0/10	2/10 1/10(1) 1/10(3)	1/10(2)

1 = minimal, 2 = slight, 3 = moderate

Toxicokinetics: TK was not conducted in this study. The rat TK data provided for 10 mg/kg (265 ng.h/ml) and 100 mg/kg (4571 ng.h/ml) in the pharmacokinetics section is not linear. Therefore it was not possible to estimate what systemic exposure would be for these doses. The multiples derived are therefore based on BSA (mg/m^2).

Summary of individual study findings: *Clinical dose = 10 mg/kg = 250 mg/m^2 (based on body surface area of a child).* In a 4-week oral toxicity study, juvenile rats (21 days old) were dosed with SUN 0588 at 5, 50 and 500 mg/kg/d. These doses represent 0.1X, 1X and 12X MRHD respectively, based on BSA (please note that these multiples were derived using the adult rat factor of 6 to convert mg/kg to mg/m^2). There were no treatment related deaths or clinical signs. Slight decreases in erythrocyte parameters (PCV, HB, RBC), clinical chemistry parameters (triglycerides, phospholipids and T. cholesterol) and urinalysis parameters (urine volume, pH, SG, Na, K and Cl) were observed with no clear dose-related effects. The target organs of toxicity include the adrenal cortex (hypertrophy), kidney (tubular dilatation and basophilia), liver (foci of leucocytes), uterus (dilatation), mandibular lymph node (follicular hyperplasia) and thymus (hemorrhage) and harderian gland (inflammation). NOAEL is 500 mg/kg/d (12X MRHD) based on the low incidence and minimal to slight severity of histopathology findings in the 500 mg/kg/d group. Incidence and severity of histopathology findings in controls are similar to those of the 500 mg/kg/d group.

Acute Toxicity Studies (Single Dose) of Tetrahydrobiopterin Metabolites

SPECIES	DOSE (mg/kg)	ADVERSE EFFECTS
Mouse	500, 1000, 2000, 4000 mg/kg of pterin, a principal catabolite of tetrahydrobiop-terin oral (gavage); 3/sex/group	• There were no treatment related clinical signs or effects on body weight following 8 days of observation. • There was no gross pathology abnormality. • LD_{50} > 4000 mg/kg.
Mouse	2000, 4000 mg/kg of pterin, a principal catabolite of tetrahydrobiopterin; oral (gavage); 6/sex/group	• There were no treatment related clinical signs or effects on body weight following 15 days of observation. • There was no gross pathology abnormality. • LD_{50} > 4000 mg/kg.
Mouse	500, 1000, 2000, 4000 in methylcellulose solution; a 2000 mg/kg dihydrobiopter-in solution with HCl; oral (gavage); 3/sex/group. 250, 500, 1000 (M); 125, 250, 500, 1000 (F), IV; 3/sex/group. Acute toxicity study of dihydrobiopterin, a principal metabolite of tetrahydrobiopterin.	• **Oral:** There were no treatment related abnormalities in all mice throughout the observation period (8 days) in terms of general condition and weight. • **IV:** All the male and female mice in the 1000 mg/kg group and 2/3 females in the 500 mg/kg group started gasping right after dosing and died within one minute after dosing. A female that survived in the 500 mg/kg group showed temporary bradypnea right after administration. There was no abnormality observed in males dosed 500 mg/kg or less and females in the 250 mg/kg or less. • There was a mild suppression of weight increase in females dosed ≥ 250 mg/kg. • Necropsy of dead mice in the 500 mg/kg and 1000 mg/kg groups revealed dark reddish or greenish lungs in all cases. • Based on these findings, the sponsor proposed 4000 mg/kg and 2000 mg/kg for future oral acute studies. For the intravenous administration, the doses proposed were 410, 480, 561, 657, 769 and 900 mg/kg for males, and 350, 410, 480, 561, 657 and 769 mg/kg for females.
Mouse	2000, 4000, Oral (gavage), 480, 561, 657, 769, 900, IV 6/sex/group. Acute toxicity study of dihydrobiopterin, a principal metabolite of tetrahydrobiopterin.	• **Oral:** There were no treatment-related clinical signs, deaths or effects on body weight throughout the 15 days observation period. • Accordingly, the LD_{50} > 4000mg/kg. • **IV:** All males and females in the 900 mg/kg group, 2/6 males and 3/6 females in the 769 mg/kg group, and 1/6 males and 2/6 cases in the 657 mg/kg group started gasping right after administration and died within one minute after administration • LD_{50} was 766 mg/kg for males and 730 mg/kg for females. • The cause of the death was assumed to be respiratory disorders because congestion and congestive edema were found in the lungs following necropsy. • Among the survivals, temporary bradypnea or panging respiration was noted in a large majority of mice at ≥ 561 mg/kg right after dosing. There was a repression or a repressive tendency of weight increase in mice dosed ≥ 480 mg/kg from day 2 of observation up to the first half of the observation period. On day 14 of observation there was no difference in weights of the treated mice relative to the control group. • Histopathology examination of the survivals revealed basophilic stained proximal tubules, calcification of tubules in the kidneys, atrophy of seminiferous tubules and hypospermia. Sponsor stated that these histological changes were not observed in a previous study where all male and female mice died at doses ≥ 400 mg/kg when SUN 0588 was intravenously administered in acute toxicity studies. However, dosages in this acute toxicity test were ≥ 480 mg/kg.

Summary: The toxicities of a principal metabolite (dihydrobiopterin) and a principal catabolite (pterin) of sapropterin HCl were evaluated following acute oral studies in mice. The LD_{50} values for the principal metabolite and catabolite were > 4000 mg/kg in both male and female mice following oral dosing. Following a single i.v administration, the LD_{50} value for of the principal metabolite (dihydrobiopterin) was 766 mg/kg in male mice and 730 mg/kg in female mice.

Histopathology Inventory for IND # 69,708

Study	66	67	70	69	71	117	115
Species	Rat	Rat	Rat	Monkey	Monkey	Mouse	Rat
Adrenals	X*	X*	X*	X*	X*	X*	X
Aorta							x
Bone Marrow smear	X	X	X	X	X	X	X
Bone (femur)	X	X	X	X	X	X	X
Brain	X*	X*	X*	X*	X*	X*	X*
Cecum	X*	X	X	X	X	X	X
Cervix							
Colon	X*	X	X	X	X	X	X
Duodenum	X*	X	X	X	X	X	X
Epididymis	X*	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X
Fallopian tube							
Gall bladder							
Gross lesions	X	X	X	X	X	X	X
Harderian gland	X	X	X	X	X	X	X
Heart	X*	X*	X*	X*	X*	X*	X*
Ileum	X*	X	X	X	X	X	X
Injection site	X*	X	X	X	X	X	X
Jejunum		X	X	X	X	X	X
Kidneys	X	X*	X*	X*	X*	X*	X*
Lachrymal gland	X*	X	X	X	X	X	X
Larynx	X	X	X	X	X	X	X
Liver		X*	X*	X*	X*	X*	X*
Lungs	X*	X*	X*	X*	X*	X*	X*
Lymph nodes, cervical	X	X	X	X	X	X	X
Lymph nodes, mandibular		X*	X*	X*	X*	X*	X
Lymph nodes, mesenteric	X	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X	X
Nasal cavity	X	X	X	X	X	X	X
Optic nerves							
Ovaries		X*	X*	X*	X*	X*	X
Pancreas	X*	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X	X
Peripheral nerve	X	X	X	X	X	X	X
Pharynx							
Pituitary		X*	X*	X*	X*	X*	X
Prostate	X*	X*	X*	X*	X*	X*	X
Rectum	X	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X	X
Seminal vesicles	X	X*	X*	X*	X*	X*	X
Skeletal muscle	X	X	X	X	X	X	X
Skin	X	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*	X*
Sternum	X	X	X	X	X	X	X
Stomach	X*	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X*	X*	X*	X*	X*	X*
Thyroid	X*	X*	X*	X*	X*	X*	X
Tongue	X	X	X	X	X	X	X
Trachea	X	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X	X
Uterus	X*	X*	X*	X*	X*	X*	X*
Vagina	X	X	X	X	X	X	X
Zymbal gland							

X, histopathology performed
*. organ weight obtained

Histopathology Inventory for IND # 69.708

Study	76-AT-SR	74-AT-SR
Species	RAT (Juv)	RAT (Juv)
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube	X	X
Gall bladder		
Gross lesions	X	X
Harderian gland	X	X
Heart	X*	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Larynx	X	X
Liver	X*	X*
Lungs	X*	X*
Lymph nodes, cervical		
Lymph nodes, mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity	X	X
Optic nerves	X	X
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve	X	X
Pharynx	X	X
Pituitary	X*	X*
Prostate	X	X
Rectum	X	X
Salivary gland	X*	X*
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X	X
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X	X
Thyroid	X	X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Reverse Mutation Test in Bacteria

Key findings:

- In the absence of S9 mix, SUN0588 tested positive in the TA100 (5000 µg/plate) and on TA98 (625 µg/plate). SUN0588 tested negative on other bacteria strains at all other concentrations in the absence of the S9 mix.
- SUN0588 tested negative in all bacteria strains in the presence of the S9 mix.
- Growth inhibition of TA98 was observed at concentrations of 1250 and 5000 µg/plate in the absence of S9 mix.
- SUN0588 tested positive under the conditions of the study.

Study no: PHN-84-AT-SR.
Study type: Bacterial mutagenesis assay.
Conducting laboratory and location: [redacted]
Date of study initiation: March 20, 1986.
GLP compliance: Yes (Japan).
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8885Y05, [redacted]
Formulation/vehicle: SUN0588 dissolved in a vehicle (0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3 mM L-cystein hydrochloride (pH 7.15).

Methods:
Strains/species/cell line: Salmonella typhimurium strains (TA98, TA100, TA1535, TA1537) and E. coli strain WP2 uvrA.

Dose selection criteria: Based on cytotoxicity (growth inhibition) and precipitation.
Basis of dose selection: The 'limit dose' or highest dose was chosen as the dose that produced no toxicity or precipitation.

Range finding studies: A Range finding study was conducted at doses of 312.5, 625, 1250, 2500 AND 5000 µg/plate with and without metabolic activation. Sponsor stated that since this preliminary test did not reveal any problem of practical concern, the preliminary test was taken to constitute the actual test.
Test agent stability: Test agent in vehicle is stable for 45 hours when kept in the dark at 4^0C.
Metabolic activation system: Commercially prepared S9 mix.

Controls:
Vehicle: 0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3 mM Lcystein hydrochloride (pH 7.15).
Negative controls: 0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3 mM L-cystein hydrochloride (pH 7.15).

Positive controls: were dissolved in DMSO.

STRAINS	TA1535	TA1537	TA98	TA100	WP2uvrA
-S9	ENNG: 5μg/plate	9AA: 80 μg/plate	AF-2: 0.1 μg/plate	AF-2: 0.01 μg/plate	AF-2: 0.01 μg/plate
+S9	2AA: 2 μg/plate	2AA: 2 μg/plate	2AA: 0.5 μg/plate	2AA: 0.5μg/plate	2AA: 80 μg/plate

ENNG = 1-ethyl-3-nitro-1-nitrosoguanidine; 9AA = 9-aminoacridine; 2AA = 2-aminoanthracene; AF-2 = 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide.

Exposure conditions:
Incubation and sampling times: Bacterial suspension in nutrient medium, test agent and S9 mix were mixed in a small tube and preincubated for 20 minutes at 37^0C. The preincubated mixture was then overlaid together with soft agar medium on a plate of Vogel-Bonner E medium. The plates were incubated for 2 days (S. typhimurium) or 3 days (E. coli) at 37^0C followed by counting revertant colonies.
Doses used in definitive study: 312.5, 625, 1250, 2500 AND 5000 μg/plate.

Study design: Preincubation method.

Analysis:
No. of replicates: 2 plates/dose.
Counting method: Counted using a stereomicroscope.
Criteria for positive results: The assay was considered positive if the number of revertant colonies induced by the test agent is at least twofold higher than the solvent negative control with an evident dose-related increase.

Summary of individual study findings:
Study validity: A limit dose of 5000 μg/plate was tested in the range-finding study which was taken to constitute the actual test. No precipitation was seen on the plates at this test concentration. The positive control compounds induced acceptable increases in revertants. The number of revertant colonies for negative controls was within acceptable limits.

Study outcome: Positive. In the absence of the S9 mix, SUN0588 tested positive in the TA 100 (5000 μg/plate) and on TA 98 (625 μg/plate). SUN0588 tested negative on the tester strains at all other concentrations in the absence of S9 mix as well as at all concentrations in the presence of S9 mix. Growth Inhibition of TA98 was observed at concentrations of 1,250 to 5,000 μg/plate in the absence of S9 mix. The positive control compounds induced acceptable increases in revertants. The number of revertant colonies for negative controls was within acceptable limits.

Reverse Mutation Tests with Sapropterin HCl in Bacteria

Test method			Preincubation method					
Metabolic activation	Test substances		Dose (μg/plate)	Revertant colony count/plate				
				TA98	TA100	TA1535	TA1537	WP2uvrA
–	Negative control[b]		0	20	147	15	14	58
	Sapropterin HCl		312.5	30	186	11	11	75
			625	45[a]	183	15	12	62
			1250	22*	197	21	17	63
			2500	29*	253	13	18	65
			5000	13*	304[a]	16	11	76
	Positive control	AF2	0.01	—	391	—	—	118
			0.1	538	—	—	—	—
		ENNG	5	—	—	166	—	—
		9AC	80	—	—	—	609	—
+	Negative control		0	36	174	18	12	67
	Sapropterin HCl		312.5	29	169	18	8	70
			625	30	192	13	14	72
			1250	31	189	14	13	63
			2500	53	223	10	12	73
			5000	45	238	17	19	76
	Positive control	2AA	0.5	388	820	—	—	—
			2	—	—	219	277	—
			80	—	—	—	—	523

*: Growth inhibition —: Not done

a: Increase in revertant colony count

b: 0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3mM L-cysteine hydrochloride (pH 7.15)

AF2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

ENNG: N-ethyl-N'-nitro-N-nitrosoguanidine

9AC: 9-aminoacridine 2AA: 2-aminoanthracene

Sapropterin HCl Lot #: 8885Y05

Study Location: [redacted]

Study Dates: March 20, 1986 – September 25, 1986

Sponsor stated that SUN0588 is stable at acidic pH but is rapidly degraded in alkaline solutions. Prior to the studies, preliminary assessments were undertaken to secure its stability. 0.1M phosphate buffer (pH 7.4) is adjusted to pH 7.15 or 7.16 when supplemented with 6 mM L-ascorbic acid and 3 mM L-cysteine hydrochloride. In the reverse mutation test without S9 Mix, the test article proved to be positive, although weakly, at the highest concentration (5,000 μg/plate) for TA100 and at a concentration (625 μg/plate) just below the growth-inhibitory concentration for TA98. The sponsor indicated that the agar medium used in these assays showed a pale yellow to yellow-orange color, depending on the concentration of SUN0588, on incubation for 2-3 days. No mutagenic effect was observed in the assays conducted in the presence of S9 Mix. According to the sponsor, the color change in the agar medium indicated that some chemical reaction took place in the medium. The remarkably weak mutagenic effect observed with SUN05S8 would suggest that the observed mutagenic effect is possibly attributable to catabolite of the test substance or to some product from actions of this agent as a biologic substance. It is also possible that SUN0588 itself possesses mutagenic activity.

Study title: Chromosome Aberration Test With Chinese Hamster Lung Cells

Key findings:

- SUN0588 tested positive for chromosome aberration in the continuous treatment (-S9) at doses of 0.25mM (48 hr) and 0.5 mM (24 and 48 hr).
- In the short term treatment, SUN0588 tested positive for chromosome aberration at doses of 0.25 and 0.5 mM (-S9) and at doses of 2.0 and 4.0 mM (+S9).
- SUN0588 tested positive for chromosome aberration under the conditions of the study.

Study no: PHN-84-AT-SR.
Study type: In vitro analysis of chromosome aberration (clastogenicity).
Conducting laboratory and location: [redacted]
Date of study initiation: March 31, 1986.
GLP compliance: Yes (Japan).
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8885Y05, [redacted]
Formulation/vehicle: SUN0588 dissolved in a vehicle (0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3 mM L-cystein hydrochloride (pH 7.15).

Methods:
Strains/species/cell line: CHL cell line.
Dose selection criteria: Based on cytotoxicity (inhibition of cell growth).
Basis of dose selection: The highest dose was selected as the dose that produced 50% inhibition of cell growth.
Range finding studies: In the preliminary toxicity assay, the range of doses tested was 0.063-4.0 mM (-S9) and 0.5-8.0 mM (+S9). The test article was soluble in the treatment medium at all dose levels tested. Substantial toxicity (i.e., at least 50% cell growth inhibition relative to the solvent control) was observed at doses $\geq$ 0.25 mM in the pulse treatment without S9 mix. In the pulse treatment with S9 mix, cytotoxicity occurred at doses $\geq$ 4.0 mM. In the continuous treatment, cytotoxicity was observed at doses $\geq$ 0.125 mM and 0.063 mM following 24 and 48 hr of treatment in the absence of S9 mix. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 0.125 to 0.5 mM (24 and 48 hr treatment) in the absence of S9 mix, 0.125-0.5 mM (6 hr treatment, -S9), and 1.0-4.0 mM (6 hr treatment, +S9).
Test agent stability: Test agent in vehicle is stable for 45 hours when kept in the dark at 4^0C.
Metabolic activation system: Commercially prepared S9 mix.
Controls:
Vehicle: 0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3 mM Lcystein hydrochloride (pH 7.15).
Negative controls: 0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3 mM L-cystein hydrochloride (pH 7.15).
Positive controls: Mitomycin C (-S9), Dimethylnitrosamine (+S9).

Exposure conditions:
Incubation and sampling times:
Short term/Pulse Treatment: Cells were treated for 6 hr with and without S9 mix at 37°C, washed once with fresh medium and incubated for further 18 hr in a fresh medium. Colcemid was added for the last 2 hr of incubation.
Continuous treatment: Cells were treated for 24 and 48 hr without S9 mix at 37°C. At 2 hr prior to termination of treatment, cells were washed once with fresh medium and colcemid was added for the last 2 hr of incubation.
Doses used in definitive study: 0.125, 0.25 and 0.5 mM for the short term and continuous treatment (-S9), and 1.0, 2.0 and 4.0 mM for the short term treatment (+S9).

Study design:

Treatment Condition	Treatment Time	Recovery Time	Dose Levels (mM)
-S9	6 hr	18	0.125, 0.25 and 0.5
	24 or 48 hr	0	0.125, 0.25 and 0.5
+S9	6 hr	18	1.0, 2.0 and 4.0

Analysis:
No. of replicates: 2 slides/dose level.
Counting method: By a trained scorer.
Criteria for positive results: For structural aberrations, including gaps and numerical aberrations, a score of $< 5\%$ was recorded as negative, $\geq 5\%$ but $< 10\%$ was recorded as inconclusive/ equivocal and a score $\geq 10\%$ was recorded as positive.

Summary of individual study findings:
Study validity: The test was considered valid if the following criteria were met: the percentage of aberrant cells must not vary greatly between the two culture dishes at each dose level; the percentage of aberrant cells must be less than 5% in the negative-control groups; the percentage of aberrant cells, excluding gaps, must be 10% or higher in the positive-control groups; and there must be no abnormalities found in the testing environment such as the culture conditions.

Study outcome: SUN0588 tested positive for chromosome aberration in the continuous treatment (-S9) at doses of 0.25 mM (48 hr) and 0.5 mM (24 and 48 hr). In the short term treatment, SUN0588 tested positive for chromosome aberration at doses of 0.25 and 0.5 mM (-S9) and at doses of 2.0 and 4.0 mM (+S9). SUN0588 tested positive for chromosome aberration under the conditions of the study.

Effect of Pulse treatment with SUM 0588 on mitotic index and ploidy of CHI cells

Time [a] Schedule (h)	S-9Mix	Compound	Concentration (mM)	Mitotic index [b]	Polyploid [c] (%)
6-18	–	N [d]	–	71	1
		C [e]	–	89	1
		SUM 0588	0.063	82	0
			0.125	77	1
			0.25	51	0
			0.50	25	0
			1.00	3	–
			2.00	7	–
			4.00	11	–
		MMC	3.74×10^{-4}	72	0
	+	N [d]	–	73	0
		C [e]	–	136	0
		SUM 0588	0.50	105	1
			1.00	102	2
			2.00	121	2
			4.00	41	1
			8.00	20	–
		DMN	3.37	115	0

Effect of Continuous treatment with SUM 0588 on mitotic index and ploidy of CHI cells

Time Schedule (h)	Compound	Concentration (mM)	Mitotic index [a]	Polyploid [b] (%)
24	N [c]	–	56	0
	C [d]	–	87	2
	SUM 0588	0.063	73	1
		0.125	50	0
		0.25	25	0
		0.50	12	0
		1.00	5	–
		2.00	0	–
		4.00	–	–
	MMC	7.48×10^{-5}	50	0
48	N [c]	–	73	1
	C [d]	–	86	1
	SUM 0588	0.0078	56	0
		0.016	55	0
		0.031	63	1
		0.063	51	0
		0.125	68	2
		0.250	37	0
		0.500	13	2
	MMC	7.48×10^{-5}	74	2

a: Treating-expression tile; b: Mitoses per 1000 cells; c: Potyploid cells 100 mitoses
d: No treatment; e: Vehicle

Results of Chromosomal Aberration Rests with Sapropterin HCl in Chinese Hamster Lung Cells

Cells used			Chinese hamster lung cells (CHL cells)									
Positive control agent			Mitomycin C (direct test) ..... DMN (test with metabc									
Test groups			Negative control[a]		Positive control		Sapropterin HCl					
Direct test	Concentration (mM)		0		7.48×10^{-5}		0.125		0.25		0.5	
	Exposure (hrs)		24	48	24	48	24	48	24	48	24	48
	Results	No. cells with chromosome aberrations (except gaps)	3	0	16**	32**	2	1	4	17**	17**	23**
		No. cells with chromosome aberrations (including gaps)	4	1	17**	33**	4	2	6	19**	18**	24**
Test with metabolic activation	Metabolic activation		□	+	−	+	−	+	−	+	−	+
	Concentration (mM)		0	0	3.74×10^{-4}	3.37	0.125	1.00	0.25	2.00	0.50	4.00
	Exposure (hrs)		6		6		6		6		6	
	Results	No. cells with chromosome aberrations (except gaps)	0	0	61**	50**	5	0	7*	7*	17**	32**
		No. cells with chromosome aberrations (including gaps)	5	0	63**	50**	5	0	7	7*	17**	33**

*: As against negative control, $p<0.05$ **: As against negative control, $p<0.01$ (χ^2 test)
a: 0.1M phosphate buffer (pH 7.4) containing 6 mM L-ascorbic acid and 3mM L-cysteine hydrochloride (pH 7.15)
Sapropterin HCl Lot #: 8885Y05
Study Location: [redacted]
Study Dates: March 20, 1986 – September 25, 1986

Induction of chromosome aberrations was observed in the presence and absence of S-9 Mix, at test article concentrations ≥ 0.25 mM. Sponsor stated that the vehicle used in the Ames assay was also used in the chromosome aberration test. If added at a concentration of 10% to assay medium, the solvent itself would inhibit the growth of indicator cells whereas reduction of the amount of the solvent added to the medium led to an instantaneous decomposition of the test article because of the alkaline pH of the medium. In view of this, the sponsor conducted the test with the vehicle added at 2.5% to the medium. The cytotoxic effect of the test article increased slightly with increasing concentration of the test article. Sponsor indicated that this may have caused the positive results observed.

Study title: Micronucleus Test in Mice

Key findings:

- The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in each of the groups given SUN0588 was not significantly higher than the value of the negative control.
- SUN0588 tested negative under the conditions of the study.

Study no: PHN-84-AT-SR.

Study type: In vivo assessment of DNA damage (clastogenicity).
Conducting laboratory and location: [redacted]

Date of study initiation: May 13, 1986.
GLP compliance: Yes (Japan).
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 8885Y05, [redacted]
Formulation/vehicle: SUN0588 dissolved in distilled water.

Methods:
Strains/species/cell line: Mouse/Slc:ddY (SPF).
Dose selection criteria: A HD of 2000 mg/kg (2 g/kg) was selected based on previous single-dose oral toxicity study of the drug in male mice.
Basis of dose selection: The highest dose was selected as a dose that produces signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce mortality.
Range finding studies: In a range finding study, doses of 0.5, 1 and 2 g/kg were administered once orally (gavage) and the animals were observed for mortality at 24, 48 and 72 hr post-dose.
Test agent stability: Test agent was stable under the conditions and over the duration of the study.
Metabolic activation system: N/A.

Controls:
Vehicle: Distilled water.
Negative controls: Distilled water.
Positive controls: Mitimycin C-2 mg/kg.

Exposure conditions:
Incubation and sampling times: For each dose level, male mice received single oral dose of SUN0588. At 24, 48 and 72 hr post-dose, the mice were sacrificed and bone marrow was aspirated for slide preparation.
Doses used in definitive study: 0.5, 1 and 2 g/kg.
Study design:

Dose (g/kg)	0	0.5	1	2
24 hr Sacrifice	6	6	6	6
48 hr Sacrifice	6	6	6	6
72 hr Sacrifice	6	6	6	6

Analysis:
No. of replicates: 2 slides/specimen.
Counting method: Microscopic evaluation of bone marrow smear slides by color differentiation of PCEs and NCEs.
Criteria for positive results: Based on detection of a positive response (statistically significant increase in MNPCEs relative to control) for at least one dose level and a statistically significant dose-related response.

Summary of individual study findings:
Study validity: For a valid study, the mean incidence of micronucleated polychromatic erythrocytes (MNPCEs) must not exceed 5/1000 PCEs (0.5%) in the negative (vehicle) control. The incidence of MNPCEs in the positive control group must be statistically significantly increased relative to the negative control.
Study outcome: The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in each of the groups given SUN0588 was not significantly higher than the value of the negative control. SUN0588 tested negative under the conditions of the study.

Micronucleus Test with Sapropterin HCl in Mice

Species, strain, age, sex, body weight			Mouse, Slc:ddy, 9 weeks old, male, 36.7-42.2 g				
No. of animals			66 (6/group)				
Route of administration			p.o. (positive control: i.p.)				
Frequency of dosing			Once				
Slide staining			Wright-Giemsa stain				
Positive control agent			Mitomycin C (MMC)				
Slide preparation periods			24, 48 and 72 hours post-dosing				
Test groups			Negative control[a]	Positive control	Sapropterin HCl		
Dose (mg/kg)			0	2	500	1000	2000
Results	Incidence of micronucleated polychromatic erythrocytes (‰)	24 hrs	3.0±1.9	74.7±15.7**	2.7±2.2	1.7±1.2	1.5±1.4
		48 hrs	—	—	3.2±2.1	2.3±2.1	2.0±1.0
		72 hrs	—	—	1.3±1.4	3.5±1.4	2.7±2.3
	Polychromatic erythrocytes, as percent of normochromatic erythrocytes	24 hrs	1.09±0.31	0.93±0.18	1.33±0.37	1.22±0.31	1.01±0.20
		48 hrs	—	—	1.42±0.51	1.45±0.74	1.54±0.38
		72 hrs	—	—	1.38±0.52	1.30±0.18	1.67±0.39

a) Distilled water **: As against negative control, p<0.01 (Kastenbaum-Bowman test)
Sapropterin HCl Lot #: 8885Y05
Study Location: [redacted]
Study Dates: March 20, 1986 – September 25, 1986

Study title: Micronucleus Test of SUN 0588 Using Mice

Key findings:
- The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in each of the groups given SUN0588 (for 2 or 4 days) was not significantly higher than the value of the negative control.
- SUN0588 tested negative under the conditions of the study.

Study no.: PHN-89-AT-SR
Conducting laboratory and location: Suntory Medicines Ltd., Gunma, Japan,

Date of study initiation: December 13, 1990
GLP compliance: yes. (Japan)
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot # 8889403, [redacted]
Formulation/vehicle: SUN0588 dissolved in distilled water.

Methods:
Strains/species/cell line: Mouse/ [redacted] CD-1 (ICR)

Dose selection criteria: In the preliminary test, doses of 250, 500, 750, 1000 and 1250 mg/kg were administered once orally (gavage) for 4 days and the animals were observed for mortality for 72 hrs. Two of 3 cases died in the 1000 and 1250 mg/kg groups, 3 of 5 cases in the 750 mg/kg group, and 1 of 5 cases in the 500 mg/kg group. The highest dosage was 500 mg/kg in the present test.
Basis of dose selection: The highest dose was selected as a dose that produces signs of toxicity such that higher dose levels would be expected to produce mortality.

Controls:
Vehicle: Distilled water.
Negative controls: Distilled water.
Positive controls: Mitomycin C- 1mg/kg. (i.p.)

Exposure conditions:
Incubation and sampling times: For each dose level, male mice received oral dose of SUN0588 once daily for 2 or 4 days. At 24hr after the end of the final administration, the mice were sacrificed and bone marrow was aspirated for slide preparation.
Doses used in definitive study: 125, 250 or 500 mg/kg.

Study design:

Name of tested Substance	Dosage(mg/kg)	Amount of Liquid administered (mL/kg)	Administration (Period days)	Number of Animals (male)
Distilled water	0	10	2	6
Distilled water	0	10	4	6
SUN 0588	125	10	2	6
SUN 0588	125	10	4	6
SUN 0588	250	10	2	6
SUN 0588	250	10	4	6
SUN 0588	500	10	2	6
SUN 0588	500	10	4	6

MMC	1	10	2	6
MMC	1	10	4	6

Analysis:
No. of replicates: 1 slides/specimen.
Counting method: Microscopic evaluation of bone marrow smear slides by color differentiation of PCEs and NCEs.
Criteria for positive results: when the frequency (%) of polychromatic erythrocytes with micronuclei increased significantly compared with the control group and dosage dependency was clearly demonstrated.

Summary of individual study findings:
Study validity: For a valid study, the mean incidence of micronucleated polychromatic erythrocytes (MNPCEs) must not exceed 5/1000 PCEs (0.5%) in the negative (vehicle) control. The incidence of MNPCEs in the positive control group must be statistically significantly increased relative to the negative control.
Study outcome: The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in each of the groups given SUN0588 was not significantly higher than the value of the negative control. SUN0588 tested negative under the conditions of the study.

Study Design and Results of a Repeated-Dose Micronucleus Test with Sapropterin in Mice

Species/Strain/Age			**Mouse/CD-1: (ICR)/9 weeks old**				
No. of animals			60 (6/group)				
Route of administration/Dose Volume			Oral Gavage, positive control: i.p. at 10 mL/kg				
Frequency of dosing			2 to 4 Days				
Slide staining			Wright-Giemsa stain				
Positive control agent			Mitomycin C (MMC)				
Slide preparation periods			24 hrs post-dosing				
Test groups			Negative control[a]	Positive control	Sapropterin		
Dose (mg/kg)			0	1	125	250	500
Results	Incidence of micronucleated polychromatic erythrocytes (‰)	2 Days	0.15 ± 0.55	2.72±1.05**	0.20±0.21	0.25±0.14	0.23±0.15
		4 Days	0.12±0.12	5.98±1.43**	0.28±0.13	0.16±0.09	0.38±0.26
	Polychromatic erythrocytes, as percent of normochromatic erythrocytes	2 Days	1.13±0.17	0.77±0.10**	0.91±0.36	0.99±0.29	0.90±0.30
		4 Days	0.93±0.45	0.53±0.22	1.03±0.44	0.87±0.19	0.98±0.15

a) Distilled water **: As against negative control, $p<0.01$ (Kastenbaum-Bowman test)
Sapropterin Lot #: 8889403
Study Location: Suntory Ltd. Medicines Center, Gunma, Japan
Study Dates: December. 13, 1990 – March 12, 1991

2.6.6.5 Carcinogenicity

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

P/T REVIEWER(s): John Colerangle (for IND); Fang Cai (for NDA)
DATE: 10/25/2004 for IND

IND/NDA: IND: 69,708. NDA: 22,181
DRUG CODE#: SUN 0588.
CAS#: 69056-38-8.
DIVISION(s): Metabolic and Endocrine Drug Products (for IND); Gastroenterology Products (for NDA)
DRUG NAME(s): Phenoptin™ [Sapropterin hydrochloride, Tetrahydrobiopterin (6R-BH4 or BH4)].
SPONSOR: BioMarin Pharmaceutical Inc., 371 Bel Marin Keys Boulevard, Suite 210, Novato, CA 94949.
LABORATORY: [redacted]
CARCINOGENICITY STUDY REPORT DATE: December 3, 1992.

THERAPEUTIC CATEGORY: Treatment of Phenylketonuria.
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Enzyme Cofactor related to tetrahydrobiopterin.

MUTAGENIC/GENOTOXIC:

Ames Test: Equivocal
Chromosome aberration test: Positive
Mouse micronucleus test: Negative

Review of the Genotoxicity studies revealed an equivocal Ames (no confirmatory study) and a positive chromosome aberration studies. The sponsor stated that sapropterin HCl/SUN0588 is stable at acidic pH but is rapidly degraded in alkaline solutions. Prior to the studies, preliminary assessments were undertaken to determine the stability of the test compound. In the reverse mutation test without S9 Mix, the test article proved to be positive, at the highest concentration (5,000 µg/plate) for TA100 and at a concentration (625 µg/plate) just below the growth-inhibitory concentration for TA98. The sponsor indicated that the agar medium used in these assays showed a pale yellow to yellow-orange color (possibly a change in pH) due a chemical reaction which possibly caused the mutagenic effect observed with SUN05S8. It was suggested that the mutagenic effect is

possibly due to a catabolite of the test substance or to some product from actions of SUN0588. It is also possible that SUN0588 itself possesses mutagenic activity.

Induction of chromosome aberrations was observed in the presence and absence of S-9 Mix, at test article concentrations ≥ 0.25 mM. Test article concentrations ≥ 0.25 mM was associated with cytotoxicity characterized by ≥ 75% decrease in mitotic index. Sponsor stated that the vehicle used in the Ames assay was also used in the chromosome aberration test. If added at a concentration of 10% to assay medium, the solvent itself inhibited the growth of indicator cells whereas reduction of the amount of the solvent added to the medium led to an instantaneous decomposition of the test article because of the alkaline pH of the medium. In view of this, the sponsor conducted the test with the vehicle added at 2.5% to the medium. The cytotoxic effect of the test article increased slightly with increasing concentration of the test article. Sponsor indicated that this may have caused the positive results observed.

RAT CARCINOGENICITY STUDY

RAT STUDY DURATION (weeks): 104 Weeks.
STUDY STARTING DATE: April 5, 1998.
STUDY ENDING DATE: April 3, 1990.
RAT STRAIN: F-344
ROUTE: Oral (gavage).

DOSING COMMENTS: The dose selection study was not reviewed by ECAC. A 4 week oral toxicity study was conducted in F-344 rats to select doses for the carcinogenicity study. In this study, rats were dosed orally, once daily with 25, 80 and 250 mg/kg/d SUN0588. These doses represent 0.4X, 1X and 4X MRHD (10 mg/kg = 370 mg/m^2) based on mg/m^2. There were no treatment-related clinical signs or effects on body weight, food consumption and gross pathology. Slight decrease in liver weight occurred in LD rats and MD females. Relative kidney weight was slightly increased in HD females. Testicular weight was slightly increased in all treated males relative to control. NOAEL could not be established since histopathology was not performed. MTD was not established in this study since the HD used did not induce any toxicity and/or mortality. Based on the findings of this study, the sponsor concluded that 250 mg/kg/d would be a suitable HD for the carcinogenicity study in rats.
Toxicokinetics: AUC for the MRHD of 10 mg/kg is 366 ng.h/ml. TK was not conducted in the rat dose selection study. Neither was TK conducted in any of the rat toxicity studies (2-, 13- and 52-Week). The rat (Sprague-Dawley) TK data provided for 10 mg/kg (265 ng.h/ml) and 100 mg/kg (4571 ng.h/ml) in the pharmacokinetics section is not linear. Therefore, it was not possible to estimate what systemic exposure would be for the doses evaluated in the dose selection study.

Sponsor stated that systemic exposure with the 40 mg/kg/d dose evaluated in the 52-week rat (Wistar rats) study was estimated to be 2000 ng.h/ml (6X the MRHD). It is not clear how this was arrived at since TK was not conducted and the only rat TK data provided in the PK section is not linear. If this is true, then systemic exposure for the 250 mg/kg/d

evaluated in the carcinogenicity study would be 12,500 ng.h/ml (34X the MRHD) which exceeds 25X AUC.

NUMBER OF RATS:
- Control: 120 (60/sex).
- Low Dose (LD): 120 (60/sex).
- Middle Dose (MD): 120 (60/sex).
- High Dose (HD): 120 (60/sex).

RAT DOSE LEVELS* (mg/kg/day):
- Low Dose: 25
- Middle Dose: 80
- High Dose: 250

BASIS FOR DOSES SELECTED: It is not clear how the sponsor selected the doses that were evaluated in the carcinogenicity study. MTD was not established in the dose selection study. Secondly, PK was not conducted to determine AUC ratio.

PRIOR FDA DOSE CONCURRENCE: There is no prior concurrence from the Division or ECAC regarding the doses evaluated in the carcinogenicity study.

RAT CARCINOGENICITY:
In the 104-week carcinogenicity study, groups of male and female rats (60/sex/group) received sapropterin HCl p.o. at dose levels of 25, 80 and 250 mg/kg/day. There was a dose-related increase in incidence of benign adrenal pheochromocytoma in males. The incidence of benign adrenal pheochromocytoma in HD males at the completion of the treatment period was significantly increased relative to the control group. The incidence of this tumor in the HD group (9/60) is almost equal to the highest incidence (8/50) observed in the historical control data provided by the sponsor. The sponsor argued that the lower incidence of adrenal pheocromocytoma in the control group in this study relative to the background data most likely accounts for the significant difference. Treatment with Sapropterin HCl had no effect on the development of tumors. Hence sapropterin HCl did not exhibit any evidence of carcinogenic potential.

RAT TUMOR FINDINGS: A high incidence of benign adrenal phaeochromocytoma was noted in HD males relative to the control group but comparable to the historical control incidence. This finding was considered to be due to the unusually low control incidence and was not attributed to treatment.

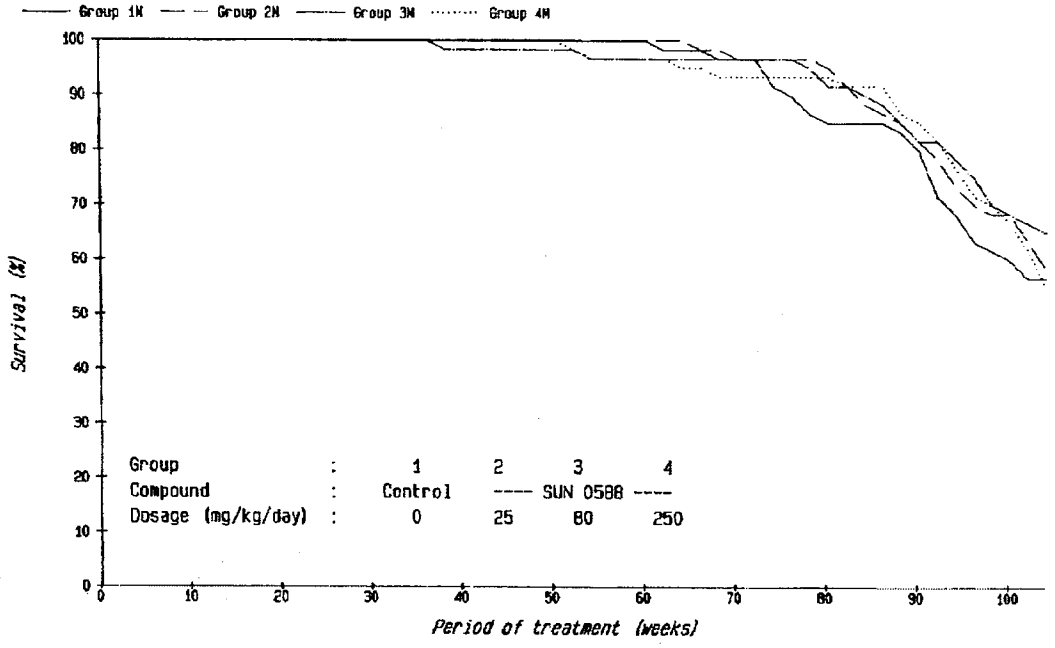
104-Week Carcinogenicity Study of Sapropterin HCl in Rats

SEX	MALES				FEMALES			
Dose (mg/kg/d)	0	25	80	250	0	25	80	250
# of animals treated	60	60	60	60	60	60	60	60
# of Deaths	27	25	23	27	15	15	13	14
Body Weight (g)	354	347	346	332* (6%↓)	208	213	210	182**(13%↓)

Necropsy	-	-	-	Granular kidney surface	-	-	-	Granular kidney surface
Histopathology	-	-	-	Dilated renal papillary ducts	-	-	-	Dilated renal papillary ducts

- No significant findings; * p < 0.05; ** p < 0.001

Survival curves for Males



Tumor distribution among animals killed or dying during the treatment period

SEX	MALES				FEMALES			
Dose (mg/kg/d)	**0**	**25**	**80**	**250**	**0**	**25**	**80**	**250**
# of animals examined	**27**	**25**	**23**	**27**	**15**	**15**	**13**	**14**
Total # of Primary Tumors	**34**	**38**	**36**	**46**	**18**	**21**	**10**	**19**
Mean # of primary tumors/animal	**1.26**	**1.52**	**1.57**	**1.70***	**1.20**	**1.40**	**0.77**	**1.36**
Total # of Benign Tumors	**14**	**13**	**16**	**24**	**10**	**11**	**4**	**11**
Mean # of benign tumors/animal	**0.52**	**0.92***	**0.70**	**0.89**	**0.67**	**0.73**	**0.31**	**0.71**
Total # of Malignant Tumors	**20**	**15**	**20**	**22**	**8**	**10**	**6**	**8**
Mean # of malignant tumors/animal	**0.74**	**0.60**	**0.87**	**0.81**	**0.53**	**0.67**	**0.46**	**0.57**

*** p < 0.05**

Tumor distribution among animals killed after 104 weeks of treatment

SEX	MALES				FEMALES			
Dose (mg/kg/d)	**0**	**25**	**80**	**250**	**0**	**25**	**80**	**250**
# of animals examined	**33**	**-**	**-**	**33**	**45**	**-**	**-**	**46**
Total # of Primary Tumors	**54**	**38a**	**49a**	**45**	**34**	**24a**	**30a**	**33**

Mean # of primary tumors/animal	1.64	-	-	1.36	0.76	-	-	0.72
Total # of Benign Tumors	38	24a	25a	32	23	19a	26a	30
Mean # of benign tumors/animal	1.15	-	-	0.97	0.51	-	-	0.65
Total # of Malignant Tumors	16	14a	24a	13	11	5a	4a	3
Mean # of malignant tumors/animal	0.48	-	-	0.39	0.24	-	-	0.07*

* $p < 0.05$; a = macroscopic abnormalities only examined

Tumor distribution among all animals

SEX	MALES				FEMALES			
Dose (mg/kg/d)	0	25	80	250	0	25	80	250
# of animals examined	60	-	-	60	60	-	-	60
Total # of Primary Tumors	88	76a	85a	91	52	45a	40a	52
Mean # of primary tumors/animal	1.47	-	-	1.52	0.87	-	-	0.87
Total # of Benign Tumors	52	47a	41a	56	33	30a	30a	41
Mean # of benign tumors/animal	0.87	-	-	0.93	0.55	-	-	0.68
Total # of Malignant Tumors	36	29a	44a	35	19	15a	10a	11
Mean # of malignant tumors/animal	0.60	-	-	0.58	0.32	-	-	0.18

a = macroscopic abnormalities only examined

Neoplastic Findings in all Rats (Early decedents and Survivors)

Sapropterin HCl Dose (mg/kg)		Control group[a]		25		80		250	
		M	F	M	F	M	F	M	F
Adrenal cortex	Adenoma	3/60	1/60	0/26	0/17	0/26	0/13	0/60	0/60
Adrenal medulla	Pheochromocytoma (malignant)	1/60	2/60	1/26	1/17	1/26	0/13	1/60	0/60
	Pheochromocytoma (benign)	1/60	1/60	0/26	0/17	4/26	0/13	9/60*	0/60
	Ganglioneuroma	0/60	0/60	1/26	0/17	0/26	0/13	2/60	0/60
Brain	Astrocytoma	1/60	1/60	1/28	0/17	0/23	1/16	0/60	0/60
	Oligodendroglioma	0/60	0/60	0/28	0/17	0/23	1/16	0/60	0/60
Duodenum	Carcinoma	0/60	0/60	0/25	0/15	0/23	0/14	0/60	1/60
Kidney	Renal carcinoma	2/60	0/60	1/32	0/17	0/27	0/15	0/60	1/60
Liver	Hepatocellular carcinoma	0/60	0/60	1/43	0/21	1/39	0/18	1/60	0/60
	Hepatocellular adenoma	0/60	1/60	0/43	0/21	0/39	0/18	1/60	0/60
Lung	Pulmonary adenoma	0/60	060	1/27	0/21	0/29	0/17	0/60	0/59
	Pulmonary carcinoma	0/60	0/60	0/27	0/21	0/29	0/17	1/60	0/59
Mammary gland	Fibroepithelioma	0/60	0/60	0/25	0/15	0/23	0/13	0/60	1/60
Pancreas	Islet carcinoma	1/60	0/60	0/25	0/15	1/23	0/13	1/59	0/59
	Islet adenoma	0/60	0/60	1/25	0/15	0/23	0/13	0/59	1/59
Parathyroid	Adenoma	0/53	0/56	0/24	0/13	0/22	0/11	2/54	1/54
Pituitary gland	Adenoma	17/60	22/60	19/35	14/29	13/32	17/30	15/58	20/59
Prostate	Adenocarcinoma	0/60	—	0/39	—	0/37	—	1/60	—
Spinal cord	Glioma	1/60	0/60	0/25	0/15	0/23	0/13	0/60	0/60
Spleen	Hemangioma	1/60	0/60	0/43	0/17	0/45	1/16	2/60	0/59
	Hemangiosarcoma	0/60	0/60	1/43	0/17	1/45	0/16	0/60	0/59
Testis	Interstitial cell tumor	54/60	—	54/59	—	50/57	—	49/60	—
Thyroid	Parafollicular cell adenoma	5/60	2/60	2/26	1/15	1/25	0/12	6/60	3/59
	Parafollicular cell carcinoma	2/60	2/60	2/26	0/15	4/25	0/12	3/60	1/59
	Follicular cell adenoma	1/60	0/60	0/26	0/15	0/25	0/12	1/60	0/59
	Follicular cell carcinoma	0/60	1/60	0/26	1/15	0/25	0/12	0/60	0/59
Tongue	Squamous cell carcinoma	0/60	1/60	0/25	0/15	0/23	0/12	0/60	0/60
Uterus	Leiomyosarcoma	—	0/60	—	0/28	—	1/30	—	1/60
	Endometrial sarcoma	—	1/60	—	1/28	—	2/30	—	0/60
	Adenocarcinoma	—	3/60	—	0/28	—	1/30	—	1/60
	Adenoma	—	0/60	—	0/28	—	1/30	—	0/60
	Leiomyoma	—	0/60	—	0/28	—	1/30	—	0/60
Vagina	Squamous cell carcinoma	—	1/60	—	0/16	—	0/13	—	0/60
Abdomen	Histiocytic sarcoma	0/1	0/2	0/1	0/0	0/1	0/0	1/1	0/0
	Sarcoma	0/1	0/2	1/1	0/0	0/1	0/0	0/1	0/0
Oral cavity	Squamous cell carcinoma	0/0	0/0	0/1	0/0	0/0	0/0	0/0	1/1
Clitoral gland	Adenoma	—	0/0	—	0/0	—	1/1	—	0/0
Diaphragm	Mesothelioma	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0
Foot	Papilloma	½	0/0	0/1	0/0	1/1	0/0	0/0	0/1
Hemopoietic system	Monocytic leukemia	23/60	7/60	17/60	9/60	28/60	2/60	21/60	4/60
	Malignant lymphoma	0/60	0/60	1/60	1/60	1/60	0/60	0/60	1/60
Head	Carcinoma	0/0	0/0	1/1	0/0	1/1	1/1	0/1	0/0
Mammary gland	Fibroepithelioma	¼	5/9	2/2	12/13	5/6	8/10	3/6	7/8
	Adenocarcinoma	0/4	0/9	0/2	1/13	0/6	1/10	0/6	0/8
Mesentery	Mesothelioma	1/1	0/0	0/0	0/1	3/4	0/1	1/2	0/0
Preputial gland	Adenoma	0/0	—	½	—	0/1	—	2/2	—
Skin, others	Fibrosarcoma	3/36	0/14	0/34	0/13	1/32	0/14	1/28	0/17
	Carcinoma	1/36	0/14	0/34	0/13	0/32	0/14	1/28	0/17
	Fibroma	11/36	0/14	11/34	0/13	5/32	1/14	6/28	2/17
	Basal cell tumor	2/36	1/14	2/34	0/13	3/32	0/14	1/28	2/17
	Keratoacanthoma	5/36	0/14	7/34	0/13	7/32	0/14	7/28	1/17
	Sarcoma	0/36	0/14	1/34	0/13	1/32	0/14	0/28	0/17
	Papilloma	2/36	0/14	1/34	1/13	1/32	0/14	1/28	1/17
	Lipoma	1/36	0/14	0/34	0/13	0/32	0/14	0/28	0/17
Tail	Keratoacanthoma	1/5	0/0	0/2	0/1	0/1	0/2	0/3	1/1
	Sarcoma	0/5	0/0	0/2	1/1	0/1	0/2	0/3	0/1

*** $p < 0.05$; a = distilled water; # of rate with tumors/# of rate examined**

Comparison of the incidence of adrenal phaeochromocytoma observed on this study with historical control data in males

Killed after 104 weeks of treatment

	This study		Historical Control Study						
Group	1M	4M	FSR 035	FSR 036	FSR 038	FSR 039	FSR 042	FSR 045	FSR 046
Commenced	-- 1988 --		1986	1986	1987	1987	1987	1987	1988
No. of animals examined	33	33	19	27	30	34	23	28	31
Phaeochromocytoma	1	7	1	5	2	2	3	2	1

All animals

	This study		Historical Control Study						
Group	1M	4M	FSR 035	FSR 036	FSR 038	FSR 039	FSR 042	FSR 045	FSR 046
Commenced	-- 1988 --		1986	1986	1987	1987	1987	1987	1988
No. of animals examined	60	60	50	50	60	55	49	50	50
Phaeochromocytoma	1	9	2	8	2	2	4	2	2

Comparison of the incidence of pituitary adenoma observed on this study with historical control data

Males

	This study		Historical Control Study						
Group	1M	4M	FSR 035	FSR 036	FSR 038	FSR 039	FSR 042	FSR 045	FSR 046
Commenced	-- 1988 --		1986	1986	1987	1987	1987	1987	1988
No. of animals examined	60	58	50	48	58	53	49	49	50
Pituitary adenoma	17	15	20	18	12	14	17	9	8

Females

	This study		Historical Control Study						
Group	1F	4F	FSR 035	FSR 036	FSR 038	FSR 039	FSR 042	FSR 045	FSR 046
Commenced	-- 1988 --		1986	1986	1987	1987	1987	1987	1988
No. of animals examined	60	59	48	48	57	54	47	50	50
Pituitary adenoma	22	20	11	21	23	17	29	26	14

Summary of Non-Neoplastic Lesions in All Rats

SEX	MALES				FEMALES			
Dose (mg/kg/d)	0	25	80	250	0	25	80	250
# of animals treated	60	60	60	60	60	60	60	60
Kidney: Progressive nephropathy	58	27	24	56	31	3	4	38
Kidney: Papilla, Distended tubules	0	1	0	4	0	0	0	7*
Thyroid: Parafollicular cell hyperplasia	3	1	4	4	3	1	1	7
Uterus: Hyperplasia, endometrial stroma	-	-	-	-	4	3	7	7
Uterus: Hyperplasia, endometrial epithelium	-	-	-	-	0	3	3	3

* $p < 0.05$

Distended renal papillary tubules were observed in 4/60 and 7/60 HD males and females respectively and in 1/60 LD male. None of the control animals was affected. The incidence was statistically significant in HD females. This finding is treatment-related. While the incidence of progressive (senile) nephropathy was similar between HD males and control males, the severity of this lesion was greatest in HD males. In HD females, the incidence and severity of progressive (senile) nephropathy were higher than in the controls. This renal pathology seems to be age-related and may not represent a toxic effect.

Incidence and severity of progressive (senile) nephropathy

Animals killed or dying during the treatment period

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Number examined	27	25	23	27	15	15	13	14
Minimal	1	0	1	2	0	1	1	0
Slight	15	8	10	4	7	1	0	4
Moderate	4	4	6	5	3	0	1	1
Marked	5	8	3	12	0	0	0	2
Total	25	20	20	23	10	2	2	7

Animals killed after 104 weeks of treatment

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Number examined	33	7	4	33	45	2	2	46
Minimal	0	0	1	0	8	1	0	5
Slight	14	0	2	4	8	0	0	14
Moderate	6	1	0	9	4	0	0	10
Marked	13	6	1	20	1	0	2	2
Total	33	7	4	33	21	1	2	31

All animals

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Number examined	60	32	27	60	60	17	15	60
Minimal	1	0	2	2	8	2	1	5
Slight	29	8	12	8	15	1	0	18
Moderate	10	5	6	14	7	0	1	11
Marked	18	14	4	32	1	0	2	4
Total	58	27	24	56	31	3	4	38

RAT STUDY COMMENTS:

MTD was not established in the dose selection study. Therefore, it is not clear if the HD evaluated in the carcinogenicity study is appropriate or not. Survival was acceptable with at least 55% of the males and 77% of the females in each group surviving for 104 weeks. An overall low decrease in body weight was observed in HD animals (6% for males; 13 % for females). The decreased body weight was not associated with a concomitant decrease in food consumption and is therefore considered to provide evidence of a toxic effect.

The incidence of benign adrenal pheochromocytoma observed in HD males was higher than in control males, but within the range of historical control data. This was not considered to represent evidence of an oncogenic potential because it was observed only in males and the incidence of malignant adrenal pheochromocytoma was unaffected. The sponsor stated that benign adrenal pheochromocytoma is a common tumor in this strain of rat. Studies of the NTP historical database for 2 year old F-344 rats report the incidence of pheochromocytoma to be 17% and 15% for males and females respectively. Malignant pheochromocytoma is 1% in males and 0.5% in females (Toxicologic Pathology Textbook).

QUESTIONS FOR ECAC:

1. Does ECAC consider the doses evaluated in male and female rats to be adequate?
2. Does ECAC agree that the benign adrenal pheochromocytoma observed in treated males was within the range of historical control data and thus does not represent evidence of an oncogenic potential?

Executive CAC Recommendations and Conclusions:

- There was no prior concurrence on selected doses, but the study was determined to be adequate.
- The Committee concludes that increased incidence of benign adrenal pheochromocytomas was drug related in males and noted that the incidence in the concurrent control was below the lowest incidence in the historical control range and that the high dose group was at the upper end of the reported historical control range.
- The Committee noted that the histopathology is incomplete because adrenal glands in more than half of the rats in the low and mid dose groups were not examined. When there is a finding, all dose groups should be examined.

APPENDIX

Group distribution, multiplicity and mean time of onset of palpable swellings recorded in vivo or at post mortem examination

Group	:	1	2	3	4
Compound	:	Control	---- SUN 0588 ----		
Dosage (mg/kg/day)	:	0	25	80	250

Group / sex	Multiplicity[ø] 0	1	2	3	4 or more	Total number of swellings[+]	Mean number of swellings per animal	Mean time of onset* (weeks)
1M	16	27	13	1	3	68	1.1	85
2M	23	23	13	1	0	52	0.9	85
3M	21	24	11	2	2	60	1.0	86
4M	31	17	7	3	2	48	0.8	85
1F	44	14	2	0	0	18	0.3	90
2F	45	11	4	0	0	19	0.3	86
3F	42	16	2	0	0	20	0.3	88
4F	41	15	4	0	0	23	0.4	85

\+ Total includes swellings which regressed *in vivo* or were not positively identified at macroscopic *post mortem* examination
ø Expressed as number of animals bearing indicated number of swellings
* To onset of first recorded swelling for each animal