CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-446

Pharmacology Review(s)

MEMORANDUM
June 24, 2004

TO: File
FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-446

I have read the primary and secondary pharmacology/toxicology reviews of the marketing application for Lyrica (pregabalin) capsules. Specifically, Dr. Jerry Cott wrote the primary review, and consult reviews were also written by Drs. Edward Fisher, Lois Freed, and Terry Peters. Dr. Daniel Mellon, the pharmacology/toxicology supervisor in the Division of Anesthetic, Critical Care, and Addiction Drug Products, wrote an extensive secondary review in order to consolidate the conclusions of the primary review team. Drs. Mellon and Cott recommended that this application should not be approved based on findings in nonclinical studies. Specifically, this recommendation was made based on uncharacterized risk to diabetic patients, specifically related to findings of dermatopathy in animal toxicology studies. Although not cited as a reason for recommending non-approval, Dr. Mellon also noted the occurrence of hemangiosarcomas in life-time bioassays conducted in mice (two strains, both sexes), and that a no effect level for this finding was not established (there were statistically significant incidences of this tumor at systemic exposures equivalent to what would be expected in humans at the recommended therapeutic dose).

Having read the reviews and considered the recommendation for non-approval, I do not concur. The dermatopathy findings are certainly of concern, especially given the indication sought by the sponsor (pain associated with diabetic peripheral neuropathy, which occurs in a patient population at increased risk for developing serious skin lesions). There are two factors that should be taken into consideration, neither of which should be addressed in the evaluation of nonclinical studies: (1) the apparent lack of an increased incidence of similar dermatopathy in clinical trials, and (2) the compelling need for the indication. Although the hemangiosarcoma findings are also of concern, the potential benefit of this drug outweigh the risk considerations. I therefore recommend that this application be approved.

I concur with the product label as amended by Dr. Mellon. In addition, I concur with the Phase 4 recommendations by Drs. Mellon and Cott. I also suggest an additional Phase 4 commitment:

The Sponsor should conduct a study (or studies) to assess the immunotoxic potential of pregabalin. Specifically, pulmonary effects were observed in nonclinical studies (lung macrophage accumulation) that could be taken to indicate that pregabalin has unintended immunosuppressive effects.

Kenneth L. Hastings, Dr.P.H.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluation II

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-446**
SERIAL NUMBER:	**N 000**
DATE RECEIVED BY CENTER:	**10/30/2003**
PRODUCT:	**Lyrica® (Pregabalin)**
INTENDED CLINICAL POPULATION:	**Pain associated with diabetic peripheral neuropathy**
SPONSOR:	**Pfizer Global Research & Development**
DOCUMENTS REVIEWED:	**Original Electronic NDA Submission N 000** **Amendments: 1/30/2004 BZ** **2/27/2004 BP** **3/30/2004 BZ** **5/4/2004 BP**
REVIEW DIVISION:	**Division of Anesthetic, Critical Care & Addiction Drug Products (HFD-170)**
PHARM/TOX REVIEWERS:	**Jerry Cott, Ph.D. (HFD-170)** **Edward Fisher, Ph.D. (HFD-120)**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D. (HFD-170)**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Lisa Malandro**

Date of review submission to Division File System (DFS): June 3, 2004

EXECUTIVE SUMMARY

The nonclinical pharmacology and toxicology studies submitted for NDA 21-446 were reviewed by a total of three primary reviewers. The Primary reviewer of this NDA for the Division of Anesthetic, Critical Care and Addiction Drug Products was Jerry Cott, Ph.D. Dr. Cott reviewed the Pharmacology and Toxicology studies pertaining to the diabetic peripheral neuropathy indication, including the acute and repeat dose toxicology studies and the genetic toxicology studies. Edward Fisher, Ph.D., was the primary reviewer for the Division of Neuropharmacological Drug Products. Dr. Fisher reviewed the reproductive toxicology studies including the juvenile toxicology studies conducted for pregabalin. In addition, Dr. Fisher reviewed the first set of carcinogenicity studies submitted by the Sponsor. Terry Peters, D.V.M., was the primary reviewer for the second series of carcinogenicity assessments as well as the mechanistic studies submitted by the Sponsor to characterize the hemangiosarcomas in mice. Dr. Peters (Division of Anti-Infective Drug Products) was specifically consulted on this NDA due to her expertise in veterinary pathology. This document serves as the secondary review for NDA 21-446, and therefore specifically examines the data in light of the indication of pain associated with diabetic peripheral neuropathy. Overall, Dr. Cott's review indicates that he does not feel that the NDA is approvable for this indication. Dr. Fisher's review specifically addresses his recommendations regarding the carcinogenicity concerns and the effects of pregabalin on reproductive and developmental toxicology. Dr. Fisher indicated that although the mechanism for hemangiosarcoma formation is plausible, the present evidence is inadequate to exclude carcinogenic risk associated with pregabalin in humans. He further notes that this obstacle to approval (hemangiosarcomas) will ultimately have to be weighed against the evidence of benefit of the drug for the indications being considered. Dr. Peters' review notes that the Sponsor's "conclusion that 'a clear association between altered respiration, acid-base imbalance, increased platelet activation, bone marrow and splenic megakaryopoiesis, circulating VEGF and PDGF, endothelial cell proliferation and the incidence of hemangiosarcoma was demonstrated in mice at carcinogenic doses' is not clear from the evidence presented." Dr. Peters suggested that the "incidence of hemangiomas and hemangiosarcomas should be addressed in the label."

I. Recommendations

A. Recommendation on approvability:

From a pure pharmacology toxicology perspective, NDA 21-446 is deemed to be **not approvable** due to uncharacterized risk associated with the exposure of diabetic patients to long-term treatment with pregabalin. I acknowledge that some of the concerns raised by the non-clinical studies (i.e., dermatopathy) have not been detected in the clinical trials to date, and that the patient database encompasses over 1800 individuals. Although data from human studies provides some degree of comfort, they do not completely eliminate the concerns that have

been raised by the nonclinical data. I concur with the reviews put forth by Drs. Jerry Cott, Ed Fisher, Lois Freed and Terry Peters.

B. Recommendation for nonclinical studies:

a) The Sponsor should characterize histologically the skin lesions noted following pregabalin treatment of rats and monkeys to provide the ability to distinguish between dermal lesions that are likely to occur in the diabetic patient population over time from the dermal lesions that are produced by pregabalin administration.

b) Conduct a drug interaction study in mice to characterize the potential interaction PPARγ agonists with pregabalin. End points should include an evaluation of the incidence of hemangiosarcomas and proposed biomarkers proposed by the Sponsor. This study should be completed with B6C3F1 mice and a diabetic rodent model.

c) Conduct a 28-day repeat-dose toxicology study in a diabetic rodent model to characterize the incidence of pregabalin-induced dermal lesions. Include a recovery group to characterize the time required for recovery and a non-diabetic control group should be included for comparison.

d) Conduct a 28-day wound healing study to characterize the effect of pregabalin in both normal and diabetic animal response.

C. Recommendations on labeling:

The following labeling was prepared by Dr. Ed Fisher (HFD-120) who reviewed the reproductive toxicology studies for the pregabalin NDAs. Dr. Fisher also reviewed the first set of carcinogenicity studies. Dr. Terry Peters reviewed the second set of carcinogenicity studies and evaluated the additional toxicology studies the Sponsor conducted in their attempt to demonstrate species specificity for the hemangiosarcomas in mice. Dr. Fisher took Dr. Peter's Review into consideration in the preparation of the carcinogenicity, mutagenicity and impairment of fertility sections of the label. In addition, Dr. Cott has proposed an Animal Toxicology section of the label to describe the dermatopathy findings.

2 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

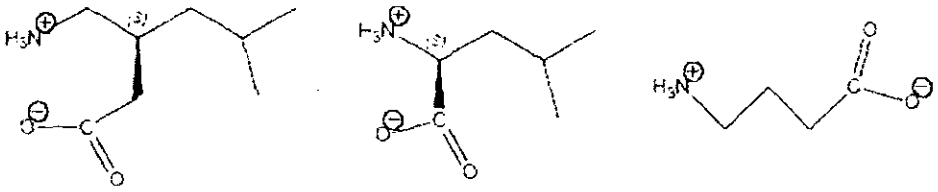
_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

Pharmacology: Pregabalin is structurally related to the naturally occurring amino acids L-leucine and γ-aminobutyric acid (GABA), as depicted in the Sponsor's diagram below:



Pregabalin L-Leucine GABA

However, pregabalin does not bind to GABA receptors. Pregabalin, like gabapentin binds to the α_2-δ protein, an auxiliary protein associated with high voltage-gated calcium channels. Evidence suggests that upon binding, pregabalin reduces but does not completely block the calcium currents.

The pharmacodynamic effects of pregabalin were assessed in models of analgesia and seizures. These studies provided evidence that pregabalin may be efficacious in the treatment of several pain states, including neuropathic pain syndromes. In straptozocin-induced diabetic neuropathic pain model, pregabalin (10 and 30 mg/kg orally) reduced dynamic allodynia. Further positive results were obtained with models of pain as a result of nerve ligation or chemotherapy as well as for the treatment of musculoskeletal pain and visceral pain.

Pregabalin was also demonstrated to prevent maximal electroshock-induced tonic seizures, pentylenetetrazole-induced threshold clonic seizures, and behavioral seizures in kindled rats. Additional studies suggested that pregabalin would have utility as an anxiolytic.

Pharmacokinetics: Pregabalin has high oral bioavailability across species (94% in mice after 50 mg/kg, 83% in rats after 50 mg/kg, 93% in monkey after 10 mg/kg but decreases with increasing dose due to saturable absorption and ≥ 90% in humans after a single oral dose of 1 to 300 mg.

Tissue distribution studies provide some indication of potential target organs of toxicity. [^{14}C]Pregabalin distribution was determined in mice, rats and monkeys. Pregabalin is widely distributed in the body following administration and crosses the blood brain barrier. [^{14}C] levels in the male B6C3F1 mouse and pregnant female rat after 10 mg/kg orally were 7-fold higher in the **pancreas** 1 hour after dosing than in the blood. Likewise, labeled materials were over 5 times higher in rat pancreas compared to bloodstream. Radiolabel did not accumulate in the

pancreas of the monkey. The observation that the mouse and rat pancreas reached significantly higher concentrations of label suggest that these species may have a greater susceptibility to pancreatic toxicity. As gabapentin (Neurontin) produced pancreatic tumors in the rat, the pancreas should be considered a potential target organ of toxicity in susceptible individuals should drug accumulation occur.

In the monkey model, pregabalin levels in the kidney and epididymis were ~2-fold greater than other tissues. In the rat, pregabalin produced testicular toxicity likely contributing to fetotoxicity in the pups. Tissue distribution studies in the pregnant rat model indicate that the developing fetus is exposed to pregabalin via the blood stream in addition to via milk. These studies also indicated that the lens in the eyes of the pups demonstrated higher and longer exposures to the labeled material than other tissues. Pregabalin/label did not accumulate in the lens of the mouse, dog or non-pregnant rat. Regardless, the finding of accumulation of label in the post-natal rat lens is of concern in light of the clear clinical signal for visual disturbances in a subset of patients taking pregabalin.

Possibly placenta

Pregabalin does not bind appreciably to mouse, rat, monkey or human plasma proteins. Pregabalin undergoes minimal metabolism in the mouse, rat or monkey and unchanged parent compound represents ≥ 90% of labeled material was detected in the urine, which is consistent with clinical observations in humans. The N-methyl metabolite constitutes only 2-3% of urinary radioactivity in mouse and rat. In contrast, the N-methyl metabolite represents approximately 45% of orally administered pregabalin in the dog. The N-methyl metabolite was not detected in the monkey. Greater than or equal to 80% of the [^{14}C] pregabalin dose was eliminated in the urine of mouse, rat and dog within the first 24 hours. In the monkey, 71 - 75% was excreted in the first 24 hours.

Safety pharmacology: Safety pharmacology studies characterized the effects of pregabalin on the nervous, cardiovascular, respiratory, renal and gastrointestinal systems. In addition, the non-clinical abuse liability studies ~~suggested at best~~ demonstrate a weak signal for increased abuse liability.

Repeat-dose toxicology: Toxicology studies were completed in the rat (4, 13 and 26/52-weeks) and the monkey (4, 13 and 65-69 weeks). The monkey was chosen as the non-rodent species due to a more similar metabolic profile to humans than the dog model.

Rats: In the 52-week rat study (with 26-week interim sacrifice), animals were treated with pregabalin via the diet at doses of 0, 50, 250 and 500 mg/kg/day. The summary of the 26-week sacrifice and the 52-week sacrifice are reproduced below: Overall, the Sponsor concluded that no clinically significant effects were detected following a dose of 50 mg/kg/day for the rat. In the 13-week study, the $AUC_{(0\text{-}24)}$ at this dose was 188 µg•hr/ml (combined males and females).

Results of 26-week interim sacrifice:

Important Findings		UC		50 mg/kg		250 mg/kg		500 mg/kg	
		M	F	M	F	M	F	M	F
N		25	25	25	25	25	25	25	25
Death or Moribund		--	--	--	--	--	1	--	--
Pyelonephritis							Week 44		
Clinical Signs									
Urine staining		--	1	1	3	1	5	1	15
Tail - Dermatopathy		1	1	--	--	2	1	6	8
Body Weight Gain[c] (g)	D	503		--	--	--	--	24%**	--
Food Consumption[c] (g)	D								
Weeks 5-52 Sporadic (Range)		185-199		--	--	1%-16%**	--	--	--
Weeks 5-52 Consistent (Range)			136-155	--	--	--	--	2%-16%**	--
Weeks 12-52 Sporadic (Range)				--	--	--	--	--	8%-24%**
N Week 26		10	10	10	10	10	10	10	10
Hematology[d]									
Red Blood Cells (10^{12}/L)	I	8.44	8.17	8.95	--	9.12	--	9.39	9.10
Percent of Control				6%**	--	8%**	--	11%**	11%**
Mean Corpuscular Volume (fL)	D	56.0	57.3	--	--	53.3	--	53.8	53.6
Percent of Control				--	--	5%**	--	4%**	6%**
Platelet Count (10^9/L)	D	1037	999	--	--	844	844	742	811
Percent of Control				--	--	19%**	16%**	28%**	19%**
N Week 26		5	5	5	5	5	5	5	5
Bone Marrow[d]									
Total Nucleated Cells (10^9/mL)		--	1.5	--	--	--	1.06	--	1.06
Percent of Control	D	--		--	--	--	29%**	--	29%**
N Week 26		10	10	10	10	10	10	10	10
Absolute Organ Weights[c]									
Salivary Gland (g)	D	0.96	0.63	--	--	--	15%**	33%**	19%**
Epididymides	D	1.71	--	--		--		19%**	
Histopathology									
Bone Marrow - Hypocellular		--	--	--	--	3	2	3	4
Urinary Bladder - Edema		--	--	--	--	--	--	3	1
- Hemorrhage		--	--	--	--	--	--	2	--
Salivary Gland – Secretory Content Decreased in Acini		--	--	--	--	2	2	8	10
N Week 26		10	10	0	0	10	10	10	10
Histopathology									
Lung – Alveolar Macrophage Accumulation		2	1	--	--	2	--	4	2

** = p<0.01(linear trend within ANOVA);
N = Number of animals; -- = No noteworthy findings; D = Decreased; I = Increased; ns = Not statistically significant.
[c] Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.
[d] Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.

Results of 52-week sacrifice:

Important Findings		UC		50 mg/kg		250 mg/kg		500 mg/kg	
		M	F	M	F	M	F	M	F
N Week 52		15	15	15	15	15	15	15	15
Hematology[d]									
Red Blood Cells (10^{12}/L)		8.14	8.13	8.88	--	9.00	8.60	8.88	8.65
Red Blood Cells (%)	I			9%**	--	11%**	6%**	9%**	6%**
Mean Corpuscular Volume (fL)		56.7	57.6	54.2	--	52.7	55.1	52.0	54.5
Mean Corpuscular Volume (%)	D			4%**	--	7%**	4%**	8%**	5%**
Platelet Count (10^9/L)		1132	876	931	--	752	709	724	749
Platelet Count (%)	D			18%**	--	34%**	19%**	36%**	14%**
Mean Platelet Volume (fL)		5.35		--	--	5.74	--	5.92	--
Mean Platelet Volume (%)	I			--	--	7%**	--	11%**	--
N Week 52		5	5	5	5	5	5	5	5
Bone Marrow[d]									
Total Nucleated Cells (10^6/femur)		75.7	--	--	--	--	--	42.7	--
Total Nucleated Cells (%)	D		--	--	--	--	--	44%**	--
N Week 52		14	15	14	15	13	14	13	14
Absolute Organ Weights[c]									
Salivary Gland (g)	D	0.89	0.65	--	--	--	--	27%**	20%**
Epididymides	D	1.45		--		--		13%**	
N Week 52		15	15	15	15	15	15	15	15
Histopathology									
Bone Marrow – Hypocellular		--	--	--	--	7	3	3	5
Lung – Alveolar Macrophage Accumulation		3	1	1	1	4	--	7	5
N Week 52		15	15	1	1	15	15	15	15
Histopathology									
Urinary Bladder - Edema		--	--	--	--	--	--	1	--
- Hemorrhage		--	--	--	--	--	--	5	--
N Week 52		15	15	1	0	15	15	15	15
Histopathology									
Salivary Gland – Secretory Content Decreased in Acini		--	--	--	--	--	--	6	3

** = p<0.01 (linear trend within ANOVA);
N = Number of animals; -- = No noteworthy findings; I = Increased. D = Decreased.

[b] Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.

[c] Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.

The three pivotal repeat-dose toxicology studies in the Cynomologous monkey were completed via oral gavage. Treatment duration was for 4, 13 and 52-weeks.

The results of the 13-week monkey study are summarized in Sponsor's table below:

Dosage (mg/kg)	VC		10		25		100		500	
Sex (M/F)	M	F	M	F	M	F	M	F	M	F
Number of Test Animals	4	4	3	3	3	3	3	3	4	4
Death or Moribund	0	0	0	0	0	0	0	0	0	0

Important Findings	VC		10 mg/kg		25 mg/kg		100 mg/kg		500 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N	4	4	3	3	3	3	3	3	4	4
Clinical Signs										
Tail dermatopathy	--	--	--	--	1	--	1	3	4	2
Tail amputation	--	--	--	--	--	--	--	--	1	1
Soft feces	--	1	--	--	1	--	1	--	4	4
Diarrhea	--	--	--	--	--	--	--	--	4	3
Nasal discharge	3	--	--	3	1	--	--	1	3	2
Heart murmur - Low grade pretest	--	1	--	1	1	--	--	1	--	--
- Low grade Wk 8	--	1	--	1	1	--	--	1	--	--
Low grade Wk 13	--	--	--	--	--	--	--	1[1]	--	--
Histopathology										
Skin (Tail)										
Neutrophilic Inflammation	--	--	--	--	--	1	1	3	4	3
Ulcer	--	--	--	--	--	--	1	3	3	2
Hyperkeratosis	--	--	--	--	--	--	--	--	1	1
- Hemorrhage	--	--	--	--	--	--	--	--	--	1
- Granulation Tissue	--	--	--	--	--	--	--	2	1	1
Bone Marrow										
- Abnormal Megakaryocytes	--	--	--	--	--	--	--	--	1	--
Heart - Ventricular Enlargement	--	--	--	--	--	--	--	1[1]	--	--
- Fibrosis in Apex or Interventricular Septum	1	1	1	2	--	--	1	--	2	--

N = Number of animals; -- = No noteworthy findings.
[1] Animal 995

The results of the chronic monkey study are summarized below:

Important Findings	VC		10 mg/kg		25 mg/kg		100 mg/kg		250/500 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3	3	3
Clinical Signs										
Tail Dermatopathy	2	--	--	2	2	1	--	2	3	3
Tail Amputation	--	--	--	--	--	--	--	--	1	--
Soft Feces/Diarrhea	--	1	--	1	1	1	1	1	3	3
Nasal Discharge	1	2	1	--	1	--	--	1	2	1
Heart Murmur – Low Grade Pretest and Throughout Study	--	--	--	--	--	--	--	--	--	1
N Week 35	3	3	3	3	3	3	3	3	3	3
Special Features - Hematology										
Antoagglutination – Slight	--	--	1	--	1	--	2	3	2	3
N Week 13	3	4	2[a]	3	3	3	3	3	4	4
Bone Marrow										
Total Megakaryocytes[b]	18.0	20.8	20,16	16.0	19.3	21.0	18.7	20.7	20.0	24.0
N Week 68 (M) /Week 65 (F)	3	3	3	3	3	3	3	2[a]	3	2[a]
Bone Marrow										
Total Megakaryocytes[b]	21.7	19.0	17.7	19.3	20.7	21.3	15.0	19, 22	14.7*	13, 16
Percent of Control							31%		32%	24%
N Week 65	3	3	3	3	3	3	3	3	3	2
Clinical Signs										
Tail Dermatopathy	--	--	--	--	--	--	--	--	--	1
Special Features - Hematology										
Platelet Aggregation – Ristocetin (I)	--	--	--	--	--	--	--	--	--	1-fold
Platelet Aggregation – Arachidonic Acid (I)	--	--	--	--	--	--	--	--	--	1-fold

* Significantly different from vehicle control mean at 5% level by linear trend test within one factor analysis of variance.
N = Number of animals; -- = No noteworthy findings; I = Increased.
[a] Individual data.
[b] Total count/5000 hematopoietic cells (mean).

Target Organs of Toxicity: Collectively, the rat and monkey toxicology studies identified several target tissues for toxicity, including the hematological system, the bone marrow, the vascular system (hemangiosarcomas), the skin (dermatopathy), the kidneys and the reproductive system.

Dermatopathy: In the 4-week rat studies, dermatological changes on the tail of the rats were described as consisting of hyperkeratosis, acanthosis, inflammation, hemorrhage, fibrosis, necrosis, ulcers, scab formation, cellular infiltrates and or bacteria. This dermatopathy was noted following doses of 250 mg/kg and higher in rats. In the 13-week monkey study, neutrophilic inflammation and ulceration of the tail skin was noted which at times was accompanied by hyperkeratosis, hemorrhage and granulation tissue following doses of 100 and 500 mg/kg. In the 52-week monkey study, tail dermatopathies occurred at all doses tested (10, 25, 100, 250/500 or 500 mg/kg) including controls, but there was an increased incidence at ≥25 mg/kg. Onset was earliest in animals treated with 500 mg/kg with lesions appearing during week 2. For almost all animals, tail lesions resolved before study termination.

Bone Marrow: In the 4-week oral repeat-dose toxicology study in rats, the high dose of pregabalin **decreased bone marrow megakaryocytes** in both males and females (30% and 25%, respectively). A retrospective analysis to evaluate

femoral bone marrow composition and peripheral blood erythrocyte and platelet morphology was completed. As in the rat, total erythroid cells decreased by 24% in males treated with ≥100 mg/kg and in both sexes at 500 mg/kg at week 68. Monkey's treated with pregabalin for over a year did not exhibit the erythrocyte and platelet morphological changes.

The standard battery of **reproductive toxicology** studies were completed, including Segment I studies in rats, Segment II studies in rats and rabbits and Segment III studies in rats. Additional studies were undertaken to evaluate the effects of pregabalin on male fertility, skull development, and fetal survival. Studies in juvenile rats were completed to support a proposed clinical development in pediatric populations. These studies were reviewed by Dr. Ed Fisher (HFD-120).

Male Reproductive System: Under conditions of the assays, pregabalin treatment had profound effects on the male reproductive system of rats and monkeys. For any nonclinical development program, the initial assessment of the effects of pregabalin on the male reproductive system is completed via gross and microscopic examination of the male sex organs from the repeat-dose toxicology studies. Two pivotal 4-week studies were completed in the **rat** model. The first study tested 500, 1250, 2500 and 5000 mg/kg. The second study examined the effects of 50, 100 and 250 mg/kg. Pregabalin treatment produced clear histological **changes in the epididymis of rats** treated for 4 weeks with doses of ≥500 mg/kg, including enlargement, hypospermia in tubules, interstitial fibrosis **and mononuclear cell infiltrates**. Sperm granulomas were noted in the high-dose group (5000 mg/kg). In the 13-week rat study, pregabalin doses of 500 mg/kg and 1250 mg/kg produced decreased epididymal weights (9 and 17%, respectively). Minimal to mild spermatogenic epithelial degeneration was noted in three of the animals treated with 1250 mg/kg.

In the repeat-dose toxicology study in the monkeys, examination of the histology slides revealed evidence of hypospermia of the testes and epididymis associated with small testes and testicular weights in 1 monkey treated with 100 mg/kg and 2 monkeys treated with 500 mg/kg bid. In the recovery group, spermatogenic epithelial degeneration was noted in animals treated with either 50 mg/kg or 100 mg/kg for 4-weeks. However, there were no alterations in reproductive organ histopathology at doses up to 500 mg/kg in the 1 year study. In light of these findings the NOAEL for pregabalin-induced male monkey effects fertility appears to be 100 mg/kg.

The Sponsor submitted several male rat fertility studies for pregabalin (Segment I), as the initial study failed to identify a NOAEL value. The first study tested pregabalin doses of 250, 1250, and 2500 mg/kg by oral gavage for 11 weeks prior to mating, throughout mating and for up to 6 weeks post-mating. In this study, the treated males were then bred with untreated females. As indicated in Dr. Fisher's review, pregabalin doses of ≥ 1250 mg/kg produced "marked

reproductive toxicity manifesting as reduced fertility, increased number of days to mating, decreased sperm counts and motility, increased abnormalities in sperm morphology, decreased implantations, and increased preimplantation loss as well as general toxicity in the form of clinical signs and decreased BW gain.

The second **Male Fertility and Early Embryonic Development study**, male rats were treated with pregabalin (100, 250 or 500 mg/kg) for 11 weeks prior to mating with untreated females. Pregabalin treatment did not alter copulation or fertility indices at any dose, nor did it alter maternal reproductive parameters, including embryonic and fetal survival, litter size, pre- or post-implantation loss (at day G21). Fetal body weights were decreased, however, following pregabalin treatment of the males, and malformation indices were increased compared to control indices (malformation indices were 3/2, 4/3, 5/5 and 11/7 fetuses/litter with malformations in the control, low-dose, mid-dose and high-dose groups, respectively). The type of malformations that appear to be increased include anal atresia, eye defects (anophthalmia, folded retina), and skeletal defects (malformed thoracic arch, thoracic hemicentra, one less presacral vertebra and fused and branched ribs). The Sponsor concluded that these results do not demonstrate male-mediated teratogenicity as there were no malformations that were dose-related and that all of the findings were similar to historical controls. Dr. Fisher notes in his review that an increase in total malformations is generally considered to indicate a teratogenic response. The collective results of the studies, however, clearly demonstrate male-mediated developmental toxicity, and are suggestive of for male-mediated teratogenicity.

The **Female Fertility study** indicated that pregabalin treatment disrupted the female estrus cycle and increased the number of days to mating all doses tested (≥500 mg/kg). At higher doses (≥500 mg/kg), pregabalin appears to decrease the fertility index.

A total of three definitive **Segment II (Embryo-Fetal Development)** reproductive toxicology studies were completed (mice, rats and rabbits). The mouse study failed to show evidence of developmental toxicity following doses up to 2500 mg/kg, however, the study can not be considered to be valid, as it did not produce clear maternal toxicity. Overall, three different rat studies were completed to evaluate the potential for pregabalin-induced embryofetal developmental toxicity. Collectively, the studies provide evidence that pregabalin may be teratogenic.

Embryofetal development studies were also completed in the rabbit model. Doses of pregabalin (250, 500 or 1250 mg/kg, gavage) were administered from gestation day 6 to 20. The incidence of malformations (total number of fetuses/litters with skeletal malformations) was 0/0, 1/1, 0/0 and 5/4 for the control, low-dose, mid-dose and high-dose groups, respectively. The Sponsor indicated that these values were within the historical control range and therefore do not represent evidence of

teratogenicity. Dr. Fisher questions this conclusion in light of the skeletal changes noted in the rat studies.

Peri- and Post-Natal Development Studies were completed in the rat model. Rats were dosed with pregabalin at 50, 100, 250, 1250 and 2500 mg/kg. Maternal toxicity was noted at the high-dose, indicating that the dosing was valid. Under these conditions, pregabalin administration to pregnant and lactating rats decreased offspring survival, growth, behavior and reproductive function at doses of 100 mg/kg and greater (AUC 601 μg•h/ml; 5-fold higher than the human AUC at 600 mg/day). Body weight deficits persisted into the post-weaning period. Further, developmental landmarks were delayed at doses of 1250 mg/kg. Decreased acoustic startle response was seen in pups from dams treated with 250 and 1250 mg/kg when examined at post-natal day 42. Dr. Fisher notes that similar responses were noted in rats directly treated with pregabalin during the early postnatal development period in juvenile developmental neurotoxicity studies. The offspring of the F1 generation were then mated

A series of **genetic toxicology** studies were completed with negative results, suggesting that under the conditions tested, pregabalin is neither mutagenic nor clastogenic *in vitro* or *in vivo*.

Carcinogenicity: The carcinogenic potential of pregabablin was evaluated in 2-year mouse and rat bioassays. Although there was no evidence for treatment-related tumors in rats, two different strains of mice developed hemangiosarcomas. In the first study, **B6C3F1 mice** were treated with 200, 1000 or 5000 mg/kg pregabalin in the diet for a total of 2 years. The dosing for this study was based upon a 13-week dose-range finding study with the high dose achieving an AUC of at least 25-fold the mean human exposure (CAC concurred with the protocol). Beginning around week 52, there was an increased incidence of distended abdomens in males treated with 200 and 1000 mg/kg and females at all doses. An increase in the incidence of internal palpable masses in the abdomen was noted in males treated with 1000 and 5000 mg/kg and in females treated with 5000 mg/kg. Food consumption and body weight increased in both sexes at all doses. Platelet counts increased 35 and 33% in males at 1000 and 5000 mg/kg, respectively, and 36%, 32% and 58% in females at 200, 1000 and 5000 mg/kg, respectively. A total of 34 tumor types were detected in males and 41 tumor types in females. The 6 tumor categories in each sex that showed a statistical significant positive dose trend are listed in the Sponsor's table 27 below:

Table 27. Tumor Categories With Statistically Significant Peto Test for Dose Trend

Tumor Type	Trend Direction	Peto Test Two-Tailed p-Value
Males		
All Tumors	-	0.001
All Malignant Tumors	-	0.001
Adenoma of Adrenal Gland, Cortex (Benign)	-	0.012
Hemangioma of Bone Marrow, Femur (Benign)	-	0.012
Interstitial Cell Tumor of Testis (Benign)	-	0.050
Hemangiosarcoma[a]	-	0.001
Females		
All Tumors	-	0.001
All Malignant Tumors	-	0.001
A Cell Carcinoma of Adrenal Gland, Cortex (Malignant)	-	0.035
Leiomyoma of Large Intestine, Cecum (Benign)	-	0.033
Adenoma of Mammary Gland (Benign)	-	0.010
Hemangiosarcoma[a]	-	0.001

[a] Multiple tissue sites

Table 28. Number of Tumor-Bearing Animals for Tumor Types With Statistically Significant Peto Test for Positive-Dose Trend

Sex	Tumor Type	CI-1008 Dose (mg/kg)			
		0	200	1000	5000
M	All Tumors	34	36	48	38
M	All Malignant Tumors	18	16	36	28
M	Adenoma of Adrenal Gland, Cortex (Benign)	0	0	0	1
M	Hemangioma of Bone Marrow, Femur (Benign)	0	0	0	1
M	Interstitial Cell Tumor of Testis (Benign)	0	0	0	1
M	Hemangiosarcoma*	2	3	19	22
F	All Tumors	44	45	51	45
F	All Malignant Tumors	24	30	40	35
F	A Cell Carcinoma of Adrenal Gland, Cortex (Malignant)	0	0	0	1
F	Leiomyoma of Large Intestine, Cecum (Benign)	0	0	0	1
F	Adenoma of Mammary Gland (Benign)	1	0	0	3
F	Hemangiosarcoma*	2	7	19	25

* Multiple tissue sites

A dose related increase in the incidence of hemangiosarcoma occurred in both sexes at 1000 and 5000 mg/kg. They occurred in multiple tissue, although were most frequently observed in **liver, spleen and bone marrow**. Hemangiosarcoma was considered to be the cause of death in 1, 3, 13 and 13 males and 1, 3, 12 and 15 females in the controls and at 200, 1000 and 5000 mg/kg respectively. The first hemangiosarcoma was diagnosed in a control female found dead at week 49, suggesting that the development of these tumors occurred within the first year of treatment in this model. The increase in hemangiosarcomas in the females treated with 200 mg/kg was not statistically significant; however, given the overall trend in the results, the results at this dose may be biologically significant. A retrospective histopathological analysis detected a dose-related increased incidence of megakaryocytic hypercellularity in both sexes and total megakaryocytes in femoral bone marrow (Sponsor's Table 30 below) at all doses.

Table 30. Total Megakaryocytes in B6C3F1 Mouse Bone Marrow

Dose (mg/kg)	Males		Females	
	N	Count[a]	N	Count[a]
UC	65	43.2 ± 1.38	62	43.0 ± 1.36
200	63	65.8 ± 2.26†	66	60.1 ± 2.53†
1000	64	77.3 ± 2.50†	62	71.2 ± 2.40†
5000	61	88.3 ± 3.37†	63	78.9 ± 2.49†

Table 2.6.7.10B, RR 745-03456

N = Number of animals; UC = Untreated control.

† Significant trend test at 0.02 (0.005 for quadratic) level of significance.

[a] Total count-5000 hematopoietic cells; mean ± standard error.

Hemangiosarcomas were not detected in the rat carcinogenicity studies.

The Sponsor examined a second strain of mouse, the CD-1 strain, to determine if the hemangiosarcomas noted in the B6C3F1 mouse were strain-specific. The results demonstrated an increase in hemangiosarcomas in CD-1 mice, although this strain was not as sensitive to pregabalin-induced tumors as the B6C3F1 mouse. The incidence is summarized in the table below, modified from the Sponsor's Tabulated Summary of the study.

Number of Tumors in All Animals Which Were Evaluated (without consideration of the causes and relevance) **Biometrical Evaluation:** Yes			**Frequency According to Dose and Sex**							
			UC		**200 mg/kg**		**1000 mg/kg**		**5000 mg/kg**	
			M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of Animals Evaluated			65	65	65	65	65	65	65	65
Number of Animals With Neoplastic Lesions			38	46	37	48	31	53	34	47
Number of Animals With Malignant Lesions			26	36	23	39	20	41	26	40
Organ	Identification of the Tumor	Malig? Y or N								
Bone Marrow Fm	Hemangioma	N	0	0	0	0	0	0	0	2
	Sarcoma	Y	0	0	0	0	0	1	0	0
Hemolymphoretic	Hemangiosarcoma[1]	Y	2	6	5	9	6	10	14§	13
	Histiocytic Sarcoma	Y	3	7	1	7	0	5	1	3
	Histiocytoma Fibrous	Y	0	2	0	0	0	0	0	0
	Lymphoma	Y	5	12	5	14	3	14	4	12

1 Multiple tissue sites, primarily liver, spleen, bone marrow and uterus.

To determine if the hemangiosarcomas were reversible, animals were treated for 12 months followed by 12 months of recovery time. As indicated in Sponsor's table 67 reproduced below, the incidence of hemangiosarcomas in the 24-month treatment group was higher than in the 12-month plus recovery group (Stop Treatment Group). However, the study clearly supports the conclusion that the B6C2F1 mouse is particularly sensitive to pregabalin-induced hemangiosarcoma formation.

Table 67. Frequency and Distribution of Hemangiosarcoma in Female B6C3F1 Mice Given Pregabalin for 12 or 24 Months

	Pregabalin (mg/kg)			
	Control	50	200	1000
24-Month Treatment Group				
Hemangiosarcoma incidence[a]	4/61	4/61	7/61	15/57¶
Distribution[b]				
Liver	1	0	3	6
Spleen	3	3	4	7
Bone marrow	0	2	2	2
Heart	0	0	0	2
Stop Treatment Group[c]				
Hemangiosarcoma incidence[a]	5/60	6/61	5/60	10/56#
Distribution[b]				
Liver	3	2	0	3
Spleen	2	4	3	6
Bone marrow	1	4	3	5

Table 2.6.7.10F, RR 745-03832

¶ $p < 0.001$

\# $p = 0.017$

[a] Number of animals with hemangiosarcoma/total number of animals

[b] Some animals had hemangiosarcoma in more than 1 tissue

[c] Mice received drug in diet for 12 months and then maintained an additional 12 months on control diet prior to necropsy.

The Sponsor conducted extensive mechanistic studies to provide evidence that the hemangiosarcomas are truly species-specific and therefore not of concern for the proposed clinical indications.

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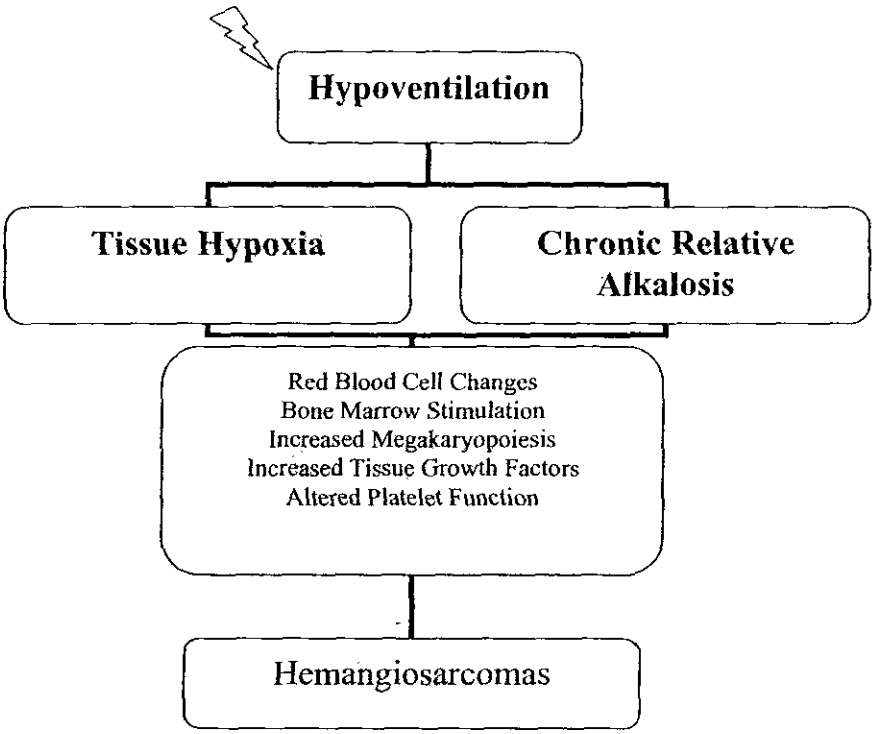
PREGABALIN



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A retrospective examination of the target organs (liver, spleen and bone/bone marrow) and non target organs (lung, kidney and lymph node) for proliferative vascular lesions and other non-neoplastic changes was completed. The incidence of minimal to mild hepatic sinusoidal cell hyperplasia was increased in the 200 and 1000 mg/kg groups with a lower incidence in the 5000 mg/kg treatment group, possibly due to increased mortality. This hyperplasia likely represents proliferation of Kupffer and or endothelial cells. There was a dose-related increase in the incidence of hypercellularity in bone marrow megakaryocytes in both males and females. Total megakaryocytes in femur bone marrow (sternal if necessary) increased 52%, 79% and 104% in males and 38%, 66% and 83% in females treated with 200, 1000 and 5000 mg/kg pregabalin, respectively. Retrospective analysis of peripheral blood smears detected a dose-related increase in RBC and platelet morphologic abnormalities. RBC abnormalities were predominately the presence of schistocytes. Platelet abnormalities were described as bizarre shape change with numerous pseudopodia, central condensation of platelet granules, platelet swelling, hypogranulation and the presence of platelet aggregates undergoing partial to complete degranulation, consistent with platelet activation. Giant platelets the size of erythrocytes were increased in a dose-dependent matter, suggesting increased platelet turnover (summarized in the Sponsor's table reproduced below).

Table 31. Peripheral Blood Abnormalities in B6C3F1 Mice Given Pregabalin for 2 Years

Dose (mg/kg)	N	Nonuniform Platelet Size (%)	Giant Platelets (%)	Platelet Aggregates (%)	Schistocytes (%)
Male					
UC	53	15.1	1.9	1.9	1.9
200	47	12.8	2.1	8.5	2.1
1000	36	75.0	63.9	13.9	16.7
5000	17	100.0	100.0	29.4	41.2
Female					
UC	42	19.0	2.4	2.4	2.4
200	41	17.1	4.9	7.3	4.9
1000	27	44.4	22.2	18.5	11.1
5000	23	91.3	52.2	30.4	26.1

Table 2.6.7.10B, RR 745-03714
UC – Untreated control.

Similar to the results in the B6C3F1 mouse, peripheral blood abnormalities were noted in the CD-1 mouse, although overall effects were less than those noted in the B6C3F1 mouse model (see Sponsor's table 35 below):

Table 35. Peripheral Blood Abnormalities in CD-1 Mice Given Pregabalin for 2 Years

Dose (mg/kg)	N	Nonuniform Platelet Size (%)	Giant Platelets (%)	Platelet Aggregates (%)	Schistocytes (%)
Male					
UC	46	2.2	2.2	4.3	0.0
200	38	0.0	5.3	7.9	5.3
1000	39	10.3	0.0	5.1	2.6
5000	39	66.7	46.2	17.9	10.3
Female					
UC	49	2.0	2.0	4.0	2.0
200	48	16.7	0.0	0.0	8.3
1000	47	25.5	8.5	12.8	4.3
5000	45	20.0	17.8	4.4	11.1

Table 2.6.7.10C, RR 745-03714
N = Number of animals; UC = Untreated control.

The incidence of pulmonary macrophage infiltrates and/or granulomatous inflammation was increased in drug-treated groups. These lesions were typically focal/multifocal and minimal to mild in severity. The Sponsor noted that these types of changes are seen spontaneously in aging mice and there was no relationship between drug treatment and presence of pulmonary neoplasia. Further there were no proliferative nonneoplastic findings in the lymph nodes or kidneys. Examination of the cell types involved in the liver changes was also completed.

Sponsor's Table 7 below (from the 1/30/2004 submission) summarizes the steps proposed to lead to hemangiosarcoma formation and provides a comparison between the mouse, rat, monkey and human platelet and endothelial functions:

Table 7. Species Specificity of Pregabalin-Induced Changes in Parameters Associated With Hemangiosarcoma Formation in Mice

Parameter	B6C3F1 Mice	CD-1 Mice	Wistar Rat	Monkey	Human
Altered Respiration	+	ND	+	ND	ND
Acid-Base Imbalance	+	ND	-	ND	-[b]
Increased Platelet Count	+	+	-	-	-
Altered Platelet Aggregation	+	+	-	-	-
Altered Platelet Morphology	+	+	-	-	-
Increased Platelet Activation	+	+	-	ND	-
Increased Circulating PDGF	+	-[a]	-	ND	-
Increased VEGF - Spleen	+	+	-	ND	ND
Increased VEGF-R2	+	ND	-	-	ND
Increased Endothelial Proliferation	+	-[a]	-	-	ND

+ = Effect; - = No effect; ND = No data.
[a] No data at relevant doses or time points.
[b] Based on absence of change in serum bicarbonate in 2 independent clinical trials.

Based upon the mechanistic studies completed and summarized in the table above, the Sponsor concluded the following:

> Results of investigational studies in mice, rats, monkeys, and humans indicate that the mode of action responsible for the increased incidence of hemangiosarcoma in mice is specific to mice. Therefore, the tumor findings in mice are not relevant to humans, and pregabalin does not represent a carcinogenic risk to humans.

B. Pharmacologic activity

Pregabalin binds to the α_2-δ **protein**, an auxiliary protein associated with voltage-gated calcium channels. High voltage-gated calcium channels (P/Q, N, R and L types) are composed of four subunits, including α_1, $\alpha_2\delta$, β and γ. The $\alpha 1$ subunit forms the pore of the channel. The other three subunits are called auxiliary subunits. Characteristics of auxiliary proteins include: 1) they are present in the purified channel complex, 2) they direct interaction with the α_1 pore, (3) they are capable of directly modulating the biophysical properties and/or trafficking of the α_1 subunit and (4) they form a stable association with the α_1 subunit. The cartoon below depicts the proposed structure of the high voltage calcium channel subunits with the $\alpha_2\delta$ subunit shown in yellow (from Arikkath and Campbell, 2003). The δ portion of the protein serves as the membrane anchor, while the α_2 portion of the protein interacts with the α_1 subunit (Arikkath and Campbell, 2003).

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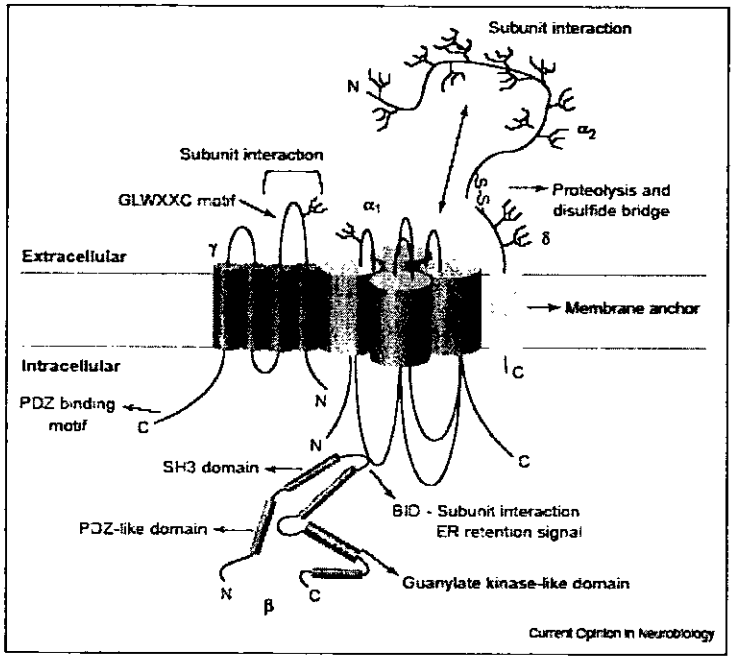
Figure 1



Predicted membrane topology, subunit interactions and structural domains of the auxiliary subunits of the voltage-gated calcium channels. The voltage-gated calcium channels are composed of the pore forming α_1 subunit and the auxiliary $\alpha_2\delta$, β and γ subunits. The $\alpha_2\delta$ and γ subunits contain transmembrane domains, whereas the β subunit is entirely intracellular. All three auxiliary subunits interact directly with the α_1 subunit, with no known inter-auxiliary subunit interactions. Each of the auxiliary subunits contains unique structural domains as shown.

There are at least 4 genetically distinct $\alpha_2\delta$ subunits described ($\alpha_2\delta$-1 through $\alpha_2\delta$-4), each with a slightly different tissue distribution. The summary table below was extracted from Arikkath and Campbell (2003).

Table 1

Chromosomal location, functional effects and tissue distribution of the auxiliary subunits of the voltage-gated calcium channels.

Subunit	Human chromosomal location	Functional effects	Tissue distribution	References
$\alpha_2\delta$-1	7q21-q22	Membrane trafficking of α_1 Increase in current amplitude activation/inactivation kinetics Voltage dependence of activation	Brain, heart, skeletal muscle	[7,9]
$\alpha_2\delta$-2	3p21.3	Increase in current amplitude	Lung, testis, brain, heart, pancreas, prostrate, skeletal muscle, spinal cord	[4,5]
$\alpha_2\delta$-3	3p21.1	Increase in current density Voltage dependence of activation Steady state inactivation	Brain, heart, skeletal muscle	[6]
$\alpha_2\delta$-4	12p13.3	Increase in current amplitude	Heart, skeletal muscle, intestine, fetal liver erythroblasts, adrenal gland, pituitary	[5]

The α_2 subunit is heavily glycosylated with different forms that are differentially glycosylated. This glycosylation is important regarding the function of the protein. The **$\alpha_2\delta$-1 subunit** is the most characterized subunit and has been implicated in the actions of **gabapentin**. Further studies indicate that the **$\alpha_2\delta$-2** is also a binding site for gabapentin.

In contrast, gabapentin does not bind to $\alpha_2\delta$-3 (Gong et al., 2001). The functional effect of pregabablin or gabapentin binding to the $\alpha_2\delta$ subunit appears to be dependent on the model examined. In vitro, **pregabablin** reduces the release of glutamate, norepinephrine, serotonin, dopamine, Substance P and CGRP from certain brain tissues. Pregabalin reduces calcium flux in isolated presynaptic terminals (synaptosomes). Pregabalin-induced reduction of calcium flux is enhanced in tissues with prior activation of the neural tissues, i.e., inflamed tissues. Pregabalin does not completely block calcium channel function or completely reduce neurotransmitter release.

The **$\alpha_2\delta$-1 subunit** has been detected in the brain, heart and skeletal muscle. The **$\alpha_2\delta$-2** subunit has been associated with an increase in current amplitude and has been detected in the lung, testis, brain, heart, pancreas, prostate, skeletal muscle and spinal cord.

C. Nonclinical safety issues relevant to clinical use

Hemangiosarcomas:

Both the Sponsor and Review Divisions agree that the findings of an increased incidence of hemangiosarcomas in both strains of mice are real findings. Based upon the results from the B6C3F1 mouse study (i.e., the most sensitive species/strain), the exposure multiples for the proposed 600 mg/day dose in humans is presented in the table below (modified from Sponsor's Table 5, Nonclinical Overview):

Incidence of Hemagiosarcoma in B6C3F1 Mice Given Pregabalin for 2 Years[a]

	Dose (mg/kg)			
	0	**200**	**1000**	**5000**
Males	2	3	19*	22*
	3%	5%	29%	34%
Females	2	7	19*	25*
	3%	11%	29%	38%
Exposure Multiple[b] (600 mg)		1.1-1.2	5-7	31
Exposure Multiple[c] (300 mg)		2.2-2.4	10-14	62

* Statistically increased compared to control, $p < 0.001$.
Historical incidence in control B6C3F1 mice: Males: 0%-13%; females: 0%-8%.
a 64-66/sex/group.
b Comparison to mean exposure in humans given the maximum recommended dose of 600 mg/day.
c Comparison to mean exposure in humans given the maximum recommended dose of 300 mg/day.

Although the incidence in the females treated with 200 mg/kg was not statistically significant, the response appears to be treatment-related. The exposure multiple at this dose is approximately equivalent to the human exposure at the maximum recommended daily dose. If the maximum daily dose were limited to 300 mg/day for this indication, the exposure ratio is still below 10. Based upon this analysis, an adequate safety margin for pregabalin has not been established.

Although the B6C3F1 mouse model is clearly the most sensitive species examined to date in terms of pregabalin-induced hemangiosarcomas, the Sponsor's position is that this model is not the most appropriate species for human risk assessment. This conclusion is based largely on extensive studies characterizing the effects of pregabalin on cells likely to be involved in the tumor production. Based upon the results of these studies, the Sponsor has proposed the following series of effects as the mostly likely mechanism for hemangiosarcoma production in mice. The studies submitted in January of 2004 provided evidence that pregabalin treatment of B6C3F1 mice decreased minute volume without an increase in tidal volume. The Sponsor states that compared to rats and humans, mice have a higher pO_2 in arterial blood and lower pO_2 in venous blood. This is consistent with the higher metabolic rate in mice than either rats or humans. Mice also have lower arterial bicarbonate and a broader range for pH than either humans or rats.

The collective results suggest that pregabalin produces respiratory depression in mice leading to a relative alkalotic state that is not compensated for. The resulting tissue hypoxia and chronic relative alkalosis are hypothesized to be the key stimuli ultimately leading the and increased incidence of hemangiosarcoma in this model.
The Although the Sponsor has conducted extensive studies to characterize the changes in the tissues described above to delineate the mechanism of the pregabalin-induced hemangiosarcomas in mice, the studies characterize effects on specific cell populations thought to contribute to tumor formation, such as the platelets, megakaryocytes and endothelial cells. Drs. Terry Peters, Edward Fisher, Lois Freed, Jerry Cott and myself have reviewed these study results. In Dr. Fisher's and Peters' reviews for this NDA, they clearly note that although a plausible explanation, there remain specific inconsistencies in the data and thus the hypothesis that has been put forth by the Sponsor is not conclusive. The proposed mechanism leading to the development of the tumors and the species specificity is correlative rather than concrete. As such, the incidence of hemangiosarcomas in mice should be described in the label. The conclusion that these findings are not related to humans is too strong a conclusion at this point in time.

A second concern related to the mechanism proposed by the Sponsor is the unique tissue pathology in advanced diabetic patients. Specifically, patients with diabetes have a high incidence of vascular abnormalities. The clinical presentation of an individual with diabetes present with unique complications including macrovascular complications (i.e., coronary disease, stroke and peripheral vascular disease) and microvascular disease (i.e., retinopathy, neuropathy, nephropathy). In addition, diabetic patients without vascular abnormalities have been shown to have enhanced basal platelet activation and decreased antioxidant status (Vericel et al., 2004), increased mean platelet volume (Hekimsoy et al., 2004), elevated fibrinogen levels and megakaryocyte ploidy (Brown et al., 1997). As such, the diabetic patient population is already sensitive to perturbations in the vasculature tissues and their platelets appear to be already sensitized similar to those described in the studies with pregabalin. Further, as the proposed biomarkers for hemangiosarcomas are commonly noted in diabetic patients, there is no method to predict impending tumor formation. As such, an additional safety margin of 10 should be placed on top of the safety margin of 10 set for the extrapolation from animals to humans.

A third concern related to the findings of hemangiosarcomas in mice related to preclinical carcinogenicity assessment of the peroxisome proliferator-activated receptor (PPAR) class of compounds in the Division of Metabolic and Endocrine Drug Products. PPARs are members of a nuclear hormone receptor superfamily. There are three PPAR subtypes (α, β/δ and γ). PPARγ is expressed in high levels in white adipose tissue, as well as many other tissues, including macrophages, B and T lymphocytes, epithelial and endothelial cells expression in the gene expression. Several PPARγ agonists have been approved for the treatment of diabetes due to their ability to sensitize cells to insulin (Rangwala and Lazar, 2004). The prototype drug, troglitazone, was shown to produce hemangiosarcomas in the B6C3F1 mouse model (males and females) but not in rats. The exposure ratio for troglitazone was 15-20 times the maximum daily human dose. Interestingly, this drug has been withdrawn from the market due to hepatic concerns, the class of drugs are being evaluated for a variety of conditions. Rosiglitazone and

pioglitazone have been approved for human use and do not show hemangiosarcomas. Two other drugs in this class have been tested for carcinogenicity and have demonstrated hemangiosarcomas in both male and female CD-1 mice. Over all, the DMEDP have 11 total drugs in the PPAR category. Of those, 8/11 display hemangiosarcomas. According to Dr. El-Hage, the French regulatory authorities held a Drugs Biomedical Research Expert Panel Meeting in December of 2003 to discuss this issue. As a result of this meeting, the French have recommended the results of carcinogenicity studies for these compounds be submitted prior to Phase III trials of > 6 month duration. This policy has also been proposed to the ECAC who concurred. As rosiglitazone and pioglitazone are approved drugs indicated for the type 2 diabetic patient population, the potential for drug interactions with pregabalin should be examined, with specific emphasis on the development of vascular abnormalities and hemangiosarcomas.

PPARγ agonists play an important role in the immune response by dampening inflammation and by attenuating macrophage/monocytes synthesis of proinflammatory cytokines and reduction in B lymphocytes. PPARγ has also been targeted for a malignant cell database by attenuating macrophages/monocytes synthesis of proinflammatory cytokines by monocytes/macrophage cell lines. PPARγ agonists have been shown to have beneficial effects on the vasculature as well by regulating the proliferation and migration of vascular smooth muscle cells and improving endothelial cell function. Clinical studies have suggested PPARγ produces improvements in blood pressure regulation of vascular inflammation, development of atherosclerosis (Collins, 2003). Recently, the approved PPARγ agonist pioglitazone was shown to suppress the increase in adhesion molecules CD11b/CD18 on fMLP-stimulated leukocytes. This suppression did not block fMLP-induced calcium influx in the leukocytes, suggesting that the chemotactic signaling cascade was not altered (Imamoto et al., 2004).

Reproduction and Developmental Toxicology:

The effect of pregabalin on the male and female reproduction and development were reviewed by Dr. Ed Fisher. Overall, the toxicities noted are not minor. I concur with Dr. Fisher's assessment that the effects of pregabalin on male fertility are significant and suggestive of male-mediated developmental toxicity, including teratogenicity. Dr. Fisher's conclusion is reproduced below (Highlights added):

In animal studies that can be considered adequate for regulatory purposes, pregabalin was shown to be a selective reproductive and developmental toxicant. Effects included male and female fertility impairment in rats, teratogenicity in rats and rabbits, embryofetal and pup lethality in rats, and developmental nervous and reproductive system functional impairment in rats. Most of these effects are fairly typical for anticonvulsants, but the degree of reproductive impairment (in both F0 and F1) and the high rates of embryofetal and pup mortality are unusual for this class. In addition, **apparent male-mediated developmental toxicity was observed, which is extremely unusual** (possibly because it is rarely examined). This is a poorly understood phenomenon that remains controversial in the teratology literature (Friedman, Adv Exp Med Biol 518:219-26,2003). However, until the sponsor is able to refute the findings described in RR 745-03322 (decreased body weights and increased malformations in the offspring of treated male rats mated with untreated females), it seems prudent, from a regulatory standpoint, to report them in labeling

(they may actually warrant highlighting in some way). The signal for *in utero* teratogenicity was not especially strong, but was present in two species. The related compound gabapentin was found to be embryotoxic (increased incidences of visceral variations in rats, decreased embryofetal and pup survival in rats, decreased ossification in mice and rats, increased postimplantation embryofetal loss in rabbits), but was not teratogenic and did not affect reproductive performance in animal studies submitted to support its approval. There was little safety margin in terms of the plasma levels of pregabalin associated with reproductive and developmental toxicity in animals relative to expected clinical drug levels. **NOELs for the most sensitive endpoints were 100 mg/kg for effects on rat sperm and 50 mg/kg for effects on growth in rats exposed pre- and postnatally, which produced AUC values of 408 and 241 ug.h/ml, respectively. Expected maximum exposure in humans at the MRD of 600 mg/day is reportedly 122 ug.hr/ml.**

Exposure Multiples for Selected Reproductive and Developmental Toxicology			
Endpoint	**Dose**	**Endpoint**	**Dose**
Rat Sperm Pathology NOEL	100 mg/kg	Growth in rats exposed pre-natally and postnatally	50 mg/kg
Exposure Multiple (600 mg)	3.3		2
Exposure Multiple (300 mg)	6.6		4

The toxicity described by Dr. Fisher will have to be evaluated in light of the risk associated with exposure to the drug and the indication being treated. The specific toxicities noted in the reproductive and developmental toxicology studies are significant and should be clearly outlined in the labeling.

Dermatopathy:

The changes noted in the tails of monkeys and rats provide a clear signal for the potential for dermatological pathology in the clinical setting. According to the Sponsor's summary, "the etiology of the skin lesions remain unknown." However, the Sponsor's overall conclusion (Submission dated 1/30/2004) states "From the available data, Pfizer concludes that the dermatopathy observed in the nonclinical studies does not appear to be relevant to humans given the low incidence of wound healing abnormalities in the DPN population and in pregabalin-treated patients across all indications, and does not suggest a need for special concern in diabetic patients treated with pregabalin." From the nonclinical pharmacology and toxicology perspective, I concur with Dr. Cott. I can not agree with the Sponsor that the lesions are not of concern, especially for the diabetic patient population.

Clinically, diabetic neuropathy could involve autonomic function thereby leading to bladder dysfunction, erectile dysfunction, dyspareunia, gastroparesis, enteropathy, exercise intolerance, postural hypotension and polyradiculopathy among other alterations. In addition, the neuropathy can produce pain and altered sensory function which can lead to the loss of protective sensations, altered biomechanics and peripheral vascular disease. Careful foot care becomes critical to avoid tissue damage that may not heal or ulcers, which may go noticed and ultimately requiring for amputation (Huntley, 1993).

A common condition of diabetes includes skin manifestations, increased viscosity of blood due to stiff red blood cell membranes leading to engorgement of post-capillary venules and papillary dermis. Dermal manifestations include Diabetic thick skin, typically in the fingers and hands ranging from pebbled skin to scleroderma-like changes. **Scleroderma of Diabetes** histologically presents as a thickened dermis with large collagen bundles separated by clear spaces. There may be an increase in the number of mast cells. Diabetics often have a yellow hue to their skin, which may be due to the deposition of glycosylation end products (Huntley, 1993).

Diabetics have a higher incidence and prevalence of large vessel disease and develop myocardial infarctions and strokes at a younger age than non-diabetics. Atheroslerosis may be present in the lower extremities and result in skin atrophy, hair loss, coldness of the toes, nail dystrophy, pallor upon elevation and mottling on dependence. Microangiopathy is one the major complications of diabetes mellitus, and can affect the small blood vessels of the retina and renal vasculature leading to blindness and kidney failure. Microvascular pathology has also been thought to contribute to diabetic neuropathy and the "diabetic foot." Histologically the skin presents with microaneurysms or hemorrhage, exudates and devascularized areas. In some cases, severe microcirculatory problems can lead to gangrene. Functional microangiopathy may be due to glycosylation of blood components such as hemoglobin, red blood cell membranes, fibronectin, fibrinogen, and platelets. The glycosylation of the red blood cell causes the cell membrane to be less pliable and thereby impedes flow through the capillaries. Diabetics also have increased concentrations of fibrinogen and capillary leakage leading to loss of albumin and water. There is an increased tendency for diabetic platelets to aggregate. Overall there can be an increase in blood or plasma viscosity.

Diabetic dermopathy, or atrophic hyperpigmented macules on the skins is one of the most common cutaneous manifestations. The etiology of these irregular round or oval, circumscribed, shallow lesions is not clear. One potential cause has been atrophy of the tissue following trauma resulting in inflammation in poorly vascularized skin. ***Pigmented purpura*** is a salt and pepper yellow-tan hyperpigmentation of the skin commonly found in older diabetics. The clinical signs are thought to result from red blood cell extravasation from superficial vascular tissues. Patients commonly demonstrate cardiac decompensation and edema in the legs (Huntley, 1993).

Diabetic bullae describes blisters that spontaneously appear on the extremities of diabetics (typically hands or feet). The lesions are not the result of trauma or infection and tend to heal without treatment. There are three types of blisters noted. Spontaneous clear, sterile blisters typically heal within 2 to 5 weeks. Histopathologically, the tissue displays an intraepidermal cleavage without acanthlysis. The second type of diabetic bullae presents with a lesion that is hemorrhagic and although it does heal, produces scarring and atrophy. Histologically, the cleavage plane is below the dermoepidermal junction. The third type of diabetic bullae presents as multiple tender nonscarring blisters on sun-exposed and deeply tanned skin. Histologically, the cleavage plan occurs at the lamina lucida (Huntley, 1993).

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The nonclinical studies conducted to date identify three major areas of concern: hemangiosarcomas, reproductive and developmental toxicity and dermal toxicity. Based upon these findings, it does not appear prudent to approve NDA 21-446 due to the significant toxicity noted. As Lyrica® appears to show efficacy for the treatment of pain associated with diabetic neuropathy, and a clear signal for dermatological toxicity has not been detected. Regardless, the risk-benefit analysis must ultimately determine the approvability of the drug. From a purely non-clinical pharmacology toxicology perspective, I believe that the toxicities associated with the drug may have significant impact on the health and welfare of diabetic patients. Should the NDA ultimately be approved, individuals taken the medication should be clearly and specifically informed about the results of the animal findings. Further, I would strongly encourage both the physician and the patient to carefully monitor the extremities for any signs of dermal toxicity.

clinical

Unresolved toxicology issues (if any):

Hemangiosarcomas: The significance of the mouse hemangiosarcomas to the human patient population is unknown. The Sponsor has conducted extensive mechanistic studies in an attempt to demonstrate that the findings in mice are not relevant to humans. These studies suggest that the mouse model is uniquely sensitive to perturbations in platelet function which contributes to the generation of these tumors. As diabetic patients are known to have platelet abnormalities which contribute to diabetic vascular pathology, it is not clear if this patient population will also be more sensitive to the effects of pregabalin treatment as well. There does not appear to be a viable biomarker for these effects, specifically for this patient population. Further studies should be completed to identify a reliable biomarker for this specific patient population. Finally, as the use of PPARγ agonists is increasing in the treatment of diabetic patients, and this group of drugs is also associated with an increase in the incidence of hemangiosarcomas in rodent models, the potential interaction of these two treatments should be characterized prior to approval.

Dermatopathy: The significance of the rat and monkey tail lesions to the human diabetic patient population is unknown. The diabetic patient population, particularly those with neuropathy, are very sensitive to tissue damage due to their inherent unsteady gait/proprioception, their decreased sensation to notice bumps bruises or painful skin abnormalities, their decreased immune function and their decreased wound healing capabilities. Further histological analysis of the dermal lesions in light of the types of lesions noted in this patient population may provide an investigator the ability to clearly distinguish the cause of the lesion. Additionally, wound healing studies should completed in a diabetic mouse model to characterize the effects of pregabalin in a model that more closely resembles the physiological status of advanced diabetic patients.

Recommendations: From a pure non-clinical pharmacology and toxicology perspective, there are significant concerns with the safety of this drug, particularly in a diabetic patient population. At this time, I must recommend that NDA 21-446 not be approved for this patient population.

Suggested labeling: The following recommendations on labeling were extracted from Dr. Fisher's Review of the Reproductive and Developmental Toxicology Studies and Carcinogenicity Studies and Dr. Cott's review of the general toxicology data:

RECOMMENDED LABELING

Carcinogenesis/Mutagenesis/Impairment of Fertility

Carcinogenesis

[redacted]

Impairment of Fertility

Preclinical Data

[redacted]

1 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

Labor and Delivery: The effects of pregabalin on labor and delivery in pregnant women are unknown. In the prenatal-postnatal study in rats, pregabalin prolonged gestation and induced dystocia [ ] times the mean human exposure [~~AUC(0-24) of 123 µg.hr/mL~~] at the maximum recommended clinical dose of 600 mg/day) [ ]

Signatures (optional):

Reviewer Signature R. Daniel Mellon, Ph.D.

Supervisor Signature _____ Concurrence Yes ___ No ___

3.7. APPENDIX/ATTACHMENTS

Reference List

Arikkath J and Campbell KP (2003) Auxiliary subunits: essential components of the voltage-gated calcium channel complex. *Curr.Opin.Neurobiol.* **13**:298-307.

Brown AS, Hong Y, de Belder A, Beacon H, Beeso J, Sherwood R, Edmonds M, Martin JF, and Erusalimsky JD (1997) Megakaryocyte ploidy and platelet changes in human diabetes and atherosclerosis. *Arterioscler.Thromb.Vasc.Biol.* **17**:802-807.

Collins AR (2003) Pleiotropic vascular effects of PPARgamma ligands. *Drug News Perspect.* **16**:197-204.

Gong HC, Hang J, Kohler W, Li L, and Su TZ (2001) Tissue-specific expression and gabapentin-binding properties of calcium channel alpha2delta subunit subtypes. *J.Membr.Biol.* **184**:35-43.

Hekimsoy Z, Payzin B, Ornek T, and Kandogan G (2004) Mean platelet volume in Type 2 diabetic patients. *J.Diabetes Complications* **18**:173-176.

Huntley AC (1993) Cutaneous manifestations of diabetes mellitus. *Diabetes Metab Rev.* **9**:161-176.

Imamoto, E., Yoshida, N., Uchiyama, K., Kuroda, M., Kokura, S., Ichikawa, H., Naito, Y., Tanigawa, T., and Yoshikawa, T. Inhibitory effect of pioglitazone on expression of adhesion molecules on neutrophils and endothelial cells. Biofactors 20(1), 37-47. 2004.

Ref Type: Journal (Full)

Rangwala SM and Lazar MA (2004) Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. *Trends Pharmacol.Sci.* **25**:331-336.

Vericel E, Januel C, Carreras M, Moulin P, and Lagarde M (2004) Diabetic patients without vascular complications display enhanced basal platelet activation and decreased antioxidant status. *Diabetes* **53**:1046-1051.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

R. Daniel Mellon
6/3/04 04:20:02 AM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-446**
SERIAL NUMBER:	**N 000**
DATE RECEIVED BY CENTER:	**10/30/2003**
PRODUCT:	**Lyrica® (Pregabalin)**
INTENDED CLINICAL POPULATION:	**Pain associated with diabetic peripheral neuropathy**
SPONSOR:	**Pfizer Global Research & Development**
DOCUMENTS REVIEWED:	**Original Electronic NDA Submission**
REVIEW DIVISION:	**Division of Anesthetic, Critical Care & Addiction Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**Jerry Cott, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Lisa Malandro**

Date of review submission to Division File System (DFS): May 24, 2004

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

This application is not recommended for approval for the indication of diabetic neuropathy from the pharmacology / toxicology perspective.

B. Recommendation for nonclinical studies

Additional studies should be conducted to determine the mechanism of dermatopathy in rats and monkeys and the potential relevance to humans.

C. Recommendations on labeling

Modifications of labeling are recommended in the *Carcinogenesis, Mutagenesis, Impairment of Fertility* section. Dr. Ed Fisher (HFD-120) revised this section of the labeling, as he reviewed the reproductive toxicology studies and HFD-120 coordinated the consult with Dr. Terry Peters to review the hemangiosarcoma mechanism studies. This reviewer (JC) added an *Animal Toxicology* section to the label to describe the development of dermatopathy in rats and monkeys and the possible relationship to human skin ulceration, particularly in diabetic patients.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pregabalin is structurally related to γ-aminobutyric acid (GABA); however, it is not active at $GABA_A$, $GABA_B$, or benzodiazepine receptors and it does not alter GABA degradation or acutely change GABA uptake. Like gabapentin (Neurontin®), pregabalin binds with high affinity to the α_2-δ auxiliary subunit of voltage-gated calcium channels and has analgesic and antiseizure activity.

Pregabalin is well absorbed following oral administration. Absolute bioavailability of pregabalin is high (>80%) in mice and rats at a 50 mg/kg dose and in monkeys at a 10 mg/kg dose. Urine is the principal route of ^{14}C excretion following [^{14}C]pregabalin administration. In mouse, rat, and dog, ≥ 80% of the [^{14}C] pregabalin PO dose is present in the 0 to 24-hr urine sample, while >71% to 75% is excreted by monkey during the same interval. More than 90% of the dose was recovered in 0-96 hour urine in rat and monkey. Pregabalin undergoes minimal metabolism in mouse, rat, and monkey with unchanged parent representing the majority (≥ 90%) of drug-derived material in urine. [^{14}C]Pregabalin is widely distributed in most tissues and crosses the blood-brain barrier in mouse, rat, and monkey after PO administration. After oral administration, the drug was

rapidly absorbed in rat and monkey with maximum plasma concentrations achieved within 1 and 2 hours postdose, respectively. Pregabalin elimination half-life ($t_{1/2}$) was 3.4, 3.9, and 5.8 hours in mouse, rat, and monkey, respectively, following intravenous (IV) administration of 50, 50, and 25 mg/kg, respectively. Absolute oral bioavailability of pregabalin was 94% and 83% in mice and rats, respectively, at a 50 mg/kg dose. Absolute PO bioavailability in monkeys was 93% at 10 mg/kg, and reduced at higher doses. Pregabalin did not bind to mouse, rat, monkey, or human plasma proteins.

The genotoxic potential of pregabalin was evaluated in a series of *in vitro* and *in vivo* tests. Pregabalin was not mutagenic up to 5000 µg/plate in *S. typhimurium* and *E. coli* in the absence and presence of metabolic activation prepared from B6C3F1 or CD-1 mouse or Wistar rat liver. In mammalian cells *in vitro*, mutation and structural chromosome aberration frequency were not increased up to 1600 µg/mL with or without metabolic activation. Single doses of pregabalin up to 2000 mg/kg to B6C3F1 or CD-1 mice and Wistar rats did not induce unscheduled DNA synthesis in hepatocytes. The micronucleus frequency was not increased in bone marrow from B6C3F1 or CD-1 mice or Wistar rats given single oral doses of pregabalin up to 2000 mg/kg. Based on negative findings in all the studies conducted, pregabalin does not exhibit genotoxic or DNA-damaging potential.

B. Pharmacologic activity

Pregabalin is structurally related to the naturally occurring amino acids L-leucine and γ-aminobutyric acid (GABA). However, it is not active at $GABA_A$, $GABA_B$, or benzodiazepine receptors and it does not alter GABA degradation or acutely change GABA uptake in brain tissue. Like gabapentin (Neurontin®), pregabalin binds with high affinity to the α_2-δ auxiliary subunit of voltage-gated calcium channels. This subtle pharmacological alteration conceivably translates into a significant inhibition of neuronal calcium influx and subsequent calcium-dependent neurotransmitter release (Dooley et al., 2002). Analgesic, anxiolytic-like, and anticonvulsant actions of pregabalin are reduced in mutant mice with defective drug binding to α_2-δ Type 1 protein. These findings support the hypothesis that the extent of binding of pregabalin to α_2-δ protein predicts the degree of pharmacological activity *in vivo*. Furthermore, the data with genetically altered mice suggest that binding at the α_2-δ site is a primary mechanism of pregabalin that is necessary for pharmacological activity in animal models.

Pregabalin potently displaces [^{3}H]gabapentin binding to pig brain membranes *in vitro* (IC_{50} value of 0.037 µM or 0.006 µg/mL. Binding of [^{3}H]gabapentin to recombinant α_2-δ protein is inhibited by pregabalin with K_i values of 0.042 µM for Type 1 α_2-δ protein cloned from pig brain and 0.044 µM for Type 2 α_2-δ protein cloned from human brain.

C. Nonclinical safety issues relevant to clinical use

Dermatopathy

Skin lesions were seen in repeated-dose toxicology studies in both rats and monkeys. They are characterized grossly by a spectrum of lesions ranging from erythema to necrosis, and histologically by hyperkeratosis, acanthosis, fibrosis, and or necrosis of the tail. In rats, the incidence of lesions began to increase in oral repeated-doses studies ≥ 50 mg/kg, with associated $AUC_{(0-24)}$ ≥ 241 µg·hr mL. Lesions typically appeared within the first 2 weeks of treatment at higher doses and resolved in most affected animals by Week 7 in the 13-week study and by Week 4 in the 52-week study. Similar skin lesions were observed in monkeys in oral repeated-dose studies. Skin alterations were prominent and common at ≥ 500 mg kg almost exclusively on the tail; one female at 500 mg/kg for 4 weeks also had skin sores on the hindpaws. In the chronic monkey study, lesions were observed at ≥ 25 mg kg, with plasma pregabalin $AUC_{(0-24)}$ values ≥ 219 µg·hr/mL. Tail amputation was necessary in 5 of 30 monkeys at > 500 mg/kg. To a lesser extent than in rats, lesions in affected monkeys sometimes healed prior to study termination. Subcutaneous tail temperature, used as an indirect measure of tail blood flow in the chronic monkey study, showed no consistent differences between control and high-dose animals, or between affected and unaffected animals within the same group. Pregabalin at 5% and 7.5% did not induce contact sensitization (allergic dermatitis) in rats in the local lymph node assay, suggesting the lack of an immune-mediated mechanism. To date, the etiology of the skin lesions in rats or monkeys remains unknown. No tail dermatopathy was observed in mice given repeated oral doses of pregabalin up to 13 g/kg up to 13 weeks. However, missing tail tips were observed in mice given up to 5000 mg/kg ($AUC_{(0-24)}$ of 3150 µg·hr/mL) in the B6C3F1 but not the CD-1 carcinogenicity study. The relationship of this lesion in B6C3F1 mice to the dermatopathy in rats and monkeys is not clear.

The clinical therapeutic dose range of 150 to 600 mg/day (3 to 12 mg/kg/day based on body weight of 50 kg) yields a pregabalin exposure ($AUC_{(0-24)}$ of 123 µg·hr/mL). As such, there is only a 2-fold safety margin in rats and monkeys for the dermatological changes. The more severe dermatopathies (Figures below) involving necrosis (not reversible) occurs at ≥ 500 mg/kg in both rats and monkeys. These doses are associated with AUC's of around 1300 and 1000 µg·hr/mL in rats and monkeys, respectively, and provide a safety margin of approximately 8 to 10-fold.

Figure: Images of dermatopathies from 4-week oral rat and 13-week oral monkey

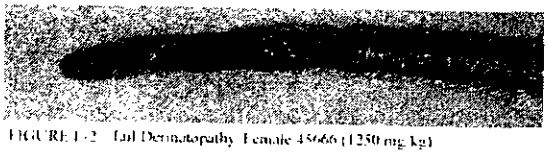


FIGURE I-2 Tail Dermatopathy Female 45666 (1250 mg/kg)

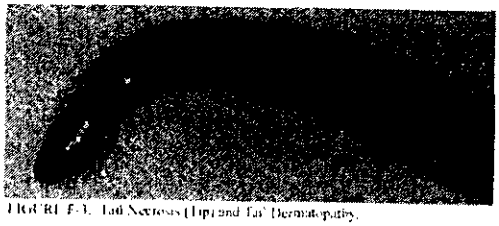


FIGURE I-3: Tail Necrosis (Tip) and Tail Dermatopathy

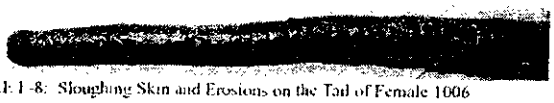


FIGURE I-8: Sloughing Skin and Erosions on the Tail of Female 1006 (500 mg/kg) on Day 15

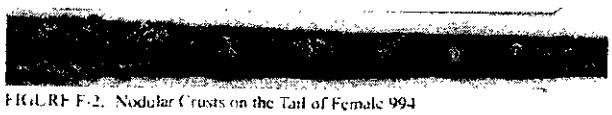
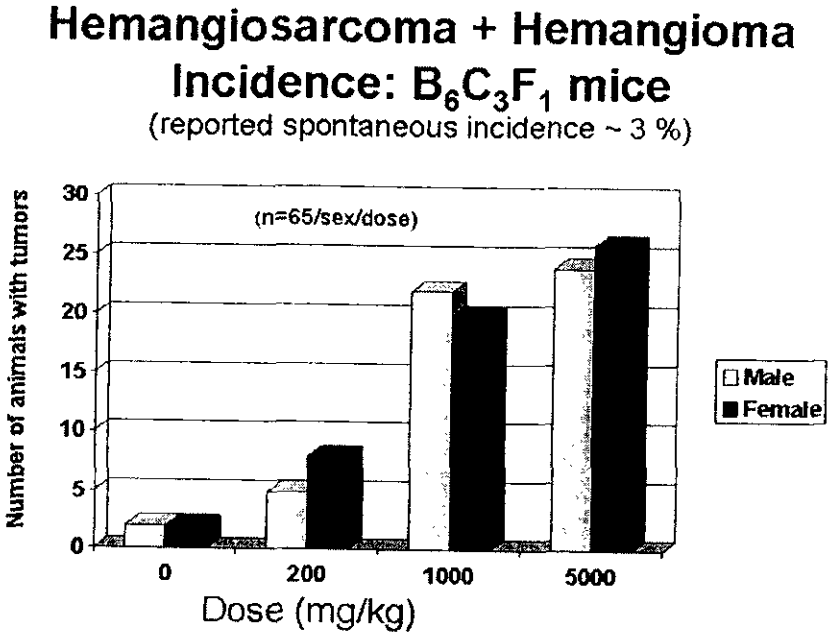


FIGURE F-2: Nodular Crusts on the Tail of Female 994 (100 mg/kg) on Day 89

Carcinogenicity

A total of 4 definitive lifetime carcinogenicity bioassays were performed: 2 in different strains of mice (B6C3F1 and CD-1) and 2 in Wistar rats. These studies are adequate from a regulatory standpoint (the initial studies in B6C3F1 mice and Wistar rats were evaluated by the Exec-CAC). Both mouse studies demonstrate dose-related increases (doses of 200, 1000, and 5000 mg/kg) in hemangiomas and hemangiosarcomas. While the 200 mg/kg dose was not considered statistically significant, the incidence was greater than controls and it was on the dose-response curve. At this dose in B6C3F1 mice, AUC exposure is 140 – 153 µg·hr/mL, providing no safety margin for clinical exposures of 123 µg·hr/mL (Figure below).



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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

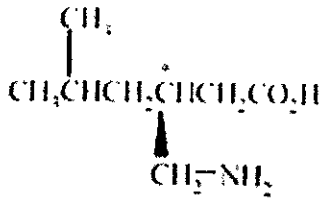
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-446
Review number: 1
Sequence number/date/type of submission: 000
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Pfizer, Inc.
Manufacturer for drug substance: Pfizer Global Manufacturing, 188 Howard Avenue, Holland, MI 49424-6596, and County Cork, Ireland

Reviewer name: Jerry M. Cott, Ph.D.
Division name: Division of Anesthetic, Critical Care and Addiction Drug Products
HFD #: 170
Review completion date: 5/24/04

Drug:

Trade name: Lyrica™
Generic name: Pregabalin
Code name: CI-1008 and PD 0144723
Chemical name: (S)-3-(Aminomethyl)-5-methylhexanoic acid
CAS registry number: 148553-50-8
Molecular formula/molecular weight: $C_8H_{17}NO_2$; MW: 159.23
Structure:



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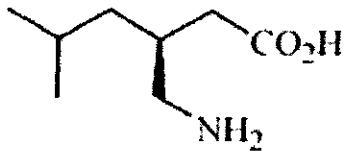

Pregabalin Stereo Structure

Relevant INDs/NDAs/DMFs:

Application	Indication
[redacted]	
N 021723	Neuropathic Pain Associated With Herpes Zoster (Postherpetic Neuralgia)
N 021724	Treatment Of Epilepsy
[redacted]	Treatment Of Generalized Anxiety Disorder

Drug class: Anticonvulsant

Indication: Neuropathic pain associated with diabetic peripheral neuropathy

Clinical formulation: The contents of each LYRICA capsule for oral use are 25, 50, 75, 100, 150, 200, 225, or 300 mg pregabalin, lactose monohydrate, cornstarch, and talc. The capsule shells contain gelatin and titanium dioxide. In addition, the orange capsule shells contain red iron oxide and the white capsule shells contain sodium lauryl sulfate and colloidal silicon dioxide. Colloidal silicon dioxide is a manufacturing aid that may or may not be present.

Route of administration: Oral

Proposed use: Pain associated with diabetic peripheral neuropathy

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

Studies reviewed within this submission:

Pharmacology Studies reviewed - See Appendix I listing. Most pharmacology studies were reviewed with the initial IND submitted to HFD-120 by T.D. Steele (review attached as Appendix II). These studies and reviews were referred to in order to compile the pharmacology section of the NDA but are usually not referred to individually.

Toxicology Studies: The following toxicology studies were reviewed with the initial IND by T.D. Steele (Appendix II) and are not summarized individually in this NDA:

Type of Study	Species/Strain	Mode of Administration	Duration	Dose (mg/kg)	Report Number
Single-Dose Toxicity	Mouse/B6C3F1	Gavage		5000	250-01674
	Rat/Wistar	Gavage		5000	250-01667
	Mouse/B6C3F1	Intravenous		300	250-01678
	Rat/Wistar	Intravenous		300	250-01675
Repeat-Dose Toxicity Nonpivotal	Rat/Wistar	Gavage or Diet	2 Weeks	500, 1250, 2500	250-01702
	Monkey/	Gavage	11 Days	Escalating 50-2000	745-02116
	cynomolgus	Gavage	2 Weeks	100, 500, 1000, 2000	250-01713
		Gavage	4 Weeks[a]	100, 1000, 2000	250-01720
		Gavage	4 Days	500, 750, 1000, 500 BID	745-02268
				Supportive TK	764-02188
	Rat/Wistar	IV Bolus	17 Days	50, 150, 300	250-01803
		IV Bolus	4 Weeks	40, 100, 300	250-01812
				Supportive TK	764-03163
		Cont Infusion	7 Days	3, 15, 75 mg/kg/hr	250-01800
		Cont Infusion	2 Weeks	3, 15, 75 mg/kg/hr	250-01818
				Supportive TK	764-03200
	Monkey/	IV Bolus	4 Weeks	Escalating 5-400	745-02970

	cynomolgus	IV Bolus	4 Days	10, 40, 200	745-03033
				Supportive TK	764-03162
		Cont Infusion	24-96 Hrs	2, 4, 6, 8 mg kg/hr	250-01801
		Cont Infusion	2 Weeks	2, 4, 6 mg/kg/hr	250-01817
				Supportive TK	764-03198

BID = Twice daily; IV = Intravenous; TK = Toxicokinetics.
* Dosing discontinued after a single dose due to death at high doses; surviving animals observed for 1 week and returned to the stock colony.

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The following toxicology studies are summarized in this NDA. Some of them were reviewed by T.D. Steele with original IND, are reproduced within, and are indicated as such. Carcinogenicity and reproduction studies were reviewed by Ed Fisher in HFD-120 (review attached as Appendix III). Others studies are new with this NDA. The Carcinogenicity studies regarding the occurrence and mechanism of hemangiosarcomas were reviewed by Terry Peters, D.V.M., and are attached as Appendix IV).

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report Number
Repeat-Dose Toxicity, Pivotal	Rat/Wistar	Diet	4 Weeks	500, 1250, 2500, 5000	250-01722
		Diet	4 Weeks	50, 100, 250	250-01730
		Diet	13 Weeks	50, 250, 500, 1250	745-02570
		Diet	52 Weeks	50, 250, 500	745-02683
	Monkey/	Gavage	4 Weeks	25, 50, 100, 500, 500 BID	745-02329
	cynomolgus	Gavage	13 Weeks	10, 25, 100, 500	745-02345
					745-02559
		Gavage	Chronic[b]	0, 25, 100, 250/500[c]	745-02646
Supplemental Report to Chronic Study in Monkeys			Bone Marrow Megakaryocytes And Peripheral RBC/Platelet Morphology		745-03746
Genotoxicity	*S. typhimurium* and *E. coli*	*In vitro* (Mouse S9[d])	312.5-5000 µg/plate		745-03418
	S.typhimurium	*In vitro* (Rat S9)	200-3200 µg/plate		745-02035
	S. typhimurium	*In vitro* (Rat S9)	312.5-5000 µg/plate		745-03320
	E. coli	*In vitro* (Rat S9)	0.996-4980 µg/plate		745-03203
	Point Mutation	*In vitro* (Rat S9)	1200-1600 µg/mL		745-02308
	SCA	*In vitro* (Rat S9)	160-1600 µg/mL		745-02393
	Mouse[d] - UDS	Gavage	One Dose 500, 718, 1510, 2000		745-03455
	Mouse[d] – MN	Gavage	One Dose 500, 1000, 2000		745-03387
	Rat[c] - UDS	Gavage	One Dose 250, 1000, 2000		745-02209
	Rat[c] - MN	Gavage	One Dose 500, 1000, 2000		745-02374

[b] Animals given 10, 25, or 100 mg/kg for 13 weeks then continued at same doses for an additional 52 weeks (Week 65); some animals given drug for up to 56 weeks due to clinical pathology scheduling (Weeks 65 to 69).
[c] Animals given 250 mg/kg for 13 weeks before dose escalation to 500 mg/kg for an additional 52 weeks (Week 65).
[d] B6C3F1 and CD-1 strains

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report #
Carcinogenicity Nonpivotal	Mouse/B6C3F1	Diet	2 Weeks	1%, 3%, 5% of Diet[f]	250-01721
			4 Weeks	100, 500, 2500	250-01768
			13 Weeks	1000, 4000, 8000	250-01744
Carcinogenicity Pivotal	Mouse/B6C3F1	Diet	104 Weeks	200, 1000, 5000	745-03275
	Mouse/CD-1	Diet	104 Weeks	200, 1000, 5000	745-03610
	Rat/Wistar	Diet	104 Weeks	(M) 50, 150, 450 (F) 100, 300, 900	745-03274
	Rat/Wistar	Diet	104 Weeks	(M) 50, 150, 450 (F) 100, 300, 900	745-03808
Carcinogenicity Supplemental Reports to 104-Week Study in B6C3F1 Mice			Reevaluation of Nonneoplastic Findings		745-03454
			Bone Marrow Megakaryocytes		745-03456

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report #
Carcinogenicity Supplemental Report to 104-Week Study in CD-1 Mice			Bone Marrow Megakaryocytes		745-03692
Carcinogenicity Supplemental Report to 104-Week Study in B6C3F1 and CD-1 Mice			Reevaluation of Peripheral Blood Morphology		745-03714
Carcinogenicity Supportive Toxicokinetic	Mouse/B6C3F1	Diet	4 Weeks	200, 1000, 5000	745-03239
				Toxicokinetic Report	764-03533
			104 Weeks	Single Time Point Report	764-03532
	Mouse/CD-1	Diet	4 Weeks	200, 1000, 5000	745-03556
				Toxicokinetic Report	764-04020
			104 Weeks	Single Time Point Report	764-04054
	Rat/Wistar	Diet	4 Weeks	(M) 50, 150, 450 (F) 100, 300, 900	745-03238
				Toxicokinetic Report	764-03534
			104 Weeks	Single Time Point Report	764-03486
	Rat/Wistar	Diet	104 Weeks	Single Time Point Report	764-03964
C			J		

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report #
Carcinogenicity Investigative Studies					
H. hepaticus DNA	Tissue from 104 Week Study in B6C3F1 Mice				745-03324
Structural Analysis	Pregabalin and N-methyl pregabalin queried in C J databases				745-03407
Gene Mutations	Tissue from 104 Week Study in B6C3F1 Mice				745-03327
Megakaryopoiesis	*In vitro* in Mouse Bone Marrow Cultures			10, 100, 1000 µg/mL	745-03461
Proliferation	*In vitro* in Mouse Endothelial Cell Cultures			10, 100, 1000 µg/mL	745-03462
Proliferation	*In vitro* in Mouse Endothelial Cell Cultures			10, 100, 1000 µg/mL	745-03769
Vascular Growth	*In vitro* in Mouse Aortic Ring Cultures			1, 10, 50, 100, 200 µg/mL	745-03398
NO Synthase	*In vitro* Mouse Endothelial/Bone Marrow Cultures			1-1000 µg/mL	745-03834
Platelet Function	*In vitro* in Mouse and Rat Plasma/WB			10, 100, 500 µg/mL	745-03566
Membrane Binding	*In vitro* in Selected Mouse and Rat Tissues			Animals Untreated	745-03740
Platelet Survival	*In vivo* in B6C3F1 Mouse			1000 mg/kg	250-01886
Proliferation	*In vivo* in B6C3F1+ CD-1 Mouse Endothelial			(M) 1000 mg/kg	745-03459
Exploratory	Mouse/B6C3F1	Diet	1-3 Months	(F) 1000	745-03460
				Proliferation Reevaluation	745-03835
				Gene Expression (1-3 Mo.)	745-03739
Exploratory			4-28 Days	(F) 1000	745-03428
Exploratory			6-24 Months	(F) 50, 200, 1000	745-03657
					745-03832
				Immunohistochemistry	745-03855
Exploratory			1 Month	(F) 50, 200, 1000	745-03660
Exploratory			1 Month	(F) 5000 mg/kg	745-03770
					764-04172
Exploratory			6 Days	(F) 750 mg/kg	745-03762
Exploratory	CD-1	Diet	3-12 Months	(F) 50, 200, 100	745-03659
Exploratory			1-6 Months	(M + F) 5000	745-03658
					745-03766
Exploratory	Rat/Wistar	Diet	1-18 Months	(F) 900	745-03463
					745-03763
					764-04171
Exploratory			1 Month	(F) 900	745-03771

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report
Reproductive and Developmental Toxicity	Rat/Wistar	Gavage	(M)[g]	250, 1250, 2500	745-02359
			(M)[g]	50, 100, 250	745-02829
			(F)[h]	500, 1250, 2500	745-02261
				Supportive TK	764-02126
	Mouse/CD-1	Gavage	(F)G6-G15	500, 1250, 2500	745-02273
	Rat/Wistar	Gavage	(F)G6-G17	500, 1250, 2500	745-02271
				Supportive TK	764-02131
	Rabbit NZW	Gavage	(F)G6-G20	250, 500, 1250	745-02285
	Rat/Wistar	Gavage	(F)G6-L20	50, 100, 250, 1250, 2500	745-02628
Juvenile Animals Single-Dose	Rat/Wistar	Gavage	One Dose[i]	500, 1250, 2500	745-03151
Juvenile Animals Pivotal	Rat/Wistar	Gavage	7 Weeks[j]	100, 250, 500	745-03294
				Supportive TK	764-03578
			7 Weeks[k]	50, 250, 500	745-03323
				50, 100, 250, 500	745-03794
			(M+F)[l]	50, 250, 500	745-03267
				Supportive TK	764-03579
			(F)[l]	50, 250, 500	745-03471
Supportive Toxicokinetic	Rat/Wistar	Gavage Juvenile	3 Weeks	50, 100, 250, 500	745-03375
				Supportive TK	764-03888
		Gavage Adult	3 Weeks	50, 100, 250, 500	745-03376
				Supportive TK	764-03887

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report #
Local Tolerance	Rabbit/NZW	Intravenous	5 Days	12 mg/min	745-02886
	Human Plasma/Whole Blood Compatibility & Human Erythrocyte Fragility			*In vitro* 0.2-10 mg/mL *In vitro* 4 mg/mL	745-02893
Antigenicity/Immunotoxicity					
Local Lymph Node	Rat/Wistar	Topical	4 Days	5%, 7.5%	745-03326
General Toxicity Investigative Studies					
Hematology and Platelet Function	Rat/Wistar	Gavage	14-18 Days	50-1562.5	250-01802
Platelet Function	Rat/Wistar	Diet	2 Weeks	500, 1250, 2500	745-03312
Microvascular	Rat/Wistar	Intradermal	One Dose	1.59, 15.9, 159	745-03317
Permeability					
Time-Course	Monkey/	Cont Infusion	96 Hours	6 mg/kg/hr	250-01888
Dermal Toxicity	cynomolgus			Supportive TK	764-03694
Reproductive Toxicity Investigative Studies					
Time/Mechanistic Evaluation of Sperm	Rat/Wistar	Gavage	3-6 Weeks	2500	745-02809
					745-02994
Sperm Motility/Morph	Rat/Wistar	*In vitro*		1600, 3200 µg/mL	745-02517
Male Embryofetal Development	Rat/Wistar	Gavage	(M)[g]	100, 250, 500	745-03322
				Supportive TK	764-03716
Skull Development	Rat/Wistar	Gavage	(F)G6-G17	50,100,250,500,1250,2500	745-03426
Skull Development Progression	Rat/Wistar	Gavage	(F)G6-G17	2500	745-03384
Skull Dev on PN21	Rat/Wistar	Gavage	(F)G6-G17	2500	745-03321
Fetal Development	Rat/Wistar	Gavage	(F)[n]	2500	745-02656
Dose Range-Finding	Rabbit/NZW	Gavage	2 Weeks	(M) 250, 750, 1250	745-03325

Type of Study	Species/Strain	Administration	Duration	Dose (mg/kg)	Report #
Studies with Impurities					
PD 0144550[o]	Rat/Wistar	Gavage[q]	13 Weeks	0.1, 0.5, 2.5	250-01833
				Supportive TK	764-03384
	S. typhimurium and *E. coli*	*In vitro*		100-5000 µg/plate	745-03197
PD 0147804[p]	Rat/Wistar	Gavage[q]	4 Weeks	0.5, 5, 10	250-01787
					745-02838
	S. typhimurium and *E. coli*	*In vitro*		312.5-5000 µg/plate	745-02952
PD [redacted]	Rat/SD	Gavage	One Dose	2000	901-00517
	Rabbit/NZW	Dermal	One Dose	2000	901-00542
	Rabbit/NZW	Dermal	4 Hours	500 mg	901-00520
	Rabbit/NZW	Ocular	One Dose	~35 mg	901-00508
	Guinea	Dermal	Induction/ Challenge	300 mg	901-00529
	Pig/Hartley *S. typhimurium*	*In vitro*		33-10000 µg/plate	901-00599
PD [redacted]	Rat/SD	Gavage	One Dose	2000	901-00717
	Rabbit/NZW	Dermal	One Dose	2000	901-00718
	Rabbit/NZW	Dermal	4 Hours	500 mg	901-00719
	Rabbit/NZW	Ocular	One Dose	~38 mg	901-00720
	Guinea Pig/ Hartley	Dermal	Induction/ Challenge	0.5 mL bulk drug	901-00721
	S. typhimurium and *E. coli*	*In vitro*		100-5000 µg/plate	901-00660

Type of Study	Report #
Critical Assessments	
Critical Assessment of Pregabalin Rat Carcinogenicity Studies	745-03710
Pregabalin Rodent Carcinogenicity Studies - Critical Assessment and Weight of Evidence	745-03370
Pregabalin Rodent Carcinogenicity Studies – Report on Vascular Neoplastic Findings in Mice	745-03221
Pregabalin Carcinogenicity and Tumor Mechanism Studies - Current Studies	745-03607
Pregabalin Rodent Carcinogenicity and Tumor Mechanism Studies – Current Status	745-03754
Assessment of Rodent Carcinogenicity, Mode of Action of Hemangiosarcoma Formation in Mice, and Human Relevance	745-03856
Critical Assessment of Skull Bone Findings in the Oral Teratology Study in Rats	745-03337
Critical Assessment of Effects on the Male Reproductive System	250-01790
Evaluation of Pregabalin (CI-1008) Toxicokinetic Data from Male Rat Fertility Studies	764-03029
Retrospective Study Reviews	
Histopathologic Evaluation of Kidney in Rats from 4-, 13-, and 52-Week Studies	745-03280
Histopathologic Evaluation of Eyes in Rats from 13- and 52-Week Studies	745-03298
Histopathologic Evaluation of Testes in Rats from a 52-Week Study	745-03359
Histopathologic Evaluation of Tissues for Vascular Proliferative Lesions in B6C3F1 Mice and Cynomolgus Monkeys	745-03431
Retrospective Evaluation of Hepatic Sinusoidal Endothelial Cells in Cynomolgus Monkeys Chronically Treated with Pregabalin	745-03828
Retrospective Histopathologic Evaluation of Eyes from Cynomolgus Monkeys Chronically Treated with Pregabalin	745-03852

Type of Study	Report #
Histopathologic Review of Skin And Mucous Membranes in Cynomolgus Monkeys from a 2-Week Continuous Infusion Study	745-02999
Updated Historical Data	
Updated Historical Control Data for Number of Days to Mating in Wistar Rats	745-03457

Pharmacokinetic Studies reviewed for this NDA are listed below. While they were reviewed for the preparation of this NDA they are not necessarily specified by study number:

Type of Study	Test System	Administration	Reference #
Absorption			
PK and Bioavailability	Mouse/B6C3F1	Gavage, IV	RR 764-03880
PK and Bioavailability	Rat/Wistar	Gavage, IV	RR 764-02203
Dose Proportionality	Rat/Wistar	Gavage	RR 764-02204
PK and Bioavailability	Monkey/Cynomolgus	Gavage, IV	RR 764-02299
Intestinal Perfusion	Rat (In situ)	In situ perfusion	RR 764-03670
Distribution			
SD WBA	Mouse/B6C3F1	Gavage	RR 764-03718
SD WBA	Rat/Wistar	Gavage	RR 764-02227
SD WBA	Rat/Wistar	Gavage	RR 764-02359
SD WBA	Monkey/Cynomolgus	Gavage	RR 764-02352
Plasma Protein Binding	Mouse/B6C3F1	*In vitro*	RR 764-02321
Plasma Protein Binding	Rat, Monkey, Human	*In vitro*	RR 764-02316
Distribution into Milk	Rat (lactating)	Gavage	RR-MEMO 764-02291
Red Blood Cell Distribution	Mouse/Rat/Monkey/Dog/Human	*In vitro*	RR 764-03885
Metabolism			
Metabolite ID in Urine	Mouse/B6C3F1	Dietary	RR 764-02681
Metabolite ID in Urine	Mouse/B6C3F1	Gavage	PSM 00157
Comparative Metabolite ID in Urine	Mouse/B6C3F1 and CD-1	Gavage	PSM 00272
Metabolite Profiling in Urine	Rat/Dog/Monkey	Gavage	RR 764-02225
Mass Balance	Rat/Wistar	Gavage	RR 764-03127
Metabolite ID in Urine	Dog/Beagle	Gavage	RR 764-02260
Mass Balance	Monkey/Cynomolgus	Gavage	RR 764-03395
Comparative Biotransformation	Rat, Dog, Monkey, Human	*In vitro*	RR 764-02235
Comparative Biotransformation	Rat, Dog, Monkey, Human	*In vitro*	RR 764-03070
In vivo Racemization of Pregabalin	Mouse/Rat/Rabbit/Monkey	*In vivo*	RR 764-02317
In vivo Racemization of PD 0144550	Rat	*In vivo*	RR 764-03384
Excretion			
Mass balance	Rat/Wistar	Gavage	RR 764-03127
Mass balance	Monkey/ Cynomolgus	Gavage	RR 764-03395

Other Pharmacokinetic Studies			
Toxicokinetics	Mouse/B6C3F1	Diet	RR-MEMO 764-02732
Toxicokinetics	Mouse/CD-1	Gavage	RR-MEMO 764-02130
Toxicokinetics	Rat	Gavage or Diet	RR-MEMO 764-02134
Toxicokinetics	Rat/SPF	Diet	RR-MEMO 764-02251
Toxicokinetics	Rat/Wistar	Gavage	RR-MEMO 764-02888
Toxicokinetics	Rat/Wistar	Gavage	RR-MEMO 764-02131
Toxicokinetics	Rat/Wistar	Diet	RR-MEMO 764-02632
Toxicokinetics	Monkey/Cynomolgus	Gavage	RR-MEMO 764-02740

Studies not reviewed within this submission:
All relevant preclinical carcinogenicity data have been reviewed previously and are discussed in the following documents:

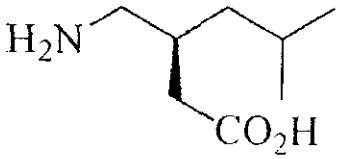
A. Pharmacology/Toxicology (HFD-120) review of initial rat and mouse carcinogenicity studies
B. Statistical review of mouse carcinogenicity studies
C. Exec-CAC evaluation of initial rat and mouse carcinogenicity studies
D. FDA Pharm/Tox consultant's review of carcinogenicity and investigative studies

All Reproductive Toxicology studies were reviewed by Edward Fisher in HFD-120 and are in a separate report dated 3/24/04 and included as Appendix III.

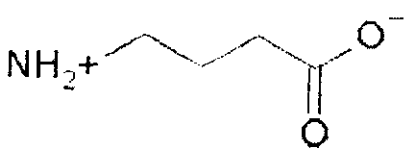
2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

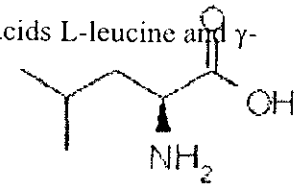
Pregabalin is structurally related to the naturally occurring amino acids L-leucine and γ-aminobutyric acid (GABA).



Pregabalin



GABA



Leucine

However, it is not active at GABAA, GABAB, or benzodiazepine receptors and it does not alter GABA degradation nor acutely change GABA uptake in brain tissue. Like gabapentin (Neurontin®), pregabalin binds with high affinity to the α2-δ auxiliary subunit of voltage-gated calcium channels. This subtle pharmacological alteration conceivably translates into a significant inhibition of neuronal calcium influx and subsequent calcium-dependent neurotransmitter release (Dooley et al., 2002). Analgesic, anxiolytic-like, and anticonvulsant actions of pregabalin are reduced in mutant mice with defective drug binding to α2-δ Type 1 protein. These findings support the hypothesis that the extent of binding of pregabalin to α2-δ protein predicts the degree of pharmacological activity *in vivo*. Furthermore, the data with genetically altered mice suggest that binding at the α2-δ site is a primary mechanism of pregabalin that is necessary for pharmacological activity in animal models.

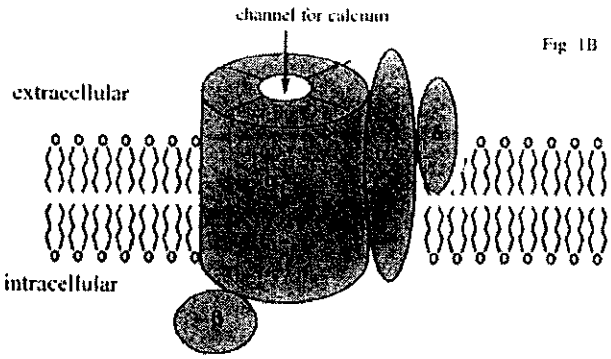
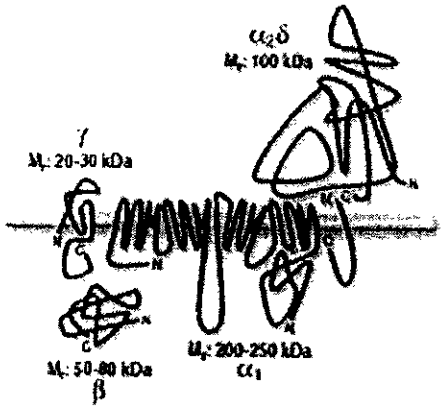


Fig 1B



Pregabalin potently displaces [^{3}H]gabapentin binding to pig brain membranes *in vitro* (IC_{50} value of 0.037 μM or 0.006 μg/mL. Binding of [^{3}H]gabapentin to recombinant α_2-δ protein is inhibited by pregabalin with K_i values of 0.042 μM for Type 1 α_2-δ protein cloned from pig brain and 0.044 μM for Type 2 α_2-δ protein cloned from human brain. Therefore, pregabalin binds with equal affinity to both subtypes of α_2-δ protein, and does not show selectivity.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Pregabalin is structurally related and also pharmacologically related to gabapentin. A specific binding interaction of [^{3}H]gabapentin with isolated rat brain membranes has been described and the binding site protein has been isolated using biochemical methods and identified as identical to the α_2-δ protein, an auxiliary part of the multi-protein assembly that comprises voltage-gated calcium channels. The α_2-δ protein is associated with cell membranes in excitable cells and is found in brain tissue, striated muscle, smooth muscle, and cardiac muscle. There are at least 3 distinct α_2-δ proteins, each coded by separate genes in mammals, but only Types 1 and 2 have high-affinity binding sites for [^{3}H]gabapentin.

[^{3}H]Gabapentin binding to α_2-δ proteins is fully displaced by unlabeled gabapentin and other 3-substituted GABA derivatives structurally related to gabapentin or pregabalin. Scatchard analysis of [^{3}H]gabapentin binding data to pig brain membranes fit a single binding site (regression coefficient R = 0.99) with an apparent affinity or K_d value close to 0.1 μM (0.016 μg/mL), and a density of binding sites or B_{max} value in neocortex brain tissues of approximately 9 pMol/mg protein (see table below).

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration	Gender and No per Group or No of Observations	Noteworthy Findings	GLP Compliance	Report No.
Brain (Membrane Homogenate)	Rat/Sprague-Dawley	In vitro	10^{-9} M to 10^{-4} M in ½ log intervals	Duplicate data points repeated at least 3 times	[^{3}H]Gabapentin labels specific site in rat brain membrane homogenates; binding was displaced by pregabalin and derivatives; IC_{50} for pregabalin was approximately 100 nM.	No	740-03239
Brain (Membrane Homogenate)	Porcine/Domestic	In vitro	10^{-9} M to 10^{-4} M in ½ log intervals	Duplicate data points repeated at least 3 times	[^{3}H]Gabapentin and ^{3}H-L-leucine were displaced by pregabalin with similar affinity, IC_{50} value was approximately 80 nM.	No	Brown et al., ref. 2
(Membrane Homogenate)	Recombinant expressed porcine or human proteins	In vitro	10^{-9} M to 10^{-4} M in 1/3 log intervals	Duplicate data points repeated at least 3 times	[^{3}H]Gabapentin was displaced by pregabalin with inhibitory concentration constant (K_i) values of 42 nM for type 1 α_2-δ protein and 44 nM for type 2 α_2-δ protein.	No	740-03602
(Membrane Homogenate)	Recombinant expressed porcine protein	In vitro	10^{-9} M to 10^{-4} M in 1/3 log intervals	Duplicate data points repeated at least 3 times	[^{3}H]Pregabalin had saturable binding by Scatchard plot analysis with equilibrium binding constant (K_d) value of 33 nM.	No	740-03614
Brain (Membrane Homogenate)	Mouse/F1 hybrid between 129/Svj and C57/Bl6 inbred strains	In vitro	3×10^{-9} M to 6.5×10^{-5} M in 1/5 log intervals	Triplicate data points repeated at least 3 times	K_d for [^{3}H]gabapentin binding to brain membranes was 23 nM in wildtype mice, and 227 nM in mutant mice defective in binding of [^{3}H]gabapentin to α_2-δ type 1.	No	740-03603
Brain (Membrane Homogenate)	Porcine/Domestic	In vitro	3×10^{-9} M to 1×10^{-5} M in ½ log intervals	Duplicate data points repeated at least 3 times	Activity of compounds in anxiety, pain or seizure tests in vivo was related significantly to potency for displacement of [^{3}H]gabapentin binding.	No	740-03576

IC_{50} = Concentration inhibiting response by 50%; K_i = Equilibrium dissociation constant for inhibitor (adjusted for radioligand concentration); K_d = Equilibrium dissociation constant of radioligand (concentration producing roughly 50% saturation of binding).

The [^{3}H]gabapentin binding site is localized heterogeneously in a number of regions of rat brain, particularly in dendritic areas of the neocortex, hippocampus, and molecular layer of the cerebellum. Additional studies show that [^{3}H]gabapentin binding is displaced stereospecifically by endogenous amino acids (e.g., L-leucine, L-isoleucine, L-methionine, L-valine, L-phenylalanine). Conversely, this binding site also is labeled specifically with [^{3}H]L-leucine. Thus, [^{3}H]L-leucine binding to brain membranes is displaced by either gabapentin or pregabalin.

Requirement of the α_2-δ site: Several findings suggest that binding activity of pregabalin at the α_2-δ site is required for pharmacological actions. The enantiomer of pregabalin (PD 0144550) is 20-fold less potent than pregabalin for displacement of [^{3}H]gabapentin binding. PD 0144550 also is less potent than pregabalin for inhibition of glutamate release from rat trigeminal nucleus slices and inhibition of calcium influx in depolarized rat brain synaptosomes. *In vivo*, PD 0144550 is virtually inactive in analgesic, anticonvulsant, and anxiolytic models with rodents. *In vivo* activity of pregabalin derivatives was usually not observed if the IC_{50} for [^{3}H]gabapentin binding was >300 nM. Furthermore, a comparison of 8 different pairs of 3-dimensional isomers (in addition to pregabalin and PD 0144550) consistently showed activity *in vivo* only with the isomer that displaced binding most potently. Results with 84 compounds that are structurally related to pregabalin indicate that potent binding activity at the α_2-δ site is sufficient to predict pharmacological activity *in vivo* (see figure below for correlation with carrageenan thermal hyperalgesia). Similar correlations were found for anticonvulsant and anxiolytic activity.

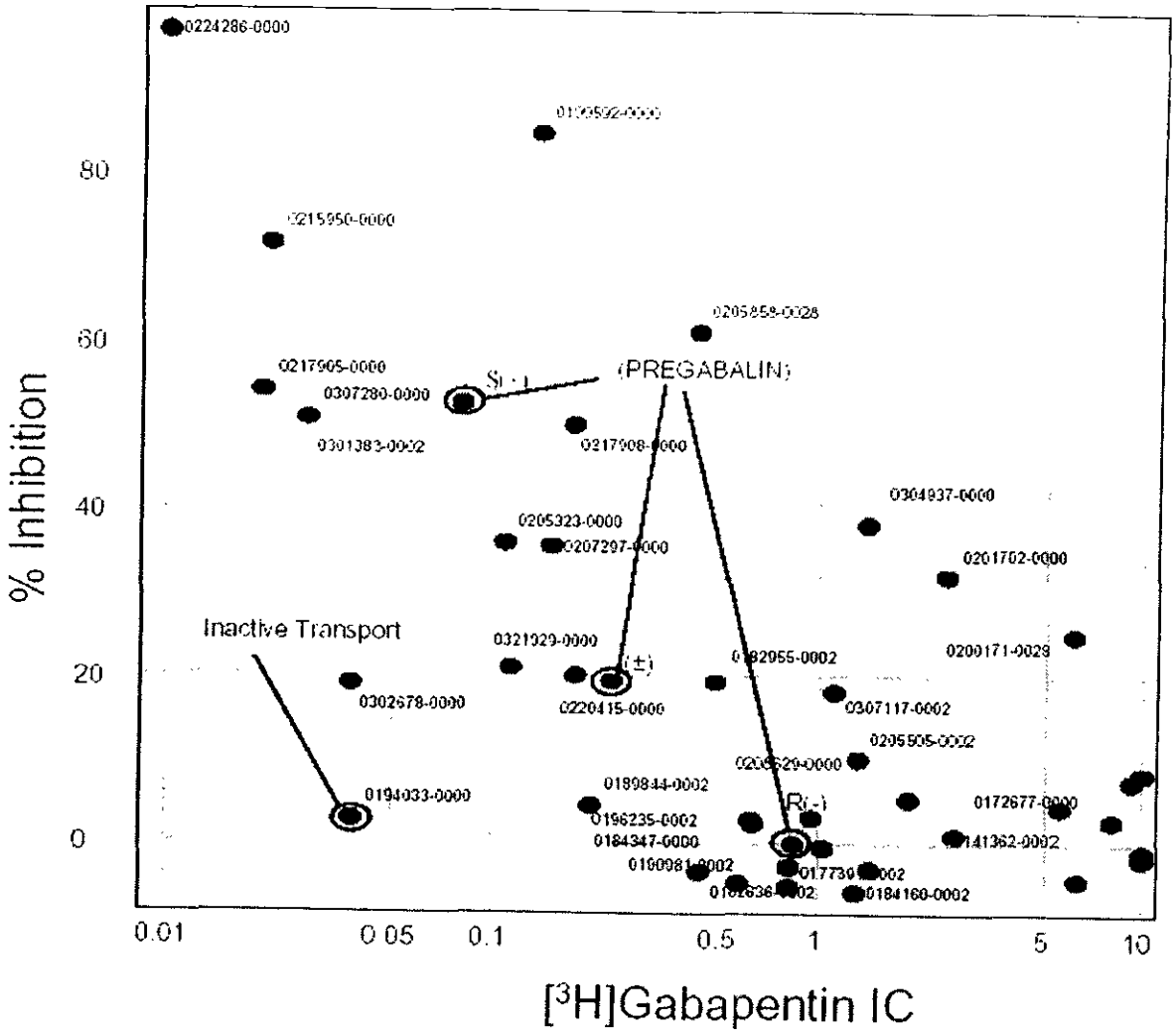


Figure 1. Relationship Between [^{3}H]Gabapentin Binding IC_{50} and Activity in the Carrageenan Thermal Hyperalgesia Test in Rats

Substance P and Glutamate: Substance P increased [^{3}H]glutamate release to 150% of control levels, and co-application of pregabalin 30 µM (4.8 µg/mL) reduced release back to control levels. Although the molecular mechanism of the inhibitory action of pregabalin on the release of glutamate is not known, it appears to require prior activation of second messenger pathways by activation of Substance P receptors. While it could be relevant for analgesic activity of pregabalin (via inhibition of voltage gated calcium channels), the concentration required is rather high.

Pregabalin also reduces release of various transmitters. The relevance of this is unclear since large concentrations are generally required for this action (See table below).

Organ Systems Evaluated	Species Strain	Method of Administration	Dose[a] (mg/kg) or Concentration	Gender and No. per Group or No. of Observations	Noteworthy Findings
Brain (Trigeminal Nucleus Tissue Slices)	Rat Hooded Lister	In vitro	30 µM (5 µg/mL)	4 assays repeated 4-7 times	Pregabalin had minimal effect on K^+-stimulated glutamate release in control conditions but reduced Substance P-enhanced K^+-evoked glutamate release by 56%.
Brain (Neocortex Tissue Slices)	Rat Sprague-Dawley	In vitro	100 µM (15 µg/mL)	9 or greater experiments	Pregabalin reduced K^+-evoked release of endogenous glutamate by 25%.
Brain (Neocortical and Striatal Tissue Slices)	Rat Sprague-Dawley	In vitro	0.3 to 1000 µM in $\frac{1}{2}$ log intervals	6 assays	Pregabalin reduced K^+-evoked [^{3}H]norepinephrine release from cortical slices with IC_{50} of 12 µM (2 µg/mL) and maximal inhibition of 30%. Effects of pregabalin and gabapentin did not add to one another
Brain (Neocortex, Cerebellum Hippocampus, Striatum or Spinal Cord Tissue Slices)	Rat Sprague-Dawley	In vitro	Various	6 assays	Pregabalin reduced K^+-evoked neurotransmitter release in a concentration-dependent manner, although changes in striatal tissues were not significant
Brain (Neocortex Tissue Slices)	Rat Sprague-Dawley	In vitro	100 µM	6 assays	Pregabalin had no effect on [^{3}H]norepinephrine release from cortical slices in the presence of 100 µM gabapentin
Brain (Striatum, Hippocampus)	Rat Long-Evans	IP	1 20 50 100	5-13 M	Pregabalin dose-dependently reduced the increase of L-3, 4-dihydroxyphenylalanine turnover (a measure of noradrenaline and dopamine synthesis) and also reduced 5-hydroxytriptophan turnover (a measure of serotonin synthesis), but only in animals pretreated with 3,4-diaminopyridine (a potassium channel blocker that increases brain activity)

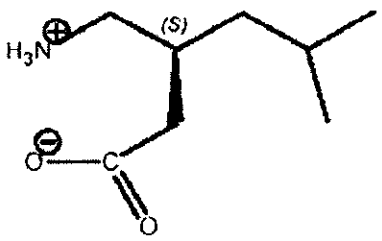
IC_{50} = Concentration inhibiting response by 50%. IP = Intraperitoneal

[a] Single dose unless specified otherwise

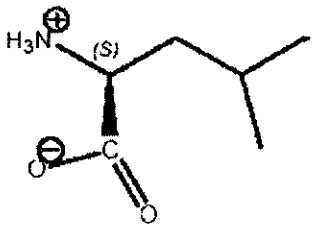
Effects on Substance P: Pregabalin was tested for changes in the release of the neuroactive peptides, Substance P and calcitonin gene-related peptide (CGRP), from isolated slices of rat spinal cord. In these studies, neuropeptides were detected with a specific radioimmunoassay, and release was triggered by application of capsaicin. Pregabalin had no effect on the release of Substance P or CGRP from tissues taken from rats without prior antigen treatment. However, if tissues were taken from rats with inflammation produced by prior peripheral injection of Freund's Complete Adjuvant, pregabalin reduced release of neuropeptides by approximately 50%.

Effects on Monoamines: Pregabalin (doses to 100 mg/kg IP) did not alter the basal accumulation of DOPA, but it caused a dose-related decrease in the enhanced accumulation of DOPA resulting from administration of 3,4-diaminopyridine (DAP) (DAP enhanced DOPA accumulation to approximately 30% above basal levels). This effect of pregabalin was statistically significant (compared to vehicle controls) ≥10 mg/kg IP. The accumulations of 5-HTP were not significantly altered by pregabalin, either with or without DAP. These results suggest that pregabalin may decrease stimulated monoamine turnover *in vivo*.

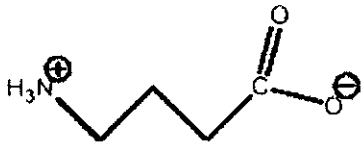
System L Amino Acid Transport in Vitro: Gamma-aminobutyric acid cannot cross most membrane barriers in the body because it mostly exists as the doubly charged form (charged at both the amine and acid moieties) at physiological pH (see figure below).



Pregabalin



L-Leucine



GABA

Specific transporter proteins have been described that are responsible for transporting metabolically important amino acids across membrane barriers. Pregabalin was tested for mutual competition with [^{3}H]L-leucine transport into primary cultures of Chinese hamster ovary (CHO) cells and rat neocortical neurons. Pregabalin completely blocked the influx of [^{3}H]L-leucine into CHO cells with an IC_{50} of 103 µM (17 µg/mL). Dixon plot analysis of this inhibition indicates that it is competitive in nature (K_I = 86 µM, 14 µg/mL). Furthermore, CHO cells that were previously loaded with [^{3}H]L-leucine had accelerated efflux of radiolabel in the presence of cold L-leucine, gabapentin or pregabalin, suggesting that all 3 amino acids share the same transport system, the system L type of transporter.

An additional study with [^{3}H]GABA in cultured astrocytes showed that pregabalin does not acutely inhibit or enhance GABA transport at concentrations up to 100 µM (16 µg/mL).

Neurokinin-1 Metabotropic Glutamate Receptors: Antagonists of neurokinin-1 (NK1) and Group I metabotropic glutamate receptors (mGluR) reduce pain-related behaviors in animal models of analgesia. To investigate whether the antihyperalgesic actions of pregabalin might be caused by interactions with protein kinases that are activated by mGluR receptors, studies were performed with recombinant cell systems *in vitro*. Pregabalin treatment of CHO cells expressing recombinant neurokinin-1 receptors decreased the activation of ELK signal transduction in a concentration-related manner. Similar results were obtained with activation of transfected mGluR5 receptors by a glutamate agonist. The effects of pregabalin were first significant at a concentration of 50 µM (8 µg/mL) and were approximately maximal with a concentration of 1.25 mM (200 µg/mL). Experiments are underway to further characterize potential upstream or downstream targets of gabapentin and pregabalin.

Drug activity related to proposed indication:
Dorsal Root Reflex Response in Rat Spinal Cord: (RR 770-00322) Because of its activity on behavioral responses related to allodynia, pregabalin was tested in anesthetized rats to measure efferent sensory nerve activity (action potentials propagating from the spinal cord to the periphery) in response to peripheral mechanical stimulation. This test system enhances sensory processing in the dorsal horn of the spinal cord by either paw injection of an immune stimulus (Freund's Complete Adjuvant) or by causing neuropathic pain from chronic constriction injury of the sciatic nerve. Both of these pretreatments cause the development of abnormal efferent activity (dorsal root reflex) in sensory nerves in response to pain-producing sensory stimuli. Stimulation was provided by either a pinch to a single toe or by application of calibrated von Frey filaments to the footpad. Intravenous treatment with pregabalin (3 mg/kg IV) did not alter nerve responses, but subsequent injection of a higher dose (10 or 30 mg/kg IV) reduced efferent activity by more than 50% in response to either stimulus. These results suggest that pregabalin reduces abnormal excitability in sensory nerve fibers that originate in the dorsal horn of the spinal cord, and this could be related to analgesic-like pharmacological actions.

Rat Model of Surgical Pain: Pregabalin, when given 1 hour before surgery, dose-dependently (3, 10, and 30 mg/kg SC) prevented hyperalgesia and allodynia with respective minimum effective doses of 3 and 10 mg/kg SC (RR 770-00296).

Substance P- or NMDA-Induced Hyperalgesia: Substance P and glutamate are co-transmitters utilized by pain-sensitive afferent neuron terminals in the spinal cord dorsal horn. Intrathecal injection via implanted catheters of Substance P (30 nmol) or the glutamate agonist N-methyl-D-aspartate (NMDA, 0.3 nmol) decreased hindpaw withdrawal latency in response to bright light irradiation. Pretreatment of rats with pregabalin given by intraperitoneal injection (1, 3, 10, 30, or 100 mg/kg IP, 60 minutes prior to Substance P) or intrathecally (3, 10, 30, or 100 µg, 15 minutes prior to Substance P) dose-dependently reduced thermal hyperalgesia (Partridge et al., Anesthesiology. 1998 88(1):196-205).

Hyperalgesia After Thermal Injury: Following a mild burn injury to the rat footpad (burn caused by application of the footpad to a 52°C hotplate for 45 seconds under halothane anesthesia), rats respond at a shorter than normal latency to intense light irradiation of the footpad. This heat hyperalgesia was reduced significantly in rats pretreated with pregabalin given intrathecally (100 or 300 µg/rat) with no change in thermal escape latency measured with the uninjured footpad (RR 770-00304).

Thermal Pain and Hyperalgesia in Rhesus Monkeys: Rhesus monkeys were restrained in a primate chair and trained to consistently withdraw the tip of their tail from water warmed to an uncomfortable temperature (50°C). In this model of thermal pain response, prior treatment with pregabalin (100, 180, or 320 mg/kg PO) significantly delayed withdrawal of the tail and delays were dose-related (RR 740-03528).

Summary of results of animal models of analgesia are shown in the sponsor's table below.

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses* (mg/kg) or Concentration	Gender and No. per Group or No. of Observations	Noteworthy Findings	GLP Compliance	Report No
Brain/Spinal Cord	Rat/Sprague-Dawley	Oral gavage	3 10 30	8 M	Pregabalin did not reduce initial phase of pain response to footpad formalin injection (0-10 minutes) but reduced the delayed phase response dose-dependently with half-maximal effective dose (ED_{50}) of 19 mg/kg PO	No	740a03479
Brain/Spinal Cord	Rat/Wistar	SC	1 3 10 30 100	8 M	Pregabalin did not reduce initial phase of pain response to footpad formalin injection (0-10 minutes) but reduced the delayed phase response dose-dependently (ED_{50} approximately 20 mg/kg SC)	No	770a00297
Brain/Spinal Cord	Mouse/F1 hybrid between 129 Sv/J and C57 Blo inbred strains	PO	100	7 M	Pregabalin had a non-significant effect in homozygous mutant mice defective in binding of [^{3}H]gabapentin to Ca^{2+} type 1 but reduced the delayed phase of response by 78% in wildtype mice	No	740a03610
Brain/Spinal Cord	Rat/Wistar	SC	1 3 10 30	8 M	Pregabalin reduced heat hyperalgesia and tactile allodynia (pain behaviors) in a dose-related manner after prior injection of carrageenan into the footpad (3 mg/kg and greater dosages SC)	No	770a00297
Brain/Spinal Cord	Rat/Sprague-Dawley	SC	3 10 30	8 M	Pregabalin reduced thermal hyperalgesia in footpad from prior surgical incision at all dosages (ED_{50} approximately 20 mg/kg SC) and also reduced tactile allodynia (10 and 30 mg/kg) dose-dependently	No	770a00296
Brain/Spinal Cord	Rat/Sprague-Dawley	IP	1 3 10 30 100	8 M	Pregabalin dose-dependently reduced thermal hyperalgesia in footpad from intrathecal administration of Substance P. ED_{50} approximately 10 mg/kg IP or 10 µg/rat intrathecal	No	Partridge et al., ref 31
		Intrathecal	1 µg/rat 3 µg/rat 10 µg/rat 30 µg/rat 100 µg/rat				

M = Male, ED_{50} = Concentration producing 50% of maximal effect, SC = Subcutaneous, IP = Intraperitoneal
* Single dose unless specified otherwise

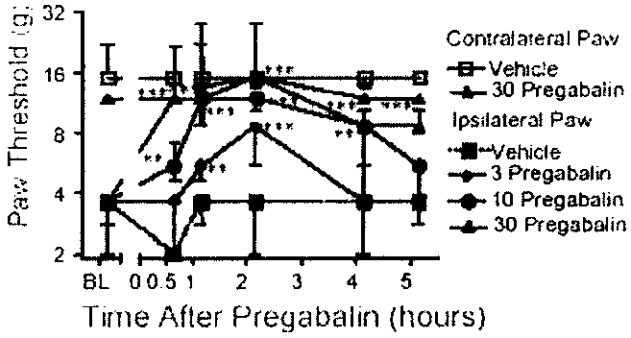
Models of Neuropathic Pain

Streptozocin-treated Diabetic Rats: In anesthetized rats, pain-induced motor reflexes were recorded with a needle electromyograph electrode in the gastrocnemius (calf) muscle (RR 770-00295). Streptozocin-treated rats develop diabetes and also neuropathic pain (tactile allodynia). Intravenous (IV) injection of pregabalin (10, 30, and 100 mg/kg in ascending doses with each rat) significantly reduced reflex activity at the 2 highest doses in rats that were untreated with streptozocin. However, in rats with neuropathy from streptozocin-induced diabetes, pain-induced reflexes were reduced significantly at 3-, 10-, and 30-mg/kg IV doses of pregabalin, with more than a 50% reduction after the 30-mg/kg dose. These effects of pregabalin in streptozocin-treated rats were more pronounced than in streptozocin-untreated rats. These results suggest that pregabalin reduces abnormal excitability in the spinal cord, and this could be related to analgesic-like pharmacological actions.

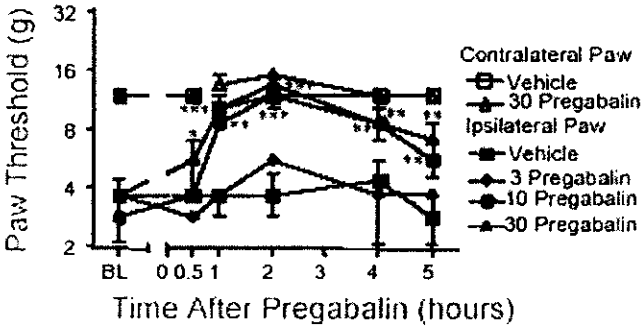
Vincristine Model: Pain from cancer can result from the disease itself, or in some cases, from the chemotherapeutic agents used to treat cancer. In particular, vinca alkaloids such as vincristine can cause pain syndromes characterized as myalgia, painful burning paresthesias, and also hyperalgesia and allodynia. This was studied in an animal model in which rats were given vincristine IV for 14 days with an osmotic minipump. The vincristine treatment caused a stable tactile allodynia that was measured with von Frey hairs. Pregabalin treatment (80 mg/kg IP, the only dose tested) reduced allodynia by more than 50% (RR 740-03529).

Nerve Ligation Models: The effect of pregabalin on static and dynamic components of mechanical hypersensitivity was examined in the rat sciatic nerve chronic constrictive injury model (CCI) and the rat Chung model of neuropathic pain from partial dorsal rhizotomy to the sciatic nerve. Pregabalin (3 to 30 mg/kg PO) reduced static allodynia significantly in both models at doses of 10 and 30 mg/kg PO and in CCI at 3 mg/kg PO (RR 770-00294; see sponsor's figure below).

a Chronic Constriction Injury Model



b Chung Model



Baseline (BL) paw withdrawal thresholds were measured in response to von Frey hairs. Loose ligation of the sciatic nerve with chromic gut sutures (CCI model) or tight ligation of the L5 and L6 dorsal roots (Chung model) caused a decrease in the withdrawal threshold compared to the unoperated (contralateral) side. Rats given vehicle PO had nerve ligation, but no analgesic drug treatment. Results are expressed as median threshold force to cause paw withdrawal in 8 to 10 rats per group (vertical bars represent first and third quartiles). Pregabalin treatment (at time = 0) caused dose-related increases in withdrawal threshold, an analgesic-like effect (doses in mg/kg PO). Asterisks, *p <0.05, **p <0.01, ***p <0.001 show significant difference from vehicle group (Mann-Whitney U-test, error bars denote 25 and 75 percentiles) (RR 770-00294).

2.6.2.3 Secondary pharmacodynamics

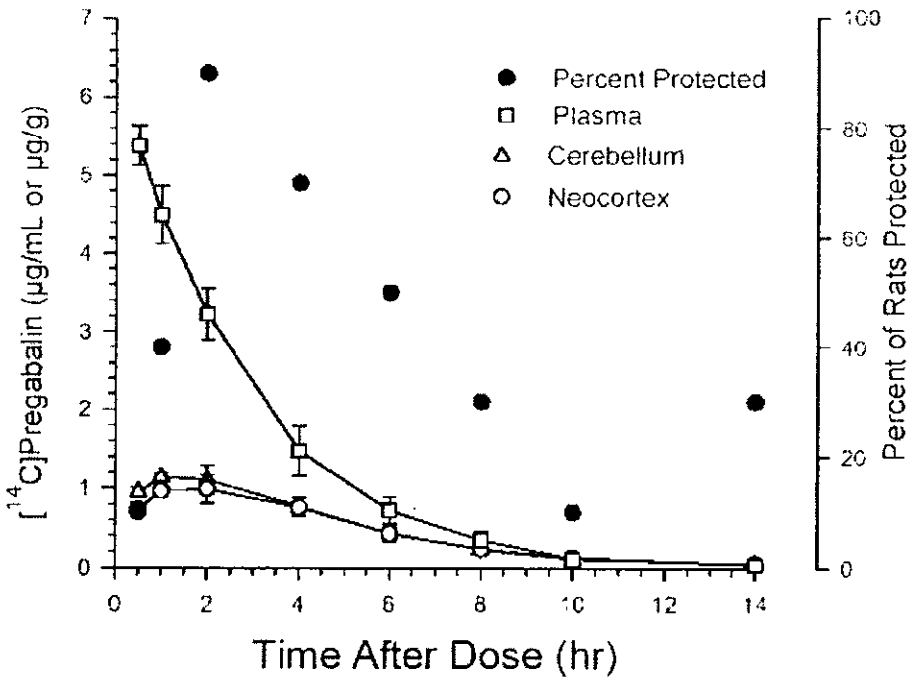
Anticonvulsant effects of pregabalin against tonic extensor seizures in mice and rats from electroshock are summarized in the table below. Lack of correlation with brain or plasma levels in rats is shown at the bottom. The significance of this is unclear.

Species	Convulsant	Dose Route	Minutes After Dosing[a]	ED_{50} (mg/kg)	Research Report	Reference No.
Mouse	Maximal electroshock	PO	120	20 (13-30)[b]	740-03090	16
Mouse	Maximal electroshock	IV	120	20 (13-29)	740-03090	16
Mouse	Maximal electroshock (low intensity)	IV	120	0.65 (0.33-1.3)	740-03172	12
Mouse	Maximal electroshock	IP	120	28 (20-37)	740-03224	75
Rat	Maximal electroshock	PO	240	1.3 (0.8-1.9)	740-03224	75
Rat	Maximal electroshock	PO	240	1.5 (1.0-2.3)	740-03081	69
Rat	Maximal electroshock	IV	240	2.2 (1.2-4.0)	740-03081	69

ED_{50} = Dose calculated to protect 50% of animals from seizures; PO = Oral; IV = Intravenous; IP = Intraperitoneal.

[a] ED_{50} values were determined at the approximate time of maximal effect after dosing, based upon results from preliminary experiments.

[b] Ninety-five percent confidence interval of ED_{50} values given in parentheses.


[14C]Pregabalin (µg/mL or µg/g)
Percent of Rats Protected
Time After Dose (hr)
Percent Protected
Plasma
Cerebellum
Neocortex
0
1
2
3
4
5
6
7
0
20
40
60
80
100
0
2
4
6
8
10
12
14

2.6.2.4 Safety pharmacology

Neurological effects:
Spontaneous Locomotor Activity in Rodents: (RR 740-03472; RR 740-03474) Pregabalin at relatively high doses (100 and 300 mg/kg PO) in rats significantly reduced locomotor activity, while lower doses caused no significant changes. In mice, administration of pregabalin (30 and 300 mg/kg PO) in 1 study and 300 mg/kg IV or 1000 mg/kg PO in a second study each appeared to reduce locomotor activity, but these changes were statistically different from vehicle only at the 300-mg/kg IV and 1000-mg/kg PO doses in the second study.

Ataxia in Rodents: The effects of pregabalin on ataxia was examined via the number of falls from the wire mesh (compared to vehicle-treated mice) was 2 of 10 with 1000 mg/kg PO and 1 of 10 with 300 mg/kg IV (RR 740-03217). On a rotorod ataxia test, 2 of 8 mice fell with 300 mg/kg IP and 1 of 8 mice fell with 500 mg/kg IP (RR 740-03224). Thus, in mice, ataxia was seen only at doses in excess of those used for analgesia or epilepsy.

In rats, the number of falls from the wire mesh (compared to vehicle-treated rats) was significantly increased with 300 mg/kg PO of pregabalin, but not with 10, 30, or 100 mg/kg PO (RR 740-03472). On a rotorod test, rats pretreated with pregabalin (100 mg/kg SC) fell in a 30% shorter time than vehicle-treated rats, while lower dosages (1, 10 and 30 mg/kg SC) caused no change in rotorod time (RR 740-00297). Ataxia assessed by a skilled observer scoring abnormal locomotor posture (in comparison to vehicle-treated rats) found pregabalin treatment caused mild locomotor ataxia in 6/8 rats at 4 hr after dosing (100 mg/kg PO) and in 3/8 rats with 50 mg/kg. Administration of pregabalin (10, 30 or 100 mg/kg PO) significantly increased the time to cross a wooden beam, the number of footslips, and the number of falls (RR 740-03224). Lower doses were not tested. These results suggest that pregabalin may be associated with reduced locomotor coordination in rats.

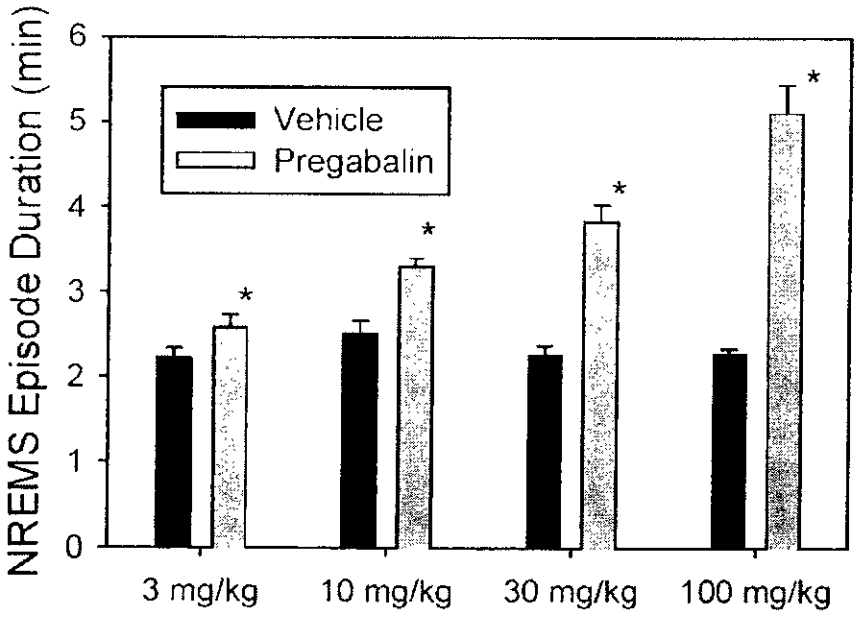
Neurofunctional Evaluation in Rodents:
Rats. While no changes were seen in muscle tone or the hindlimb placing response in rats given pregabalin at 25, 50, 100, or 125 mg/kg PO, a dose-related increased incidence of abnormal gait while walking on a flat surface was seen 4 hours postdose at all doses with an ED_{50} of 61 mg/kg (RR 740-03224). Righting reflex was assessed in groups of 8 rats given pregabalin at 100, 300, or 1000 mg/kg PO (RR 740-03215). One rat at 100 mg/kg, 3 rats at 300 mg/kg, and all rats at 1000 mg/kg had righting times (from a supine posture) of $\geq$ 1 second with an ED_{50} of 257 mg/kg PO.

Mice. Behavioral and physiologic effects (Irwin test) were monitored 30 minutes postdose in mice given pregabalin at 100, 300, or 1000 mg/kg PO or 30, 100, or 300 mg/kg IV (RR 740-03074). Mice were examined for normal pinnal and corneal reflexes, pupillary size, tail pinch response, catalepsy, body temperature, and ECS seizure protection. Slightly decreased spontaneous activity at 300 mg/kg IV was the only effect noted.

Ataxia and Incoordination in Squirrel Monkeys: In the Sidman avoidance procedure with squirrel monkeys the ability of monkeys to climb, grab, and balance on the perch in their home cage was reduced by pregabalin 30 and 100 mg/kg PO for several hours after dosing (RR 740-03483).

Pregabalin doses of 3 or 10 mg/kg PO were not associated with imbalance or other signs. These effects were dose-related in duration and mild to moderate in severity. Therefore, pregabalin has moderate sedative-like activity and reduces motor coordination at these doses.

Sleep in Rats: Pregabalin produced a significant dose-related increase in nonrapid eye movement sleep (non-REM sleep) when administered to rats just prior to the onset of the light phase of the light-dark cycle (RR 740-03527). The minimum effective dose for increasing non-REM sleep during the overall 12-hour period was 10 mg/kg PO. Even at 3 mg/kg PO (see Figure), pregabalin increased the average length of non-REM sleep episodes and decreased the number of non-REM sleep episodes. These data suggest that pregabalin treatment caused a consolidation of episodes of non-REM sleep into a smaller number of episodes, each with a longer duration than normal. Pregabalin at 30 and 100 mg/kg PO, also significantly decreased the total duration of rapid eye movement sleep (REM sleep) with little or no effect on the total duration of all sleep. This was characterized by a decrease in the duration of REM sleep episode at 30 mg/kg PO and by decreases in both episode duration and number of REM sleep episodes at 100 mg/kg PO. None of the effects of pregabalin carried over into the dark phase of the light-dark cycle (i.e., no drug effects were observed >12 hours after drug administration). Pregabalin did not affect the latency to onset of non-REM sleep or the sleep-cycle length.



Black bars denote the mean duration of spontaneous non-REM sleep episodes in rats given vehicle PO just prior to the light (inactive) portion of the light-dark cycle. Grey bars denote the mean duration of spontaneous non-REM sleep episodes in rats given pregabalin PO (dose as indicated). Each dose of pregabalin caused a significant increase in episode duration (* denotes $p < 0.05$ by ANOVA). Error bars denote SEM.

Cardiovascular effects:
Cardiovascular effects were characterized in the rat, dog and monkey models.

Rats: In conscious catheterized rats, oral administration of ascending doses of pregabalin at 10, 100, and 300 mg/kg did not significantly affect heart rate and blood pressure up to 20 hours postdose (RR 740-03115).

Potential cardiovascular effects of intravenous pregabalin were also evaluated in rats (RR 740-02986). Each animal received vehicle or pregabalin at 15 and 150 mg/kg IV, with 7 days between treatments, and served as its own control. Doses were based on an exploratory 7-day IV toxicity study in rats where the maximum plasma pregabalin concentration (C_{max}) at 150 mg/kg was 210 µg/mL, approximately 23 times the anticipated human therapeutic concentration of 9 µg/mL with 300 mg BID. Heart rate, blood pressure (mean arterial, systolic, and diastolic), and electrocardiography (ECG) were measured continuously for 24 hours postdose. No clinical signs or effects on cardiovascular parameters were noted at 15 mg/kg. At 150 mg/kg, heart rate increased 9% to 19% within the first 9 hours postdose. Parameters returned to control levels by 11 hours postdose.

Dogs: Arterial blood pressure, heart rate, cardiac output, peripheral resistance, left ventricular contractility, ECG, and plasma drug concentrations were measured hourly for 6 hours postdose, and peripheral resistance was calculated in conscious dogs given pregabalin at 50 mg/kg PO (RR 742-00010). Mean plasma concentration 1 to 6 hours postdose was approximately 100 µg/mL. No changes in cardiovascular parameters were observed.

Monkeys: Potential cardiovascular effects of pregabalin were evaluated at 10 and 40 mg/kg IV in monkeys (RR 745-02988). Doses were based on a 4-week IV toxicity study in monkeys where plasma pregabalin C_{max} values were approximately 60 and 200 µg/mL at 10 and 40 mg/kg, respectively. These concentrations are 7 and 23 times the proposed human efficacious therapeutic plasma concentration of 9 µg/mL (for a 300 mg/day maximum dose). Heart rate, blood pressure (mean arterial, systolic, and diastolic), and ECG were measured continuously for 24 hours postdose with a telemetry system. There were no clinical signs and no drug-related cardiovascular effects at 10 or 40 mg/kg. No separate oral studies were conducted of cardiovascular effects in monkeys.

Pulmonary effects: Pulmonary airflow and transpulmonary pressure were measured continuously for 50 minutes in anesthetized dogs given pregabalin intravenously at a cumulative dose of 200 mg/kg at 4 mg/kg/min (RR 760-00073). Total pulmonary resistance, dynamic compliance, tidal volume, respiratory rate, and minute volume were calculated. Pregabalin did not alter pulmonary function in anesthetized dogs given a cumulative IV dose of 200 mg/kg. The effect of oral pregabalin on pulmonary function in dogs was not evaluated.

Renal effects: Potential renal effects of pregabalin were evaluated in rats (RR 740-02986). Each animal received vehicle or pregabalin at 15 and 150 mg/kg IV, with 7 days between treatments, and served as its own control. Doses were based on an exploratory 7-day IV toxicity study in rats where the maximum plasma pregabalin concentration (C_{max}) at 150 mg/kg was 210 µg/mL, approximately 23 times the anticipated human efficacious therapeutic concentration of 9 µg/mL (300 mg/day). Potential renal effects were evaluated by monitoring water consumption and urine volume for 24 hours postdose. Urinary sodium, potassium, and chloride were analyzed on 24-hour collection samples. No clinical signs or effects on renal parameters were noted at 15 mg/kg.

Water consumption increased 29% and urine volume increased 82% at 150 mg/kg. Since urine electrolyte elimination was unaffected, the effects of pregabalin on water consumption and urine volume were not considered an adverse renal effect.

Gastrointestinal effects: Gastric emptying and intestinal transit time were measured after oral administration of 30, 100 or 300 mg/kg pregabalin (providing exposure levels of ~ 0.5 – 8 times the human therapeutic exposure) to rats (RR 6051-00006). Administration of pregabalin 2 hours prior to testing, decreased the percentage of a gastric meal that was emptied at 15 minutes after feeding (inhibition of 39% and 64% in comparison to vehicle, respectively). However, pregabalin at 30 mg/kg PO caused only a 12% (insignificant) decrease in gastric emptying. The decrease in gastric emptying caused by pregabalin was not reversed by treatment with naloxone (a mu-opiate antagonist), indicating that reduced gastric emptying was not caused by an opioid receptor-dependent mechanism. In addition, the weighted mean distance of meal progression into the small intestine after 15 minutes was not altered by pregabalin at 30 mg/kg PO, but was reduced 14% at 100 mg/kg PO and 38% at 300 mg/kg PO.

Using a different procedure with a charcoal meal in rats, the percentage of the length of small intestine that contained charcoal (measured 15 minutes after gavage administration of charcoal and 40 minutes after administration of pregabalin at 10, 30, or 100 mg/kg PO) was not significantly altered in comparison to administration of vehicle. In contrast, morphine (given 5 mg/kg SC, 30 minutes prior to charcoal) reduced the length of charcoal meal progression by 45% in this procedure.

Pregabalin at 30 and 100 mg/kg PO significantly increased mean colonic retention time from 5.9 hours (vehicle control) to 8.9 and 16.4 hours, respectively. These results indicate that pregabalin may reduce both gastric emptying and intestinal motility by a non-opioid receptor mechanism.

Abuse liability: The Sponsor conducted multiple nonclinical studies to examine the potential abuse liability of pregabalin. The binding profile and mechanism of action of pregabalin is similar to the unscheduled drug, gabapentin, and is unlike that of any compound currently scheduled in the United States. Conditioned place preference studies in the rat model failed to suggest that pregabalin had rewarding properties. Pregabalin also did not maintain IV self-administration studies in rhesus monkeys. However, there was some evidence of withdrawal signs in rats upon cessation of treatment, although this effect was not clearly statistically significant. Overall, the preclinical data would suggest that pregabalin has a low abuse liability. Regardless, the Controlled Substances Staff (CSS) was consulted to evaluate the existing abuse liability package. Based largely upon an increased incidence of reported feelings of "euphoria" in the clinical studies for Generalized Anxiety Disorder (GAD), CSS concluded that pregabalin should be scheduled.

Other:

2.6.2.5 Pharmacodynamic drug interactions

No direct animal pharmacology studies were conducted to address potential pharmacodynamic drug interactions with pregabalin. However, one indirect study (from RR 740-03224) is summarized here.

Hexobarbital-Induced Sleep Time in Rats: The effect of pregabalin on hexobarbital-induced sleep was assessed. Rats were given a single dose or 5 daily doses of pregabalin at 1.3 mg/kg PO, a nonsedative dose, followed 24 hours later by hexobarbital at 100 mg/kg IV. Multiple doses of pregabalin did not affect hexobarbital-induced sleep time suggesting that pregabalin did not induce hepatic metabolism of hexobarbital. A single dose of pregabalin followed by hexobarbital did increase sleep slightly (16%) suggesting either a pharmacodynamic sedative effect or a metabolic effect.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The absorption, distribution, metabolism, and elimination of pregabalin were investigated in the species used in pharmacology and/or toxicology studies (mouse, rat, dog, and monkey), using similar or identical formulations. Dose proportionality and accumulation were assessed using multiple-dose pregabalin toxicokinetic (TK) data in mouse, rat, rabbit and monkey. The key findings from these studies are listed below:

- Pregabalin is well absorbed following oral administration. Absolute oral bioavailability of pregabalin is high (>80%) in mice and rats at a 50-mg/kg dose and in monkeys at a 10-mg/kg dose.

- Urine is the principal route of ^{14}C excretion following [^{14}C]pregabalin administration. In mouse, rat, and dog, ≥ 80% of the absorbed [^{14}C] pregabalin PO dose is present in the 0 to 24-hr urine sample, while >71% to 75% is excreted by monkey during the same interval. More than 90% of the orally absorbed label was recovered in 0-96 hour urine in rat and monkey.

- Pregabalin undergoes minimal metabolism in mouse, rat, and monkey with unchanged parent representing the majority (≥ 90%) of drug-derived material in urine. A minor metabolite representing 2% to 3% of the urinary radioactivity in mouse and rat is identified as the N-methyl metabolite. In monkey, only 1 minor (<1%) unidentified component is detected in the urine. In dog, however, approximately 45% of the pregabalin dose is excreted in urine as N-methyl metabolite suggesting greater metabolism in this model.

- No significant inhibition of major cytochrome CYP450 isoforms is observed up to a pregabalin *in vitro* concentration of 1 mM. The potential for metabolism related drug-drug interaction is low at therapeutic concentrations of pregabalin.

- In general, pregabalin exposure is dose-proportional up to 2500 mg/kg (oral gavage/dietary) in mice and rats (except for gravid and lactating rats), and up to 50 mg/kg

in monkeys (nasogastric intubation). At higher doses, exposure to pregabalin was less than dose proportional in these species, possibly due to saturation of absorption. In pregnant rabbits, pregabalin exposure was less than dose proportional from 250 to 1250 mg/kg (oral gavage).

- [^{14}C]Pregabalin is widely distributed in most tissues and crosses the blood-brain barrier in mouse, rat, and monkey after PO administration. Radioequivalents concentrate in the pancreas of mice and rats, but not in primates. Pregabalin does not bind to mouse, rat, monkey, or human plasma proteins. [^{14}C]Pregabalin red blood cell (RBC)/plasma partition coefficients range from 0.69 to 0.80 for all species tested.

Pregabalin is highly soluble (>30 mg/mL) in aqueous media. After oral (PO) administration, the drug was rapidly absorbed in rat and monkey with maximum plasma concentrations achieved within 1 and 2 hours postdose, respectively. Pregabalin elimination half-life ($t_{1/2}$) was 3.4, 3.9, and 5.8 hours in mouse, rat, and monkey, respectively, following intravenous (IV) administration. Absolute PO bioavailability of pregabalin was 94% and 83% in mice and rats, respectively, at a 50-mg/kg dose. Absolute PO bioavailability in monkeys was 93% at 10 mg/kg, and reduced at higher doses.

Oral dose-proportionality and accumulation were assessed using multiple-dose pregabalin TK data. In general, pregabalin exposure was dose-proportional up to 2500 mg/kg in the mouse and rat, except for gravid and lactating rats, and up to 50 mg/kg in monkey. Exposure was less than dose-proportional at higher doses in these species. There were no apparent gender differences of pregabalin toxicokinetics in mouse, rat, or monkey. Pregabalin exposure did not change after repeated administration to rats for up to 48 weeks or to monkeys for up to 65 weeks. In pregnant rabbits, pregabalin exposure was less that dose-proportional from 250 to 1250 mg/kg.

[^{14}C]Pregabalin was widely distributed in most tissues and crossed the blood-brain barrier in mouse, rat, and monkey after PO administration. Radioequivalents concentrated ~7-fold in the pancreas of mice and rats compared to plasma levels. This observation was not found in primates. Pregabalin did not bind to mouse, rat, monkey, or human plasma proteins. [^{14}C]Pregabalin red blood cell (RBC)/plasma partition coefficients ranged from 0.69 to 0.80 for all species tested.

Renal excretion was the principal route of elimination following PO administration of [^{14}C]pregabalin. In mouse, rat and dog, ≥ 80% of the [^{14}C]pregabalin PO dose was present in the 0- to 24-hr urine, while >71% to 75% was excreted by monkey during the same interval. Higher than 90% of the dose was recovered in 0-96 hour urine in rat and monkey.

Pregabalin underwent minimal metabolism in mouse, rat, and monkey with unchanged parent representing the majority (≥90%) of drug-derived material in urine. A minor metabolite representing 2% to 3% of the urinary radioactivity in mouse and rat was identified as the N-methyl metabolite (PD 0155083). In monkey, only one minor (<1%) unidentified component was detected in the urine. In dog, approximately 45% of the pregabalin dose was excreted in urine as N-methyl metabolite. Since monkey *in vivo* metabolic profile was similar to that of human, rat, and mouse, with unchanged parent representing the majority of drug-derived material in urine, monkey was used as non-rodent species for pharmacokinetics and safety evaluation.

In 2- and 4-week rat toxicology studies, a minor induction of hepatic cytochrome P450 isozymes, CYP2B1/2 and CYP2E1, was observed at doses ≥1250 mg/kg/day with pregabalin $AUC_{(0-24)}$ >3300 µg·hr/mL. Enzyme induction is not anticipated in humans in the clinical therapeutic dose range of 150 to 600 mg/day (3 to 12 mg/kg/day based on body weight of 50 kg). At those doses, the maximum pregabalin exposure ($AUC_{(0-24)}$ of 123 µg·hr/mL) is at least 10-fold lower than the value observed at 1250 mg/kg in toxicology studies.

No significant inhibition of human CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1, or 3A4 was observed *in vitro* at pregabalin concentrations of 40, 200, and 1000 µM, suggesting low probability for pregabalin to elicit drug-drug interactions through inhibition of CYP450 isozymes.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Pregabalin is highly soluble (>30 mg/mL) in aqueous media. In rat and monkey, maximum plasma concentrations following PO gavage administration were achieved by 1 and 2 hours postdose, respectively. Absolute PO bioavailability of pregabalin was 94% and 83% in mice and rats, respectively, at a 50-mg/kg dose. Absolute PO bioavailability in monkeys was 93% at 10 mg/kg, and reduced at higher doses. The decrease in bioavailability at higher doses was most likely due to saturable absorption. Results from studies, using *in situ* intestinal perfusion or brush-border membrane vesicles, suggested that multiple amino acid transport systems may be involved in the small intestinal absorption of pregabalin, and saturation of transporters may have occurred at high doses. However, no saturation of pregabalin absorption was observed in clinical trials. In humans, pregabalin exposure was proportional after single (1-300 mg) and repeated oral doses (75-900 mg/day; see table below).

Pregabalin Absorption After a Single Dose

Species:	Mouse (B6C3F1)*	Rat (Wistar)	Rat (Wistar)	Monkey (Cynomolgus)
Gender (M/F)/Number of Animals	3/Time point (M)	5/Dose (M)	5-6/dose (M)	3/Sex/dose (M+F)
Feeding Condition	Fed	Fasted	Fasted	Fasted
Sample (eg, Whole Blood, Plasma, Serum)	Plasma (heparin)	Plasma (heparin)	Plasma (heparin)	Plasma (heparin)
Assay	LC/MS/MS	HPLC/UV	HPLC/UV	HPLC/UV
Mean (SD) PK Parameters, PO				
Vehicle/Formulation	0.5% Methylcellulose	0.9% NaCl	5% Dextrose/water	Distilled water
Method of Administration	Gavage	Gavage	Gavage	Nasogastric intubation
Pregabalin Dose (mg/kg)	50	50	5, 25, 50, 100, 150	10, 25, 50, 100
Cmax (µg/mL)	51.0	52.7(2.9)	6.1(0.3), 28(1.5), 65(12), 92(11), 127(13)	12.3(3.1), 20.5(5.5), 40.6(5.0), 47.0(14.7)
tmax (hours)	0.25	0.6(0.2)	0.5(0), 0.5(0), 1.1(0.5), 1.1(0.4), 1.2(0.4)	0.9(0.4), 2.2(1.5), 2.3(1.3), 2.3(1.4)
$t_{1/2}$(hours)	ND	2.7(0.02)	2.9(0.5), 4.2(1.3), 4.4(0.6), 3.1(0.4), 3.7(1.2)	5.0(2.0), 4.9(1.1), 8.8(2.6), 6.0(1.4)
AUC(0-24) (µg·hr/mL)	74.8	ND	ND	ND
AUC(0-∞) (µg·hr/mL)	ND	243(20)	25(2.2), 133(9.4), 317(78), 488(97), 686(90)	91.1(23), 171(42), 376(106), 400(163)
Mean (SD) PK Parameters, IV				
Vehicle/Formulation	0.9% NaCl	0.9% NaCl	NA	0.9% NaCl
Pregabalin Dose (mg/kg)	50	50	NA	25
CL (mL/min/kg)	10.4	2.90(0.37)	NA	1.76(0.35)
Vss (L/kg)	1.04	0.994(0.287)	NA	0.837(0.203)
t½ (hours)	3.4	3.9(0.9)	NA	5.8(2.0)
AUC(0-24) (µg·hr/mL)	79.6	289(36)	NA	ND
AUC(0-∞) (µg·hr/mL)	79.7	292(38)	NA	246(57)
F	94%	83.3(7.0)%	NA	92.8(10.7)%, 70.3(14.8)%, 76.8(15.9)%, 41.4(18.1)%
Study No.	764-03880	764-02203	764-02204	764-02299
Location in *t*	M 4, 15, V 006	M 4, 15, V 006	M 4, 15, V 006	M 4, 15, V 006

2.6.4.4 Distribution

Tissue distribution studies were conducted in the mouse, rat, and monkey. The volume of distribution at steady state (Vss) is greater than total body water in mice, rats, and monkeys (1.0, 0.99, and 0.84 L/kg, respectively), indicating that pregabalin is widely distributed in the body (from RR 764-02203 and 764-02299). See results in table above.

Autoradiography

In mouse, rat, and monkey, radioactivity was widely distributed at 1 to 4 hours postdose, and concentrations in most tissues were equivalent to that in blood. Overall concentrations in mouse tissues (RR 764-03718) were lower than those found in rats (RR 764-02227165) at equal [^{14}C]pregabalin PO doses of 10 mg/kg, although the relative distribution pattern was similar. There was also evidence of biliary excretion in mice demonstrated by presence of activity in gallbladder. [^{14}C]Pregabalin was rapidly eliminated from the body in mouse and rat. At 8 hours postdose, [^{14}C]pregabalin concentrations in the blood decreased to approximately 10% of the peak levels in rat. Radioactivity in the blood was undetectable in mouse at 8 hours and in rat at 24 hours postdose, suggesting that there is no minor, slowly eliminated metabolite circulating systemically. In both mice and rats, [^{14}C] radioactivity was low or undetectable in most of the tissues at 24 hours postdose. See autoradiography tables from mice below.

Distribution of [^{14}C]pregabalin Radioequivalents in Mice

Species:	Mouse (B6C3F1)
Gender (M/F)/Number of Animals:	2 Males/time point
Feeding Condition:	Fed
Vehicle/Formulation:	0.9% Saline
Method of Administration:	PO
Pregabalin Dose (mg/kg), (µCi):	10 mg/kg, 3 µCi/mouse
Radionuclide:	^{14}C
Specific Activity:	10.8 µCi/mg

	Mean (n=2) Concentration (µg equivalents/g)					
Sampling Time:	1 Hour	2 Hours	4 Hours	8 Hours	24 Hours	48 Hours
Tissues/Organs (WBA)						
Adrenal	4.93	3.37	0.38	BLQ	ND	ND
Blood	4.00	2.42	0.28	BLQ	ND	ND
Brain	0.92	0.93	0.35	BLQ	ND	ND
Brown Fat	2.41	1.43	BLQ	BLQ	ND	ND
Epididymis	2.70	1.94	0.47	BLQ	ND	ND
Fat	BLQ	BLQ	BLQ	BLQ	ND	ND
Harderian Gland	3.19	1.82	0.24	BLQ	ND	ND
Heart	4.42	2.56	0.28	BLQ	ND	ND
Kidney	6.45	4.05	0.49	BLQ	ND	ND
Liver	4.44	2.79	0.34	BLQ	ND	ND
Lung	3.63	2.29	0.23	BLQ	ND	ND
Marrow	3.66	1.70	BLQ	BLQ	ND	ND
Muscle	4.40	2.69	0.35	BLQ	ND	ND
Pancreas	30.1	20.4	1.99	0.23	ND	ND
Preputial	3.47	3.13	ND	ND	ND	ND
Salivary	3.92	2.35	0.32	BLQ	ND	ND
Seminal Vesicles	0.52	0.54	0.20	BLQ	ND	ND
Spleen	4.65	2.94	0.16	BLQ	ND	ND
Testis	3.13	2.82	0.52	BLQ	ND	ND

BLQ = Below limit of quantitation ([redacted] µg equivalents/g); ND = Not detected.

From the table above, radioactivity accumulated in the pancreas of mice compared to the blood levels. Although label was detected at 8 hours in this tissue, levels were below the level of quantitation by the 24 h time point.

Distribution of [^{14}C]Pregabalin Radioequivalents in Rats

Species:	Rat (Wistar)											
Gender (M/F)/No. of Animals:	2/sex/timepoint											
Feeding Condition:	Fasted											
Vehicle/Formulation:	5% Dextrose											
Method of Administration:	PO											
Pregabalin Dose (mg/kg), (µCi):	10 mg/kg, 40µCi/rat											
Radionuclide:	^{14}C											
Specific Activity:	31.6 µCi/mg											
	Mean (n=2) Concentration (µg equivalents/g)											
Sampling Time:	1 Hour		2 Hours		4 Hours		8 Hours		24 Hours		48 Hours	
Tissues/Organs (WBA)	M	F	M	F	M	F	M	F	M	F	M	F
Adrenal	12.2	11.5	8.98	10.5	6.92	6.20	1.49	1.29	BLQ	BLQ	BLQ	BLQ
Blood	11.4	11.2	8.76	10.1	6.84	6.36	1.46	1.34	BLQ	BLQ	BLQ	BLQ
Brain	2.55	1.97	2.74	2.97	2.60	2.91	1.23	1.02	BLQ	BLQ	BLQ	BLQ
Brown Fat	9.23	9.94	5.32	8.40	5.52	5.42	1.14	0.97	BLQ	BLQ	BLQ	BLQ
Epididymis	11.2	NA	10.3	NA	8.08	NA	2.38	NA	BLQ	NA	BLQ	NA
Fat	3.10	2.58	2.22	1.52	1.46	1.66	0.29	0.28	BLQ	BLQ	BLQ	BLQ
Harderian	7.21	7.43	5.51	6.72	4.25	4.89	1.08	0.96	BLQ	0.04	BLQ	BLQ
Heart	11.8	11.6	9.19	11.8	7.05	6.85	1.64	1.39	BLQ	BLQ	BLQ	BLQ
Kidney	20.1	16.8	15.6	14.4	11.0	9.55	2.82	2.56	BLQ	0.04	BLQ	BLQ
Lacrimal	18.5	19.6	13.7	22.8	8.04	11.3	1.65	2.12	BLQ	0.05	BLQ	BLQ
Lens	0.61	0.72	0.52	0.53	1.73	1.23	1.75	1.10	0.43	0.49	0.11	0.13
Liver	11.8	10.4	9.44	8.66	7.44	6.31	2.05	1.42	0.05	0.04	BLQ	BLQ
Lung	11.2	10.8	8.14	10.8	6.42	5.96	1.54	1.72	BLQ	BLQ	BLQ	BLQ
Lymph Node	11.1	11.5	8.27	12.4	6.58	6.59	1.49	1.39	BLQ	BLQ	BLQ	BLQ
Marrow	9.08	7.80	6.56	7.01	5.62	4.75	1.25	0.96	BLQ	BLQ	BLQ	BLQ
Muscle	11.0	11.2	8.88	11.1	6.72	6.44	1.56	1.34	BLQ	BLQ	BLQ	BLQ
Ovary	NA	13.3	NA	11.9	NA	6.62	NA	1.35	NA	BLQ	NA	BLQ

BLQ = Below limit of quantitation — µg equivalents/g), NA = Not applicable

Distribution of [^{14}C]Pregabalin Radioequivalents in Rats (Continued)

	Mean (n=2) Concentration (µg equivalents/g)											
Sampling Time:	1 Hour		2 Hours		4 Hours		8 Hours		24 Hours		48 Hours	
Tissues/Organs (WBA)	M	F	M	F	M	F	M	F	M	F	M	F
Pancreas	48.7	45.2	51.6	44.0	26.8	27.2	4.41	4.14	0.05	0.07	0.03	BLQ
Pineal	8.43	8.50	6.52	6.19	5.21	5.56	1.42	1.17	BLQ	BLQ	BLQ	BLQ
Pituitary	7.98	7.04	6.61	6.92	4.67	4.93	1.37	1.09	BLQ	BLQ	BLQ	BLQ
Preputial	14.6	13.3	19.3	22.7	14.2	20.0	7.73	12.0	1.25	0.90	0.17	0.32
Prostate	9.76	NA	9.67	NA	6.70	NA	1.61	NA	0.10	NA	0.06	NA
Salivary	10.5	11.1	8.15	10.8	6.62	6.27	1.52	1.28	BLQ	BLQ	BLQ	BLQ
Seminal Vesicles	5.40	NA	10.8	NA	8.65	NA	1.88	NA	0.17	NA	BLQ	NA
Skin	11.0	8.06	8.49	5.89	6.58	4.33	1.63	0.90	BLQ	BLQ	BLQ	BLQ
Spleen	12.3	12.6	8.78	12.0	6.75	6.25	1.47	1.33	BLQ	BLQ	BLQ	BLQ
Testis	6.07	NA	7.22	NA	6.41	NA	2.22	NA	BLQ	NA	BLQ	NA
Thymus	12.3	12.7	9.06	13.4	6.97	6.88	1.61	1.45	BLQ	BLQ	BLQ	BLQ
Brain Stem	2.82	1.95	2.91	2.97	2.53	2.90	1.19	0.95	BLQ	BLQ	BLQ	BLQ
Cerebellum	2.96	1.97	3.15	2.96	2.52	2.72	0.98	0.82	BLQ	BLQ	BLQ	BLQ
Colliculus	3.16	2.03	3.45	3.30	2.69	3.15	1.21	1.01	BLQ	BLQ	BLQ	BLQ
Cortex	2.55	1.73	2.80	2.95	2.67	2.92	1.24	1.02	BLQ	BLQ	BLQ	BLQ
Hippocampus	2.31	1.91	2.69	2.68	2.58	2.65	1.28	1.12	BLQ	BLQ	BLQ	BLQ
Olfactory	2.73	1.75	3.45	3.08	2.64	3.15	1.41	1.10	BLQ	BLQ	BLQ	BLQ
Striatum	1.91	1.60	2.99	2.88	2.49	2.75	1.23	1.13	BLQ	BLQ	BLQ	BLQ
Thalamus	2.40	1.69	2.91	2.97	2.39	3.00	1.50	1.20	BLQ	BLQ	BLQ	BLQ

BLQ = Below limit of quantitation — µg equivalents/g), NA = Not applicable

As was the case in the mouse model, the radioactivity was greatest in pancreas, kidney and lacrimal glands of rats. By 48 hours, the radioactivity was largely below the level of qualification.

Distribution of [^{14}C]Pregabalin Radioequivalents in Monkeys

Species:	Monkey (Cynomolgus)
Gender (M/F)/Number of animals:	1/sex/timepoint
Feeding condition:	Fasted
Vehicle/Formulation:	Not Available
Method of Administration:	PO
Pregabalin Dose (mg/kg), (μCi/kg):	24.5 mg/kg, 82.5 μCi/Kg
Radionuclide:	^{14}C
Specific Activity:	3.4 μCi/mg

	Concentration (μg Equivalents/g)			
Sampling Time:	**4 Hours**		**10 Hours**	
Tissues/Organs (WBA)	**Male**	**Female**	**Male**	**Female**
Adrenal	11.7	18.8	5.83	3.74
Blood	12.6	18.2	6.48	3.49
Brain	3.49	4.07	1.41	1.44
Cerebellum	3.51	4.19	1.65	1.32
Gray Matter	3.80	4.52	1.41	1.69
White Matter	2.47	2.45	0.86	1.24
Epididymis	21.0	NA	10.1	NA
Heart	13.2	18.5	6.61	4.12
Kidney	26.4	36.0	16.9	11.0
Lens	1.61	0.91	0.82	2.01
Liver	13.0	19.3	6.93	3.79
Lung	12.7	13.7	5.80	3.42
Lymph Node	13.0	16.6	6.85	3.76
Marrow	10.6	10.0	4.15	2.99
Muscle	13.9	16.2	7.24	3.84
Pancreas	11.7	14.7	5.15	3.38
Pituitary	6.63	10.1	5.42	3.01

In contrast to the rodent models, the monkeys do not demonstrate elevated pancreatic drug concentrations compared to blood levels. In the monkey, however, pregabalin levels were higher in the kidney following a 24.5-mg/kg [^{14}C]pregabalin PO dose. This blood level, however, is similar to the rat. Also of potential significance, the epididymis also showed a high concentration of radioactivity in the monkey.

The cross-species differences of pancreatic distribution have not been fully investigated. Overall concentrations in mouse tissues were lower than those found in rats at equal [^{14}C]pregabalin PO doses of 10 mg/kg, although the relative distribution pattern was similar. There was also evidence of biliary excretion in mice demonstrated by presence of activity in gallbladder. [^{14}C]Pregabalin was rapidly eliminated from the body in both mouse and rat. At 8 hours postdose, [^{14}C]pregabalin concentrations in the blood of the mouse decreased to approximately 10% of the peak levels in rat. Radioactivity in the blood was undetectable in mouse at 8 hours and in rat at 24 hours postdose, indicating that there is no minor, but slowly eliminated metabolite circulating systemically. In both mice and rats, [^{14}C] radioactivity was low or undetectable in most of the tissues by 24 hours postdose.

In pregnant rats, distribution of [^{14}C]pregabalin radioequivalents in maternal tissues was essentially identical to that in nonpregnant rats (see table below). [^{14}C]Pregabalin radioequivalents were able to cross the placental barrier, and were taken up in fetal tissues. While elevated concentrations were not found in fetal pancreas, fetal lens was the most highly labeled structure at 1 hour postdose and at subsequent time points. [^{14}C]Pregabalin was also taken up in maternal lens, but at a considerably lower rate. Access to a vascular lenticular tissue is limited to

some extent by transporters in its vascular supply. Thus it can be speculated that slow uptake of [^{14}C]pregabalin in adult lens may reflect the presence of specific transporter systems. Incomplete development of these systems in the fetus could result in the observed rapid uptake and persistence of the radioactivity in the fetal lens. Radioequivalents in fetal tissues were detectable at 24 hours postdose. In addition, appreciable drug concentrations were detected in the milk of lactating rats, indicating that neonatal exposure occurred.

Distribution of [^{14}C]Pregabalin Radioequivalents in Pregnant Rats

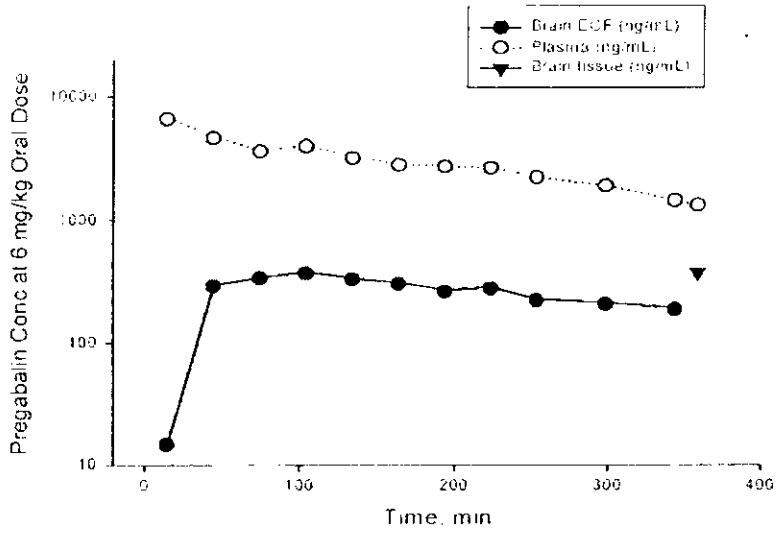
Species:	Rat (Wistar, pregnant)
Gender (M/F)/Number of Animals:	2 F timepoint
Feeding Condition:	Fasted
Vehicle/Formulation:	5% Dextrose
Method of Administration:	PO
Pregabalin Dose (mg/kg), (µCi):	10 mg kg, 50µCi rat
Radionuclide:	^{14}C
Specific Activity:	31.6 µCi mg

	Mean (n=2) Concentration (µg equivalents/g)				
Sampling Time:	1 Hour	2 Hours	4 Hours	8 Hours	24 Hours
Tissues Organs (WBA)					
Blood	10.5	9.93	5.39	1.87	0.04
Brain	2.20	3.05	2.61	1.20	0.05
Fetal Brain	8.22	12.9	14.3	7.11	0.16
Fetal Liver	14.7	18.5	15.0	5.01	0.20
Fetus	13.8	18.7	16.5	5.72	0.19
Kidney	17.9	13.1	11.3	3.96	0.14
Liver	11.9	9.91	5.79	2.41	0.13
Pancreas	69.2	49.2	29.0	6.51	0.11
Placenta	12.9	12.9	11.0	3.74	0.10
Preputial	18.7	30.2	32.1	16.7	1.74
Amniotic Fluid	ND	ND	ND	ND	0.94
Lens	ND	ND	ND	ND	0.45

ND = Not detected

[^{14}C]pregabalin radioequivalents crossed the blood-brain barrier and was present in brain of mouse, rat, and monkey. Although the time to disappearance of quantifiable pregabalin radioequivalents in the rat is similar between blood and brain, pharmacokinetic/pharmacodynamic assessments in rat indicated that there was a negative hysteresis relationship between CNS concentrations and effect. As such, there was a lag in both the onset and offset of effect relative to concentration over time (see figure below). The significance of this finding is unclear.

Mean pregabalin concentrations in plasma, brain tissue, and brain extracellular fluid (ECF) following a 6 mg/kg oral dose of pregabalin to rats



Protein Binding and Red Blood Cell Distribution: In vitro studies with pregabalin (0.1, 0.5, 1, 2, 5, 10, and 20 µg/mL) were conducted to determine the level of protein binding of the drug to rat, monkey and human plasma proteins at 37°C. Binding was also determined in mouse plasma at 0.1 and 20 µg/mL under similar conditions. The results indicated that pregabalin concentrations in the ultrafiltrate were essentially identical to those spiked in plasma indicating little to no binding of drug to mouse, rat, monkey or human plasma proteins. Thus, potential drug-drug interactions through displacement of protein-bound drug are unlikely.

In mouse, rat, dog, monkey, and human whole blood, the partitioning of [^{14}C]pregabalin between plasma and RBC did not show any dose dependency with increases in drug concentration. These data suggest that pregabalin does not become sequestered inside RBC, and support the conclusion that the drug does not bind to intracellular proteins under the conditions of the assay. RBC/plasma partition coefficients were 0.80 in mouse, 0.78 in rat, 0.71 in dog, 0.79 in monkey, and 0.69 in human. The pattern and extent of distribution were relatively similar between species.

2.6.4.5 Metabolism

In Vivo Metabolism
Pregabalin undergoes minimal metabolism in mouse, rat, and monkey with unchanged parent compound representing the majority (≥90%) of drug-derived material in urine. This finding is consistent with observations in humans. A minor metabolite representing 2% to 3% of the urinary radioactivity in mouse and rat was identified as the N-methyl metabolite (PD 0155083). In dog, approximately 45% of the pregabalin dose was excreted in urine as N-methyl metabolite. Since monkey *in vivo* metabolic profile was similar to that of human, rat, and mouse, with unchanged

parent representing the majority of drug-derived material in urine, the monkey was used as non-rodent species for pharmacokinetics and safety evaluation.

Pregabalin (the S-enantiomer) did not undergo racemization *in vivo*. The *in vivo* racemization of pregabalin (the S-enantiomer) to PD 0144550 (the R-enantiomer) was evaluated in plasma samples from mouse and rat (by diet) and from rabbit and monkey (by gavage) toxicokinetic studies, using an [ ] assay. A single plasma sample/gender/species was selected for analysis, except for rabbits (pregnant dams only). Plasma pregabalin concentrations were previously determined and ranged from [ ] µg/mL. R-enantiomer (PD 0144550) concentrations were below the limit of quantitation (— µg/mL) in the samples examined. Conversion of R-enantiomer (PD 0144550) to pregabalin was not observed in rats after oral doses of PD 0144550 at 0.1 to 2.5 mg/kg.

In general, mouse, rat, and monkey *in vivo* metabolic profiles were consistent with those observed *in vitro*. Pregabalin is the major component detected in the plasma and primarily eliminated in the urine unchanged in mouse, rat, and monkey. A minor metabolite representing 2% (mouse) and 3% (rat) of the urinary radioactivity was identified as the N-methyl metabolite (PD 0155083). The N-methyl metabolite was not detected in monkey. Pregabalin undergoes minimal metabolism in the monkey with only one minor (<1%) unidentified component detected in the urine.

In dog, approximately 45% of the pregabalin dose was excreted in urine as N-methyl metabolite (PD 0155083). Three minor polar metabolites (<5% each) were also detected in dog urine, but not identified. The differences in metabolism in dog suggest that it is not a good model for study.

Gender (M/F) No. of Animals:	$B_6C_3F_1$ Mice: [ ]	$B_6C_3F_1$ and CD-1 Mice: [ ] M/F each	Rats: [ ] M or F	Dogs: [ ] M, [ ] F	Monkeys: [ ] M, [ ] F	Humans[a]: [ ] M
Feeding Condition:	Fed	Fed	Fed	Fed	Fed	Fed
Vehicle/Formulation:	Solution	Diet admix[b] or [ ] solution	Solution/water	Solution 5% Dextrose	Solution/water	Solution/water
Method of Administration:	Gavage	Gavage	Gavage	Gavage	Gavage	Oral
Pregabalin Dose (mg/kg):	25 mg/kg (single dose)	500 mg/kg	25 mg/kg	10 mg/kg	10 mg/kg	100 mg
Radionuclide, ^{14}C (µCi):	36 µCi/mouse (single dose)	25 µCi/mouse	22 µCi/rat	50 µCi/dog	45 µCi/monkey	308 nCi/subject
Specific Activity:	22.5 µCi/mg	22.5 µCi/mg	22.5 µCi/mg	22.5 µCi/mg	22.5 µCi/mg	1.05 µCi/mg

Species	Sample[c]	Sampling Time or Period	% of Dose in Sample	% of Total Component in Sample: Parent	M1	M2	M3[d]	M4	Research Report Ref	Location in CFD
$B_6C_3F_1$ Mouse	Urine[e]	0-24 hour	87	92	--	--	--	--	[ ]	M 4, 1 5, V 006
		24-48 hour	9	--	--	--	--	--		
$B_6C_3F_1$ Mouse	Urine	0-24 hour	98	94	--	--	2	--	[ ]	M 4, 1 5, V 006
CD-1 Mouse	Urine	0-24 hour	87	93	--	--	2	--		
Rat	Plasma	1 and 6 hours	NA	100	--	--	--	--	[ ]	M 4, 1 5, V 006
	Urine	0-24 hours	93	90	--	2.5	2.6	--		
Dog	Urine[f]	0-24 hours	84	52.0	3.0	1.5	4.0	2.0	[ ]	M 4, 1 5, V 006
Monkey	Plasma	2 and 8 hours	NA	100	--	--	--	--	[ ]	M 4, 1 5, V 007
	Urine[g]	0-24 hours	76	92.9	<1	--	--	--		

a Pregabalin undergoes minimal metabolism in the human with only three minor (<5% ea.) identified components detected in the urine Reference RR 744-00510
b Mice were dosed 1000mg/kg/day by diet admixture for 2 weeks prior to the single radioactive dose of 25µCi via 500mg/kg
c In those studies where feces was collected there was insufficient radioactivity in the matrix for profiling
d M3 was identified as the N-methyl metabolite (PD 0155083). M1, M2, M4 were unidentified
e Pooled urine was profiled
f An individual dog urine sample was profiled
g Selected monkey urine samples were profiled and the data averaged
-- Indicates no data NA - Not applicable

In vivo Metabolism of Pregabalin

In Vitro Metabolism
[^{14}C]Pregabalin was not metabolized in rat, dog, monkey, or human liver cytosolic and microsomal preparations incubated at 37°C for up to 2 hours. An N-methyl metabolite (PD 0155083) was detected in rat, dog, and human hepatocyte incubates.
The results from rat, monkey and human *in vitro* and *in vivo* metabolism studies were consistent, which indicated that pregabalin undergoes minimal metabolism in those species and provided justification for selection of rat and monkey for toxicology studies. The N-methyl derivative was detected as a minor component in dog hepatocyte incubates, but a major metabolite in dog urine, suggesting inconsistency of *in vitro* and *in vivo* results for this species.

2.6.4.6 Excretion

Urine is the principal route of ^{14}C excretion following [^{14}C]pregabalin administration. In mouse, rat, and dog ≥ 80% of a [^{14}C] pregabalin PO dose was present in the 0- to 24-hour urine sample, while >71% to 75% was excreted by monkey during the same interval (see table on previous page).

Mouse: CD-1 and B6C3F1 mice received a single 300-mg/kg PO dose of [^{14}C]pregabalin after 2 weeks of pregabalin in the diet at 1000 mg/kg/day. Recovery of radioactivity in the 0-24 hr pooled urine was 98% of dose for B6C3F1 mice and 87% of dose for CD-1 mice. Metabolic profiling of the pooled 0-24 hr urine from B6C3F1 and CD-1 mice showed similar profiles with one major and several minor components accounting for essentially 100% of the urinary radioactivity. The major component representing 93% of the urinary radioactivity in both B6C3F1 (90% of dose) and CD-1 (81% of dose) mice was identified as unchanged pregabalin. A minor metabolite representing 2% of the urinary radioactivity in both B6C3F1 (2% of dose) and CD-1 (1% of dose) mice was identified as the N-methyl metabolite of pregabalin. No other single metabolite accounted for greater than 2% of the urinary radioactivity.

Rat and monkey: Zero- to 96-hr [^{14}C]pregabalin elimination in rat and monkey are summarized in the table below. These data suggest that [^{14}C]pregabalin is well absorbed following oral administration. No gender differences in excretion or metabolism were apparent. In the 0-24 hr urine samples, the major component, representing 84% (rat) and 71% (monkey) of the administered radioactive dose, was identified as unchanged pregabalin.

Pregabalin Elimination: Mean Recovery of Radioactivity Over 96 Hours Following a Single [^{14}C]Pregabalin PO Dose of 25 mg/kg in Rats and 10 mg/kg in Cynomolgus Monkeys

Elimination Route	Mean % Recovery of Radioactivity
Rat (mean N=12)	
Urine	95%
Feces	4%
Total	99%

Monkey (mean N=12)	
Urine	91.0%
Feces	4.9%
Total	95.9%

Following IV administration, pregabalin systemic plasma clearance is 10.5, 2.9, and 1.8 mL/min/kg, and elimination half-life is 3.4, 3.9, and 5.8 hours in mouse, rat, and monkey, respectively.

2.6.4.7 Pharmacokinetic drug interactions

Microsomal Enzyme Activities in Rats:
Rats were given pregabalin at 1.3 or 50 mg/kg PO daily for 7 days (RR 740-03224). Livers were excised, weighed, and homogenized 24 hours after the last dose. Microsomal protein content and cytochrome P450, P-nitroanisole O-demethylase, NADPH cytochrome C reductase, and UDP-glucuronosyltransferase activities were assessed. There were no effects on microsomal protein content or cytochrome P450. UDP glucuronosyltransferase activity was increased 17% at 1.3 mg/kg but not significantly changed at 50 mg/kg. NADPH cytochrome C reductase activity decreased 24% at 50 mg/kg. P-nitroanisole O-demethylase activity decreased 22% at 1.3 mg/kg and 28% at 50 mg/kg.

In 2- and 4-week rat toxicology studies, a minor induction of hepatic cytochrome P450 isozymes, CYP2B1/2 and CYP2E1, was observed at doses $\geq$ 1250 mg/kg/day with pregabalin $AUC_{(0\text{-}24)}$ >3300 µg·hr/mL. Enzyme induction is not anticipated in humans in the clinical therapeutic dose range of 150 to 600 mg/day (3 to 12 mg/kg/day based on body weight of 50 kg). At those doses, the maximum pregabalin exposure [$AUC_{(0\text{-}24)}$ of 123 µg·hr/mL] is at least 20-fold lower than the value observed at 1250 mg/kg in toxicology studies. The exposure multiple for the proposed maximal daily dose in humans of 300 mg/day would therefore be 40-fold lower than the concentration of drug that produced minor induction liver enzymes.

The potential for pregabalin to inhibit 7 major cytochrome P450 enzymes that mediate drug and xenobiotic metabolism in humans (CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4) was investigated using isoform selective marker substrates and human liver microsomal preparations. No significant inhibition was observed *in vitro* at pregabalin concentrations of 40, 200, and 1000 µM. These results suggest that a metabolically-based clinical interaction between pregabalin and other drugs, whose clearance is dependent upon one of the P450 enzymes tested, is highly unlikely at therapeutic concentrations of pregabalin.

2.6.4.9 Tables and Figures to Include Comparative TK Summary

The PK data are summarized below.

Mean Pregabalin Pharmacokinetic Parameter Values in Mice, Rats, and Monkeys Following Single-Dose Administration of Pregabalin

Species/Strain (N)	Dose (mg/kg)	Route	PK Parameters						
			Cmax	tmax	t½	AUC $_{(0-\infty)}$	CL	Vss	F
Mouse/B6C3F1 (3/sex/time point)	50	IV	--	--	3 44	79.7	10.5	1.04	--
	50(G)	PO	51.0	0.25	--	ND	--	--	94.0
Rat/Wistar (5/treatment)	50	IV	--	--	3.9	292	2.90	0.99	--
	50 (G)	PO	52.7	0.6	2.7	243	--	--	83.3
Rat/Wistar (5-6/dose)	5 (G)	PO	6.1	0.5	2.9	25.2	--	--	ND
	25 (G)	PO	28.4	0.5	4.2	133	--	--	ND
	50 (G)	PO	64.8	1.1	4.4	317	--	--	ND
	100 (G)	PO	91.8	1.2	3.1	488	--	--	ND
	150 (G)	PO	127	1.2	3.7	686	--	--	ND
Monkey/Cynomolgus (3/sex/dose)	25	IV	--	--	5.8	246	1.76	0.84	--
	10 (G)	PO	12.3	0.9	5.0	91.1	--	--	92.8
	25 (G)	PO	20.5	2.2	4.9	171	--	--	70.3
	50 (G)	PO	40.6	2.3	8.8	376	--	--	76.8
	100 (G)	PO	47.0	2.3	6.0	400	--	--	41.4

C_{max} = Maximum observed plasma concentration (µg/mL)
t_{max} = Time to reach C_{max} (hr)
t½ = Apparent terminal elimination half-life (hr)
AUC = Area under plasma concentration-time curve (µg·hr/mL)
CL = Total plasma clearance (mL/min/kg)
Vss = Volume of distribution at steady state (L/kg)
F = Absolute PO bioavailability (%)
G = Dose administered by gavage
ND = Not determined
-- = Not applicable

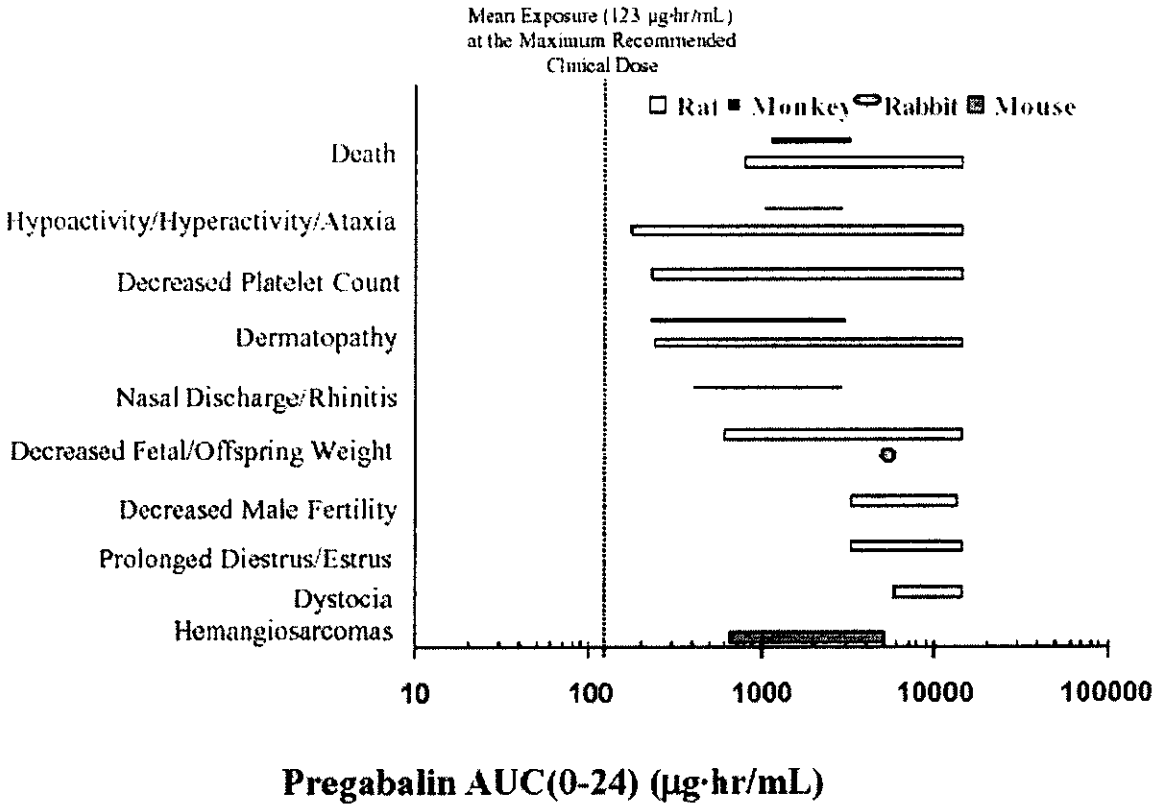
2.6.6 TOXICOLOGY

2.6.6.1 Overall Toxicology Summary

General toxicology:

In summary, toxicologic findings after oral dosing of pregabalin included hypoactivity, hyperactivity, and ataxia in rats at ≥ 1.5 times, in rabbits at ≥ 11 times, and in monkeys at ≥ 8 times the mean human exposure ($AUC_{[0-24]}$ of 123 µg·hr/mL) at the maximum recommended clinical dose of 600 mg/day. Dermatopathy was observed in rats and monkeys at ≥ 2 times the mean human exposure at the maximum recommended clinical dose. Decreased platelet count occurred in rats at ≥ 2 times and nasal discharge/rhinitis occurred in monkeys at ≥ 3 times the mean human exposure at the maximum recommended clinical dose. Note: at a maximum clinical dose of 300 mg/day, an $AUC_{(0-24)}$ of ~ 75 µg·hr/mL would provide a somewhat greater safety margin.

Figure: Pregabalin Oral Toxicodynamics from Sponsor's View



Genetic toxicology:
The genotoxic potential of pregabalin was assessed in both *in vitro* and *in vivo* studies. Pregabalin was not mutagenic under the conditions of the assays in bacteria using metabolic activation provided by mouse or rat liver. Pregabalin did not induce point mutations or structural chromosome aberrations in Chinese hamster ovary cells *in vitro*. Pregabalin did not induce unscheduled deoxyribonucleic acid (DNA) synthesis in mouse or rat hepatocytes and was not clastogenic in mouse or rat bone marrow *in vivo*.

Carcinogenicity: (from Ed Fisher's review)
A dose-dependent increase in the incidence of malignant vascular tumors (hemangiosarcomas) was observed in two strains of mice ($B_6C_3F_1$ and CD-1) given pregabalin in the diet for 2 years at doses of 200, 1000, or 5000 mg/kg/day. Plasma pregabalin exposures (based on AUC) in mice receiving the lowest dose that increased hemangiosarcoma incidence were approximately equal to the mean exposure in humans receiving a daily dose of 600 mg. No evidence of carcinogenicity was seen in two studies in rats (Wistar strain) following oral administration of pregabalin for 2 years at doses of up to 450 (males) and 900 mg/kg/day (females), which were associated with plasma exposures approximately 14 and 24 times, respectively, human exposure at a daily dose of 600 mg/day. If the maximum daily dose in humans were limited to 300 mg/day, the NOAEL values provide approximately a 28 fold and 48 fold exposure ratios in males and females, respectively. The Sponsor's tables 5 and 6 summarize the data obtained in the B6C3F1 mice.

Table 5. Incidence of Hemangiosarcoma in B6C3F1 Mice Given Pregabalin for 2 Years[a]

	Dose (mg/kg)			
	Control	200	1000	5000
Males	2 (3%)	3 (5%)	19* (29%)	22* (34%)
Females	2 (3%)	7 (11%)	19* (29%)	25* (38%)
Exposure Multiple[b]		1.1-1.2	5-7	31

* Statistically increased compared to control, $p < 0.001$.
Historical incidence in control B6C3F1 mice: Males: 0%-13%; females: 0%-8%.
[a] 64-66/sex/group.
[b] Comparison to mean exposure in humans given the maximum recommended dose.

Table 6. Platelet and Bone Marrow Megakaryocyte Counts in B6C3F1 Mice Given Pregabalin for 2 Years[a]

	Dose (mg/kg)			
	Control	200	1000	5000
	Platelet Count (10^9/L)			
Male	1182 ± 299 (52)	1342 ± 226 (46)	1599 ± 466* (35)	1577 ± 476* (17)
Female	631 ± 203 (40)	856 ± 216 (40)*	830 ± 237 (23)*	997 ± 267 (20)*
	Megakaryocyte Count (total/5000 hematopoietic cells)			
Male	43.2 ± 11.2 (65)	65.8 ± 18.0** (63)	77.3 ± 19.9** (64)	88.3 ± 26.3** (61)
Female	43.0 ± 10.7 (62)	60.1 ± 20.3** (66)	71.2 ± 18.9** (62)	78.9 ± 19.7** (63)

* Significantly different from control, $p < 0.01$.

** Significant trend test at $p < 0.02$ ($p < 0.005$ for quadratic).

[a] Values represent mean ± standard deviation (N).

Reproductive toxicology: (from Ed Fisher review)
In a fertility study in which male rats were administered pregabalin (250, 1250, or 2500 mg/kg) prior to and during mating, a number of adverse reproductive effects were observed, primarily at doses ≥ 1250 mg/kg; these included: increased number of days to mating, decreased sperm counts and motility, increased sperm abnormalities, reduced fertility, increased preimplantation loss, and decreased litter size. Decreased sperm motility was also seen at 250 mg/kg. Because a no-effect dose was not established, a follow-up study was conducted using lower doses (50, 100, or 250 mg/kg). No significant reproductive or other toxic effects were observed in this study. Based on the finding of decreased sperm motility at the low dose in the original study, the no effect dose for male reproductive impairment in rats was 100 mg/kg, which was associated with plasma pregabalin exposures (AUC) approximately 3 times human exposures at the maximum recommended dose (MRD) of 600 mg/day (this would produce a 6-fold exposure ratio if the maximum daily dose in humans were limited to 300 mg/day).

In a fertility study in which female rats were given pregabalin (500, 1250, or 2500 mg/kg) prior to and during mating and early gestation (males were not treated), the drug treatment appeared to disrupt estrous cyclicity during the premating treatment period. In addition, there was an increase in the number of days to mating, and increased embryonic death were seen at all doses of pregabalin tested. The low effect dose for female reproductive impairment and embryolethality was 500 mg/kg (plasma exposure approximately 10 times those in humans receiving the MRD of 600 mg/day or 20 times a maximum human daily dose of 300 mg/day).

Segment II Reproductive Toxicology Studies. Increased incidences of fetal structural abnormalities and other manifestations of developmental toxicity (lethality, growth retardation, nervous and reproductive system functional impairment) were observed in the offspring of animals treated with pregabalin during pregnancy.

When pregnant rats were given pregabalin (500, 1250, or 2500 mg/kg) throughout the period of organogenesis, incidences of specific skeletal malformations (fusion of the jugal bone and maxilla and fusion of the nasal bones) were increased at ≥ 1250 mg/kg, and incidences of skeletal variations and retarded ossification were increased at all doses. Fetal body weights were

decreased at the highest dose. The low effect dose for developmental toxicity in rats was 500 mg/kg, which was associated with a plasma pregabalin exposures (AUC) approximately 17 times human exposures at the maximum recommended dose [MRD] of 600 mg/day. This low effect dose provides approximately 34 times the human exposure if the maximum daily dose is limited to 300 mg/day.

When pregnant rabbits were given pregabalin (250, 500, or 1250 mg/kg) throughout the period of organogenesis, total incidences of skeletal malformations, visceral variations, and ossification retardation were increased and fetal body weights were decreased at the highest dose. The no effect dose for developmental toxicity in rabbits was 500 mg/kg (plasma exposures approximately 17 times human exposures at the MRD or 34 times the predicted plasma levels if the maximum daily dose were limited to 300 mg/day).

Segment III Reproductive Toxicology. Pregabalin treatment of rats produced reproductive and developmental effects in the peri- and post-natal periods. In a study in which female rats were dosed with pregabalin (50, 100, 250, 1250, 2500 mg/kg) throughout gestation and lactation, offspring growth was reduced at $\geq$ 100 mg/kg, offspring survival was decreased at $\geq$ 250 mg/kg, and offspring neurobehavioral (decreased auditory startle responding) and reproductive function (decreased fertility, decreased litter size) were impaired at 1250 mg/kg. The effect on offspring survival was pronounced at doses $\geq$ 1250 mg/kg, with 100% mortality in high dose litters. The no effect level for pre- and postnatal development was 50 mg/kg (plasma exposures approximately 2 times human exposures at the MRD of 600 mg/day or 4 times the MRD of 300 mg/day).

Special toxicology:

Dermatopathy
Skin lesions characterized clinically by a spectrum of lesions ranging from erythema to necrosis, and histopathologically by hyperkeratosis, acanthosis, fibrosis, and/or necrosis of the tail, were observed in rats given $\geq$ 50 mg/kg in oral repeated-dose studies, with associated $AUC_{(0\text{-}24)} \geq 241$ μg·hr/mL. Lesions typically appeared within the first 2 weeks of treatment at higher doses and resolved in most affected animals by Week 7 in the 13-week study and by Week 4 in the 52-week study. Similar skin lesions were observed in monkeys in oral repeated-dose studies, and were located primarily on the tail in most animals. In the chronic monkey study, lesions were observed at $\geq$ 25 mg/kg, with plasma pregabalin $AUC_{(0\text{-}24)}$ values $\geq$ 219 μg·hr/mL. As in rats, lesions in affected animals in the chronic monkey study generally resolved prior to study termination. Subcutaneous tail temperature, used as an indirect measure of tail blood flow in the chronic monkey study, showed no consistent differences between control and high-dose animals, or between affected and unaffected animals within the same group. Pregabalin at 5% and 7.5% did not induce contact sensitization (allergic dermatitis) in rats in the local lymph node assay. The etiology of the skin lesions remains unknown. No tail dermatopathy was observed in mice given repeated oral doses of pregabalin up to 13 g/kg up to 13 weeks. Missing tail tips were observed in mice given up to 5000 mg/kg ($AUC_{(0\text{-}24)}$ of 3150 μg·hr/mL) in the B6C3F1 but not the CD-1 carcinogenicity study, however, the relationship of this lesion to dermatopathy in rats and monkeys is unknown.

The clinical therapeutic dose range of 150 to 600 mg/day (3 to 12 mg/kg/day based on body weight of 50 kg) yields a maximal pregabalin exposure (AUC_{0-24}) of 123 μg·hr/mL in humans. Therefore this results in a 2-fold safety margin for dermatopathy in humans compared to rats and monkeys.

2.6.6.2 Single-dose toxicity

All acute studies were reviewed with the original IND by T.D. Steele. They are summarized here. The acute oral and IV toxicity of pregabalin was assessed in adult mice and rats. All animals were observed for clinical signs and mortality daily for 14 days, body weights were recorded weekly, and selected clinical laboratory parameters were measured. All animals were examined for gross pathologic changes at necropsy and selected tissues were examined histopathologically.

Maximum tolerated doses were ≥ 5000 mg/kg orally and ≥ 300 mg/kg i.v. The Sponsor's summary table of acute oral and intravenous toxicology studies is reproduced below:

Species (Strain) Animals Sex/Group Total	Route (Vehicle) [Dose Volume]	Dose (mg/kg)	Observation Period (Days)	Significant Findings	Maximum Nonlethal Dose (mg/kg)	Report Number
Oral						
Mouse (B6C3F1) 2F, 3M + 3F 14	Oral (0.5% MC) [50 mL/kg]	VC 5000	2 or 14[a]	Hypoactivity in drug-treated males. Body weight and clinical biochemical parameters not affected. No gross or histopathologic changes.	5000	250-01674
Rat (Wistar) 2F, 3M + 3F 14	Oral (0.5% MC) [30 mL/kg]	VC 5000	2 or 14[a]	Hypoactivity, diarrhea, and urine staining in drug-treated animals. Body weight and biochemical parameters not affected. No gross or histopathologic changes.	5000	250-01667
Intravenous						
Mouse (B6C3F1) 2F, 3M + 3F 14	IV[b] (0.9% NaCl) [10 mL/kg]	VC 300[c]	1 or 14[d]	No clinical signs. Decreased body weight in drug-treated females 24 hours postdose. Clinical biochemical parameters not affected. No gross or histopathologic changes.	300	250-01678
Rat (Wistar) 2F, 3M + 3F 14	IV[b] (0.9% NaCl) [10 mL/kg]	VC 300[c]	1 or 14[d]	Hypoactivity, mild ataxia, and urine staining in drug-treated animals. Decreased body weight in 1 treated female 24 hours postdose. Clinical biochemical parameters not affected. No gross or histopathologic changes.	300	250-01675

MC = Methylcellulose; VC = Vehicle control; IV = Intravenous.

[a] Two females received 5000 mg/kg and were observed for 14 days; 3 animals per sex received vehicle or 5000 mg/kg and were observed for 48 hours.

[b] Dose solution concentration of 30 mg/mL and injection rate of 1 mL/min resulted in dose rate of 30 mg/kg/min.

[c] Highest dose achievable based on solubility and dose volume limitations

[d] Two females received 300 mg/kg and were observed for 14 days; 3 animals per sex received vehicle or 300 mg/kg and were observed for 24 hours.

2.6.6.3 Repeat-dose toxicity

Only the studies considered pivotal are reviewed and are included with the NDA (even if they were previously reviewed with the IND). This review does not include range-finding and some non-GLP studies. These studies, and other repeated-dose studies that were reviewed as part of the original IND by T.D. Steele and are available.

Study title: Four-Week Daily Repeated Dose Oral Toxicity Study of Pregabalin in Rats.

Key study findings:

- Two males and 1 female at 5000 mg/kg died or were euthanized in moribund condition during Weeks 3 or 4.
- Treatment-related clinical signs were CNS (hypoactivity, ataxia, hyper-reactivity) in all treated groups of both sexes.
- Urine staining and tail lesions were noted in both sexes at all doses.
- Body weight gain was suppressed by 18%, 26%, 42%, and 60% in males at 500, 1250, 2500, and 5000 mg/kg, respectively, and 34%, 60%, and 70% in females at 1250, 2500, and 5000 mg/kg, respectively, relative to controls. Food consumption was reduced throughout the treatment phase at 1250, 2500, and 5000 mg/kg in both sexes.
- Urinary bladder dilatation, and epididymal changes (hypospermia, fibrosis, cellular debris, mononuclear cell infiltrates) were observed at all doses.
- C_{max} and $AUC_{(0\text{-}24)}$ values showed no gender difference and were dose proportional between 500 and 2500 mg/kg, but less than dose proportional at 5000 mg/kg. The average C_{max} following 500, 1250, 2500, and 5000 mg/kg was 106, 270, 494, and 799 µg/mL in males, and 82, 229, 476, and 606 µg/mL in females, respectively. $AUC_{(0\text{-}24)}$ averaged 1.7, 4.7, 9.0, and 14.6 µg-hr/mL in males, and 1.4, 3.8, 8.4, and 11.3 µg·hr/mL in females at 500, 1250, 2500, and 5000 mg/kg, respectively.
- This study did not identify a no-effect dose. Target organs were male reproductive tissues, thymus, and skin.

Study no.:	SP1554
Volume #, and page #:	EDR M 4, I 5, V 015
Conducting laboratory and location:	Warner-Lambert Canada, Inc., Mississauga, Ont.
Date of study initiation:	Apr 12, 1995.
GLP compliance:	yes
QA report:	yes (x) no ( )
Drug, lot #, and % purity:	CI-1008, Lot XH340993, — purity

Methods

Doses:	0, 500, 1250, 5000 mg/kg
Species/strain:	[ ] Wistar rat
Number/sex/group or time point (main study):	10/sex/dose
Route, formulation, volume, and infusion rate:	In diet, adjusted weekly
Satellite groups:	5/sex/dose for 4-week recovery and 5/sex/dose for TK

Age: 44 days
Unique study design or methodology (if any): Bone marrow samples from 5/sex high dose and controls examined microscopically, and liver tissue from 5/sex/dose evaluated for microsomal parameters in Weeks 4 and 8.

Observation times and results

Mortality and Clinical Signs: Observed daily; see table.

Important Findings	UC		500 mg/kg		1250 mg/kg		2500 mg/kg		5000 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N	18	18	21	21	21	21	21	21	21	21
Death or Moribund Cystitis or pyelonephritis	0	0	0	0	0	0	0	0	2 Weeks 3-4	1 Week 4
Clinical Signs										
Hypoactivity in Week 1	--	--	8	7	12	3	18	6	15	12
Ataxia in Week 1	--	--	4	3	7	8	15	9	12	14
Hyperreactivity in Week 1	--	--	4	5	6	7	4	6	8	3
Urine Stain/Scald to Week 4	--	--	1	1	2	3	6	14	14	16
Tail Dermatopathy to Week 4	--	--	11	12	16	17	17	11	19	18

A breakdown of the tail dermatopathy by severity and by sex is found in the following 2 tables:

Males

Week	Dose (mg/kg)	Ataxia	Hypoactivity	Hyper-Reactivity	Urine Staining (Scalding)	Tail Lesions				Feces Reduced	Death
						Erythema	Dermatopathy	Necrosis	Missing		
1	500	4/21	8/21	4/21	1/21	11/21	9/21	1/21	0/21	0/21	0/21
	1250	3/21	12/21	6/21	1/21	17/21	14/21	4/21	0/21	0/21	0/21
	2500	15/21	18/21	4/21	4/21	17/21	12/21	7/21	1/21	1/21	0/21
	5000	12/21	15/21	8/21	13/21	19/21	17/21	13/21	1/21	2/21	0/21
2	500	0/21	0/21	0/21	0/21	0/21	4/21	0/21	0/21	0/21	0/21
	1250	0/21	0/21	0/21	2/21	0/21	8/21	3/21	3/21	0/21	0/21
	2500	0/21	0/21	0/21	4/21	0/21	10/21	4/21	0/21	0/21	0/21
	5000	0/21	1/21	0/21	8/21	0/21	16/21	6/21	0/21	5/21	0/21
3	500	0/21	0/21	0/21	0/21	0/21	2/21	0/21	0/21	0/21	0/21
	1250	0/21	0/21	0/21	0/21	0/21	4/21	0/21	1/21	0/21	0/21
	2500	0/21	0/21	0/21	1/21	0/21	5/21	1/21	4/21	0/21	0/21
	5000	0/21	1/21	0/21	8/21	0/21	14/21	4/21	0/21	1/21	1/21
4	500	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21
	1250	0/21	0/21	0/21	0/21	0/21	2/21	1/21	2/21	0/21	0/21
	2500	0/21	0/21	0/21	1/21	0/21	2/21	2/21	4/21	0/21	0/21
	5000	0/20	0/20	0/20	0/20	0/20	2/20	1/20	3/20	0/20	1/20

* Shaded cells indicate occurrence of clinical signs

Females

Week	Dose (mg/kg)	Ataxia	Hypoactivity	Hyper-reactivity	Urine Staining (Scalding)	Tail Lesions				Feces Reduced	Death
						Erythema	Dermatopathy	Necrosis	Missing		
1	500	3/21	7/21	5/21	1/21	9/21	9/21	3/21	0/21	1/21	0/21
	1250	8/21	3/21	7/21	3/21	15/21	17/21	4/21	3/21	5/21	0/21
	2500	9/21	6/21	6/21	13/21	11/21	7/21	0/21	3/21	5/21	0/21
	5000	14/21	12/21	3/21	14/21	16/21	14/21	7/21	2/21	2/21	0/21
2	500	0/21	0/21	0/21	1/21	1/21	7/21	1/21	0/21	0/21	0/21
	1250	0/21	0/21	0/21	1/21	0/21	12/21	2/21	5/21	2/21	0/21
	2500	0/21	0/21	0/21	2/21	1/21	4/21	0/21	3/21	1/21	0/21
	5000	0/21	0/21	0/21	14/21	2/21	13/21	6/21	3/21	2/21	0/21
3	500	0/21	0/21	0/21	1/21	0/21	6/21	0/21	0/21	0/21	0/21
	1250	0/21	0/21	0/21	1/21	0/21	10/21	1/21	5/21	0/21	0/21
	2500	0/21	0/21	0/21	7/21	0/21	2/21	0/21	4/21	0/21	0/21
	5000	0/21	0/21	0/21	12/21	1/21	10/21	1/21	3/21	1/21	0/21
4	500	0/21	0/21	0/21	0/21	0/21	2/21	0/21	0/21	0/21	0/21
	1250	0/21	0/21	0/21	1(1)/21	1/21	4/21	1/21	3/21	1/21	0/21
	2500	0/21	0/21	0/21	3/21	0/21	1/21	0/21	3/21	0/21	0/21
	5000	0/21	1/21	0/21	8/21	0/21	2/21	1/21	3/21	3/21	1/21

* Shaded cells indicate occurrence of clinical signs

Body weights: Body weight gain was suppressed by 18%, 26%, 42%, and 60% in males at 500, 1250, 2500, and 5000 mg/kg, respectively, and 34%, 60%, and 70% in females at 1250, 2500, and 5000 mg/kg, respectively, relative to controls.

Food consumption: Food consumption was determined weekly and was decreased in males from 1250 mg/kg and above and females at 2500 and 5000 mg/kg. See summary table at end of section.

Ophthalmoscopy: Ophthalmic examinations on animals designated for toxicologic assessment conducted pretest and in Weeks 4 and 8; no effects were noted.

EKG: None performed

Hematology: Pregabalin treatment produced statistically significant increases in RBC, hemoglobin, hematocrit, and a significant decrease in platelet counts (table).

	Control		500 mg/kg		1250 mg/kg		2500 mg/kg		5000 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Hematology[a]										
Red Blood Cells (10^{12} L/l	[illegible]	[illegible]	[illegible]**		[illegible]**		[illegible]**	[illegible]**	[illegible]**	[illegible]**
Percent of Control			[illegible]%	--	[illegible]%	--	[illegible]%	[illegible]%	[illegible]%	[illegible]%
Hemoglobin (g/L)	145	146	154**	--	157**	--	158**	--	158**	--
Percent of Control			6%	--	8%	--	9%	--	9%	--
Hematocrit (L/L)	0.44	0.43	0.47**	--	0.48**	--	0.48**	--	0.48**	--
Percent of Control			7%	--	9%	--	9%	--	9%	--
Platelet Count (10^9 L/L)	1153	989	933**	895*	829**	816**	789**	810**	852**	757**
Percent of Control			19%	10%	28%	17%	32%	18%	28%	23%

Best Possible Copy

-- *no noteworthy findings*

Clinical chemistry: There was a small (4-6%) decrease at week 4 in chloride concentration in males at 1250 and 5000 mg/kg and females at 2500 and 5000 mg/kg that were not apparent at week 8.

In view of the increases in red blood cell parameters in treated animals, differential cell counts were conducted on randomized blind-coded bone marrow smears from the first 5 surviving animals in high dose (5000 mg/kg) and control groups. Bone marrow smears were prepared for each animal at termination (moribund or Week 4 or 8) and examined microscopically for the high dose Group V (5000 mg/kg) and control (Group I) animals. Bone marrow megakaryocytes decreased 30% in males and 25% in females at 5000 mg/kg, the only dose evaluated (see table below).

Total hepatic cytochrome P450 content increased 30% to 107% in both sexes at ≥ 1250 mg/kg. Aniline hydroxylase increased up to 4-fold, and nitrosodimethylamine N-demethylase increased up to 2-fold in females at 1250 mg/kg and in both sexes at 2500 and 5000 mg/kg. EROD activity increased up to 3-fold in males and females at ≥ 1250 mg/kg. PROD activity increased up to 16-fold in males and up to 30-fold in females at ≥ 1250 mg/kg. Increased amounts of CYP 2B1/2 and CYP 2E1 were detected by immunoblotting in both sexes at 2500 and 5000 mg/kg correlating with increases in microsomal enzyme activities. No effects on hepatic microsomal enzymes occurred at 500 mg/kg. All changes in hematological parameters and hepatic microsomal isoenzymes were reversible (see table below).

	Control		500 mg/kg		1250 mg/kg		2500 mg/kg		5000 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N Week 4	5	5	0	0	0	0	0	0	5	5
Bone Marrow										
Megakaryocytes[d]	98.9	89.4	--	--	--	--	--	--	69.7	67.1
Megakaryocytes (%) D			--	--	--	--	--	--	30%**	25%**
Liver Biochemistry[b]										
CYP Total (mg/g liver)	0.50	0.34	--	--	30%**	31%[ns]	36%**	59%**	68%**	107%**
AH (nmol/min/mg prot) I	0.06	0.10	--	--	--	2-fold**	1-fold**	2-fold**	4-fold**	4-fold**
NMND (nmol/min/mg p) I	0.39	0.48	--	--	--	1-fold**	1-fold[ns]	1-fold**	1-fold**	2-fold**

EROD (nmol/min/mg p) l	94.9	63.9	--	1-fold**	1-fold**	1-fold**	1-fold**	2-fold**	2-fold**	3-fold**
PROD (nmol/min/mg p) l	6.84	2.45	--	--	1-fold**	1-fold^{ns}	4-fold**	7-fold^{ns}	16-fold**	30-fold**
Immunoblot CYP 2B1/2	--	--	--	--	--	--	I	I	I	I
CYP 2E1	--	--	--	--	--	--	I	I	I	I

** p<0.01 (linear trend within ANOVA).
N = Number of animals; I = Increased; -- = No noteworthy findings; D = Decreased; ns = Not statistically significant; CYP =Cytochrome P450; AH = Aniline hydroxylase; NMND = Nitrosodimethylamine N-demethylase; EROD = Ethoxyresorufin O-deethylase; PROD = Pentoxyresorufin O-dealkylase; NA = Not applicable.
b Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.
c Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.
d Group mean number of megakaryocytes from 10 high power fields per animal of sternal bone marrow section are shown for controls. Statistical significance is based on actual data and not on the percent differences.

Urinalysis: There were no treatment-related differences at the end of week 4 or week 8.

Gross pathology: Group-related effects were seen with regard to tail dermatopathy, consisting of hyperkeratosis, acanthosis, inflammation, hemorrhage, fibrosis, necrosis, ulcers, scab formation, cellular infiltrates and/or bacteria, was seen in females at 500 mg/kg and in both sexes at ≥ 1250 mg/kg. Tail dermatopathy persisted in females at 1250 mg/kg and in both sexes at 2500 and 5000 mg/kg after the reversal period. Urinary bladder dilatation was noted at necropsy in males at all doses, and pyelonephritis was observed in 1 F each at 500 and 1250 mg/kg in Week 4. These changes, however, did not appear to be dose-related.

Organ weights (specify organs weighed if not in histopathology table): brain, kidney, heart, adrenal, pituitary, liver, pancreas, prostate, testes, uterus, ovary, epididymides, spleen and thymus.

Statistically significant trends towards reduced renal, cardiac, hepatic, splenic, pancreatic (decreased 27% in females at 2500 mg/kg and 25% to 48% in both sexes at 5000 mg/kg), thymic, and brain weights occurred in both males and females during the treatment phase. Adrenal and pituitary weights were similarly reduced in both sexes, although these changes were statistically significant only in the females. Prostate weights in the males (46% at 5000 mg/kg) and uterine weights in the females were also significantly reduced. These organ weight changes were consistent with reduced body weights and, except for the pancreatic weights in the females, were partially or fully reversed by the end of the withdrawal period. Pancreatic weights in the females were not reversed, although the differences between treated and control groups at the end of the withdrawal period were not statistically significant. See table below of absolute weights.

TABLE 3. Summary of Organ Weight Changes

Parameter (Weight)	MALES Treatment (% control)*	MALES Withdrawal (% control)	FEMALES Treatment (% control)	FEMALES Withdrawal (% control)
Body	69-90****	82-93***	74-100***	95-103
Brain	95-98**	97-103	93-100*	99-102
Kidney	72-84****	90-98	83-106**	101-107
Heart	68-88***	92-109	79-104**	98-107
Adrenal	71-81	110-134	75-99*	80-101
Pituitary	64-82	109-136	45-73***	100-127
Liver	76-92**	90-94	82-97**	102-107
Pancreas	52-74*	86-100	73-96**	74-78
Spleen	64-95***	104-117	64-95***	97-108
Thymus	51-104***	80-90	47-77***	121-147
Prostate	54-100*	88-93		
Testis	98-112	90-107		
Epididymis	89-102	100-107		
Uterus			68-97*	99-130
Ovary			91-117	108-117

* Range of absolute weights of treated groups as % of control.

Trend statistically significant at * 5000 mg/kg, ** 2500 mg/kg, *** 1250 mg/kg, **** 500 mg/kg

Histopathology: Adequate Battery: yes (x), no ( )--explain
Peer review: yes ( ), no (x)

Epididymal changes, including epididymal enlargement, hypospermia in tubules, and interstitial fibrosis and mononuclear cell infiltrates, were observed at all doses and the incidence and severity were increased at 2500 and 5000 mg/kg. An increased incidence of sperm granulomas was seen at 5000 mg/kg (see table below).

	Control		500 mg/kg		1250 mg/kg		2500 mg/kg		5000 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N Week 4	10	10	10	6	10	8	10	6	10	10
Histopathology Epididymis										
Hypospermia	--	NA	1	NA	3	NA	6	NA	7	NA
Interstitial Fibrosis	--	NA	4	NA	7	NA	8	NA	5	NA
Infiltrates Mononuclear	1	NA	6	NA	7	NA	8	NA	7	NA
Sperm Granuloma	1	NA	2	NA	1	NA	1	NA	3	NA

No drug-related histopathological changes were observed in testes. Prostatic atrophy and/or attenuation of prostatic epithelium was observed in 7 males at 5000 mg/kg. Single-cell necrosis, reduced zymogen granules, and/or acinar atrophy was noted in the pancreas in 3 males and 2 females at 5000 mg/kg. The incidence and severity of these histopathologic changes were decreased at the end of the reversal period. Tail dermatopathy, consisting of hyperkeratosis, acanthosis, inflammation, hemorrhage, fibrosis, necrosis, ulcers, scab formation, cellular infiltrates and/or bacteria, was seen in females at 500 mg/kg and in both sexes at ≥ 1250 mg/kg. Tail dermatopathy persisted in females at 1250 mg/kg and in both sexes at 2500 and 5000 mg/kg after the reversal period.

Important Findings	**Control**		**500 mg/kg**		**1250 mg/kg**		**2500 mg/kg**		**5000 mg/kg**	
Histopathology (continued)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N Week 4	10	10	10	6	10	8	10	6	10	10
Pancreas										
Necrosis	--	--	--	--	--	--	--	--	3	1
Reduced Zymogen	--	--	--	--	--	--	--	--	3	1
Acinar Atrophy	--	--	--	--	--	--	--	--	1	--
Skin (Tail)										
Hyperkeratosis	--	--	--	--	2	1	--	1	6	3
Acanthosis	--	--	--	--	3	2	2	1	5	4
Inflammation	--	--	--	--	--	--	2	--	--	--
Hemorrhage	--	--	--	--	1	--	1	--	--	--
Fibrosis	--	--	--	--	3	1	2	--	5	2
Necrosis	--	--	--	--	--	--	--	--	1	2
Ulceration	--	--	--	--	--	--	2	--	--	--
Scab Formation	--	--	--	--	--	--	--	--	2	1
Infiltrates (mononuclear & neutrophilic)	--	--	--	2	3	2	--	1	3	3
Bacteria	--	--	--	--	--	--	--	--	1	--
N Week 8	5	5	5	2	5	5	5	4	3	4
Histopathology Epididymis										
Interstitial Fibrosis	--	NA	--	NA	--	NA	--	NA	1	NA
Infiltrates Mononuclear	1	NA	1	NA	2	NA	--	NA	--	NA
Sperm Granuloma	--	NA	--	NA	--	NA	--	NA	1	NA
Skin (Tail)										
Fibrosis	--	--	--	--	--	2	1	1	--	--
Infiltrates (mononuclear & neutrophilic)	--	--	--	--	--	--	2	--	1	1

N = Number of animals; -- No noteworthy findings; NA = Not applicable.

Toxicokinetics: Plasma samples obtained for drug concentration analyses 0, 4, 7, and 12 hours after the initiation of the dark cycle during Week 4 from 3 controls/sex and 6/dose/sex; each drug-treated animal used for 2 time points (beginning and 7 hours, or 4 and 12 hours after initiation of the dark cycle; N = 3/time point. A single concentration-time curve was constructed from the mean of individual plasma drug concentrations at each sampling time. Control animals were all below the limits of detection (see table below).

Dose (mg/kg)	Sex	Plasma CI-1008 (μg/mL) and Sampling Time (hours after initiation of dark cycle)				Cmax (μg/mL)	AUC(0-24) (μg·hr/mL)
		0	4	7	12		
500	Males	24.3	55.1	99.7	106	106	1690
	Females	32.8	37.9	81.9	75.6	81.9	1370
1250	Males	85.0	172	267	270	270	4650
	Females	68.8	132	204	229	229	3770
2500	Males	246	332	494	456	494	8980
	Female	206	252	476	468	476	8410
5000	Males	376	571	799	753	799	14600
	Females	305	359	602	606	606	11300

Summary: *(from original T.D. Steele review)*

The most significant toxicological finding in this study was the death of 3 animals following 3-4 weeks of treatment with pregabalin. The deaths were attributed to inflammation/dysfunction of the urinary system.* In survivors, hypoactivity, ataxia, decreased body weight and food consumption, and tail dermatopathies were the primary clinical signs of pregabalin treatment. These findings are consistent with the 2-week study, as were increases in RBC parameters, decreases in platelets, male reproductive changes, and reductions in spleen, thymus, heart, kidney and liver weights. Of the non-reproductive tissues which decreased in weight, only the thymus and pancreas showed notable histopathological changes. Pregabalin markedly induced PROD (2B1/2) activity. As in the dead animals, some pathologies in the bladder were evident in pregabalin-treated rats. A "No Effect" level was not established.

* Added note: Urinary bladder dilatation and cystitis or pyelonephritis were seen histopathologically in these animals.

Study title: Repeated-Dose Toxicity: 4-Week Oral in Rats - Lower Doses

Key study findings: Rats were given pregabalin at 50, 100, and 250 mg/kg in the diet daily for 4 weeks since effects were seen at all doses in the previous 4-week study, with the following findings:

- No deaths were observed at any dose.
- No clinical signs were observed at 50 or 100 mg/kg.
- Tail dermatopathy was observed at 250 mg/kg throughout the treatment period but not during the reversal period.
- Body weight and clinical biochemical parameters were not affected. Food consumption increased 9% to 13% in males and females at 100 and 250 mg/kg. Food consumption was similar to controls in the reversal period.
- Platelet count decreased 14% to 20% in females at 100 mg/kg and in both sexes at 250 mg/kg, but was similar to controls after the reversal period.
- Bone marrow megakaryocyte count was not affected at 250 mg/kg; however, this was the only dose evaluated.
- Pancreas weight in males decreased 37% at 100 mg/kg and 44% at 250 mg/kg, and prostate weight decreased 18% at 100 mg/kg and 23% at 250 mg/kg; these decreases were reversible.
- No drug-related histopathological changes were noted after the treatment or reversal periods.
- NOAEL = 50 mg/kg (C_{max} ~11 µg/mL; 180 µg·h/mL).

Study no.:	1566
Volume #, and page #:	EDR M 4, I 5, V 007
Conducting laboratory and location:	Warner-Lambert Canada, Inc., Mississauga, Ont.
Date of study initiation:	15 Sept. 1994
GLP compliance:	yes
QA report:	yes (x) no ( )
Drug, lot #, and % purity:	CI-1008, Lot XH340993, purity [ ]

Methods

Doses:	0, 50, 100, 250 mg/kg
Species/strain:	Wistar rat
Number/sex/group or time point (main study):	10/sex/dose
Route, formulation, volume, and infusion rate:	oral in diet
Satellite groups used for toxicokinetics or recovery:	5/sex/dose: 4-week recovery
Age:	6 weeks
Weight (nonrodents only):	
Unique study design or methodology (if any):	

Observation times and results

Mortality: Observed daily. There were no deaths during the study.

Clinical signs: Observed daily. No clinical signs were observed at 50 or 100 mg/kg. Tail dermatopathy was observed at 250 mg/kg throughout the treatment period (see table below) but not during the reversal period.

Important Findings	Control		50 mg/kg		100 mg/kg		250 mg/kg	
	M	F	M	F	M	F	M	F
N	18	18	21	21	21	21	21	21
Clinical Signs								
Tail dermatopathy to Week 4	--	--	--	--	--	--	8	2
N Week 4	10	10	10	10	10	10	10	10
Food Consumption[b] (g)	681	445	--	--	9%**	10%[ns]	9%**	13%[ns]
I								
Hematology[c]								
Platelet Count (10^9/L)	1119	1003	--	--	--	867[ns]	946**	804**
D								
Percent of control			--	--	--	14%	15%	20%
Absolute Organ Weights[b]								
Pancreas (g)	1.30	--	--	--	37%**	--	44%**	--
D								
Prostate (g)	1.26	--	--	--	18%**	--	23%**	--
D								
Gross Pathology								
Skin (Tail) - Discoloration	--	--	--	--	--	--	4	--
- Abnormal Surface	--	--	--	--	--	--	2	1

** = $p<0.01$ (linear trend within ANOVA);
N = Number of animals; -- = No noteworthy findings; I = Increased; ns = Not statistically significant; D = Decrease.
[b] Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.
[c] Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.

Body weights: Determined weekly. There were no treatment-related effects.

Food consumption: Determined weekly. Mean food consumption increased 9% to 13% in males and females at 100 and 250 mg/kg (see table above). Food consumption was similar to controls in the reversal period.

Ophthalmoscopy: Ophthalmic examinations on animals designated for toxicologic assessment conducted pretest and in Weeks 4 and 8. There were no ophthalmic findings.

EKG: Not done

Hematology: At termination (week 4 or 8). Mean platelet count decreased 14% in females at 100 mg/kg and in both sexes (15 to 20%) at 250 mg/kg (see table above), but was similar to controls after the reversal period. Bone marrow megakaryocyte count was not affected at 250 mg/kg, the only dose evaluated.

Clinical chemistry: At termination (week 4 or 8). There were no treatment-related differences.

Urinalysis: At termination (week 4 or 8). There were no treatment-related differences.

Gross pathology: Treatment-related gross pathologic changes were limited to minimal lesions (discoloration, abnormal surface) of the skin of the distal tail in 4/10 males and 1/10 females at 250 mg/kg at the end of the 4-week treatment phase. Skin lesions of the distal tail were not observed in either sex at the end of the 4-week withdrawal period.

Organ weights (specify organs weighed if not in histopathology table): Brain, pituitary, heart, liver, spleen, thymus, kidney, adrenal, pancreas, prostate, uterus (including cervix), ovary, epididymis, and testis. Mean pancreas weight in males decreased 37% at 100 mg/kg and 44% at 250 mg/kg, and prostate weight decreased 18% at 100 mg/kg and 23% at 250 mg/kg; these decreases were not evident in the animals allowed a 1-month recovery period.

Histopathology: Adequate Battery: yes ( ), no (x)—explain (only tissue identified in previous study as targets) Peer review: yes ( ), no (x)
Only potential target organs (brain, heart, pancreas, testis, epididymis, prostate, seminal vesicle, bone marrow, and tail), and gross abnormalities from all groups were examined microscopically. No drug-related histopathological changes were noted after the treatment and reversal periods.

Toxicokinetics: Heparinized blood samples were collected during Week 4 from the last 3 animals per sex from the control group and from the last 6 animals per sex from each pregabalin treated group. Control animals were bled 4 hours after initiation of the dark cycle. Three treated animals per dose group were bled at 0 and 7 hours or 4 and 12 hours after initiation of the dark cycle. Plasma was assayed for pregabalin using a validated [ ] procedure. In general, there was no apparent gender difference in pregabalin pharmacokinetics. Increases in pregabalin C_{max} and $AUC_{(0-24)}$ values were dose proportional up to 250 mg/kg.

Toxicokinetic Parameters in Rats Given Pregabalin Daily in the Diet for 4 weeks

	Male		Female	
Dose (mg/kg)	Cmax	$AUC_{(0-24)}$	Cmax	$AUC_{(0-24)}$
50	11.2	181	11.3	172
100	23.9	360	18.1	325
250	60.2	923	42.7	729

Other: Bone marrow samples from 5/sex high dose and controls examined microscopically in Weeks 4 and 8. No effects were noted.

Study title: **Repeated-Dose Toxicity: 13-Week Oral in Rats**

Key study findings: Rats were given pregabalin at 50, 250, 500, or 1250 mg/kg in the diet daily for 13 weeks with the following findings:

- One female at 250 mg/kg died in Week 12; death was attributed to pyelonephritis. In addition to this animal, pyelonephritis was observed in 1 female at 1250 mg/kg.
- Urine staining and hypoactivity occurred in females at 1250 mg/kg.
- **Tail dermatopathy** was noted in both sexes at 1250 mg/kg and resolved by Week 7 in most animals.
- Body weight gain at Week 13 decreased 12% at 250 mg/kg, 25% at 500 mg/kg and 44% at 1250 mg/kg in males, and 42% at 1250 mg/kg in females. Food consumption decreased 10% at 250 mg/kg, 17% at 500 mg/kg, and 28% at 1250 mg/kg in males, and 18% at 500 mg/kg and 15% at 1250 mg/kg in females.
- **RBC count** increased 5% to 11% and **platelet count** decreased 18% to 34% in both sexes at ≥ 250 mg/kg.
- **Bone marrow total nucleated cell (TNC) count** decreased 29% at 500 mg/kg and 40% at 1250 mg/kg in males, and 22% at 250 mg/kg, 18% at 500 mg/kg, and 32% at 1250 mg/kg in females.
- Serum phosphorus increased 49% in females at 1250 mg/kg.
- Organ weights of animals at 500 and 1250 mg/kg were decreased secondary to decreased body weight gain.
- **Edema and hemorrhage of urinary bladder lamina propria** were seen in males at 250 and 1250 mg/kg and in females at 1250 mg/kg
- **Epithelial hyperplasia of bladder mucosa** occurred in both sexes at 1250 mg/kg.
- **Bone marrow hypocellularity** seen histopathologically in males at 500 and 1250 mg/kg and in females at ≥ 250 mg/kg correlated with flow cytometric results.
- 9% and 17% **decreases in epididymal weight** at 500 and 1250 mg/kg, respectively, considered treatment-related.
- Minimal to mild spermatogenic epithelial degeneration was observed in 3 males at 1250 mg/kg.
- Accumulation of **alveolar foamy macrophages** was observed in both sexes at 500 and 1250 mg/kg; incidence and severity were dose-related.
- Target tissues: bladder, skin, blood, bone marrow, male reproductive system, lung.
- NOAEL = 50 mg/kg

Study no.:	1994
Volume #, and page #:	M 4, I 5, V 017
Conducting laboratory and location:	Pfizer Global Research & Development, Ann Arbor Laboratories, Ann Arbor, Michigan
Date of study initiation:	28-Feb-1995
GLP compliance:	yes
QA report:	yes (x) no ( )
Drug, lot #, and % purity:	pregabalin, Lot XH340993, [redacted] % active

Methods

Doses: 50, 250, 500, 1250 mg/kg
Species/strain: Rat/Wistar
Number/sex/group or time point (main study): 10/sex/dose
Route, formulation, volume, and infusion rate: oral in diet
Satellite groups used for toxicokinetics or recovery: no
Age: 7 weeks
Weight (nonrodents only):
Unique study design or methodology (if any): This study was actually an interim sacrifice for the 52-week study.

Observation times and results

Mortality: Observed daily. One female at 250 mg/kg died in Week 12; death was attributed to pyelonephritis.

Clinical signs: Observed daily. Urine staining and hypoactivity occurred in females at 1250 mg/kg. Tail dermatopathy was noted in both sexes at 1250 mg/kg and resolved by Week 7 in most animals. See table next page.

Body weights: Determined weekly. There was a dose-related decrease on body weight gain at Week 13: 12% at 250 mg/kg, 25% at 500 mg/kg and 44% at 1250 mg/kg in males, and 42% at 1250 mg/kg in females. See table next page.

Food consumption: Determined weekly. Food consumption decreased 10% at 250 mg/kg, 17% at 500 mg/kg, and 28% at 1250 mg/kg in males, and 18% at 500 mg/kg and 15% at 1250 mg/kg in females. See table next page.

Ophthalmoscopy: Ophthalmic examinations conducted pretest on all animals and on animals designated for toxicologic assessment in Week 13; no effects noted.

EKG: none

Hematology: Mean RBC count increased 5% to 11% and platelet count decreased 18% to 34% in both sexes at ≥ 250 mg/kg. Mean bone marrow total nucleated cell (TNC) count decreased 29% at 500 mg/kg and 40% at 1250 mg/kg in males, and 22% at 250 mg/kg, 18% at 500 mg/kg, and 32% at 1250 mg/kg in females. These decreases occurred in all cell types, so no changes were noted in myeloid:erythroid (M:E) ratio. See table next page.

Clinical chemistry: Mean serum phosphorus was increased in treated females: outside historical control range (49%) at 1250 mg/kg. There were no changes in males.

Urinalysis: No changes occurred in urinalysis parameters.

Gross pathology: Treatment related gross and histopathological changes were seen urinary bladder, bone marrow, and lung. Edema and hemorrhage of urinary bladder lamina propria were

seen in males at 250 and 1250 mg/kg and in females at 1250 mg/kg; epithelial hyperplasia of bladder mucosa occurred in both sexes at 1250 mg/kg. See table below.

Important Findings		Control		50 mg/kg		250 mg/kg		500 mg/kg		1250 mg/kg	
		M	F	M	F	M	F	M	F	M	F
	N	10	10	10	10	10	10	10	10	22	22
Death – Pyelonephritis		0	0	0	0	0	1 Week 12	0	0	0	0
Clinical Signs											
Urine Staining		--	--	--	--	--	--	--	--	--	7
Hypoactivity		--	--	--	--	--	--	--	--	--	2
Tail – Dermatopathy		--	--	--	1	1	--	--	1	17	10
	N	10	10	10	10	10	10	10	10	10	10
Body Weight Gain[b] (g)	D	343	126	--	--	12%**	--	25%**	--	44%**	42%**
Food Consumption[b] (g)	D	196	123	--	--	10%[ns]	--	17%**	18%**	28%**	15%**
Hematology[c]											
Red Blood Cells (10^{12}/L)	I	8.38	8.23	--	--	9.11**	8.99**	9.04**	8.64**	9.26**	9.09**
Percent of Control				--	--	9%	9%	8%	5%	11%	10%
Platelet Count (10^9/L)	D	945	1013	--	--	772**	829**	626**	816**	671**	735**
Percent of Control				--	--	18%	18%	34%	19%	29%	27%
Bone Marrow[c]	N	5	5	5	5	5	5	5	5	5	5
Total Nucleated Cells (10^9/L)	D	1.74	1.48	--	--	--	1.16**	1.24**	1.22**	1.04**	1.00**
Percent of Control				--	--	--	22%	29%	18%	40%	32%
Clinical Chemistry[c]	N	10	10	10	10	10	10	10	10	10	10
Phosphorus (mg/dL)	I	--	8.3	--	8.9	--	10.3	--	9.8	--	12.4**
Percent of Control				--	--	--	--	--	--	--	49%
Histopathology											
Pyelonephritis		--	--	--	--	--	--	--	--	--	1
Urinary Bladder											
Edema Lamina Propria		--	--	--	--	1	--	--	--	2	1
Hemorrhage Lamina Propria		--	--	--	--	1	--	--	--	2	1
Epithelial Hyperplasia Mucosa		--	--	--	--	--	--	--	--	2	2
Bone Marrow - Hypocellular		--	--	--	--	--	1	2	2	3	6
Absolute Organ Weights[b]	N	10		10		10		10		10	
Epididymides	D	1.57		--		--		9%**		--	17%**
Histopathology	N	10		0		0		10		10	
Testis – Spermatogenic Epithelial Degeneration		--	NA	--	NA	--	NA	--	NA	3	NA
Histopathology	N	10	10	0	0	10	10	10	10	10	10
Lung - Alveolar Foamy Macrophage Accumulation		--	1	--	--	--	1	2	2	8	6

** $p<0.01$ (linear trend within ANOVA).
N = Number of animals; -- = No noteworthy findings; D = Decreased; ns = Not statistically significant; I = Increased.
[b] Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.
[c] Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.

Organ weights (specify organs weighed if not in histopath table): brain, pituitary, adrenals, gonads, prostate, epididymides, heart, lung, spleen, thymus, liver, pancreas, submandibular salivary glands, kidneys were recorded. Absolute organ weights of animals at 500 and 1250

mg/kg were decreased secondary to decreased body weight gain. The 9% and 17% decreases in epididymal weight at 500 and 1250 mg/kg, respectively, were initially considered due to decreased body weight gain, based on subsequent studies in rats, these changes are now considered treatment-related. See absolute and relative male organ weights below.

	0 mg/kg	50 mg/kg	250 mg/kg	500 mg/kg	1250 mg/kg
Body Wt (g)	536.8+ 5.54	520.4+ 4.24	491.9+ 7.31*	468.1+ 11.95*	403.4+ 12.52*
Brain	2.159+ 0.0178	2.157+ 0.0253	2.092+ 0.0273	2.086+ 0.0244	1.982+ 0.0514*
g/100g BW	0.403+ 0.0060	0.415+ 0.0064	0.426+ 0.0093	0.448+ 0.0123*	0.495+ 0.0180*
Kidney	4.036+ 0.0829	3.781+ 0.1047	3.416+ 0.0995*	3.229+ 0.1315*	2.902+ 0.1023*
g/100g BW	0.752+ 0.0140	0.727+ 0.0214	0.697+ 0.0256	0.690+ 0.0227	0.720+ 0.0156
g/g Brain	1.871+ 0.0433	1.756+ 0.0577	1.633+ 0.0438*	1.547+ 0.0568*	1.472+ 0.0599*
Heart	1.595+ 0.0462	1.550+ 0.0217	1.536+ 0.0445	1.440+ 0.0299*	1.348+ 0.0550*
g/100g BW	0.297+ 0.0081	0.298+ 0.0049	0.313+ 0.0100	0.308+ 0.0056	0.334+ 0.0081*
g/g Brain	0.740+ 0.0246	0.719+ 0.0130	0.734+ 0.0198	0.691+ 0.0153	0.683+ 0.0292
Lung	2.083+ 0.0744	2.037+ 0.1288	1.993+ 0.1196	2.086+ 0.0822	2.049+ 0.0981
g/100g BW	0.387+ 0.0139	0.392+ 0.0247	0.403+ 0.0244	0.447+ 0.0172	0.507+ 0.0178*
g/g Brain	0.970+ 0.0325	0.941+ 0.0519	0.957+ 0.0474	1.001+ 0.0420	1.045+ 0.0716
Thyroid-Para	0.034+ 0.0017	0.032+ 0.0018	0.030+ 0.0022	0.031+ 0.0011	0.030+ 0.0020*
g/100g BW	0.006+ 0.0003	0.006+ 0.0004	0.006+ 0.0004	0.007+ 0.0003	0.007+ 0.0005
g/g Brain	0.016+ 0.0008	0.015+ 0.0010	0.015+ 0.0011	0.015+ 0.0006	0.015+ 0.0011
Adrenal	0.072+ 0.0028	0.069+ 0.0052	0.063+ 0.0023	0.070+ 0.0042	0.065+ 0.0041
g/100g BW	0.013+ 0.0005	0.013+ 0.0010	0.013+ 0.0005	0.015+ 0.0009	0.016+ 0.0011
g/g Brain	0.033+ 0.0014	0.032+ 0.0024	0.030+ 0.0010	0.034+ 0.0020	0.033+ 0.0031
Pituitary	0.015+ 0.0006	0.013+ 0.0005	0.011+ 0.0004*	0.011+ 0.0008*	0.010+ 0.0005*
g/100g BW	0.003+ 0.0001	0.003+ 0.0001	0.002+ 0.0001	0.002+ 0.0001	0.003+ 0.0001
g/g Brain	0.007+ 0.0003	0.006+ 0.0003	0.005+ 0.0002*	0.005+ 0.0004*	0.005+ 0.0003*
S. Salv. Gld	0.823+ 0.0251	0.845+ 0.0453	0.756+ 0.0586	0.665+ 0.0437*	0.651+ 0.0408*
g/100g BW	0.154+ 0.0052	0.162+ 0.0088	0.154+ 0.0131	0.143+ 0.0102	0.162+ 0.0097
g/g Brain	0.381+ 0.0109	0.390+ 0.0172	0.360+ 0.0243	0.319+ 0.0214*	0.333+ 0.0274*
Liver	16.297+ 0.4099	17.144+ 0.4390	15.669+ 0.4144	15.071+ 0.5644	13.467+ 0.5258*
g/100g BW	3.034+ 0.0569	3.296+ 0.0850	3.187+ 0.0775	3.214+ 0.0634	3.336+ 0.0644
g/g Brain	7.555+ 0.2067	7.959+ 0.2353	7.490+ 0.1713	7.223+ 0.2476	6.834+ 0.3189*
Pancreas	1.682+ 0.1092	1.534+ 0.0873	1.417+ 0.0733	1.298+ 0.0824*	1.194+ 0.0729*
g/100g BW	0.314+ 0.0208	0.294+ 0.0155	0.288+ 0.0143	0.277+ 0.0148	0.300+ 0.0216
g/g Brain	0.778+ 0.0483	0.712+ 0.0418	0.679+ 0.0361	0.621+ 0.0379	0.604+ 0.0358*
Prostate	1.490+ 0.0649	1.461+ 0.0605	1.574+ 0.1114	1.281+ 0.0862	1.267+ 0.0584*
g/100g BW	0.277+ 0.0107	0.281+ 0.0128	0.320+ 0.0228	0.273+ 0.0158	0.315+ 0.0136
g/g Brain	0.691+ 0.0311	0.676+ 0.0235	0.750+ 0.0475	0.614+ 0.0418	0.643+ 0.0335
Testes	4.003+ 0.1204	3.765+ 0.1009	4.068+ 0.4120	3.787+ 0.0966	3.706+ 0.1278
g/100g BW	0.747+ 0.0269	0.725+ 0.0238	0.829+ 0.0866	0.815+ 0.0314	0.923+ 0.0354*
g/g Brain	1.855+ 0.0556	1.746+ 0.0474	1.952+ 0.2101	1.816+ 0.0446	1.871+ 0.0513
Epididymides	1.567+ 0.0442	1.520+ 0.0300	1.450+ 0.0357	1.427+ 0.0371*	1.306+ 0.0758*
g/100g BW	0.292+ 0.0081	0.292+ 0.0073	0.295+ 0.0078	0.307+ 0.0108	0.326+ 0.0213
g/g Brain	0.726+ 0.0214	0.705+ 0.0136	0.693+ 0.0144	0.685+ 0.0208	0.664+ 0.0433
Spleen	1.047+ 0.0208	0.969+ 0.0267	0.952+ 0.0354	0.993+ 0.0433	0.888+ 0.0274*
g/100g BW	0.195+ 0.0032	0.186+ 0.0052	0.193+ 0.0064	0.214+ 0.0108	0.222+ 0.0090
g/g Brain	0.486+ 0.0119	0.450+ 0.0145	0.456+ 0.0181	0.476+ 0.0204	0.450+ 0.0167
Thymus	0.531+ 0.0248	0.450+ 0.0255	0.457+ 0.0397	0.520+ 0.0353	0.413+ 0.0245
g/100g BW	0.099+ 0.0046	0.087+ 0.0051	0.093+ 0.0082	0.111+ 0.0068	0.103+ 0.0065
g/g Brain	0.246+ 0.0116	0.208+ 0.0109	0.220+ 0.0205	0.249+ 0.0156	0.211+ 0.0152

For each dose group, values expressed are means + standard errors.
* Mean value significantly different from control mean at 1% level by sequential trend test within one-factor analysis of variance.

See absolute and relative female organ weights in the table below:

Study 1994 - Females - Week 13 Sac.

	0 mg/kg	50 mg/kg	250 mg/kg	500 mg/kg	1250 mg/kg
Body Wt (g)	284.3+ 6.37	320.0+ 11.57	302.1+ 8.68	273.5+ 8.71	228.9+ 8.51*
Brain	1.972+ 0.0329	1.959+ 0.0445	1.953+ 0.0292	1.918+ 0.0376	1.895+ 0.0404
g/100g BW	0.697+ 0.0198	0.617+ 0.0211	0.650+ 0.0168	0.706+ 0.0229	0.837+ 0.0318*
Kidney	2.309+ 0.0648	2.413+ 0.0729	2.162+ 0.0645	1.981+ 0.0355*	2.066+ 0.0751*
g/100g BW	0.814+ 0.0212	0.758+ 0.0205	0.717+ 0.0169	0.731+ 0.0270	0.908+ 0.0347
g/g Brain	1.173+ 0.0349	1.236+ 0.0427	1.108+ 0.0311	1.036+ 0.0250*	1.093+ 0.0437*
Heart	1.016+ 0.0575	1.065+ 0.0234	1.059+ 0.0458	0.924+ 0.0244	0.899+ 0.0307*
g/100g BW	0.356+ 0.0162	0.335+ 0.0098	0.352+ 0.0161	0.339+ 0.0086	0.395+ 0.0120
g/g Brain	0.516+ 0.0293	0.545+ 0.0114	0.542+ 0.0215	0.483+ 0.0138	0.477+ 0.0193
Lung	1.621+ 0.1212	1.691+ 0.0897	1.583+ 0.0766	1.468+ 0.0527	1.621+ 0.1064
g/100g BW	0.571+ 0.0418	0.530+ 0.0249	0.524+ 0.0244	0.539+ 0.0197	0.713+ 0.0483*
g/g Brain	0.824+ 0.0629	0.862+ 0.0389	0.816+ 0.0328	0.768+ 0.0322	0.855+ 0.0496
Thyroid-Para	0.028+ 0.0018	0.030+ 0.0019	0.034+ 0.0029	0.027+ 0.0013	0.028+ 0.0026
g/100g BW	0.010+ 0.0006	0.010+ 0.0007	0.011+ 0.0009	0.010+ 0.0007	0.012+ 0.0010
g/g Brain	0.014+ 0.0009	0.015+ 0.0009	0.017+ 0.0015	0.015+ 0.0007	0.015+ 0.0016
Adrenal	0.113+ 0.0064	0.112+ 0.0042	0.111+ 0.0050	0.093+ 0.0051*	0.088+ 0.0030*
g/100g BW	0.040+ 0.0024	0.036+ 0.0019	0.037+ 0.0019	0.035+ 0.0029	0.039+ 0.0022
g/g Brain	0.057+ 0.0033	0.058+ 0.0028	0.057+ 0.0024	0.049+ 0.0035	0.047+ 0.0014*
Pituitary	0.015+ 0.0008	0.015+ 0.0005	0.014+ 0.0011	0.014+ 0.0004	0.010+ 0.0013*
g/100g BW	0.005+ 0.0003	0.005+ 0.0003	0.005+ 0.0004	0.005+ 0.0002	0.004+ 0.0005
g/g Brain	0.008+ 0.0004	0.008+ 0.0003	0.007+ 0.0006	0.007+ 0.0002	0.005+ 0.0006*
S. Salv. Gld	0.655+ 0.0491	0.661+ 0.0598	0.518+ 0.0344*	0.463+ 0.0213*	0.436+ 0.0193*
g/100g BW	0.231+ 0.0185	0.205+ 0.0147	0.172+ 0.0116	0.172+ 0.0130	0.193+ 0.0109
g/g Brain	0.335+ 0.0294	0.341+ 0.0356	0.267+ 0.0205	0.243+ 0.0146*	0.232+ 0.0129*
Liver	9.213+ 0.2739	9.873+ 0.3864	9.204+ 0.3465	8.315+ 0.1109*	8.062+ 0.2859*
g/100g BW	3.245+ 0.0849	3.100+ 0.1102	3.049+ 0.0812	3.065+ 0.0976	3.535+ 0.0977
g/g Brain	4.683+ 0.1614	5.064+ 0.2271	4.721+ 0.1852	4.348+ 0.0872	4.272+ 0.1769*
Pancreas	1.007+ 0.0850	0.999+ 0.0898	0.956+ 0.0662	0.813+ 0.0859	0.825+ 0.0701
g/100g BW	0.354+ 0.0291	0.312+ 0.0275	0.315+ 0.0162	0.297+ 0.0293	0.364+ 0.0339
g/g Brain	0.515+ 0.0465	0.518+ 0.0526	0.491+ 0.0351	0.423+ 0.0433	0.434+ 0.0342
Uterus	0.829+ 0.0921	0.756+ 0.0779	0.598+ 0.0285	0.603+ 0.0485	0.570+ 0.0513*
g/100g BW	0.291+ 0.0303	0.237+ 0.0247	0.200+ 0.0121	0.224+ 0.0206	0.251+ 0.0240
g/g Brain	0.421+ 0.0464	0.386+ 0.0380	0.308+ 0.0184	0.314+ 0.0239	0.301+ 0.0260
Ovary	0.176+ 0.0121	0.186+ 0.0159	0.199+ 0.0175	0.183+ 0.0167	0.175+ 0.0138
g/100g BW	0.062+ 0.0047	0.059+ 0.0053	0.066+ 0.0061	0.069+ 0.0083	0.077+ 0.0059
g/g Brain	0.090+ 0.0072	0.096+ 0.0086	0.102+ 0.0089	0.097+ 0.0103	0.092+ 0.0059
Spleen	0.699+ 0.0292	0.684+ 0.0377	0.629+ 0.0253	0.567+ 0.0222*	0.536+ 0.0254*
g/100g BW	0.247+ 0.0108	0.213+ 0.0082	0.208+ 0.0063	0.208+ 0.0076	0.236+ 0.0123
g/g Brain	0.355+ 0.0145	0.350+ 0.0180	0.322+ 0.0108	0.296+ 0.0121*	0.283+ 0.0125*
Thymus	0.465+ 0.0864	0.488+ 0.1241	0.332+ 0.0315	0.270+ 0.0267*	0.257+ 0.0178*
g/100g BW	0.161+ 0.0269	0.152+ 0.0384	0.109+ 0.0082	0.099+ 0.0096	0.112+ 0.0052
g/g Brain	0.240+ 0.0499	0.244+ 0.0567	0.170+ 0.0149	0.141+ 0.0138*	0.136+ 0.0106*

For each dose group, values expressed are means + standard errors.
* Mean value significantly different from control mean at 1% level by sequential trend test within one-factor analysis of variance.

Histopathology: Adequate Battery: yes (x), no ( ) explain
Peer review: yes ( ), no (x)

Tissues evaluated: brain (optic nerve, optic tract/hippocampus, substantia nigra, and lateral vestibular nucleus), spinal cord, sciatic nerve, pituitary, thyroid, parathyroid, adrenal, pancreas, liver, tongue, submandibular salivary gland, esophagus, stomach, small intestine, large intestine, trachea, larynx, lung, heart, thymus, spleen, mesenteric lymph node, eye (6% glutaraldehyde), Harderian gland, skin, mammary gland, skeletal muscle, bone, bone marrow, kidney, urinary bladder, testis (Bouin's), epididymis (Bouin's), prostate (Bouin's), seminal vesicle (Bouin's), ovary, uterus, vagina, and gross lesions. Tissues from the high-dose groups (Groups 4 and 8) and controls, tissues from animals found dead, and tissues with gross lesions were examined histopathologically.

Minimal to mild spermatogenic epithelial degeneration was observed in 3 males at 1250 mg/kg. Accumulation of alveolar foamy macrophages was observed in both sexes at 500 and 1250 mg/kg; incidence and severity were dose-related. In addition to the animal that died, pyelonephritis was observed in 1 female at 1250 mg/kg. Edema and hemorrhage of urinary bladder lamina propria were seen in males at 250 and 1250 mg/kg and in females at 1250 mg/kg; epithelial hyperplasia of bladder mucosa occurred in both sexes at 1250 mg/kg.

TABLE 3. Incidence of Pathologic Changes[a] in Selected Tissues After 13-Week Oral Administration of CI-1008 in Rats

Tissue/Lesion	Dose (mg/kg)									
	VC		50		250		500		1250	
	M	F	M	F	M	F	M	F	M	F
Kidney: Marked pyelonephritis	0	0	0	0	0	1	0	0	0	1
Urinary bladder lamina propria:										
minimal to moderate edema and/or										
minimal to mild hemorrhage	0	0	0	0	1	0	0	0	3	1
minimal to mild mixed cell infiltrates	0	0	0	0	1	0	0	0	3	2
Urinary bladder mucosa:										
minimal to moderate hyperplasia	0	0	0	0	0	0	0	0	2	2
Submandibular salivary gland acini:										
minimal to mild secretory depletion	0	0	0	-	2	0	3	3	7	7
Thymus:										
minimal to mild lymphoid depletion	0	0	0	0	1	0	0	2	0	3
Femoral bone marrow:										
minimal hypocellularity	0	0	0	0	0	1	2	2	3	6
Testicular spermatogenic epithelium:										
minimal to mild degeneration	0	-	-	-	-	-	0	-	3	-
Lung alveoli:										
minimal to marked alveolar										
macrophage infiltrates	0	1	-	-	0	1	2	2	8	6

[a] n = 10 unless noted otherwise.

Toxicokinetics: Plasma samples obtained for drug concentration analyses 0, 4, 7, and 12 hours after the initiation of the dark cycle during Week 13 from 3/controls/sex and 12/dose/sex; each drug-treated animal used for 1 time point; N = 3/time point. A single curve was constructed from individual samples for males and females at each sampling time. Animals used for plasma drug concentrations at 50, 250, and 500 mg/kg not included in total number of test animals. Pregabalin toxicokinetic parameters increased proportionally to dose, females appeared to have lower levels than males. See table.

Toxicokinetic Parameters –Week 13[a]

Dose (mg/kg)	50		250		500		1250	
	M	F	M	F	M	F	M	F
C_{max} (µg/mL)	12.7	8.9	56.8	54.0	99.3	74.7	248	258
AUC (µg·hr/mL)	228	149	1210	802	2270	1280	5370	4040

Other: Bone marrow samples from 5/sex/dose evaluated by flow cytometry in Week 13. Bone marrow hypocellularity seen histopathologically in males at 500 (-29%) and 1250 (-40%) mg/kg and in females at ≥ 250 mg/kg (-22 to 32%) correlated with flow cytometric results.

Study title: **52-Week Oral Toxicity Study of pregabalin in Male and Female Wistar Rats**

Key study findings: Rats were given pregabalin at 50, 250, or 500 mg/kg in the diet daily for 52 weeks Ten animals/sex/group were euthanized at Week 26, and the remaining 15 animals/sex/group were euthanized at Week 52, with the following results:

- One female at 250 mg/kg died in Week 44 due to pyelonephritis.
- Clinical signs were limited to tail lesions and urine staining.
- Tail dermatopathy occurred from Weeks 1 to 4 at 250 and 500 mg/kg with dose-related incidence.
- An increased incidence of urine staining occurred in females 250 and 500 mg/kg. Some animals exhibited urine staining for up to 39 weeks and others for only 1 or 2 weeks.
- Body weight gain decreased 24% from Weeks 0 to 52 in males at 500 mg/kg; body weight was unaffected in females. Food consumption decreased up to 16% in males at 250 and 500 mg/kg from Weeks 5 to 52 and up to 24% in females at 500 mg/kg sporadically throughout the study.
- RBC count increased 6% to 11%, and mean corpuscular volume decreased 4% to 8% in males at all doses and in females at 250 and 500 mg/kg in Weeks 26 and/or 52. Platelet count decreased 14% to 36% in males at all doses and in females at $\geq$ 250 mg/kg in Weeks 26 and/or 52. Mean platelet volume (MPV) in males decreased 7% at 250 mg/kg and 11% at 500 mg/kg at Week 52.
- Bone marrow changes at Week 26 included a 29% decrease in TNC count in females at 250 and 500 mg/kg reflecting decreases in myeloid, erythroid, and lymphoid cell lines with no change in M:E ratio. At Week 52, males at 500 mg/kg had a 44% decrease in TNC and no change in M:E ratio.
- Drug-related gross and/or histopathologic changes were observed in bone marrow, urinary bladder, submandibular salivary gland, and lung. Bone marrow hypocellularity in both sexes at 250 and 500 mg/kg in Weeks 26 and 52 correlated with decreased TNC counts.
- Minimal to moderate hemorrhage and/or edema in the urinary bladder lamina propria occurred at 500 mg/kg in both sexes at Week 26 and in males only at Week 52. Pyelonephritis was observed in 1 female at 500 mg/kg in Week 26 and in 1 female at 50 mg/kg in Week 52.
- Minimal to mild accumulation of macrophages in alveoli occurred with greater incidence in males at 500 mg/kg at Weeks 26 and 52 with no evidence of lesion progression.
- Minimally decreased secretory content in salivary gland acini of males and females at 250 and 500 mg/kg in Week 26 and at 500 mg/kg in Week 52 correlated with 15% to 33% decreases in salivary gland weight.
- A 19% and 13% decrease in epididymal weight at 500 mg/kg at Weeks 26 and 52, respectively, are now considered treatment-related
- Target organs: skin, bladder, kidney, lung, bone marrow, blood, salivary gland
- NOAEL = 50 mg/kg

Study no.:	1994
Volume #, and page #:	EDR - M 4, I 5, V 018
Conducting laboratory and location:	Pfizer Global Research & Development, Ann Arbor Laboratories, Ann Arbor, Michigan

Date of study initiation: 28-Feb-1995
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Pregabalin, Lot XH340993, τ J% active

Methods

Doses: 50, 250, 500 mg/kg/day
Species/strain: Rat/Wistar
Number/sex/group or time point (main study): 35/sex/dose
Route, formulation, volume, and infusion rate: oral in diet
Satellite groups used for toxicokinetics or recovery: 10/sex/dose used in 13-week study
Age: 47-49 days
Unique study design or methodology (if any): Ten animals/sex/group were euthanized at Week 26, and the remaining 15 animals/sex/group were euthanized at Week 52.

Observation times and results

Mortality: Observed daily. One female at 250 mg/kg died in Week 44 due to pyelonephritis.

Dose (mg/kg)	0		50		250		500	
Sex (M/F)	M	F	M	F	M	F	M	F
# of Test Animals	25	25	25	25	25	25	25	25
# Euthanized Week 26	10	10	10	10	10	10	10	10
# Euthanized Week 52	15	15	15	15	15	15	15	15
Death or Moribund	1[a]	0	1[a]	0	2[a]	1	2[a]	1[a]

[a] *deaths not considered drug-related*

Sponsor's table of intercurrent deaths:

Animal	Sex	Dose (mg/kg)	Week Died	Description	Gross Pathology	Histopathology/ Cause of Death
69017	M	0	33	Moribund sac	Hydrothorax	Chronic progressive cardiomyopathy
69041	M	50	51	Moribund sac	Ulcerated thoracic mass	Fibroma
69075	M	250	46	Found dead	Pulmonary edema	Pulmonary edema
69089	M	250	48	Found dead	Kidney mass	Malignant mesenchymal tumor
69121	M	500	32	Found dead	Kidney mass/ Hemoperitoneum	Malignant mesenchymal tumor
69122	M	500	32	Moribund sac	Hemothorax	Malignant mesothelioma
69223[a]	F	250	12	Found dead	Hydronephrosis	Hydronephrosis with pyelonephritis and urinary calculi
69244	F	250	44	Found dead	Hydronephrosis	Hydronephrosis with pyelonephritis and urinary calculi
69274	F	500	52	Found dead	Uterine hemorrhage	Hemangiosarcoma

[a] Results reported previously in 13-week interim report (RR 745-02570).

Clinical signs: Observed daily. Physical exams performed monthly. Clinical signs were limited to tail lesions and urine staining. Tail dermatopathy occurred from Weeks 1 to 4 at 250 and 500 mg/kg with dose-related incidence. The affected tails appeared normal in all animals by Week 5. An increased incidence of urine staining occurred in females 250 and 500 mg/kg. Some animals exhibited urine staining for up to 39 weeks and others for only 1 or 2 weeks. See table below.

Body weights: Determined weekly. Body weight gain decreased 24% from Weeks 0 to 52 in males at 500 mg/kg; body weight was unaffected in females.

Food consumption: Determined weekly. Food consumption decreased up to 16% in males at 250 and 500 mg/kg from Weeks 5 to 52 and up to 24% in females at 500 mg/kg sporadically throughout the study.

Important Findings	**Control**		**50 mg/kg**		**250 mg/kg**		**500 mg/kg**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Death or Moribund	--	--	--	--	--	1	--	--
Pyelonephritis						Week 44		
Clinical Signs								
Urine staining	--	1	1	3	1	5	1	15
Tail - Dermatopathy	1	1	--	--	2	1	6	8
Body Weight Gain[c] (g)	503		--	--	--	--	24%**	--

D								
Food Consumption[c] (g) D								
Weeks 5-52 Sporadic (Range)	185-		--	--	1%-16%**	--	--	--
Weeks 5-52 Consistent (Range)	199	136-	--	--	--	--	2%-16%**	--
Weeks 12-52 Sporadic (Range)		155	--	--	--	--	--	8%-24%**

** = p<0.01(linear trend within ANOVA);
N = Number of animals; -- = No noteworthy findings; D = Decreased; I = Increased; ns = Not statistically significant.
c Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.
d Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.

Ophthalmoscopy: Ophthalmic examinations conducted pretest on all animals and at termination. There were no drug-related ophthalmic findings.

EKG: none performed

Hematology: RBC count increased 6% to 11%, and mean corpuscular volume decreased 4% to 8% in males at all doses and in females at 250 and 500 mg/kg in Weeks 26 and/or 52. Platelet count decreased 14% to 36% in males at all doses and in females at ≥250 mg/kg in Weeks 26 and/or 52. Mean platelet volume (MPV) in males decreased 7% at 250 mg/kg and 11% at 500 mg/kg at Week 52. See table below.

Clinical chemistry: No changes occurred in biochemical parameters

Urinalysis: No changes occurred in urinalysis parameters.

Gross pathology: Drug-related gross and/or histopathologic changes were observed in bone marrow, urinary bladder, submandibular salivary gland, and lung. See table below and next page.

Organ weights: brain, pituitary, adrenals, gonads, prostate, epididymides, heart, lung, spleen, thymus, liver, pancreas, submandibular salivary glands, kidneys. Although 19% and 13% decreases in epididymal weight at 500 mg/kg at Weeks 26 and 52, respectively, were not originally considered treatment-related, subsequent studies suggested that they are. There were no drug-related effects on spermatogenesis. Several absolute and/or relative organ weights including kidney, pituitary, and salivary gland were significantly decreased at ≥ 250 mg/kg in the 26-week phase and at 500 mg/kg in the 52-week phase. No gross or histologic findings correlated with these organ weight changes, except for histologic changes in salivary glands. No other organ weight changes were considered toxicologically significant.

52-Week (26-week sac) Rat Primary Findings

Important Findings	**UC**		**50 mg/kg**		**250 mg/kg**		**500 mg/kg**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N Week 26	10	10	10	10	10	10	10	10

Hematology[d]								
Red Blood Cells (10^{12} L) ↑	8.44	8.17	8.95	--	9.12	--	9.39	9.10
Percent of Control			6%**	--	8%**	--	11%**	11%**
Mean Corpuscular Volume (fL) ↓	56.0	57.3	--	--	53.3	--	53.8	53.6
Percent of Control			--	--	5%**	--	4%**	6%**
Platelet Count (10^9/L) ↓	1037	999	--	--	844	844	742	811
Percent of Control			--	--	19%**	16%ns	28%**	19%**
N Week 26	5	5	5	5	5	5	5	5
Bone Marrow[d]								
Total Nucleated Cells (10^9/mL)	--	1.5	--	--	--	1.06	--	1.06
Percent of Control ↓	--		--	--	--	29%**	--	29%**
N Week 26	10	10	10	10	10	10	10	10
Absolute Organ Weights[c]								
Salivary Gland (g) ↓	0.96	0.63	--	--	--	15%**	33%**	19%**
Epididymides ↓	1.71	--	--		--		19%**	
Histopathology								
Bone Marrow - Hypocellular	--	--	--	--	3	2	3	4
Urinary Bladder - Edema	--	--	--	--	--	--	3	1
- Hemorrhage	--	--	--	--	--	--	2	--
Salivary Gland – Secretory								
Content Decreased in Acini	--	--	--	--	2	2	8	10
N Week 26	10	10	0	0	10	10	10	10
Histopathology								
Lung - Alveolar macrophage accumulation	2	1	--	--	2	--	4	2

** = p<0.01(linear trend within ANOVA);
N = Number of animals; -- = No noteworthy findings; D = Decreased; I = Increased; ns = Not statistically significant.
c Group means are shown for controls. Percent differences from control are shown for treated groups. Statistical significance is based on actual data and not on the percent differences.
d Group means are shown followed by percent differences from control below. Statistical significance is based on actual data and not on the percent differences.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Tissues evaluated: brain (optic nerve, optic tract/hippocampus, substantia nigra, and lateral vestibular nucleus), spinal cord, sciatic nerve, pituitary, thyroid, parathyroid, adrenal, pancreas, liver, tongue, submandibular salivary gland, esophagus, stomach, small intestine, large intestine, trachea, larynx, lung, heart, thymus, spleen, mesenteric lymph node, eye (6% glutaraldehyde), Harderian gland, skin, mammary gland, skeletal muscle, bone, bone marrow, kidney, urinary bladder, testis (Bouin's), epididymis (Bouin's), prostate (Bouin's), seminal vesicle (Bouin's), ovary, uterus, vagina, and gross lesions. Tissues from the high-dose groups (Groups 4 and 8) and controls, tissues from animals found dead, and tissues with gross lesions were examined histopathologically.

As shown in sponsor's table below, minimal to moderate hemorrhage and/or edema in the urinary bladder lamina propria occurred at 500 mg/kg in both sexes at Week 26 and in males only at Week 52. Pyelonephritis was observed in 1 female at 500 mg/kg in Week 26 and in 1 female at 50 mg/kg in Week 52. There was no evidence of progressive hyaline droplet accumulation in male rats as has been observed with gabapentin.

In the lung, minimal to mild accumulation of macrophages in alveoli occurred with greater incidence in males at 500 mg/kg at Weeks 26 and 52 with no evidence of lesion progression. Minimally decreased secretory content in salivary gland acini of males and females at 250 and 500 mg/kg in Week 26 and at 500 mg/kg in Week 52 correlated with 15% to 33% decreases in salivary gland weight. See summary table.

Sponsor's Summary Table of Pathological Findings from 26 and 52 Weeks:

TABLE 3. Incidence of Pathologic Changes in Selected Tissues After 26[a]- and 52[b]-Week Oral Administration of CI-1008 in Rats

Tissue/Lesion	Dose (mg/kg)							
	Control		50		250		500	
	M	F	M	F	M	F	M	F
Bone Marrow								
Week 26:								
Minimal Hypocell Femur	0	0	0	0	1	2	3	4
Week 52:								
Minimal Hypocell Femur	0	0	0	0	3	2	3	4
Minimal Hypocell Sternum	0	0	0	0	3	1	0	1
Mild Hypocell Femur	0	0	0	0	4	1	0	1
Urinary Bladder								
Week 26:								
Minimal to Mild Mucosal Hyperplasia	0	0	0	0	0	0	0	2
Minimal to Mild lp Edema	0	0	0	0	0	0	3	1
Minimal lp Hemorrhage	0	0	0	0	0	0	2	0
Week 52:								
Marked Mucosal Hyperplasia	0	0	0[d]	1[d]	0	0	0	0
Mild lp Edema	0	0	0	0	0	0	1	0
Minimal lp Hemorrhage	0	0	0	0	0	0	4	0
Moderate lp Hemorrhage	0	0	0	0	0	0	1	0
Lung								
Week 26:								
Minimal to Mild Alveolar Foamy Macrophage Infiltrate	2	1	--	--	2	0	4	2
Week 52:								
Minimal to Mild Alveolar Foamy Macrophage Infiltrate	3	1	1	1	4	0	7	5
Submandibular Salivary Gland								
Week 26:								
Minimal Secretory Depletion	0	0	0	0	2	2	8	10
Week 52:								
Minimal Secretory Depletion	0	0	0[d]	--	0	0	6	3
Kidney								
Week 26:								
Minimal CPN	6	2	0[d]	--	4	3	7	4
Mild CPN	2	0	0	--	1	0	2	2
Week 52:								
Minimal CPN	2	8	2[c]	0[d]	7	9	4	9
Mild CPN	9	1	0	0	5	2	3	3
Moderate CPN	2	0	1	0	3	2	4	0
Marked CPN	1	0	0	0	0	0	1	1

VC = Vehicle control; Hypocell = hypocellularity (pancytic); lp = lamina propria; CPN = chronic progressive nephropathy.

[a] n = 10, unless noted otherwise

[b] n = 15, unless noted otherwise

[c] n = 3

[d] n = 1

Toxicokinetics: In Week 48, blood samples were obtained for plasma drug concentration determination 12 hours after initiation of the dark cycle from 3 rats/sex/dose group to monitor exposure. See table.

Plasma Concentrations in Rats Given Pregabalin for 52 Weeks		
Dose (mg/kg)	Male	Female
50	10.6 ± 1.35	10.9 ± 2.57
250	53.0 ± 7.37	49.6 ± 13.5
500	130 ± 20.0	108 ± 16.8

Other: Bone marrow samples obtained at terminal sacrifice from 5 animals per group were evaluated by flow cytometry. An alternate flow cytometric methodology was used at Week 52. Bone marrow changes at Week 26 included a 29% decrease in total nucleated cell (TNC) count in females at 250 and 500 mg/kg reflecting decreases in myeloid, erythroid, and lymphoid cell lines with no change in M:E ratio. At Week 52, males at 500 mg/kg had a 44% decrease in TNC and no change in M:E ratio.

Study title: 4-Week Oral Toxicity Study Of pregabalin in Cynomolgus Monkeys

(taken verbatim from original IND review by Tom Steele except where noted)

Note: seems to have been performed at Parke-Davis in Ann Arbor, MI

Groups of cynomolgus monkeys (4/gender/dose) were treated by gavage once daily with 25, 50, 100, 500 mg/kg, or twice daily (BID) with 500 mg/kg of CI (Lot# XH340993 on 0.5% methylcellulose). BID doses were separated by 4 hrs. Control animals (4/sex) received 0.5% methylcellulose. (Note: the 25 and 50 mg/kg groups and an additional control group were added after the completion of the other groups since a NOEL was not established in that dosage range). At the end of four weeks of treatment, three animals were sacrificed ; the remaining animals was kept for an additional four-week reversal study. Clinical observations were made daily. Physical and ophthalmic exams were done pretest and at termination. Blood pressure and ECGs were recorded pretest, and 2 h postdose during week 4. Hematology, clinical chemistry, and urinalysis were done at pretest, and weeks 4 and 8. Liver microsome biochemistry, bone marrow analyses, gross pathology and histopathology were done at termination.

Mortality :

Monkey:	**Dose (#)**	**C_{max}**	**AUC**	**Comment**
M898	500 BID (2)		--	died 24 hr after Day 2 dose
F914	500 BID (6)		2630	euthanized 4.5 hrs after Day 3/Dose 2
F916	500 BID (2)		1640	died 24 hrs after Day 1/Dose 2
M900	500 BID(44)		2060	euthanized on Day 23 due to tail sores

The following refers to M898, F914, F916:		
Clinical :	All Fs	ataxia, hypoactivity, hypothermia, cyanotic mucous membranes, no ECG abnormalities, and BP normal until just prior to death
Histopath :	M898 F914	lymphoid necrosis (spleen, tonsil, lymph nodes) lymphocytolysis (spleen, tonsil, thymus, lymph nodes); atrophy of splenic pulp; centrilobular hepatocellular swelling
	F916	lymphocytolysis (spleen, tonsil, thymus, lymph nodes); myocyte vacuolation and necrosis ; centrilobular hepatocellular swelling
Cause(s) of Death:	Uncertain	

Surviving Animals:			
Clinical:	Ataxia, hypoactivity	-	1 500BID M ; 1 500BID F; 1 500 F (generally on Day 1 and 2)
	Soft Feces, diarrhea	-	all animals at 500 and 500 (bid) (started day 1 and 4; continued for duration of study; reversible)
	Nasal Discharge (red)	-	1 0M; 1 100M, 2 100F; 3 500M, 3 500F; 2 500(bid)M, 1 500(bid)F
	Tail dermatopathies	-	1 500M, 1 500F; 2 500(bid)M, 1 500(bid)F
	Body weight	-	No drug-related changes
	Ophthalmic Exam	-	No drug-related changes
	ECG	-	No drug-related changes
	BP	-	No drug-related changes
Hematology:	No statistically significant changes (P< 0.01). White blood cell counts were below the reference range in 4 control and 2 25 mg/kg animals. RBC counts were below reference in 1 500 (bid) animal.		
Bone Marrow:	No statistically significant (p<0.01) drug-related effects were evident. However, the mean absolute lymphoid cell count in 500(bid) males was decreased by 60% relative to controls.		
Biochem:	Significant reductions (p<0.01) in serum albumin were found in 500 and 500(bid) males and 500 females; values were below the reference range only in males. Significant reductions (p<0.01) were also noted in inorganic phosphorus (100 and 500(bid) males) and glucose (50 males), but values were not outside the reference range. Some of the more crucial data, LDH measurements in particular, were difficult to interpret. Almost and all data (including control animal and pretest measurements) fell above the reference range. Frequently, 4 week data values appeared to double the pretest measurement, but again this occurred in control and treated animals. Several AST and ALT measurements at 4 weeks exceeded the reference range, also frequently in control animals . Inexplicably, in the individual data tables, the clinical enzyme (LDH, AST, ALT) determinations that fell outside the reference range were not marked (#) similarly to other deviating clinical measurements. Serum creatinine phosphokinase was a notable oversight in the clinical chemistry determinations.		
Urinalysis	No significant changes in urine specific gravity or pH. Blood was detected in several animals including controls.		
Liver Microsomes	CI did not induce ethoxyresorufm-O-deethylase or erthyromycin-N-demethylase activities.		
Organ Weight:	Evaluation of organ weight is limited by the small sample size, high variability among animals, and absence of a historical control range. Notable trends were:		
	Heart:brain increase	35% in 500(bid)M 42-45% in 500* and 500(bid)F	
	Low testis weight	1 100M, 1 500M	
	Thymus decrease	48% in 500M	

Gross Path:	Heart	see histopath table
	Spleen small	1 100F
	Thymus enlarged	1 100F, 1 500F
	Small	1 500M, 1 500(bid)F (reversal)
	Testes, small	1 100M, 1 500 M
	Tail dermatopathy	1 0M, 2 50M, 1 500M, 1 500F, 1 500(bid)M

Histopath: Some histopathological changes in the heart accompanied gross path and organ weight changes:

8.10. TABLE T-10 Significant Heart Changes in Monkeys Treated With CI-1008[a]

Treatment Group (mg/kg)	Sex	Animal Number	Ht Wt/ Body Wt[b] (g/100 g)	Ht Wt/ Brain Wt[c] (g/100 g)	Gross Changes	Microscopic Myocardial Changes
50	M	927	0.423	31.669	--	Infiltrate[d]
		928	0.447	31.624	--	Infiltrate[d]
		929	0.417	28.666	--	Infiltrate[d]
100	M	891	0.420	30.836	--	IVS and LV Degeneration
500 BID	M	899	0.418	31.278	--	--
		900	0.355	33.854	--	Infiltrate[d]
100	F	908	0.454	24.201	Enlarged	IVS and LV Degeneration
		909(R)	0.405	23.158	--	IVS Necrosis and LV Fibrosis
500	F	910	0.458	27.110	Enlarged	--
		912	0.329	29.343	RV Focus	Epicardial Fibrosis; Myocardium Normal
500 BID	F	915	0.539	27.741	Enlarged	LV Necrosis
		916	NA	NA	--	IVS Necrosis
		917(R)	0.406	22.076	--	IVS Fibrosis

-- = No changes.
IVS = Interventricular septum.
LV = Left ventricle.
R = Reversal animal.
RV = Right ventricle.
NA = Not applicable (no organ weights taken; animal found dead).
[a] Incidental findings considered biologically insignificant are not included.
[b] Heart/body weight ratio (combined control range); males: 0.316-0.396; females: 0.306-0.423
[c] Heart/brain weight ratio (combined control range); males: 21.867-32.375; females: 17.030-28.348
[d] Same as controls.

Other notable histopath changes were:

Thyroid atrophy	1 100F, 1 500(bid)H (R)
Liver fibrosis	1 25M, 1 100F
Swelling	1 500F
Vacuolation	1 25F, 1 50M, 1 50F
Lymph node histiocytosis	1 500(bid)F, 1 500(bid)F (R)
Thymus atrophy	1 0M, 1 0F(R), 1 500M, 1 500M(R), 1 500(bid)F(R)
Spleen, atrophy	1 500(bid)F(R)
Epididymis, hypospermia	1 100M, 1 500(bid)M
seminiferous tubules, hypospermia	1 100M, 1 500(bid)M
testes, degeneration	1 100M (R), 1 50M (R)
kidney, atrophy	1 100F
dilatation	1 100F, 1 500(bid)M; 1 500(bid)F, 1 500(bid)M (R); 1 500(bid)F (R)
fibrosis	1 0F, 1 100F, 1 25F(R), 1 100F (R)
adrenal, hyperplasia	1 25F
vacuolation	1 25F
abnormal	1 500(bid)F (R)
hypertrophy	1 100M (R)
pancreas, hyperplasia	1 500(bid)M (R)
lung, granuloma	1 25M

Summary: The most significant toxicological finding in this study was lethality in 3 animals at 500 mg/kg, bid (a fourth animal was euthanized due to tail sores). As in previous studies, the cause of death was undetermined. Also consistent with previous studies, lymphocytolysis or lymphoid depletion occurred in the animals that died early in the study. The possible link between lymphocytolysis and death was not discussed. Clinical signs of ataxia and hypoactivity preceded death, and was also seen in surviving animals. As in the previous rat and monkey studies, male reproductive tissues were affected by repeated CI treatment. Also as in rats, the thymus appears to be a target of CI toxicity in monkeys, although the atrophy noted in this study was equivocal because of a similar finding in control animals.

Myocardial degeneration/necrosis was evident at ≥ 100mg/kg. Heart:brain or body weight ratios appeared elevated at ≥ 50 mg/kg. However, CI did not acutely effect ECG or blood pressure. The sponsor states that there was no evidence of a drug-induced inflammatory response, which may indicate myocardial ischemia. However, a high incidence of myocardial tissue infiltration by lymphocytes and monocytes was evident in most of the animals (including controls) in this study, which made it difficult to identify a "drug induced" inflammatory response.

The NOAEL in this study was 25 mg/kg.

(J. Cott Note: Pregabalin was found in plasma of 5 control females at day 1 and in 1 at week 4. Sponsor summary table from NDA submission is added below.)

Important Findings	VC		25 mg/kg		50 mg/kg		100 mg/kg		500 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N	8	8	4	4	4	4	4	4	4	4
Clinical Signs										
Nasal Discharge	1	--	--	--	--	--	1	2	3	3
Ataxia on Days 1 or 2	--	--	--	--	--	--	--	--	--	1
Soft Feces	--	--	--	--	--	--	--	--	4	4
Diarrhea	--	--	--	--	--	--	--	--	4	4
Tail Dermatopathy Nonhealing	--	--	--	--	--	--	--	--	1	1
N Week 4	6	6	3	3	3	3	3	3	3	3
Organ Weights[b]										
Heart (g/g Brain) I	--	0.19	--	--	--	--	--	--	--	42%**
Gross Pathology										
Heart - Enlarged	--	--	--	--	--	--	--	1	--	--
- Hypertrophy Left Ventricle	--	--	--	--	--	--	--	--	--	1
Histopathology										
Heart - Degeneration Left Ventricular Wall and Interventricular Septum	--	--	--	--	--	--	1	1	--	--
- Fibrosis Left Ventricle	--	--	--	--	--	--	--	--	--	1
Nasal Cavity - Neutrophilic Inflammation	--	2	--	--	--	--	1	--	3	3
Tail Extremities - Neutrophilic Inflammation	--	--	--	--	--	--	--	--	1	2
N Week 8	2	2	1	1	1	1	1	1	1	1
Histopathology										
Heart - Necrosis Interventricular Septum and Fibrosis Left Ventricle	--	--	--	--	--	--	--	1	--	--
- Fibrosis in Apex	--	1	--	--	--	--	--	--	--	--
- Fibrosis Left Ventricle	--	--	--	--	1	--	--	--	--	--
Nasal Cavity - Neutrophilic Inflammation	--	--	--	--	--	--	--	--	1	--

** p<0.01 (linear trend within ANOVA).
N Number of animals; -- = No noteworthy findings; I - Increased.
[b] Group mean is shown for controls. Percent difference from control is shown for treated group. Statistical significance is based on actual data and not on the percent differences.

Important Findings	500 mg/kg BID		Comments
	M	F	
N	4	4	
Death or Moribund			
Found Dead on Day 2	1	0	Ataxic and hypoactive 2 hours postdose on Day 1.
Died on Day 2	0	1	Preceded by ataxia, hypoactivity, cyanosis and hypothermia.
Euthanized Moribund on Day 3	0	1	Preceded by ataxia, hypoactivity, cyanosis and hypothermia.
Euthanized Moribund in Week 4	1	0	Due to tail dermatopathy.
N	2	2	
Clinical Signs			
Nasal Discharge	2	2	
Ataxia on Days 1 or 2	--	1	
Soft Feces	2	2	
Diarrhea	2	2	
Tail Dermatopathy - Nonhealing	1	1	
Tail Amputation	--	1	
N Week 4	1	1	
Gross Pathology			
Heart - Enlarged	--	1	
Histopathology			
Heart - Necrosis Left Ventricle	--	1	Necrosis of interventricular septum also noted in the female that died on Day 2
Nasal Cavity - Neutrophilic Inflammation	1	1	Changes in nasal cavity also noted in male euthanized in Week 4.
Tail - Neutrophilic Inflammation	1	--	
N Week 8	1	1	
Histopathology			
Heart - Fibrosis Interventricular Septum	--	1	
Nasal Cavity - Neutrophilic Inflammation	--	1	
Tail - Neutrophilic Inflammation	--	1	

BID - Dosed twice daily with 4 hours between doses; N - Number of animals; -- - No noteworthy findings.

Study Title: 52-Week Oral Toxicity Study: 13-Week Interim Report (10, 25, 100, 250, 500 mg/kg/day, gavage) *(taken verbatim from original IND review by Tom Steele except where noted)*

Since "equivocal" cardiac effects were observed in the 4-week oral toxicity study, this portion of the chronic toxicity study evaluated potentially cardiotoxic effects of CI. Cynomolgus monkeys (3-6/gender/dose) were administered 0, 10, 25, 50, 100, 250 or 500 mg/kg CI (Lot unspecified) in 0.5% methylcellulose. Resting and ambulatory ECGs and blood pressures were recorded pretest, prates, and 2 hrs postdose at Weeks 4, 8, and 13. Echocardiograms were recorded pretest and at Week 13. Serum LDH and CK were determined pretest and at Weeks 1, 2, 3, 4, 6, 8 and 13. An interim sacrifice on animals from the 10, 25, 100 and 500 mg/kg dose groups was conducted at Week 13 for microscopic examination of hearts.

Mortality: None

Clinical:	tail dermatopathy	≥ 25 mg/kg in males, ≥ 100 mg/kg in females
	ECG:	No significant drug-related effect
	BPs:	No significant drug-related effect
	Echocardiogram	Increase aortic diameter at week 13 (100 = 29%, 250 = 19%, 500 = 14%)

BioChem: The LDH and CK data were difficult to interpret. Although the testing appears to have been conducted at the same facilities as the previous toxicity studies, the reference range data are substantially different than in previous studies (most all of the LDH data in this study would exceed the reference range in previous studies). As the data stands, LDH and CK elevations were sporadic and distributed among all treatment groups. No animals appeared to have consistently high or progressively higher levels over the course of the study. Mean levels of total CPK were significantly elevated in 100, 250 and 500 mg/kg males at Week 13 primarily due to increases in the MM (skeletal muscle) isozyme.

Organ Weights: There were no statistically significant ($p<0.01$) differences in organ weights. The following trends in means were noted:

liver:brain	increase 47% in 500M; increase 32% in 500F
spleen:brain	increase 50% in 500M; increase 50% in 25F, 27% in 500F

Gross Path: Mean heart weights were not increased by CI. One 100F had mild ventricular enlargement. Two 25F had enlarged spleens.

Histopath: heart, fibrosis: 1 0M, 1 0F, 1 10M, 2 10F, 1 100M, 1 500M

Lymphocytic or mononuclear cell infiltrates were present in cardiac tissue of most animals. The ventricular enlargement in the 100F was accompanied by myocyte hypertrophy, anisokaryosis, karyomegaly, and myofiber disarray, and attributed to spontaneous hypertrophic cardiomyopathy.

Summary: This study failed to identify drug-induced cardiac abnormalities that may be associated with the lethal effects of CI. The drug did not acutely alter resting or ambulatory ECGs,

echocardiograms, or blood pressures. Cardiac fibrosis was found at a similar incidence rate in control and treated animals at the end of 13 weeks of treatment.

(Cott note: 3/4 animals with the worst tail lesions had high blood levels of drug, esp. 2/4 HDM.)

Study title: **Chronic Oral Toxicity Study of Pregabalin in Cynomolgus Monkeys**
(Note: this is a continuation of the same, previous, 13-week study.)

Key study findings: Monkeys were given pregabalin at 10, 25, or 100 mg/kg by gavage daily for 65 to 69 weeks or 250 mg/kg for 13 weeks followed by 500 mg/kg for an additional 52 weeks to assess chronic toxicity and found:

- Soft feces or diarrhea was observed in all animals at 500 mg/kg throughout the study
- One female at 500 mg/kg was found dead during Week 39; abdominal distension and pathologic findings were consistent with acute gastric dilatation
- In another female at 500 mg/kg, clinical signs of abdominal distension, dyspnea, hypothermia, and cyanosis in Week 65 were due to colonic dilatation with moribundity resulting from cardiopulmonary collapse.
- Tail dermatopathy occurred at all doses including controls with increased incidence at ≥ 25 mg/kg. Lesions were similar to those seen in the 13-week study. In general, onset was earliest at 500 mg/kg with lesions initially appearing during Week 2. In all animals except 1 female at 500 mg/kg, tail lesions resolved before study termination.
- There were no effects on body weight, food consumption, or ophthalmic parameters.
- No clinically significant effects were noted in resting and ambulatory ECG, echocardiographic, or blood pressure parameters.
- Clinical laboratory parameters and bleeding time were unaffected.
- No differences were noted in semen analyses conducted pretest and at Weeks 13, 40, and 65.
- Slight erythrocyte autoagglutination was present at all doses at Week 35 with a higher incidence at 100 and 500 mg/kg. Autoagglutination was not present at study termination.
- Platelet aggregation in the presence of ristocetin and/or arachidonic acid was enhanced in females at 500 mg/kg. (sponsor suggests ADP-induced aggregation is not biologically relevant in the absence of changes in collagen).
- There were no drug-related organ weight changes or histopathologic findings.
- NOAEL = 10 mg/kg

Study no.:	1992
Volume #, and page #:	M 4, I 5 V 022
Conducting laboratory and location:	Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company Ann Arbor, Michigan
Date of study initiation:	04/03/95
GLP compliance:	yes
QA report:	yes (x) no ( )

Drug, lot #, and % purity: Pregabalin (PD 144723-0000), Lots: XH340993, XH330993; [ ] and [ ], respectively.

Methods

Doses: 0, 10, 25, 100, 250/500, and 500 mg/kg

Species/strain: wild-caught cynomolgus monkeys from [ ] and [ ] Primates

Number/sex/group or time point (main study): 3-6/sex/group, control: 7/sex

Route, formulation, volume, and infusion rate: oral gavage, suspended in 0.5% methylcellulose in a dose volume of 5 mL/kg

Satellite groups used for toxicokinetics or recovery: main animals used

Age: 3 to 14 years

Weight (nonrodents only): 2.8 to 8.8 kg

Unique study design or methodology (if any): At the end of Week 13, 3 monkeys/sex at 10, 25, and 100 mg/kg, all monkeys at 500 mg/kg, and 4 controls/sex were sacrificed. The remaining animals continued on study for an additional 52 to 56 weeks, with animals previously dosed at 250 mg/kg given 500 mg/kg.

Observation times and results

Mortality: Observed daily. One HDF (#1001) was found dead during the Week 39 ambulatory ECG procedure with a bloated abdomen and prolapsed rectum. Diarrhea was the only significant clinical sign noted the day prior to death. A second HDF (#1000) developed a gas-distended abdomen with dyspnea, cyanosis, and hypothermia (body temperature 36.2°C) approximately 24 hours after ambulatory ECG procedures during Week 65 and was subsequently euthanized in moribund condition.

Clinical signs: Daily. Physicals at ~ 1-month intervals. Tail dermatopathy occurred in 1 LDF, 2 M and 1 F at 25 mg/kg, 1 M and 5 F at 100 mg/kg, 3 M and 2 F at 250 mg/kg, and 4 M and 3 F at 500 mg/kg. The female (1001) at 250 mg/kg that did not have tail dermatopathy during Weeks 1 to 13 subsequently developed the lesion at 500 mg/kg during Week 18. Tail dermatopathy was usually characterized by single or multiple erosive lesions often associated with sloughing skin or crusts. Lesions were most severe in 1 male (971) and 1 female (1006) at 500 mg/kg whose distal tails became necrotic and required amputation by Week 10. Lesions appeared initially on the distal tail and often progressed proximally, but were otherwise randomly distributed. The onset of the lesion was generally dose-related ranging from Week 2 at 500 mg/kg to Week 54 at 25 mg/kg. Except for 1 female at 500 mg/kg (250 mg/kg during Weeks 1 through 13) with tail dermatopathy during Weeks 8 to 65, tail dermatopathy in animals treated beyond Week 13 resolved before the end of the study. No drug-related changes occurred in subcutaneous tail temperatures.

Soft feces/diarrhea occurred in all animals at 500 mg/kg throughout the study. It occurred with relatively low incidence in other treated and control groups.

Emesis, generally associated with dosing, occurred mainly in drug-treated groups. However, weekly frequency was highly variable among individuals within groups and no drug-related effects occurred with respect to onset, severity, or duration.

Nasal discharge occurred in 1 to 3 animals in most groups, including controls, and was characterized as bloody, purulent, or serous. Nasal discharge was also noted pretest in some affected animals. It generally began and ended during Weeks 1 to 13; however, intermittent

episodes, usually serous, occurred during Weeks 14 to 62 in 1 female control, 1 female at 100 mg/kg, and 2 males and 1 female at 500 mg/kg. At Week 27, nasal discharge became bloody and was associated with skin sores at the nostrils in Female 1000 at 500 mg/kg. Staphylococcus aureus and Corynebacterium species were isolated from a nasal swab culture taken at that time.

Low-grade heart murmurs were detected pretest in 1 control female, 1 female at 10 mg/kg, 1 male at 25 mg/kg, 1 female at 100 mg/kg, and 1 female at 250 mg/kg. Split first heart sounds were ausculted pretest in 1 male at 500 mg/kg. Except for the 250 and 500 mg/kg female (1000) whose murmur detected pretest was generally audible throughout the study, abnormal heart sounds were not detected in any animal after Week 13.

Body weights: No drug-related effects occurred in mean weekly body weights.

Food consumption: Reduced daily (visual only) food consumption occurred with relatively equal incidence in all groups.

Ophthalmoscopy: Pretest, weeks 13, 26, 65. There were no drug-related changes.

EKG:
Methods: BP and resting ECGs at pretest and at Weeks 4, 8, 13, 26, 39, 52, and 65; Holter ECGs recorded pretest and Weeks 4, 8, 13, 26, 39, and 65. Parameters measured were incidence of ventricular ectopic complexes (singles, pairs, bigeminy, ventricular tachycardia, R-on-T forms, morphologies, total incidence, and incidence 2 to 4 hours postdose, approximating tmax) and supraventricular ectopic complexes, and the presence of second or third degree atrioventricular block.

Echocardiographic examinations were conducted on anesthetized animals pretest and Weeks 13, 25 or 26, 39, and 65. The following parameters were obtained using M-mode and/or 2-D echocardiographic functions: left ventricular internal dimension diastole (LVIDd), left ventricular internal dimension systole (LVIDs), systolic and diastolic left ventricular posterior wall thickness, systolic and diastolic interventricular septum thickness, E point to septal separation, and aortic diameter. Fractional shortening (calculated based on LVIDd and LVIDs) measures contractility of the left ventricle and is a simple measure of left ventricular function.

Results: No differences occurred between treated and control groups in resting and ambulatory ECG parameters. In ambulatory ECG, the prevalence of ventricular ectopic complexes (total and approximately 2-4 hours postdose) in treated animals was not different from controls. The range of abnormal ECG complex types was similar in treated and control groups, was consistent with pretest findings, and was typical of findings reported in healthy cynomolgus monkeys.

No statistically significant differences occurred between treated and control groups in echocardiographic parameters except for increases in aortic diameter in 100, 250, and 500 mg/kg males at Week 13 and increased left ventricular internal dimension (systole) in 500 mg/kg males at Week 39.

There were no drug-related changes occurred in systolic and diastolic blood pressures.

Hematology: Pretest and Weeks 4, 8, 13, 26, 39, 52, and 65. Statistically significant differences between treated and control groups in hematologic parameters were not considered clinically significant because of their low magnitude, intermittent nature, and/or lack of a dose response. Anemia and/or thrombocytopenia were noted during Weeks 27 through 32 in Female 1000 at 500 mg/kg.

Marked agglutination of erythrocytes was noted on a peripheral blood smear from Female 1000 at 500 mg/kg at Week 26. Slight autoagglutination of erythrocytes occurred in at least one animal at each dose except controls at Week 35, with a higher incidence in animals at 500 mg/kg. Autoagglutination was not apparent in blood smears at termination (Weeks 65-69). No inhibitory effects occurred on collagen, ADP, ristocetin, or arachidonic acid-induced platelet aggregation or ATP secretion. Enhanced aggregation of platelets in the presence of ristocetin and/or arachidonic acid occurred in females at 500 mg/kg. No drug-related effects occurred in bleeding time.

No clinically significant changes occurred. Female 1000 at 500 mg/kg showed a profoundly decreased total nucleated cell count at termination. However, histologic review of a sternal section from this animal revealed normal bone marrow cellularity and cellular distribution. A sampling error was suggested to account for the decreased total nucleated cell count.

Clinical chemistry: Pretest and Weeks 4, 8, 13, 26, 39, 52, and 65. Statistically significant differences between treated and control groups were generally considered clinically insignificant because of their intermittent nature, low magnitude, direction of the change was not relevant, lack of a dose response, and values that generally remained within historical ranges. Elevated serum enzymes in Female 1000 at 500 mg/kg at termination were attributed to its moribund condition.

Urinalysis: Pretest and termination. No clinically significant changes occurred

Gross pathology: Complete necropsies were performed. At the 13-week necropsy, the skin of the tail had sores in several animals at 100 and 500 mg/kg. Histologically, inflammatory lesions were observed containing neutrophils and accompanied by hyperkeratosis, hemorrhage, and granulation tissue. Tail lesions were treatment-related, but the incidence and severity of inflammation were not clearly dose related. The axial skeletal muscle of the amputated portion of the tail from a high-dose female (1006) demonstrated necrosis in addition to ulceration and inflammation of the overlying epidermis. One control animal had a focal mild ulcer and mixed cell infiltrates noted at abrasion sites; these changes were less severe than skin changes in animals administered 100 and 500 mg/kg. The lesions appear to have gone away, since animals sacrificed at termination did not have these tail lesions.

Based on a previous 4-week study with monkeys (RR 745-02329), and 4- and 13-week studies in rats administered pregabalin (RR 250-01730 and RR 745-02570), the cardiovascular and male reproductive systems represent potential targets of toxicity. However, in the present study, no treatment-related changes were observed in heart or aortic tissue at termination. Pathologic changes noted in myocardial tissues of treated animals at the 13-week sacrifice included mononuclear cell infiltrates. See table below.

Histopathology Group Incidence Summary
Week 13 Sacrifice

9.21. Table T-21 (Page 1 of 9)

Group	1 / 7		2 / 8		3 / 9		4 / 10		6 / 12	
Dose(mg/kg)	V.C.		10		25		100		500	
Sex	M	F	M	F	M	F	M	F	M	F
Animals On Study	7	7	6	6	6	6	6	6	4	4
Animals Logged	4	4	3	3	3	3	3	3	4	4
Cardiovascular										
Aorta	4	4	3	3	3	3	3	3	4	4
Not Remarkable	4	3	3	3	3	3	3	3	4	3
Remarkable Observations	0	1	0	0	0	0	0	0	0	1
Inflammation, eosinophilic	0	0	0	0	0	0	0	0	0	1
Pigmentation	0	1	0	0	0	0	0	0	0	0
Heart	4	4	3	3	3	3	3	3	4	4
Not Remarkable	3	1	0	1	0	1	1	0	1	1
Remarkable Observations	1	3	3	2	3	2	2	3	3	3
Atrophy; Ventricle, heart	0	0	0	0	0	0	0	1	0	0
Fibrosis; Apex of heart	1	0	0	2	0	0	1	0	2	0
Fibrosis; Interventricular septum	0	1	1	0	0	0	0	0	0	0
Hypertrophy; Ventricle, heart	0	0	0	0	0	0	0	1	0	0
Infiltrate, lymphocytic; Atrium, heart	0	0	1	0	0	0	0	1	0	0
Infiltrate, lymphocytic; Interventricular septum	0	0	0	0	0	0	0	1	0	0
Infiltrate, lymphocytic; Ventricle, heart	0	2	1	0	0	0	0	0	1	0
Infiltrate, mixed cell; Apex of heart	0	0	0	0	0	1	0	0	0	0
Infiltrate, mixed cell; Interventricular septum	0	0	0	0	0	0	0	0	1	0
Infiltrate, mononuclear; Atrium, heart	0	0	0	0	1	0	0	0	0	0
Infiltrate, mononuclear; Interventricular septum	1	1	0	0	1	2	1	0	0	1
Infiltrate, mononuclear; Ventricle, heart	0	0	1	1	2	1	2	1	2	3
Thrombus, canalized; Atrium, heart	0	0	0	0	1	0	0	0	0	0

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No treatment-related changes occurred in the testes; small testes noted grossly were confirmed as immature histologically.

There were no treatment-related findings in skin or other tissues of animals sacrificed at study termination. Two females did not survive to study termination. Found dead, Female 1001, administered 500 mg/kg, had gastric dilation and rupture with septic peritonitis secondary to the rupture. Female 1000, receiving 500 mg/kg and sacrificed moribund, had abdominal swelling due to obesity and colonic dilatation with mucus-filled large intestine. Tail epidermis exhibited ulceration, hyperkeratosis, and had neutrophil infiltrates.

Organ weights (specify organs weighed if not in histopath table): Brain, pituitary, thyroid, adrenals, gonads, prostate, uterus, epididymides, heart, lung, spleen, thymus, liver, kidneys, and mandibular salivary glands were weighed. Rel spleen wt. slightly less in HDM.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Tissues obtained were: brain, spinal cord, sciatic nerve, pituitary, thyroid, parathyroid, adrenal, pancreas, liver, gallbladder, tongue, esophagus, stomach, small intestine, large intestine, parotid salivary gland, mandibular salivary gland, thymus, spleen, tonsil, mesenteric lymph node, tracheobronchial lymph node, axillary lymph node, trachea, lung, heart, aorta, skin, mammary gland, costochondral junction, sternebra, skeletal muscle, kidney, urinary bladder, prostate, seminal vesicle, ovary, uterus, vagina, and gross lesions. There were no drug-related effects.

Sponsor's Summary:

Important Findings	VC		10 mg/kg		25 mg/kg		100 mg/kg		250/500 mg/kg	
	M	F	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3	3	3
Clinical Signs										
Tail Dermatopathy	2	--	--	2	2	1	--	2	3	3
Tail Amputation	--	--	--	--	--	--	--	--	1	--
Soft Feces/Diarrhea	--	1	--	1	1	1	1	1	3	3
Nasal Discharge	1	2	1	--	1	--	--	1	2	1
Heart Murmur – Low Grade Pretest and Throughout Study	--	--	--	--	--	--	--	--	--	1
N Week 35	3	3	3	3	3	3	3	3	3	3
Special Features - Hematology										
Autoagglutination – Slight	--	--	1	--	1	--	2	3	2	3
N Week 13	3	4	2[a]	3	3	3	3	3	4	4
Bone Marrow										
Total Megakaryocytes[b]	18.0	20.8	20,16	16.0	19.3	21.0	18.7	20.7	20.0	24.0
N Week 68 (M) /Week 65 (F)	3	3	3	3	3	3	3	2[a]	3	2[a]
Bone Marrow										
Total Megakaryocytes[b]	21.7	19.0	17.7	19.3	20.7	21.3	15.0	19, 22	14.7*	13, 16
Percent of Control							31%		32%	24%
N Week 65	3	3	3	3	3	3	3	3	3	2
Clinical Signs										
Tail Dermatopathy	--	--	--	--	--	--	--	--	--	1
Special Features - Hematology										
Platelet Aggregation – Ristocetin (I)	--	--	--	--	--	--	--	--	--	1-fold
Platelet Aggregation – Arachidonic Acid (I)	--	--	--	--	--	--	--	--	--	1-fold

* Significantly different from vehicle control mean at 5% level by linear trend test within one factor analysis of variance.
N = Number of animals; -- = No noteworthy findings; I = Increased.
[a] Individual data.
[b] Total count/5000 hematopoietic cells (mean).

Toxicokinetics: Heparinized blood samples were collected prior to dosing and 2 hours postdosing at Weeks 13 and 65. No sex difference was observed in predose or 2-hour postdose plasma pregabalin concentrations. Mean combined-sex plasma pregabalin concentrations increased with increasing dose and were comparable between Weeks 13 and 65. (see sponsor's table and Figure below).

Table 21. Plasma Concentrations in Monkeys Given Pregabalin Daily by Gavage for 65 Weeks[a]

Dose (mg/kg)	Male	Female
10	12.8 ± 2.57	15.1 ± 5.00
25	28.2 ± 2.01	25.0 ± 6.66
100	46.6 ± 6.07	34.4 ± 5.59
500	74.4 ± 8.97	68.2, 84.2[b]

Table 2.6.7.7G, RR 764-02740 appended to RR 745-02646.

[a] Samples obtained 2 hours postdose in Week 65 at 10, 25, and 100 mg/kg and in Week 52 at 500 mg/kg (µg/mL); mean ± standard deviation; N = 3.

[b] Individual data

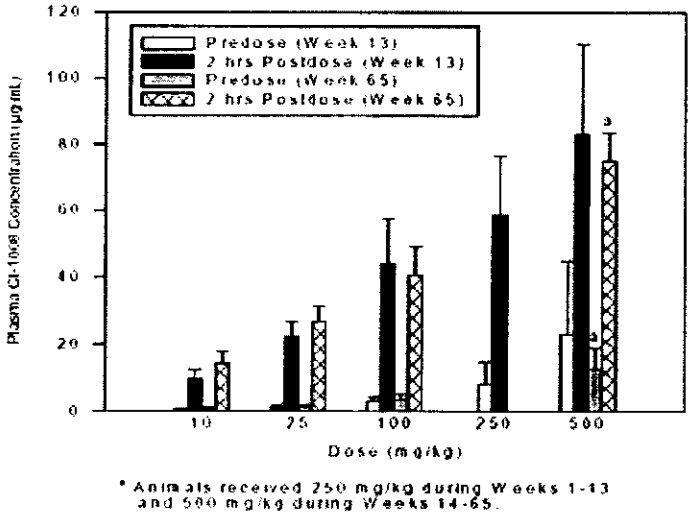

FIGURE 1. Mean Plasma CI-1008 Concentrations (Combined-Sex)

Other: To investigate potential hemodynamic changes as a possible cause of the tail lesions that occurred in monkeys during the 4-week oral toxicity study with pregabalin (RR 745-02329), a biocompatible temperature transponder was implanted subcutaneously in each monkey's tail. Temperature measurements were recorded from all monkeys 3 times during the pretest period, and from control monkeys and all monkeys at 250 and 500 mg/kg at approximately 2 hours postdose each day during Weeks 2 through 4.

Semen samples were collected by electroejaculation pretest and approximately at Weeks 13, 40, and 65 for analysis of ejaculate weight (including coagulum), color, sperm count, sperm motility, and sperm morphology. Testis volume was determined pretest and at termination by measuring length and largest diameter of each testis. No statistically significant differences occurred between groups in testes volume, ejaculate weight, total sperm count per ejaculate, sperm motility (including progressiveness), and percent normal sperm morphology.

2.6.6.4 Genetic toxicology *(taken verbatim from original review by Tom Steel)*

(Mutagenicity studies complied with GLP.)

Standard Ames Bacterial Mutagenicity

The mutagenicity of pregabalin (0.32 to 3200 µg/plate; Lot R; 3200 = solubility limit) with and without an S9 fraction from Arochlor-induced rats was evaluated in strains of *S. typhimurium*:

Test strains: TA-100, TA-1535 (detect base-pair substitution mutations)
TA-98, TA-1537, TA-1538 (detect frameshift mutations)

Vehicle/Negative Control: water

Positive Controls:

TABLE 2. Positive Control Compounds

Tester Strain	S9[-a]	S9[+]
TA-1535	sodium azide, 1 µg/plate	2-aminoanthracene, 2 µg/plate
TA-1537	9-aminoacridine, 75 µg/plate	2-aminoanthracene, 2 µg/plate
TA-1538	2-nitrofluorene, 1 µg/plate	2-aminoanthracene, 2 µg/plate
TA-98	2-nitrofluorene, 1 µg/plate	2-aminoanthracene, 2 µg/plate
TA-100	sodium azide, 5 µg/plate	2-aminoanthracene, 2 µg/plate

[a] Not used in the initial mutagenicity assay

Historical Revertant Ranges:

TABLE 3. Background Historical Ranges

Strain	Revertant Ranges
TA-1535	5-50
TA-1537	3-25
TA-1538	5-40
TA-98	15-75
TA-100	60-220

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Initial testing was done with a preincubation method wherein drug was combined with bacteria and S9 and incubated for 20 min at 37°C before adding agar overlay and plating. Confirmatory testing was done using the standard plate incorporation method. Triplicate samples at 5 dosage levels (200, 400, 800, 1600 and 3200 µg/plate) were run in each tester strain in the absence or presence of S9.

No toxicity to the background lawn occurred at concentrations up to 3200 µg/plate (the solubility limit) with or without activation in either initial or confirmatory testing.

Positive control values for increases in revertant frequency were within historical control range. No significant increases in revertant frequency occurred in any strain under any condition.

STUDY 1780. STANDARD AMES BACTERIAL MUTAGENICITY ASSAY OF PD 144723

10.4. TABLE T-4 Confirmatory Plate Incorporation Trial

	Revertant Frequency[a]									
	TA-1535		TA-1537		TA-1538		TA-98		TA-100	
	S9-	S9+	S9-	S9+	S9-	S9+	S9-	S9+	S9-	S9+
Vehicle Control (Water)	7.3	11.0	5.0	6.7	10.3	16.0	32.3	31.3	60.0	62.3
PD 144723 (µg/plate)										
200	8.3	10.0	5.3	8.0	8.3	12.0	38.3	25.3	50.7	64.3
400	9.3	6.0	4.7	6.0	5.3	17.3	36.3	21.0	59.7	70.0
800	9.3	9.0	9.3	10.0	9.7	17.0	41.3	22.3	52.0	60.3
1600	6.3	9.7	3.7	8.0	7.3	15.7	35.0	17.0	59.0	63.3
3200	8.7	8.7	5.0	7.0	10.3	13.3	40.7	20.7	51.3	82.0
Positive Control	401.0*	199.0*	48.3*	102.7*	944.7*	530.3*	1616.7*	1730.0*	530.0*	1487.3*
p-Value for Positive Linear Slope	0.498	0.727	0.552	0.533	0.417	0.568	0.370	0.998	0.742	0.625
p-Value for Negative Quadratic Effect	0.290	0.826	0.220	0.326	0.910	0.251	0.435	0.912	0.492	0.296

* Statistically significant: p <0.05 for positive control effect; p <0.05 for positive linear slope; p <0.01 for negative quadratic effect

[a] Revertant colony counts; mean of three dishes

Unscheduled DNA Synthesis in Hepatocytes from Rats Treated with Pregabalin

Hepatocytes were isolated from Wistar rats (3-4/dose/time) treated with pregabalin (250, 1000, 2000 mg/kg, p.o.; Lot XH090393) 2 and 16 hrs after dosing. Positive controls (10 mg/kg dimethylnitrosamine) were assessed at 2 hrs, and vehicle controls (0.5% methyleellulose) at 16 hrs. Incorporation of [3H]-thymidine was measured by autoradiography in 100 cells per animal. A positive response was a count of ≥ 5 net grains (total - background) per nucleus, or 20% of cells in repair.

CI did not induce unscheduled DNA synthesis/repair in rat hepatocytes. Positive and vehicle control responses were in the historical control range.

TABLE 2. UDS Summary Data by Treatment Group

Treatment	Time (hr)	Number of Animals Evaluated	Mean NG[a] (SE)		% Cells in Repair[b]
Vehicle Control (0.5% methylcellulose)	16	3	0.17	(0.02)	0
Positive Control (DMN)	2	3	15.4*	(2.72)	91.7
CI-1008 (mg/kg)					
250	2	3	0.32	(0.14)	0.3
250	16	3	0.32	(0.07)	0
1000	2	3	0.45	(0.33)	0.3
1000	16	3	0.18	(0.03)	0
2000	2	3	0.40	(0.14)	0.3
2000	16	3	0.19	(0.11)	0

SE = Standard error.
[a] Net grains/nucleus
[b] Mean net grains ≥5.
* Significance $p \leq 0.05$.

In vitro Mutation Assay in Chinese Hamster Ovary Cells

CHO cells were incubated with CI (1200, 1300, 1400, 1500, 1600 µg/mL; Lot# XH340993) for 3 hrs in the absence or presence of an S9 fraction from Arochlor-induced rat. Cells were subsequently cultured for 8 days to allow for expression of mutations in the hypoxanthine-guanine phosphoribosyl transferase (hprt) locus. Trials were done in duplicate. Mutants are identified by growth in medium containing 6-thioguanine. Positive controls were ethyl methanesulfonate (EMS; 800 µg/ ml; no activation) or benzo(a)pyrene (BP; 10 µg/mL; with activation). Water was the negative control. A result was considered positive if the test compound produced a reproducible, concentration-related increase in mutant frequency at 2 adjacent concentrations, and a mean mutant frequency of 20 mutant/10^6 surviving cells above the negative control frequency.

CI did not inhibit plating efficiency and did not significantly induce hprt mutations with or without activation according to the established criteria. The positive controls significantly increased mutants under both activation and non-activation conditions:

STUDY 1950. In Vitro Mutation Assay of CI-1008 in Chinese Hamster Ovary Cells

10.2. TABLE T-2 Mutant Frequency - S9-

	Treatment	Concentration (μg/mL)	Plating Efficiency[a]		Mutant Frequency[c]
			PE_1[b]	PE_2	
Trial 1					
	Water	10%	100	84	11.6
	CI-1008	1200	105	84	11.6
	CI-1008	1300	107	89	6.6
	CI-1008	1400	101	75	16.9
	CI-1008	1500	109	80	8.5
	CI-1008	1600	98	84	10.1
	EMS	800	42	60	739.6

Contrast	p-Value
Positive control - Negative control	<0.0001*
Linear dose trend - NC and 5 concentrations	0.5483
Quadratic trend - NC and 5 concentrations	0.5440

Trial 2					
	Water	10%	100	77	6.7
	CI-1008	1200	102	84	7.7
	CI-1008	1300	103	83	22.3
	CI-1008	1400	101	75	7.9
	CI-1008	1500	106	86	11.2
	CI-1008	1600	108	84	12.4
	EMS	800	63	51	795.3

Contrast	p-Value
Positive control - Negative control	<0.0001*
Linear dose trend - NC and 5 concentrations	0.5455
Quadratic trend - NC and 5 concentrations	0.0282

EMS = Ethyl methanesulfonate.
[a] Based on 200 cells per plate (10 plates), expressed as a percentage.
[b] Relative to negative control, as a percentage
[c] Mutants per 10^6 viable cells, mean of duplicate cultures
* p <0.05 (positive control versus vehicle control or linear dose trend); <0.01 (quadratic trend).

STUDY 1950. In Vitro Mutation Assay of CI-1008 in Chinese Hamster Ovary Cells

10.3. TABLE T-3 Mutant Frequency - S9+

	Treatment	Concentration (μg/mL)	Plating Efficiency[a] PE_1[b]	Plating Efficiency[a] PE_2	Mutant Frequency[c]
Trial 1					
	Water	10%	100	83	5.3
	CI-1008	1200	126	79	14.4
	CI-1008	1300	117	84	11.7
	CI-1008	1400	93	72	21.9
	CI-1008	1500	102	81	9.9
	CI-1008	1600	102	80	8.6
	BP	10	74	63	300.8

Contrast	p-Value
Positive control - Negative control	<0.0001*
Linear dose trend - NC and 5 concentrations	0.9591
Quadratic trend - NC and 5 concentrations	0.0352

Trial 2					
	Water	10%	100	76	5.7
	CI-1008	1200	87	82	13.8
	CI-1008	1300	106	77	11.4
	CI-1008	1400	87	80	23.8
	CI-1008	1500	101	91	5.3
	CI-1008	1600	108	83	10.7
	BP	10	30	62	338.7

Contrast	p-Value
Positive control - Negative control	<0.0001*
Linear dose trend - NC and 5 concentrations	0.8356
Quadratic trend - NC and 5 concentrations	0.0023*

BP = Benzo(a)pyrene.

[a] Based on 200 cells per plate (10 plates), expressed as a percentage

[b] Relative to negative control, as a percentage

[c] Mutants per 10^6 viable cells, mean of duplicate cultures

* $p < 0.05$ (positive control versus vehicle control or linear dose trend); < 0.01 (quadratic trend).

Additional Mutagenicity Studies:

Since the initial IND filing, some additional mutagenicity studies have been peformed and are reviewed here.

Study title: **Bacterial Mutagenicity - Mouse Metabolic Activation**

Key findings: In the initial and definitive assays with B6C3F1 or CD-1 metabolic activation, pregabalin was not cytotoxic to the background lawn and did not increase revertant frequency in any bacterial strain up to 5000 µg/plate. Under the conditions of this study, pregabalin was not mutagenic in bacteria in the presence of metabolic activation provided by B6C3F1 or CD-1 mouse liver. However, OECD Guidelines and CFSAN Redbook state it is not acceptable to use 2-aminoanthracene as the sole +S9 positive control. While the sponsor claims to be in accord with OECD Guidelines, they used 2-aminoanthracene as the sole +S9 positive control. The sponsor was contacted regarding this and did provide asked the necessary information.

Study no.:	Protocol AA2734
Volume #, and page #:	M 4, I 5, V 023
Conducting laboratory and location:	Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company Ann Arbor, Michigan
Date of study initiation:	01/16/01
GLP compliance:	yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	pregabalin, Lot XH020100 with an active moiety of

Methods

Strains/species/cell line: Bacterial mutagenicity was assessed using metabolic activating systems provided by B6C3F1 or CD-1 mice treated with Aroclor 1254. Four histidine auxotrophs of *S. typhimurium* TA-98, TA-100, TA-1535 and TA-1537, and a tryptophan auxotroph of *E. coli* (WP2*uvr*A) were exposed to pregabalin.

Doses used in definitive study: Initial and definitive assays were conducted to assess cytotoxicity and mutagenicity at 312.5, 625, 1250, 2500, and 5000 µg/plate.

Basis of dose selection: This is the highest recommended concentration.

Negative controls: Vehicle - sterile distilled water

Positive controls: 2-Aminoanthracene was used as a positive control for both S9 fractions and for all bacterial strains. OECD Guidelines and CFSAN Redbook state it is not acceptable to use 2-aminoanthracene as the sole +S9 positive control.

Incubation and sampling times: In the exploratory phase of this study, the preincubation method did not exhibit advantages over the plate incorporation method. Therefore, the trials were performed by the plate incorporation method.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Negative controls were within reference ranges. Positive control 2-aminoanthracene increased mean revertant frequency 2- to 23-fold above corresponding negative controls with B6C3F1 metabolic activation, and 1- to 35-fold above corresponding negative controls with CD-1 metabolic activation. OECD Guidelines and CFSAN Redbook state it is not acceptable to use 2-aminoanthracene as the sole +S9 positive control.

Study outcome: In the initial and definitive assays with B6C3F1 or CD-1 metabolic activation, pregabalin was not cytotoxic to the background lawn and did not increase revertant frequency in any bacterial strain up to the maximum concentration tested of 5000 µg/plate.

Study title: **Bacterial Mutagenicity – Additional Rat Metabolic Activation Study**

Key findings: In the initial and confirmatory assays, no cytotoxicity was observed up to 5000 µg/plate in any strain. In both assays, there were no significant increases in mutation frequency with or without S9. Positive controls sodium azide, 9-aminoacridine, and 2-nitrofluorene increased mean revertant frequency 7- to 63-fold above corresponding vehicle controls, indicating valid assay conditions. Therefore, results of the first mutagenicity assay were confirmed, and pregabalin was not mutagenic in bacteria under the current conditions. However, OECD Guidelines and CFSAN Redbook state it is not acceptable to use 2-aminoanthracene as the sole +S9 positive control.

Study no.:	Protocol # AA2670
Volume #, and page #:	M 4, I 5, V 024
Conducting laboratory and location:	Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company Ann Arbor, Michigan
Date of study initiation:	05/15/00
GLP compliance:	yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	Pregabalin, Lot XH020100, [illegible] 1 parent

Methods

Strains/species/cell line: Mutagenicity was evaluated by exposing 4 strains of *S. typhimurium* (TA-98, TA-100, TA-1535, and TA-1537) to pregabalin with and without S9 from the livers of rats treated with Aroclor 1254.

Doses used in definitive study: A confirmatory assay was conducted at 312.5, 625, 1250, 2500, and 5000 µg/plate.

Basis of dose selection: Since the first mutagenicity assay was conducted early in pregabalin development and the highest concentration tested was 3200 µg/plate, the assay was repeated up to 5000 µg/plate to confirm those results. An initial assay was conducted to assess cytotoxicity and mutagenicity at 0.5, 1.581, 5, 15.81, 50, 158.1, 500, 1581, and 5000 µg/plate with and without S9. The highest dose to be tested is 5 mg/plate.

Negative controls: Sterile distilled water served as the negative control.

Positive controls: For trials with metabolic activation, 2-aminoanthracene was used as a positive control. For trials without metabolic activation, 9-aminoacridine in ethanol and 2-nitrofluorene in DMSO were used as positive controls for TA-1537 and TA-98, respectively. Sodium azide in DMSO was used as a positive control in the absence of metabolic activation for TA-100 and TA-1535.

Incubation and sampling times: A preincubation trial was performed concurrently with the plate incorporation method using the same concentrations of test article. All 4 bacterial strains were

used in the preincubation trial. In the preincubation method, test article, bacteria, and S9 were combined and incubated for 20 minutes at 37°C before adding the agar overlay and plating. After agar solidification, the plates were incubated at 37°C for 48 to 72 hours.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears to be valid based on the following assumptions: The — Counter was used to count the number of colonies, and revertants and recorded as colonies/plate. Negative controls were within historical reference ranges. Revertant frequencies were 13 to 66.5-fold above respective negative controls for the positive control compounds. OECD Guidelines and CFSAN Redbook state it is not acceptable to use 2-aminoanthracene as the sole +S9 positive control. The sponsor was contacted regarding this and did provide asked the necessary information.

Study outcome: In the initial and confirmatory assays, no cytotoxicity was observed up to 5000 µg/plate in any strain. In both assays, there were no significant increases in mutation frequency with or without S9. Positive controls sodium azide, 9-aminoacridine, and 2-nitrofluorene increased mean revertant frequency 7- to 63-fold above corresponding vehicle controls, indicating valid assay conditions. Therefore, results of the first mutagenicity assay were confirmed, and pregabalin was not mutagenic in bacteria under the current conditions.

Study title: Mutagenicity Test With pregabalin in Escherichia coli WP2uvrA

Key findings: There was no cytotoxicity to the background lawn and pregabalin did not increase the number of revertants/plate in the absence or presence of S9. Under these conditions, pregabalin was not mutagenic in *E. coli*.

Study no.: 2477

Volume #, and page #: M 4, I 5, V 024
Conducting laboratory and location: [redacted]

Date of study initiation: 6/10/99
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: pregabalin, Lot XH230695, start: — end: —

Methods

Strains/species/cell line: In an additional mutagenicity assay, a tryptophan auxotroph of *E. coli*, WP2*uvr*A able to detect base-pair substitution, was exposed to pregabalin with or without S9.

Doses used in definitive study: Doses tested in the initial and confirmatory assays were 0.996, 3.32, 9.96, 33.2, 99.6, 332, 996, 3320, and 4980 µg/plate.

Basis of dose selection: Maximum recommended is 5 mg/plate.

Negative controls: Vehicle with or without S9 mix.

Positive controls:

Tester Strain	S9 Mix	Positive Control	Conc. per plate
WP2*uvr*A	+	2-aminoanthracene	25.0 µg
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0 µg

Incubation and sampling times: Following incubation at 37 ± 2°C for 52 ± 4 hours, revertant colonies were counted.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study appears to be valid based on: 1) the tester strain culture exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions; 2) the density of tester strain cultures was greater than or equal to 0.5 x 10^9 bacteria/mL based on a target level of turbidity; 3) positive controls 2-aminoanthracene and 4-nitroquinoline-N-oxide

increased mean revertant frequency ≥15-fold above corresponding vehicle controls, confirming assay conditions. However, OECD Guidelines state: "2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S9-mix. If 2-aminoanthracene is used, each batch of S9 should also be characterized with a mutagen that requires metabolic activation by microsomal enzymes, e.g., benzo(a)pyrene, dimethylbenzanthracene." This study with pregabalin used 2-aminoanthracene as the sole agent without specifying how the activity of each batch was characterized. The sponsor was contacted regarding this and did provide asked the necessary information.

Study outcome: In the initial mutagenicity assay, all data were acceptable and no positive increases were observed in the mean number of revertants per plate with tester strain WP2*uvr*A in either the presence or absence of S9 mix. In this experiment, a 2.1-fold increase was observed at 100 µg per plate and a 2.3-fold increase was observed at 3320 µg per plate with tester strain WP2*uvr*A in the absence of S9 mix. However, these increases were not dose-responsive and therefore did not meet the criteria for a positive evaluation.

In the confirmatory assay, all data were acceptable and no positive increases were observed in the mean number of revertants per plate with tester strain WP2*uvr*A in either the presence or absence of S9 mix.

Study title: ***In vitro* Structural Chromosome Aberration in Chinese hamster ovary cells**

Key findings: In the absence of S9 at 3- or 20-hour exposures, no effects on plating efficiency (PE) or proliferation index (PI) were observed. In the presence of S9, no effect on PI was observed although PE decreased 25% at 1600 µg/mL. Pregabalin marginally increased the percentage of cells with aberrations (6.5%) with S9 at the highest concentration (10 µM; 1600 µg/mL). As this was close to the historical control frequency (up to 6.3%) it was not considered biologically relevant. Thus, pregabalin was not considered to be clastogenic under the conditions of this assay. Sponsor's reviews of this study incorrectly state that no increases in aberration frequency were seen at any concentration, which is not technically correct. The reviewer agrees with overall conclusion, however.

Study no.:	1940
Volume #, and page #:	M 4, I 5, V 024
Conducting laboratory and location:	Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company Ann Arbor, Michigan
Date of study initiation:	08/30/94
GLP compliance:	yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	Pregabalin Lot XH340993, — active content

Methods

Strains/species/cell line: CHO cells (ovary; Chinese hamster, *Cricetulus griseus*) were obtained from [redacted]

Doses used in definitive study: Concentrations of 160, 300, 500, 900, and 1600 µg/mL were tested with and without S9 after exposure for 3 hours and concentrations of 160, 300, 500, and 900 µg/mL were tested without S9 after exposure for 20 hours.

Basis of dose selection: The highest concentration used, 1600 µg/mL, is equal to 10 mM and is the maximum recommended test concentration for cytogenic studies.

Negative controls: The diluent for the test substance (water) served as the negative control. Activation phase solvent control included S9 preparation at the level used in the assay.

Positive controls: Mitomycin C (MMC; CAS 50-07-7, MW 334.33) served as the positive control for the nonactivation portion (S9-) of the SCA assay at 1.0 and 1.25 µg/mL for the 3-hour exposure and 0.1 and 0.2 µg/mL for the 20-hour exposure. MMC was dissolved in distilled water.

Cyclophosphamide (CP; CAS 50-18-0, MW 279.1) served as the positive control for the activation portion (S9+) of the SCA assay at 4 and 8 µg/mL. CP was dissolved in distilled water.

Incubation and sampling times: After the 3-hour incubation period with drug, cells were rinsed and incubated an additional 17 hours prior to harvesting. After the 20-hour incubation period, cells were immediately harvested. Plating efficiency (PE) and aberration frequencies from 200 cells/concentration were analyzed statistically for elevation over the solvent control aberration frequency and dose response. Cell cycle kinetics also were evaluated using [illegible] technique to obtain a proliferation index (PI).

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was considered valid for the following reasons: 1) the positive controls mitomycin C and cyclophosphamide increased the incidence of cells with chromosome aberrations ≥ 9-fold above corresponding solvent controls, confirming assay validity; 2) at least 1 concentration of the positive control yielded a statistically significant increase in the SCA frequency over the solvent control frequency at any time point; 3) the PI data indicated that a substantial portion of cells in at least 3 drug-treated groups had undergone at least 1 cell division after treatment; 4) at least 3 drug-treated groups were scorable for at least 1 time point.

Study outcome: In the absence of S9 at 3- or 20-hour exposures, no effects on PE or PI were observed. In the presence of S9, no effect on PI was observed although PE decreased 25% at 1600 µg/mL. Pregabalin treatment resulted in a range of 3.0% to 6.5% cells with aberrations compared to 2.5% in the negative control. The results indicated a significant increase in the mean total number of aberrations per cell and the percent of cells with aberrations at 1600 µg/mL, respectively. See table below.

2.6.7.8F Genotoxicity – In Vitro Structural Chromosome Aberration in Chinese Hamster Ovary Cells **Pregabalin**

Test Article	Concentration	Relative Proliferation Index	Relative Plating Efficiency	Total Abs	Abs Cell	% Cells With Abs
3 Hour Treatment - 17 Hour Incubation With S9						
Water	0	100	100	5	0.025	2.50
Pregabalin	160	108	113	6	0.030	3.00
	300	107	85.0	13	0.065	6.00
	500	96.0	61.0	7	0.035	3.50
	900	115	87.0	8	0.040	4.00
	1600	98.0	75.0	13	0.065§	6.50‖
Cyclophosphamide	4	Not Determined	79.0	73	0.365†	24.5‡
	8	Not Determined	38.0	94	0.537†	33.1‡

† Fisher's exact test, ($p < 0.025$).
‡ t-test, ($p < 0.025$).
§ Armitage sequential trend test, ($p < 0.05$).
‖ ANOVA sequential trend test, ($p < 0.05$).

This was a marginal effect just outside of the historical control range for the assay (0%-6.3%) and it occurred at the maximum recommended test concentration for cytogenetic studies (10 mM). Since statistical significance was not observed at 2 consecutive concentrations, the criterion set for a positive effect to be established for this assay. Therefore, the statistical result for treatment in the presence of S9 was not considered by te sponsor to have biological significance. This seems a reasonable conclusion.

Study title: ***In Vivo* Unscheduled DNA Synthesis - Mouse**

Pregabalin was not carcinogenic and did not induce unscheduled DNA synthesis (UDS) in rats. However, the incidence of hemangiosarcoma was increased in pregabalin-treated B6C3F1 mice. This study was designed to evaluate the potential of pregabalin to induce UDS in a species and strain that developed tumors. In addition, because the carcinogenic potential of pregabalin was then being evaluated in CD-1 mice (2001) in an ongoing study, UDS was also evaluated in this strain (it was also carcinogenic in CD-1's).

Key findings: Under the conditions of this assay, pregabalin did not induce unscheduled DNA synthesis in female B6C3F1 or CD-1 mouse hepatocytes at doses of up to the limit of 2000 mg/kg.

Study no.:	Protocol AA 2792 RR 745-03455
Volume #, and page #:	M 4, I 5, V 024
Conducting laboratory and location:	[redacted]
Date of study initiation:	19-Jun-01
GLP compliance:	yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	Pregabalin, Lot # XH020100; purity was stated as [redacted]

Methods

Strains/species/cell line: Female B6C3F1 and CD-1 Mice

Doses used in definitive study: Mice were given a single dose of pregabalin at 500, 718, 1510, or 2000 mg/kg by gavage.

Basis of dose selection: Current ICH and OECD guidelines recommend that the high-dose selected for the rodent MN assay should produce some toxicity, be conducted at maximum tolerated dose, or be administered at a limit dose of 2000 mg/kg.

Negative controls: Vehicle control (VC) given 0.5% methylcellulose at 20 mL/kg.

Positive controls: Positive control given dimethylnitrosamine in water at 10 mg/kg for 2-4 hour time points and fast garnet GBC in corn oil at 200 mg/kg for 14-16 hour time points by gavage at 20 mL/kg.

Incubation and sampling times: At approximately 2 to 4 hours and 14 to 16 hours postdose, hepatocytes were isolated and incubated with tritiated thymidine.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The assay was considered valid for the following reasons: 1) the vehicle control animals had a group mean NNG that did not exceed the upper limit of the historical reference range and 2) the positive control groups had a group mean value of ≥ 5 NNG with $\geq 50\%$ of the cells having ≥ 5 NNG. Cultures were prepared on microscope slides (6 slides/animal) from at least 3 animals/dose group (6 animals/group from B6C3F1 vehicle and positive control groups). From 2 of the 3 slides (100 cells/animal with the exception of 1 B6C3F1 animal which only had 10 cells analyzed), microscopic evaluation determined the net nuclear grain count (NNG), the number of cells in DNA repair, and the net nuclear grain count of cells in DNA repair.

In B6C3F1 mice, the vehicle control mean net grains/nucleus ranged from -2.7 to 0.1, and mean percentage of cells in repair ranged from 0.2% to 3.7%. Mean net grains/nucleus ranged from 16.7 to 47.1, and mean percentage of cells in repair ranged from 83.5% to 99.8% in B6C3F1 positive controls.

In CD-1 mice, the vehicle control mean net grains/nucleus ranged from -1.5 to 0.2, and mean percentage of cells in repair ranged from 0.3% to 1.3%. Mean net grains/nucleus ranged from 7.7 to 40.1 and mean percentage of cells in repair ranged from 51.0% to 100% in CD-1 positive controls.

Study outcome: In B6C3F1 mice, mean net grains/nucleus ranged from -3.7 to -0.2, and mean percentage of cells in repair ranged from 1.0% to 3.0%. In CD-1 mice, mean net grains/nucleus ranged from -1.8 to 0.3, and mean percentage of cells in repair ranged from 0.7% to 1.3%.

These results indicate that pregabalin did not induce unscheduled DNA synthesis in female B6C3F1 or CD-1 mice when given orally at up to 2000 mg/kg.

Study title: ***In vivo* Micronucleus Study in B6C3F1 and CD-1 Mice**

Key findings: Pregabalin was not clastogenic in B6C3F1 or CD-1 mouse bone marrow *in vivo*.

Study no.:	Protocol # AA2657
Volume #, and page #:	M 4, I 5, V 024 RR 745-03387
Conducting laboratory and location:	Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company Ann Arbor, Michigan
Date of study initiation:	22-Jan-01
GLP compliance:	yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	Pregabalin, XH020100; purity —

Methods

Strains/species/cell line: Male and female B6C3F1 and CD-1 mice C J

Doses used in definitive study: 0, 500, 1000, 2000 mg/kg by oral gavage

Basis of dose selection: Current ICH and OECD guidelines recommend that the high-dose selected for the rodent MN assay should produce some toxicity, be conducted at maximum tolerated dose, or be administered at a limit dose of 2000 mg/kg, and that multiple-dose levels be used which cover a range from maximum to little or no toxicity.

B6C3F1 Mice = Hypoactivity on the day of dosing in 3 males at 1000 mg/kg and 2 males at 2000 mg/kg.

CD-1 Mice = Hypoactivity on the day of dosing in 2 males at 1000 mg/kg and 1 female at 500 mg/kg.

The percentage of polychromatic erythrocytes (PCE) to total erythrocytes (TE) was an indicator of cytotoxicity to the bone marrow and the frequency of micronucleated polychromatic erythrocytes (MNPCE) assessed clastogenicity.

Negative controls: Vehicle: 0.5% methylcellulose at 10 mL/kg.

Positive controls: Positive control given cyclophosphamide at 60 and 80 mg/kg in distilled water at 10 mL/kg intraperitoneally.

Incubation and sampling times: 24 and 48 hours postdose.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was considered valid for the following reasons: 1) Approximately 100,000 cells were

analyzed per animal by flow cytometry. 2) Cyclophosphamide (CP) at both doses on both sampling days decreased the percentage of PCE up to 76% in B6C3F1 mice and up to 69% in CD-1 mice, and increased the percentage of MNPCE up to 4.5-fold in B6C3F1 mice and up to 3.9-fold in CD-1 mice.

Study outcome: While CP decreased PCE and increased MNPCE, pregabalin was not cytotoxic to the bone marrow (did not decrease PCE) and did not increase the percentage of MNPCE in either sex or strain up to 2000 mg/kg after 24 or 48 hours of exposure, except for one group. One significant (by one measure) increase (0.78%) was seen at the 2000 mg/kg dose at 24 hr in B6C3F1 males only. A supplemental analysis using nonrank-transformed data disagreed, indicating no statistical significance. In addition, since this was the first study conducted that quantified micronucleus formation in mouse bone marrow using flow cytometry, historical control data were not available for comparison, though concurrent controls were very close ().76%). The sponsor's summaries all claim no significant differences were seen , and though the result may not be clinically meaningful, it is technically inaccurate. In summary, pregabalin was not found to be clastogenic in B6C3F1 or CD-1 mouse bone marrow *in vivo* under the conditions of this study.

Study title: ***In vivo* Micronucleus Study in Rats**

Key findings: In pregabalin-treated (up to 2000 mg/kg) animals, no biologically significant effect on PCE/TE ratio was noted. In addition, pregabalin did not induce a statistically significant increase in MNPCE frequency in either sex at either time point studied. Under the conditions of this assay, pregabalin was not clastogenic to rat bone marrow *in vivo*.

Study no.:	1945
Volume #, and page #:	M 4, I 5, V 025
Conducting laboratory and location:	Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company Ann Arbor, Michigan
Date of study initiation:	08/01/94
GLP compliance:	yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	Pregabalin, XH340993; purity —

Methods

Strains/species/cell line: Rat/Wistar

Doses used in definitive study: 0, 500, 1000, 2000 mg/kg oral by gavage

Basis of dose selection: Ataxia in all animals given pregabalin, and hypoactivity in 6 males and 5 females at 500 mg/kg and all animals at 1000 and 2000 mg/kg on the day of dosing.

Negative controls: Vehicle: 0.5% methylcellulose at 20 mL/kg

Positive controls: Positive control given cyclophosphamide at 20 mg/kg in distilled water at 10 mL/kg intraperitoneally.

Incubation and sampling times: 24 and 48 hours postdose (24 hours only for positive control)

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was considered valid for the following reasons: 1) Cyclophosphamide produced a slight but statistically significant decrease in the mean PCE/TE ratios; though still within the historical control range; 2) in the same animals, the MNPCE frequency increased over 9-fold (combined sexes) in comparison with vehicle controls.

Study outcome: In pregabalin-treated animals, no biologically significant effect on PCE/TE ratio was noted. In addition, pregabalin did not induce a statistically significant increase in MNPCE frequency in either sex at either time point studied. Thus, under the conditions of this study, pregabalin was not clastogenic.

2.6.6.5 Carcinogenicity

The Sponsor conduced a total of 4 2-year carcinogenicity bioassays in rodents. Drs. Edward Fisher and Terry Peters reviewed these studies. As noted in the executive summary, an increase in the incidence of hemagiosarcomas was detected in the B6C3F1 mouse and also in the CD-1 mouse models. There were no tumors detected in two different rat studies. Specific details of these studies can be found in the NDA reviews from Drs. Fisher and Peters.

2.6.6.6 Reproductive and developmental toxicology

The Sponsor conduced a standard battery of reproductive toxicology studies. Dr. Edward Fisher reviewed these studies. Specific details can be found in the NDA review prepared by Dr. Fisher.

2.6.6.7 Local tolerance

Irritation in Rabbits
Rabbits were given 1-mL injections of formulated pregabalin (20 mg/mL) in 0.9% NaCl into the lateral auricular vein of the left ear for 5 consecutive days. The injection rate was 0.6 mL/min resulting in a dose rate of 12 mg/min. Animals were observed daily for signs of local irritation and euthanized 4 hours after dosing on Day 5. Ears were evaluated at necropsy and tissue was obtained for histopathologic examination.

Injection site discoloration and swelling scores were similar in control and drug-treated animals. There were no drug-related gross or histopathologic findings. Under the conditions of this study, pregabalin was not an IV irritant in rabbits.

In Vitro Compatibility in Human Blood
Formulated pregabalin (20 mg/mL) was tested for compatibility with human plasma and whole blood at 0.2, 0.4, 1, 2, 4, 6, and 10 mg/mL. Erythrocyte fragility in the presence of pregabalin at 20 mg/mL also was assessed; final concentration in this system was 4 mg/mL.

No precipitation, coagulation, or hemolysis was noted in plasma or whole blood incubated with formulated pregabalin at any concentration tested. Pregabalin, at 20 mg/mL in 0.9% NaCl, had no effect on erythrocyte fragility. Under the conditions of this study, pregabalin was compatible with human blood up to 10 mg/mL.

Species (Strain) Animals/Sex/Group Total	Route (Vehicle) [Dose Rate]	Dose or Concentration	Treatment Duration	Significant Findings	Report Number
In Vivo					
Rabbit (NZW) 5F 10	Intravenous (0.9% NaCl) [0.6 mL/min]	VC 12 mg/min	5 Days	No vascular irritation.	745-02886
In Vitro[a]					
Human Plasma Compatibility Human Whole Blood Compatibility Human Erythrocyte Fragility		0.2 to 10 mg/mL 0.2 to 10 mg/mL 4 mg/mL	Not applicable	Formulated pregabalin compatible with human plasma and whole blood up to 10 mg/mL. No effects on erythrocyte fragility.	745-02893

NZW New Zealand White; VC Vehicle control.
[a] Formulated pregabalin contained active drug at 20 mg/mL in 10 mL sterile 0.9% NaCl.

2.6.6.8 Special toxicology studies

Study title: Chronic Investigative Study of Pregabalin in Female Wistar Rats

Key study findings: Responses of the Wistar rat to pregabalin appear to differ from those associated with mice. In contrast to the mouse, pregabalin treatment at 900 mg/kg for up to 12 months did not stimulate liver sinusoidal endothelial cell proliferation. Decreased bone marrow cellularity associated with increased fatty infiltration was observed in the rat as opposed to increased cellularity in the mouse. Pregabalin had no effect on platelet number, structure, or function in rat, in contrast to increased platelet count, increased platelet activation, and altered platelet aggregation in the mouse.

Study no.: Protocol # AA2796
Volume #, and page #: Module M 4, I 5, V 063
Conducting laboratory and location: Pfizer Global Research & Development
Ann Arbor Laboratories, Ann Arbor, Michigan
Date of study initiation: 08/22/01
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, and % purity: Lot: XH020100

Methods

Species/strain: Female Wistar (— (WI)BR) rats (C
J

Unique study design or methodology (if any): To evaluate potential mitogenic effects relevant to pregabalin-induced hemangiosarcoma, proliferation of liver sinusoidal endothelial cells, as well as hepatocytes and Kupffer cells, was evaluated in 5 control and 5 pregabalin-treated rats after 1, 3, 6, and 12 months of treatment (900 mg/kg) by using bromodeoxyuridine (BrdU) labeling and image analysis techniques. Liver, lung, spleen, and bone marrow from 10 control and 8 treated animals at 1-month sacrifice were examined microscopically and tissues from 10 or 5 animals per group were examined at 3 and 6-month sacrifice, respectively. At 1-year, lung and

liver from 10 animals per group and spleen and bone marrow from 5 animals were examined. Growth factors with known proliferative influence on endothelium, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and thrombopoietin (TPO) were measured in platelet-rich and platelet-poor plasma collected from 5 rats per group after 3, 6, and 12 months of treatment. Basic fibroblast growth factor (bFGF) was assayed in platelet-rich plasma only, after 3, 6, and 12 months of treatment and in urine after 6 and 12 months.

Conclusion:
Responses of the Wistar rat to pregabalin appear to differ from those associated with mice. In contrast to the mouse, pregabalin treatment at 900 mg/kg for up to 12 months did not stimulate liver sinusoidal endothelial cell proliferation. Decreased bone marrow cellularity associated with increased fatty infiltration was observed in the rat as opposed to increased cellularity in the mouse. Pregabalin had no effect on platelet number, structure, or function in rat, in contrast to increased platelet count, increased platelet activation, and altered platelet aggregation in the mouse.

Antigenicity/Immunotoxicity - Local Lymph Node Assay in Rats (report 745-03326)		
Species (strain)	**Design**	**Findings**
Rat (Wistar) 6M 36	Dose: Topical application of vehicle (acetone and DMSO), negative control (methyl salicylate), positive control (oxazolone) or pregabalin at 5% and 7.5% in 75 µL to dorsum of each ear daily for 4 consecutive days. Parameters: Ears examined daily for erythema. Local lymph nodes excised on Day 7 and weighed; cellularity, ^{3}H-thymidine incorporation, BrdU labeling, and phenotypic analysis obtained. Samples of pinna examined histopathologically.	No effects on cellularity, ^{3}H-thymidine incorporation, BrdU labeling, or phenotypic analysis, and no drug-related histopathologic findings with pregabalin. Oxazolone increased lymph node weight, cellularity, ^{3}H-thymidine incorporation, BrdU labeling and percentage of blast cells; moderate to marked mononuclear cell infiltrates seen histopathologically.

Hematologic Parameters and Platelet Function in Rats (report 250-01802)		
Species (strain)	**Design**	**Findings**
Rat (Wistar) 20-25M 135	Dose: Oral doses of 0.5% MC (vehicle) or pregabalin at 50 or 1250 mg/kg daily for 14 days. Additional animals given 62.5 or 1562.5 mg/kg daily for 14 to 18 days followed by sulfo-NHS-biotin (label) at 35 mg/kg on Day 14 to study platelet kinetics. Parameters: Hematology and bone marrow parameters, bleeding time, PT, aPTT, platelet aggregation, thromboxane, platelet lifespan, and erythropoietin measured at designated intervals. Platelets and megakaryocytes examined ultrastructurally.	No deaths. Tail dermatopathy at all doses; hypoactivity, ataxia, and erythema of extremities at ≥62.5 mg/kg. RBC count, Hb, and Hct increased and platelet count decreased at 1250 mg/kg. Tail-tip bleeding time increased at 50 and 1250 mg/kg. No effects on bone marrow parameters, erythropoietin, platelet lifespan, thromboxane, PT, aPTT, platelet aggregation, or morphology. Mean plasma concentration 4 hours postdose of 25.7 and 546 µg/mL at 50 and 1250 mg/kg, respectively.
Hematologic Parameters and Platelet Function in Rats (report 745-03312)		
Species (strain)	**Design**	**Findings**

Rat (Wistar) 10M + 10F 82[a]	Dose: Oral doses of pregabalin in the diet at 500, 1250, or 2500 mg/kg or untreated diet daily for 14 days. Parameters: Hematology and bone marrow parameters, platelet morphology, reticulated platelet, platelet activation, clot retraction, and platelet aggregation assays measured at termination, template bleeding time measured pretest and at termination.	No deaths. Tail dermatopathy, hypoactivity, red staining of muzzle and/or urine staining at all doses. RBC count increased in males at all doses. Platelet count decreased at all doses but not significantly. Slight platelet morphologic abnormalities at all doses. No effects on reticulated platelet number, activated platelet and clot retraction percentages, or template bleeding time. No effects on platelet aggregation at ≤1250 mg/kg; mild decrease at 2500 mg/kg.

Species (Strain)	Study Design	Significant Findings
Microvascular Permeability in Rats		
Rat (Wistar) 8M 8	Dose: Single ID dose of vehicle (0.9% NaCl), pregabalin at 0.01, 0.1, or 1 mM, and positive control (histamine) at 1 mM in 100 µL in the dorsal area. Parameters: Plasma extravasation of ^{125}I-labeled albumin in skin sites measured 60 minutes postdose.	No effects on plasma extravasation in pregabalin-treated skin sites. Histamine induced a 46% increase in plasma extravasation.
Time-Course Evaluation of Dermal Toxicity Following Continuous Intravenous Infusion in Monkeys		
Monkey (cynomolgus) 8M + 8F 16	Dose: Continuous IV infusion of vehicle (sterile saline) or pregabalin at 6 mg/kg/hr for up to 96 hours. Parameters: Hematology, biochemical and urinalysis parameters including lymphocyte subsetting, direct, antiplatelet and antinuclear antibody tests, C3, IgG, IgM, IgA, C-reactive protein, E-selectin, P-selectin, ICAM-1, VCAM-1, cholinesterase, and cryoglobulins evaluated at 24, 48, 72, and 96 hours. Bone marrow cytospin preparations evaluated at 96 hours. Gross and histopathologic examinations at each time point including ultrastructural and immunocytochemical evaluation of selected tissues.	Pregabalin at 6 mg/kg/hr induced vascular lesions in the skin. Histopathologic changes noted at 24 hours; gross pathologic changes noted at 48 hours; and clinical changes noted at 72 hours. Vascular lesions and edema preceded skin sores and were not immune mediated.

Impurities

Toxicity Studies were performed with the significant impurities (occurring at [ ] of pregabalin: PD 0144550, the (R)-enantiomer of pregabalin, PD 0147804, a lactam degradation product in the marketed formulation, and [ ] pregabalin. [ ] [ ] They were all found to be non-mutagenic under the conditions tested. The studies are summarized below.

PD 0144550

PD 0144550 is the (R)-enantiomer of pregabalin. Racemization of pregabalin does not occur with storage of bulk drug. The proposed specification limit for the (R)-enantiomer in drug substance, [ ] exceeds the ICH qualification threshold of 0.1%. Therefore, the safety of the (R)-enantiomer was evaluated in a 13-week study in rats, and genotoxic potential was evaluated in bacterial mutagenicity and rat micronucleus assays.

Rats were given PD 0144550 at 0.1, 0.5, or 2.5 mg/kg by gavage daily for 13 weeks (RR 250-01833). With clinical use of pregabalin at 600 mg, the dose of PD 0144550 in a 50 kg human

would not exceed — mg/kg. The low dose approximated the anticipated human exposure, the high dose was approximately 100 times the anticipated maximum human dose, and the mid dose evaluated the dose response.

There were no drug-related deaths. Convulsions of <1 minute in duration were noted within approximately 15 minutes postdose in 1 female at 2.5 mg/kg during 6 of the last 8 days of dosing. This female also had a convulsion approximately 1 hour postdose on the last of dosing after completion of the ophthalmic examination. No other drug-related clinical signs or effects on body weight and food consumption were noted. PD 0144550 did not induce any changes in clinical laboratory parameters or organ weights. There were no drug-related gross or histopathologic findings. Plasma PD 0144550 toxicokinetic parameters increased dose proportionally and were similar between males and females (RR 764-03384).

PD 0147804

PD 0147804 is a lactam degradation product formed by [redacted] of pregabalin. The proposed specification limits are — % in drug substance and — % in drug product, are equal to or exceed the applicable ICH qualification thresholds of 0.1% and 0.2%, respectively. Subchronic toxicity was evaluated in a 4-week study in rats, and bacterial mutagenicity and rat micronucleus assays evaluated genotoxic potential.

Rats were given oral doses of PD 0147804 at 0.5, 5, or 10 mg/kg daily for 4 weeks (RR 250-01787). With clinical use of pregabalin at 600 mg, the dose of PD 0147804 in a 50 kg human would not exceed — mg/kg. The low dose, 0.5 mg/kg, is approximately 8 times and the high dose, 10 mg/kg, was approximately 170 times the anticipated maximum human dose. There were no deaths, clinical signs, or drug-related gross or histopathological findings. Increases in plasma PD 0147804 kinetic parameters were dose-proportional.

Mutagenicity was evaluated by exposing 4 histidine auxotrophs of *S. typhimurium* and a tryptophan auxotroph of *E. coli* to PD 0147804 with or without S9 from the livers of rats treated with Aroclor 1254 (RR 745-02952). In the initial and confirmatory assays, cytotoxicity was observed at 5000 mg/plate in strains TA-1537, TA-1535, TA-98, and TA-100. Positive controls 2-nitrofluorine, sodium azide, 9-aminoacridine, methyl methanesulfonate, and 2-aminoanthracene increased mean revertant frequency 6- to 38-fold above corresponding vehicle controls, indicating valid assay conditions. There were no significant increases in revertant frequency in any strain with or without S9 at any concentration of PD 0147804. Therefore, PD 0147804 was not mutagenic in bacteria under the conditions of this study.

A micronucleus assay was performed concurrently with the 4-week study of PD 0147804 in rats. Rats were given 0.5, 5, or 10 mg/kg by gavage (RR 745-02838). Additional animals were given a single oral dose of 0.5% MC and served as negative controls or a single IP dose of cyclophosphamide at 20 mg/kg and served as positive controls. The frequency of MNPCE in bone marrow was assessed in vehicle- and drug-treated animals 24 hours after the last dose. Cyclophosphamide induced a significant decrease in PCE in both sexes and an 8-fold increase in MNPCE frequency in males and a 9- fold increase in MNPCE in females, thus confirming assay validity. PD 0147804 had no effect on the percentage of PCE or MNPCE frequency in either sex

at any dose. Therefore, under the conditions of this study, PD 0147804 was not clastogenic in rat bone marrow *in vivo*.

PD [ ] and PD [ ]

Studies were conducted to identify potential occupational safety hazards associated with the manufacture of pregabalin. PD [ ] the racemic mixture and PD [ ] pregabalin [ ] in the synthesis of pregabalin used to resolve the racemic mixture to the S-enantiomer. Occupational safety studies assessed acute toxicity in rats, acute dermal toxicity in rabbits, dermal and ocular irritation in rabbits, skin sensitization in guinea pigs, and bacterial mutagenicity of the racemic mixture and synthesis intermediate.

PD [ ]

After oral treatment with 2000 mg/kg in corn oil, no deaths occurred (RR 901-00517). Diarrhea was noted in 1 male and 2 females on Day 1 and perinasal staining was observed in 2 males on Day 2. There were no effects on body weight and no drug-related gross pathologic changes. The oral lethal dose of PD [ ] was >2000 mg/kg. After acute dermal application of 2000 mg/kg in rabbits, and observation for 14 days, no deaths occurred, and there were no clinical signs, effects on body weight or gross pathologic findings (RR 901-00542). The dermal lethal dose of PD [ ] was >2000 mg/kg.

Dermal irritation was assessed with 500 mg of PD [ ] applied to the shaved skin of the dorsal trunk area of rabbits RR 901-00520. There were no deaths and no effects on body weight. One animal showed very slight erythema 30 to 60 minutes after patch removal but not at 48 or 72 hours postexposure. No skin reactions were observed in the remaining animals at any time point. Therefore, the Primary Dermal Irritation Index was 0.11, corresponding to a negligible irritant.

Ocular irritation of PD 0140410 was assessed in rabbits (RR 901-00508). Conjunctivitis, characterized by redness, discharge, and/or swelling, occurred in all animals given PD [ ] in both nonirrigated and irrigated eyes. The conjuctival irritation diminished during the remainder of the test period and resolved completely in all animals by 48 hours. The maximum mean total score was 4.67 for each group. No corneal opacity, iritis, or conjunctivitis was observed in the control eyes. There were no effects on body weight. Based on the ocular evaluation criteria, PD [ ] is considered minimally irritating to ocular tissue with or without irrigation.

The skin sensitization potential of PD [ ] was evaluated in guinea pigs (RR 901-00529). Positive control animals were given dinitrochlorobenzene (DNCB). Increasing redness was observed in DNCB-treated animals during the first 5 induction applications prompting reduction of the dose from 0.1% to 0.05% for the sixth application. Redness was noted in all DNCB-treated animals after challenge with 0.05% DNCB and the severity score was 2.6 at 24 and 48 hours each. However, no skin reactions were observed in untreated animals given the challenge application of DNCB. No dermal reactions were observed in animals treated with PD [ ] in the induction or after the challenge phase indicating PD [ ] is not a skin sensitizer.

Mutagenicity was evaluated by exposing 5 histidine auxotrophs of *S. typhimurium* to PD [ ] with and without metabolic activation from a postmitochondrial supernatant fraction (S9) from the livers of rats treated with Aroclor 1254 (RR 901-00599). In the initial and confirmatory assays, no

cytotoxicity was observed up to 10,000 mg/plate in any strain. There were no significant increases in revertant frequency in any strain with or without S9 at any concentration of PD [ ] in any assay. Therefore, PD [ ] was not mutagenic in bacteria under the conditions of this study.

PD [ ]

To assess acute toxicity, adult rats were given a single oral dose of PD [ ] at 2000 mg/kg in distilled water and observed for 14 days (RR 901-00717). No deaths occurred. Piloerection was observed in all animals within 3 minutes postdose. Later on Day 1, hunched posture, hypoactivity, and unsteadiness were noted in all animals, and ptosis was noted in all females. Also on Day 1, increased salivation was seen in 1 female, and decreased respiratory rate was present in 1 male. Piloerection persisted through Day 3 in all animals, and no clinical signs were noted after Day 3. There were no effects on body weight and no gross pathologic changes. The oral median lethal dose of PD [ ] was >2000 mg/kg.

To assess acute dermal toxicity, 2000 mg/kg of PD [ ] was applied to the shaved skin of the dorsal trunk area of rabbits (RR 901-00718). A gauze was placed over the treated area for 24 hours then removed. No deaths occurred, and there were no clinical signs. There were no dermal reactions observed immediately after removal of the gauze 24 hours postdose. Slight erythema became apparent in 1 male and 2 females on Day 5 resolving by Day 8 or 9 and was also apparent in 1 male on Day 9 resolving by Day 14. Desquamation characterized by dryness, sloughing, or scaling was noted in 3 males and 1 female from Days 5 to 10. Body weight was unaffected, and there were no gross pathologic changes. The dermal lethal dose of PD [ ] was >2000 mg/kg.

To assess a dermal irritation, a single dose of pregabalin PD [ ] at 500 mg/kg was applied to the shaved skin of the dorsal trunk area of rabbits (RR 901-00719) and covered with gauze for 4 hours. The treated skin was scored for erythema and eschar or edema 60 minutes after removal of the dressings and 24, 48, and 72 hours postexposure. There were no clinical signs of toxicity and any erythema or edema in any animal. The mean Primary Dermal Irritation Index of PD [ ] was 0.

Ocular irritation of PD [ ] was assessed in rabbits (RR 901-00720). Primary ocular irritation was evaluated 1, 24, 48, and 72 hours and 4, 7, and 14 days posttreatment; cornea, iris, conjunctiva, and ocular discharge were graded separately. No clinical signs of toxicity were noted. In animals with nonirrigated eyes, dulling of the cornea was seen in 1 animal 1 hour after drug instillation, and corneal opacification developed in 2 animals. No iridial inflammation was observed. A crimson coloration of the conjunctivae accompanied by swelling with partial closure eversion of the eyelids was seen in all 3 animals. Blanching on the nictating membrane was present in 1 animal, and discharge with moistening of the lids and hair adjacent to the lids was seen in 2 animals. The maximum mean total score for ocular toxicity was 18, and no irritation was seen by Day 4. In animals with irrigated eyes, no corneal damage, or iridial inflammation was seen in any animal. Temporary mild conjunctival irritation was present in all 3 animals; the maximum mean total score for ocular toxicity was 3.3. No irritation was observed after 48 hours. PD [ ] was moderately irritating when instilled without irrigation and minimally irritating with irrigation.

The skin sensitization potential of PD [ ] was evaluated in guinea pigs (RR 901-00721). No dermal reactions were observed in the test or control group during the induction or after the challenge phase. PD [ ] did not induce dermal sensitization in guinea pigs under the conditions of this study.

Mutagenicity was evaluated by exposing 4 histidine auxotrophs of *S. typhimurium* and a tryptophan auxotroph of *E. coli* to PD [ ] with and without S9 from the livers of rats treated with Aroclor 1254 (RR 901-00660). In the initial and confirmatory assays, no cytotoxicity was observed up to 5000 µg/plate in any strain. There were no significant increases in revertant frequency in any strain with or without S9 at any concentration of PD [ ] Therefore, PD [ ] was not mutagenic in bacteria under the conditions of this assay.

2.6.6.9 Discussion and Conclusions

In summary, toxicologic findings after oral dosing of pregabalin included hypoactivity, hyperactivity, and ataxia in rats at ≥ 1.5 times, in rabbits at ≥ 11 times, and in monkeys at ≥ 8 times the mean human exposure ($AUC_{[0-24]}$ of 123 µg·hr/mL) at the maximum recommended clinical dose of 600 mg/day. Dermatopathy was observed in rats and monkeys at ≥ 2 times the mean human exposure at the maximum recommended clinical dose. Decreased platelet count occurred in rats at ≥ 2 times and nasal discharge/rhinitis occurred in monkeys at ≥ 3 times the mean human exposure at the maximum recommended clinical dose. Note: at a maximum clinical dose of 300 mg/day, an $AUC_{(0-24)}$ of ~ 75 µg·hr/mL would provide a somewhat greater safety margin.

The genotoxic potential of pregabalin was assessed in both *in vitro* and *in vivo* studies. Pregabalin was not mutagenic under the conditions of the assays in bacteria using metabolic activation provided by mouse or rat liver. Pregabalin did not induce point mutations or structural chromosome aberrations in Chinese hamster ovary cells *in vitro*. Pregabalin did not induce unscheduled deoxyribonucleic acid (DNA) synthesis in mouse or rat hepatocytes and was not clastogenic in mouse or rat bone marrow *in vivo*.

A dose-dependent increase in the incidence of malignant vascular tumors (hemangiosarcomas) was observed in two strains of mice ($B_6C_3F_1$ and CD-1) given pregabalin in the diet for 2 years at doses of 200, 1000, or 5000 mg/kg/day. Plasma pregabalin exposures (based on AUC) in mice receiving the lowest dose that increased hemangiosarcoma incidence were approximately equal to the mean exposure in humans receiving a daily dose of 600 mg. No evidence of carcinogenicity was seen in two studies in rats (Wistar strain) following oral administration of pregabalin for 2 years at doses of up to 450 (males) and 900 mg/kg/day (females), which were associated with plasma exposures approximately 14 and 24 times, respectively, human exposure at a daily dose of 600 mg/day. If the maximum daily dose in humans were limited to 300 mg/day, the NOAEL values provide approximately a 28 fold and 48 fold exposure ratios in males and females, respectively.

In a fertility study in which male rats were administered pregabalin (250, 1250, or 2500 mg/kg) prior to and during mating, a number of adverse reproductive effects were observed, primarily at doses ≥ 1250 mg/kg; these included: increased number of days to mating, decreased sperm counts and motility, increased sperm abnormalities, reduced fertility, increased preimplantation loss, and

decreased litter size. Decreased sperm motility was also seen at 250 mg/kg. Because a no-effect dose was not established, a follow-up study was conducted using lower doses (50, 100, or 250 mg/kg). No significant reproductive or other toxic effects were observed in this study. Based on the finding of decreased sperm motility at the low dose in the original study, the no effect dose for male reproductive impairment in rats was 100 mg/kg, which was associated with plasma pregabalin exposures (AUC) approximately 3 times human exposures at the maximum recommended dose (MRD) of 600 mg/day (this would produce a 6-fold exposure ratio if the maximum daily dose in humans were limited to 300 mg/day).

In a fertility study in which female rats were given pregabalin (500, 1250, or 2500 mg/kg) prior to and during mating and early gestation (males were not treated), the drug treatment appeared to disrupt estrous cyclicity during the premating treatment period. In addition, there was an increase in the number of days to mating, and increased embryonic death were seen at all doses of pregabalin tested. The low effect dose for female reproductive impairment and embryolethality was 500 mg/kg (plasma exposure approximately 10 times those in humans receiving the MRD of 600 mg/day or 20 times a maximum human daily dose of 300 mg/day).

Segment II Reproductive Toxicology Studies. Increased incidences of fetal structural abnormalities and other manifestations of developmental toxicity (lethality, growth retardation, nervous and reproductive system functional impairment) were observed in the offspring of animals treated with pregabalin during pregnancy.

When pregnant rats were given pregabalin (500, 1250, or 2500 mg/kg) throughout the period of organogenesis, incidences of specific skeletal malformations (fusion of the jugal bone and maxilla and fusion of the nasal bones) were increased at ≥ 1250 mg/kg, and incidences of skeletal variations and retarded ossification were increased at all doses. Fetal body weights were decreased at the highest dose. The low effect dose for developmental toxicity in rats was 500 mg/kg, which was associated with a plasma pregabalin exposures (AUC) approximately 17 times human exposures at the maximum recommended dose [MRD] of 600 mg/day. This low effect dose provides approximately 34 times the human exposure if the maximum daily dose is limited to 300 mg/day.

When pregnant rabbits were given pregabalin (250, 500, or 1250 mg/kg) throughout the period of organogenesis, total incidences of skeletal malformations, visceral variations, and ossification retardation were increased and fetal body weights were decreased at the highest dose. The no effect dose for developmental toxicity in rabbits was 500 mg/kg (plasma exposures approximately 17 times human exposures at the MRD or 34 times the predicted plasma levels if the maximum daily dose were limited to 300 mg/day).

Segment III Reproductive Toxicology. Pregabalin treatment of rats produced reproductive and developmental effects in the peri- and post-natal periods. In a study in which female rats were dosed with pregabalin (50, 100, 250, 1250, 2500 mg/kg) throughout gestation and lactation, offspring growth was reduced at ≥ 100 mg/kg, offspring survival was decreased at ≥ 250 mg/kg, and offspring neurobehavioral (decreased auditory startle responding) and reproductive function (decreased fertility, decreased litter size) were impaired at 1250 mg/kg. The effect on offspring survival was pronounced at doses ≥ 1250 mg/kg, with 100% mortality in high dose litters. The no

effect level for pre- and postnatal development was 50 mg/kg (plasma exposures approximately 2 times human exposures at the MRD of 600 mg/day or 4 times the MRD of 300 mg/day).

Skin lesions characterized clinically by a spectrum of lesions ranging from erythema to necrosis, and histopathologically by hyperkeratosis, acanthosis, fibrosis, and/or necrosis of the tail, were observed in rats given ≥ 50 mg/kg in oral repeated-dose studies, with associated $AUC_{(0-24)}$ ≥ 241 µg·hr/mL. Lesions typically appeared within the first 2 weeks of treatment at higher doses and resolved in most affected animals by Week 7 in the 13-week study and by Week 4 in the 52-week study. Similar skin lesions were observed in monkeys in oral repeated-dose studies, and were located primarily on the tail in most animals. In the chronic monkey study, lesions were observed at ≥ 25 mg/kg, with plasma pregabalin $AUC_{(0-24)}$ values ≥ 219 µg·hr/mL. As in rats, lesions in affected animals in the chronic monkey study generally resolved prior to study termination. Subcutaneous tail temperature, used as an indirect measure of tail blood flow in the chronic monkey study, showed no consistent differences between control and high-dose animals, or between affected and unaffected animals within the same group. Pregabalin at 5% and 7.5% did not induce contact sensitization (allergic dermatitis) in rats in the local lymph node assay. The etiology of the skin lesions remains unknown. No tail dermatopathy was observed in mice given repeated oral doses of pregabalin up to 13 g/kg up to 13 weeks. Missing tail tips were observed in mice given up to 5000 mg/kg ($AUC_{(0-24)}$ of 3150 µg·hr/mL) in the B6C3F1 but not the CD-1 carcinogenicity study, however, the relationship of this lesion to dermatopathy in rats and monkeys is unknown.

2.6.6.10 Tables and Figures

Sponsor's non-pivotal studies:

Species (Strain) Animals Sex Group Total	Route (Vehicle) [Dose Volume]	Daily Dose (mg/kg)	Treatment Duration (Withdrawal)	Significant Findings	No Adverse Effect Dose (mg/kg)	Report Number
RAT						
Rat (Wistar) 5M + 5F 280[1]	Oral (Diet) Oral (0.5% MC) [25 mL/kg]	UC 500 1250 2500 VC 500 1250 2500	2 Weeks	One female given 1250 mg/kg by gavage died in Week 2 attributed to pyelonephritis. Hypoactivity and ataxia at all doses by gavage and >1250 mg/kg by diet. Decreased body weight gain and nondose-related increases in RBC count at all doses by gavage and/or diet. Hb and Hct increased at 1250 mg/kg by gavage. Decreased platelet count at 2500 mg/kg by gavage and at all doses by diet. Inflammation of epididymis in males at 2500 mg/kg by diet. Tail dermatopathy at all doses by diet. Drug exposure similar by diet and gavage.	None	250-01702
Rat (Wistar) 5M + 5F 118[c]	Bolus IV[b] (0.9% NaCl) [2 mL/min][d]	VC 50 150 300	7 Days	No deaths. Hypoactivity and/or ataxia in both sexes at all doses up to Day 5. Decreased body weight gain in males at 300 mg/kg. Decreased platelet count in males at 300 mg/kg and in females at all doses. No histopathologic findings.	None	250-01803
Rat (Wistar) 10M + 10F 140[e]	Bolus IV[b] (0.9% NaCl) [2 mL/min][d]	VC 40 100 300	4 Weeks	No deaths. Hyperactivity and/or ataxia in both sexes at all doses through Week 4. Decreased platelet count in both sexes at all doses. No histopathologic findings.	None	250-01812 764-03163

MC = Methylcellulose; UC = Untreated control; VC = Vehicle control; RBC = Red blood cells; Hb = Hemoglobin; Hct = Hematocrit; IV = Intravenous

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Sponsor's non-pivotal studies continued:

Species (Strain) Animals/Sex/Group Total	Route (Vehicle) [Dose Volume]	Daily Dose (mg/kg)	Treatment Duration (Withdrawal)	Significant Findings	No Adverse Effect Dose (mg/kg)	Report Number
Rat (continued)						
Rat (Wistar) 3M + 3F 24	Continuous Infusion[f] (0.9% NaCl) [4 mL/kg/hr][g]	VC 3 15 75	4 Days	No deaths. Ataxia in both sexes at 75 mg/kg; hypoactivity and/or urine staining on Day 4 in 1 male at 15 mg/kg/hr and both sexes at 75 mg/kg/hr. No clinical signs at 3 mg/kg/hr. Urinary bladder dilatation in males at all doses.	None	250-01800
Rat (Wistar) 10M +10F 80	Continuous Infusion[b] (0.9% NaCl) [2-4 mL/kg/hr][h]	VC 3 15 75	2 Weeks	No drug-related deaths. Chromodacryorrhea and urine staining at all doses; catalepsy in males at all doses; hypoactivity in females at ≥15 mg/kg/hr. Decreased platelet count in males at ≥15 mg/kg/hr. Foamy macrophage accumulation in alveolar lumen and degeneration of urinary bladder muscularis at 75 mg/kg/hr.	None	250-01818 764-03200
Monkey						
Monkey (cynomolgus) 1M + 1F 2	Oral (0.5% MC) [5 mL/kg]	50-2000	11 Days[i]	Emesis in the male at 100 mg/kg, diarrhea and/or soft feces in both sexes at ≥800 mg/kg, and hypoactivity in the male at 1250 mg/kg. Body weight, food consumption, ophthalmic, blood pressure, ECG, and clinical laboratory parameters not affected. No drug-related gross or histopathologic changes	400	745-02116

VC = Vehicle control; ECG = Electrocardiographic parameters.
[b] Bulk drug at 20 mg/mL
[f] Formulated pregabalin contained active drug at 20 mg/mL in 10 mL sterile 0.9% NaCl.
[g] Drug concentrations of 0.75, 3.75, and 18.75 mg/mL resulted in dose rates of 3, 15, and 75 mg/kg/hr.
[h] Drug concentrations of 1.5, 7.5, and 18.75 mg/mL resulted in dose rates of 3, 15, and 75 mg/kg/hr.
[i] Escalating-dose regimen; animals received 50 (Day 1), 100 (Day 2), 200 (Day 3), 400 (Day 4), 800 (Day 8), 1000 (Day 9), 1250 (Day 10), and 2000 mg/kg (Day 11); animals not dosed on Days 5 to 7.

Sponsor's non-pivotal studies continued:

Species (Strain) Animals/Sex/Group Total	Route (Vehicle) [Dose Volume]	Daily Dose (mg/kg)	Treatment Duration (Withdrawal)	Significant Findings	No Adverse Effect Dose (mg/kg)	Report Number
Monkey (continued)						
Monkey (cynomolgus) 2M + 2F 18[j]	Oral (0.5% MC) [5 mL/kg]	VC 100 500 1000 2000[l]	2 Weeks	One female at 1000 mg/kg died; death not clearly drug-related. Soft feces and/or diarrhea at all doses; hypoactivity at 1000 mg/kg; and bloody nasal discharge at 1000 and 2000 mg/kg. Body weight loss at 1000 mg/kg; decreased food consumption at 100, 1000, and 2000 mg/kg. Decreased RBC count, Hb, Hct, and increased neutrophils at ≥500 mg/kg. No drug-related gross or histopathologic changes.	None	250-01713
Monkey (cynomolgus) 4M + 4F 32	Oral (0.5% MC) [10 mL/kg]	VC 100 1000 2000	4 Weeks (4 Weeks)	Two males at 1000 mg/kg and 1 animal/sex at 2000 mg/kg died after 1 dose. Ataxia and/or hypoactivity at 1000 and 2000 mg/kg; stereotypic behavior at 2000 mg/kg. Food consumption and ECG not affected. No gross or histopathologic changes in animals that died.	Study Terminated Prior to Completion[m]	250-01720
Monkey (cynomolgus) 2M + 2F 16[d]	Oral (0.5% MC) [5 mL/kg]	VC 500 750 1000 500 BID	4 Days[n]	Soft feces/diarrhea at all doses; ataxia and/or hypoactivity at 1000 mg/kg and 500 mg/kg BID. One animal per sex at 1000 mg/kg died or was moribund and euthanized on Day 2. Body weight and food consumption not affected. No gross or histopathologic changes in animals that died. Similar pregabalin exposure at 1000 mg/kg and 500 mg/kg BID.	None	745-02268 764-02188

MC = Methylcellulose; VC = Vehicle control; RBC = Red blood cells; Hb = Hemoglobin; Hct = Hematocrit; ECG = Electrocardiographic parameters; BID = Dosed twice daily.

[j] Vehicle controls = 1/sex.

[k] Dose volume = 10 mL/kg at 2000 mg/kg.

[l] Animals given 2000 mg/kg returned to stock colony at completion of study.

[m] Dosing discontinued after a single dose due to death at high doses; surviving animals observed for 1 week and returned to the stock colony.

[n] Due to death or moribundity, animals given daily doses at 1000 mg/kg for 2 days only.

[o] Same animals given 500 and 500 mg/kg BID

Sponsor's non-pivotal studies continued:

Species (Strain) Animals/Sex/Group Total	Route (Vehicle) [Dose Volume]	Daily Dose (mg/kg)	Treatment Duration (Withdrawal)	Significant Findings	No Adverse Effect Dose (mg/kg)	Report Number
Monkey (continued)						
Monkey (cynomolgus) 1M + 1F 2	Bolus IV[b] (0.9% NaCl) [1 mL/kg/min][q]	5-400[p]	17 Days	No deaths. No clinical signs at ≤300 mg/kg. After repeated dosing at 400 mg/kg, hunched posture, hypoactivity, somnolence, tremors and epistaxis in the male, fecal changes in the female, and reduced food consumption both animals. Decreased RBC count, Hb, and Hct in both sexes; compensatory increased reticulocyte count in the female. Skin sores around nostrils and on the foot in the male.	300 mg/kg	745-02970
Monkey (cynomolgus) 3M + 3F 24	Bolus IV[f] (0.9% NaCl) [1 mL/kg/min][q]	VC 10 40 200	4 Weeks	No deaths. Ataxia in both sexes and tremors and nasal discharge in males at 200 mg/kg. Convulsions in 1 male at 40 mg/kg on Day 22 and in 1 female at 200 mg/kg on Day 1. Decreased RBC count, Hb, and Hct and increased reticulocyte count in both sexes at 200 mg/kg.	10 mg/kg	745-03033 764-03162
Monkey (cynomolgus) 1M + 2F 3	Continuous Infusion[b] (0.9% NaCl) [2 mL/kg/hr][s]	8 4 2 6	1 Day[r] 4 Days 4 Days 3 Days	One female given 8 mg/kg/hr for 24 hours died; no signs in the male. No signs in the female given 2, 4, or 6 mg/kg/hr; tremors in the male at all doses not clearly drug-related. Tail dermatopathy in both surviving animals.	None	250-01801

IV = Intravenous; RBC = Red blood cell count; Hb = Hemoglobin; Hct = Hematocrit; VC = Vehicle control.

[b] Bulk drug at 20 mg/mL

[f] Formulated pregabalin contained active drug at 20 mg/mL in 10 mL sterile 0.9% NaCl.

[p] Escalating-dose regimen; animals received 5 (Day 1), 10 (Day 2), 25 (Day 3), 50 (Day 4), 75 (Day 7), 125 (Day 8), 200 (Day 9), 300 (Day 10), 400 (Day 11), and 400 mg/kg (Days 14-17); animals not dosed on Days 5, 6, 12, and 13.

[q] Drug concentration of 20 mg/mL resulted in a dose rate of 20 mg/kg/min.

[r] Escalating-dose regimen; animals received 8 (Day 1), 4 (Days 8-11), 2 (Days 15-18), and 6 mg/kg (Days 23-25); animals not dosed on Days 3 to 7, 12 to 14, and 19 to 22.

[s] Drug concentrations of 1, 2, 3, and 4 mg/mL resulted in dose rates of 2, 4, 6, and 8 mg/kg/hr.

Sponsor's non-pivotal studies continued:

Species (Strain) Animals/Sex/Group Total	Route (Vehicle) [Dose Volume]	Daily Dose (mg/kg)	Treatment Duration (Withdrawal)	Significant Findings	No Adverse Effect Dose (mg/kg)	Report Number
Monkey (continued)						
Monkey (cynomolgus) 3M + 3F 24	Continuous Infusion[b] (0.9% NaCl) [2 mL/kg/hr][c]	VC 2 4 6	2 Weeks	One female at 6 mg/kg/hr died and 1 female each at 4 and 6 mg/kg/hr were moribund and euthanized after Day 6. Edema, dermatopathy, and red nasal discharge at all doses; ataxia and hypoactivity at ≥4 mg/kg/hr. Vascular lesions in skin localized to the extremities and oral mucous membrane, subcutaneous edema, and lesions in the nasoturbinates at all doses; changes in clinical laboratory parameters secondary to pathologic findings.	None	250-01817 764-03198

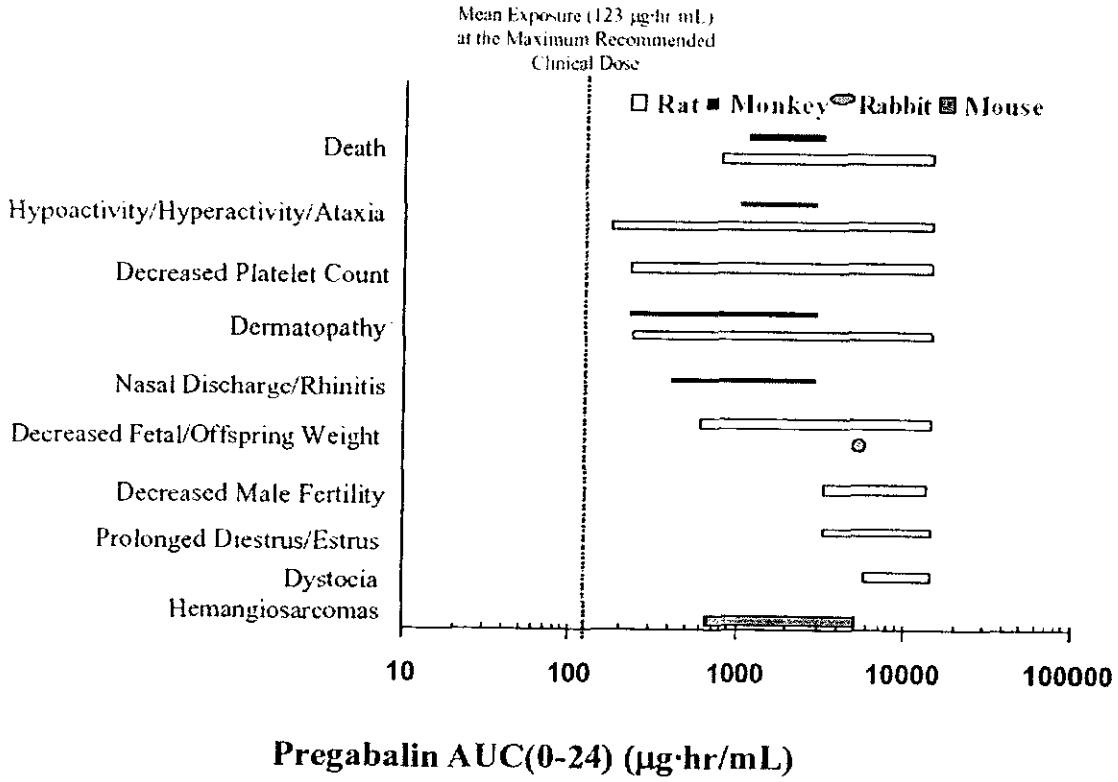
VC = Vehicle control.
[b] Bulk drug at 20 mg/mL
[c] Drug concentrations of 1, 2, or 3 mg/mL resulted in dose rates of 2, 4, or 6 mg/kg/hr.

Species (Strain) Animals/Sex/Group Total	Route (Vehicle) [Dose Volume]	Daily Dose (mg/kg)	Treatment Duration	Significant Findings	No Adverse-Effect Dose (mg/kg)	Report Number
Mouse (B6C3F1) 10M + 10F 300[b]	Oral (Diet)[a]	M: UC 2174 7538 12685; F: UC 2607 8152 13607	2 Weeks	No deaths. Urine staining and decreased body weight gain at mid and high doses Minimal increases in RBC, Hb, and Hct at mid and high dose. Decreased prostate weight at mid and high dose. Urinary bladder dilatation in males at all doses. No histopathologic changes.	None	250-01721
Mouse (B6C3F1) 21M + 21F 132[c]	Oral (Diet)	UC 100 500 2500	4 Weeks	No deaths, clinical signs, or effects on body weight or food consumption. AUC(0-24) increased linearly and ranged from 46.8 to 2130 µg·hr/mL.	Toxicokinetic	250-01768
Mouse (B6C3F1) 10M + 10F 194[d]	Oral (Diet)	UC 1000 4000 8000	13 Weeks	Sporadic deaths at all doses; not clearly drug-related. MPV increased at all doses. In females, increased kidney weight at ≥4000 mg/kg; decreased thymic weight at 8000 mg/kg; mild dilatation and basophilia of renal cortical tubules at 8000 mg/kg; and increased vacuolation of adrenal X-zone at all doses. No proliferative vascular changes.	None	250-01744

UC =Untreated control; RBC = Red blood cell; Hb = Hemoglobin; Hct = Hematocrit; MPV = Mean platelet volume.
[a] Pregabalin given in diet at 1%, 3%, or 5%; doses estimated based on actual body weight and food consumption.
[b] Two hundred twenty additional animals used for determination of toxicokinetic parameters (5 controls/sex and 35/dose/sex).
[c] Three animals/sex in control group; all animals used for toxicokinetic analyses.
[d] One hundred fourteen additional animals used for determination of toxicokinetic parameters (3 controls/sex and 18/sex/dose).

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

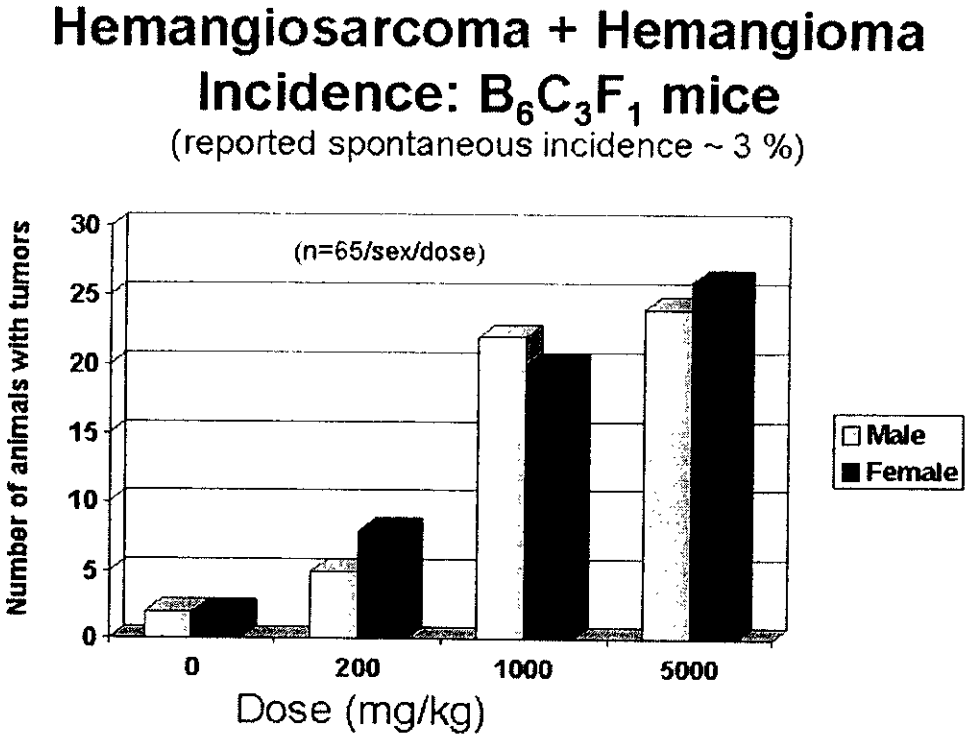


Sponsor's summary table:

Significant Effect	Species	No-Effect Dose AUC(0-24)	Lowest-Effect Dose AUC(0-24)
Hypoactivity Hyperactivity-Ataxia	Rat	Effects at all doses studied.	50 mg/kg 173 µg·hr/mL[a]
	Monkey	≤100 mg/kg ≤388 µg·hr/mL	500 mg/kg 990 µg·hr/mL
Decreased Platelets	Rat	Effects at all doses studied.	50 mg/kg 228 µg·hr/mL[b]
Dermatopathy	Rat	Effects at all doses studied.	50 mg/kg 241 µg·hr/mL[c]
	Monkey	10 mg/kg ≤91.1 µg·hr/mL	25 mg/kg 219 µg·hr/mL
Nasal Discharge/Rhinitis	Monkey	50 mg/kg ≤385 µg·hr/mL	100 mg/kg 388 µg·hr/mL
Developmental Toxicity Decreased Fetal Weight	Rat	1250 mg/kg 6590 µg·hr/mL	2500 mg/kg 9470 µg·hr/mL
	Rabbit	500 mg/kg ≤2020 µg·hr/mL	1250 mg/kg 4750 µg·hr/mL
Developmental Toxicity Decreased Offspring Wt	Rat	50 mg/kg ≤241 µg·hr/mL[c]	100 mg/kg 601 µg·hr/mL
Male Fertility	Rat	250 mg/kg ~1320 µg·hr/mL[d]	1250 mg/kg 3320 µg·hr/mL
Prolonged Diestrus/Estrus	Rat	500 mg/kg ≤1020 µg·hr/mL	1250 mg/kg 3340 µg·hr/mL
Dystocia	Rat	250 mg/kg ≤1380 µg·hr/mL[c]	1250 mg/kg 5760 µg·hr/mL[c]
Carcinogenicity - No Drug-Related Tumors	Rat	450 mg/kg (M) ≤1740 µg·hr/mL 900 mg/kg (F) ≤2960 µg·hr/mL	None
Carcinogenicity- Hemangiosarcomas	Mouse B6C3F1	200 mg/kg ≤153 µg·hr/mL	1000 mg/kg 653 µg·hr/mL
Carcinogenicity- Hemangiosarcomas	Mouse CD-1	1000 mg/kg ≤558 µg·hr/mL	5000 mg/kg 3150 µg·hr/mL
Death	Rat	50 mg/kg ≤228 µg·hr/mL[b]	250 mg/kg 802 µg·hr/mL[b]
	Monkey	≤500 mg/kg ≤1090 µg·hr/mL	1000 or 500 BID mg/kg 1100 µg·hr/mL[e]

[a] Value obtained from a supportive toxicokinetic study by gavage.
[b] Value obtained from a 13-week toxicity study by diet.
[c] Value obtained from a prenatal-postnatal study by gavage.
[d] Value approximated from the first male fertility and early embryonic development study by gavage.
[e] Individual animal value.

Reviewer summary table:



OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Pregabalin is a ligand at gabapentin receptors and has a pharmacologic profile that is very similar to the prototype for this class, gabapentin. The absorption of pregabalin is less limited than gabapentin resulting in greater bioavailability. This fact, together with the higher affinity for the $\alpha_2\delta$ receptor, mean that pregabalin is likely to have greater oral potency than gabapentin. The toxicology of pregabalin in animal studies is much greater than gabapentin, however. In addition to the dose-related increase in occurrence of angiosarcomas in both B6C3F1 and CD-1 mice, there is a dermatopathy that is very evident in monkeys and rats. This later toxicity is particularly problematic for the therapeutic indication of diabetic neuropathy, since these patients are already prone to skin injuries and impaired healing.

Unresolved toxicology issues (if any): The dermatopathy in rats at $\geq$ 50 mg/kg in oral repeated-dose studies, with associated $AUC_{(0\text{-}24)} \geq 241$ µg·hr/mL. Skin lesions typically appeared within the first 2 weeks of treatment at higher doses and resolved in most affected animals by Week 7 in the 13-week study and by Week 4 in the 52-week study. Similar skin lesions were observed in monkeys in oral repeated-dose studies, and were located primarily on the tail in most animals. In the chronic monkey study, lesions were observed at $\geq$ 25 mg/kg, with plasma pregabalin $AUC_{(0\text{-}24)}$ values $\geq$ 219 µg·hr/mL.

Recommendations: This application is not recommended for approval for the indication of diabetic neuropathy from the pharmacology / toxicology perspective.

Suggested labeling: (from Ed Fisher review)

Carcinogenesis/Mutagenesis/Impairment of Fertility

Carcinogenesis

(redacted)

Impairment of Fertility

1 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ **Concurrence Yes** ___ **No** ___

APPEARS THIS WAY
ON ORIGINAL

APPENDIX/ATTACHMENTS

Appendix 1. Pharmacology Studies reviewed for this NDA

PHARMACOLOGY STUDIES

Type of Study Test System	**Administration**	**Study No.**
2.6.3.1.1.A. Primary Pharmacodynamics Radioligand Binding		
Rat brain (membrane homogenate) [^{3}H]gabapentin radioligand binding	*In vitro*	740-03239
Porcine brain (membrane homogenate) [^{3}H]gabapentin radioligand binding	*In vitro*	Brown et al.
Recombinant mammalian cells (membrane homogenate) [^{3}H]gabapentin radioligand binding	*In vitro*	740-03602
Porcine brain (membrane homogenate) [^{3}H]gabapentin radioligand binding	*In vitro*	740-03576
Mutant mouse brain deficient in [^{3}H]gabapentin binding to $\alpha^2\delta$ type 1 protein (membrane homogenate) [^{3}H]gabapentin radioligand binding	*In vitro*	740-03603
Correlation between [^{3}H]gabapentin radioligand binding to rat brain membrane homogenate and *in vivo* pharmacology of GABA derivatives in rats	*In vitro*	740-03576
Recombinant mammalian cells (membrane homogenate) [^{3}H]pregabalin radioligand binding	*In vitro*	740-03614
2.6.3.1.1.B. Neurotransmitter Release and Turnover		
Rat brain tissue slices (trigeminal nucleus) [^{3}H]glutamate release	*In vitro*	770-00311
Rat brain tissue slices (neocortex) glutamate release	*In vitro*	Dooley et al.
Rat brain tissue slices (neocortex; striatum) [^{3}H]noradrenaline release	*In vitro*	740-03489
Rat brain tissue slices (neocortex; striatum; cerebellum; hippocampus; spinal cord) [^{3}H]noradrenaline release; [^{3}H]serotonin release	*In vitro*	740-03578
IV = Intravenous; PO = Oral gavage; SC = Subcutaneous; IP = Intraperitoneal; ICV = Intracerebroventricular. [a] Denotes GLP study		

Type of Study Test System	**Administration**	**Study No.**
2.6.3.1.1.B. Neurotransmitter Release and Turnover (continued)		
Rat brain tissue slices (neocortex) [^{3}H]noradrenaline release in presence of gabapentin	*In vitro*	740-03579
Rat brain L-3, 4-dihydroxyphenylalanine turnover and 5-hydroxytriptophan turnover	IP	740-03470
Rat brain or monkey brain synaptosome preparation calcium influx (fluorescence endpoint)	*In vitro*	740-03538
Rat spinal cord tissue slices immunoreactive Substance P or calcitonin gene-related peptide release	*In vitro*	740-03537
2.6.3.1.1.C. Transporter Activity		
Rat brain cells (primary culture) or mammalian cell line [^{3}H]L-leucine uptake	*In vitro*	761-00007
Rat brain cells (primary cell culture) [^{3}H]GABA transporter location and function	*In vitro*	740-03516
2.6.3.1.1.D. Protein Kinase Activation		
Chinese hamster transformed cell line activation of transcription factor proteins	*In vitro*	761-00006
2.6.3.1.1.E. Electrophysiology		
Anesthetized rat dorsal root electrophysiological reflex activity	IV	770-00322
Anesthetized rat ventral root electrophysiological reflex activity	IV	770-00326

Type of Study Test System	**Administration**	**Study No.**
Anesthetized rat electrophysiological seizure activity in hippocampus & evoked potentials	IP	740-03518
2.6.3.1.1.F. Analgesia		
Rat formalin footpad test analgesia	PO	740-03479
Rat formalin footpad test analgesia	SC	770-00297
Mouse (mutant, deficient in [^{3}H]gabapentin binding) formalin footpad test analgesia	PO	740-03610
Rat carrageenan footpad heat hyperalgesia	SC	770-00297
Rat postsurgical footpad heat and tactile allodynia	SC	770-00296
Rat intrathecal Substance P-induced footpad thermal hyperalgesia	IP, intrathecal	Partridge et al.
Rat thermal injury-induced footpad thermal hyperalgesia	Intrathecal	Jun & Yaksh
Rat ultraviolet irradiation footpad thermal hyperalgesia	SC	770-00304
Rhesus monkey tail immersion thermal hyperalgesia from capsaicin	PO	740-03528
Rat diabetes-induced footpad tactile allodynia	PO, intrathecal	770-00295
Rat diabetes-induced footpad tactile allodynia	PO, intrathecal	770-00312
Rat vincristine-induced footpad tactile allodynia	IP	740-03529
Rat sciatic nerve ligation or dorsal root ligation footpad tactile allodynia	PO	770-00294
Rat footpad tactile allodynia from prior injection of acidic saline into gastrocnemius muscle	PO	740-03589
2.6.3.1.1.G. Epileptic Seizures		
Mouse tonic extensor seizures from maximal electroshock	PO	740-03090
Mouse (mutant, deficient in [^{3}H]gabapentin binding) tonic extensor seizures from maximal electroshock	PO	740-03610
Mouse tonic extensor seizures from low-intensity electroshock	PO	740-03172
Mouse tonic extensor seizures from maximal electroshock	PO (repeated dosing)	740-03216
Rat tonic extensor seizures from maximal electroshock	IV, PO	740-03081
Rat tonic extensor seizures from maximal electroshock	IV, PO	740-03263
Rat tonic extensor seizures from maximal electroshock (estimation of plasma concentration)	IV, PO	740-03268
Rat tonic extensor seizures from maximal electroshock	PO (repeated dosing)	740-03108
Rat tonic extensor seizures from maximal electroshock (time course and pharmacokinetic comparison)	PO	740-03225
Mouse clonic seizures from pentylenetetrazole	PO	740-03214
Mouse clonic seizures from pentylenetetrazole	IP	740-03224
Mouse clonic seizures from bicuculline	PO	740-03224
Mouse clonic seizures from picrotoxin	PO	740-03224
Mouse clonic seizures from strychnine	PO	740-03224
Rat electrographic and behavioral seizures in kindled rats	IP	740-03222
Rat electrographic absence seizures	IP	740-03136
DBA/2 inbred mouse strain seizures induced by sound	PO	740-03365
DBA/2 inbred mouse strain seizures induced by sound (effect of benzodiazepine antagonist)	PO	740-03551

Type of Study Test System	Route	Report #
2.6.3.1.1.H. Anxiety Disorders		
Mouse tail suspension behavior (anxiolytic/sedative effects)	PO	740-03464
Mouse (mutant, deficient in [^{3}H]gabapentin binding) tail suspension behavior (anxiolytic/sedative effects)	PO	740-03610
Rat Geller conflict test (anxiolytic activity)	SC	770-01316
Monkey Geller conflict test (anxiolytic activity)	PO	740-03526
Rat Vogel conflict test (anxiolytic activity)	PO	740-03464
Rat brain primary cultured neurons; uptake of [^{3}H]GABA	*In vitro*	761-00007
Rat Vogel conflict test (anxiolytic activity; effect of benzodiazepine antagonist)	PO	740-03551
Rat elevated X-maze (anxiolytic activity)	PO	740-03464
Rat elevated X-maze (anxiolytic activity)	SC	770-01316
2.6.3.1.2.A. Secondary Pharmacodynamics Radioligand Binding		
Various membrane-bound radioligand binding assays	*In vitro*	740-03076
Rat and mouse blood platelet (membrane homogenate) radioligand binding for [^{3}H]pregabalin	*In vitro*	740-03614
Rat neocortex brain (membrane homogenate) [^{3}H]-CGP54626A radioligand binding ($GABA_B$ receptors)	*In vitro*	740-03547
Rat neocortex brain (membrane homogenate) [2,3,4-^{3}H(N)]-CP 55,940 radioligand binding (Cannabinoid 1 receptors)	*In vitro*	740-03548
Human recombinant CB1 and CB2 cannabinoid receptors expressed *in vitro* with [^{3}H]WIN 55212-2 as radioligand	*In vitro*	770-00350
2.6.3.1.2.B. Neurotransmitter Transporters		
Rat brain synaptosomes uptake of [^{3}H]noradrenaline, [^{3}H]dopamine and [^{3}H]serotonin	*In vitro*	740-03545
Rat brain cultured neurons [^{3}H]GABA uptake	*In vitro*	761-00007
2.6.3.1.2.C. Enzyme Assays		
Porcine brain glutamic acid decarboxylase enzyme activity	*In vitro*	Taylor et al.
Rat brain GABA transaminase enzyme activity	*In vitro*	761-00012
Human blood platelet cyclooxygenase 1 and blood macrophage transformed cell line cyclooxygenase 2 activity	*In vitro*	760-00132
Mouse J774A.1 macrophage cell line; cyclooxygenase enzyme activity	*In vitro*	760-00132
2.6.3.1.2.D. Secondary Pharmacodynamics -GABA Tissue Content		
Rat isolated optic nerve segments; GABA content	*In vitro*	740-03515
Rat whole forebrain; GABA content	Ex vivo	Errante & Petroff,
2.6.3.1.2.E. Electrophysiology		
Chinese hamster ovary tumor cells stably transfected with rat brain type IIA sodium channels; voltage-clamp electrophysiology of sodium channel currents	*In vitro*	740-03220
Rat primary cultured autonomic ganglion neurons; voltage clamp electrophysiology of sodium and potassium channel currents	*In vitro*	740-03519
Chinese hamster ovary tumor cells stably transfected with rat brain type IIA sodium channels; voltage-clamp electrophysiology of sodium channel currents	*In vitro*	740-03519
Human embryonic kidney tumor cell line stably transfected with B-class calcium channels; voltage-clamp electrophysiology of calcium channel currents	*In vitro*	740-03519
Rat primary cultured neocortex neurons; $GABA_A$ receptor pharm by voltage-clamp	*In vitro*	740-03539
Rat hippocampal brain tissue slices; physiology of long-term synaptic potentiation and both glutamate-mediated and GABA-mediated synaptic potentials	*In vitro*	740-03517
2.6.3.1.2.F. Other Types of Analgesia		
Mouse intraperitoneal acetic acid-induced abdominal constriction analgesia test	PO	740-03479
Rat abdominal constriction responses from colonic distension following colonic infusion of trinitrobenzene sulfonic acid	SC, PO	6051-00002

Type of Study Test System	Route	Report #
Rat abdominal constriction responses after systemic administration of lipopolysaccharides and rectal distension	IP, PO	6051-00003
Rat abdominal constriction responses following intracolonic formalin infusion	SC, ICV, Intrathecal	6051-00004
Rat abdominal constriction responses following intracolonic glycerol infusion	PO	6051-00008
Rat abdominal constriction responses following immobilization stress	PO	6051-00009
Guinea pig abdominal constriction responses in anesthetized animals following rectal distension	IP	6051-00005
2.6.3.1.2.G. Arthritis and Gastric Cytoprotection		
Rat ankle swelling after antigen-induced monoarthritis	PO	760-00177
Mouse arthritis rate of onset of symptoms and symptom severity after systemic collagen antigen challenge	PO	760-00178
Rat gastric mucosal damage (surface area) caused by oral administration of indomethacin	PO, IP, cisterna magna	760-00138
2.6.3.1.2.H. Subjective Properties and Physiologic Dependence		
Rat operant response with training to discriminate morphine injection SC from saline injection SC	SC	770-00297
Rhesus monkey operant response with training to discriminate midazolam injection SC from saline injection SC	PO	740-03524
2.6.3.1.2.I. Subjective Properties and Physiologic Dependence (continued)		
Rats trained to prefer one chamber over another in response to drug injection (conditioned place preference)	PO	770-00314
Rat increased locomotor activity from administration of cocaine or amphetamine	IP	740-03441
Rhesus monkey intravenous self administration of pregabalin in animals trained to self-administer pentobarbital	IV	745-03278
Rhesus monkey intravenous self administration of pregabalin in animals trained to self-administer methohexital	IV	740-03525
Rats given continuous infusion of pregabalin for 12 days and then discontinued to determine possible weight loss and behavioral withdrawal signs in comparison to infusion of pentobarbital	IP	740-03540
2.6.3.3.A. Safety Pharmacology - Spontaneous Locomotor Activity and Ataxia		
Rat spontaneous locomotor activity	PO	740-03472
Mouse spontaneous locomotor activity	PO	740-03472
Mouse spontaneous locomotor activity	PO, IV	740-03074
Mouse inverted screen ataxia	PO	740-03472
Mouse inverted screen ataxia	PO, IV	740-03217
Mouse inverted screen ataxia	PO	740-03074
Mouse rotorod ataxia	IP	740-03224
Rat rotorod ataxia	PO	770-00297
Rat ataxia (observation of walking)	PO	740-03224
Rat inverted screen ataxia	PO	740-03472
Rat beam walking ataxia	PO	770-01317
2.6.3.3.B. Safety Pharmacology - Central Nervous System		
Rat hindlimb placing response	PO	740-03224
Rat impaired righting reflex	PO	740-03215
Mouse observation for central nervous system signs (modified Irwin test)	PO, IV	740-03074
Mouse observation for central nervous system signs and spontaneous activity	IV	745-02928[a]
Rat observation for central nervous system signs and spontaneous activity	IV	745-02928[a]
Monkey central nervous system signs	PO	740-03483
Rat spontaneous sleep	PO	740-03527

Type of Study Test System	Route	Report #
2.6.3.3.C. Gastrointestinal System		
Rat gastric emptying and intestinal transit time	PO	6051-00006
Rat intestinal transit at a fixed time after feeding in rats	PO	770-00297
Rat colonic transit time	PO	6051-00007
2.6.3.3.D. Cardiovascular System		
Rat cardiovascular parameters	PO	740-03115
Rat cardiovascular and renal parameters	IV	745-02986[a]
Monkey cardiovascular parameters	IV	745-02988[a]
Dog cardiovascular parameters	PO	742-00010
2.6.3.3.D. Pulmonary System		
Dog pulmonary function	IV	760-00073
2.6.3.3.E. Changes in Drug Metabolism		
Rat hexobarbital sleeping time	PO	740-03224
Activity of rat hepatic microsomal enzymes ex vivo	PO	740-03224

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Jerry Cott
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PHARMACOLOGIST

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R. Daniel Mellon
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PHARMACOLOGIST

Ed Fisher Review
5/20/04
Carc | Reprotox

March 24, 2004

Review and Evaluation of Pharmacology and Toxicology
Original NDA Review

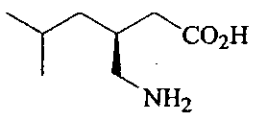
NDA #s: NDA 21-446 (neuropathic pain associated with diabetic peripheral neuropathy)
NDA 21-723 (neuropathic pain associated with herpes zoster)
NDA 21-724 (epilepsy)
NDA C J

Sponsor: Pfizer
Ann Arbor, MI

Drug:

Generic Name:	Pregabalin
Trade Name:	Lyrica
Code Name:	CI-1008
Chemical Name:	(S)-3-(aminomethyl)-5-methylhexanoic acid
Molecular Formula:	$C_8H_{17}NO_2$
Mol. Wt.:	159.23

Structure:



Related IND: C J 53,763 (neuropathic pain)

Table of Contents

Note: Portions of this review were excerpted from the sponsor's submission.

I. CARCINOGENICITY

All relevant preclinical carcinogenicity data have been reviewed previously and are discussed in the following documents:

A. Pharmacology/Toxicology review of initial rat and mouse carcinogenicity studies (IND — Ed Fisher, HFD-120; dated 12/12/00)
B. Statistical reviews of carcinogenicity studies (IND — . Roswitha Kelly, HFD-710; 6/13/03)
C. Exec-CAC evaluation of initial rat and mouse carcinogenicity studies (IND — 12/12/00)
D. Supervisory pharmacologist's memorandum on mouse tumor findings (IND 53,763; Tom Papoian, HFD-170; 3/19/01)
E. FDA Pharm/Tox consultant's review of carcinogenicity and investigative studies (NDA 21-446; Terry Peters, HFD-520; 2/9/04)

II. REPRODUCTIVE TOXICITY

A. ORAL FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY IN MALE RATS WITH CI-1008 (RR 745-02359, dated 11/95; conducted by Parke-Davis; GLP; Vol. 066)

1. Methods

Male rats (25/grp) received 0 (0.5% methylcellulose vehicle), 250, 1250, or 2500 mg/kg by oral gavage for 11 weeks prior to mating, throughout mating, and for up to 6 weeks after mating. After 11 weeks of treatment, each male was cohabitated with an untreated female until evidence of mating (sperm positive vaginal smear) had occurred or 19 days had elapsed. Animals were monitored for clinical signs, body weight, and food consumption. All females bred to treated males underwent cesarean section on Gestation Days 13, 14, or 15, or 13 days after the end of the mating period, and maternal term sacrifice parameters were evaluated. Blood was collected from 5 males/group during treatment Week 15 for plasma level determination. Ten males/group were sacrificed during Week 15 for evaluation of male reproductive indices, which included (appropriate) histological evaluation of testis and epididymis. Because of observed reproductive effects, the remaining males were assigned to a recovery phase of the study. Recovery group males were cohabitated for 5 days with 2 untreated females each week for 9 consecutive weeks for evaluation of reproductive outcome and then sacrificed during Recovery Week 10 for evaluation of reproductive indices.

Strain: Wistar [(WI)BR-VAF/Plus]
Drug lot: XH330993

2. Results

a. Mortality and Clinical Observations

There were no deaths considered treatment-related; however, 2 MD males died (64903 died during trmt week 1 and was found to have a liver mass; 64921 died during trmt week 13 and had lung congestion and abnormal color of the heart but no heart histopathology) and 1 C animal was sacrificed moribund (trmt week 15). T-R clinical signs consisted primarily of hypoactivity at the MD and HD.

b. Body Weight and Food Consumption

Statistically significant reductions in BW gain occurred during the premating treatment period in MD (28% compared to C, over 11 week period) and HD (51%) males. There was no effect during the postmating trmt period. Food consumption was decreased by 6 and 17% compared to C in these groups during the premating period. Mean BWs were 5, 16, and 30% below C at the end of the 11 week premating trmt period and 6, 15, and 26% below C at the end of the total trmt period (week 17) in LD, MD, and HD groups, respectively.

c. Plasma drug levels

Mean 4-hour plasma concentrations of CI-1008 during Treatment Week 15 were 128, 465, and 669 ug/mL at 250, 1250, and 2500 mg/kg, respectively.

d. Male Reproductive Indices

Decreases in epididymal weights (15 and 24% below C), epididymal sperm counts (28 and 36%), vas deferens sperm motility (43 and 94%), and the percent sperm with normal morphology (8 and 59%) were seen in MD and HD males after 15 weeks of treatment (**Table A.1**). There was also a statistically significant decrease (6%) in sperm motility at the LD. The overall incidence of detached sperm heads was increased at the MD and HD, and incidences

of abnormal sperm tails were increased at all doses. None of these effects on reproductive parameters would be expected due to the decreased body weights alone. There were no drug-related gross pathological findings (no effect on testes weights). T-R histological findings consisted of cell debris in the epididymal tubule lumina (minimal to mild) in MD and HD males. Testicular degeneration was seen in 1, 2, 2, and 2 animals, and testicular atrophy was seen in 0, 1, 1, and 3 males, in the C, LD, MD, and HD groups, respectively; but neither finding was considered T-R. Although the copulation index was comparable between control and treated groups, the fertility index was decreased at the MD and HD (**Table A.2**). No female mated to a HD male became pregnant. The group mean number of days to mating was increased 1 and 2.7 days at the MD and HD, respectively, compared to C.

e. Maternal term sacrifice parameters

Litters of females mated to males treated with 1250 mg/kg had statistically significantly reduced numbers of implant sites and live fetuses and increased percent preimplantation loss (**Table A.3**).

f. Recovery

During the 10-week recovery period, BW gain was increased at the MD (61%) and HD (188%), but mean BW was still significantly lower in all groups at the end of that period . Differences in sperm parameters at the recovery sacrifice were not statistically significant and not considered biologically significant. No drug-related gross pathological or histological findings were observed in recovery males. The recovery copulation index was comparable for all doses for all weeks. The fertility (pregnancy) index was lower for recovery week 1 at the HD, and the total number of pregnant females was reduced at the MD and HD mg/kg for week 1 and at the HD for week 2. Mean time to first pregnancy was increased by more than a week at the HD, and was slightly increased at the MD (1.0, 1.0, 1.2, and 2.1 weeks in C, LD, MD, and HD, respectively). At the HD only 1 female out of 25 with evidence of a positive mating was gravid and had a single implant site (live fetus) during week 1. Statistically significant reductions in the number of implant sites and increases in percent preimplantation loss were seen at the MD during weeks 1 and 2 and during weeks 1 through 4 at the HD. By recovery week 5,all reproductive parameters were comparable to C.

3. Conclusions

At doses ≥ 1250 mg/kg, administration of pregabalin to male rats caused marked reproductive toxicity as indicated by reduced fertility, increased number of days to mating, decreased sperm counts and motility, increased abnormalities in sperm morphology, decreased implantations, and increased preimplantation loss as well as general toxicity in the form of clinical signs and decreased BW gain. There was no no-effect dose for reproductive toxicity, as decreased sperm motility was also seen at the LD of 250 mg/kg. The adverse effects on male reproduction and embryonic development appeared to be reversible.

Table A.1

F_0 Male Reproductive Indices - Treatment Week 15[a]

Treatment	Vehicle		CI-1008					
Dose (mg/kg)	0		250		1250		2500	
Terminal Body Weight (g)	(10)	527 ± 20.8	(10)	526 ± 12.0	(10)	451 ± 15.0*	(10)	401 ± 9.1*
Absolute Organ Weight (g)								
Testes	(10)	3.81 ± 0.114	(9)	3.89 ± 0.136	(10)	3.86 ± 0.125	(10)	3.73 ± 0.103
Epididymides	(10)	1.47 ± 0.046	(9)	1.46 ± 0.047	(10)	1.23 ± 0.036**	(10)	1.12 ± 0.037**
Accessory Organs	(10)	3.20 ± 0.241	(9)	3.30 ± 0.142	(10)	3.17 ± 0.189	(10)	2.99 ± 0.278
Relative Organ Weight (g/100 g body weight)								
Testes	(10)	0.73 ± 0.028	(9)	0.74 ± 0.017	(10)	0.87 ± 0.044**	(10)	0.95 ± 0.022**
Epididymides	(10)	0.28 ± 0.009	(9)	0.28 ± 0.006	(10)	0.28 ± 0.009	(10)	0.28 ± 0.006
Accessory Organs	(10)	0.60 ± 0.039	(9)	0.62 ± 0.022	(10)	0.70 ± 0.039	(10)	0.74 ± 0.057
Sperm (x 10^6)								
Per g Cauda Epididymis	(10)	799 ± 35.5	(9)	800 ± 47.7	(10)	579 ± 43.0**	(10)	509 ± 49.8**
Per Cauda Epididymis	(10)	212 ± 7.7	(9)	192 ± 10.5	(10)	111 ± 13.3**	(10)	92 ± 11.5**
Per g Left Testis	(10)	153 ± 4.9	(9)	163 ± 8.3	(10)	152 ± 4.7	(10)	164 ± 5.0
Per Left Testis	(10)	365 ± 10.2	(9)	282 ± 18.1	(10)	264 ± 9.3	(10)	273 ± 10.9
Sperm Motility (%)	(10)	90.7 ± 1.36	(9)	85.7 ± 2.40*	(10)	52.0 ± 9.14*	(10)	5.00 ± 2.16*
Sperm Morphology[b]								
% Normal[c]	(10)	86.6 ± 3.05	(9)	84.9 ± 5.21	(10)	79.5 ± 2.63*	(10)	56.2 ± 3.99*
% Detached Normal Head	(10)	6.19 ± 1.136	(9)	5.61 ± 0.832	(10)	10.84 ± 1.926	(10)	41.29 ± 4.036
% Detached Abnormal Head	(10)	1.00 ± 0.540	(9)	0.11 ± 0.074	(10)	0.50 ± 0.197	(10)	3.45 ± 0.887
% Abnormal Head, Normal Tail	(10)	2.54 ± 0.825	(9)	1.94 ± 0.733	(10)	2.75 ± 0.712	(10)	2.80 ± 0.700
% Abnormal Head, Abnormal Tail	(10)	0.10 ± 0.066	(9)	0.56 ± 0.328	(10)	0.20 ± 0.111	(10)	0.85 ± 0.553
% Normal Head, Abnormal Tail	(10)	3.99 ± 1.112	(9)	6.83 ± 4.365	(10)	6.25 ± 1.928	(10)	15.40 ± 2.535

[a] (N) Mean ± SE
[b] Approximately 200 sperm per animal individually classified into 1 of 6 categories. Values are group means of the percent of sperm within each category.
[c] Statistical comparisons for sperm morphology conducted only for % normal
* $p < 0.05$, different from vehicle control for trend test
** $p < 0.0204$, different from vehicle control for trend test
*** $p < 0.0250$, different from vehicle control for trend test

Table A.2

Reproductive Performance - Treatment Phase

Treatment	Vehicle	CI-1008		
Male Daily Dose (mg/kg)	0	250	1250	2500
No. of Pairs Cohabitated	25	25	23	25
Copulation Index (%)[a,d]	100.0	96. 0	100.0	100.0
Fertility Index (%)[b,d]	84.0	95.8	65.2	0.0*
No. of Days to Mating[c,d]	2.6 ± 0.62	2.3 ± 0.23	3.6 ± 0.81	5.3 ± 1.01*
No. With Undetected Mating	0	0	1	0
No. Unmated	0	1	0	0
No. Gravid	21	23	15	0
No. Nongravid	4	2*	8	25
No. Total Resorption	0	0	0	0
No. With Viable Litters	21	23	15	0

[a] (Number females with positive mating divided by number cohabitated) x 100
[b] (Number pregnant females divided by number with positive mating) x 100
[c] Mean ± SE.
[d] Statistical comparison of treated groups to vehicle control.
[e] Includes 1 sperm negative not pregnant animal (64983)
* $p < .0289$ (.05/sq rt 3) for trend test, one-tailed

Table A.3

F0 Maternal Term Sacrifice Parameters - Treatment Phase a,c

Treatment		Vehicle		CI-1008							
Dose (mg/kg)		0		250		1250		2500			
Corpora lutea	b	(21)	17.7 + 0.45	(23)	16.6 + 0.41*	(15)	14.4 + 1.14*	(.)	. + .		
Implant sites	b	(21)	16.0 + 0.63	(23)	15.4 + 0.53	(15)	9.4 + 1.60*	(.)	. + .		
Live fetuses	b	(21)	14.8 + 0.64	(23)	14.7 + 0.61	(15)	9.0 + 1.49*	(.)	. + .		
Dead fetuses	b	(21)	0.0 + 0.00	(23)	0.0 + 0.00	(15)	0.0 + 0.00	(.)	. + .		
Resorptions	b	(21)	1.2 + 0.38	(23)	0.7 + 0.21	(15)	0.4 + 0.16	(.)	. + .		
Preimplantation loss (%)	b	(21)	9.54 + 2.888	(23)	7.20 + 1.974	(15)	35.66 + 8.950*	(.)	. + .		
Postimplantation loss (%)	b	(21)	7.52 + 2.084	(23)	4.96 + 1.599	(15)	2.58 + 1.029	(.)	. + .		

a (N) mean + standard error
b Statistical comparison of treatment group to vehicle control
*: $p < 0.0189$, different from vehicle control for trend test
c Although females were untreated, they are referred to by the dose groups of the males with which they cohabitated

B. ORAL FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY IN MALE RATS WITH LOWER DOSES OF CI-1008 (RR 745-02829, dated 6/10/98; conducted by Parke-Davis; GLP; Vol. 068)

Summary

Because a no-effect dose was not established in the initial definitive fertility and early embryonic development study in male rats (RR 745-02359), a follow-up study was conducted using lower doses. In the original study, male reproductive toxicity in the form decreased sperm motility was seen at the low dose of 250 mg/kg. In the current study, male Wistar rats (25/grp + 5/grp for TK) were treated with 0 (0.5% methylcellulose), 50, 100, or 250 mg/kg by gavage according to the same protocol used in the original study (11 weeks premating, throughout mating, and until necropsy after 15 weeks). There were no treatment-related deaths. Clinical signs consisted of red fur staining at all doses and increased red nasal discharge at the HD. For the overall premating treatment period, body weight gain was decreased 9% at HD compared to C. There were no treatment-related effects on fertility and copulation indices, number of days to mating, or male reproductive parameters (the decreased sperm motility effect observed in the previous study at 250 mg/kg was not reproduced). There were no treatment-related gross or microscopic pathological findings. There were also no effects on maternal reproductive parameters, including numbers of corpora lutea and implants, embryonic survival, and pre- and postimplantation loss, in untreated females mated with treated males. Cmax values were 44.5, 71.5, and 185 ug/mL and AUC(0-24) values were 232, 408, and 1280 ug·hr/mL at LD, MD, and HD, respectively.

C. ORAL FERTILITY AND EARLY EMBRYONIC DEVELOPMENT STUDY IN FEMALE RATS WITH CI-1008 (RR 745-02261, dated 10/27/94; conducted by Parke-Davis; GLP, Vol. 069)

1. Methods

Female rats (25/grp + 5/grp TK) received daily doses of 0 (0 .5 % methylcellulose, vehicle), 500, 1250, or 2500 mg/kg CI-1008 by gavage for 15 days prior to mating with untreated males, and throughout mating until Gestation Day 7. Fo females were monitored for clinical signs, body weight, and food consumption throughout the study. Blood was collected from 5/grp approximately 4 hours posttreatment on premating Day 13 for plasma drug level determinations. Evaluation of Fo reproductive potential included monitoring of estrous cycles (prior to and throughout mating). Fo females underwent cesarean section on Gestation Days 13 to 15 and maternal term sacrifice parameters were evaluated.

Strain: Wistar [(WI)BR VAF/Plus]
Drug lot: XH330993

2. Results

a. Mortality and Clinical Observations

There were 2 C deaths attributed to gavage error and 1 HD death during the mating period for which a cause was not determined. Treatment-related clinical observations consisted of transient hypoactivity on the first day of treatment at the HD; increased urine staining at all doses; tail and skin sores at the MD and HD; and rough pelage, alopecia, dacryorrhea, and chromodacryorrhea at the HD.

b. Body Weight and Food Consumption

There were no clearly treatment-related changes in BW gain during the treatment period; however, BW gain was significantly decreased during the posttreatment period of gestation

(GDs 8-13) at the MD and HD (68 and 57%). Food consumption was comparable among groups during the treatment period, but was decreased in all treatment groups during the posttreatment period of gestation (16, 26, and 18%, respectively).

c. Plasma drug levels

Mean 4-hr plasma concentrations were 269, 559, and 769 ug/mL at the LD, MD, and HD, respectively.

d. Female Reproductive Parameters

A treatment-related disruption of estrus cyclicity was observed: the number of animals with 4 or more consecutive days of diestrus and 3 or more consecutive days of estrus was increased at the MD and HD, the number of animals with 2 or more consecutive days of proestrus was increased at all doses, and the mean number of estrous cycles completed during the 15-day premating period was dose-dependently decreased at all doses (**Table C.1**). The fertility index was decreased slightly at the MD and HD, and the number of days to mating was increased at all doses compared to C (**Table C.2**). Pre- and postimplantation loss were increased at all doses (**Table C.3**). (It should be noted that numbers of corpora lutea were higher in treatment groups compared to C.)

3. Conclusion

Administration to female rats resulted in reproductive toxicity in the form of disrupted estrous cyclicity during the premating treatment period and an increase in the number of days to mating at all doses tested (≥500 mg/kg). An possible effect on fertility index was also seen at doses ≥1250 mg/kg, and embryolethality was indicated by increased pre- and postimplantation loss at all doses.

Table C.1

Estrous Cycle

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Pretreatment Period				
No. of Females[a]	30	30	30	30
No. of Females with 4 or More Consecutive Days of Diestrus	0	0	1	0
No. of Females with 3 or More Consecutive Days of Estrus	0	0	0	0
No. of Females with 2 or More Consecutive Days of Metestrus	0	0	0	0
No. of Females with 2 or more Consecutive Days of Proestrus	0	0	0	0
No. of Estrous Cycles Completed[b,c]	2.87 ± 0.070[g]	2.96 ± 0.040	2.76 ± 0.087	2.80 ± 0.082
Treatment Period (Premating Days 0-15)				
No. of Females[d]	30	30	30	30
No. of Females to Complete Premating Period	23	25	25	25
No. Gravid	22	25	22	21
No. Nongravid	1	0	3	3
No. of Females with 4 or More Consecutive Days of Diestrus[e]	4	4	8	16
No. of Females with 3 or More Consecutive Days of Estrus	0	0	2	4
No. of Females with 2 or More Consecutive Days of Metestrus	0	1	0	0
No. of Females with 2 or More Consecutive Days of Proestrus	0	2	1	7
No. of Estrous Cycles Completed[b,f]	2.87 ± 0.095[g]	2.64 ± 0.122	2.40 ± 0.183	2.04 ± 0.196

a Includes animals from TDM subgroup.
b Estrous cycle defined as the period from 1 stage of estrus to the next, with at least 1 day of nonestrus intervening.
c Includes only the number of estrous cycles completed for the pretreatment period for animals that completed the premating period; N = 25/group.
d Includes animals from TDM subgroup until time of sacrifice and animals that died or were sacrificed up until group.
e Females with estrus theoretically occurring during pretreatment period not included.
f Includes only animals that completed pretreatment period.
g Mean ± SE; statistical analysis not done.

Table C.2

F_0 Generation Reproductive Performance

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
No. of Females to Complete Cohabitation Period	23	25	25	24
Copulation Index (%)[a]	100	100	100	100
Fertility Index (%)[b]	95.7	100	88.0	87.5
No. of Days to Mating[c]	1.9 ± 0.19	3.6 ± 0.79	3.0 ± 0.56	3.0 ± 0.62
No. with Undetected Mating	1	0	0	2
No. with Viable Litters	22	25	21	20

[a] (No. females with positive mating divided by number cohabitated) x 100.
[b] (No. pregnant females divided by number with positive mating) x 100.
[c] Mean ± SE.

Table C.3

F_0 Maternal Term Sacrifice Parameters[a]

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Corpora lutea	(22) 16.6 ± 0.40	(25) 18.2 ± 0.58	(21) 19.2 ± 0.57	(20) 18.8 ± 0.57
Implant sites	(22) 16.4 ± 0.40	(25) 16.9 ± 0.86	(22) 16.6 ± 0.99	(21) 17.0 ± 0.84
Live fetuses	(22) 15.7 ± 0.43	(25) 15.4 ± 0.84	(22) 15.4 ± 0.94	(21) 14.1 ± 1.22
Dead fetuses	(22) 0.0 ± 0.00	(25) 0.0 ± 0.00	(22) 0.0 ± 0.00	(21) 0.0 ± 0.00
Resorptions	(22) 0.7 ± 0.15	(25) 1.4 ± 0.30	(22) 1.2 ± 0.21	(21) 2.8 ± 0.76[c]
Preimplantation loss (%)[b]	(22) 1.4 ± 0.67	(25) 8.2 ± 3.77	(21) 9.6 ± 2.45[c]	(20) 10.2 ± 3.87[c]
Postimplantation loss (%)[c]	(22) 4.3 ± 0.98	(25) 8.3 ± 1.76	(22) 11.2 ± 4.97	(21) 19.8 ± 5.63[c]

[a] (N) mean ± standard error
[b] [(Number of corpora lutea - implant sites) / corpora lutea] x 100
[c] [(Number of implant sites - viable fetuses) / implant sites] x 100
[c] $p < 0.0189$, different from vehicle control for trend test

D. ORAL EMBRYO-FETAL DEVELOPMENT STUDY IN MICE WITH CI-1008 (RR 745-02273, dated 11/1/94, conducted by Parke-Davis, GLP, Vol 070)

1. Methods

Pregnant mice (CD-1; 25/grp) were given doses of 0 (vehicle: 0.5 % methylcellulose), 500, 1250, or 2500 mg/kg by gavage on Gestation Days 6 through 15. Additional animals were dosed for plasma level determinations on GD 11. Maternal clinical observations, body weight, and food consumption were monitored throughout the study. Cesarean sections were performed on day 18 of gestation and the following parameters were evaluated: uterine weights; numbers of corpora lutea, implantations, resorptions, live and dead fetuses; fetal weights; and fetal external, visceral (all fetuses by fresh dissection), and skeletal (2/3 examined for skeletal malformations and variations including ossification; head from 1/3 sliced examined, and discarded) structural abnormalities.

Strain: CD-1 (C .CD-1(ICR)BR VAF/PLUS)
Drug lot: XH330993

2. Results

a. No treatment-related clinical signs or deaths occurred during the study. Maternal body weight gain and food consumption were similar among groups during the overall treatment period. On GD 11, mean plasma Cmax values were 291, 640, and 1310 ug/mL and AUCs were 706, 1680, and 3790 ug.h/mL at the LD, MD, and HD, respectively. At term sacrifice, maternal and litter parameters were comparable among groups.

b. Incidences of fetal abnormalities were comparable between treated and control groups, although fetal malformation percentages were slightly higher in the HD group (**Table D.1**). The specific findings were: in the C group, 1 fetus (32976-7) with cleft face, 1 (32979-10) with cleft palate, 1 (32967-1) with reduced number of presacral vertebrae, 1 (32981-5) with malformed vertebrae, and 2 (32967-4 and 32987-3) with fused sternebrae; in the MD group, 2 fetuses (33049-10 and 33088-2) with fused sternebrae; and in the HD group, 1 fetus (33129-5) with exencephaly and diaphragmatic hernia, 3 littermates (33119-4, -10, -11) with ablepharia, 1 (33119-4) with malformed vertebrae, and 3 fetuses from 2 litters (33120-7, -11 and 33122-8) with fused sternebrae. Ossification retardations appeared to be increased somewhat in treated groups, but there was not a dose relationship.

3. Conclusion

When given to pregnant mice throughout organogenesis at doses of up to 2500 mg/kg, pregabalin did not induce clear maternal or developmental toxicity

Table D.1

Incidence of Fetal Malformations and Variations

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Number of Term Fetuses/Litters With Malformations[a]	6/6	0/0	2/2	8/5
Percent Fetuses per Litter With:[b]				
External/Visceral Malformations	1.1 ± 0.75	0.0 ± 0.00	0.0 ± .0.00	1.7± 1.34
Skeletal Malformations	2.2 ± 1.01	0.0 ± 0.00	1.3 ± 0.86	2.6 ± 1.23
External/Visceral Variations	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Anatomic Skeletal Variations	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0± 0.00
Ossification Retardations	1.1 ± 1.14	2.3 ± 2.27	2.2 ± 1.20	2.0 ± 1.41
Percent Litters With:[c]				
External/Visceral Malformations	9.1	0.0	0.0	8.7
Skeletal Malformations	18.2	0.0	10.0	17.4
External/Visceral Variations	0.0	0.0	0.0	0.0
Anatomic Skeletal Variations	100.0	100.0	100.0	100.0
Ossification Retardations	4.6	4.6	15.0	8.7

[a] Includes external, visceral, and skeletal malformations from all five fetuses.
[b] Mean ± SE; statistical comparisons for treated versus vehicle control.
[c] Group mean; statistical comparisons for treated versus vehicle control.

E. ORAL TERATOLOGY STUDY OF CI-1008 IN RATS (RR 745-02271, dated 12/16/94, conducted by Parke-Davis, GLP, Vol 071)

1. Methods

Assumed pregnant female rats (25/grp) were given oral (gavage) doses of 0 (0.5 methylcellulose), 500, 1250, or 2500 mg/kg on Gestation Days 6 through 17. Dams were monitored for clinical signs, body weight, and food consumption. On GD 15, blood was obtained from 5/grp for plasma level determinations. Cesarean sections were performed on day 21 of gestation. The following parameters were evaluated: numbers of corpora lutea, implantations, resorptions, live and dead fetuses; fetal and placental weights; and external (all fetuses), visceral (all by fresh dissection; head from 1/3 fixed and sliced), and skeletal (2/3) fetal structural abnormalities.

Strain: Wistar [C 'WI)BR VAF/Plus])
Drug lot #: XH330993

2. Results

a. Effects on the dam

There were no T-R deaths. T-R clinical signs noted at all doses included hypoactivity, tail sores, and urine staining. In addition, chromodacryorrhea and alopecia were seen at the MD and HD. Food consumption and BW gain were significantly reduced (14 and 26% compared to C) throughout treatment in HD dams. On GD15, Cmax values were [C] ug/ml and AUCs were [C 1 ug.h/ml at the LD, MD, and HD, respectively. At term sacrifice, no T-R effects were observed on maternal parameters, including corpora lutea, litter size, and pre- and postimplantation loss.

b. Fetal evaluations

i. Fetal body weights were decreased (SS) in HD litters; mean weights were 5.3, 5.4, 5.3, and 4.9 gm in males and 5.1, 5.0, 5.1, and 4.6 gm in females in C, LD, MD, and HD groups, respectively.

ii. Fetal and litter incidences of total skeletal malformations were increased (SS) at the HD, and fetal incidences of skeletal variations were increased at all doses (SS at MD and HD; **Table E.1**). Retarded ossification was also increased somewhat at all doses (NS). There was no clear effect on incidences of external/visceral malformations, but fetal and litter percentages of external/visceral variations were increased at the HD.

iii. Specific external/visceral alterations are shown in **Table E.2**. Three C fetuses were malformed: 1 (63585-1) with short face, 1 (63592-14) with micrognathia and micromelia, and 1 (63602-11) with fused liver lobes. One LD (63623-3) and 1 MD (63628-6) fetus had anal atresia and a short, thread-like tail. Another MD fetus (63647-1) had exencephaly. Encephalocele was observed in 2 HD littermates (63654-6, 63654-10), and omphalocele was observed in another HD fetus (63671-20). Due to the low incidence of malformations, none were considered related to treatment; however, the occurrence of related cranial defects only in MD and HD fetuses should be noted.

iv. Specific skeletal malformations elevated in treated groups relative to C were (premature) fusion of the jugal bone and maxilla (MD and HD) and fusion of the nasal bones (HD; **Table E.3**). Variations increased in incidence in treated groups were (increased) ossification of the middle phalanges and calcaneus, extra well-formed lumbar ribs, and rudimentary cervical and thoracic ribs. Unossified ventral tubercle of the atlas was also found in increased incidences at all doses, and retarded ossification in cervical centra was seen at the HD. Fetal and litter percentages of skeletal abnormalities are shown in **Table E.4**.

3. Conclusions

When given orally to pregnant rats throughout organogenesis at doses of 500, 1250, or 2500 mg/kg, pregabalin produced developmental toxicity at all doses and was teratogenic at the MD and HD. Significant maternal toxicity was seen primarily at the HD.

Table E.1

Incidence of F_1 Fetal Malformations and Variations

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
No. of Fetuses/Litters With Malformations[a]	11/8	2/2	12/6	29/11
Percent Fetuses With:[b]				
Ext/Visc Malformations	5.6 ± 4.99	0.3 ± 0.31	0.6 ± 0.44	1.2 ± 0.90
Skeletal Malformations	10.7 ± 5.31	1.9 ± 1.85	5.7 ± 2.54	14.7 ± 4.37*
Ext/Visc Variations	0.4 ± 0.36	0.4 ± 0.37	0.3 ± 0.29	1.4 ± 0.77
Anatomic Skeletal Variations	34.0 ± 8.11	47.3 ± 7.5	55.9 ± 6.48*	54.9 ± 8.01*
Ossification Retardations	21.6 ± 5.58	25.6 ± 4.70	30.2 ± 5.38	30.0 ± 5.83
Percent Litters With:[c]				
Ext/Visc Malformations	15.0	5.6	10.5	11.1
Skeletal Malformations	42.1**	5.6	33.3	61.1*
Ext/Visc Variations	5.0	5.6	5.3	16.7
Anatomic Skeletal Variations	84.2	100	100	100
Ossification Retardations	57.9	77.8	88.9	83.3

[a] Includes external (ext), visceral (visc), and skeletal malformations
[b] Mean ± SE; statistical comparisons for treated versus vehicle control.
[c] Group means; statistical comparisons for treated versus vehicle control.
* $p < 0.025$ for trend test, one-tailed.
** $p < 0.005$ for nonlinearity test.

Table E.2

External and Visceral Findings in F_1 Fetuses

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Fetuses Examined	274	244	270	261
Litters Examined	20	18	19	18
Malformed Fetuses/Litters	3/3	1/1	2/2	3/2
	Number of Fetuses/Number of Litters Affected[a]			
Malformations				
Anal Atresia	–	1/1[b]	1/1[b]	–
Face - Shorter than Normal	1/1	–	–	–
Head - Encephalocele	–	–	–	2/1
- Exencephaly	–	–	1/1	–
Jaw (Lower) - Micrognathia	1/1[c]	–	–	–
Limbs (Fore and Hind) - Micromelia	1/1[c]	–	–	–
Liver - Fused Lobes	1/1	–	–	–
Omphalocele	–	–	–	1/1
Tail - Thread-Like	–	1/1[b]	1/1[b]	–
Variations				
Head - Hematoma	–	–	1/1	–
Kidney - Dilated Pelvis	–	1/1	–	1/1
- Reduced Papilla and Dilated Pelvis	1/1	–	–	–
Liver - Discoloration	–	–	–	2/2
Ureter - Dilated	1/1	–	–	1/1

[a] Some fetuses included in more than 1 category.
[b] Fetuses 63623-3 (500 mg/kg) and 63628-6 (1250 mg/kg); skeletal evaluation revealed malformation and agenesis of sacral vertebrae in Fetus 63628-6; Fetus 63623-3 was not evaluated for skeletal abnormalities.
[c] Fetus 63592-14; skeletal evaluation revealed malformation of multiple skull bones, as well as bent humerus, radius, ulna, scapula, clavicle, femur, tibia, fibula, and ilium bones.
– Finding not observed.

Table E.3

Skeletal Findings in F_1 Fetuses

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Fetuses Examined	180	168	174	181
Litters Examined	19	18	18	18
Malformed Fetuses/Litters	11/8	1/1	12/6	26/11
	Number of Fetuses/Number of Litters Affected[a]			
Malformations				
Limbs (Fore and Hind, All Bones) - Bent	1/1[b]	–	–	–
Pectoral Girdle (Scapula, Clavicle) - Bent	1/1[b]	–	–	–
Pelvic Girdle (Ilium) - Bent	1/1[b]	–	–	–
Ribs - Fused	1/1[c]	–	–	–
Skull - Agenesis	–	–	1/1	–
- Jugal Bone Fused to Maxilla	3/2	1/1	10/4	18/10
- Malformed Bone(s)	2/2[b]	–	1/1	–
- Nasal Bones Fused	–	–	–	10/4
Sternum - Fused Sternebrae/Costal Cartilage	1/1[c]	–	–	–
- Malformed (Sternoschisis)	1/1	–	–	–
Vertebral Column - Agenesis of Sacral Vertebrae	–	–	1/1	–
- Fusion of Thoracic Arches	1/1[c]	–	–	–
- Malformed	2/2[c]	–	1/1	–
- One Less Presacral vertebra	3/2	–	–	–
Anatomical Variations				
Digits - Middle Phalanges Ossified	–	1/1	14/3	26/6
Limbs - Calcaneus Ossified	28/5	27/8	52/9	42/6
Ribs - Wavy	4/3[b]	8/3	13/6	5/3
- Extra Well-Formed Lumbar	–	3/3	4/2	11/7
- Rudimentary Cervical Rib	–	7/2	6/4	4/4
- Rudimentary Thoracic Rib	21/10	37/13	41/16	62/16
Sternum - Asymmetric Form	2/2	5/5	2/2	7/5
Vertebral Column - Extra Presacral Vertebrae	–	1/1	–	–
Ossification Retardations				
Ribs - Hypoplastic	1/1	–	–	–
Skull - Unossified Hyoid Bone	3/2[b]	–	–	–
- Hypoplastic	12/8[b]	3/2	6/3	1/1
Vertebral Column - Bifid Thoracic Centra	1/1[c]	–	1/1	1/1
- Concave/Convex Thoracic Centra	4/3[c]	1/1	2/2	1/1
- Figure-8-Shaped Thoracic Centra	–	1/1	–	1/1
- Hypoplastic Thoracic, Lumbar Centra	1/1[c]	–	–	–
- Unossified Ventral Tubercle of the Atlas	32/9[b,c]	42/14	47/15	55/15
- Unossified Thoracic Centrum	1/1[c]	–	–	–
- Thoracic Vertebrae Misaligned	1/1[c]	–	–	–
- Sacral Centrum Bifid	–	–	1/1	–

[a] Some fetuses included in more than 1 category.
[b] Includes Fetus 63592-14; external observations of micrognathia and micromelia
[c] Includes Fetus 63602-11
– Finding not observed.

Table E.4

Percentage of Fetuses and Litters with Treatment-Related Skeletal Malformations

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Jugal bone fused to maxilla				
% Fetuses	1.7	0.6	5.7	9.9
% Litters	10.5	5.6	22.2	50.0
Nasal bones fused				
% Fetuses	0	0	0	5.5
% Litters	0	0	0	22.2

Percentage of Fetuses and Litters with Treatment-Related Skeletal Variations

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	500	1250	2500
Middle Phalanges Ossified				
% Fetuses	0	0.6	8.0	14.4
% Litters	0	5.6	16.7	33.3
Calcaneus Ossified				
% Fetuses	15.6	16.1	29.9	23.2
% Litters	26.3	44.4	50.0	33.3
Extra Well-Formed Lumbar Ribs				
% Fetuses	0	1.8	2.3	6.1
% Litters	0	16.7	11.1	38.9
Rudimentary Cervical Rib				
% Fetuses	0	4.2	3.4	2.2
% Litters	0	11.1	22.2	22.2
Rudimentary Thoracic Rib				
% Fetuses	11.7	22.0	23.6	34.3
% Litters	52.6	72.2	88.9	88.9
Unossified Ventral Tubercle of the Atlas				
% Fetuses	17.8	25.0	27.0	30.4
% Litters	47.4	77.8	83.3	83.3

F. ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF CI-1008 IN RABBITS (RR 745-02285, dated 1/12/95, conducted by Parke-Davis, GLP, Vol 072)

1. Methods

Presumed pregnant female rabbits (24/grp) were given oral (gavage) doses of 0 (vehicle=0.5% CMC), 250, 500, or 1250 mg/kg on days 6 through 20 of gestation. Maternal mortality, clinical signs, food consumption, and body weight were recorded. On GD 14, blood was obtained from 4/grp for plasma level determinations. Cesarean sections were performed on GD 30 . The following parameters were evaluated: uterine weights; numbers of corpora lutea, implantations, resorptions, live and dead fetuses; fetal weights; and external, visceral, and skeletal fetal structural abnormalities (all fetuses).

Strain: New Zealand White (Hra:(NZW)SPF)
Drug lot #: XH330993

2. Results

a. Maternal effects

One T-R death occurred at the HD (#5996 euthanized moribund with total resorption on GD 21) and 1 HD animal (#5991) was euthanized after aborting on GD 20. Clinical signs occurring in a D-R incidence and severity at all doses included ataxia, hypoactivity, cool to touch, and reduced feces. BW gain was significantly increased (25-27% compared to C) in all treated groups during the overall treatment period. Food consumption was not different among groups. On GD14, Cmax values were [ ] ug/ml and AUCs were [ ] ug.h/ml at the LD, MD, and HD, respectively. Except for the above 2 does with abortion and total resorption, no other T-R effects on maternal reproductive parameters (corpora lutea, implants, live and dead fetuses, resorptions) were observed.

b. Developmental effects

i. Fetal body weights were decreased at all doses (but not dose-dependently), reaching statistical significance in HD males and females (**Table F.1**).

ii. Total incidences of skeletal malformations, external/visceral variations, and ossification retardation were increased (SS) at the HD (**Table F.2**). Incidences of external/visceral malformations were also increased somewhat (NS) at this dose.

iii. Individual fetuses with external/visceral malformations (**Table F.3**) consisted of 1 C fetus (5925-3) with gallbladder agenesis, 1 dead LD fetus (5945-3) with multiple malformations, and 4 HD fetus from 3 litters with the following malformations: 1 (5980-2) with a short tail; 1 (5990-1) with an intravenous thrombus; and 2 littermates (5988-1,5) with agenesis of the gallbladder, 1 of which (5988-5) also had a diaphragmatic hernia. Visceral variations also increased at the HD included gallbladder and blood vessel alterations.

iv. Individual fetuses with skeletal malformations (**Table F.4**) included 1 LD fetus (5932-7) with fused ribs and 5 HD fetuses from 4 litters with the following malformations: 2 fetuses (5985-2 and 5988-5) with fused sternebrae; 1 fetus (5995-2) with 1 less presacral vertebra than normal; 1 (5988-3) with malformed cervical centra; and 1 (5980-2) with agenesis and fusion of caudal vertebrae (also had short tail externally). The incidence of unossified digits was also increased in HD fetus compared to C. Various fetal ossification parameters were decreased in treated groups, with ossification of the olecranon statistically decreased at all doses (**Table F.5**).

3. Conclusions

When given orally throughout organogenesis to pregnant rabbits at daily doses of 250, 500, or 1250 mg/kg, pregabalin produced developmental toxicity including teratogenicity at the HD. Although maternal toxicity at the HD was otherwise limited to clinical signs of pharmacological activity, 2 does lost their litters at this dose, indicating that dose selection was appropriate.

Table F.1

Fetal Term Sacrifice Parameters[a]

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	250	500	1250
Survival at Term (%)	(15) 100 ± 0.00	(16) 98.8 ± 1.25	(16) 100 ± 0.00	(15) 99.2 ± 0.83
Sex Ratio (%)				
Males (live)	(15) 45.8 ± 7.72	(16) 45.1 ± 5.88	(16) 48.4 ± 6.73	(15) 46.1 ± 5.37
Females (live)	(15) 54.2 ± 7.72	(16) 54.9 ± 5.88	(16) 51.6 ± 6.73	(15) 53.9 ± 5.37
Placenta Weight (g)	(15) 3.854 ± .3434	(16) 3.558 ± .1566	(16) 3.953 ± .2202	(15) 3.275 ± .1015
Body Weight (g)				
Males (live)	(13) 56.44 ± 1.726	(14) 50.74 ± 1.457	(15) 53.31 ± 1.651	(15) 49.10 ± 1.223*
Females (live)	(14) 54.28 ± 1.387	(15) 49.81 ± 1.262	(15) 50.88 ± 1.756	(15) 44.84 ± 1.232*

a (N) mean ± standard error
* $p < 0.0204$, different from vehicle control for trend test

Table F.2

Incidence of Fetal Malformations and Variations

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	250	500	1250
Number of Term Fetuses/Litters With Malformations[a]	1/1	2/2[b]	0/0	7/5
Percent Fetuses per Litter With[c]				
External/Visceral Malformations	1.7 ± 1.67	1.3 ± 1.25	0.0 ± 0.00	3.9 ± 2.21
Skeletal Malformations	0.0 ± 0.00	0.9 ± 0.89	0.0 ± 0.00	4.4 ± 2.19*
External/Visceral Variations	2.1 ± 1.41	3.9 ± 3.17	8.5 ± 4.37	15.8 ± 4.37+
Anatomic Skeletal Variations	15.2 ± 5.80	68.4 ± 5.79	78.3 ± 7.81	74.3 ± 5.65
Ossification Retardation	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	3.2 ± 2.15*
Percent Litters With[d]				
External/Visceral Malformations	6.7	6.3	0.0	20.0
Skeletal Malformations	0.0	6.3	0.0	26.7
External/Visceral Variations	13.3	12.5	25.0	60.0+
Anatomic Skeletal Variations	100.0	100.0	93.8	100.0
Ossification Retardation	0.0	0.0	0.0	13.3

* $p < 0.025$ for trend test, one-tailed.
+ $p < 0.0054$ for trend test, one-tailed.
a Includes external, visceral, and skeletal malformations from all fetuses
b Includes 1 dead fetus
c Mean ± standard error; statistical comparisons for treated versus vehicle control
d Group mean; statistical comparisons for treated versus vehicle control

Table F.3

Fetal External and Visceral Findings

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	250	500	1250
No. Fetuses/No. Litters Examined	82/15	97/16[a]	84/16	98/15[a]
No. Fetuses Malformed/No. Litters	1/1	1/1	0/0	4/3
		Number of Fetuses/Number of Litters Affected		
Malformations:				
Eye - Ablepharia, Unilateral	–	1/1[b]	–	–
Head - Domed-shaped with Dilated Ventricles	–	1/1[b]	–	–
Jaw - Agnathia	–	1/1[b]	–	–
Mouth - Atresia	–	1/1[b]	–	–
Palate - High Arch	–	1/1[b]	–	–
Tongue - Aglossia	–	1/1[b]	–	–
Digits - Brachydactyly/Ectrodactyly		1/1[b]	–	–
Tail - Short	–	–	–	1/1[a]
Aorta - Bulbous	–	1/1[b]	–	–
Diaphragm - Hernia	–	–	–	1/1[d]
Gallbladder - Agenesis	1/1	–	–	2/1[d]
Heart - Interventricular Septal Defect	–	1/1[b]	–	–
Vessel - Thrombus	–	–	–	1/1
Variations:				
Gallbladder - Bilobed	1/1	–	–	–
- Dislocation	–	–	–	1/1
- Decreased Size	–	–	1/1	5/3
Lung - Azygous Lobe Absent	1/1	–	4/1	5/3
Vessel - Left Carotid Arising from Innominate	–	4/2	3/2	6/4

[a] Includes dead fetus
[b] Fetus 5945-3 (dead); skull and digit malformations confirmed by skeletal examination
[c] Fetus 5980-2
[d] Fetus 5988-5

Table F.4

Fetal Skeletal Findings

Treatment	Vehicle	CI-1008		
Dose (mg/kg)	0	250	500	1250
No. Fetuses Examined/No. Litters	82/15	96/16	84/16	97/15
No. Fetuses Malformed/No. Litters	0/0	1/1	0/0	5/4
		Number of Fetuses/Number of Litters Affected		
Malformations:				
Ribs - Fused	–	1/1	–	–
Sternebrae - Fused	–	–	–	2/2
Vertebrae - Agenesis	–	–	–	1/1[a]
- Fused	–	–	–	1/1[a]
- Malformed	–	–	–	1/1
- One less Presacral	–	–	–	1/1
Anatomic Variations:				
Limbs - Epiphysis of Femur (Advanced)	11/7	4/1	7/5	4/2
Ribs - Short 12th	–	–	–	1/1
- Extra Well-Formed Lumbar	50/13	47/16	55/14	50/13
- Extra Rudimentary Lumbar	29/12	28/12	20/12	26/12
Skull Bone - Bent	1/1	6/5	2/2	7/6
Sternebrae - Epiphysis (Advanced)	–	–	2/2	–
- Extra Ossification Site	–	2/2	3/2	1/1
- Focal Fusion	–	–	2/2	–
- Asymmetric Form	–	–	2/2	1/1
Vertebrae - Focal Fusion	–	–	–	1/1
- Extra Presacral	17/7	16/10	21/9	28/11
- Misshapen Centra	–	–	–	1/1
Ossification Retardation:				
Digits - Unossified	–	–	–	4/2

[a] Fetus 5980-2 (external correlate, short tail)

Table F.5

Fetal Skeletal Ossification Parameters[a]

Treatment Dose (mg/kg)	Vehicle 0	C1.1008 250	 500	 1250
Sternebrae	(15) 5.9 ± 0.03	(16) 6.0 ± 0.00	(16) 6.0 ± 0.00	(15) 6.0 ± 0.02
Olecranon	(15) 1.3 ± 0.12	(16) 0.8 ± 0.14*	(16) 0.6 ± 0.14*	(15) 0.2 ± 0.07*
Tuberosities of Humerus	(15) 4.0 ± 0.01	(16) 3.8 ± 0.07	(16) 3.8 ± 0.07	(15) 3.3 ± 0.24*
Epiphyses				
Forelimbs	(15) 2.0 ± 0.00	(16) 2.0 ± 0.00	(16) 2.0 ± 0.00	(15) 2.0 ± 0.02*
Hindlimbs	(15) 3.9 ± 0.04	(16) 3.9 ± 0.06	(16) 3.9 ± 0.08	(15) 3.2 ± 0.23*

[a] (N) mean ± standard error of mean ossified sites per litter
* $p < 0.0224$, different from vehicle control for trend test

G. ORAL PRE- AND POSTNATAL DEVELOPMENT STUDY OF CI-1008 IN RATS (RR 745-02628, dated 4/17/98, conducted by Parke-Davis, GLP, Vol. 1.72)

1. Methods

Pregnant rats (25/group) were treated with 0 (vehicle: 0.5% CMC), 250, 1250, or 2500 mg/kg orally (gavage) from gestation day 6 through lactation day 20. The dams were allowed to deliver and rear offspring. Due to excessive offspring mortality at the two highest doses, 2 additional groups of females received 50 or 100 mg/kg according to the same regimen and an additional concurrent C group was added. Satellite TK animals (6/grp) were included at each dose level for determination of plasma and milk concentrations on lactation days 12, 13, or 14. F0 mortality, clinical signs, food consumption, and body weight were recorded. F1 offspring were evaluated for survival, growth, physical and behavioral development, and reproductive performance.

Strain: Wistar C (WI)BR VAF/Plus])
Drug lot #: XH330993

2. Results

a. Effects on the dam

i. Mortality and clinical observations - There were 2 HD moribund sacrifices (dam # 66020 on GD 21 and 66030 during delivery on GD22) which were considered related to treatment. T-R clinical signs during the gestational treatment period included ataxia, bruxism, and tail chewing and/or sores (all doses); hypoactivity (≥100); urine staining, vaginal discharge, and chromodacryorrhea (≥1250); and dry red material around the nose (2500). During parturition, dystocia occurred in 1 female each at 1250 and 2500 (65997 and 66026, 1 dead pup removed from the vaginal canal of each); both completed delivery but had no viable pups. During lactation, T-R clinical signs were hypoacitivity and urinary stain (≥1250) and abnormal maternal care (not nesting or nursing; 3 animals at HD).

ii. Body weight and food consumptions - During the overall gestational treatment period, BW gain was decreased at the HD (31% below C) and food consumption was decreased at 1250 (8%) and 2500 (21%). There was no clear effect on BW gain during the lactational period, but food consumption was reduced at 250 (12%) and 1250 (21%). No data were available at 2500 due to euthanasia of all dams after total litter death.

iii. Parturition and lactation parameters – One C female (with1 live fetus in utero) and 3 HD females (66018 with11 live, 1 dead, 4 resorbed fetuses; 66033 with 9 fetuses, 7 resorptions; 66038 with 6 live, 14 resorbed fetuses) were euthanized on GD 24 because they were unable to deliver (**Table G.1**). An increased number (4) of females classified as nonpregnant at the HD probably reflects early resorption. One C female, 1 250 group female, and 16 females each at 1250 and 2500 were euthanized between postpartum days 0-14 after complete litter death. At the HD, all litters were lost by postpartum day 2. Statistically significant increases in gestation duration (0.5 days at HD), number of stillborn and cannibalized pups at birth (21 and 26X at 1250 and 2500), and postimplantation loss (5 and 8X at 1250 and 2500) and decreases in live pups/litter (32 and 56% at 1250 and 2500) and litter size (21% at HD) were seen in treated groups (**Table G.2**).

iv. Drug concentrations - Cmax values were determined to be C and 1 ug/ml at 50, 100, 250, 1250, and 2500 mg/kg, respectively; AUCs were 241, 601, 1380, 6170, and 8930 ug.h/ml, respectively; and 4-hr milk concentrations were 20.8, 53.1, 187, 438, and 766 ug/ml, respectively.

b. Offspring evaluations

i. Survival - Pup survival at birth and during the postnatal period until weaning were severely reduced at 1250 and 2500 (**Table G.3**). All offspring died in 16/24 litters at 1250 and 16/16 at 2500. Survival at birth and from PND 0-4 were also decreased at 250. Survival was decreased during the postweaning period at 1250. No correlations were apparent between litter death and maternal clinical signs.

ii. Body Weight - Pup weights were decreased in treated litters at birth (5, 13, 23% compared to C at 250, 1250, and 2500, respectively), PND 4 (10 and 33% at 250 and 1250), and PND 21 (9, 10, and 37% at 100, 250, and 1250). BW gain during PND 4 -21 was significantly decreased at ≥100 (10, 10, 39% at 100, 250, and 2500). This BW deficit persisted into the postweaning period, with BW gain for the period PN weeks 3-13 statistically decreased in males at 1250 (14% compared to C), and PNW 13 BWs decreased in females at 250 and in males and females at 1250 (3, 16, and 4%, respectively).

iii. Developmental Landmarks - There were significant delays in the attainment of pre- and postweaning developmental landmarks at 1250 (approximately 1, 2, and 3 days for pinna detachment, testes descent, and preputial separation, respectively; **Table G.4**).

iv. Offspring Behavior - When behavioral evaluations were performed of F1 animals (rotorod on PND 28, acoustic startle on PND 42, motor activity on PND 49, shuttle avoidance during PN Week 9 or 10; same animals used for all behavioral testing), no clear effects were observed on rotorod, activity, or avoidance learning, but a decrease in startle response was seen treated females at 250 and 1250 (**Table G.5**). A tendency was also seen in males at these doses. The deficit was considered due to attenuated reactivity to the acoustic stimulus, rather an effect on hearing or the motor component of the startle response.

v. Offspring Reproductive Performance - When F1 offspring were mated, the fertility index was decreased (**Table G.6**); numbers of corpora lutea, implants, live fetuses, and litter size were decreased (39, 72, 71, and 71%, respectively, compared to C); and preimplantation loss was significantly increased (9X) at 1250 (**Table G.7**). Pre- and postimplantation loss were also increased somewhat at 250 (2X). It could not be determined whether one or both sexes was responsible for the effects on reproductive function.

C. Conclusions

When given orally to female rats throughout pregnancy and lactation at daily doses of 50, 100, 250, 1250, 2500 mg/kg, pregabalin produced developmental toxicity at doses ≥100 mg/kg. This included adverse effects on offspring survival, growth, behavior, and reproductive function. The effect on offspring survival was marked at doses ≥ 1250 mg/kg, with 100% mortality at the HD. Significant maternal toxicity was seen at the HD.

Table G.1

F_0 Female Status at Parturition

Treatment	Vehicle[a]	CI-1008		Vehicle[b]	CI-1008		
Dose (mg/kg)	0	50	100	0	250	1250	2500
No. of Females	25	25	25	25	25	25	25
No. Died/Euthanatized Prior to Scheduled Termination[c]	0	1	0	0	1	0	5
No. Gravid	25[d]	24[e]	24[f]	25	25[g]	24	21[h]
No. Nongravid	0	1	1	0	0	1	4
No. With Total Resorption	0	0	0	0	0	0	0
No. Unable to Deliver[i]	1	0	0	0	0	0	3
No. With Viable Litters at Birth	24	23	23	25	24	24	16
No. Euthanatized Due to Total Litter Death	1	0	0	0	1	16	16

[a] Concurrent with 50 and 100 mg/kg
[b] Concurrent with 250, 1250, and 2500 mg/kg
[c] Excludes dams euthanatized due to total litter death
[d] Includes gravid Animal 69917, euthanatized on Gestation Day 24 due to inability to deliver
[e] Includes gravid Animal 69956, found dead on Gestation Day 10
[f] Includes Animal 69967, identified as gravid by Kopf's stain (no fetuses present)
[g] Includes gravid Animal 65975, found dead on Gestation Day 20
[h] Includes gravid Animals 66018, 66033, and 66038, euthanatized on Gestation Day 24 due to inability to deliver; gravid Animal 66020, euthanatized moribund on Gestation Day 21; and Animal 66030, euthanatized moribund during delivery
[i] Euthanatized on Gestation Day 24

Table G.2

F0 Dam Delivery Maternal Parameters

Treatment / Dose (mg/kg)		Vehicle 0			250			CI-1008 1250			2500		
Duration of gestation	n	(25)	22.2 ±	0.07	(24)	22.2 ±	0.12	(24)	22.4 ±	0.12	(16)	22.9 ±	0.13*
Live pups/litter at birth	n	(25)	13.9 ±	0.50	(24)	13.2 ±	0.78	(24)	9.5 ±	0.66*	(16)	6.1 ±	1.01*
Stillborn + cannibalized at birth	n	(25)	0.2 ±	0.10	(24)	0.7 ±	0.22	(24)	4.2 ±	0.69*	(16)	5.1 ±	0.75*
Litter size	n	(25)	14.1 ±	0.51	(24)	13.8 ±	0.69	(24)	13.6 ±	0.41	(16)	11.2 ±	0.87*
Implant sites	n	(25)	15.1 ±	0.50	(24)	14.8 ±	0.64	(24)	15.8 ±	0.44	(16)	15.9 ±	0.66
Postimplantation loss (%)	n	(25)	7.6 ±	1.56	(24)	11.5 ±	3.34	(24)	39.3 ±	4.29*	(16)	58.7 ±	6.88*

Values expressed as (N) mean ± standard error
n: Statistical comparison of treatment groups to vehicle control
*: p < 0.0204, different from vehicle control for trend test

Table G.3

F_1 Neonatal Survival[a]

Treatment	Vehicle[b]	CI-1008		Vehicle[c]	CI-1008		
Dose (mg/kg)	0	50	100	0	250	1250	2500
At birth	95.6 ± 2.94	99.6 ± 0.40	98.6 ± 0.98	98.7 ± 0.67	93.7 ± 2.04	69.9 ± 5.05*	51.4 ± 7.68*
Neonatal (PN Days 0 through 4)	95.4 ± 4.16	96.0 ± 1.67	97.8 ± 0.73	99.2 ± 0.65	86.1 ± 5.04*	32.4 ± 7.22*	0*
Weaning (PN Days 4 through 21)	100	98.9 ± 0.75	98.9 ± 0.75	97.7 ± 2.29	97.3 ± 1.10	56.7 ± 13.47*	–[d]

[a] Values expressed as mean ± standard error (%)
[b] Concurrent with 50 and 100 mg/kg
[c] Concurrent with 250, 1250, and 2500 mg/kg
[d] No data available, all litters dead
* p <0.0289, different from concurrent vehicle control for trend test

F_1 Maturation % Survival (PN Weeks 3 to 13)[a]

Treatment	Vehicle[b]	CI-1008		Vehicle[c]	CI-1008	
Dose (mg/kg)	0	50	100	0	250	1250
Males	100	100	100	98.0 ± 2.00	97.7 ± 2.27	92.9 ± 7.14
Females	100	97.8 ± 2.17	100	100	100	93.8 ± 6.25*

[a] Values expressed as mean ± standard error; Litter N = 23 for the 50 and 100 mg/kg groups and their concurrent control, and 22 or 23, 7 or 8, and 25 for the 250 and 1250 mg/kg groups and their concurrent control, respectively.
[b] Concurrent with 50 and 100 mg/kg.
[c] Concurrent with 250 and 1250 mg/kg.
* p <0.0500, different from vehicle control for trend test.

Table G.4

F1 Developmental Landmarks (days)

Treatment / Dose (mg/kg)		Vehicle 0			250			CI-1008 1250			2500		
Eye Opening	a	(25)	15.4 ± 0.09	(23)	15.7 ± 0.11	(8)	15.9 ± 0.30	(.)	.	+	.		
Incisor Eruption	a	(25)	11.6 ± 0.16	(23)	11.5 ± 0.21	(10)	11.7 ± 0.27	(.)	.	+	.		
Pinnae Detachment	a	(26)	2.7 ± 0.08	(23)	2.8 ± 0.10	(13)	3.6 ± 0.19*	(.)	.	+	.		
Testes Descent	a	(25)	22.8 ± 0.27	(22)	21.9 ± 0.17	(7)	23.8 ± 0.60*	(.)	.	+	.		
Preputial Separation	a	(25)	45.3 ± 0.25	(22)	44.6 ± 0.24	(7)	46.0 ± 0.55*	(.)	.	+	.		
Vaginal Opening	a	(25)	31.7 ± 0.37	(23)	31.5 ± 0.40	(8)	32.9 ± 0.51	(.)	.	+	.		

Values expressed as (N) mean ± standard error
a: Statistical comparison of treatment groups to vehicle control
*; p < 0.0204, different from vehicle control for trend test

Table G.5

Acoustic Startle Parameters for F_1 Males and Females[a]

Treatment	Vehicle[b]	CI-1008		Vehicle[c]	CI-1008	
Dose (mg/kg)	0	50	100	0	250	1250
Mean MIV, NL Trials 1-3[d,e]						
Males	1762 ± 167	1345 ± 108	1628 ± 124	1417 ± 119	1196 ± 101	1261 ± 346
Females	1746 ± 132	1593 ± 94	1662 ± 156	1756 ± 193	1343 ± 154	911 ± 65*
MIV, PRE Trial 1[f]						
Males	739 ± 122	847 ± 97	797 ± 87	784 ± 111	689 ± 102	667 ± 272
Females	909 ± 83	853 ± 117	875 ± 138	860 ± 151	698 ± 119	455 ± 144
MIV, PRE Trial 2[f]						
Males	542 ± 88	588 ± 91	662 ± 101	438 ± 68	435 ± 89	630 ± 358
Females	725 ± 71	741 ± 90	743 ± 132	704 ± 173	474 ± 77	258 ± 76
MIV, PRE Trial 3[f]						
Males	384 ± 68	541 ± 91	609 ± 98	415 ± 72	374 ± 58	312 ± 160
Females	589 ± 60	578 ± 88	626 ± 100	684 ± 117	527 ± 92	214 ± 94
% Response Inhibition[g,h]						
Males	49.2 ± 13.03	33.2 ± 9.10	50.7 ± 4.46	56.6 ± 4.92	39.3 ± 11.75	56.6 ± 9.47
Females	41.3 ± 11.03	53.7 ± 3.89	46.0 ± 7.00	40.2 ± 11.07	44.3 ± 9.32	51.8 ± 14.77

[a] Values expressed as mean ± standard error; N = 23 or 24 for the 50 and 100 mg/kg groups and their concurrent control, and 20 or 22, 7 or 8, and 25 for the 250 and 1250 mg/kg groups and their concurrent control, respectively.
[b] Concurrent with 50 and 100 mg/kg.
[c] Concurrent with 250 and 1250 mg/kg.
[d] Maximum Input Voltage (MIV), mean of the group means of the first 3 Noise Level (NL) trials.
[e] Statistical comparison of treatment groups to concurrent vehicle control.
[f] Group mean MIV for the specified Prepulse (PRE) trial.
[g] Calculated using the formula 100 - (MIV specified PRE trial × 100/MIV corresponding NL trial); values represent a mean of the group means.
[h] $p < 0.05$, different from vehicle control for trend test.

Table G.6

F_1 Generation Reproductive Performance

Treatment	Vehicle[a]	CI-1008		Vehicle[b]	CI-1008	
Dose (mg/kg)	0	50	100	0	250	1250
No. Pairs Cohabited	23	22	23	25	22	6
Copulation Index (%)[c]	100	100	95.7	96.0	100	100
Fertility Index (%)[d]	91.3	100	95.5	100	100	83.3*
No. Days to Mating[e]	3.7 ± 0.78	2.2 ± 0.26	3.7 ± 1.03	2.7 ± 0.50	2.6 ± 0.56	2.4 ± 0.93
No. Unmated	0	0	1	1	0	0
No. Gravid	21	22	21	24	22	5
No. Nongravid[f]	2	0	2	1	0	1
No. Died	0	0	0	0	0	0
No. Euthanized Prior to Term	0	0	0	0	0	0
No. Dams With Undetected Mating	2	3	2	0	0	1
No. Dams With Total Resorption	0	0	0	0	1	0
No. Dams Delivered Early	0	0	0	0	0	0

[a] Concurrent with 50 and 100 mg/kg
[b] Concurrent with 250 and 1250 mg/kg
[c] (Number females with positive mating divided by number cohabited) × 100
[d] (Number pregnant females divided by number with positive mating) × 100
[e] Mean ± standard error
[f] Includes unmated female
* $p < 0.0289$, different from vehicle control for trend test

Table G.7

F1 Maternal Term Sacrifice Parameters

Treatment / Dose (mg/kg)		Vehicle 0			CI-1008 250			1250		
Corpora lutea	a	(24)	16.8	± 0.41	(21)	16.9	± 0.52	(4)	10.3	± 1.44*
Implant sites	a	(24)	15.6	± 0.51	(22)	14.6	± 0.79	(4)	4.3	± 1.89*
Live fetuses	a	(24)	14.8	± 0.52	(22)	13.8	± 0.89	(4)	4.3	± 1.85*
Dead fetuses	a	(24)	0.8	± 0.08	(22)	0.8	± 0.00	(4)	0.0	± 0.00
Resorptions	a	(24)	0.9	± 0.17	(22)	1.4	± 0.28	(4)	0.0	± 0.00
Litter size	a	(24)	14.8	± 0.52	(22)	13.8	± 0.89	(4)	4.3	± 1.85*
Preimplantation loss (%)	a	(24)	5.69	± 3.026	(21)	12.09	± 3.082	(4)	62.14	± 15.102*
Postimplantation loss (%)	a	(24)	5.63	± 1.066	(22)	12.80	± 4.498	(4)	0.00	± 0.000

Values expressed as (N) mean ± standard error
a: Statistical comparison of treatment groups to vehicle control
*: p < 0.0177, different from vehicle control for trend test

III. SUMMARY AND EVALUATION

CARCINOGENICITY

A total of 4 definitive lifetime carcinogenicity bioassays were performed: 2 in different strains of mice (B6C3F1 and CD-1) and 2 in Wistar rats. These studies are adequate from a regulatory standpoint (the initial studies in B6C3F1 mice and Wistar rats were evaluated by the Exec-CAC; minutes dated 12/12/00). Results are summarized below.

Mouse

Two definitive 2-year carcinogenicity studies were conducted in mice.

B6C3F1

Mice (64-66/sex/group) were given doses of 0, 200, 1000, or 5000 mg/kg in the diet for 2 years. The HD was expected to produce an AUC 25 times the human exposure at the maximum therapeutic dose (122 ug.hr/ml; 600 mg/day). The doses used were those recommended by the Division and CAC. A dose-related decrease in survival was seen at the MD and HD in both sexes. Overall survival percentages at Week 104 were 88, 80, 62, and 34% in males and 69, 68, 44, and 38% in females from the C, LD, MD, and HD groups, respectively. Statistically significant increases in BW were seen in males and females from all treatment groups compared to C (not D-R); at 104 weeks, the differences from C were 15, 21, and 15% in males and 19, 31, and 19% in females at the LD, MD, and HD, respectively. D-R increases in average food consumption were also seen in both sexes at all doses compared to C. An increased incidence of missing tail tips occurred in both sexes at all doses. At termination, platelet count was increased 35% and 33% in MD and HD males and 36, 32, and 58% in LD, MD, and HD females, respectively.

Incidences of hemangiosarcomas were dose-dependently increased in treated males and females, reaching statistical significance (Fisher's exact test) at the MD and HD in both sexes (3.1, 4.7, 29.2, and 34.4% in males and 3.1, 10.6, 29.7, and 38.5% in females from C, LD, MD, and HD groups, respectively; historical control range: 0-12% in males, 0-8% in females). These tumor findings correlated with clinical signs (palpable masses) and macroscopic findings (liver masses and enlarged spleens). Hemangiosarcomas occurred at multiple sites, but were most frequently found in the liver, spleen, and bone marrow. Hemangiosarcomas were considered the cause of death in 1, 3, 13, and 13 males and in 1, 3, 12, and 15 females in the C, LD, MD, and HD groups, respectively.

AUCs determined in a separate TK study using the same doses were 135, 800, and 3840 ug.h/ml in males and 148, 598, and 3740 ug.h/ml in females, respectively. Thus, mouse exposures at the LD, which were associated with increased incidences of hemangiosarcomas, are similar to exposures expected in humans (mean AUC of 122 ug.hr/ml at MRD of 600 mg/day).

CD-1

Mice (65/sex/group) were given doses of 0, 200, 1000, or 5000 mg/kg/d in the diet for 2 years. The doses were the same as those used in the previous mouse study. Survival among the male mice was not statistically different among groups. At study end at week 104, there were about 51, 48, 42, and 43 percent of animals alive in the C, LD, MD, and HD groups, respectively. Increased mortality (SS in FDA statistical review) was seen in HD females and dosing was stopped in this group during week 99. The survival of C, LD, and MD animals was 43, 46, and 42 percent at week 104. The HD animals had 21 survivors or 32 percent at week 99. Body weight gains were increased (29-62%) in treated animals compared to C (but not dose-related), and statistical significance was reached for all groups. Significantly, there were no drug-related effects on platelet counts at Week 65/66 or Week 78/79, but platelet counts were increased in treated males (16, 10 and 32%, respectively; SS at HD) and females (6, 13 and 24%, respectively) at 24 months. Platelet volumes were also increased (SS) in all treatment groups in males and in MD and HD females at study termination. MCH was

increased in HD males and MCV was increased in MD and HD males. The only non-neoplastic finding reported was an increased incidence and severity of alveolar macrophage infiltration with associated changes in treated females (primarily HD, but increased severity in MD).

A highly statistically significant increase in the incidence of hemangiosarcoma was seen in pregabalin-treated male mice. Numbers of tumor-bearing males were 2 (3%), 5 (8%), 6 (9%), and 14 (22%) in C, LD, MD, and HD groups, respectively (historical control range: 0-12% in males; mean: 1.1%). There was a statistically significant difference at the HD compared to C in the pair-wise comparison ($p< 0.005$). Treated females also had higher incidences of hemangiosarcomas than controls: numbers with tumors were 6 (9%), 9 (14%), 10 (15%), and 13 (20%) in the C, LD, MD, and HD groups, respectively (historical control range: 0-12% in females; mean: 0.9%). (In the FDA consultant's review, 2 additional females with hemangiosarcoma were identified, so that the numbers became 7, 9, 10, and 14, respectively.) However, neither the trend ($p= 0.0058$) nor the pair-wise comparison quite reached statistical significance. The lack of statistical significance can be attributed, at least in part, to a higher incidence among female than among male controls (and considerably higher than the historical control mean), as well as to the early termination of HD females, which rendered further manifestations of hemangiosarcomas impossible. The FDA statistician considered it possible that the early termination of the HD females, which did not seem clearly warranted, resulted in a loss of observable hemangiosarcomas which otherwise might have been statistically significant (see Stat review). But this mouse study was considered valid since a sufficient numbers of animals were exposed sufficiently long and the increased mortality among HD females indicated that this dose was close to the MTD.

No TK analysis was included in this study other than 4 hr plasma concentrations at 104 weeks, which were 14.3, 49.7, and 429 µg/mL in males and 11.0, 61.9, and 473 µg/mL in females in the respective dose groups. In a separate 4-week dietary TK study, AUCs were 110, 550, and 3290 ug.h/ml in LD, MD, and HD mice (no sex difference). Thus, AUCs were 15 to 25% lower in CD-1 as compared to B6C3F1 mice.

Rat

Two definitive 2-year carcinogenicity studies were conducted in rats.

Wistar Study #1

Rats (65/sex/group) were given 0, 50, 150, and 450 mg/kg (males) or 0, 100, 300, and 900 mg/kg (females) in the diet for 2 years. The doses used were those recommended by the Division and CAC. They were primarily based on the results of a 13-week rat study, with the HD considered an estimated MTD. Survival was increased in HD males and in females from all treatment groups at the end of the study. At week 104, overall survival was 49, 45, 51, and 65% in males and 54, 74, 82, and 69% in females from the C, LD, MD, and HD groups, respectively. Overall BW gain was increased at the LD (13 and 30% in M and F, respectively), similar at the MD, and significantly decreased at the HD (22 and 41% in M and F, respectively), compared to C. BWs were significantly lower in HD males and females compared to C throughout the study (mean wts 13 and 24% below C at termination, in M and F, respectively). Food consumption followed the same pattern (8.9 and 13% below C in HDM and HDF, respectively, at 104 weeks). At the end of the study, erythrocyte counts were increased (up to 14%) and MCV, MCH, and platelets were decreased (up to 9, 11, and 20%, respectively) in treated males and females.

There was no clear evidence of a T-R effect on the frequency of neoplasms in animals that died or were sacrificed moribund. Two rare tumors showed a positive trend: meningioma of the brain in males and squamous cell carcinoma of the skin in females. These were seen in 2 HD animals (3%) each and were not found in other groups. Historical control incidences of these tumor types in Wistar rats have been reported to range from 0-4%.

AUCs determined in a separate TK study using the same doses were 157, 600, and 1718 ug.h/ml in males and 306, 944, and 2930 ug.h/ml in females, respectively.

Wistar Study #2

Rats (65/sex/group) were given 0 (untreated control), 50, 150, or 450 mg/kg (males) or 0, 100, 300, or 900 mg/kg (females) in the diet for 2 years. Doses were the same as those used in a previous 2-year rat study. Overall survival at Week 104 was 58%, 58%, 71%, and 69% in males and 52%, 55%, 74%, and 51%, in females from C, LD, MD, and HD groups, respectively. There was no statistically significant dose trend in mortality. There were no T-R clinical signs except possibly fur staining. Mean body weights were consistently lower in HD males and females throughout the study. BW in MD females was also somewhat lower during most of the study, but the difference was only 2% at Week 104. BWs were significantly higher at study termination in LD males and females. BW gain was decreased 22% in HD males and 40% in HD females over the course of the study. Food consumption was consistently lower in HD males (1%-19%) and females (2%-24%). At termination, small, but dose-related increases in RBCs (5%-18%) and HGB (2%-7%) were seen in both sexes at all doses. In addition, there was a dose-related decrease in platelet counts in both sexes at all doses (12%-30%). HD males also had an increased mean white cell count (12%). Keratitis with neovascularization and pallor of the fundus of the eye was seen in MD and HD males and females upon ophthalmologic exam. T-R macroscopic findings included opaque/opalescent eyes in MD and HD males and uterine enlargement and distended uterus in MD and HD females. Retinal atrophy and corneal inflammation were observed with high incidences in MD and HD males and females. An increased incidence of angiectasis of the adrenal cortex was observed in males at all doses, and there was an increased incidence of atrophy of the ovary at the HD and increased incidence of uterine dilatation and inflammation at the MD and HD. Atrophy and degeneration of the testicular germinal epithelium were increased at all doses.

Tumor incidences were similar across groups. Two rare tumor types, granular cell tumor of the brain and schwannoma of the heart were identified as having a positive dose trend by the Peto analysis (0, 0, 0, and 2 males; 0,1, 0, and 3 males, respectively), but the exact-trend test using Haseman's rule detected no statistically significant positive dose trend for these tumor types. Plasma concentrations were consistent with the previous study based on single time point determinations.

Mechanism studies

The sponsor conducted a series of investigational studies intended to elucidate the mechanism of pregabalin-induced hemangiosarcomas in mice in an effort to provide a better basis for assessing their human relevance. These consist primarily of collections of correlative data in the two mouse strains in which increased incidences of hemangiosarcoma were found in 2-year studies as well as some corresponding data from rats (which did not exhibit a tumorigenic response to pregabalin in lifetime studies), monkeys, and humans. Endpoints were selected to test the hypothesis, based on an initial observation of increased platelets in mice treated with pregabalin, that increased proliferation following exposure of endothelial cells to elevated levels of growth factors resulting from increased megakaryopoiesis is responsible for hemangiosarcoma formation in pregabalin-exposed mice. The sponsor suggests that mice differ markedly from other species including humans in factors that affect endothelial homeostasis (eg, tissue distribution of hematopoiesis, background platelet counts, platelet turnover and activation, and endothelial turnover) and that these differences may contribute to a higher incidence of spontaneous hemangiosarcoma and a unique sensitivity for developing increased incidences of hemangiosarcoma when given xenobiotics that alter those factors. The sponsor attempted to provide evidence for a progression of events leading to tumors in mice that could be monitored in other species in order to rule out human risk. The major components of the proposed pathway leading to hemangiosarcoma in pregabalin-treated mice are: 1) increased platelet activation and/or alterations in platelet aggregation and morphology consistent with increased activation, 2) increased megakaryopoiesis, 3) increased circulating and tissue concentrations of endothelial cell growth factors, and 4) increased endothelial cell proliferation. So, these would have to be seen in mice but not other species. In addition, as stated by the sponsor, "the temporal relationship between these (platelet and growth factor) changes and increased endothelial cell proliferation must be consistent with a causal association." A summary of data provided in support of this proposed mechanism, organized according to the four components, follows.

1) According to the sponsor, increased platelet activation, as assessed by flow cytometric evaluation of increased P-selectin expression on the platelet surface, is a key mechanistic feature thought to be relevant to increased endothelial cell proliferation. P-selectin is a membranous glycoprotein adhesin present within platelet alpha granules that is expressed on the platelet surface only after activation and alpha granule extrusion of contents, including endothelial growth factors. Platelet activation results in conformational changes in platelet membrane proteins that facilitate binding to endothelium. Thus, according to the sponsor, demonstration of increased platelet activation would be consistent with increased binding of platelets to endothelial cells and platelet granule release. The results for this parameter in investigational studies are suggestive, at least, of a treatment-related increase in platelet activation, first occurring relatively early in the course of treatment (**Figures 1** and **2**), although the data are quite variable and not always consistent within and between the 2 mouse strains. The magnitude of the increase ranged from 40-60% at 1000 mg/kg in B6C3F1 females and from 50-100% at 5000 mg/kg in CD-1 mice, ie, at clearly turmorigenic doses in the 2 species. No consistent effects were observed at 200 mg/kg, a dose associated with a marginal increase in tumor incidence. Similar changes were not seen in rats; platelet activation was not assessed in monkeys.

Figure 1

Platelet Activation in Female B6C3F1 Mice Given Pregabalin

Values are mean ± standard deviation, N = 4 to 9/group; *significantly different from control, p <0.05. Data from References 159 (1 Month) and 76 (6,12 Months).

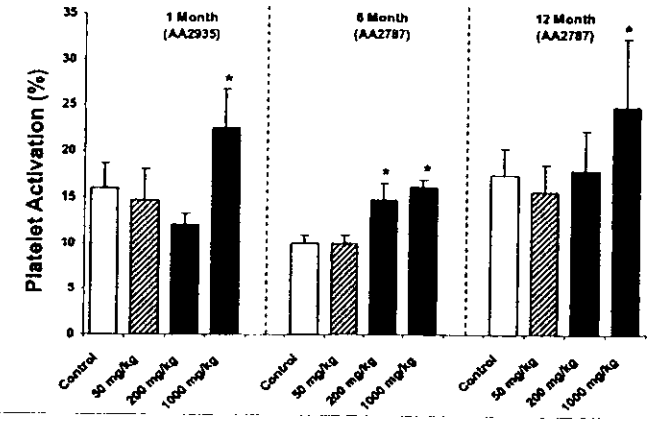
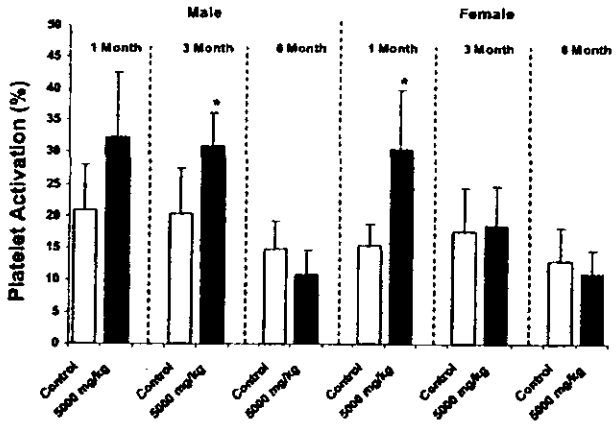


Figure 2

Platelet Activation in CD-1 Mice Given 5000 mg/kg for up to 6 Months

Values are mean ± standard deviation, N = 5; *significantly different from control, p <0.05. Data are from Reference 96.



Pregabalin also induced what was considered abnormal *ex vivo* platelet aggregation patterns characterized by defective secondary ADP-dependent aggregation in B6C3F1 mice. These findings were thought to suggest that platelet ADP content is depleted, consistent with increased platelet activation and *in vivo* release of dense granule content of ADP and alpha granule content of growth factors. Significant decreases in platelet aggregation endpoint (secondary ADP-dependent aggregation) were observed in female B6C3F1 mice beginning at 6 months (**Figure 3**). No significant changes were seen in male or female CD-1 mice given 5000 mg/kg for up to 6 months, but there appeared to be a trend for values to be somewhat lower (**Figure 4**). No comparable effect was seen in rats; however, an increase in platelet aggregation was seen after 12 months at 900 mg/kg. There was no apparent effect on this endpoint in monkeys given up to 500 mg/kg for 69 weeks.

Figure 3

Changes in Platelet Aggregation Endpoint (Secondary ADP-Dependent Aggregation) in Platelet Rich Plasma of Female B6C3F1 Mice Given Pregabalin

Values are mean ± standard deviation, N = 5 to 7/group, *significantly different from control, p <0.05. Data from References 159 (1 Month) and 76 (6,12 Months).

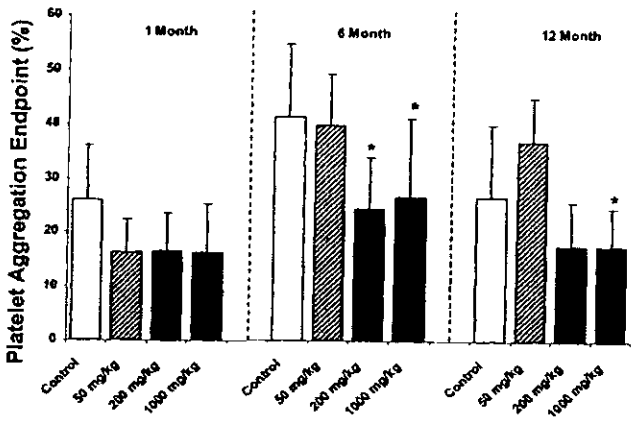
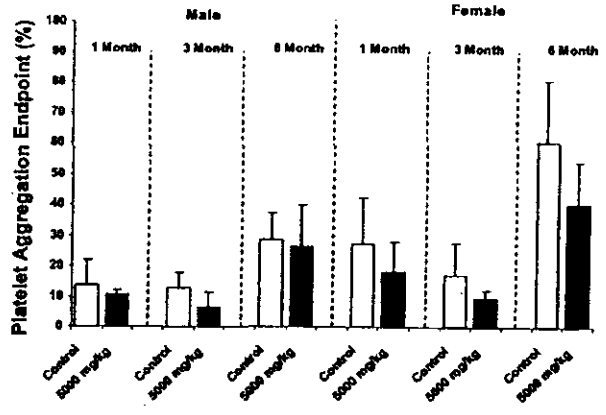


Figure 4

Changes in Platelet Aggregation Endpoint in Platelet Rich Plasma of CD-1 Mice Given 5000 mg/kg for up to 6 Months

Values are mean ± standard deviation, N = 4 to 5/group. Data from Reference 96.



Platelet morphological changes, which were considered by the sponsor to be a surrogate marker of platelet activation, were also seen in mice, but not in rats or monkeys. Alterations in platelet morphology (nonuniform platelet size, giant platelets) and increased circulating aggregates of degranulated platelets in peripheral blood were observed in pregabalin-treated female B6C3F1 and male CD-1 mice at early time points, although sometimes based on very limited sampling (**Tables 1** and **2**), and seemed to correlate fairly well with tumor incidence at the end of the 2-year mouse studies (**Tables 3** and **4**). In contrast, the presence of schistocytes (rbc fragments), also increased in pregabalin-treated animals, was considered an effect secondary to the presence of hemangiosarcoma and the resulting intravascular sheer stress in tumor blood vessels. It is difficult to separate primary form secondary events.

Table 1

Peripheral Blood Abnormalities in Female B6C3F1 Mice Given Pregaba

Dose (mg/kg)	N	Nonuniform Platelet Size (%)	Giant Platelets (%)	Platelet Aggregates (%)	Schistocytes (%)
			1 Month		
0	37	0.0	0.0	0.0	0.0
1000	37	54.1	54.1	18.9	0.0
			6 Months		
0	9	0.0	0.0	8.0 (one sample)	0.0
50	10	0.0	0.0	10.0 (one sample)	0.0
200	10	30.0	60.0	40.0	0.0
1000	10	30.0	70.0	40.0	0.0
			12 Months		
0	5	0.0	0.0	0.0	0.0
50	5	20.0	0.0	20.0 (one sample)	0.0
200	5	40.0	40.0	20.0	0.0
1000	5	20.0	80.0	20.0	0.0
			2 Year		
0	42	19.0	2.4	2.4	2.4
200	41	17.1	4.9	7.3	4.9
1000	27	44.4	22.2	18.5	11.1
5000	23	91.3	52.2	30.4	26.1

References 33, 76, 160

Table 2

Peripheral Blood Abnormalities in Male CD-1 Mice Given Pregabalin

Dose (mg/kg)	N	Nonuniform Platelet Size (%)	Giant Platelets (%)	Platelet Aggregates (%)	Schistocytes (%)
			1 Month		
Control	5	0.0	0.0	0.0	0.0
5000	5	0.0	0.0	60.0	0.0
			3 Month		
Control	5	0.0	0.0	20.0	0.0
5000	5	40.0	40.0	60.0	0.0
			6 Month		
Control	5	0.0	0.0	20.0	0.0
5000	3	33.3	33.3	33.3	0.0
			2 Year		
Control	46	2.2	2.2	4.3	0.0
200	38	0.0	5.3	7.9	5.3
1000	39	10.3	0.0	5.1	2.6
5000	39	66.7	46.2	17.9	10.3

References 33, 96

Table 3

Relationship of Hemangiosarcoma Incidence, Platelet, and Bone Marrow Changes in B6C3F1 Mice Given Pregabalin for 2 Years

Finding		Control	Pregabalin (mg/kg)		
			200	1000	5000
Male					
Hemangiosarcoma Incidence		2/65	3/64	19/65*	22/64*
Platelet Count (10^3/L)		1182 ± 299	1342 ± 226	1599 ± 466†	1577 ± 476†
	N	52	46	35	17
Peripheral Blood Abnormalities					
	N	53	47	36	17
Nonuniform Platelet Size (%)		15 1	12 8	75	100
Giant Platelets (%)		1.9	2.1	63.9	100
Platelet Aggregates (%)		1 9	8.5	13 9	29 4
Schistocytes (%)		1 9	2 1	16 7	41.2
Bone Marrow Megakaryocyte Count (per 5000 hematopoietic cells)		43 2 ± 11 2	65 8 ± 18 0‡	77 3 ± 19 9‡	88 3 ± 26 3‡
	N	65	63	64	61
Female					
Hemangiosarcoma Incidence		2/65	7/66	19/64*	25/65*
Platelet Count (10^3/L)		631 ± 203	856 ± 216†	830 ± 237†	997 ± 267†
	N	40	40	23	20
Peripheral Blood Abnormalities					
	N	42	41	27	23
Nonuniform Platelet Size (%)		19 0	17.1	44.4	91 3
Giant Platelets (%)		2 4	4 9	22.2	52.2
Platelet Aggregates (%)		2 4	7.3	18 5	30 4
Schistocytes (%)		2 4	4 9	11 1	26 1
Bone Marrow Megakaryocyte Count (per 5000 hematopoietic cells)		43 0 ± 10.7	60.1 ± 20.3‡	71 2 ± 18.9‡	78.9 ± 19.7‡
	N	62	66	62	63

References 31, 22, 33
N = Number of mice.
Statistically different from control, *p <0 001, †p<0 01, ‡p<0 02 (p<0.005 for quadratic)

Table 4

Relationship of Hemangiosarcoma Incidence, Platelet, and Bone Marrow Changes in CD-1 Mice Given Pregabalin for 2 Years

Finding		Control	Pregabalin (mg/kg)		
			200	1000	5000
Male					
Hemangiosarcoma Incidence		2/65	5/65	6/65	14/65*
Platelet Count (10^3/L)		1553 ± 630	1809 ± 950	1715 ± 759	2047 ± 760†
	N	27	26	22	22
Peripheral Blood Abnormalities					
	N	46	38	39	39
Nonuniform Platelet Size (%)		2 2	0 0	10 3	66 7
Giant Platelets (%)		2.2	5.3	0.0	46.2
Platelet Aggregates (%)		4.3	7.9	5 1	17.9
Schistocytes (%)		0.0	5.3	2 6	10.3
Bone Marrow Megakaryocyte Count (per 5000 hematopoietic cells)		37.6 ± 13 4	44 4 ± 17 9	53.7 ± 25.5‡	78.2 ± 29.0‡
	N	59	61	59	55
Female					
Hemangiosarcoma Incidence		6/65	9/65	10/65	13/65
Platelet Count (10^3/L)		958 ± 216	1019 ± 263	1087 ± 418	1186 ± 636
	N	22	25	23	16
Peripheral Blood Abnormalities					
	N	49	48	47	45
Nonuniform Platelet Size (%)		2.0	16.7	25.5	20.0
Giant Platelets (%)		2.0	0.0	8.5	17.8
Platelet Aggregates (%)		4.0	0 0	12.8	4.3
Schistocytes (%)		2.0	8.3	4.3	11.1
Bone Marrow Megakaryocyte Count (per 5000 hematopoietic cells)		28.4 ± 9.59	39.6 ± 16.9‡	51.2 ± 16.4‡	51.2 ± 17.7‡
	N	57	62	59	54

References 37, 38
N = Number of mice.
* Statistically different from control, p <0.001, †p<0.01, ‡p<0.02 (p<0.005 for quadratic)

Pregabalin did not affect megakaryopoiesis *in vitro* in cultures of bone marrow isolated from B6C3F1 mice, did not bind with high affinity to platelets, and did not affect platelet function in vitro. Findings from these latter 2 studies indicate that pregabalin-induced changes in platelet function are not directly mediated by alterations in calcium homeostasis as a result of the drug's pharmacologic activity. Hypoxia and acid-base balance can reportedly affect platelet count and function, and the sponsor hypothesized that pregabalin induces depression of respiratory function in mice leading to development of low-grade hypoxia and acid-base imbalances. Recent investigative studies in B6C3F1 mice indicated that pregabalin treatment (5000 mg/kg) induced respiratory depression (decreased respiratory rate and weight-corrected minute volume), increased pCO2, and increased venous and arterial pH, without changing oxygen saturation. These changes were seen over the course of 1 month of treatment. (The effect on weight-corrected minute volume was also seen in a chronic study, but there are no pH data for longer-term treatment.) It was postulated that these changes could alter pHi in platelets as a result of ion exchange effects and result in an increased level of platelet activation. An experiment conducted by the sponsor showed that alkalinization of blood produced an increase in platelet activation in vitro. Although similar changes in respiration, bicarbonate, and pCO2 were seen in pregabalin-treated rats, there were no apparent changes in blood pH. The sponsor stated that B6C3F1 mice may be uniquely susceptible to hypoxia based on their hypoxic hypoventilatory phenotype, but this would not explain the tumors in CD-1 mice, which were not investigated for pregabalin-induced changes in pH. Furthermore, there was no evidence of hypoxia, which is known to affect platelets, in B6C3F1 mice.

2) The sponsor considered an increase in platelet counts to be an early marker of pregabalin-induced hematological effects, ie, increased megakaryopoiesis. However, while increased platelet counts were seen after 12 months (i.e., when tumors were starting to appear), there was really no consistent effect on platelet count at early time points in the investigational studies (**Figures 5** and **6**), and the magnitude of the changes was generally small (30% or less). Megakaryocyte counts tended to be increased at tumorigenic doses in pregabalin-treated mice at earlier times in the investigational studies, however (**Tables 5** and **6**). In a reversal study, increased platelet counts seen after 12 months persisted for at least 10 weeks, indicating a reactive response to a lasting tissue injury or neoplastic changes, rather than a response to hypoxia, as proposed. Although much was made of an early effect on M:E ratio and megakaryocytes, it is interesting to note that in the original 13 week study in B6C3F1 mice (RR 250-01744) in which doses up to 8000 mg/kg were given and relatively large Ns were available, no effects on platelet count or M:E ratio were seen. A non-dose-related increase in platelet volume in treated animals (up to 20% compared to C) was reported in that study, however.

Figure 5

Platelet Count in Female B6C3F1 Mice Given Pregabalin at up to 5000 mg/kg for up to 2 Years

Values are mean ± standard deviation, N = 8 to 40/group; * significantly different from control, p <0.05 Data from Reference 76 (3,6,12 Months) and 31 (2 Year)

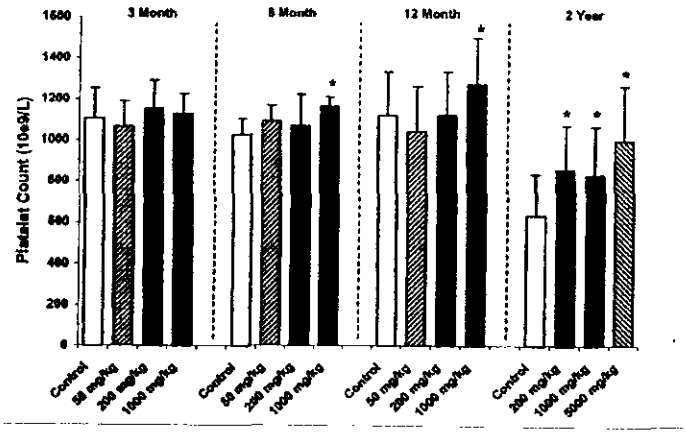


Figure 6

Platelet Count in CD-1 Mice Given 5000 mg/kg for up to 2 Years

Values are mean ± standard deviation, N = 5 to 22, * significantly different from control, p <0.05 Data from References 97 (1,3,6 Month) and 37 (2 Year)

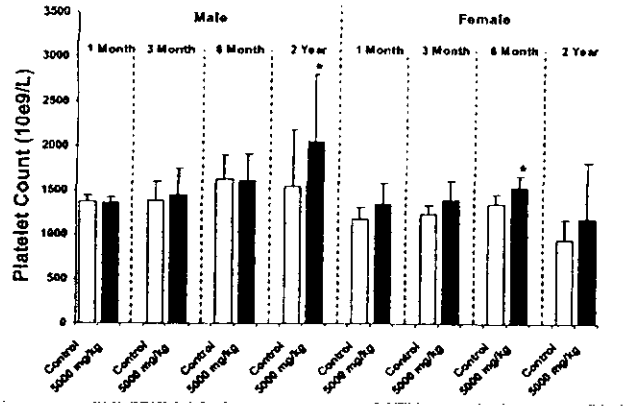


Table 5

Bone Marrow Megakaryocyte Counts in Female B6C3F1 Mice

Study	Time	Control	Pregabalin (mg/kg)			
			50	200	1000	5000
			10^4/Femur			
AA2795	1 Month	0.02 ± 0.042	--	--	0.09 ± 0.035*	--
	3 Month	0.03 ± 0.050	--	--	0.07 ± 0.071	--
AA2787	6 Month	0.07 ± 0.048	0.03 ± 0.048	0.07 ± 0.048	0.06 ± 0.052	--
	12 Month	0.05 ± 0.053	0.07 ± 0.048	0.08 ± 0.079	0.17 ± 0.082*	--
	2 Year	0.07 ± 0.057	0.04 ± 0.059	0.09 ± 0.075	0.18 ± 0.144*	--
			Per 5000 Hematopoietic Cells			
AA2795	1 Month	36.1 ± 6.39	--	--	55.1 ± 11.71*	--
	3 Month	41.6 ± 5.68	--	--	62.4 ± 11.19*	--
AA2236	2 Year	43.0 ± 10.7	--	60.1 ± 20.3*	71.2 ± 18.9*	78.9 ± 19.7*

References 22, 76, 95
Mean ± standard deviation, N = 5 to 66 animals/group
* Significantly different from control, p <0.01

Table 6

Bone Marrow Megakaryocyte Count in CD-1 Mice Given Pregabalin

Study	Time	Control	Pregabalin (mg/kg)			
			50	200	1000	5000
Male						
			10^4/Femur			
AA 2934	1 Month	0.06 ± 0.055	--	--	--	0.08 ± 0.084
	3 Month	0.00 ± 0.000	--	--	--	0.02 ± 0.045
	6 Month	0.03 ± 0.050	--	--	--	0.07 ± 0.050
			Per 5000 Hematopoietic Cells			
AA 2658	2 Year	37.6 ± 13.4	--	44.4 ± 17.9	53.7 ± 25.5*	78.2 ± 29.0*
Female						
			(10^4/femur)			
AA 2892	3 Month	0.04 ± 0.052	0.01 ± 0.032	0.03 ± 0.048	0.04 ± 0.052	--
	6 Month	0.02 ± 0.042	0.00 ± 0.000	0.04 ± 0.052	0.02 ± 0.063	--
	12 Month	0.05 ± 0.053	0.03 ± 0.048	0.05 ± 0.071	0.08 ± 0.063	--
AA 2934	1 Month	0.08 ± 0.084	--	--	--	0.10 ± 0.071
	3 Month	0.02 ± 0.045	--	--	--	0.00 ± 0.000
	6 Month	0.04 ± 0.052	--	--	--	0.12 ± 0.042*
			Per 5000 Hematopoietic Cells			
AA 2658	2 Year	28.4 ± 9.59	--	39.6 ± 16.9*	51.2 ± 16.4*	51.2 ± 17.7*

References 38, 96, 97
* Significantly different from control, p <0.05
Mean ± standard deviation, N = 5 to 62 animals/group

In the sponsor's most extensive investigational study (RR 745-03832), in which female B6C3F1 mice were treated for either 12 or 24 months with 0, 50, 200, or 1000 mg/kg, there were no statistically significant differences in mean platelet counts at any dose in animals treated with pregabalin for 24 months (**Table 7**). Mean platelet volume (MPV) was statistically significantly increased 13% and 38% at 200 and 1000 mg/kg, respectively, at week 104. These were the same doses that produced increased hemangiosarcoma incidences (6.6, 6.6, 11.5, and 26.3% in C, LD, MD, and HD, respectively). Although platelet aggregates were increased in all dose groups, there was no difference between the response at a tumorigenic dose and what was considered the no-effect dose of 50 mg/kg. Degranulated (abnormal) platelet aggregates were only observed at 200 and 1000 mg/kg, however. Abnormal platelet morphology was observed in a single animal each at 50 and 200 mg/kg and 8 of 20 animals at 1000 mg/kg. Platelet abnormalities included lack of uniform platelet size, presence of giant platelets, and platelet hypogranularity. These platelet abnormalities would be expected in animals with hemangiosarcomas. Incidences of hemangiosarcoma at 24 months were lower in mice that were removed from pregabalin treatment after 12 months (5/60, 6/61, 5/60, and 10/56 in C, LD, MD, and HD, respectively), but the incidence at the HD was still increased compared to C and was not statistically different from that in the HD 24-month treatment group. Therefore, it cannot be concluded that there was significant reversal of tumor induction following the 12-month treatment period.

Platelet and megakaryocyte counts tended to be decreased or unchanged in rats and monkeys treated with pregabalin on a subchronic or chronic basis. However, in a chronic investigative study in female rats (0 vs 900 mg/kg) platelet volume was consistently increased (at 6 [14%], 12 [17%], and 18 months [35%]; SS at all times) in pregabalin-treated animals.

Table 7

Relationship of Hemangiosarcoma Incidence, Platelet, and Bone Marrow Changes in Female B6C3F1 Mice Given Pregabalin in an Investigational 2-Year Study

Finding		Control	Pregabalin (mg/kg)		
			50	200	1000
Hemangiosarcoma Incidence		4/61	4/61	7/61	15/57*
Platelet Count (10^9/L)		834 ± 296	927 ± 200	876 ± 212	989 ± 380
	N	40	39	35	24
Peripheral Blood Abnormalities					
	N	20	21	20	20
Nonuniform Platelet Size (%)		0.0	4.8	5	40
Giant Platelets (%)		0.0	0.0	0.0	40
Platelet Aggregates (%)		20	48	35	50
Schistocytes (%)		4.8 (N = 21)	13.6 (N = 22)	10	15
Bone Marrow Megakaryocyte Count (10^6/femur)		0.07 ± 0.057	0.04 ± 0.059	0.09 ± 0.075	0.18 ± 0.144‡
	N	20	20	20	20

Reference 42
N = Number of mice.
* Statistically different from control, $p < 0.001$, ‡$p < 0.01$ ($p < 0.005$ for quadratic)

3) Pregabalin-treatment did not affect vascular endothelial growth factor (VEGF) concentrations in platelet-rich or platelet-poor plasma of mice. Concentrations of platelet-derived growth factor (PDGF) in platelet rich plasma (PRP) were variable, and were generally unchanged or decreased in pregabalin-treated groups. Although the sponsor claimed that PDGF concentrations in platelet-poor plasma (PPP) were increased in B6C3F1 mice after 12 months of treatment (1257 pg/mL in C vs 4882 pg/mL at 1000 mg/kg), this appears to have been a random finding (**Table 8**), and levels were significantly decreased at other times. PDGF concentrations were increased 47% at 1000 mg/kg after 24 months of treatment (N=20), but given the failure

to find increases in PDGF at earlier times, it is likely that PDGF secreted from tumors contributed to the increase at 2 years. Interestingly, in a chronic investigative study in rats (0 and 900 mg/kg), concentrations of basic fibroblast growth factor (bFGF) in PRP were 3.3-, 2.9-, 7.1-, and 2.9-fold greater in pregabalin-treated rats after 3, 6, 12, and 18 months, respectively; a statistically significant increase (27%) in PDGF concentration in PRP was observed in pregabalin-treated rats at 3 months (but not at 6, 12, or 18 months); and quantifiable levels of PDGF were detected only in pregabalin-treated rats at 3 months (not at 6, 12, or 18 months; PDGF was detected in control platelets at 18 months only).

Table 8. Plasma PDGF Concentrations in Serum of Female B6C3F1 Mice Treated for 2 Years

0 mg/kg	50 mg/kg	200 mg/kg	1000 mg/kg
Plt-Derived Growth Factor PRP (pg/mL)			
Week 24 4155.19+2817.835 (9)	3768.05+1254.274 (10)	3722.50+1574.462 (10)	3829.29+1579.895 (10)
Week 51 11987.28+3422.300 (5)	4108.94+1547.435** (5)	2745.04+1073.658** (5)	12144.76+4285.382 (5)
Plt-Derived Growth Factor PPP (pg/mL)			
Week 24 939.14+ 216.755 (9)	959.84+ 578.838 (10)	1202.97+ 927.786 (10)	612.21+ 224.162* (10)
Week 51 1257.28+ 535.141 (5)	1668.24+ 813.996 (5)	1448.92+ 748.410 (5)	4882.08+4966.474 (5)
Week 65 2645.09+ 955.589 (8)	2352.64+ 939.609 (10)	981.38+1458.206** (10)	1614.98+2067.812** (10)

For each group, values expressed are mean + standard deviation (N)

Immunohistochemistry showed increased VEGF immunostaining in the spleen, which correlated with the increased splenic extramedullary hematopoiesis and megakaryocyte number in pregabalin-treated CD-1 (after 6 months at 5000 mg/kg) and B6C3F1 (12 months at 1000 mg/kg) mice. This would be expected with an increase in hematopoietic cells, which are recognized sources of angiogenic growth factors. No effect on VEGF immunolabeling was seen in the spleen of female CD-1 mice given 1000 mg/kg for 12 months, however, and there were no increases in immunostaining for VEGF or other growth factors in hepatocytes or liver endothelial cells. There was a slight trend towards increased VEGF receptor 2 (Flk-1) immunolabeling on endothelial cells in liver of pregabalin-treated B6C3F1 mice after 12 months of treatment (**Table 9**). There was no apparent change in growth factor immunolabeling in rats or monkeys (only Flk-1 examined in monkeys).

Table 9. Incidence of Flk-1 Immunostaining in Liver of Mice

Study No.	Dose (mg/kg)	Staining Intensity and Confluency 1a	2	3
2787:12 month Sacrifice	0	2b	6	2
	50	2	7	1
	200	3	6	0
	1000	1	5	4

a 0: negative staining, 1: minimal, 2: mild, 3: moderate

b 10 animals/group. Immunostaining in less than 10 animals is due to unsuitability of section or loss of section during staining.

4) Cell proliferation was quantitated by morphometric analysis of cell type-specific bromodeoxyuridine (BrdU) incorporation. In the initial evaluations of proliferation, the number of proliferating (BrdU-labeled) endothelial cells was expressed as a function of total nuclei (endothelial cell + hepatocyte + Kupffer cell) per image. Data generated by this method were quite variable, and endothelial cell proliferation did not consistently appear related to dose or duration of treatment. In addition, increases in hepatocyte and Kupffer cell proliferation were frequently seen. Subsequently, given the relative proportions of cells (hepatocytes>>endothelial cells>Kupffer cells) and the potential confounding effects of any changes in the proportion of these cells, the assessment of hepatic endothelial cell proliferation was refined so that the number of proliferating endothelial cells was expressed as a function of total endothelial cells per image. Based on these analyses, pregabalin induced a statistically significant increase in hepatic endothelial cell proliferation in B6C3F1 mice given 200 or 1000 mg/kg after 12 months of treatment (**Figure 7**). However, while not statistically different from control, proliferation at the supposed no-effect level of 50 mg/kg was higher than in controls, and similar to that seen at higher doses. In addition, hepatocyte and Kupffer cell proliferation was also increased at 200 and 1000 mg/kg after 12 months. Hepatic endothelial cell proliferation was assessed using the refined method in female CD-1 mice given up to 1000 mg/kg for up to 12 months and in male and female CD-1 mice given 5000 mg/kg for up to 6 months, and was not increased (**Figures 8** and **9**). Hepatic endothelial cell proliferation was also not increased in female Wistar rats given 900 mg/kg, the highest dose evaluated in the carcinogenicity studies, for up to 18 months.

Figure 7

Hepatic Endothelial Cell Proliferation (Proliferating Endothelial Cells/Total Endothelial Cells) in Female B6C3F1 Mice Given Pregabalin at up to 1000 mg/kg

Values are mean ± standard error, N = 4 to 5/group; *significantly different from control, p <0.05 Data from Reference 161.

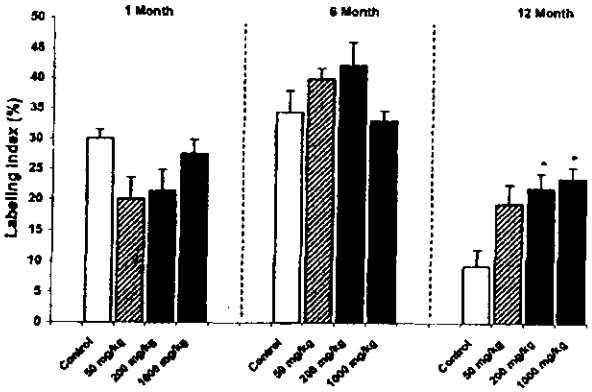


Figure 8

Hepatic Endothelial Cell Proliferation (Proliferating Endothelial Cells/Total Endothelial Cells) in Female CD-1 Mice Given Pregabalin at up to 1000 mg/kg

Values are mean ± standard error, N = 4 to 5/group. Data from Reference 97

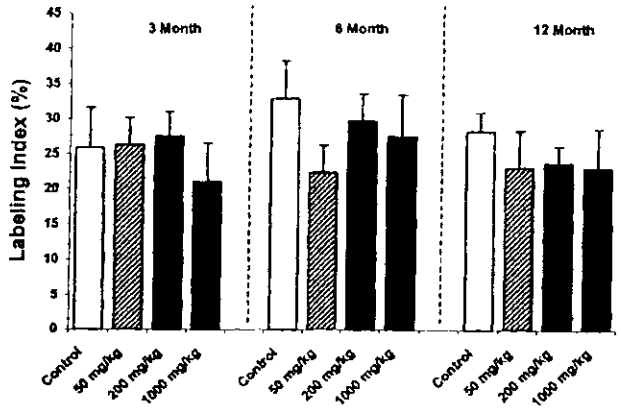
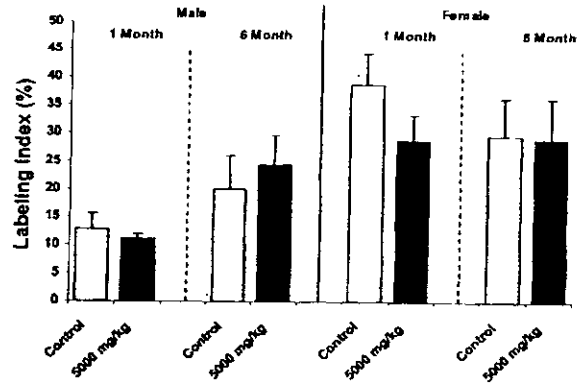


Figure 9

Hepatic Endothelial Cell Proliferation (Proliferating Endothelial Cells/Total Endothelial Cells) in CD-1 Mice Given Pregabalin at 5000 mg/kg for 6 Months

Values are mean ± standard error, n = 4 to 5/group. Data from Reference 96



OVERALL CARCINOGENICITY CONCLUSIONS

In the initial 2-year mouse carcinogenicity study in the B6C3F1 strain, pregabalin administration increased incidences of hemangiosarcoma at all doses: incidences were 3.1, 4.7, 29.2, and 34.4% in males and 3.1, 10.6, 29.7, and 38.5% in females from C, LD, MD, and HD groups, respectively (SS at MD and HD in both sexes). According to published data, spontaneous hemangiosarcomas occur with a prevalence of 3.0% in males and 3.6% in female B6C3F1 mice (NTP 2002). Plasma drug AUCs were 135, 800, and 3840 ug.h/ml in males and 148, 598, and 3740 ug.h/ml in females, at doses of 200, 1000, and 5000 mg/kg, respectively. Thus, mouse exposures at the LD, which were associated with non-statistically significant increased incidences of hemangiosarcoma, are similar to exposures expected in humans (122 ug.hr/ml at 600 mg/day). A second study in CD-1 mice, using the same doses, essentially replicated the previous findings, although the incidences of hemangiosarcoma were somewhat lower: total incidences were 3, 8, 9, and 22% in males and 9, 14, 15, and 20% in females from C, LD, MD, and HD groups, respectively (SS in HD males). Exposures were also lower in CD-1 mice: AUCs were 110, 550, and 3290 ug.h/ml in LD, MD, and HD mice, respectively (male and female combined).

The biological characteristics of vascular neoplasms in mice seem to differ in some respects from those in humans. In humans, benign and malignant vascular neoplasms are distinct: hemangiomas develop principally in children and generally regress spontaneously, while hemangiosarcomas usually affect the elderly and are aggressive malignant tumors with a poor prognosis (Enzinger and Weiss, Soft Tissue Tumors, Mosby, 3rd edition, 1995). Malignant transformation of benign vascular tumors in humans is unusual. Hemangiosarcomas are rare in humans, representing less than 1% of all sarcomas. Vascular tumors are also relatively rare in mice but apparently appear as a morphologic continuum from benign to malignant, with a similar onset and latency, and occur primarily in the second year of life (Herman et al., Toxicol Sci 68:226-36, 2002). Only hemangiosarcomas were increased by pregabalin, however. According to a recent publication, chemical-related increased incidences of vascular neoplasms in B6C3F1 mice have been found in 23 NTP studies (Abdo et al, Arch Toxicol 77:702-11,2003). Of those, the majority were mutagenic in the Ames test, but several were not; these included 2-butoxyethanol, chloroprene, and Elmiron, a heparin-like macromolecular carbohydrate derivative, used clinically in the treatment of interstitial cystitis. A drug-related increased incidence of hemangiosarcoma appears to be an uncommon finding in rodent carcinogenicity studies submitted to the FDA. Based on product labeling, the only drug approved with this finding in recent years was the antidiabetic agent troglitazone, which was subsequently removed from the market due to hepatotoxicity. Troglitazone belongs to the group of peroxisome proliferator–activated receptor (PPAR) agonists, a relatively new class of insulin sensitizers used clinically to treat diabetes. Interestingly, a number of related compounds currently in development also produced hemangiosarcomas in rodents in preclinical studies submitted to the Agency. PPAR agonists appear to affect endothelial function independent of their insulin sensitization effects, and it has been reported that PPAR agonists increase VEGF expression in human vascular smooth muscle and increase blood VEGF concentrations in patients with diabetes (Wang et al., Circulation 109:r67-r75,2004; Yamakawa et al., Biochem Biophys Res Commun 271:571-4, 2000; Emoto et al., Diabetes 50:1166-70,2001).

The pathogenesis of hemangiosarcoma in rodents is unknown, although the involvement of sustained local production of angiogenic factors, such as VEGF, has recently been suggested in some cases of chemical-induced hemangiosarcoma (Herman et al., Toxicol Sci 68:226-36, 2002; Nyska et al., Toxicol Appl Pharmacol 184:153-64, 2002). Data from the investigational studies do not provide strong support for the sponsor's proposed mechanism of pregabalin-induced hemangiosarcoma in mice. As pointed out by the FDA consultant, Dr. Terry Peters (review dated 2/9/04), the proposed key drug-related effects are generally too variable and inconsistent, within and between strains, and their magnitude too small, to allow a conclusion that they contributed to the tumorigenic response. Furthermore, the data are inadequate to establish a causal linkage between the proposed early markers of pregabalin-induced preneoplastic changes and the tumors. While there were group correlations in long-term studies between changes in some hematological parameters and hemangiosarcoma incidences (correlations for individual animals were not provided), there was no evidence of a causal relationship. The proposed early markers were never validated, since preneoplastic changes were not conclusively demonstrated. Thus, while there was some evidence that some of the hematological changes preceded the identifiable tumors, their temporal relationship to presumed preneoplastic changes is unknown.

Some if not all of the hematological changes would be expected to accompany tumors. For example, secondary or reactive thrombocytosis is associated clinically with inflammatory states of either infectious or noninfectious origin, such as trauma and malignancy. And according to Greaves (Histopathology of Preclinical Toxicity Studies, Elsevier, 1990), changes in platelet counts in preclinical toxicity studies are most commonly the result of disease or tissue damage repair occurring in other organs. Hyperplasia of the bone marrow occurs in a variety of reactive states, particularly when there is increased red cell or platelet turnover or destruction. And it is well known that tumors can stimulate the bone marrow. Hemorrhage, which would be expected with hemangiosarcoma, is also a known stimulus of thrombocytosis. A megakaryocyte response has been reported in posthemorrhagic thrombocytosis of mice (Krizsa et al., Acta Haematol 39(2):112-7, 1968). And as stated in the original mouse B6C3F1 carcinogenicity study report, extramedullary hematopoiesis was considered a "secondary response to blood loss that was likely associated with the hemangiosarcoma."

Activation of platelets normally occurs after vessel injury, but the sponsor states that there was no histopathologic evidence of endothelial or other tissue damage in mice given pregabalin. A dermatopathy characterized primarily by tail lesions was seen in rats and monkeys following oral administration of pregabalin. Skin lesions in a 2-week iv infusion study in monkeys (RR 250-01817), which were found to be similar to the lesions in the oral studies, although more extensive, were thought to be secondary to hypoxia following injury to the vascular endothelium. Missing tail tips were seen in B6C3F1 mice in the first 2-year study at all doses, but it is unclear how or if these tail lesions relate to those observed in other species. The sponsor has not proposed direct endothelial injury as a cause of the hemangiosarcomas in mice. Instead, the sponsor has hypothesized a mechanism involving increased platelet activation occurring in pregabalin-treated mice in response to respiratory depression and increased blood pH, a response they were able to demonstrate *in vitro*. There appears to be no evidence reported in the literature, however, to support a mechanism involving platelet activation in the induction of hemangiosarcoma or other tumors. Verheul and Pinedo (Oncologist 3(2):II,1998) have proposed a functional role for platelets in tumor angiogenesis, based on a number of preclinical and clinical findings, but even this role is controversial (Verheul and Pinedo, Clin Cancer Res 9:3219-21,2003). They and others have shown that platelets stimulate the proliferation of endothelial cells and endothelial cell tube formation *in vitro*, that platelets store and transport VEGF, and that patients with soft tissue sarcomas and other tumors show the presence of platelet activation and elevated levels of VEGF. The mechanism of angiogenic growth factor release from platelets is not entirely clear, but *in vitro* experiments have demonstrated that it is dependent on platelet activation and aggregation. But it is not clear that increasing platelet numbers or platelet activation *per se* would be sufficient to promote endothelial cell proliferation resulting in tumor formation. *In vitro*, a 1000-fold increase in platelet concentration was required to produce about a 2-fold increase in endothelial cells, and there was no difference between activated and nonactivated platelets (Verheul et al., Blood 96:4216-21,2000). The promoting effect of platelets on matrigel tube formation *in vitro* was also independent of activation, and was thought to involve a direct cell-to-cell interaction between platelets and the proliferating endothelium, since the platelet release contents (VEGF, etc.) alone had no effect on tube formation (Pipili-Synetos et al., Br J Pharmacol 125:1252-7,1998). Normally the endothelium produces autocoids which prevent the adhesion and aggregation of platelets, but it is thought that the tumor microcirculation may present a thrombogenic environment owing to compromised blood flow and discontinuous endothelial layers. In addition, under physiological conditions platelet-derived antiangiogenic factors such as angiostatin appear to serve as a counterbalance to proangiogenic molecules as VEGF, which are both released from platelets upon activation. Thus a complex interaction between platelet factors and cellular and extracellular matrix angiogenesis-regulating molecules at sites of tissue or vascular damage would determine the net angiogenic signal. The balance is thought to be tipped toward angiogenesis under some incompletely understood sets of circumstances leading to tumor growth and metastasis (Hanahan and Folkman, Cell 86:353-64,2004). There appears to be no evidence for a primary role of platelets in tumorigenesis, however.

So, even if the data presented were less ambiguous, the proposed mechanism would remain highly speculative. The sponsor made a considerable effort, but given the current state of knowledge concerning mechanisms of chemical carcinogenesis, they were facing a daunting task. Short of developing an intervention strategy that would have conclusively demonstrated the involvement of a specific element in their proposed pathway (as was recommended by the Agency), it is hard to imagine how they could have succeeded in

establishing this novel mechanism. While their hypothesis is plausible, other possibilities remain untested. For example, multiple signal transduction pathways have been implicated in the regulation of angiogenesis and tumorigenesis, including MAP kinases. A structural analog of pregabalin, the marketed drug gabapentin, induced pancreatic acinar cell tumors in rats (see Neurontin labeling for details); and investigative studies have shown that gabapentin activates MAP kinase, both in pancreatic tissue sections from *in vivo* rat studies and in rat primary acinar cells in culture (other cell types were not investigated). Potential cellular interactions leading to gabapentin-induced MAP kinase activation postulated by the sponsor included an effect on calcium channels, which is thought to be involved in the pharmacological mechanism of action of gabapentin and pregabalin. It was suggested that by increasing intracellular calcium gabapentin might trigger proliferative signaling pathways, thus acting as a tumor promoter (Dethloff et al., Toxicol Sci 55:52-9, 2000). Another possibility involves the binding of pregabalin to $\alpha_2\delta$ subunits of voltage activated calcium channels. An association of the $\alpha_2\delta$-2 subunit with tumor pathogenesis has been suggested based on the chromosomal location of the gene and its expression patterns (Gao et al, J Biol Chem 275:12237-42, 2000).

The sponsor has also argued that because pregabalin produced only one tumor type in a single species and appears to be non-genotoxic, it is unlikely to represent a risk to humans. While it may be true that some experts in the field consider non-genotoxic, single-species carcinogens of less concern than the genotoxic multiple-species kind (Tennant, Mutat Res 286:111-18,1993), there is no reason to think that such agents should be considered safe. Part of the reason for the lowered risk assessment for single-species, single-site carcinogens has to do with the recognition that inbred rodent strains, such as those used by NTP in their 2-year bioassays, "possess an allelic distribution that is uncharacteristic of the type and frequency found in feral or outbred populations " (Tennant and Spalding, Environ Health Persp 104(Suppl 5):1095-100,1996). As a result of inbreeding, rodent lines lose many of the polymorphic alleles found with variable frequencies in outbred populations and consequently possess a more limited number of specific alleles which are more uniformly distributed. Two outcomes of this so-called allelic enrichment are thought to be that inbred strains demonstrate specific patterns of spontaneous tumors and can exhibit strain-specific responses to chemicals (Ibid.). It is thought that such chemicals may only be modulating the expression of a genetic disease characteristic of a specific rodent strain and therefore have little effect in other species or strains (except possibly in certain susceptible individuals). The ability of pregabalin to induce hemangiosarcomas in both the inbred B6C3F1 and outbred CD-1 strains of mice, then, obviously heightens concern according to this line of reasoning. However, since the genetic and biochemical bases of strain-specific effects induced by carcinogens are not well understood and cannot be reliably predicted (Ibid.), this categorization should probably not be used as a basis for predicting carcinogenic risk to humans. The discounting of nongenotoxic animal carcinogens seems to derive in large part from the notion that humans would be unlikely to be exposed chronically to the high doses of some environmental chemicals necessary to produce tumors in animals. Clearly, nongenotoxic carcinogens are of concern when tumors are produced in animals at exposures expected in humans for a chronic-use drug, as is the case with pregabalin.

In conclusion, while the sponsor has proposed a plausible mechanism and one that certainly warrants further investigation, the present evidence is inadequate to exclude a carcinogenic risk associated with the use of pregabalin in humans. The preamble to IARC (International Agency for Research on Cancer) Monographs on the Evaluation of Carcinogenic Risks to Humans highlights the public health view that "in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans." Sufficient evidence of carcinogenicity in animals is defined in the document as existing when "a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols." The findings for pregabalin satisfy these criteria. This preclinical evidence of carcinogenicity certainly constitutes a significant obstacle to approval, but as always the assumed carcinogenic risk to humans must be weighed against the evidence of benefit of the drug in the various indications for which it is being considered.

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Fertility and Reproductive Performance

Two definitive fertility and early embryonic development studies were conducted in Wistar rats, one in which only males were dosed (250, 1250, or 2500 mg/kg) and one in which only females were dosed (500, 1250, or 2500 mg/kg). Dose selection was appropriate in males based on clinical signs and BW effects, and in females based on clinical signs and disrupted estrous cyclicity.

Males

A number of adverse reproductive effects were seen in the male fertility study, primarily at doses ≥ 1250 mg/kg; these included: increased number of days to mating, decreased sperm counts and motility, increased sperm abnormalities, reduced fertility, increased preimplantation loss, and decreased litter size. Marked decreases in epididymal weights, epididymal sperm counts, and vas deferens sperm motility were seen in the absence of changes in testicular weight or testicular sperm count. There was a virtual absence of motility in sperm collected from the vas deferens and complete lack of fertility at the HD of 2500 mg/kg. Decreased sperm motility was also seen at the LD of 250 mg/kg (extrapolated AUC: 1320 ug.h/ml; human exposure at 600 mg/day: 122 ug.hr/ml). The adverse male reproductive effects appeared to be reversible, however. Because a no-effect dose was not established, a follow-up study was conducted using lower doses (50, 100, or 250 mg/kg). No significant reproductive or other toxic effects were observed in this study.

In general toxicity testing in rats, epididymal enlargement, epididymal tubular hypospermia, and fibrosis and mononuclear cell infiltrates in the interstitium were observed at ≥ 500 mg/kg in a 4-week toxicology study (no testicular changes); and spermatogenic epithelial degeneration of the testis was observed after 13 weeks at 1250 mg/kg. However, no treatment-related effects on spermatogenic epithelia or other reproductive tissues were reported in rats after 52 weeks at up to 500 mg/kg. In the first 2-year rat carcinogenicity study (50, 150, and 450 mg/kg), decreased reproductive organ size (ie, gross findings of small testes and seminal vesicles) and weight and increased incidences of atrophy of the seminiferous tubules and aspermatogenesis in the testes and aspermia in the epididymides were seen at all doses. In the second rat carcinogenicity study (same doses), atrophy and degeneration of the testicular germinal epithelium were increased at all doses. In monkeys, hypospermia of the testis and epididymis in association with small testes and testicular weights was found in 1 monkey at 100 mg/kg and 2 monkeys at 500 mg/kg BID and spermatogenic epithelial degeneration was seen in recovery monkeys at 50 and 100 mg/kg in a 4 week toxicity study. But there were no reported effects on sperm parameters or reproductive organ histopathology at up to 500 mg/kg in the 1-year monkey study.

In an investigative study of pregabalin effects on male rat reproductive parameters (RR 745-02809), administration of 2500 mg/kg for up to 6 weeks produced decreased sperm motility in the cauda epididymis and vas deferens, decreased cauda epididymal sperm count, and abnormal sperm morphology within 2 to 3 weeks of treatment. The time course of appearance of these effects was thought to indicate an effect on epididymal sperm maturation. However, the additional observation of decreased sperm count and increased sperm abnormalities in the caput epididymis after 4 weeks of treatment also suggested a testicular effect, since sperm produced by the testis are normally morphologically mature and remain in the caput for only a short period of time. The effects on sperm parameters were reversed within 4 to 6 weeks after treatment was discontinued.

Another investigative study of male rat reproductive effects with electron microscopic evaluation (RR 745-02994) found ultrastructural changes in sperm and luminal contents in the cauda and caput epididymis of rats receiving 2500 mg/kg for 1 to 6 weeks, but no ultrastructural effects in the testis. Epididymal changes consisted primarily of multiple mitochondrial defects in the sperm, frayed sperm tails/midpieces, excessive debris in the lumen, multiple sperm tails in the same membrane, and tailless sperm heads. These changes correlated with decreased sperm motility and with morphological changes observed in sperm smears (ie, detached sperm heads, thread-like tail connections, sperm apparently stuck together). It was concluded that

the nature and location of the sperm abnormalities were consistent with an effect primarily involving sperm maturation in the epididymis. However, the presence of so-called cytoplasmic lobes in the epididymal lumen again suggested a possible testicular effect, since these lobes are thought to only come from the testis and it is unusual for the testis to release cytoplasmic lobes into the epididymis.

In the definitive male fertility study, litters of females mated to males treated with pregabalin (≥1250 mg/kg) had reduced numbers of implant sites and live fetuses and increased percent preimplantation loss, effects considered evidence of developmental toxicity in the study report. No fetal examinations were performed at C-section on GDs 13 to 15, however. In another supplemental study to investigate possible male-mediated embryo-fetal developmental toxicity (RR 745-03322; not described in detail in this review), male rats were dosed (100, 250, or 500 mg/kg) for 11 weeks prior to mating with untreated females (N=25). There were no treatment-related effects on copulation or fertility indices and no effects on maternal reproductive parameters, including embryofetal survival, litter size, and pre- and postimplantation loss, when litters were evaluated on GD 21. However, fetal body weights were decreased (5.50, 5.35, 5.25, and 5.26 g in males, and 5.19, 5.09, 4.98, and 5.02 g in females in C, LD, MD, and HD groups, respectively; statistically significant in MD and HD males) and malformation incidences were increased in treated groups compared to C (total numbers of fetuses/litters with malformations were 3/2, 4/3, 5/5, and 11/7 in respective groups). Malformations that appeared to be increased in treated groups included external (fetal/litter incidences: 0/0, 0/0, 2/2, and 1/1 in C, LD, MD, and HD, respectively), skeletal (0/0, 0/0, 3/3, 2/2), and visceral (3/2, 4/3, 2/2, 9/5). The study report concluded that the results did not meet the criteria for teratogenicity because there were no particular malformations that appeared to increase with dose and all malformations occurred at a low incidence with similar frequencies to historical controls. These are not universally accepted criteria for teratogenicity, however. An increase in total malformations is generally considered to indicate a teratogenic response. And, while it is true that incidences of external and skeletal defects were similar to historical controls, the fetal and litter incidences of visceral malformations (primarily CNS defects: exencephaly, anophthalmia, folded retina) at the HD were not (5.2/21 vs 0.2/2%, respectively). The results of the study are suggestive, at least, of male mediated developmental toxicity, including teratogenicity.

Females

In the female fertility study, disrupted estrous cyclicity during the premating treatment period and an increase in the number of days to mating were seen at all doses tested (≥500 mg/kg; AUC in females given 500 mg/kg in 4-week toxicity study: 1370 ug.h/ml). A possible effect on fertility index was also seen at doses ≥1250 mg/kg. As also seen in the developmental toxicity studies, pregabalin was embryolethal as indicated by increased pre- and postimplantation loss at all doses.

Embryo-Fetal Development

Definitive embryofetal development studies were conducted in mice (500, 1250, 2500 mg/kg), rats (500, 1250, 2500 mg/kg), and rabbits (250, 500, 1250 mg/kg). The mouse study was of questionable value, since no maternal toxicity was observed at the highest dose tested. Dose selection in the rat and rabbit studies was appropriate, based on the presence of at least minimal maternal toxicity at the high doses.

Mice

Doses of up to 2500 mg/kg (mean maternal AUC: 3790 ug.h/mL) did not induce clear maternal or developmental toxicity.

Rat

Developmental toxicity was observed at all doses in rats (≥500 mg/kg); specifically, fetal body weights were decreased in HD litters, fetal and litter incidences of skeletal malformations were increased at the MD and HD, and incidences of skeletal variations and retarded ossification were increased at all doses (SS at MD and HD).

Cmax values were [ ] ug/ml and AUCs were 2060, 6590, and 9470 ug.h/ml at the LD, MD, and HD, respectively. While the malformation incidence was reported increased at the MD and HD in the study report, this was due primarily due to increased findings of fusion of the jugal bone and maxilla and fusion of the nasal bones. It was later argued that since fusion of the jugal and nasal sutures (specifically: closure of the suturae zygomaticomaxillaris and nasofrontalis) occurs normally during development, these skull findings represent advanced ossifications, or variations, rather than malformations.

In a supplemental study of fetal skull effects in rats (RR 745-03426), administration of pregabalin to pregnant rats on GD 6 through 17 reproduced the previous findings of an increased incidence of fusion of the jugal suture at ≥1250 mg/kg (9 and 15X C at 1250 and 2500 mg/kg, respectively) and fusion of the nasal suture at 2500 mg/kg. Light microscopic examination of the sutures did not reveal any abnormalities. Another study on the progression of skull development in rats following gestational exposure to pregabalin (RR 745-03384) found that while treatment of dams with 2500 mg/kg pregabalin from GD 6 to 17 produced an increase in the incidence of closed jugal and nasal sutures from PM (postmating) Days 19 to 26, closed sutures were first observed in both control and pregabalin-treated animals on PM Day 19 (corresponding to GD 19), and the incidence of closed sutures increased with the age of the offspring but was still incomplete in most pups from both the control and treated groups on PM Day 26.

In another study (RR 745-03321), in which the 21-day-old offspring of dams treated with vehicle or pregabalin (2500 mg/kg) during gestation were examined, percentages with closure of the jugal suture were 99.6% and 94.9%, respectively, and with closure of the nasal suture were 1.1% and 4.7%. In general this drug-related increase in closure of the nasal sutures was reportedly not associated with malformation of the skull; however, malocclusion and an asymmetrically shaped head were observed in one treatment group pup on PN Days 19 to 21. The same pup had a corresponding external finding of misshapen head and a skeletal finding of misshapen left frontal skull and parietal bones. No other remarkable findings were reported upon external and skeletal evaluation. However, it should be noted that only surviving pups were examined, and that dead, cannibalized, and missing offspring occurred at a 3-, 4-, and 13-fold higher incidence, respectively, in the pregabalin-treated group relative to controls. In addition offspring from treated dams were smaller than controls. Both factors, pup death and growth retardation, complicate the interpretation of these studies.

Seen in the context of a general increase in skeletal variations at all doses in the original rat study (which was also seen with gabapentin), these alteration in skull ossification should probably be considered evidence of a teratogenic effect of pregabalin. As stated in the original study report, "Although fusion of these bones would be expected to occur later in postnatal development, premature closure might result in possible distortion of bone development and/or potentially impair the growth of soft tissue structures underneath; these fusions were therefore considered malformations."

Rabbit

Evidence of developmental toxicity was seen in rabbits (decreased fetal body weight, increased incidences of malformations, variations, and ossification retardation, abortion and total litter resorption), at a dose (1250 mg/kg) that was not otherwise very maternally toxic (1 moribund sacrifice associated with total litter resorption and 1 abortion, but no effect on maternal BW). Although, in the study report, the sponsor did not view the increased incidence of malformations in the HD group (total number of fetuses/litters with skeletal malformations was 0/0, 1/1, 0/0, and 5/4, in respective groups) as evidence of teratogenicity because it was within the historical control range, this conclusion is certainly debatable, particularly in light of the effects on skeletal development seen in rats. Cmax values were [ ] ug/ml and AUCs were 1400, 2020, and 4750 ug.h/ml at the LD, MD, and HD, respectively.

Pre- and Postnatal Development

A standard ICH pre- and postnatal development study was conducted in rats, with an expanded dose range (50, 100, 250, 1250, 2500 mg/kg). Dose selection was appropriate based on the presence of significant

maternal toxicity at the HD. When administered to pregnant and lactating rats, pregabalin produced adverse effects on offspring survival, growth, behavior, and reproductive function at doses ≥100 mg/kg (AUC: 601 ug.h/ml; human AUC at 600 mg/day: 122 ug.h/ml). Significant, but not excessive, maternal toxicity in terms of clinical signs and BW gain reduction was seen at the HD of 2500 mg/kg. However, the moribund sacrifice of 2 HD dams as well as other peripartum observations was probably related to the high rate of (late) embryofetal death (postimplantation loss increased at ≥250 mg/kg; 8X C at HD). Pup survival at birth and during the postnatal period until weaning was markedly reduced at ≥1250, with all HD offspring dying by PND 3. Significantly, no correlations were apparent between litter death and maternal clinical signs. Pup weights were decreased in treated litters at birth and during the lactation period at ≥100 mg/kg, and BW deficits persisted into the postweaning period. There were significant delays in the attainment of pre- and postweaning developmental landmarks at 1250 mg/kg. A decrease in acoustic startle response was seen in offspring from the 250 and 1250 mg/kg groups when they were tested on PND 42, ie, after cessation of dosing. This deficit was considered to be due to attenuated reactivity to the acoustic stimulus, rather than to an effect on hearing or the motor component of the startle response. Interestingly, a similar long-term deficit in startle response was seen in rats directly exposed to pregabalin during early postnatal development in the juvenile developmental neurotoxicity studies (RRs 745-03323 and 745-03794, not described in detail in this review). When offspring from the pre- and postnatal development study were mated, there were a number of effects on reproductive parameters (decreases in fertility index, numbers of corpora lutea, implants, live fetuses, and litter size and increased preimplantation loss) at 1250 mg/kg. Since both sexes of offspring were exposed through the dam, it could not be determined which was responsible for the effects on reproductive function. However, the juvenile rat reproductive toxicity studies (RRs 745-03267 and 745-03471, not described in detail in this review) indicate that reproductive function is impaired in both males and females following early postnatal exposure to pregabalin. In a follow-up investigative study relating developmental toxicity to stage of exposure (RR 745-02656, not described in detail in this review), female rats received vehicle or 2500 mg/kg during the organogenesis, fetal, perinatal, or neonatal period, then were allowed to deliver and rear their offspring through Lactation Day 10. Results indicated that perinatal mortality was greatest in groups treated during late gestation. Both *in utero* and neonatal death were increased in the fetal and perinatal groups, ultimately resulting in no offspring survival. Neonatal survival was also reduced in the organogenesis and neonatal period groups, although some offspring survived in the majority of the litters. Maternal toxicity occurred in all groups, but review of individual data did not provide a correlation between maternal toxicity and offspring death: maternal clinical signs and body weight gain suppression were associated with litters that survived, as well as those that died. Gestation length was not affected, contrary to the prolongation of gestation in the pre- and postnatal development study. It was concluded that the potential exists for pregabalin to affect a variety of functions necessary for perinatal survival.

OVERALL REPRODUCTIVE AND DEVELOPMENTAL TOXICITY CONCLUSIONS

In animal studies that can be considered adequate for regulatory purposes, pregabalin was shown to be a selective reproductive and developmental toxicant. Effects included male and female fertility impairment in rats, teratogenicity in rats and rabbits, embryofetal and pup lethality in rats, and developmental nervous and reproductive system functional impairment in rats. Most of these effects are fairly typical for anticonvulsants, but the degree of reproductive impairment (in both F0 and F1) and the high rates of embryofetal and pup mortality are unusual for this class. In addition, apparent male-mediated developmental toxicity was observed, which is extremely unusual (possibly because it is rarely examined). This is a poorly understood phenomenon that remains controversial in the teratology literature (Friedman, Adv Exp Med Biol 518:219-26,2003). However, until the sponsor is able to refute the findings described in RR 745-03322 (decreased body weights and increased malformations in the offspring of treated male rats mated with untreated females), it seems prudent, from a regulatory standpoint, to report them in labeling (they may actually warrant highlighting in some way). The signal for *in utero* teratogenicity was not especially strong, but was present in two species. The related compound gabapentin was found to be embryotoxic (increased incidences of visceral variations in rats, decreased embryofetal and pup survival in rats, decreased ossification in mice and rats, increased postimplantation embryofetal loss in rabbits), but was not teratogenic and did not affect reproductive performance in animal studies submitted to support its approval. There was little safety margin in terms of the plasma levels of pregabalin associated with reproductive and developmental toxicity in animals relative to expected clinical drug levels. NOELs for the most sensitive endpoints were 100 mg/kg for effects on rat sperm and 50 mg/kg for effects on growth in rats exposed pre- and postnatally, which produced AUC values of 408 and 241 ug.h/ml, respectively. Expected maximum exposure in humans at the MRD of 600 mg/day is reportedly 122 ug.hr/ml.

Pregabalin appears to have a variety of pharmacological actions, any or several of which could play a role in its reproductive and developmental toxicity. However, the ability of pregabalin to selectively bind to the alpha2-delta subunit of high-voltage calcium channels is thought to be particularly important pharmacologically. Calcium influx through voltage-gated calcium channels orchestrates many key biological functions, including neuronal excitability, neurotransmitter release, and gene expression; and there is also evidence that voltage-dependent calcium influx is important during development, including neurodevelopment (Schmid and Guenther, J Neurosci 19:3486-3494,1999; Kocsis et al., J Neurobiol 25:252-264,1994. In addition, calcium ions reportedly play a vital role in sperm function during various stages of fertilization, including a prominent role in facilitating sperm motility (Ren et al, Nature 413, 603-9, 2001). So given the apparent pharmacological action of pregabalin on voltage-gated calcium channels, some of these effects may not be unexpected, and there is no reason to think they would be species specific. But there were no reported effects on sperm parameters or reproductive organ histopathology in the 1-year monkey study at doses of up to 500 mg/kg, which was associated with an AUC of 1040 ug.h/ml. And in a double-blind, placebo-controlled clinical trial in which 30 healthy subjects were given 600 mg/day for a complete sperm cycle of 3 months, no effect of pregabalin on the primary outcome measure of sperm motility was found. Although 5 subjects had a reduction of sperm motility of more than 15%, 2 of these received placebo. There were no apparent effects on secondary outcome measures of sperm motility, morphology, sperm count, testes and breast measurement, and laboratory endocrine values (the adequacy of this clinical study for labeling is being evaluated by Dr. Mark Hirsch of HFD-580). In all controlled studies combined, adverse events related to sexual dysfunction, which included impotence, decreased libido, anorgasmia, and abnormal ejaculation, were more common in patients treated with pregabalin than in those given placebo. However, these are likely to be CNS-mediated effects. So, while human data are limited, it appears that there may be some safety margin for the male reproductive effects of pregabalin, presumably as a result of an exposure threshold and possible species differences in sensitivity. Human data addressing the potential for pregabalin-related female reproductive effects and developmental toxicity were not available. There are two case reports of gabapentin-exposed infants born with defects in skull formation: one with cyclopic holoprosencephaly (no nose and one eye) and one with an absence of the opening for one ear canal, but no definite conclusions can be drawn about a causal relationship (Reprotox teratogen information system).

2 Page(s) Withheld

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_____ § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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5/20/04 04:44:19 PM
PHARMACOLOGIST

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Terry Peters' Review 2/9/04
-Carcinogenicity/Mechanistic DATA

Reviewer: Terry S. Peters, D.V.M. NDA: 21446, 21723, 21724, 21-725

PHARMACOLOGY/TOXICOLOGY REVIEW
PATHOLOGY CONSULT for HFD-120 and 170

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-446, 21-723, 21-724, [redacted]
Sponsor and/or agent: Parke-Davis Pharmaceutical Research, Ann Arbor, MI
Reviewer name: Terry S. Peters, D.V.M.
Division name: Anti-Infective Drug Products
HFD #: 520
Review completion date: 2/9/04

Drug:

Trade name: Lyrica
Generic name: Pregabalin
Code name: CI-1008
Chemical name: (S)-3-(aminomethyl)-5-methylhexanoic acid: S-(+)-3-isobutyl GABA
Molecular formula/molecular weight: $C_8H_{17}NO_2$

Indication: Epilepsy (IIFD-170) and peripheral diabetic neuropathy. [redacted]

Studies reviewed within this submission:

1) 2-Year Dietary Carcinogenicity Study of CI-1008 in CD-1 Mice
2) 2-Year Dietary Oncogenicity Study in Rats with CI-1008
3) Retrospective Histopathologic Evaluation of Non-neoplastic Findings in Selected Tissues from B6C3F1 Mice Treated with CI-1008 for 2 Years
4) Analysis of Hemangiosarcoma and Liver Samples from the CI-1008 B6C3F1 Mouse Carcinogenicity Study for the Presence of *Heliobacter hepaticus* DNA
5) Exploratory Study of Pregabalin on Megakaryocyte Development in Female B6C3F1 Mice
6) Exploratory Study of the In Vitro Effects of Pregabalin on B6C3F1 Mouse Spleen Endothelial Cell Proliferation
7) Analysis of [^{3}H]Pregabalin Binding and α2• •Protein Expression in Selected B6C3F1 Mouse and Wistar Rat Tissue
8) Exploratory 1-Week Investigation of the Effects of Pregabalin on Endothelial Cell Factors in Platelet-Depleted B6C3F1 Mice
9) Chronic Investigative Study of Pregabalin in Female Wistar Rats
10) [^{3}H] Pregabalin Does Not Bind to Membrane Proteins of Mouse or Rat Platelets
11) Exploratory Investigation of the Effects of Pregabalin on Endothelial Cell Factors in Female CD-1 Mice, Interim Report
12) Exploratory Investigation of the Effects of Pregabalin on Endothelial Cell Factors in Male and Female CD-1 Mice- Interim Report
13) Exploratory Investigation to the Effects of Pregabalin on Red Cell Factors in Female B6C3F1 Mice
14) Exploratory Investigation of the Effects of Pregabalin on Red Cell Factors in Female Wistar Rats
15) Retrospective Evaluation of Hepatic Sinusoidal Endothelial Cells in Cynomolgus Monkeys Chronically Treated with Pregabalin

16) Chronic Investigative Study of Pregabalin in Female B6C3F1 Mice- Interim Report of Tumor Analysis, Clinical Pathology and Pulmonary Function
17) Exploratory Study of the Effects of Pregabalin on Nitric Oxide Synthetase Activity and Isoenzyme Levels in Cultured Mouse Endothelial and Bone Marrow Cells
18) Reanalysis and Refinement of Quantitation of Hepatic Proliferative Indices
19) Immunohistochemical and/or Immunofluorescent Evaluation of Growth Factors in Mice and Rats Given Pregabalin in the Diet for 12 Months

20) Pregabalin: Assessment of Rodent Carcinogenicity, Mode of Action of Hemangiosarcoma Formation in Mice and Human Relevance

21) Retrospective Evaluation of Bone Marrow Megakaryocytes from B6C3F1 Mice and Wistar Rats

22) Retrospective Evaluation of Bone Marrow Megakaryocytes from CD-1 Mice Treated with Pregabalin for 2 Years

23) Retrospective Evaluation of Peripheral Blood Morphology in B6C3F1 and CD-1 Mice Treated with Pregabalin for 2 Years

24) Analysis of p53 and *Ras* Gene Mutation Frequencies in Hemangiosarcomas from a 2-Year Carcinogenicity Study of CI-1008 in B6C3F1 Mice

25) Effect of Pregabalin on Endothelial Cell Proliferation in Monolayer Cultures Exploratory Study of CI-1008 Effects on Vascular Growth of Mouse Aortic Rings

26) Exploratory Study of CI-1008 Effects on the In Vitro Function of Platelets from B6C3F1 Mice, CD-1 Mice and Wistar Rats

27) 1-Month Investigative Study of the Effects of Pregabalin on Platelet Survival in B6C3F1 Mice

28) Exploratory Study of Pregabalin Effects on Endothelial Cell Proliferation and Apoptosis in B6C3F1 and CD-1 Mice

29) 3-Month Exploratory Study of CI-1008 in Female B6C3F1 Mice

30) Reanalysis and Refinement of Quantitation of Hepatic Proliferative Indices

31) 1 Month Exploratory Time-Course Study of Pregabalin on Endothelial Cell Proliferation in Female B6C3F1 Mice

32) Exploratory 4-Week Investigation of the Effects of Pregabalin on Endothelial Cell Proliferation and Platelet Function in B6C3F1 Mice

3.4.5. Carcinogenicity

Study title: 2-Year Dietary Carcinogenicity Study of CI-1008 in CD-1 Mice

Key study findings: Pregabalin administered to CD-1 mice elicited hemangiomas and hemangiosarcomas and changes in the myeloid:erythroid ratio in bone marrow. Significant increases in body weight and body weight gains were associated with treatment. The presence of active compound in the sera of the control animals of both sexes is of concern.

Adequacy of the carcinogenicity study and appropriateness of the test model: The test model is appropriate and the carcinogenicity study appears to have been conducted in compliance with the GLP regulations.

Evaluation of tumor findings: A biologically significant increase in hemangiomas and hemangiosarcomas was noted in a dose-related fashion in both male and female mice in this study.

Study no.: RR 745-03610 or 19836
Volume #, and page #: 33, page 1
Conducting laboratory and location: [redacted]
Date of study initiation: 3/30/00
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: Lot #XH020100
CAC concurrence: No

Methods

Doses: 0, 200, 1000 or 5000 mg/kg/d
Basis of dose selection (MTD, MFD, AUC etc.): Previous study in B6C3F1 mice
Species/strain: CD-1 [redacted] CD-1®(ICR)BR SPF mice from [redacted]
Number/sex/group (main study): 65
Route, formulation, volume: Dietary administration
Frequency of dosing: Daily
Satellite groups used for toxicokinetics or special groups: None
Age: 8 weeks of age at study initiation.
Animal housing: Individual housing in polycarbonate cages with autoclaved sawdust
Restriction paradigm for dietary restriction studies: Not applicable
Drug stability/homogeneity: Drug was admixed in the powdered [redacted] maintenance diet. There was adequate correlation between nominal and achieved dose at all time points tested.
Dual controls employed: No
Interim sacrifices: None
Deviations from original study protocol: High dose females were euthanized during Week 100.

Observation times

Mortality and clinical signs: Twice daily for morbidity and mortality, once/day for clinical signs
Body weights: Weekly for the first 13 weeks, then monthly
Food consumption: Weekly for the first 13 weeks, then monthly
Ophthalmoscopy: Prior to study initiation and every 6 months thereafter
Hematology: All moribund animals and at study termination. Additional thrombocyte counts were performed at Week 65/66 and 78/79 with blood taken from the tail vein.
Gross pathology: All animals at sacrifice and all premature decedents

Organ weights: All terminal sacrifice animals: brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, and uterus
Histopathology: Preserved tissues were transferred to [ ] for slide preparation. All tissues listed were examined from all animals by [ ] by Drs. [ ] A peer review was performed by 2 additional pathologists from Pfizer. It is evident from the individual animal histopathology sheets that there were different study pathologists for males and females.
Toxicokinetics: Samples were taken from the orbital sinus at the end of the dosing period from the first 5 animals/sex/group at approximately 4 hours after the start of the dark cycle (Week 100 for high dose females, Week 104 for all others).

Results
Mortality: No treatment-related significant effects were reported during the first year on study. High dose females were terminated during Week 100 due to high mortality. Dietary administration was stopped for these animals during Week 99. The remaining animals were treated for the duration specified in the protocol.

% Survival for Mice Treated with CI-1008

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose	0	200	1000	5000	0	200	1000	5000
Week 26	95.4	100	100	98.5	100	100	100	100
52	93.8	93.8	92.3	96.9	95.4	96.9	95.4	95.4
78	84.6	84.6	76.9	80.0	75.4	80.0	81.5	67.7
99	64.6	55.4	44.6	52.3	46.2	56.9	52.3	32.3
103	50.8	47.7	41.5	43.1	43.1	46.2	41.5	-
# surviving at term. sac.	33	31	27	28	28	30	27	21

Clinical signs: Swollen/hard abdomens were found in mid and high dose animals when compared to controls.
One mid dose female (V11218) was not given feed for 3 days during Week 46. She was emaciated during Weeks 46-48 but apparently recovered without sequelae.

Body weights: Body weights were increased in treated animals during the first year on study when compared to controls but no dose-relationship was appreciated. No significant differences from controls were reported during the second year on study. Overall, body weight gains were increased for the treated animals 29-62% when compared to controls but not in a dose-related increment. Statistical significance was reached for all groups.

Food consumption: Feed consumption was • 16% higher in treated animals when compared to controls, especially for the final year on study.

Ophthalmoscopy: No treatment-related differences from controls were observed.

Hematology: Platelet counts were increased in dosed males (16, 10 and 32%, respectively) and females (6, 13 and 24%, respectively) at the 24 month evaluation only, and increased platelet volumes were also reported in all males and mid and high dose female groups.

Myeloproliferative or lymphoproliferative disorders are not uncommon findings by the end of carcinogenicity studies. Primary thrombocytosis is an unusual finding and is usually reported when the test article is a hematopoietic growth factor. Reactive or secondary thrombopoiesis may be secondary to catecholamine-induced splenic contraction or generalized bone marrow stimulation (as in hemolytic anemia), blood loss or other etiologies. While increased platelet production may be demonstrated as younger, larger platelets, these may cause an increased platelet volume (MPV), but changes in MPV are usually inconsistently present. The lifespan of platelets in circulation is relatively short (~ 5 days in rats and mice) and the platelet production time from megakaryocytes is also relatively short (4-5 days). In general, increased MPV indicates marrow responsiveness. Many xenobiotics have been shown to elicit immune-mediated platelet destruction (Aster in Comprehensive Toxicology, Vol. 4, pp. 263-284, Elsevier Science, Oxford). No clinically significant alterations in coagulation parameters or increases in thromboembolic events were reported. Thus, the biological significance of these platelet findings is uncertain.

MCH was increased (<4%) in high dose males and MCV was increased (• 5%) in mid dose males and all high dose animals. These increases are not considered biologically significant.

Platelet aggregation was decreased by 12 months in the high dose animals. No differences from controls were appreciated after 6 months of dosing. The alterations noted were characterized by defective secondary aggregation. This may be due to depleted ADP content and indicative of increased platelet activation and release of growth factors. Similar alterations were reported in the B6C3F1 carcinogenicity study at doses of 200 and 1000 mg/kg/d starting after 6 months of dosing. No comparable findings were found in the rat studies (18 or 24 months) or the monkey study (69 weeks). While changes in some but not all of the platelet and megakaryocyte parameters were found in the 200 mg/kg/d animals in both the B6C3F1 and CD-1 mice, there was an increased incidence of hemangioma/hemangiosarcoma at this dose. At the clearly carcinogenic 1000 and 5000 mg/kg/d doses, there was good correlation between these effects. This finding may be supportive of an indirect carcinogenic effect but determining a definitive species specific mode of action would be extremely difficult. Additionally, in the "investigational" carcinogenicity study where the animals were treated for 12 or 24 months, an increased incidence of hemangiosarcomas was reported in both groups in spleen and bone marrow.

Organ weights: Males: Mean brain/body weight ratios were decreased in the mid and high dose groups. Absolute and relative liver weights were increased in the same groups. The mean absolute testes weights were decreased in the high dose males and the relative testes: body weight ratio and prostate: body weight ratio were decreased in the mid and high dose males.

Females: Increased mean and absolute heart weights were increased in low and mid dose and relative heart weights were increased in the high dose. An increase in absolute and relative liver weights was appreciated in all dosed females and an increase in absolute and relative kidneys weights were found in mid and high dose animals.
Decreased ovarian weights were reported for the mid and high dose females but were correlated with decreased numbers of ovarian cysts in these animals so are not considered biologically significant.

Gross pathology: Gross lesions were generally correlated with the histologic findings.

Histopathology: In the pathologist's report for the male mice, there is an unusual statement: "A certain degree of autolysis was considered to be common in mice that died during the study, and therefore, was not recorded. Autolysis was only recorded if it severely impaired or prevented microscopic evaluation." Additionally, they state that "malignant lymphoma, histiocytic sarcoma and hemangiosarcoma often involved more than one organ in an animal but was counted only once under "Hemolymphoreticular system." It appears that they 'split' "malignant lymphomatous infiltration" from "lymphoid cell infiltration" as whether they determined the infiltration was "clearly" neoplastic or not. In addition, "infiltration of an organ by histiocytic sarcoma was termed "histiocytic sarcomatous infiltration", while hemangiosarcoma was termed "hemangiosarcoma, multicentric."

Non-neoplastic: An increased incidence of pulmonic lesions, to include alveolar macrophage infiltration with associated findings (cholesterol cleft, lymphoid infiltration) was noted in high dose females.

Neoplastic:

	Males				**Females**			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Hemangiosarcoma#	104	79	72	56	47	69	48	45
Lung	0	0	0	1/ 1.5*				
Liver	1/ 1.5	5/ 7.7	4/ 6.2	8/ 12.3	1/ 1.5	0	2/ 3.1	5/ 7.7
Spleen	1/ 1.5	1/ 1.5	2/ 3.1	4/ 6.2	1/ 1.5	0	2/ 3.1	1/ 1.5
Bone marrow	0	0	1/ 1.5	8/ 12.3	1/ 1.5	0	0	0
Lymph node	0	0	1/ 1.5	0				
Skin	0	0	0	3/ 4.6	0	0	1/ 1.5	0
Ovaries					1/ 1.5	0	0	1/ 1.5
Uterus					3/ 4.6	9/ 14	6/ 9.2	7/ 10.7
Total hemoreticular hemangiosarcoma	**2/ 3.1**	**5/ 7.7**	**6/ 9.2**	**14/ 21.5**	**7/ 9**	**9/ 14**	**10/ 15**	**14/ 24**
Hemangioma								
Liver	0	1/ 1.5	0	0				
Testes	0	0	0	1/ 1.5				
Bone marrow								2/ 3.1
Uterus					0	1/ 1.5	1/ 1.5	0
Total hemangioma and hemangiosarcoma	**2**	**6**	**6**	**14**	**7**	**10**	**11**	**15**

Time of first diagnosis in weeks

*Number of animals/% of animals with tumor

From the statistical review by R. Kelly, M.S.:

	[illegible]	CONTROL	LOW	MED	HIGH
59-78	1	0	1	0	3
59-78	2	6	9	9	9
79-91	1	0	3	4	1
79-91	2	6	7	7	10

92-103	1	0	0	2	2
92-103	2	16	8	12	6
FINALKILL104-105	1	2	3	2	5
FINALKILL104-105	2	31	29	26	24
72	1	0	0	1	0
72	2	59	56	52	55
77	1	0	0	0	1
77	2	56	56	51	52
93	1	0	0	0	1
93	2	48	41	41	37
99	1	0	0	0	1
99	2	43	36	30	33
101	1	0	1	0	0
101	2	41	34	28	31
Total		2	5	6	14

Table 1 above is incidence table for hemangiosarcoma of hemolymphoreticular system in male mice. First row of each time interval in the number of tumor bearing animals. The second row is the number of animals without tumor but were at risk (which is the number of animals that died without that tumor during this interval for incidental tumors and the number of animals at the beginning of that week for fatal tumors). E.g.: for time interval 53-78 weeks, the percentage of hemangiosarcomas were 0/6 1/7 0/9 and 3/12, for control to HD respectively.

Table 2: Male mice, combining hemangiosarcomas with hemangiomas regardless of tissue:

Organ Code	Organ Name	Tumor Code	Tumor Name	CTRL	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
1800	[C]LIVER	999	Hemangioma	2	6	6	14	0.0008	0.0004

For the above tables, the p-value from the asymptotic is the more relevant one. However, results are consistent with either approach, i.e. all are statistically significant.

For Female Mice on Pregabalin

Table 5: Female Mice Detailed Table for Hemangiosarcoma in Hemolymphatic System

Time Interval	Table Rows	CTRL	LOW	MED	HIGH
0-52	1	1		0	0
0-52	2	0		2	2
53-78	1	2		1	2

53-78	2	11	11	9	14
79-99	1	2	0	1	1
79-99	2	16	14	16	20
FINALKILL100-105	1	1	7	6	5
FINALKILL100-105	2	29	29	28	16
45	1	0	0	0	1
45	2	65	63	65	63
47	1	0	0	1	0
47	2	65	63	64	62
62	1	0	0	0	1
62	2	60	60	56	58
65	1	0	0	0	1
65	2	59	57	56	57
69	1	0	1	0	0
69	2	53	53	55	52
77	1	0	0	0	1
77	2	49	53	54	45
81	1	0	0	0	1
81	2	48	51	49	43
90	1	0	0	1	0
90	2	44	43	42	33
92	1	0	0	1	0
92	2	43	41	41	31
95	1	0	0	0	1
95	2	39	39	41	27
101	1	0	1	0	0
101	2	28	35	33	0
Total		6	9	10	13

p-value did not reach statistical significance with 0.0122 versus alpha 0.005

Table 6: Female Mice Combined Hemangiosarcoma with Hemangioma Regardless of Tissue:

Organ Code	Organ Name	Tumor Code	Tumor Name	CTRL 0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
34007	C UTERUS	999	Hemangioma	7	10	11	15	0.0092	0.0068

Approach statistical significance with 0.0068 vs. alpha 0.005

In the retrospective "look" into the femoral bone marrow of the mice on study, a significant change in marrow myeloid:erythroid ratio was described in the pregabalin-treated animals. The treated animals showed a predominantly erythroid environment compared to the controls that had a predominantly myeloid environment.

Toxicokinetics: In a separate report (RR-MEMO 764-03532), the sponsor presented data to

demonstrate that only 2 of 5 sex plasma samples analyzed from control mice (4/10 total tested) were below the level of quantitation. Reanalysis of the samples confirmed the presence of drug and no analytical explanation was demonstrated. In control males, the values were [ ] • g/mL, and in control females the values were [ ] • g/mL. Overall, it appears that this variability persisted through all dose groups (high dose male values were [ ] • g/mL; high dose female values were [ ] • g/mL). This brings into question the validity of the results. The only reasonable conclusion to be drawn from these data is that the mice were exposed to drug at some level.

The sponsor concluded that there was no carcinogenic effect of administration of CI-1008 in the diet to CD-1 mice for 2 years. This is not a reasonable conclusion from the data presented in this submission.

Study title: **2-Year Dietary Oncogenicity Study in Rats with CI-1008**

Key study findings: Test compound administration had significant effects on body weight, body weight gain and minor histopathologic findings. No evidence of carcinogenic activity was seen in this study in Wistar rats at doses of up to 450 mg/kg/d in males and 900 mg/kg/d in females.

Adequacy of the carcinogenicity study and appropriateness of the test model: The study was adequate and appropriate.
Evaluation of tumor findings: No treatment-related tumors were found.

Study no.: RR 745-03274 or — 116-219 or 2235
Volume #, and page #: 40, page 1
Conducting laboratory and location: [ ]
Date of study initiation: 7/17/97
GLP compliance: Yes
QA report: yes
Drug, lot #, and % purity: Lots # XH411095, XH350995, XH2200399 and XH210398
CAC concurrence: No

Methods

Doses: 0, 50, 150 or 450 mg/kg/d in males, and 0, 100, 300 and 900 mg/kg/d in females
Basis of dose selection (MTD, MFD, AUC etc.):
Species/strain: [ ] (WI)BR VAF/Plus® (Wistar) rats
Number/sex/group (main study): 65
Route, formulation, volume: Dietary administration with fresh chow prepared weekly
Frequency of dosing: Daily
Satellite groups used for toxicokinetics or special groups: None
Age: 8 weeks of age at study initiation
Animal housing: Individually in stainless steel cages with wire mesh floors
Restriction paradigm for dietary restriction studies:
Drug stability/homogeneity: Samples were analyzed weekly during the first 4 weeks on study and every month thereafter. All samples were within • 11% of theoretical dose.
Dual controls employed: No
Interim sacrifices: None

Observation times

Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Pre-study, weekly for 13 weeks, monthly thereafter
Food consumption: Weekly
Ophthalmoscopy: Pre-study and at 6 month intervals
Hematology: Erythrocyte and leukocyte counts were taken from survivors at study termination
Histopathology: Peer review: Yes to include livers from all animals and all neoplasms. All tissues from all animals were evaluated microscopically. No animals were lost to autolysis. This is an unusual finding in carcinogenicity studies.
Toxicokinetics: Near the end of the treatment period, blood was collected 7 hrs. into the dark cycle from the first 5 surviving animals/group.

Results
Mortality: Survival was comparable across dose groups.

Group	Dose (mg/kg/d) Males	Females	Survivors Males	Females
1	0	0	38	34
2	50	100	38	36
3	150	300	46	48
4	450	900	45	33

Clinical signs: Clinical signs were comparable across groups except for dose-related urogenital staining in all dosed females. Thin appearances and pallor were reported for high dose males and females but obesity was noted in the mid dose animals.

Body weights: High dose animals had significantly reduced body weights throughout the study. Mean body weights of males were decreased 13% and females by 24% when compared to controls at study termination. Mid dose animals' weights were comparable to controls. Low dose animals had higher body weights throughout the study when compared to controls.

Food consumption: Feed consumption was decreased in the high dose animals throughout the study (9% for males, 13% for females). In the low dose animals, consumption was higher at many time points.

Hematology: No biologically significant treatment-related effects were detailed.

Ophthalmoscopy: Keratitis with neovascularization was noted in mid and high dose males from mid-study until study termination. In females, the finding was only reported in a few high dose females at study termination. It is unclear if this is a treatment-related finding but progression to keratoconjunctivitis sicca was not appreciated so the biological significance is questionable.

Gross pathology: Small testes were reported for all treated males with no evident dose response. Dose-related increases in the incidence of dilated/distended uterine horns were reported for the high dose females.

Organ weights: Significant decreases in epididymal and testicular weights (both relative and

absolute) were reported for the high dose males. All other findings, even when statistically significant, were related to the decreased lower mean final body weights.

In females, a statistically significant increase in uterus/cervix: body weight was found at 900 mg/kg/d. Absolute uterine weights were also increased but did not reach statistical significance. All other findings, even when statistically significant, were related to the decreased lower mean final body weights.

Pituitary weights were decreased for mid and high dose animals of both sexes due primarily to the decreased incidence of pituitary adenomas and hyperplasia in these groups when compared to controls.

Histopathology:

Non-neoplastic: Testicular effects to include small testes, decreased relative and absolute testicular weights correlated with the seminiferous tubular atrophy and aspermatogenesis noted in high dose males. The sponsor attributed these changes to normal aging changes in rats. However, the incidence and severity were increased in the high dose males so the normal changes are considered exacerbated by treatment. The sponsor separately considered unilateral and bilateral changes so concluded that there was no treatment-related effect. However, the lesions were usually seen in physiologically compromised animals so it is unclear whether it's a direct toxic effect or secondary to the physiologic alterations.

Dose group	**1**	**2**	**3**	**4**
Testis-atrophy- bilateral	15	25	36	26
Trace	4	6	11	6
Mild	1	6	5	2
Moderate	3	6	6	5
Severe	7	7	13	13

Similarly, the bilateral retinal atrophy noted in mid and high dose females is considered an exacerbation of the normal aging in rats. It was only reported in the last quarter of the study.

Uterine dilatation was reported in high dose females (11 of moderate severity, 10 of severe degree), reportedly associated with "intrauterine hemorrhage of unknown origin." Ovarian atrophy was an associated finding as was active inflammation. There was no evidence of neoplastic change associated with this finding.

Neoplastic: Although the Peto analysis showed a positive trend for malignant meningioma in males and squamous cell carcinoma of the skin in females, the incidence is within historical range and no dose-relationships were appreciated.

Due to the findings in the mouse carcinogenicity study, the following information for tumors of the hemolymphoreticular system is provided.

Sex	**Male**				**Female**			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
# exam.	65	65	65	65	65	65	65	65
Hemangioma	5	2	4	1	1	2	1	0

Hemangiosarc.	2	1	2	1	3	0	1	2
Total neoplasms	7	3	6	2	4	2	2	2

Toxicokinetics: In a separate report (RR-MEMO 764-03486), the sponsor presented data to demonstrate that only 4 of 5 sex plasma samples analyzed from control mice (6/10 total tested) were below the level of quantitation. Reanalysis of the samples confirmed the presence of drug and no analytical explanation was demonstrated. In the control male with drug above 0, the value was — • g/mL, and in control female, the value was — • g/mL. Overall, it appears that, as in the CD-1 mouse study, variability persisted through all dose groups (high dose male values were — • g/mL; high dose female values were — • g/mL).

In yet another separate report (RR-MEMO 764-03964), the sponsor provided data to support the conclusion that exposure to pregabalin increased with increasing dose and that exposure in females was consistently higher than in males. The sponsor concluded that: "Exposures at these dose levels in males were 1.3, 5 and 14 times the human exposure at the maximum clinical doses of 600 mg daily (— g.hr/mL) and in females were 2.5, 8 and 24 times the estimated human exposure." Given the drug detected in controls and the variability of the results for each dose group, the only reasonable conclusion is that the animals were exposed to drug at some level.

3.4.8 Special toxicology studies

Study title: Retrospective Histopathologic Evaluation of Non-neoplastic Findings in Selected Tissues from B6C3F1 Mice Treated with CI-1008 for 2 Years

Key study findings: Increased extramedullary hematopoiesis and increased thrombopoiesis and hepatic sinusoidal cell hyperplasia were reported as treatment-related lesions in this study. However, these findings were reported to be of "limited severity" and no consistent dose-relationship was appreciated.

Study report no.: RR-MEMO 745-03454
Conducting laboratory and location: Pfizer Global Research, Ann Arbor, MI
Date of study initiation: "Periods covered 5/22/01- 9/28/01"
GLP compliance: Not necessary
QA reports: No
Drug, lot #, and % purity: CI-1008, Lots XH200399, XH350995, XH411095 and XH210398

Methods: This retrospective "look" at the non-neoplastic findings in selected tissues from the mouse carcinogenicity study was done to evaluate the changes in hematopoietic cells and the vasculature. It was done to help select endpoints for further studies with the test compound.

In the original interpretation of the histologic slides, a biologically and statistically significant increase in the incidence of hemangiomas and hemangiosarcomas was found in the 1000 and 5000 mg/kg/d male and female mice. When they retrospectively looked at the bone marrow sections from those animals, a dose-related increase in megakaryocytes was noted.

In the current evaluation, the sponsor's pathologist reevaluated sections of bone marrow, kidney, liver, lung, lymph node, and spleen from all animals on study.

Results:

Upon re-review of the tissues, the following tables were generated by the sponsor:

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Megakaryocytic hypercellularity	N= 61	N=60	N=59	N=53	N=62	N=60	N=52	N=48
Minimal	12	18	13	12	10	13	17	19
Mild	3	6	18	25	2	3	8	9
Moderate	0	1	8	9	0	1	2	5
Splenic extramedullary hematopoiesis	N=61	N=59	N=58	N=51	N=60	N=62	N=51	N=53
Mild	7	5	6	19	7	9	10	13
Moderate	5	3	10	7	7	5	9	7
Severe	0	1	4	0	3	4	8	6

N= number of animals evaluated

Their conclusions were that the following were drug-related phenomena:

1) Increased thrombopoiesis
2) Increased extramedullary hematopoiesis
3) Hepatic sinusoidal cell hyperplasia
4) Pulmonary macrophage infiltrates/granulomatous inflammation

They considered these non-neoplastic findings of limited severity. Similar changes were noted in controls but of lesser incidence and/or severity. They stated that these changes are indicative of "self-limited cellular proliferation" and "indirectly linked to the pathogenesis of hemangiosarcoma".

While their conclusions are interesting, it would be necessary to do further studies to "prove the hypothesis". Additionally, it is a significant concern that these "lesions" were not reported in the original study report, even though a peer review was reportedly performed.

Study title: **Analysis of Hemangiosarcoma and Liver Samples from the CI-1008 B6C3F1 Mouse Carcinogenicity Study for the Presence of *Heliobacter hepaticus* DNA**

Key study findings: None of the samples were positive for the presence of *H. hepaticus*.

Study no.: RR-MEMO 745-03324
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
GLP compliance: Not necessary

Methods: This analysis was performed on preserved tissues from the 2 year carcinogenicity study in B6C3F1 mice. The purpose was to determine if *H. hepaticus* was present in the hemangiosarcomas and/or liver sections from that study. In the literature, there are references to *H. hepaticus* as the etiologic agent involved in an increased incidence of hepatic hemangiosarcomas in mice.

A total of 45 tumor samples were analyzed: 1/sex/control group, 1 low dose female, 4 males and 5 females from the mid dose and 20 males and 13 females from the high dose groups. Of these,

the majority (40) were hepatic hemangiosarcomas. The remainder were hemangiosarcomas from other sites that were not expected to contain the microbe.

Tumor cells or liver samples were dissected from sections by [redacted]
DNA was extracted and the [redacted] products were purified. PCR was performed to detect the presence of *H. hepaticus* DNA,
Results: None of the samples was positive for the presence of *H. hepaticus*. This eliminates one of the hypotheses for *H. hepaticus* as the etiologic agent for the hepatic hemangiosarcomas in mice from the 2 year bioassay.

Study title: **Exploratory Study of Pregabalin on Megakaryocyte Development in Female B6C3F1 Mice**

Key study findings: Pregabalin at 1000 • g/mL in cultured cells has some inhibitory effect on cell growth in vitro.

Study no.: RR-MEMO 745-03461 or Protocol # AA3045
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
This study was initiated on 9/4/01

Methods: The sponsor determined that increased megakaryocytosis in bone marrow and increased platelet counts seen in the B6C3F1 mouse studies may be "key elements" in the elicitation of hemangiosarcomas. To prove this hypothesis, they used isolated bone marrow cells from female B6C3F1 mice.

Doses: 0 (media without pregabalin), 10, 100 and 1000 • g/mL of pregabalin, lot #XH020100 at [redacted] purity. The 10 • g/mL dose was calculated to be approximately 6x the minimally effective concentration in human plasma (1.6 • g/mL that is the intended clinical target concentration).

Study design: There were 2 phases to this study:
1) Short term bone marrow cultures: Bone marrow cells [stromal cell depleted] (3x 10^5 cells/mL) were incubated with 25 ng/mL thrombopoietin (TPO) and 12.5 ng/mL stem cell factor (SCF) for 5 days to expand the megakaryocyte lineages. The number of megakaryocytes (CD41+) and DNA content were measured by flow cytometry. The number of total nucleated cells (TNC) was measured. Cells were then incubated for 5 days with pregabalin.

2) Long term bone marrow cultures: Whole bone marrow cells (5x 10^5 cells/mL) were incubated for up to 3 weeks with pregabalin. TNCs were measured. The suspension-phase and adherent-phase cell portions were determined separately and then combined to evaluate the effects of pregabalin on megakaryocyte counts and cell ploidy (DNA content) by flow cytometry.

Results: The concentration of pregabalin remained essentially constant throughout the incubation periods as evidenced by measurement of supernatant media.

1) Short term assay- The CD41+ cells were approximately 21.9% of the total population in control cultures after 5 days of incubation. The majority of these cells were immature with DNA content • 4C. There was a statistically significant decrease (13.9% of total population) in the high dose and the TNC counts were also significantly decreased (10.9 x 10^5 for controls, 7.3x 10^5 for high dose). This may suggest an inhibition of growth but

it occurred only at the highest dose tested.

2) Long term assay: Incubation of control cultures showed significant increases in TNC that peaked at 2 weeks and increased numbers of mature megakaryocytes (47% at 48 hrs, 84% at Week 3). Addition of pregabalin did not increase the megakaryocyte numbers or maturation of these cells. Dose-related decreases (37-80%) were appreciated in TNC but statistical significance was only reached at the highest dose. The percentage of megakaryocytes at the end of the 3 weeks was decreased by >50% but did not reach statistical significance.

Taken together, it appears that pregabalin has an inhibitory effect on cell growth at 1000 • g/mL but the biological significance of this finding is uncertain.

Study title: **Exploratory Study of the In Vitro Effects of Pregabalin on B6C3F1 Mouse Spleen Endothelial Cell Proliferation**

Key study findings: Direct and/or indirect exposure of splenic endothelial cells to pregabalin did not elicit *in vitro* proliferation.

Study no.: RR-MEMO-745-03769 or Protocol AA3033
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI

Methods: The purpose of this study was to determine if pregabalin had a direct and/or an indirect effect on spleen endothelial cell (SEC) proliferation.

SEC cultures from female B6C3F1 mice were prepared and monolayers were grown. After 7-9 days, the cells were removed from the culture flasks and resuspended. The suspensions were plated into microtiter plates and allowed to adhere for 18 hrs.

Study design: To measure the potential of a direct effect of pregabalin, the growth medium was exchanged for medium supplemented with ^{14}C-thymidine, and 0, 1, 10, 50, 100, 500, 750 or 1000 • g/mL pregabalin. This study was duplicated but the amount of fetal calf serum in the medium was 5x greater. Cultures were harvested after 72 hours and levels of ^{14}C-thymidine were measured. There were 4 wells/dose and each dose was repeated 4 times.

To measure the potential indirect effect of pregabalin on SEC proliferation, 5 female B6C3F1 mice were fed diet containing 0, 200, 1000 or 5000 mg/kg pregabalin for 2 weeks. The animals were euthanized, serum samples taken and SEC cultures were created as above. On the day of the experiment, ^{14}C-thymidine and 2.5% serum from the treated animals was added to the medium. Cultures were incubated for 72 hours and radioactivity was quantitated. As above, 4 wells/experiment and 4 repetitions were performed.

Results: Increasing the fetal calf serum concentration to 20% elicited a 2x increase in SEC when compared to controls, thus validating the procedures.

In the direct action portion of the study, neither biologically nor statistically significant differences from controls were appreciated at any dose tested.

In the indirect portion of the study, neither biologically nor statistically significant differences from controls were appreciated at any dose tested.

Study title: Analysis of [^{3}H]Pregabalin Binding and α2•••Protein Expression in Selected B6C3F1 Mouse and Wistar Rat Tissues

Key study findings: No high affinity binding of pregabalin was found to rat bone marrow, mouse bone marrow, or mouse SEC cells.

Study no.: RR-MEMO-745-03740
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI

Methods: This study was performed to determine if pregabalin interacts directly with mouse or rat membrane proteins for endothelial cell tumor development. The tissues were taken from male and female B6C3F1 mice and male Wistar rats. Blood samples were taken from the same animals. Plasma was separated from the blood samples as were platelets. The platelets were pelleted and frozen until the membranes were fractionated (for binding assays) or lysates (for Western blot analysis).

[^{3}H]Pregabalin binding assay: A [ ] Assay [ ] was used on the bone marrow membrane preparations from mice and rats and SEC membranes. Competition binding was carried out on the rat membranes using a fixed concentration of radioactive pregabalin competed by 7 concentrations of unlabeled pregabalin from 0.001 to 10 • M.

Results: Pregabalin binds to α2••1 cell membranes with high affinity with results consistent with a single binding site.
In the competitive binding assay, there was no specific binding of [^{3}H]pregabalin to rat bone marrow. Specific binding to positive control porcine α2••1 exceeded 90% of total binding. In the mouse bone marrow and SEC pellets, no specific binding was noted across the dose range tested but nonspecific binding was increased • 125x at the highest dose tested.

Thus, no high affinity binding of pregabalin was found to rat bone marrow, mouse bone marrow,

or mouse SEC cells.

Study title: **Exploratory 1-Week Investigation of the Effects of Pregabalin on Endothelial Cell Factors in Platelet-Depleted B6C3F1 Mice**

Key study findings: While statistically significant increases in platelet activation were reported in the non-platelet depleted animals when compared to appropriate controls, no biological significance can be attributed to this isolated finding.

Study no.: RR 745-03762 or Protocol AA2990
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI

Methods: The purpose of this study was to determine the effects of pregabalin on endothelial cell proliferation and platelet activation after 1 week of treatment in the diet with a "carcinogenic dose" of pregabalin in platelet-depleted B6C3F1 mice.

Doses: Diet control/non-platelet depleted vehicle control, diet control/platelet depleted vehicle control, 750 mg/kg pregabalin/non-platelet depleted vehicle control and 750 mg/kg pregabalin/platelet depleted animals. The intended dose was 1000 mg/kg but the actual intake provided only 750 mg/kg/d as a dose.

Study design: On Day -1 as well as Days 2, 4 and 6, all platelet depleted animals were administered 0.1 mL of neuraminidase in 1% normal female B6C3F1 mouse serum by i.p. injection to ensure platelet depletion. Non-platelet depleted animals were administered 0.1 mL sterile saline in 1% normal female B6C3F1 mouse serum on the same days.

Animals were observed for clinical signs, body weight, and feed consumption. Blood samples were taken on Day -2, Day 1 prior to drug exposure and on Day 7 for platelet counts. Samples were taken from the "pedal vein". On Day -1 and Day 3, samples were taken for evaluation of platelet activation. On Day 3, osmotic pumps containing BrdU were implanted subcutaneously. On Day 7, all animals were perfused with physiologic saline. Necropsies were performed and liver samples were collected. Routine histopathology was performed. The first 5 livers/group were assessed for proliferation of endothelial cells, Kupffer cells, and hepatocytes by morphometry for BrdU specific incorporation and a BrdU labeling index.

Results:
No significant differences from controls were appreciated in clinical signs, body weight or feed consumption. Mean body weight gains were increased in the pregabalin-treated animals. The actual drug intake was approximately 75% of the intended dose (1000 mg/kg).

On Day 1, platelet counts in depleted animals were decreased ~86% when compared to non-platelet depleted controls.

Mean platelet activation was 26% in non-platelet depleted, pregabalin-treated animals when compared to non-platelet depleted controls on Day 3. This was a statistically significant increase but it was of questionable biological significance. In the platelet depleted animals, platelet activation could not be evaluated.

In the platelet depleted controls and the pregabalin-treated animals, there was a significant mean

increased in platelet microparticles.

Mean hepatic endothelial cell proliferation was increased 15% (not statistically significant) in the non-platelet depleted pregabalin-treated mice, and by 31% (statistically significant) in the platelet-depleted pregabalin-treated mice when each was compared to their appropriate controls. Additionally, mean hepatic endothelial cell proliferation in platelet-depleted, dietary controls was increased 11% when compared to non-platelet depleted dietary controls and increased proliferation by 28% was found in platelet depleted pregabalin animals when compared to non-platelet depleted pregabalin animals.

Mean hepatocyte proliferation was increased in non-platelet depleted and platelet depleted pregabalin-treated animals when compared to respective dietary controls. However, no significant histologic alterations were appreciated in the B6C3F1 carcinogenicity studies so the biological significance of these findings is undetermined at this point.

No significant drug-related effects were appreciated on Kupffer cells, gross necropsy findings or histologic alterations in livers of pregabalin-treated animals, regardless of platelet status.

Study title: **Chronic Investigative Study of Pregabalin in Female Wistar Rats**

Key study findings: Pregabalin did not elicit hepatic endothelial cell proliferation, megakaryopoiesis, or effects on platelet structure or function in Wistar rats treated for up to 18 months in the diet.

Study no.: RR-MEMO 745-03463 and RR745-03763
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 8/22/01 to 2/17/03" and "8/22/01 to 8/18/02"
GLP compliance: Exploratory study so not required
Drug, lot #, and % purity: Lot XH020100

Doses: 0 or 900 mg/kg/d in the diet "to coincide with the maximum dose used in the previous rat carcinogenicity study".

Study design: Sixty female Wistar rats/group were given 0 or 900 mg/kg/d of pregabalin admixed in the diet for up to 18 months.

Four days prior to necropsy, 10 rats/group (months 1 and 3) or five control and 5 treated rats were implanted with osmotic pumps were evaluated via BrdU labeling at 6, 12 and 18 months of treatment to determine mitogenic effects potentially relevant to hemangiosarcoma induction by pregabalin. This was done by evaluating proliferation of liver sinusoidal endothelial cells, hepatocytes and Kupffer cells.

To determine the potential effect of pregabalin on specific growth factors (vascular endothelial [VEGF], platelet derived [PDGF] and thrombopoietin [TPO]), these factors were measured in platelet-rich and/or platelet-poor plasma from 10 rats/group after 3 months and 18 months. VEGF exerts biological effect by binding to tyrosine kinase receptors; Flk-1 is expressed on endothelial cells, and bFGF is an angiogenic factor expressed on platelets, white blood cells, stromal cells in several tissues and on megakaryocytes.

Additionally, platelet samples were taken from 5/group during Month 2 for ultrastructural analysis. Platelet aggregation studies were conducted on whole blood and platelet rich plasma from 5/group after 1 month of dosing and in platelet rich plasma only after 6, 12 and 18 months on study. Platelet counts were determined at the same time points. P-selectin (a measure of platelet activation) was evaluated in plasma from 5/group after 6, 12 and 18 months on study. Bone marrow from 5/group was evaluated by flow cytometry at the same times.

"Cursory gross necropsies" were performed at designated time points and liver, lung, spleen, and bone marrow were examined histologically from 10 control and 8 treated animals after 1 month and 10 or 5/group after 3 and 6 months, respectively. After 12 months, lung and liver were examined from 10/group and spleen and bone marrow were evaluated from 5/group.

Results: Clinical signs to include urine staining, fecal changes (primarily reduced/absent feces), tail injuries and reduced body weight (29% less than controls) were appreciated in the pregabalin-treated animals. One control and 3 treated animals were euthanized in moribund condition.

During the first 12 months on study, pregabalin elicited decreased bone marrow cellularity (all cell lines). By the end of the study, the bone marrows were comparable across groups, probably due to age-related hypocellularity. Increased fatty infiltrate into the bone marrow correlated with the hypocellularity. Total nucleated counts were 40% and 36% less than controls at 6 and 12 months, respectively, but by 18 months, the counts were comparable to controls.

At the 18 month evaluation, increased (• 17%) red cell parameters (counts, hemoglobin, and hematocrit) were noted in the treated animals. No differences from controls were noted at earlier evaluations. However, mean reticulocyte counts were decreased 19% and 35% at the 12 and 18 month time points, respectively.

While platelet counts did not differ between the groups, mean platelet volume was increased up to 14%, 17% and 35% by the 6, 12 and 18 month evaluations, respectively. No biologically relevant effects of pregabalin were found in platelet ultrastructure, platelet activation or platelet aggregation.

No significant treatment-related effects were found in endothelial cell, hepatocyte or Kupffer cell labeling or in VEGF, PDGF or TPO or in 8-hydroxydeoxyguanosine concentration (marker of oxidative stress) in pregabalin-treated rats when compared to controls.

The sponsor contends that the homeostatic relationship between platelets, endothelial cells, platelet derived cytokines and growth factors indicates a causal link between the platelet effects and the incidence of hemangiosarcomas in mice. While the mouse carcinogenicity study demonstrated increased mean hepatocyte proliferation in non-platelet depleted and platelet depleted pregabalin-treated animals when compared to respective dietary controls, no significant hepatic histologic alterations were appreciated in the B6C3F1 carcinogenicity studies so the biological significance of these findings is undetermined at this point. In the Wistar rat, no effects were appreciated on endothelial cell proliferation or hemangiosarcoma elicitation. Therefore, they conclude that the responses of the Wistar rat are distinct from those in B6C3F1 mice. While the conclusions are reasonable, given the lack of significant hepatic lesions seen in the mice, the biological significance of the findings remains to be clarified as does the choice of the appropriate species to make the extrapolation to humans.

The sponsor also contends that "induced cell proliferation is universally accepted as the common denominator in epigenetic carcinogenesis in rodents, regardless of target tissue." While generally this is a true statement, the increased hepatocyte proliferation seen in the mouse studies did not result in histologically altered tissues. Thus, it is unclear whether the endothelial proliferation and the hepatocyte proliferation seen in the mouse studies identified by BrdU labeling and not histologic alteration are directly related to the development of hemangiosarcomas.

Study title: **[^{3}H] Pregabalin Does Not Bind to Membrane Proteins of Mouse or Rat Platelets**

Key study findings: High affinity binding of tritiated pregabalin to the α_2-••1 cell membrane did not occur. Low affinity binding has not been ruled out.

Study no.: RR 740-03614
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: 8/1/03

Study design: The purpose of this study was to determine if tritiated pregabalin directly binds to membrane proteins of mice or rats with high affinity. Pregabalin binds specifically to α_2-••1 (Ki= 42 nM) and α_2-••2 (Ki= 44 nM) receptor subunits of calcium channels, according to the sponsor's analysis. They also state that pregabalin does not bind to $GABA_A$ or $GABA_{B2A}$ receptors in vitro.

Platelets from male and female B6C3F1 mice (samples pooled) and male Wistar rats were collected and pelleted, then frozen until membrane fractionation for binding assays. Once the membrane fractionation process was completed, a [ ] Assay was performed using [ ] Membranes from HEK cells expressing α_2-••1 cDNA were used for the competitive inhibition. Seven concentrations of unlabeled pregabalin (0.001-10 • M) were tested. Tritiated pregabalin at 60 nM was used to saturate binding sites. Samples were run in triplicate experiments.

Results: No specific binding of the tritiated pregabalin was noted to either species' platelets at competitive pregabalin levels up to 10 • M. However, the tritiated pregabalin was highly bound to recombinant α_2-••1 cell membranes..

Unfortunately, the low level of tritiated pregabalin in the study did not allow for evaluation of low affinity binding. Therefore, the sponsor performed saturation binding and Scatchard plot analysis using platelet membranes from mice to evaluate the potential. They did not detect specific binding at any concentration from 5-2500 nM of the tritiated product tested. This does not rule out the presence of a low affinity binding interaction between the drug and platelets.

Using the HEK cells to validate the variability of the ligand, specific binding of tritiated pregabalin to porcine α_2-••1 protein was examined. Specific binding was >90%. The saturation binding experiment correlated with a single exponential, consistent with a single binding site. The sponsor suggests that since the dissociation constant is similar to that of tritiated gabapentin that they bind to the same recombinant porcine α_2-••1 cell membranes.

Study title: **Exploratory Investigation of the Effects of Pregabalin on Endothelial Cell Factors in Female CD-1 Mice, Interim Report**

Key study findings: Due to the variability seen in the data and the inconsistent findings at 6 months, no definitive conclusions can be reached concerning the significance of the data when extrapolated to humans. Growth factor analyses were inconsistent and no consistent dose relationship was found. No biologically significant differences from controls were noted in any of the dose groups with respect to endothelial cell proliferation.

Study no.: RR-MEMO- 745-03659
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 4/1/02 to 4/4/03"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: The objective of this study was to compare the effects of dietary administration of pregabalin to female CD-1 mice to female B6C3F1 mice with respect to endothelial cell factors.

150 female CD-1 mice were assigned to groups receiving 0 (control diet only), 50, 200 or 1000 mg/kg/d for 3, 6 or 12 months. At 12 months, all surviving animals were changed to control diet to evaluate potential reversibility of the drug-induced changes. This report addresses only the 12 month data. They include information from 10 animals/group/time point and include hematology parameters, effects on bone marrow by evaluation of cytospin slides, growth factor concentrations in platelet-rich and platelet-poor plasma (VEGF, PDGF and TPO) as well as histologic evaluation of liver, lung, spleen and bone marrow from scheduled euthanasias and premature decedents at the 3 and 6 month time points. Kidneys from these animals were collected to serve as a "negative control" for the labeling portion of the study.

As in previous studies, 4 days prior to euthanasia, 5 animals/group were implanted with osmotic pumps and administered BrdU to measure endothelial cell proliferation. Liver samples from these animals were embedded and stained immunohistologically with rabbit anti-BrdU antibody and rat anti-mouse F4/80 Macrophage. Proliferation indices were generated as the # of positive cells/ total # of cells of the same type.

Platelet aggregation was measured in 5 animals/time point using ADP as the agonist in platelet-rich plasma. Platelet activation was evaluated from 5 animals/group at the 3 month time point by flow cytometry to measure the number of platelets showing surface P-selectin. However, the sponsor reports that these data were too variable (controls too high) and did not report the results. For the Week 17 evaluation, 7 animals were tested and at the 6 and 12 month time points, 6-7 animals were evaluated.

Results: Six premature decedents were reported and 9 animals were sacrificed moribund. The sponsor did not attribute any of these deaths to treatment-related effects.

Mean body weights and body weight gains were increased in the treated animals in a dose-related fashion at each time point.

Mean platelet volumes were increased in the high dose animals only at 6 and 12 months but the 6-10% increases are not considered biologically significant by this reviewer. No significant differences from controls were appreciated with respect to platelet counts, red cell counts, hemoglobin, or platelet morphology.

However, platelet aggregation was markedly decreased in the high dose animals (58% at 3 months and 47% at 12 months) in the high dose group. No differences from controls were noted at 6 months. Due to the variability seen in the data and the inconsistent findings at 6 months, no definitive conclusions can be reached concerning the significance of the data when extrapolated to humans.

Growth factor analyses were inconsistent and no consistent dose relationship was found.

A significant decrease in myeloid to erythroid ratio was found in the high dose animals but remained within reference range. The erythroid cell lines were increased and there was no appreciable increase in the myeloid lines. Total erythroid cells were increased at the high dose.

No biologically significant differences from controls were noted in any of the dose groups with respect to endothelial cell proliferation.

No gross pathologic findings were related to drug treatment in any of the premature decedents or any animal euthanized by the 6 month time point.

In non-BrdU-treated animals, no evident drug-related histopathologic lesions were found at the 3 month time point.

Increased fatty change was reported in femoral marrow (1/10 in control and 50 mg/kg/d groups, 2/10 in the 200 mg/kg/d, and 5/10 in the 1000 mg/kg/d group). This is probably related to the obesity and the 16% weight gain over controls noted in the high dose group. By 6 months, 2/10 and 7/10 for the mid and high dose groups, respectively, reported increased fatty change. While increased pigmented macrophages were noted in bone marrow at 6 months, no increased severity of the finding was reported so the biological significance is uncertain given the myeloid changes and potential for the "brown pigment" to be iron stores.

Pregabalin elicited similar changes in B6C3F1 and CD-1 mice strains with respect to decreased myeloid:erythroid ratios. Neither changes in red cell parameters nor megakaryocytes were seen in the CD-1 mice, while significant increases were found in the B6C3F1 mice. The B6C3F1 mice also showed slight alterations in platelet morphology and "abnormal" aggregation but the biological significance is uncertain.

The sponsor contends that pregabalin at 1000 mg/kg/d is carcinogenic in B6C3F1 mice and elicited hepatic endothelial cell proliferation but that this dose was not carcinogenic in CD-1 mice. This conclusion is not supported by the data.

Study title: **Exploratory Investigation of the Effects of Pregabalin on Endothelial Cell Factors in Male and Female CD-1 Mice- Interim Report**

Key study findings: The increased megakaryocytes and decreased myeloid:erythroid ratios were due to drug administration but the biological significance of these mild changes is uncertain.

Study no.: RR 745-03766 and RR-MEMO 745-03658
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 6/17/02- 12/12/02"
GLP compliance: Not required

Drug, lot #, and % purity: XH020100

Methods: The purpose of this study was to determine the effects of dietary pregabalin on endothelial cell factors in male and female CD-1 mice after exposure to drug for 1, 3, or 6 months at 5000 mg/kg/d (dose that elicited statistically significant tumors in the carcinogenicity study).

Doses: 0 or 5000 mg/kg/d

Study design: 60 CD-1 mice/sex were administered pregabalin in the diet for 1, 3 or 6 months. At each of the time points, 20 mice/sex were divided into groups of 5 each and evaluated for one of the following:
1) Proliferation subgroup (hepatic endothelial cell proliferation)- At 4 days prior to scheduled euthanasia, the first 5/group were implanted with osmotic pumps containing BrdU to evaluate effects on cell proliferation. Only data from the 1 month time point are included in this report.
2) Platelet activation subgroup
3) Platelet aggregation subgroup
4) Hematology subgroup (hematology, growth factors, bone marrow composition, histopathology)

At the 6 month time point, an additional 9 or 10 animals were evaluated for #2 and 3 above.

Animals were evaluated for body weights, clinical signs and feed consumption. For the platelet aggregation portion, samples were collected in citrate and ADP was used as the agonist. Samples for hematology and evaluation of growth factors, and bone marrow were collected in EDTA. Platelet activation samples were collected in heparin for analysis by flow cytometry for P-selectin expression.

Results: Body weights were increased in treated animals when compared to controls by 25% in males and 44% in females at 3 months and 9% in males, 16% in females after 6 months on study. Feed consumption was similarly increased.

Males showed slight increases in circulating red cell parameters and decreases in relative reticulocytes.

Increases in mean platelet volume (9-18%) were seen in both sexes at all time points. Platelet counts were only increased in females at the end of the study (• 14%). This is not considered a biologically significant increase. The megakaryocytes were increased in the females at 6 months but not at other time points.

At all time points, statistically significant decreases in the myeloid:erythroid ratio (• 45%) were discussed. This difference was due to decreased relative percentages of myeloid cells. Erythrophages were present in a few animals after 3 months but in • 70% by 6 months.

Growth factors (VEGF, PDGF or TPO) in circulation did not show consistent changes. An increased incidence of abnormal platelet aggregation was found in drug treated animals. Platelet activation was increased at 1 and 3 months in males and 1 month in females. No differences from controls were enumerated at the 6 month sampling.

No treatment-related effects were appreciated in the proliferative indices.

In the bone marrow, increased megakaryocytes were reported at all time points and at 6 months, the macrophages were increased 13x in females and 1x in males. In the spleen, extramedullary hematopoiesis and increased megakaryocytes were noted. Increased VEGF was seen in the spleen, supporting the histologic findings. However, in the vast majority of the animals, the histologic findings were listed as minimal to mild.

The sponsor concluded that this study shows that "Pregabalin treatment effected changes in peripheral blood, bone marrow and platelet function in CD-1 mice that are considered potential factors related in endothelial cell proliferation." While this conclusion is perhaps valid, the lack of increase in incidence and/or severity of the findings make the biologic significance uncertain.

Study title: **Exploratory Investigation to the Effects of Pregabalin on Red Cell Factors in Female B6C3F1 Mice**

Key study findings: Myeloid:erythroid ratios were decreased (decrease in the absolute number of myeloid cells and an increase in absolute erythroid cells) as in previous studies and macrophages in marrow were increased. Respiratory parameters were affected by pregabalin treatment which may be partially responsible for the changes in red cell parameters (increased numbers of mature red cells in marrow and spleen) noted.

Study no.: RR 745-03770
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 11/18/02 to 4/14/03"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Methods: The purpose of this study was to determine the short-term effect on B6C3F1 of pregabalin on erythrocyte parameters in blood, bone marrow and spleen, signs of endothelial cell proliferation and the numbers of macrophages in bone marrow.

Doses: 0 (groups 1 and 4) or 5000 mg/kg/d (groups 2 and 5) for 1 month. Thirty additional animals were given 2 i.p. injections (48 hours apart) of phenylhydrazine (groups 3 and 6) to serve as positive controls for accelerated erythropoiesis. The serum from these animals was inadvertently thawed so 10 additional animals were added to serve as the positive controls.

Study design: Female B6C3F1 mice were treated with pregabalin and/ or pregabalin and phenylhydrazine for 1 month and animals were evaluated for clinical signs, body weight, splenic weights (groups 1 and 2 only), feed consumption, hematology, bone marrow differentials, growth factors (bFGF [basic fibroblast growth factor], EPO [erythropoietin], IL-3), clinical chemistries, lactic acid, soluble P-selectin, E-selectin, reactive oxygen species, total iron binding capacity, and routine histopathology on liver, lung, spleen, and bone marrow. 8-hydroxydeoxyguanosine was measured to determine DNA adduct formation. Serum bicarbonate quantitation was added in the Amendment VII as the sponsor noted "increased serum bicarbonate values observed in another on-going pregabalin study in rats."

Plethysmography was performed on 10 unrestrained mice/group. Pulmonary function was evaluated for 1 hour on Days 2, 3, 8, 9, 25 and 26 and for 2 hours on Days 15 and 16. To this reviewer's knowledge, respiratory parameters have not been previously measured in animals

treated with pregabalin.

Samples were taken from groups 1 and 2 on Days 2, 3, 8, 15 and 29 and groups 4 and 5 on Days 2, 8, 15 and 29. Phenylhydrazine samples were taken on Day 4. Spleens were taken from the last 5 animals/group from groups 1-3.

Results: Interestingly, there were a total of 10 amendments to this study protocol after study initiation.

Neither increased drug-related mortality nor morbidity was observed. Body weights for treated animals were, as in other studies, increased (• 8%) when compared to controls. Feed consumption was correlatively increased.

No changes in reticulocytes were reported and erythrocyte morphology was comparable across groups. Slight increases in rbc counts, hemoglobin and hematocrit were appreciated in treated animals early in the study but the increases were minimal by the end of the study.

Platelet counts increased by 12% by Day 29 and mean platelet volume was also increased (• 15% over controls). White cell counts were significantly increased at all time points, reaching a peak on Day 8. The increases were due primarily to neutrophils.

The myeloid:erythroid ratios were mildly decreased within 24 hours of study initiation with ranges of 0.91- 1.01 in pregabalin-treated mice and 1.2- 2.2 in controls. This was due to a decrease in the absolute number of myeloid cells and an increase in absolute erythroid cells. No increase was appreciated in the number of proliferating myeloid cells. The increases and decreases did not change significantly with the longer duration of dosing. Additionally, acridine orange staining of marrow showed an increased PCE (polychromatic erythrocytes): NCE (nonnucleated polychromatic erythrocytes) ratio due to an accumulation of mature erythrocytes in the marrow of pregabalin-treated animals. Similar findings were found in the spleen with total erythrocytes increased 53%, 3%, 19%, 19% and 46% compared to controls on Days 2, 3, 8, 15 and 29, respectively. While there appears to be an increase, the 3% and 19% increases are not biologically significant and call into question the overall validity of the finding.

Flow cytometry of marrow did not reveal histograms with discernible populations so macrophages in marrow were only evaluated histologically. Macrophages in the bone marrow increased after 14 days of dosing and were 7x higher than controls by the end of the study. Erythrophages were present at all time points in several animals from the treated groups and only 1 control.

No treatment-related effects were seen on growth factors, soluble P-selectin, E-selectin or oxidative radicals as measured by osmotic fragility. No Heinz bodies were found and serum iron, transferring and iron binding were comparable across groups.

Respiratory parameters were significantly decreased in the pregabalin-treated animals at all time points. Tidal volumes showed an increase in all groups when respiratory rates were increased and decreases when the rates were decreased. Corresponding increases in sodium bicarbonate and decreases in sodium chloride were consistent with a physiologic response to pH changes due to respiratory challenge. Increased erythropoiesis may be part of that physiologic response.

Study title: Exploratory Investigation of the Effects of Pregabalin on Red Cell Factors in Female Wistar Rats

Key study findings: Plasma basic fibroblast growth factor (bFGF) levels were increased during the study but not in a time-related fashion. Platelet and megakaryocyte numbers were decreased in pregabalin-treated animals but the normal variability in these parameters is quite large.

Study no.: RR 745-03771
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Methods: The objective of this study was to determine if short-term administration of pregabalin to Wistar rats would affect red cell parameters in target tissues (spleen, bone marrow or peripheral blood), soluble markers of erythropoiesis, macrophages in bone marrow or red cell membranes (reactive oxygen species, osmotic fragility or Heinz body formation). Histopathologic evaluation of tissues was not performed as per Amendment III: "There were no findings in microscopic evaluation of cytocentrifuge preparations of rat bone marrow in either drug-treated or control animals in this study. Additionally, since histopathology has previously been assessed in liver, spleen and bone marrow at this dose and approximate exposure time, histopathologic examination of tissues in this study has been cancelled." This was an unusual decision as the sponsor reports megakaryocyte effects of pregabalin exposure that reached statistical significance.

Doses: 0 or 900 mg/kg/d in the diet for up to 1 month.

Study design: Forty female Wistar rats/group were treated and 10/group were sacrificed on Days 2, 8, 15 and 29. Positive controls (administered 50 mg/kg phenylhydrazine i.p. x2) were used to determine effects on hemolysis-induced accelerated erythropoiesis. In all other respects, this study was similar in design and conduct to the study in B6C3F1 mice described above. Urinalyses were performed only on Day 29.

Results: No drug-related mortalities or clinical signs were reported. Mean body weights of pregabalin-treated animals were significantly reduced when compared to controls (• 13% by Day 29). Mean body weight gains were comparably reduced. This is unusual as in other species tested, pregabalin-treated animals showed increased weights and weight gains. Feed consumption was comparable across groups.

Slight, statistically significant increases in red cell parameters were noted by Day 2 but were not biologically significant (4- 11%). Relative and absolute reticulocyte counts were decreased by Day 8 (36% and 42%, respectively) but given the normal variability in this parameter, the biological significance is unclear. No difference in proliferating myeloid cell lines was found.

Platelet counts were statistically decreased (• 27%) at all time points but given the huge normal variability in this parameter, the biological significance is probably nil. Megakaryocyte counts were significantly decreased (66%) in treated animals by Day 29. Given the large variability in these counts, the biological significance of this finding is also questionable.

No peripheral leukocyte effects of pregabalin treatment were appreciated.

Total nucleated cell counts in bone marrow were statistically significantly affected/decreased at Day 8 and increased at Day 29 in pregabalin-treated animals, but remained within historical control values so are not considered consistently affected by treatment. Thus, no biological significance is assigned to the finding. Erythroid numbers were comparable across the groups. Additionally, no effect was found on the polychromatic erythrocyte: normochromic erythrocyte ratio in bone marrow or spleen. Bilirubin, osmotic fragility, reactive oxygen species, Heinz body formation and lactic acid levels were not affected by pregabalin. No effect on 8-hydroxydeoxyguanosine levels in the liver was reported for pregabalin in Wistar rats. No effect on erythropoietin levels was discovered.

Plasma basic fibroblast growth factor levels (bFGF) were increased during the study but not in a time-related fashion.

Serum bicarbonate levels were increased in pregabalin-treated animals early in the study but by Day 29 were comparable across groups.

Study title: **Retrospective Evaluation of Hepatic Sinusoidal Endothelial Cells in Cynomolgus Monkeys Chronically Treated with Pregabalin**

Key study findings: No significant effects of pregabalin treatment were appreciated in staining of hepatic sinusoidal endothelial cells when compared to controls.

Study no.: RR-MEMO- 745-03828
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
GLP compliance: Not required
Drug, lot #, and % purity: XH330993 and XH340993

Study design: The cynomolgus monkeys (3-6/group) in this study were given pregabalin in 0.5% methylcellulose at 0 (vehicle controls), 10, 25, 100, 250 or 500 mg/kg by oral gavage once/day for 13 weeks. This study has been reviewed previously (IND — , Submission date: 12/1/95).

At the end of the 13 week dosing period, 3 monkeys/sex/all groups except all 500 mg/kg/d animals were euthanized. The remaining animals were treated for an additional year with the 250 mg/kg/d animals given 500 mg/kg/d.

Liver sections were taken from the surviving 500 mg/kg/d animals (3 males, 1 female) and controls for immunohistochemical staining [redacted] Slides were evaluated for antibody signal and color-coded images were collected at 1• M intervals. Ten projection composite images were collected from each liver section.

No BrdU labeling was performed.

Results: Composite images showed no significant differences from controls at any of the dose levels. Variability within sections and within animals was significant.

Study title: **Chronic Investigative Study of Pregabalin in Female B6C3F1 Mice- Interim Report of Tumor Analysis, Clinical Pathology and Pulmonary Function**

Key study findings: Hemangiosarcoma incidence was increased in the 200 and 1000 mg/kg/d animals treated for 24 months and the 1000 mg/kg/d animals treated for 12 months and untreated for the remainder of the study.

Study no.: RR-MEMO 745-03832 and RR-MEMO 745-03657
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 7/2/01 to 7/18/03"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Doses: 0 (control diet), 50, 200 or 1000 mg/kg/d for 24 months or 12 months followed by a 12 month recovery/reversibility period. The doses were selected to coincide with the low and mid doses of the prior carcinogenicity study in this strain and the 50 mg/kg/d group was added to "explore a potential no-effect dose".

Study design: Female B6C3F1 mice (56-61/group) were treated as described above in the diet. During Week 95, 10 animals from the control and high dose groups were evaluated for pulmonary function via unrestrained whole body plethysmographs fitted with flow transducers. Function was measured for 60 minutes.

After 24 months on study, all animals were euthanized and complete necropsies were performed. Routine hematologic parameters were evaluated as were growth factors (VEGF, PDGF, bFGF) from 5 animals/group at 6 and 12 months and 20 animals/group treated for 24 months. Erythrocyte and platelet morphologies were examined on blood smears and bone marrow differentials were determined from cytocentrifuged preparations from 19-21 animals/group. Analysis was done by flow cytometry. Endothelial cell proliferation was examined in 5 mice/group after 6 and 12 months of dosing and in 5/group after 10 weeks of recovery after 6 months of dosing via BrdU label administered through an implanted osmotic pump.

Histopathology was performed on spleen, heart, liver, skin and bone marrow (target tissues in previously reviewed B6C3F1 carcinogenicity study). The tissues were evaluated for proliferative vascular changes and angiectasis. Peer review was done on these tissue samples.

Results:
Body weight gain and feed consumption increased in treated animals for the first 12 months. After 4 weeks of the reversibility period, the treated animals' body weights were comparable to controls.
Pulmonary function: No significant differences from controls were appreciated in respiratory rate, minute volume or tidal volume. Mean weighted minute volume was decreased in treated animals at both 5 minutes and 60 minutes. These results are consistent with the prior study in young B6C3F1 mice treated for 1 month with pregabalin. Whether the respiratory depression is centrally mediated or not has not been determined.

Hematologic parameters: Lots of variability was seen in these parameters, as expected in a 2 year rodent carcinogenicity study. Increased numbers of schistocytes (fragmented erythrocytes) were found in the 1000 mg/kg/d for 24 months group. In the reversibility animals, these changes were reduced. After 12 months, in the reversibility animals, no effects on red cell parameters were appreciated.

Mean platelet volume and degranulated platelet aggregates were increased for the 200 and 1000 mg/kg/d for 24 month animals with increased platelet aggregates at all doses and at the earlier time points but the increases were not statistically significant. In the reversibility animals, the mean platelet volume remained elevated in the 1000 mg/kg/d group but counts and platelet activation were comparable across groups.

When treated for 6 or 12 months, no significant effects on PDGF or VEGF were noted in treated animals. In animals treated for 24 months, serum PDGF was increased at the high dose when compared to controls but no significant differences were noted in bFGF or VEGF. There was no apparent correlation between specific animals with tumors and animals with high levels of growth factors.
Bone marrow from animals treated for 12 or 24 months showed decreased total nucleated cells and decreased myeloid: erythroid ratios. However, the amount of change was minimal and consistent with normal aging changes in mice. Total myeloid cells were decreased while erythroid cells were increased. Therefore, the pregabalin-treated animals maintained a profile more ordinarily seen in young animals. Interestingly, the effect on M:E ratio were reversed within 10 weeks of the recovery period after 6 months of dosing.

Numbers of macrophages were increased 2-4 x at the 200 and 1000 mg/kg/d doses. Megakaryocyte numbers were increased by only 1.6x. This finding was reversed during the early recovery period.

The numbers of erythrophages were increased in a dose-dependent fashion (15% for controls and low dose, 30% for mid dose and 60% for high dose) but these are an occasional finding in normal, untreated mice. Similarly, increases in "intact blood vessels" were reported (10% from control and high dose, 40% from mid dose and 75% from high dose) from bone marrow flushes. This is an unusual finding from flushes and the etiology is not clear. It may be due to an increased number of vessels in treated animals and/or a change in the marrow architecture that enabled the vessels to be flushed out. No further investigation of the finding was performed by the sponsor.

In the reversibility animals, no significant differences from controls were appreciated in the treated animals. No increase in megakaryocytes was found. No increase in blood vessels in marrow was determined in the treated animals. Thus,

Tumor findings: An increased incidence in hemangiosarcomas was reported in the high dose animals, both from the main study and the reversibility animals.

Hemangiosarcoma Incidence in Female B6C3F1 Mice Treated with Pregabalin

Dose (mg/kg/d)	0	50	200	1000
Main study (24 months of rx)	**4/61**	**4/61**	**7/61**	**15/57***
Liver	1	0	3	6
Spleen	3	3	4	7
Bone marrow	0	2	2	2
Heart	0	0	0	2
Reversibility	**5/60**	**6/61**	**5/60**	**10/56****

Liver	3	2	0	3
Spleen	2	4	3	6
Bone marrow	1	4	3	5

* $p<0.001$; ** $p<0.017$

Although the 200 mg/kg/d main study animals did not reach statistical significance in the increased numbers of hemangiosarcomas, it supports the conclusion that the findings are real and that pregabalin elicits hemangiosarcomas in mice. As only limited tissues were evaluated and only "vascular tumors" were enumerated, it is not clear whether hemangiomas were also part of the determinations. Additionally the reversibility animals showed a slightly lower incidence of hemangiosarcomas but the difference was neither statistically nor biologically significant. There was a lessening of hematologic effects (schistocytes, degranulated platelets, etc.) but not an elimination of those effects. The sponsor suggested that the lack of increased hemangiosarcomas in the liver might be due to "Pregabalin-induced changes (postulated growth factor stimulation of hepatic endothelial proliferation as a result of persistent increases in platelet activation) resulting in the increased incidence of hemangiosarcoma in liver at 24 months are reversible." Since the incidence was increased in 2 target tissues, the data do not seem to support their conclusion.

Study title: **Exploratory Study of the Effects of Pregabalin on Nitric Oxide Synthetase Activity and Isoenzyme Levels in Cultured Mouse Endothelial and Bone Marrow Cells**

Key study findings: Increased eNOS was detected in pregabalin-treated bone marrow cells at 2-3x control levels. However, a consistent concentration-response relationship was not demonstrated.

Study no.: RR-MEMO 745-03834
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 8/30/01 to 3/19/02"
GLP compliance: Not required
Drug, lot #, and % purity: XH230695

Study design: Splenic endothelial cells and bone marrow cells were isolated from female B6C3F1 mice. The cells were exposed *in vitro* to 0, 10, 100 or 1000 • g/mL of pregabalin. Endothelial cells were exposed for 24-48 hours and bone marrow cells were exposed for 48 hours and 1, 2 or 3 weeks. The sponsor stated that the 1000 • g/mL level was ~100x and the 10 • g/mL level was approximately equivalent to the targeted human maximal therapeutic plasma concentration.
NOS levels were determined for nNOS(neuronal origin), eNOS (endothelial origin) and iNOS(inducible) protein. Once the preliminary evaluations of nitric oxide synthase (NOS) levels were done via Western blot analysis, additional pregabalin concentrations of 1, 3, 30 and 300 • g/mL were added to the bone marrow cultures to help characterize the concentration-response relationship.

Results: Neither nNOS nor iNOS were detected in lysates from control or pregabalin-treated endothelial or bone marrow cells. Levels of eNOS were comparable at 24 and 48 hour time points in endothelial cells. Increased eNOS was detected in pregabalin-treated bone marrow cells at 2-3x control levels. However, a consistent concentration-response relationship was not demonstrated.

Study title: **Reanalysis and Refinement of Quantitation of Hepatic Proliferative Indices**

Key study findings: No biologically significant information was derived in this study.

Study no.: RR-MEMO 745-03835
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 7/2/01 to 9/3/03"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: This study was simply a reanalysis of the previously reported (#2787, 2795 and 2935) studies using BrdU incorporation to determine cell proliferation indices. Here the sponsor reports the number of proliferating endothelial cells expressed as a function of total endothelial cells/image.

Results: Using this [redacted] process, the sponsor concluded that there were significantly increased numbers of proliferating hepatic endothelial cells at 1 month of dosing in 1 of 2 studies at 1000 mg/kg/d and at 12 months of dosing at 200 and 1000 mg/kg/d.

Study title: Immunohistochemical and/or Immunofluorescent Evaluation of Growth Factors in Mice and Rats Given Pregabalin in the Diet for 12 Months

Key study findings: B6C3F1 mice treated for 1 year with pregabalin had increased VEGF expression in spleen and femoral bone marrow when compared to controls. Similar expression was not seen in the rats.

Study no.: RR-MEMO 745-03855
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 6/25/01 to 9/8/03"
GLP compliance: Not required
Drug, lot #, and % purity: XH330993 and XH340993

Study design: The purpose of this study was to determine if 12 month dosing with pregabalin altered expression of VEGF, bFGF and vascular endothelial growth factor receptor 2 (Flk-1) in liver, spleen, and bone marrow of mice and rats.

Tissues evaluated were from previously reviewed studies (RR 745-03770, -03763, -03771 and AA2787, AA2892). Immunohistochemistry was performed on liver (VEGF, bFGF), and bone marrow (bFGF) from mice and rats. Confocal microscopy was performed on 8• m thick sections of liver from mice and rats after 1 year on pregabalin.

Results: Increased VEGF staining was described in the spleens of mice treated with 1000 or 5000 mg/kg/d for 1 or 6 months but no increased staining was noted in bone marrow or liver and no bFGF staining was noted in any of the tissues.

After 12 months of dosing, increased VEGF staining was found at 50, 200 and 1000 mg/kg/d and intensity increased somewhat with increasing dose. The staining was primarily in hematopoietic cells in the red pulp of the spleen. Increased intensity staining was found in the femoral bone marrow of all treated animals but the staining was less intense than in the spleen.

No increased VEGF staining was found in the spleen or bone marrow of CD-1 mice or the liver of B6C3F1 mice treated at 1000 mg/kg/d when compared to controls. Macrophages from these animals in bone marrow did take up the bFGF stain but an increase was also seen in spleen of mice treated with 50 mg/kg d and not the higher doses. This calls into question the significance of these findings.

No increases in VEGF, bFGF or Flk-1 staining were found in treated rat livers, spleens or bone marrows.

Study title: **Pregabalin: Assessment of Rodent Carcinogenicity, Mode of Action of Hemangiosarcoma Formation in Mice and Human Relevance**

Study no.: RR 745-03856
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: Not specified
GLP compliance: Not required

This summary of the sponsor's interpretations of the carcinogenicity and mechanistic data essentially says that pregabalin is a single species, single tumor type, epigenetic mouse carcinogen. They believe that the exposure of endothelial cells to increased levels of growth factors as a result of increased megakaryocyte production and increased platelet activation is the "most plausible" mode of action of endothelial cell proliferation in mice. They suggest that the interactions of platelet factors, platelet aggregation changes, growth factors, and endothelial cell proliferation have been shown to elicit hemangiosarcoma formation in mice and the findings are consistent with a "causal association". However, hemangiosarcomas were elicited in B6C3F1 mice and CD-1 mice, even though the changes in the parameters above were more variable but less severe overall. The sponsor contends that "In a species given pregabalin that does not have similar alterations (in platelets, growth factors, etc.), no endothelial proliferation or hemangiosarcoma formation would be expected." This would seem to be a very large "leap of faith" given the epigenetic nature of the elicitation of this neoplasm.

Species Specificity of Pregabalin-Induced Changes in Parameters Potentially Associated with Hemangiosarcoma Formation in Mice

Parameter	B6C3F1 mice	CD-1 mice	Wistar rat	Monkey	Human
• platelet count	+	+/-	-	-	-
Altered platelet aggregation	+	+	-	-	-
Altered platelet morphology	+	+	-	-	-
• platelet activation	+	+	-	ND	-
Megakaryocyte proliferation	+	+	-	ND	ND
Endothelial proliferation	+	-	-	-	ND
Increased circulating PDGF	+/-	-	-	-	ND

The sponsor suggests that the decreased platelet aggregation seen in female B6C3F1 mice was characterized by "defective secondary ADP-dependent aggregation" While this may be true, and the platelet aggregation effects were only seen in mice, it is difficult to assign a causative relationship to hemangioma/hemangiosarcoma.

When considering the megakaryocyte proliferative effect of pregabalin treatment, it is difficult to determine the mechanism responsible for the effect. No effect of pregabalin was noted *in vitro* on bone marrow cultures from B6C3F1 mice, it did not bind with high affinity to platelets and there was no effect on platelet function *in vitro*. Thus, there may be an indirect mechanism but it is impossible to determine that such a mechanism would be species specific.

The issue of circulating growth factors is an interesting one. While increased PDGF was found in platelet rich plasma in the female B6C3F1 mice given 1000 mg/kg/d for 12 and 24 months, no changes were reported in CD-1 mice at the same dose for 12 months or 5000 mg/kg/d for 6 months. Additionally, increased platelet counts were seen in some doses and some time points where no increases in PDGF were found. In the 12/24 month dosing study in B6C3F1 mice, no significant effects on PDGF or VEGF were noted in treated animals. In animals treated for 24 months, serum PDGF was increased at the high dose when compared to controls but no significant differences were noted in bFGF or VEGF. There was no apparent correlation between specific animals with tumors and animals with high levels of growth factors. Unfortunately, these parameters were not always evaluated from the same animals but given the increased tumors in both strains of mice in multiple studies, it weakens their argument that the circulating growth factors were causative for tumor production. Another consideration is that humans normally produce significantly more PDGF and VEGF than do mice or rats and the platelet and red cell lifespans in humans are longer than in mice.

The hypoxia elicited in the B6C3F1 mice by pregabalin is potentially an exacerbation of the normal hypoventilation due to small lung volume (from the C57Bl/6 progenitor) and low ventilatory responses (from the C3H progenitor). This may contribute to the high endothelial cell proliferation reported here but this phenomenon does not help to explain the increased incidence of hemangiomas/hemangiosarcomas in the CD-1 mice which has a very different phenotype and background.

Study title: **Retrospective Evaluation of Bone Marrow Megakaryocytes from B6C3F1 Mice and Wistar Rats**

Key study findings: Increased megakaryocytes at all stages of development were found in B6C3F1 mouse femoral bone marrow. Decreased megakaryocytes at all stages of development were found in Wistar rat femoral bone marrow.

Study no.: RR-MEMO 745-03456
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 2/9/01 to 4/30/01"
GLP compliance: Not required
Drug, lot #, and % purity: XH200399, XH350995, XH411095, XH210398

Study design: This report covers a retrospective "look" at the femoral bone marrow sections from B6C3F1 mice treated at 200, 1000 and 5000 mg/kg/d and Wistar rats treated at 50, 150 and 450 mg/kg/d (males) or 100, 300 and 900 mg/kg/d (females). Quantitative megakaryocyte number and morphology are discussed. Elements examined included megakaryocyte stages, cytoplasm and nuclei.

Results: Male and female mice had significant increases in the numbers of megakaryocytes of all stages and increased total megakaryocytes (52-104% in males, 40-80% in females). Male rats

had • 37% decrease in early megakaryocytes and • 24% decrease in total megakaryocytes. Female rats had 27% fewer late megakaryocytes. These findings are consistent with the peripheral blood findings in these species.

Study title: **Retrospective Evaluation of Bone Marrow Megakaryocytes from CD-1 Mice Treated with Pregabalin for 2 Years**

Key study findings: The myeloid:erythroid ratio was significantly altered in the treated animals. In controls, the myeloid cell lines were predominant and in the pregabalin-treated animals, the erythroid cell lines predominated. These findings are indicative of a treatment-related phenomenon but the significance is uncertain.

Study no.: RR-MEMO 745-03692
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 4/13/00 to 4/16/02"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: This report covers a retrospective "look" at the femoral bone marrow sections from CD-1 mice treated at 200, 1000 and 5000 mg/kg/d. Quantitative megakaryocyte number and morphology are discussed. Elements examined included megakaryocyte stages, cytoplasm and nuclei.

Results: Increased numbers of total megakaryocytes were reported for male (18-108%) and female (39-80%) mice in a dose-related fashion. Increases were 18-43% in males (dose related increases) and 39-80% in females (non-dose related increases). In the B6C3F1 mice, some level of increase was also reported in the control animals. Early stages of megakaryocyte production (megakaryoblasts, numbers of megakaryocyte nuclei) were decreased, unlike the findings in the B6C3F1 mice. It is difficult to understand how the total numbers increased but the normal progression from stage-to-stage was not present.

Of interest is the finding of increased numbers of mitotic figures in megakaryocytes of pregabalin-treated CD-1 males (3-22%) and females (10-28%). The significance of this finding is uncertain, but brings into question the possibility of arrested maturation of the megakaryocyte line.

The myeloid:erythroid ratio was significantly altered in the treated animals. In controls, the myeloid cell lines were predominant and in the pregabalin-treated animals, the erythroid cell lines predominated. Additionally, increased numbers of pigment-laden macrophages (hemocyanin, hemosiderin and erythrophages) were reported in a dose-related fashion. These findings were not reported in the original study report for this carcinogenicity study. The sponsor proposes an interaction between the increased incidence of bone marrow macrophages and erythroid predominance seen in these mice and they suggest that erythropoietin may be a contributing entity as well. They also suggest that the microenvironment in mouse bone marrow is significantly different than that found in human bone marrow, thus adding to their "weight of the evidence" approach and conclusion that the carcinogenic potential of pregabalin is species specific and not extrapolatable to humans. While it is accepted that rats and humans undergo fatty replacement of marrow with age and mice do not, the change in the microenvironment described above suggests that the "normal" aging effects are altered with pregabalin dosing. This

makes their conclusions more questionable as the "usual" did not occur in this study.

Study title: **Retrospective Evaluation of Peripheral Blood Morphology in B6C3F1 and CD-1 Mice Treated with Pregabalin for 2 Years**

Key study findings: As many of the mice were premature decedents and no smears were made from them, no correlation between the incidence of abnormal red cells and hemangioma/hemangiosarcoma was determinable.

Study no.: RR-MEMO 745-03714
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 9/24/97 to 4/16/02"
GLP compliance: Not required
Drug, lot #, and % purity: XH200399, XH350995, XH411095, XH210398, XH020100

Study design: This report covers a reanalysis of the peripheral blood schistocytes and platelet (uniformity, size, granularity and aggregation) morphologies from the carcinogenicity studies in B6C3F1 and CD-1 mice previously described. Some of the smears (<5%) were uninterpretable due to poor staining quality or leukemic conditions in the animals.

Results: In the B6C3F1 mice: Schistocytes were increased in a dose-dependent fashion (1.9-41.2% in males and 2.4- 26.1% in females for the respective groups). However, there were a large number of animals that were premature decedents so in the mid and high dose groups, the sponsor was unable to make a direct comparison between the incidences of schistocytes and hemangiosarcomas.

Giant platelets and abnormalities (bizarre shape, swelling, hypogranulation) in platelets were appreciated in the animals in a dose-dependent manner. These changes might be entirely attributable to the presence of altered endothelial cells in animals with hemangiosarcomas or other effects directly or indirectly related to treatment with pregabalin.

In the CD-1 mice: Similar alterations in platelets and red cells were discovered but the incidence of these changes, as well as the incidence of hemangiosarcomas, was less but still biologically significant.

Study title: **Analysis of p53 and *Ras* Gene Mutation Frequencies in Hemangiosarcomas from a 2-Year Carcinogenicity Study of CI-1008 in B6C3F1 Mice**

Key study findings: No significant p53 or Ha or Ki-*ras* oncogene mutations were found in the hemangiosarcomas.

Study no.: RR-MEMO 745-03327
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 11/1/99 to 8/14/00"
GLP compliance: Not required

Study design: Hemangiosarcoma sections were taken from all animals determined to have the tumors on routine histopathologic evaluation. These samples were processed by [redacted] for mutation analysis. DNA was extracted from the samples and lysates were

prepared by primer extension preamplification and purified. Aliquots of these products were analyzed for Ha and Ki-*ras* oncogenes and p53 suppressor gene by PCR and DNA sequencing.

Results: Analyzable DNA sequences were found in 84% of the tumors for each of the 8 loci evaluated and 109/174 tumors had definitive sequencing for all 8 loci.

No Ha- or Ki- *ras* mutations were detected. Fourteen p53+ tumors were found and 12 of them (7% of total hemangiosarcomas) had mutational inactivation of the p53. This low incidence did not allow the frequencies to contribute to a mechanistic discussion of hemangiosarcoma development in the B6C3F1 mouse. The sponsor contends that, since human hemangiosarcomas have ~50% *ras* and/or p53 mutations, the mouse is a poor predictor of human health risk for development of hemangiosarcomas. While this is an interesting premise, the fact remains that ~50% of human hemangiosarcomas are negative for *ras* and/or p53 mutations.

Study title: **Effect of Pregabalin on Endothelial Cell Proliferation in Monolayer Cultures**

Key study findings: Pregabalin did not increase endothelial cell proliferation in splenic monolayers.

Study no.: RR-MEMO 745-03462
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: This study was conducted to determine if pregabalin had an effect on endothelial cell monolayers from B6C3F1 female spleens.

Results: The addition of pregabalin to the cultures did not induce increased proliferation when compared to controls.

Study title: **Exploratory Study of CI-1008 Effects on Vascular Growth of Mouse Aortic Rings**

Study no.: RR 745-03398
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 5/11/00 to 11/21/00"
GLP compliance: Not required
Drug, lot #, and % purity: Not specified

Study design: Sections of aortic ring from male B6C3F1 mice were prepared into cultures and preincubated for 4 days prior to drug addition into the media. Doses tested were 1, 10, 50, 100 and 200 • g/mL and fetal bovine serum was used as the positive control.

Results: Pregabalin did not elicit a significant increase in vascular growth when compared to controls but addition of fetal bovine serum increased the growth• 20%.

Study title: **Exploratory Study of CI-1008 Effects on the In Vitro Function of Platelets from B6C3F1 Mice, CD-1 Mice and Wistar Rats**

Key study findings: No significant platelet functional alterations were attributed to pregabalin exposure.

Study no.: RR 745-03566
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 8/16/01 to 11/12/01"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: Female B6C3F1 and CD-1 mice, and Wistar rats' platelets were examined *in vitro* after incubation with 0, 10, 100 or 500 • g/mL of pregabalin. Exposure times were from 10 minutes to 2 hours. Effects on platelet function were examined.

Results: There were no significant effects of pregabalin exposure on platelet function. Some species differences were noted, i.e. rats showed more degenerative changes than mice and CD-1 mice had more rapid adhesion to glass surfaces but baseline parameters differed slightly with respect to bFGF (nearly undetectable in mice) and PDGF and thrombopoietin (higher in mice).

Study title: 1-Month Investigative Study of the Effects of Pregabalin on Platelet Survival in B6C3F1 Mice

Key study findings: No effect on platelet survival was seen at any time point.

Study no.: RR 250-01886
Conducting laboratory and location: [redacted]
Date of study initiation: "Periods covered: 6/12/02 to 7/13/02"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: Pregabalin was administered to 25 female B6C3F1 mice for 28-32 days at 1000 mg/kg/d in the diet. On Day 27, a label for cell surface glycoproteins [redacted] was given i.v. to all animals on study. At 24, 48, 72, 96 and 120 hours after this, 5/group were euthanized and platelet rich plasma was prepared from their blood. A flourochrome was added to aid in flow cytometry and determining the fluorescent platelets/time point.

Results: No effect on platelet survival was seen at any time point.

Study title: Exploratory Study of Pregabalin Effects on Endothelial Cell Proliferation and Apoptosis in B6C3F1 and CD-1 Mice

Key study findings: B6C3F1 mice showed proliferative effects of pregabalin on hepatic endothelial cells in 1 month of dietary dosing while CD-1 mice showed less proliferative effect than the controls.

Study no.: RR 745-03459
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 7/31/00 to 4/26/02"
GLP compliance: Not required
Drug, lot #, and % purity: XH230695

Study design: 20 male B6C3F1 and CD-1 mice were given 0 or 1000 mg/kg/d pregabalin in the diet. Four days prior to euthanasia, the animals (15/group) were implanted with osmotic pumps with BrdU. The livers were perfused in situ and liver, spleen, and bone marrow were collected. Five animals/fixation method (formalin, Zamboni's fixative or cryosectioning) were evaluated.

Results: No biologically significant clinical signs or body weight effects were appreciated. Pregabalin did not appear to have an effect on apoptosis in the liver when compared to controls but the levels of apoptosis found were extremely low overall (• 0.21%).

Nonendothelial cell proliferation was ~70% greater at the initial time point in the pregabalin-treated B6C3F1 mice (morphologic criteria only) when compared to the CD-1 mice and endothelial proliferation was 38% greater than in controls (morphologic and histochemical evaluation). Hepatocyte and Kupffer cell proliferation were not affected by pregabalin dosing.

In the CD-1 mice, nonendothelial cell proliferation was decreased (37%) when compared to controls but this is not considered biologically significant. Endothelial cell proliferation in these mice was less (32%) than controls and Kupffer cell proliferation was decreased. Hepatocytes were not affected.

The importance of the findings in this study is questionable as both strains of mice developed hemangiosarcomas in response to pregabalin exposure.

Study title: **3-Month Exploratory Study of CI-1008 in Female B6C3F1 Mice**

Key study findings: No consistent growth factor increases were found in this study. Endothelial cell and Kupffer cell proliferation were found at the 3 month evaluation.

Study no.: RR 745-03460
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 7/17/01 to 10/19/01"
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Doses: 0 or 1000 mg/kg/d in the diet

Study design: 60 female B6C3F1 mice/group were treated. Standard parameters (clinical signs, body weights, etc.) were evaluated as were VEGF, PDGF, TPO and bFGF and bone marrow. Platelet rich plasma was obtained at 1 and 3 months to look at platelet ultrastructure and reticulated platelets. Ten animals/group were implanted with osmotic pumps containing BrdU 4 days prior to euthanasia to evaluated cell proliferation in liver endothelial cells, hepatocytes and Kupffer cells. Urine bFGF was examined at 1, 2 and 3 months. Histopathologic examination was limited to liver, lung, spleen and bone marrow at 1 and 3 months.

Results: No treatment-related effects were noted on clinical signs, mortality, platelet counts or reticulated platelets. Body weights, body weight gains and feed consumption of treated animals were significantly higher than controls.

Slightly increased (3-8%) red cell counts, hemoglobin and hematocrit were reported for treated

animals. As in other studies, bone marrow erythroid cells were increased in treated animals (36% at 1 month, 48% at 3 months) but this finding was due to both increased numbers of proliferating and mature red cells.

While they reported a 350% increase in megakaryocytes in cytospin preparations at 1 month, the 3 month finding was of a modest increase that was not biologically significant. The sponsor described an increase in megakaryocytes in histopathologic sections of bone marrow but this increase is not considered biologically significant. VEGF levels were increased at 1 month but were comparable to controls by 3 months. PDGF decreased at 1 month and increased at 3 months. The inconsistencies in these factors make the significance questionable and this finding is consistent with findings from other studies.

Giant platelets were increased in treated animals after 1 month on study and platelet hypogranularity was also seen in the same group. These findings are consistent with the platelet aggregation seen in most of the treated animals at 1 and 3 month time points.

In platelet rich plasma, rapid platelet disaggregation was seen in treated animals and an increase in P-selectin levels was reported for the treated animals at 3 months.

Endothelial cell proliferation in the liver, as measured by BrdU staining, was significantly increased at both time points tested but no increased staining was reported for either hepatocytes or Kupffer cells (1 month time point only). By 3 months, Kupffer cell proliferation was ~300% increased in treated mice. While this is an interesting finding, none of the other studies looking at this parameter found significant increases.

Study title: **Reanalysis and Refinement of Quantitation of Hepatic Proliferative Indices**

Key study findings: Inconsistencies and lack of similar findings across studies make this information of questionable significance.

Study no.: RR-MEMO 745-03835
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: This report describes the reanalysis of liver cell proliferation from the exploratory studies in B6C3F1 mice (1 month, 3 month and chronic study).

Results:
1) Study AA2787: The reanalysis demonstrated "significant" increases in endothelial cell proliferation, hepatocytes and Kupffer cells at 200 and 1000 mg/kg/d at 12 months. However, total hepatocytes were decreased by 12 months. The initial analysis showed endothelial cell proliferation at 200 mg/kg at 6 months and 200 and 1000 mg/kg/d at 12 months, hepatocyte proliferation only at 12 months (200 mg/kg/d) and Kupffer cell proliferation at 12 months (200 and 1000 mg/kg/d).

2) Study AA2795: The original analysis showed significant increases in endothelial cell proliferation at 1 and 3 months and increased Kupffer cells at 3 months. The reanalysis demonstrated increased endothelial cells at 1 month and 3 months, increased total endothelial

cells at 3 months and increased proliferating and decreased total numbers of hepatocytes at 3 months. No effects on Kupffer cells were noted.

3) Study 2935: The original analysis showed no effects on any of the parameters. The reanalysis described significant proliferation of Kupffer cells and decreased total hepatocytes and no effects on endothelial cells.

Overall the results of these reanalyses show how inexact and inconsistent the findings from these studies are.

Study title: 1 Month Exploratory Time-Course Study of Pregabalin on Endothelial Cell Proliferation in Female B6C3F1 Mice

Key study findings: Inconsistencies and lack of similar findings across studies make this information of questionable significance.

Study no.: RR 745-03428
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
GLP compliance: Not required
Drug, lot #, and % purity: XH020100

Study design: Female B6C3F1 mice were given 0 or 1000 mg/kg/d of dietary pregabalin. Interim sacrifices on ten animals/group were performed at 4, 7, 14 and 28 days of dosing. Four days prior to each euthanasia time point, osmotic pumps with BrdU were implanted in 5 animals/group. Livers only were examined histologically. The five animals/group that were not implanted were used to evaluate PDGF, VEGF, platelet counts and eNOS protein.

Results: As in other studies, the mean body weights and body weight gains were increased in the pregabalin-treated animals.

Endothelial cell proliferation was increased by 90% on Day 4 in treated animals. At the later time points, the percentage of proliferating cells decreased (15% on Day 28). Increased hepatocyte proliferation was reported on Days 4, 14 and 28 but not on Day 7. However, the variability within the control animals was very high, making conclusive decisions about the biological significance impossible.

As with other studies in B6C3F1 mice, the VEGF and PDGF levels did not show any effect of treatment. Mean platelet counts did not differ significantly from controls. No eNOS protein was detected in bone marrow lysates.

No histopathologic treatment-related findings were described.

Study title: Exploratory 4-Week Investigation of the Effects of Pregabalin on Endothelial Cell Proliferation and Platelet Function in B6C3F1 Mice

Key study findings: Inconsistencies and lack of similar findings across studies make this information of questionable significance.

Study no.: RR 745-03660

Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: "Periods covered: 6/10/02 to 7/9/02"
GLP compliance: The sponsor considered this to be an exploratory study so not required.
Drug, lot #, and % purity: XH020100

Doses: 0, 50, 200 or 1000 mg/kg/d by dietary admixture to female B6C3F1 mice (18-29/group).
Study design: The study was conducted to further determine effects of pregabalin on platelet function, growth factors and endothelial cell proliferation. Five-six mice/group were evaluated for each of the parameters. "Cursory gross necropsies" were performed and livers were taken from the "Platelet Activation" subgroup and examined histologically.

Results: Body weights and body weight gains, as well as feed consumption, were increased as in all other B6C3F1 studies. High dose animals showed an increase in the P-selectin staining when compared to controls. Platelet aggregation was not sustained in the treated animals, as in previously reviewed studies. However, in this study, hepatic endothelial cell proliferation was comparable across groups.

Study title: **Pregabalin: Mode of Action of Hemangiosarcoma Formation and Pathophysiology of Platelet Changes in Mice**

Key study findings:

Study no.: RR-MEMO 745-03923
Conducting laboratory and location: Pfizer Global Research & Development, Ann Arbor, MI
Date of study initiation: Not specified
GLP compliance: Not required

The purpose of this report was to summarize the information collected as of the date of this memo (1/29/04) as to the sponsor's proposed mechanism of tumor formation in B6C3F1 mice. They also present results from additional studies demonstrating the functional "consequences" of pregabalin-induced alterations to respiration in rats and mice. There is also a discussion of the effects of pregabalin on acid-base balance and platelet function. The sponsor considered the effects to be a "key element in hemangiosarcoma formation in mice."

Results: In this summary, there is a statement that suggests that mice differ markedly in factors that affect endothelial homeostasis: tissue distribution of hematopoiesis, increased platelet counts, increased platelet turnover and activation and an increased rate of endothelial turnover. They contend that these differences "between mice and other species including humans may contribute to the higher incidence of spontaneous hemangiosarcoma in mice. These predisposing factors also may cause mice to be uniquely sensitive and develop increased incidences of hemangiosarcoma when given xenobiotics that alter those factors." While all of these premises may be correct, it does not negate the fact that both B6C3F1 and CD-1 mice showed an increased incidence of hemangioma/hemangiosarcoma when treated with pregabalin for 12 or 24 months. Increased cell proliferation may potentiate spontaneous mutations or select clonal growth of spontaneously initiated cells. **Unfortunately, endothelial cell proliferation in target tissues for hemangioma/hemangiosarcoma was not a constant or consistent finding in the mechanistic studies.**

In vitro work did not support the mechanistic premise proposed by the sponsor in that pregabalin

did not affect megakaryocytes in bone marrow cultures or bind to platelets or affect platelet function. In histopathologic evaluations of endothelial or other tissues, no significant evidence was shown for activation of the coagulation cascade or inflammation in any tissues.

The sponsor proposes that the effect of pregabalin on respiration and acid-base balance in mice leads to the changes in platelet function which they consider essential in the pathophysiology of these effects. Given the differences in metabolic rate and normally high variability in mice for pO_2, pCO_2, pH and bicarbonate when compared to other species, it is unclear how this explains the pathophysiologic basis for hemangiosarcoma from pregabalin administration.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Pregabalin administered to CD-1 mice elicited hemangiomas and hemangiosarcomas and changes in the myeloid:erythroid ratio in bone marrow. Significant increases in body weight and body weight gains were associated with treatment. The presence of active compound in the sera of the control animals of both sexes is of concern.

Table 2: Male mice, combining hemangiosarcomas with hemangiomas regardless of tissue:

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR 0	LO W	ME D	HIG H	P-Value (Exact Method)	P-Value (Asymptotic Method)
1800	[C]LIVER	999	Hemangioma	2	6	6	14	0.0008	0.0004

For the above table, the p-value from the asymptotic is the more relevant one. However, results are consistent with either approach, i.e. all are statistically significant.

Table 6: Female Mice Combined Hemangiosarcoma with Hemangioma Regardless of Tissue:

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR 0	LO W	ME D	HIG H	P-Value (Exact Method)	P-Value (Asymptotic Method)
3400	[C]UTERUS	999	Hemangioma	7	10	11	15	0.0092	0.0068

Approach statistical significance with 0.0068 vs. alpha 0.005

Wistar rats did not show any increased incidence attributable to pregabalin of any tumor type.

While persistent increases in platelet activation (P-selectin expression) were reported in female B6C3F1 mice treated with 1000 mg/kg/d for up to 12 months (last time point tested) of dosing, no consistent effects were noted at 200 mg/kg/d where increased tumors were also found. Increased activation at 1000 mg/kg/d was reversible 4 weeks after stop dosing, but in the 12 month reversibility animals, an increased incidence of hemangiosarcoma was still found. In CD-1 mice treated for 6 months at 5000 mg/kg/d, no consistent increases in platelet activation were appreciated. However, there was a definite increase in hemangiosarcoma incidence in these animals. Thus, it does not appear that this mechanistic basis for tumor formation is supported by

the data.

Platelet aggregation was decreased at 200 and 1000 mg/kg/d in B6C3F1 mice and at 1000 mg/kg/d in CD-1 mice by 6 months of dosing. No changes in platelet aggregation were noted after 6 months of dosing at 5000 mg/kg/d in the CD-1 mice. No changes were reported in rats at 900 mg/kg/d for 18 months or monkeys at 500 mg/kg/d for 69 weeks. Thus, the platelet aggregation effects appear to be unique to the mouse.

Increased megakaryocyte counts in bone marrow and increased platelet counts in blood were found only in mice. However, these changes were not reported in the original study reports and on reanalysis were generally considered mild in severity.

Of interest is the finding of increased PDGF in platelet poor plasma from B6C3F1 female mice at 1000 mg/kg/d at 12 and 24 months. Similar increases were not reported for CD-1 female mice treated for the same time. No VEGF increases were reported at these doses or times. This makes this mechanistic explanation somewhat useless as both strains of mice had increased hemangioma/hemangiosarcoma incidences. Rats do not show increases in PDGR or VEGF. In mice, VEGF labeling was increased in spleen and bone marrow and increased VEGF receptor 2 labeling in livers. Whether this proximate mediator of endothelial cell proliferation is significant in the formation of hemangioma/hemangiosarcoma remains to be seen. The sponsor suggests that monitoring these platelet function indices (platelet activation, aggregation, morphology) and that doing so might serve as "peripheral biomarkers in the process responsible for the increased incidence of hemangiosarcoma in mice." As these markers are inconsistent and not necessarily correlated to the tumors, it would not seem to provide the non-invasive biomarker to monitor human risk from pregabalin.

The sponsor proposes that the effect of pregabalin on respiration and acid-base balance in mice leads to the changes in platelet function which they consider essential in the pathophysiology of these effects. Given the differences in metabolic rate and normally high variability in mice for pO_2, pCO_2, pH, oxygen saturation and bicarbonate when compared to other species, it is unclear how this explains the pathophysiologic basis for hemangiosarcoma from pregabalin administration. Additionally, the B6C3F1 mice did not demonstrate the "expected" changes to the relative metabolic alkalosis (respiratory tract compensatory effects, epithelial effects in nasal cavity and respiratory tract, fibrosis, emphysema, loss of cells, increased inflammation, proliferation of endothelial cells in the lung, in addition to effects on renal tubules, etc.) that one would expect with a persistently impaired oxygen delivery to tissues. In a similar study in rats given 900 mg/kg/d for 30 days, bicarbonate, pO_2, and pCO_2 values in controls were comparable to humans but pregabalin administration elicited increased arterial and venous bicarbonate and pCO_2 as in the mice. Decreased oxygen saturation was reported in treated rats. The pH was not affected by pregabalin administration.

An indirect mechanism has been proposed for the platelet changes as pregabalin did not bind with high affinity to platelets from any species tested. The sponsor suggests that *in vitro* experiments "confirm" that changes in pH elicited by bicarbonate are "consistent with and sufficient to account for pregabalin-induced effects on platelet activation in vivo." However, a decreased secondary platelet aggregation response was not seen in the mouse platelets used for this experiment.

The sponsor's conclusion that "a clear association between altered respiration, acid-base

imbalance, increased platelet activation, bone marrow and splenic megakaryopoiesis, circulating VEGF and PDGF, endothelial cell proliferation and the incidence of hemangiosarcoma was demonstrated in mice at carcinogenic doses" is not clear from the evidence presented.

Suggested labeling: The increased incidence of hemangiomas and hemangiosarcomas should be addressed in the label.

Signatures:

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Deputy Division Director Signature: _____Concurrence Yes____ No ___

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/s/

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