CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-723

Pharmacology Review(s)

Memorandum

To: NDA 21-723

From: R. Daniel Mellon, Ph.D., Pharmacology Toxicology Supervisor,
Division of Anesthetic, Critical Care and Addiction Drug Products

Date: August 6, 2004

Re: Secondary Review of Pharmacology Toxicology Review

Lyrica® (pregabalin)

Indication: Post-herpetic Neuralgia (PHN)

The nonclinical pharmacology and toxicology studies submitted for NDA 21-723 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 finding of hemangiosarcomas in mice. Dr. Peters (Division of Anti-Infective Drug Products) was specifically consulted on this NDA due to her expertise in veterinary pathology.

Overall, there are three major toxicological concerns raised by the nonclinical data: Dermatopathy, reproductive toxicology and the development of hemangiosarcomas in mice. These issues were described in the Supervisory Review for NDA 21-446. Based exclusively upon the nonclinical findings, I concur with Dr. Cott that it does not appear prudent to approve NDA 21-723 due to the narrow safety margins. I also agree with Dr. Cott that further clarification of the dermatological findings should be conducted. However, my concern about this finding is reduced for the post-herpetic pain population.

However, Lyrica® appears to show efficacy for the treatment of pain associated with post-herpetic neuropathy, and a clear signal for dermatological toxicity has not been detected. Ultimately, the clinical benefit of the drug must be considered in light of the potential risks predicted by the non-clinical data. I agree with the revised labeling describing the dermatological findings, the reproductive toxicities and the findings of hemangiosarcomas.

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
8/6/04 05:28:14 PM
PHARMACOLOGIST

NDA 21-723, pregabalin

Additional information pertaining to this section can be found in the action package for NDA 21-446.

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:	**21-723**
SERIAL NUMBER:	**N 000**
DATE RECEIVED BY CENTER:	**10/30/2003**
PRODUCT:	**Lyrica® (Pregabalin)**
INTENDED CLINICAL POPULATION:	**Pain associated with post-herpetic 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): July 27, 2004

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

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

B. Recommendation for nonclinical studies

Additional studies should be conducted to investigate the mechanism of dermatopathy in rats and monkeys in order to assist in determining the potential relevance to humans.

C. Recommendations on labeling

Modifications of labeling were made for NDA 21-446, and will be the same for all nonclinical subject matter for NDA 21-723.

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 $AUC_{(0\text{-}24)}$ ≥ 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 (top) and 13-week oral monkey (bottom)

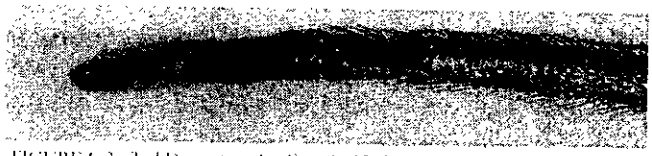


FIGURE F-2: Tail Dermatopathy, Female 45666 (1250 mg/kg)

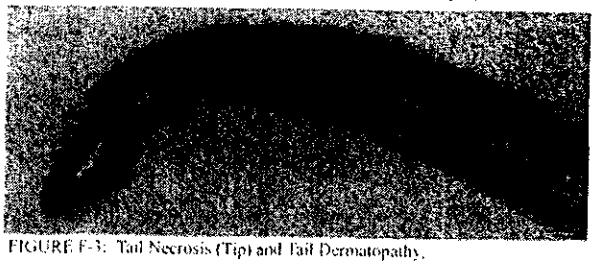


FIGURE F-3: Tail Necrosis (Tip) and Tail Dermatopathy.

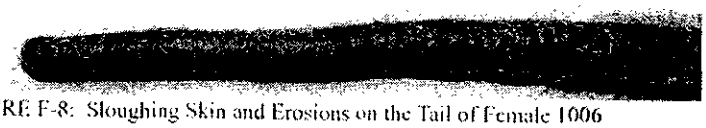


FIGURE F-8: Sloughing Skin and Erosions on the Tail of Female 1006 (500 mg/kg) on Day 15

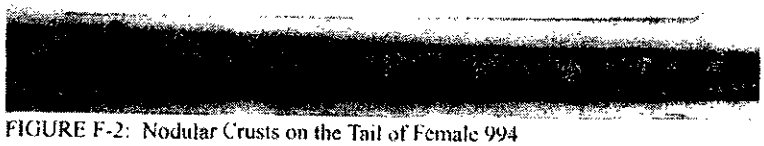
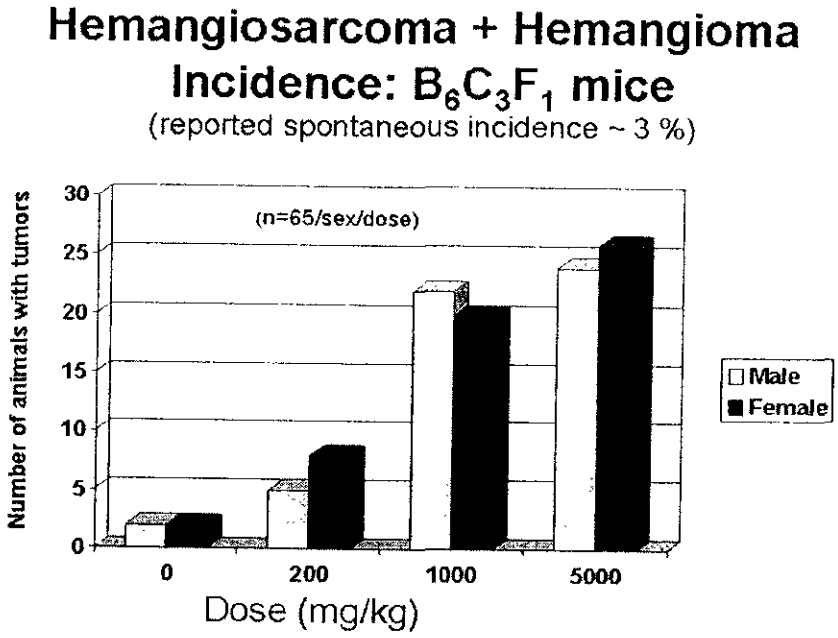


FIGURE F-2: Nodular Crusts on the Tail of Female 994 (100 mg/kg) on Day 89

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).



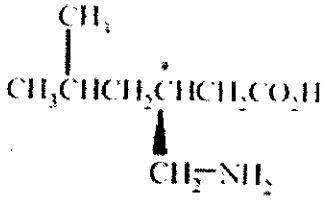
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

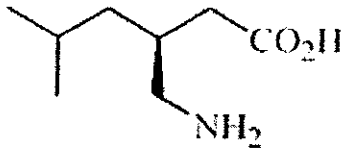
NDA number:	21-723
Review number:	1
Sequence number/date/type of submission:	000
Information to sponsor:	Yes (X) No ( )
Sponsor and/or agent:	Pfizer, Inc.
Manufacturer for drug substance:	Pfizer Global Manufacturing, 188 Howard Avenue, Holland, MI 49424-6596, and County Cork, Ireland
Reviewer name:	Jerry M. Cott, Ph.D.
Division name:	Division of Anesthetic, Critical Care and Addiction Drug Products
HFD #:	170
Review completion date:	7/26//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:	



* Chiral Center



Pregabalin Stereo Structure

Relevant INDs/NDAs/DMFs:

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

Drug class: Anticonvulsant

Indication: Neuropathic pain associated with post-herpetic 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 post-herpetic 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.

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[e] - UDS	Gavage	One Dose 250, 1000, 2000		745-02209
	Rat[e] - 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

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 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
Histopathologic Review of Skin And Mucous Membranes in Cynomolgus Monkeys from a 2-Week Continuous Infusion Study	745-02999
Updated Historical Data	

Type of Study	Report #
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:

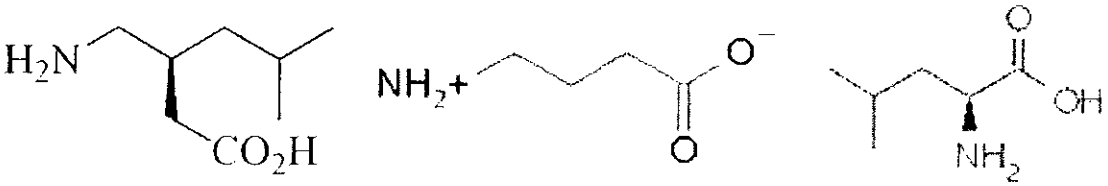
A. Pharmacology/Toxicology (HFD-120) review of initial rat and mouse carcinogenicity studies
B. Statistical review of mouse carcinogenicity studies
C. Exec-CAC evaluation of initial rat and mouse carcinogenicity studies
D. FDA Pharm/Tox consultant's review of carcinogenicity and investigative studies

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.

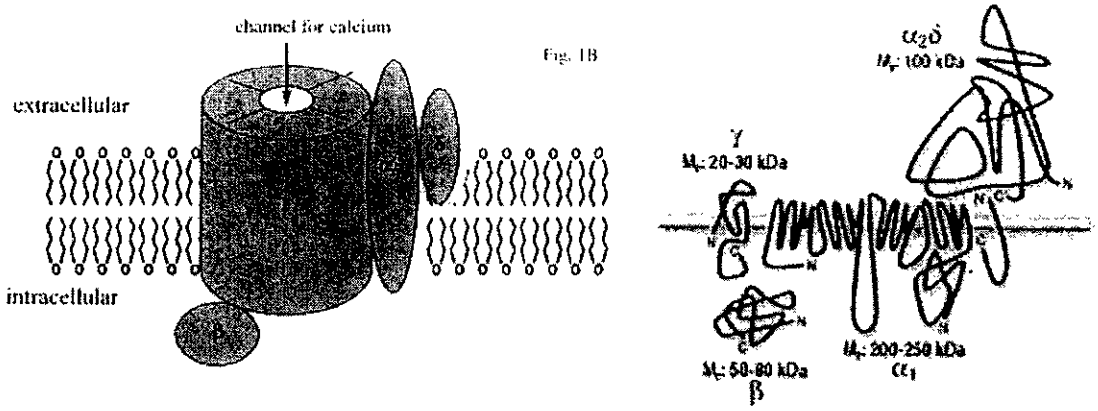


Fig. 1B

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 wild-type 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.

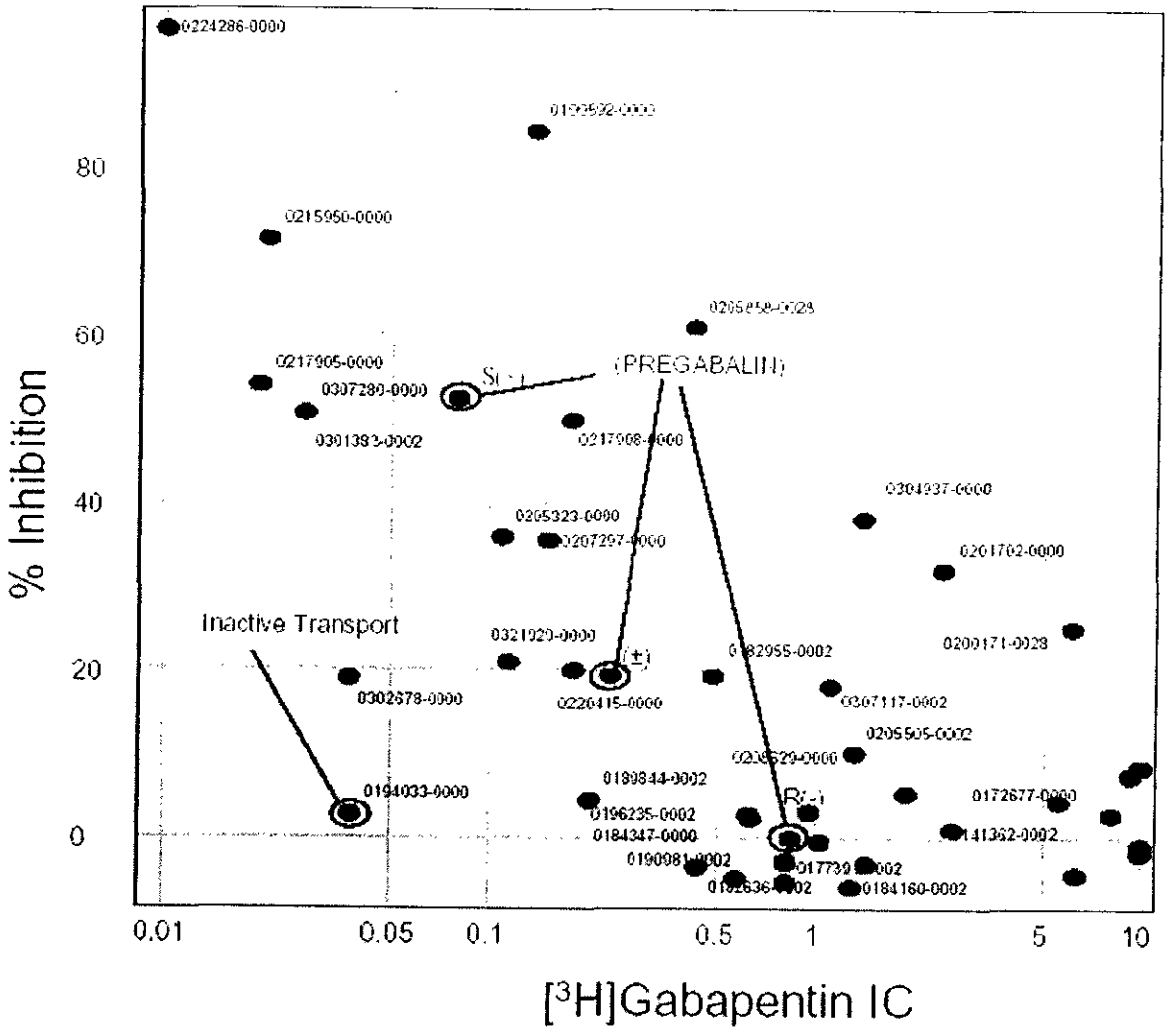


Figure 1. Relationship Between [^{3}H]Gabapentin Binding IC_{50} and Activity in the Carrageenan Thermal Hyperalgesia Test in Rats

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	Doses[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	50 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 50%.
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 ½ 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 30 100	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)

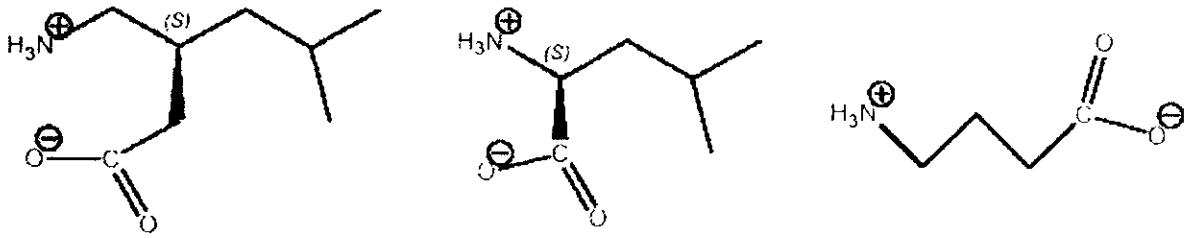
IC_{50} = Concentration inhibiting response by 50%, IP = Intraperitoneal
[a] Single dose unless specified otherwise

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	740-03479
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	770-00295
Brain/Spinal Cord	Mouse/F1 hybrid between 129/Sv] and C57/BL6 inbred strains	PO	100	7 M	Pregabalin had a non-significant effect in homozygous mutant mice defective in binding of [^{3}H]gabapentin to $\alpha_2\delta$ type 1 but reduced the delayed phase of response by 78% in wild-type mice	No	740-03610
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	770-00297
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	770-00296
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 30 mg/kg IP or 10 µg/rat intrathecal	No	Partridge et al., ref. 54
		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

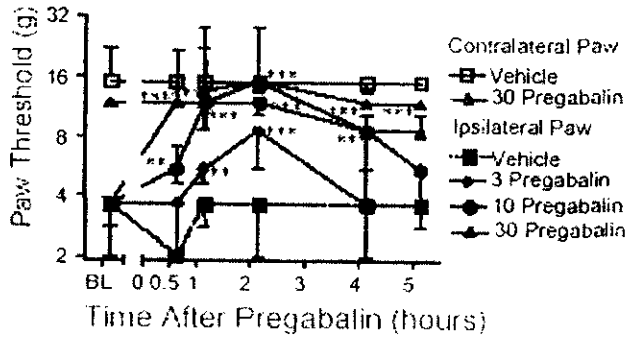
Models of Neuropathic Pain

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.

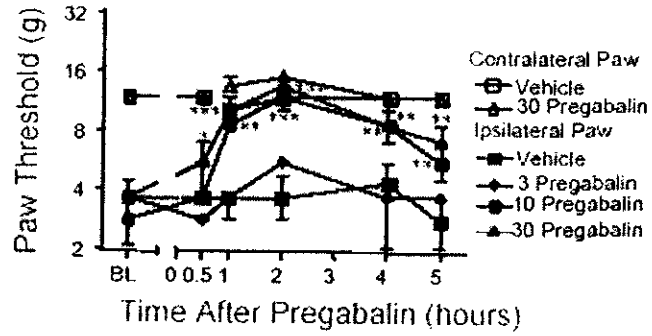
Vincristine Model: Pain from cancer can result from the disease itself, or in some cases, from the chemotherapeutic agents used to treat cancer. In particular, vinca alkaloids such as vincristine can cause pain syndromes characterized as myalgia, painful burning paresthesias, and also hyperalgesia and 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



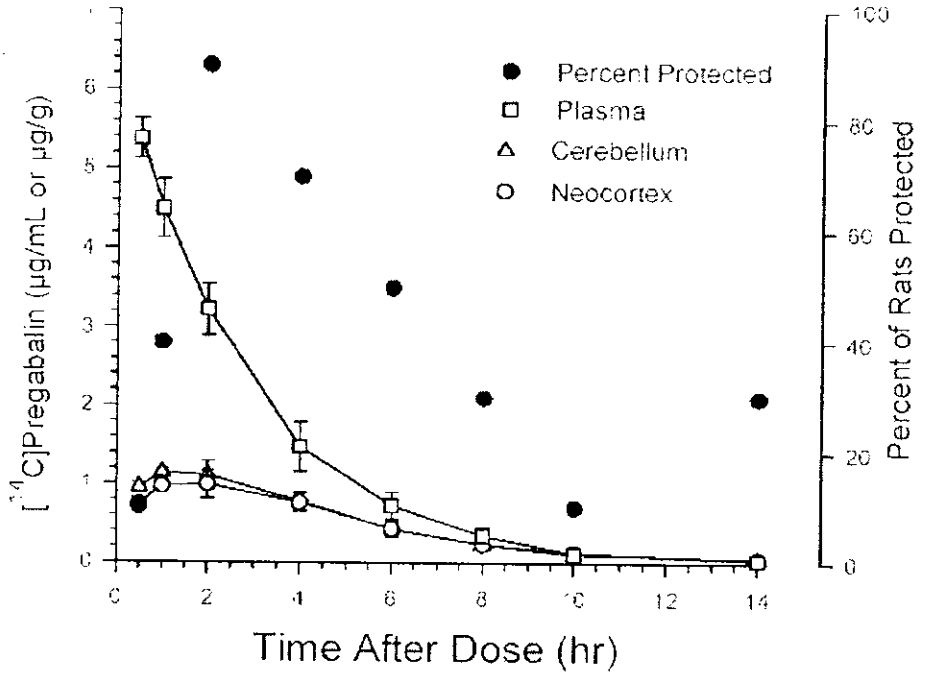
b. Chung Model



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.

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.

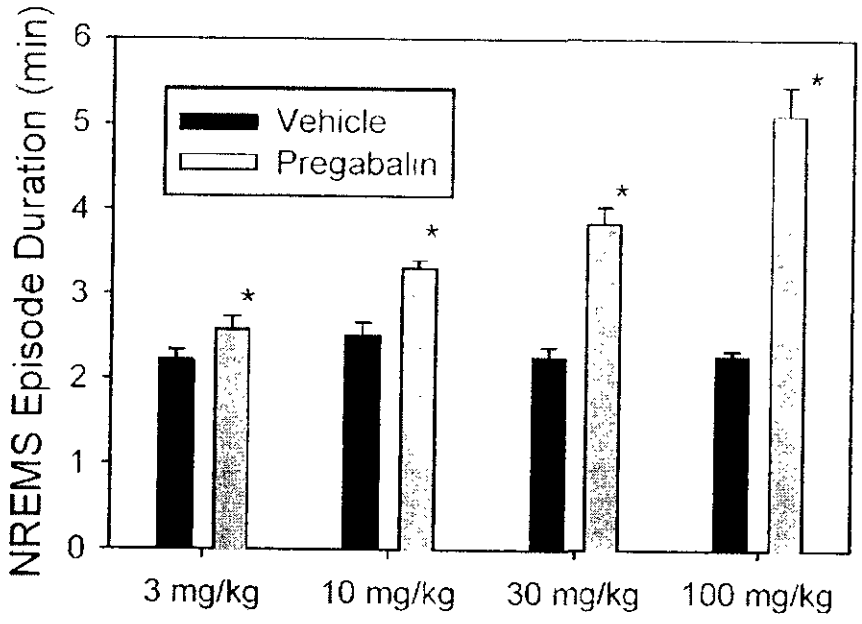
Neurofunctional Evaluation in Rodents:
Rats. While no changes were seen in muscle tone or the hindlimb placing response in rats given pregabalin at 25, 50, 100, or 125 mg/kg PO, a dose-related increased incidence of abnormal gait while walking on a flat surface was seen 4 hours postdose at all doses 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) [a]	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 CTD	M 4, 1 5, V 006	M 4, 1 5, V 006	M 4, 1 5, V 006	M 4, 1 5, 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 (Balb/c)
Gender (M/F) Number of Animals:	2 M/sex/time point
Feeding Condition:	Fed
Vehicle/Formulation:	Saline
Method of Administration:	IV
Pregabalin Dose (mg/kg), (µCi):	10 mg/kg, 5µCi/mouse
Radionuclide:	^{14}C
Specific Activity:	0.5 µCi/mg

Sampling Time:	Mean (n=2) Concentration (µg equivalents/g)					
	1 Hour	2 Hours	4 Hours	8 Hours	24 Hours	48 Hours
Tissues/Organs (WBA)						
Adrenal	4.95	3.37	0.38	BLQ	ND	ND
Blood	2.01	2.42	0.28	BLQ	ND	ND
Brain	0.92	0.65	0.35	BLQ	ND	ND
Brown Fat	2.31	1.43	BLQ	BLQ	ND	ND
Epididymis	2.50	1.64	0.47	BLQ	ND	ND
Fat	BLQ	BLQ	BLQ	BLQ	ND	ND
Harderian Gland	3.19	1.82	0.23	BLQ	ND	ND
Heart	3.42	2.56	0.28	BLQ	ND	ND
Kidneys	6.45	4.05	0.59	BLQ	ND	ND
Liver	3.34	2.59	0.53	BLQ	ND	ND
Lung	2.63	2.50	0.23	BLQ	ND	ND
Marrow	0.90	1.73	BLQ	BLQ	ND	ND
Muscle	1.50	2.69	0.35	BLQ	ND	ND
Pancreas	10.1	20.4	1.99	0.25	ND	ND
Preputial	6.27	3.13	ND	ND	ND	ND
Salivary	3.02	2.78	0.32	BLQ	ND	ND
Seminal Vesicles	0.62	0.84	0.20	BLQ	ND	ND
Spleen	2.05	2.03	0.16	BLQ	ND	ND
Testes	2.13	2.82	0.52	BLQ	ND	ND

BLQ = Below limit of quantitation (0.15 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:	IV
Pregabalin Dose (mg/kg), (µCi):	10 mg/kg, 40µCi/rat
Radionuclide:	^{14}C
Specific Activity:	31.6 µCi/mg

Sampling Time:	Mean (n=2) Concentration (µg equivalents/g)											
	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	11.1	6.84	6.36	1.56	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	3.89	1.08	0.96	BLQ	0.01	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	1.95	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	0.39	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	2.01	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	1.55	NA	BLQ	NA	BLQ

BLQ = Below limit of quantitation (0.03 µg equivalents/g), NA = Not applicable

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

Sampling Time:	Mean (n=2 or 3) Concentration (µg equivalents/g)											
	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	3.44	0.08	0.07	0.02	BLQ
Pineal	8.45	8.30	6.52	6.19	5.21	5.86	1.32	1.17	BLQ	BLQ	BLQ	BLQ
Pituitary	7.98	7.04	6.64	6.92	4.67	4.11	1.37	1.06	BLQ	BLQ	BLQ	BLQ
Preputial	14.6	13.5	19.1	22.7	14.2	9.00	[illegible]	1.16	2.37	0.98	0.47	0.55
Prostate	9.76	NA	9.67	NA	6.79	NA	1.61	NA	0.30	NA	0.06	NA
Salivary	10.5	11.1	8.45	10.8	6.62	6.27	1.52	1.38	BLQ	BLQ	BLQ	BLQ
Seminal Vesicles	5.40	NA	10.8	NA	8.69	NA	1.88	NA	0.17	NA	BLQ	NA
Skin	11.0	8.06	8.49	5.80	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	1.34	BLQ	BLQ	BLQ	BLQ
Testis	6.07	NA	7.52	NA	6.11	NA	2.22	NA	BLQ	NA	BLQ	NA
Thymus	12.3	12.7	9.16	13.4	6.67	6.88	1.61	1.35	BLQ	BLQ	BLQ	BLQ
Brain Stem	2.82	1.95	2.94	2.97	2.43	2.50	1.19	0.95	BLQ	BLQ	BLQ	BLQ
Cerebellum	2.96	1.97	3.13	2.56	2.42	2.72	0.98	0.82	BLQ	BLQ	BLQ	BLQ
Colliculus	3.16	2.03	3.48	3.50	2.69	3.15	1.21	1.01	BLQ	BLQ	BLQ	BLQ
Cortex	2.55	1.73	2.80	2.93	2.67	2.92	1.24	1.02	BLQ	BLQ	BLQ	BLQ
Hippocampus	2.31	1.91	2.69	2.68	2.58	2.66	1.28	1.12	BLQ	BLQ	BLQ	BLQ
Olfactory	2.73	1.75	3.45	3.68	2.64	3.15	1.11	1.01	BLQ	BLQ	BLQ	BLQ
Striatum	1.91	1.60	2.99	2.88	2.49	2.78	1.25	1.13	BLQ	BLQ	BLQ	BLQ
Thalamus	2.40	1.69	2.94	2.97	2.39	2.06	1.50	1.20	BLQ	BLQ	BLQ	BLQ

BLQ = Below limit of quantitation (0.05 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

Sampling Time:	Concentration (µg Equivalents/g)			
	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	1.33
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	1.23
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	3.83
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 (unspecified)
Feeding Condition:	Fasted
Vehicle/Formulation:	5% Dextrose
Method of Administration:	PO
Pregabalin Dose (mg/kg), (µCi/g):	10 mg/kg, 50µCi/rat
Radionuclide:	^{14}C
Specific Activity:	31.6 µCi/mg

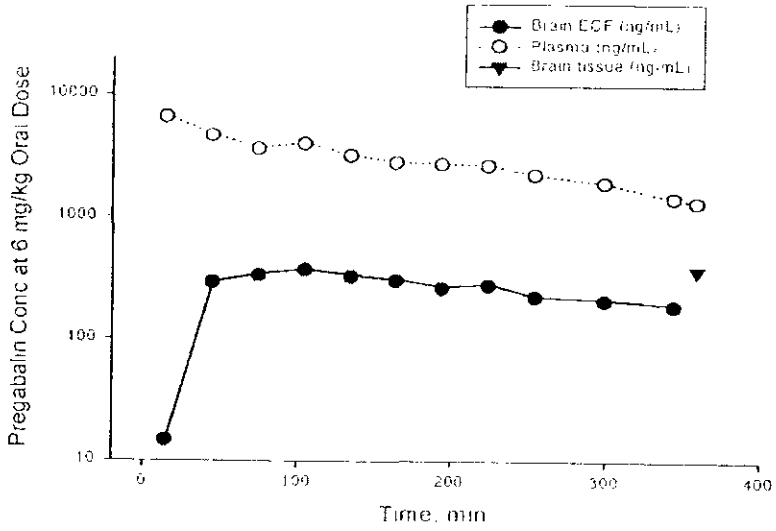
	Mean (n=2) Concentrations (µg equivalents/g)				
Sampling Time:	1 Hour	2 Hours	4 Hours	8 Hours	24 Hours
Tissues/Organs (WBA)					
Blood	10.5	9.93	5.39	1.87	0.01
Brain	2.20	3.05	2.61	1.20	0.05
Fetal Brain	8.22	12.9	11.3	7.11	0.16
Fetal Liver	14.7	18.5	15.0	5.61	0.20
Fetus	13.8	18.7	16.5	5.72	0.19
Kidney	17.9	13.1	11.3	3.96	0.11
Liver	11.9	9.91	5.79	2.41	0.13
Pancreas	69.2	19.2	2.66	0.51	0.11
Placenta	12.9	12.9	11.0	3.74	0.10
Preputial	18.7	36.2	32.1	16.7	1.21
Amniotic Fluid	ND	ND	ND	ND	0.04
Lens	ND	ND	ND	ND	0.15

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.

In vivo Metabolism of Pregabalin

Gender (M/F)/No. of Animals:	$B_6C_3F_1$ Mice [illegible]	$B_6C_3F_1$ and CD-1 Mice: [illegible] M [illegible]	Rats: [illegible] M 6 [illegible]	Dogs: [illegible] M [illegible]	Monkeys: [illegible] M [illegible]	Humans[a]: [illegible] M
Feeding Condition:	Fed	Fed	Fed	Fed	Fast	Fed
Vehicle/Formulation	Solution	Diet admixture[b] [illegible]	Solution in water	Solution in 5% Dextrose	Solution in water	Solution in [illegible]
Method of Administration	Gavage	Gavage	Gavage	Gavage	Gavage	Oral
Pregabalin Dose (mg/kg):	25 mg/kg (single dose)	300 mg/kg	25 mg/kg	10 mg/kg	10 mg/kg	100 mg
Radionuclide, ^{14}C (µCi):	70 µCi/mouse (single dose)	25 µCi/mouse	22 µCi/rat	50 µCi/dog	45 µCi/monkey	108 µCi/subject
Specific Activity:	22.5 µCi/mg	22.5 µCi/mg	22.5 µCi/mg	22.5 µCi/mg	22.5 µCi/mg	1.07 µCi/mg

Species	Sample[c]	Sampling Time or Period	% of Dose in Sample	% of Compound in Sample: Parent	M1	M2	M3[d]	M4	Research Report Ref	Location in CTD
$B_6C_3F_1$ Mouse	Urine[e]	0-24 hr 24-48 hour	87	92		-			[redacted] [illegible]-57	M 4, 1.5, V 006
$B_6C_3F_1$ Mouse	Urine	0-24 hours	98	[illegible]	-	-	2	--	[redacted] 00272	M 4, 1.5, V 006
CD-1 Mouse	Urine	0-24 hours	87	[illegible]			2	-		
Rat	Plasma	[illegible] hours	NA	[illegible]	-	-	-	-	764-[illegible]127	M 4, 1.5, V 006
	Urine	0-24 hours	[illegible]	[illegible]	-	2.[illegible]	2.6	[illegible]		
Dog	Urine[f]	0-24 hours	84	[illegible]	3.0	1.5	44.6	[illegible]	764-[illegible]226	M 4, 1.5, V 006
Monkey	Plasma	2 and 8 hours	NA	100	--	-	--	--	764-[illegible]368	M 4, 1.5, V 007
	Urine[g]	0-24 hours	7[illegible]	[illegible]	[illegible]4	--	--	--		

a Pregabalin undergoes minimal metabolism in the human with only three minor [illegible] unidentified components detected in the urine. Reference RR 7[illegible]
b Mice were dosed 1000mg/kg/day by diet admixture for 2 weeks prior to the single radioactive dose of 2[illegible]µCi or 300mg/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 not identified
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 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 ≥ 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 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

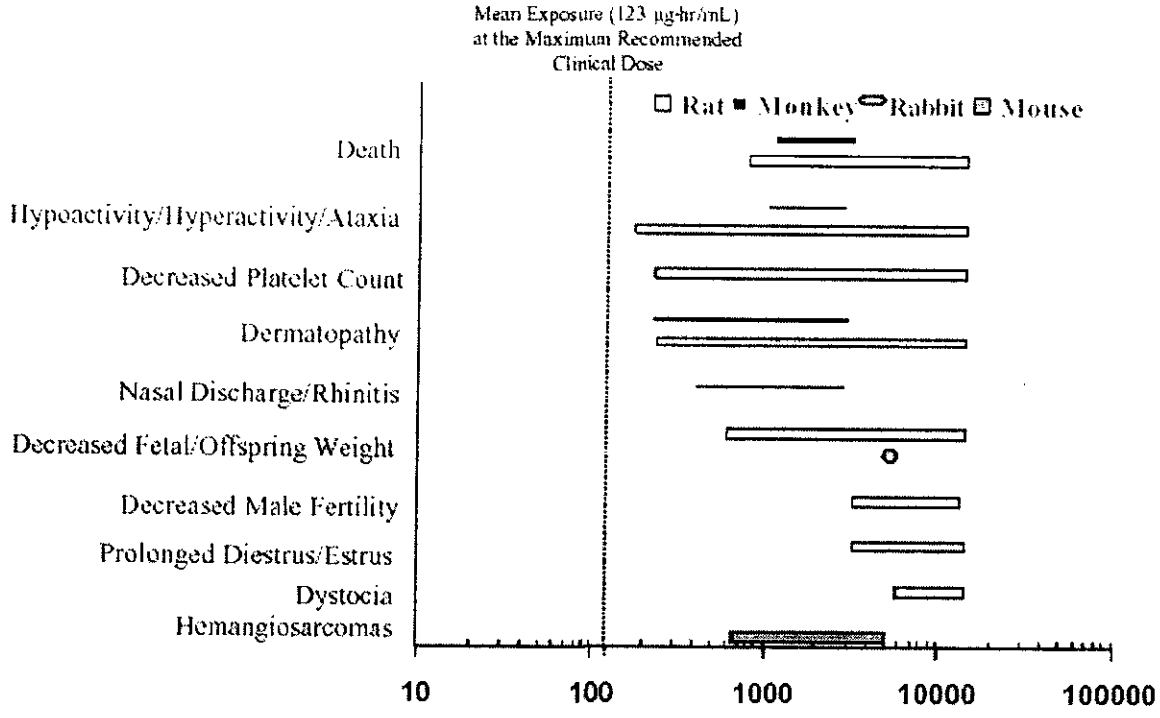
2.6.6 TOXICOLOGY

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 ≥ 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).

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 ≥ 50 mg/kg in oral repeated-dose studies, with associated $AUC_{(0\text{-}24)}$ ≥ 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 $AUC_{(0\text{-}24)}$ 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 ($AUC_{(0\text{-}24)}$ 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 ($AUC_{0\text{-}24}$) 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) [50 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/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	0	0	0	0	0	0	0	0	2	1
Cystitis or pyelonephritis									Weeks 3-4	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	7/21	12/21	6/21	1/21	17/21	14/21	3/21	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	12/21	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	1/21	0/21	0/21
	2500	0/21	0/21	0/21	4/21	0/21	10/21	3/21	3/21	0/21	0/21
	5000	0/21	1/21	0/21	8/21	0/21	16/21	4/21	2/21	2/21	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	2/21	1/21	0/21	0/21
	2500	0/21	0/21	0/21	1/21	0/21	5/21	3/21	4/21	0/21	0/21
	5000	0/21	1/21	0/21	9/21	0/21	14/21	4/21	2/21	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	2/20	1/21	2/21	0/21	0/21
	2500	0/21	0/21	0/21	1/21	0/21	2/21	3/21	4/21	0/21	0/21
	5000	0/20	1/20	0/20	6/1/20	0/20	2/20	3/20	3/20	2/20	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	7/21	1/21	9/21	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	4/21	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	5/21	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*										
Red Blood Cells (10^{12}/L)	7.7	7.6	8.4**	--	8.7**	--	8.8**	8.2**	8.9**	8.3**
Percent of Control			9%	--	13%	--	14%	8%	16%	9%
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)	1153	989	937**	893**	829**	816**	789**	810**	827**	757**
Percent of Control			19%	10%	28%	17%	32%	18%	28%	23%

-- *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	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) I	94.9	63.9	--	1-fold**	1-fold**	1-fold**	1-fold**	2-fold**	2-fold**	3-fold**
PROD (nmol/min/mg p) I	6.84	2.45	--	--	1-fold**	1-fold[ns]	4-fold**	7-fold[ns]	16-fold**	30-fold**
Immunoblot CYP2B1/2	--	--	--	--	--	--	I	I	I	I
CYP2E1	--	--	--	--	--	--	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

	MALES		FEMALES	
Parameter (Weight)	Treatment (% control)*	Withdrawal (% control)	Treatment (% control)	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 Histopathology (continued)	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
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, 1 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 $\geq$ 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+ 0.0356	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 ≥ 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, [illegible] % 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) D	503		--	--	--	--	24%**	--
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	I 500(bid)F, I 500(bid)F (R)
Thymus atrophy	I 0M, I 0F(R), I 500M, I 500M(R), I 500(bid)F(R)
Spleen, atrophy	I 500(bid)F(R)
Epididymis, hypospermia	I 100M, I 500(bid)M
seminiferous tubules, hypospermia	I 100M, I 500(bid)M
testes, degeneration	I 100M (R), I 50M (R)
kidney, atrophy	I 100F
dilatation	I 100F, I 500(bid)M; I 500(bid)F, I 500(bid)M (R); I 500(bid)F (R)
fibrosis	I 0F, I 100F, I 25F(R), I 100F (R)
adrenal, hyperplasia	I 25F
vacuolation	I 25F
abnormal	I 500(bid)F (R)
hypertrophy	I 100M (R)
pancreas, hyperplasia	I 500(bid)M (R)
lung, granuloma	I 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 $\geq$ 100mg/kg. Heart:brain or body weight ratios appeared elevated at $\geq$ 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) ↑	--	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; ↑ 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			Necrosis of interventricular septum also noted in the female that
Heart - Necrosis Left Ventricle	--	1	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, 1 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; [redacted] and [redacted] respectively.

Methods

Doses: 0, 10, 25, 100, 250/500, and 500 mg/kg
Species/strain: wild-caught cynomolgus monkeys from [redacted]
[redacted]: 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	6	6
Animals Logged	6	6	3	3	3	3	3	3	6	6
Cardiovascular										
Aorta	6	6	3	3	3	3	3	3	6	6
Not Remarkable	6	5	3	3	3	3	3	3	6	5
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	6	6	3	3	3	3	3	3	6	6
Not Remarkable	5	1	0	1	0	1	1	0	1	1
Remarkable Observations	1	5	3	2	3	2	2	3	5	5
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

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

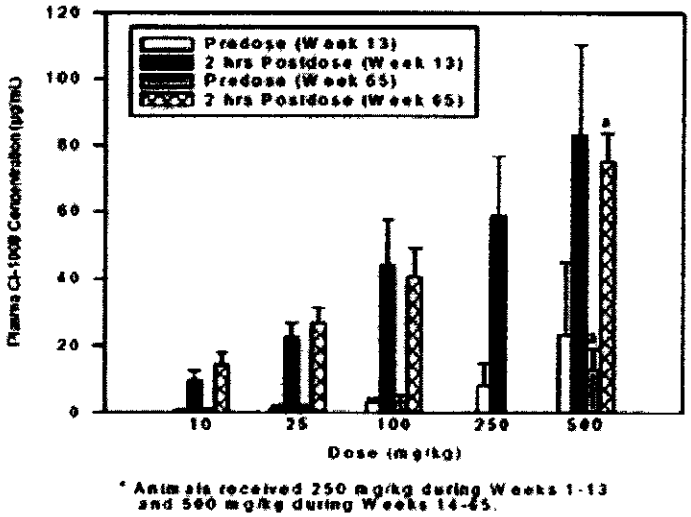


FIGURE 1. Mean Plasma CI-1008 Concentrations (Combined-Sex)

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

Appears This Way
On Original

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

10.4. TABLE 3-4 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	19.3	16.0	32.3	31.3	60.0	62.3
FD 144723 (µg/plate)										
200	8.3	10.0	5.7	8.0	8.3	12.0	38.3	25.3	50.7	54.3
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	3.3	10.0	9.7	17.3	41.3	22.3	62.0	50.3
1600	6.3	9.7	3.7	8.0	7.3	16.7	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	52.0
Positive Control	941.0*	159.0*	68.3*	102.7*	944.7*	530.3*	1616.7*	1730.0*	530.0*	1487.3*
p-Value for Positive Linear Slope	0.496	0.727	0.652	0.533	0.417	0.558	0.370	0.998	0.742	0.625
p-Value for Negative Quadratic Effect	0.290	0.825	0.220	0.325	0.910	0.251	0.435	0.912	0.492	0.256

* 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

Appears This Way
On Original

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% methylcellulose) 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 $p \leq 0.05$.

Appears This Way
On Original

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] PE$_1$[b]	PE$_2$	Mutant Frequency[c]
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]		Mutant Frequency[c]
		PE_1[b]	PE_2	
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 [redacted]

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, 1 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] 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: [redacted] end: [redacted]

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 0.5×10^9 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 [ ]

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 [ ] 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	0.0125	2.50
Pregabalin	1600	108	113	16	0.0380	3.00
	3000	107	85.0	13	0.0665	6.00
	5000	96.0	61.0	7	0.0135	3.50
	9000	115	87.0	8	0.0140	4.00
	16000	98.0	75.0	13	0.0665§	6.50
Cyclophosphamide	4	Not Determined	79.0	73	0.2653†	24.5†
	8	Not Determined	38.0	94	0.5537†	33.5†

† 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, 1 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 ≥ 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 4.1 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 [illegible]

Methods

Strains/species/cell line: Male and female B6C3F1 and CD-1 mice [illegible]

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 (0.76%). 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 four, 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 hemangiosarcomas 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

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 [ ] 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 [ ] is 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 [ ] was assessed in rabbits (RR 901-00508). Conjunctivitis, characterized by redness, discharge, and/or swelling, occurred in all animals given PD [ ] 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 $AUC_{(0\text{-}24)}$ ≥ 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 $AUC_{(0\text{-}24)}$ 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 ($AUC_{(0\text{-}24)}$ 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.

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[a]	Oral (Diet)	UC 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
	Oral (0.5% MC) [25 mL/kg]	VC 500 1250 2500				
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.

[a] Two hundred additional animals used for determination of toxicokinetic parameters (5 controls/sex and 15/dose/sex each for diet and gavage administration).

[b] Bulk drug at 20 mg/mL

[c] Seventy-eight additional animals used for determination of toxicokinetic parameters (3 controls/sex and 12/dose/sex).

[d] Drug concentration of 20 mg/mL resulted in a dose rate of 40 mg/min.

[e] Sixty additional animals used for determination of toxicokinetic parameters (3 controls/sex and 9/dose/sex).

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[h] (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[o]	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.

[l] Vehicle controls = 1/sex.
[k] Dose volume = 10 mL/kg at 2000 mg/kg.
[j] 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

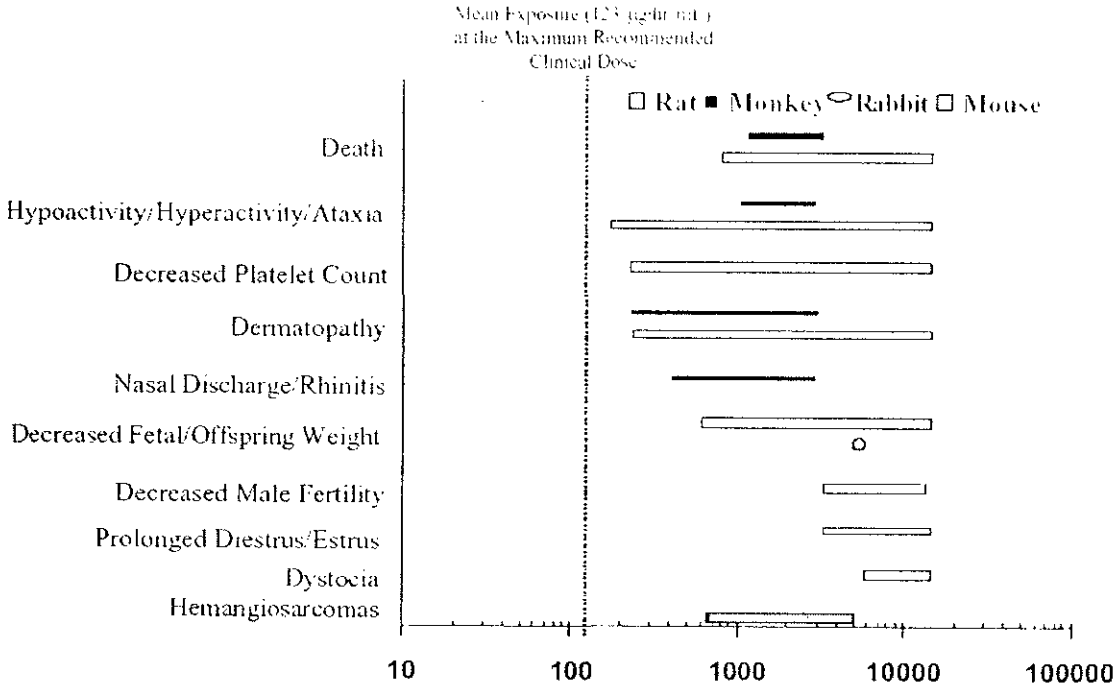
VC = Vehicle control.
[b] Bulk drug at 20 mg/mL
[c] Drug concentrations of 1, 2, or 3 mg/mL resulted in dose rates of 2, 4, or 6 mg/kg/hr.

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[a]	Oral (Diet)[a]	M F UC UC 2174 2607 7538 8152 12685 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]



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Sponsor's summary table:

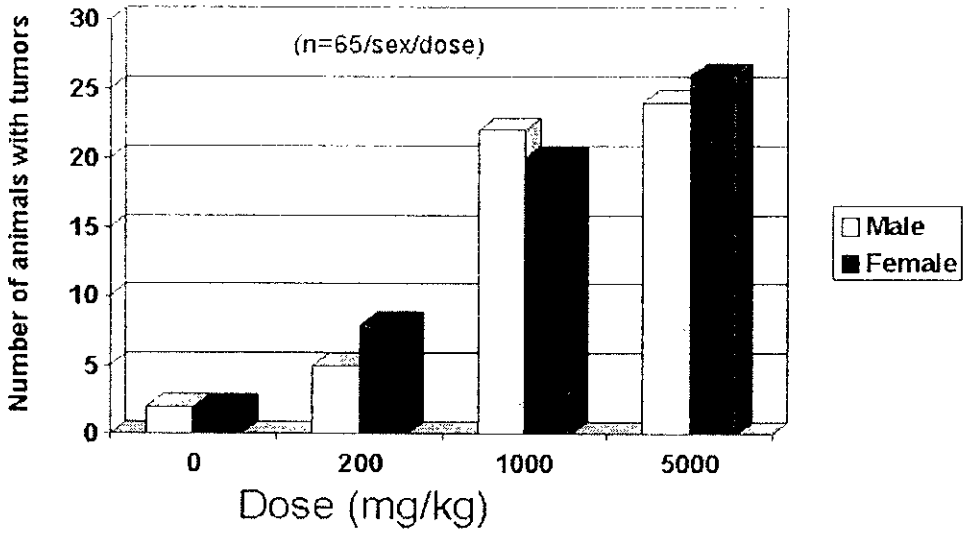
Significant Effect	Species	No-Effect Dose AUC (0-24)	Lowest Effect Dose AUC (0-24)
Hyperactivity Hyperactivity, Ataxia	Rat	Effects at all doses studied	50 mg/kg 173 µg hr/mL[a]
	Monkey	100 mg/kg 1388 µg hr/mL	500 mg/kg 9001 µg hr/mL
Decreased Platelets	Rat	Effects at all doses studied	50 mg/kg 228 µg hr/mL[b]
Dermatopathy	Rat	Effects at all doses studied	50 mg/kg 241 µg hr/mL[c]
	Monkey	100 mg/kg 2911 µg hr/mL	25 mg/kg 219 µg hr/mL
Nasal Discharge/Exudate	Monkey	500 mg/kg 3885 µg hr/mL	100 mg/kg 388 µg hr/mL
Developmental Toxicity Decreased Fetal Weight	Rat	1250 mg/kg 6566 µg hr/mL	2500 mg/kg 9470 µg hr/mL
	Rabbit	500 mg/kg 2202 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 1523 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 1389 µg hr/mL[e]	1250 mg/kg 5760 µg hr/mL[e]
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[d]

[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.

J. Cott summary table:

Hemangiosarcoma + Hemangioma Incidence: $B_6C_3F_1$ mice

(reported spontaneous incidence ~ 3 %)



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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 clear dose-related dermatopathy in monkeys and rats.

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: Based on the low safety margins between human therapeutic dose and animal toxicology findings, this application is not recommended for approval for the indication of post-herpetic neuropathy from the pharmacology / toxicology perspective.

Suggested labeling: For all pharmacology / toxicology issues, the labeling from NDA 21-446, pregabalin for the treatment of diabetic neuropathy, will be used.

Carcinogenesis/Mutagenesis/Impairment of Fertility

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ **Concurrence Yes** ___ **No** ___

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.
[P] 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
Anesthetized rat electrophysiological seizure activity in hippocampus & evoked potentials	IP	740-03518

Type of Study Test System	Administration	Study No.
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

Type of Study Test System	Route	Report #
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
Rat abdominal constriction responses after systemic administration of lipopolysaccharides and rectal distension	IP, PO	6051-00003

Type of Study Test System	Route	Report #
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
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

Type of Study Test System	Route	Report #
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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/s/

Jerry Cott
7/29/04 09:23:11 AM
PHARMACOLOGIST

converted and ready

Mamata De
7/29/04 09:49:44 AM
PHARMACOLOGIST
I concur (acting supervisor, pharmacology toxicology, for Dr. Daniel R.Mellon)

Executive CAC
December 12, 2000

Committee: Joseph DeGeorge, Ph.D., Chair
Joseph Contrera, Ph.D., HFD-901, Member
Robin Huff, Ph.D., HFD-570,Alternate Member
Glenna Fitzgerald, Ph.D., Team Leader
Ed Fisher, Ph.D., Presenting Reviewer

Author of Draft: Ed Fisher

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # [ ]
Drug Name: pregabalin
Sponsor: Parke-Davis

Background:

Pregabalin is a GABA analogue being investigated for the treatment of epilepsy, pain, [ ] It is also chemically related to the approved antiepileptic gabapentin. It was negative in the gentox battery.

Mouse Study

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.

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.

Rat Study

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. They were based on the results of a 13-week rat study, with the HD considered an estimated MTD. The doses used were those recommended by the Division and CAC. 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).

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.

Executive CAC Recommendations and Conclusions:

The Committee concluded that both studies were adequate. They thought that the increased incidence of hemangiosarcomas in treated mice represented a true tumorigenic response to the drug. And they considered this finding to be of concern, since based on the present information, they could not say that it is not relevant to humans. Thus, they strongly disagreed with the following statement proposed by the sponsor for the pregabalin [

]

Furthermore, they did not consider the LD a no-effect dose for hemangiosarcomas, since the incidence in females mice was outside the historical control range at this dose.

The Committee suggested that if the sponsor believes that the tumor findings are specific to this strain, they could conduct a second 2-year bioassay in a different strain of mouse.

The Committee recommended that additional statistical analysis of the rat findings be conducted in which incidences of tumors histologically-related to the hemangiosarcomas found in mice be appropriately combined across tissues.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:
/Division File, HFD-120
/EFisher, HFD-120
/GFitzgerald, HFD-120
/JWare, HFD-120
/ASeifried, HFD-024

/s/

Joseph DeGeorge
12/21/00 04:49:51 PM

/s/

Jackie Ware
12/29/00 02:10:16 PM
Signed for John S. Purvis