CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20646

PHARMACOLOGY REVIEW(S)

July 12, 1996

Review and Evaluation of Pharmacology and Toxicology

NDA: 20-646

Sponsor: Abbott Laboratories
Abbott Park, IL 60064

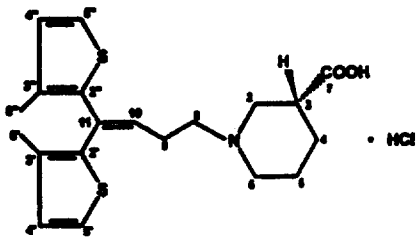
Drug: Tiagabine HCl tablets

Chemical Name: (R)-(-)-1-[4,4-Bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid

Molecular Formula: $C_{20}H_{25}NO_2S_2 \cdot HCl$

Code name(s): Abbott-70569·HCl, NO-05-0328 (Novo)

Structure:



Mol. Wt.: 412.0 (375.5, free base)

Category: Antiepileptic

Related IND(s):

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Table of Contents

APPEARS THIS WAY
ON ORIGINAL

I. PHARMACODYNAMICS

A) ANTICONVULSANT ACTIVITY (studies conducted by . NDA Vol. 1.30).

1. Tiagabine generally resembled other drugs that enhance GABAergic neurotransmission in standard anticonvulsant screening tests (**Table I.1**, ip administration). It was potent against sc PTZ-induced clonic seizures in mice (ip ED50: 1.3 mg/kg) but like other GABA uptake inhibitors (eg, SKF 100330A) exhibited a U-shaped dose-response curve, never reaching 100% protection, although tonic seizures were almost completely prevented (**Figure I.1**). It showed activity against MES in rats only at neurotoxic doses (ED50: 40 mg/kg, ip).
2. Tiagabine also potently inhibited DMCM-induced clonic seizures in mice (DMCM is a full inverse agonist at the BDZ receptor), although a U-shaped dose-response curve was again seen, ie, higher doses (30 or 100 mg/kg, ip) failed to block seizures. At the ED50 dose for inhibition of DMCM-induced seizures, 20-30% of the GABA uptake sites were occupied by tiagabine; at the ED50 for ataxia, 50-60% of sites were occupied. PIs for tiagabine based on various indices of anticonvulsant activity and neurotoxicity are compared with those of other AEDs in **Tables I.3** and **I.4**.
3. In a study designed to evaluate tolerance development in mice (2 mg/kg, ip, bid for 8 days), a significant reduction in anticonvulsant effect against iv PTZ was seen after 2 doses, but recovery appeared to occur during continued dosing so that after 8 days anticonvulsant efficacy was similar to that seen acutely. Cross tolerance with BDZs was also observed in this study. In a study reported only in abstract form, tolerance did not develop to the acute anticonvulsant effects of tiagabine after administration to mice for 21 days at 15 or 30 mg/kg bid, po [acute ip ED50s for inhibition of DMCM-induced clonic convulsions were 1.7 (vehicle control group), 1.9 (15 mg/kg group), and 2.0 mg/kg (30 mg/kg group)]; and there were no signs of withdrawal after discontinuation of treatment (no changes in PTZ seizure threshold, body weight, or general behavioral observations).
4. Tiagabine potently blocked audiogenic seizures in DBA/2 mice (ED50 = 0.4 mg/kg, ip). Plasma and brain concentrations in DBA/2 mice at the ED50 were 141 ng/ml and 46 ng/g, respectively.
5. Tiagabine blocked sound-induced seizures in genetically epilepsy prone rats (GEPRs) with ED50 values of 11 and 30 mg/kg, ip, for the tonic and clonic seizure components, respectively.
6. Tiagabine decreased seizure severity and after-discharge duration in amygdala-kindled rats with an ED50 of 3 mg/kg, ip; a dose of 10 mg/kg ip completely blocked kindled seizures.
7. Tiagabine was ineffective against bicuculline-induced seizures, and exacerbated absence-like spike wave discharges in rat models of non-convulsive epilepsy (WAG/Rij, GAERS).

B) MECHANISM OF ACTION (studies conducted by Vol. 1.30).

1. Tiagabine was about 25 times more potent than nipecotic acid in inhibiting the uptake of 3H-GABA into rat forebrain-derived synaptosomes (IC50 = 67 nM). Tiagabine (R-) was approximately 4 times more potent than the S+ enantiomer, indicating a stereospecific interaction at the GABA uptake carrier.
2. GABA uptake carriers on neurons and glia were both affected by tiagabine, as demonstrated in studies using primary cultures of neurons and astroglia in which tiagabine IC50s of 446 and 182 nM, respectively, were determined for inhibition of 3H-GABA uptake (ie, 2.5 times more potent in inhibiting glial uptake). Tiagabine did not stimulate the release of 3H-GABA from cerebral cortical neurons in culture.
3. Tiagabine had no significant affinity (IC50>100 uM) for DA, ACh, adrenoceptors, 5HT, adenosine, histamine (H2 and H3), opiate, glycine, glutamate, or GABA-A receptors, nor did it interact with DA, NE, 5HT, ACh, or GLU uptake sites. It also lacked affinity for sodium or calcium channels. It did have weak affinity for histamine H1 (IC50=6.6 uM) and BDZ (IC50=15 uM) receptors (>98-fold higher than IC50 for inhibition of [3H]GABA uptake).

4. 3H-tiagabine appeared to bind to a single class of high affinity binding sites *in vitro* (Kd=18 nM, Bmax=669 pmol/g), in a sodium-dependent manner.
5. Tiagabine produced a dose-dependent increase in extracellular levels of GABA in rat brain as measured by *in vivo* microdialysis. Doses of 11.5 and 21 mg/kg, ip, significantly elevated extracellular GABA levels in the globus pallidus and ventral pallidus of unanesthetized rats to approximately 250 and 350% of basal levels. These doses are equivalent to the ED50 and ED85 doses of tiagabine, respectively, for inhibiting PTZ-induced tonic seizures in rats.
6. In electrophysiological studies in rat hippocampal slices, tiagabine increased the duration and amplitude of responses to exogenously applied GABA, and prolonged GABA-mediated inhibitory postsynaptic potentials (IPSPs).
7. *In vivo*, tiagabine bound (Kd of 72.5 mM and Bmax of 640 pmol/g) to a single class of binding sites that were regionally distributed in the CNS: hippocampus>occipital cortex>midbrain>parietal cortex>frontal cortex>cerebellum>pons-medulla>striatum.

C) GENERAL PHARMACOLOGY

1. *Behavioral pharmacology* - Tiagabine was active in the acetic acid-induced writhing (ED50=0.18 mg/kg, ip), hot plate (3.7 mg/kg, ip), and grid shock avoidance (1.75 mg/kg, ip) tests for analgesic activity in mice, but was inactive in the tail flick test in rats (up to 30 mg/kg ip). It was active in the Vogel water lick conflict test for anxiolytic activity in rats at 10 mg/kg ip, and potentiated DA functioning as reflected in its ability to enhance methylphenidate-induced gnawing in mice (ED50=0.9 mg/kg, ip). Tiagabine doses up to 30 mg/kg, ip, did not substitute for d-amphetamine, diazepam, PTZ, or CGS-9896 in drug discrimination testing. Tiagabine (0.1 mg/kg, iv infusion) was not self administered by rats.
2. *EEG* - Tiagabine doses up to 30 mg/kg, po, did not affect rat EEG recordings and did not affect the appearance of alpha waves in the EEG from sensory and motor areas of the neocortex.
3. *GI* - In isolated guinea pig ileum, tiagabine antagonized histamine-induced contractions with an EC50 of 1.5 ug/ml and antagonized acetylcholine-induced contractions with an EC50 of 20 ug/ml. Concentrations of 3 and 30 ug/ml caused reductions in spontaneous motility. Doses of 3 and 30 mg/kg, iv, produced a significant D-R increase in gi transit time in mice, indicating an inhibitory effect on gi motility.
4. *Blood* - Concentrations of 1, 10, and 100 uM of tiagabine did not affect prothrombin time or platelet aggregation in rat-derived blood specimens.

D) NEUROTOXICITY

1. Tiagabine reduced exploratory locomotor activity, traction, and rotarod performance in mice and rats at doses greater than those required for anticonvulsant activity (**Table I.2**, ip administration). The PI based on the (ip) ED50s for decreased locomotor activity and inhibition of seizures was 14 in mice for DMCM-induced seizures and 10 in rats for PTZ-induced seizures (**Table I.4**).
2. In a test of cognitive function in mice (one-trial avoidance learning), tiagabine impaired memory after acute administration of 3 or 10 mg/kg, ip, or after 10 mg/kg following pretreatment with 3 mg/kg, ip, twice daily for 3 days. The ratio of cognitive impairment to anticonvulsant efficacy against DMCM-induced convulsions was 7, and tolerance to cognitive impairment, but not to anticonvulsant efficacy was observed after subchronic administration.
3. *Mice* - Tiagabine decreased locomotor activity and caused piloerection and hypothermia in mice at 10 mg/kg, po and ip. Larger doses decreased grip strength and muscle tone, increased respiration, and caused cyanosis, opisthotonos, tremors, limb abduction, and clonic convulsions. The estimated LD50 values were 200 mg/kg, po, and 75 mg/kg, ip.
4. *Rats* - In rats, doses of 100 and 200, po, produced decreased locomotor activity, piloerection, dyspnea, slight ataxia, and wet dog shaking.
5. *Dogs* - In dogs, tiagabine produced mild sedation and slight ataxia at a dose of 0.5 mg/kg,

po, and deep sedation, ataxia, decreased muscle tone, tremors, increased respiration, salivation, ptosis, and mydriasis at a dose of 2 mg/kg. When tiagabine was administered to dogs for 5 days at 0.5 mg/kg, po, adverse behavioral signs (sedation, mydriasis, salivation, ataxia, disorientation, and vocalization) were observed only on the first 2 days. Administration of 1 mg/kg for 5 days produced mild sedation, ataxia, tremors, disorientation, and restlessness for several after dosing on each day of treatment.

E) CARDIOVASCULAR AND RENAL PHARMACOLOGY

1. Pentobarbital anesthetized rats (n=4) were treated consecutively with vehicle and 3 tiagabine doses (0.1, 1, or 10 mg/kg, iv) at intervals of about 15 min, and systemic arterial pressure and heart rate were measured continuously. BP and HR were unaffected following 0.1 and 1 mg/kg. Administration of 10 mg/kg produced an acute and short-lasting (1 min) hypotension followed by an increase in BP. BP was maximally elevated 2-5 min after administration and returned to normal after 15 min. The hypertension was associated with a decrease in HR, suggesting that the BP increase was due to increased peripheral vascular resistance. It was concluded that tiagabine had little CV activity in rats at clinically relevant doses. (Table I.5)
2. Isoflurane-anesthetized male beagle dogs (n=1-6 dogs/dose) were evaluated for CV and pulmonary effects following intraduodenal administration of tiagabine. Doses of 10 and 30 mg/kg produced marked respiratory depression, including complete apnea, which prevented completion of the studies at these dose. Doses of 0.3 and 3 mg/kg had no effects on HR, mean aortic pressure, left ventricular end-diastolic pressure, or maximum left ventricular dP/dt. At 0.3 mg/kg, tiagabine produced a statistically significant increase in cardiac output, due to increased stroke volume, and a small reduction in systemic vascular resistance. Dogs dosed at 3 mg/kg showed significant (42%) reduction in spontaneous respiratory rate (Fig I.2). No significant changes in CV parameters were observed in dogs dosed with 3 mg/kg, possibly due to the respiratory depression. The reduction in spontaneous respiration rate observed in anesthetized dogs was not seen in unanesthetized dogs (below), suggesting a possible drug-drug interaction, although behavioral effects interfered with data collection in the unanesthetized dogs.
3. In a study in unanesthetized dogs, tiagabine was administered at doses of 0.3, 1, and 3 mg/kg, po. Mean blood pressure and limb lead II ECG did not change at any dose. Respiration was increased 30 to 120 min after administration of the high dose. HR tended to decrease 30 to 60 min after administration of 0.3 or 1 mg/kg, but 3 mg/kg had no effect on HR. Rigidity of limb muscles, clonic seizures, and "disappearance of attention to man" occurred intermittently in 2 of 4 dogs between 30 and 180 min after each dose of 3 mg/kg.
4. In another study conducted in unanesthetized dogs, tiagabine was administered to dogs (2/dose) at doses of 0.25 and 0.5 mg/kg, ig. These doses produced mild to moderate sedation. Changes in arterial pressure and respiratory rate could not be dissociated from the behavioral changes. One dog tested at a dose of 5 mg/kg, ig, exhibited pronounced initial excitation (vocalization, thrashing, salivation, rapid respiration) followed by deep sedation. The behavioral effects in conscious animals precluded obtaining meaningful CV and pulmonary data.
5. In a pilot study in male rats, tiagabine displayed diuretic and saluretic activity at a dose of 100 mg/kg, po. When doses of 0.5, 5, or 50 mg/kg, po, were administered to male rats (8/group), urine volume was significantly increased during the first 4 hr following the HD; mean urine sodium, chloride, and potassium excretion were increased at all doses during the 8-24 hr period.

E) DRUG INTERACTION STUDIES

1. Doses of 0.3 or 3 mg/kg, iv, produced non-significant increases in hexobarbital and ethanol-induced sleep times in mice. The acute administration of 3 mg/kg, iv, prolonged hexobarbital-induced sleep time in mice after subchronic (21 day) treatment with vehicle or valproate, but

not after tiagabine, hydantoin, phenobarbital, clonazepam, carbamazepine, or ethosuximide, indicating tolerance development with the later drugs. Subchronic treatment with these AEDs did not change the effect of acute administration of tiagabine on ethanol-induced sleep time.

2. Coadministration of tiagabine (1 or 10 mg/kg, ip) increased the anticonvulsant efficacy of ethosuximide, valproate, phenobarbital, carbamazepine, and clonazepam against DMCM-induced seizures in mice. Coadministration of 1 mg/kg, ip, increased the PI of these compounds, while 10 mg/kg decreased the ratio.

3. There was no significant change in the iv PTZ seizure threshold 2, 4, or 5 days after termination of subchronic (21 day) administration of tiagabine (15 or 30 mg/kg, po, BID), suggesting an apparent lack of withdrawal effects. No residual tiagabine was present in brain tissue on these days.

4. The acute ED50 of tiagabine against DMCM-induced seizures following subchronic treatment (21 days) with vehicle was 1 mg/kg, po, and the sedation ratio (PI) was 10. Following subchronic administration of tiagabine (5 mg/kg), there was a small decrease in tiagabine anticonvulsant efficacy (ED50=1.5 mg/kg) without a significant change in the sedation ration (8.7). Tiagabine (6 mg/kg, po) was able to completely block the DMCM-induced seizures. Following subchronic administration of hydantoin (25 mg/kg), there was also a decrease in efficacy of tiagabine (ED50=3 mg/kg) with a decrease in PI (1.5). Following subchronic treatment with either phenobarbital (20 mg/kg, po) or carbamazepine (100 mg/kg, po), partial tolerance to the effects of tiagabine were seen (ED50= 10 and 5 mg/kg, respectively), and tiagabine was unable to completely inhibit seizures. Following ethosuximide (350 mg/kg, po), valproate (350 mg/kg, po), or clonazepam (2.2 mg/kg, po), full tolerance to the anticonvulsant effect of tiagabine was observed.

5. In female mice, no overt differences in deaths, convulsions, decreased motor activity, decreased body temperature, exophthalmus, or motor incoordination/abnormal gait) were produced by acute administration of tiagabine (1-150 mg/kg, iv) following subchronic administration of tiagabine and ethosuximide, valproate, clonazepam, hydantoin, phenobarbital, or carbamazepine. However, after subchronic administration of clonazepam, tiagabine (10 mg/kg, iv, or greater) increased aggressiveness. After subchronic administration of phenobarbital, clonazepam, ethosuximide, or tiagabine, acute administration of tiagabine produced vocalization upon touch. After subchronic administration of clonazepam, valproate, carbamazepine, ethosuximide, or tiagabine, administration of tiagabine produced shivering upon touch. The mice were observed only after the acute dose of tiagabine, so it is not known whether these findings were the result of subchronic administration of the AEDs or only appeared upon acute challenge with tiagabine.

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Table I.1

Anticonvulsant properties of tiagabine and reference compounds. Three to four doses of each compound were administered. N = 5-10/dose.

Compound	Model/seizure type: ED_{50}, mg/kg						
	DBA/2 (mice) audiogenic tonic	PTZ (mice) Clonic	PTZ (mice) Tonic	DMCM (mice) clonic	PTZ (rats) Clonic	PTZ (rats) Tonic	MES (rats)
Carbamazepine	7	400	4.7	(15)	>64	8.5	11
Clonazepam	0.004	0.035	0.02	0.9	0.55	0.1	-
Diazepam	0.17	0.7	0.2	2.5	2.5	2	-
Tiagabine	0.4	(1.3)	1.2	(1)	6	4	40
NO-329	1	1.5	1.5	(1.2)	12	6.5	-
Phenobarbital	3.6	32	32	18	30	7	-
Phenytoin	11	>300	6	~1000	>64	60	8
SKF 100330A	2.5	(150)	(7)	4	12	7	-
Valproic acid	38	285	100	200	310	215	-

Table I.2

Motor impairment by tiagabine and reference compounds. ED_{50} values for tiagabine and reference compounds for inhibition of rotarod, traction and exploratory behavior (rearing and loc = locomotion) in mice and rats.

Compound	DBA/2 mice		NMRI mice		Rats			
	Rotarod	Traction	Rotarod	Loc	Rotarod	Traction	Rearing	Loc
Carbamazepine	85	80	36	70	64	70	40	30
Clonazepam	0.5	1	0.45	0.2	0.5	0.6	0.2	0.25
Diazepam	3.2	6	2.8	6	4	4.1	0.9	1.3
Tiagabine	4	6.5	6	14	15	15	15	40
Phenobarbital	60	90	55	120	32	>48	23	36
Phenytoin	70	70	360	30	64	70	15	40
SKF 100330A	12	14	15	30	41	24	30	43
Valproic acid	300	600	400	380	400	420	160	300

Table I.3 *Protective effects of tiagabine HCl and prototypic antiepilepsy drugs against maximal electroshock and pentylenetetrazol-induced convulsions in mice*[a]

	Effective dose 50% (mg/kg, i.p., 30 min before testing)				
	Rotarod failure	MES[b]	Pentylenetetrazol Clonic	Pentylenetetrazol Tonic	Protection index[c]
Tiagabine	6	40	1.3	1.2	4.6
Phenytoin	360	8	>300	9	<1
Carbamazepine	38	11	>200	4.7	<1
Phenobarbital	55		32	32	1.7
Clonazepam	0.45		0.04	0.02	11.2
Valproic acid	400		285	100	1.5

[a]From Nielsen et al. (20).
[b]Maximal electroshock.
[c]The effective dose 50% for rotarod failure divided by the effective dose 50% for protection against pentylenetetrazol-induced clonic convulsions.

Table I.4

Protective indices. Ratios of ED_{50} values for inhibition of exploratory locomotor activity and inhibition of DMCM-induced convulsions in mice and rats (ratio of ED_{50} values for inhibition of exploratory locomotor activity and PTZ-induced tonic seizures. Some of the drugs showed inverted U-shape dose-response curves for their anti-convulsant actions.

	Ratio of motor/anti-convulsant effects	
	Mice	Rats
Carbamazepine	4.6	5.9
Clonazepam	0.22	2.5
Diazepam	2.4	0.65
Tiagabine	14	30
Phenobarbital	6.6	>4.2
Phenytoin	0.88	0.66
SKF 100330A	7.5	6.1
Valproic acid	1.9	1.4

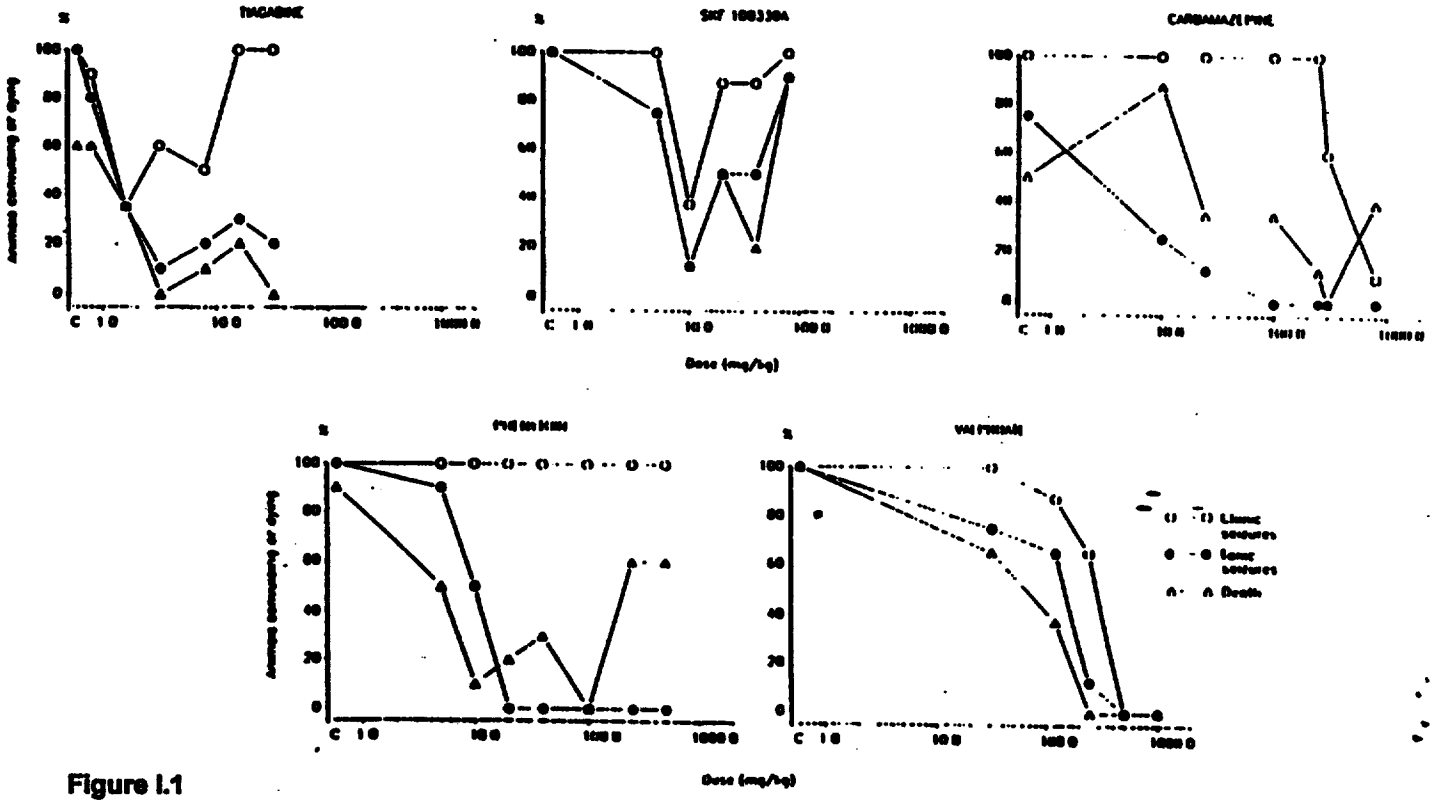


Figure I.1

Ability of selected reference compounds to antagonize convulsions and lethality induced by 120 mg/kg of pentylenetetrazol s.c. in male NMRI mice. The test drugs were given i.p. t – 30 min to groups of 8-10 mice. 'C' denotes control (saline).

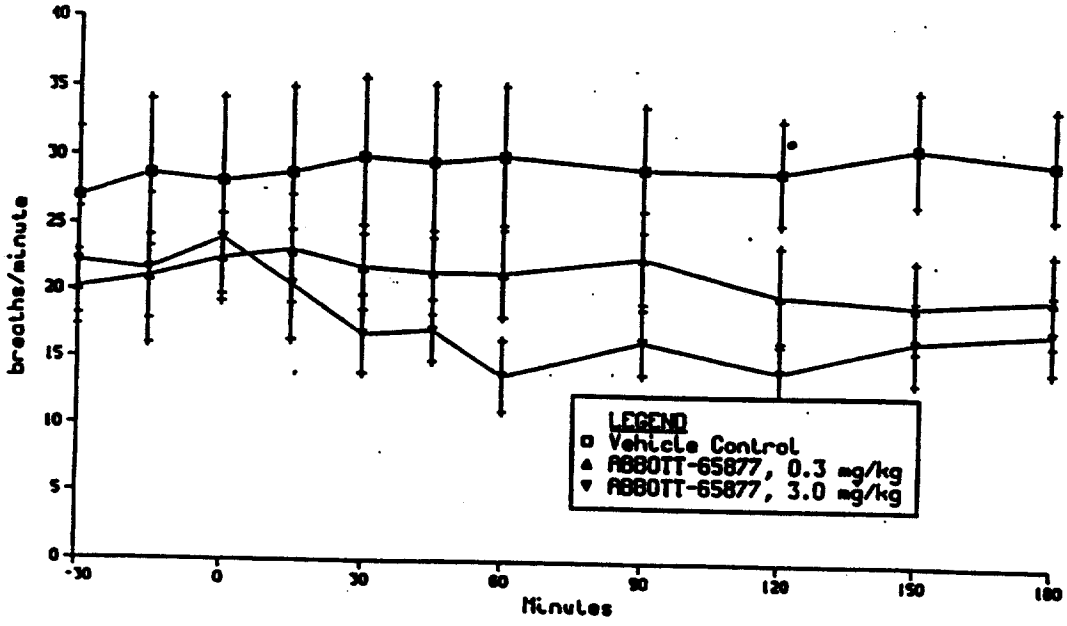
Table I.5: Cardiovascular Effects of Tiagabine in Anesthetized Rats

Dose	Time	Mean blood pressure (mmHg) as differences from baseline mean (SD) (n = 4)		Mean heart rate (beats/min.) as differences from baseline mean (SD) (n = 4)	
	Baseline	138	(14)	395	(21)
Placebo	1 min.	0.5	(2.4)	- 2.0	(5.0)
	2 min.	- 2.3	(2.6)	- 2.0	(5.0)
	5 min	- 2.8	(2.2)	- 4.5	(7.0)
	10 min	- 4.0	(4.5)	- 1.0	(9.6)
	Baseline	134	(15)	393	(21)
NO-05-0328 0,1 mg/kg b.w.	1 min.	2.8	(2.5)	- 2.0	(2.3)
	2 min.	0.5	(1.7)	- 1.0	(3.8)
	5 min.	- 1.3	(1.3)	- 2.3	(10.4)
	10 min.	- 1.8	(5.0)	0.0	(17.5)
NO-05-0328 1.0 mg/kg b.w.	1 min.	4.0	(4.9)	- 4.5	(3.7)
	2 min.	0.0	(1.6)	- 5.5	(2.4)
	5 min.	- 1.0	(1.2)	- 7.8	(2.5)
	10 min.	- 1.5	(2.6)	-10.0	(5.8)
	Baseline	132	(15)	389	(31)
NO-05-0328 10 mg/kg b.w.	1 min.	-15.0	(22.3)	-20.0	(9.1) *
	2 min.	19.3	(13.4) **	-25.5	(14.5) **
	5 min.	20.5	(15.8) **	-32.3	(9.7) *
	10 min.	16.0	(16.8) **	-32.0	(13.8) *
	15 min.	9.8		-26.3	

* Statistically significant (analysis of variance (one-way) and Tukeys multiple comparison test)

** Statistically significant (Kruskal Wallis one-way analysis of variance and Mann-Whitney U-test).

Figure I.2: Effect of Tiagabine on Respiration in Anesthetized Dogs



II. ADME

Taken largely from sponsor's summary

A) PHARMACOKINETICS

Pharmacokinetic, metabolic, and excretion data for tiagabine in mice, rats, and dogs are compared to those in humans in **Table II.1.**

After iv administration, clearance showed a 3- to 4-fold variation between mice (2.8-3.1 L/h/kg at 5 mg/kg), rats (0.8-2.2 L/h/kg at 10 mg/kg), and dogs (1.1 L/h/kg at 1 mg/kg) but was at least 10 times slower in humans (0.09 L/h/kg at 0.08 mg/kg). The iv t1/2 values exhibited similar interspecies differences, averaging 0.8 hr in mice (5 mg/kg) and dogs (1 mg/kg), 0.5-1.8 hr in rats (9.1-10 mg/kg), but 10 hr in humans (0.08 mg/kg). The volumes of distribution appeared to be similar in rats, dogs, and humans (1.3-1.6 L/kg) but were slightly greater in mice (~3 L/kg).

Tiagabine was rapidly absorbed when administered orally as an aqueous solution of the hydrochloride salt; peak plasma concentrations were seen within 10 min in mice (20 mg/kg), 30 min in rats (9-40 mg/kg), 1 hr in dogs (1 mg/kg), and 30 min in humans (8 mg). Longer Tmax values of 2-7 hr were seen in dogs given tiagabine HCl capsules (0.5-10 mg/kg/day). Following oral administration of solid dosage forms to fasting humans, Tmax values averaged 0.5-2 hr. Absorption of an oral dose appeared to be nearly quantitative in all species studied. The bioavailability of tiagabine was also high in mice (92% at 20 mg/kg) and humans (90% at 10 mg), but was lower in rats (25-30% at 9-40 mg/kg) and dogs (50% at 1 mg/kg).

Dose-adjusted Cmax and AUC values from single oral doses were lowest in mice and rats, with slightly higher values in dogs, and considerably higher values in humans. The half-life of orally administered tiagabine was estimated to be 3.3-5.8 hr in mice, 1.6-4.5 hr in rats, and 0.8-2.3 hr in dogs. Following single or daily oral administration of tiagabine HCl to healthy subjects, mean t1/2 values ranged from 5 to 9 hr, although patients receiving AED polytherapy (ie, induced) displayed shorter half-lives for tiagabine (2-5 hr). Small, meal-related, secondary peaks were seen in both dogs and humans, suggesting enterohepatic recirculation in these species.

B) PROTEIN BINDING

The *in vitro* protein binding of [14C]tiagabine was independent of concentration over a range from 0.1 to 10 ug/ml and averaged 89.3% in mouse plasma, 92.6% in rat plasma, 90.8% in rabbit plasma, 91.6% in dog plasma, and 96.2% in human plasma. Thus, the unbound concentrations were approximately 2-3 times greater in mouse (10.7% unbound), rat (7.4%), rabbit (9.2%), and dog (8.4%) plasma than in human plasma (3.8%).

C) DISTRIBUTION

Mice: During the first 2 hr after oral administration of a dose of 1 mg/kg [14C]tiagabine to mice, the levels of radioactivity in the liver, kidneys, and muscle decreased in parallel with the decline in plasma concentrations, giving tissue to plasma ratios of approximately 9 in liver, 4 in kidney, and 1 in muscle. The muscle to plasma ratios continued to average about 1 between 4 and 24 hr, but the hepatic and renal ratios increased to 16-22 and 5-7, respectively. In contrast to these tissues, concentrations in brain remained constant or increased during the first 2 hr after dosing, with corresponding T/P ratios of 1 at 0.5 hr and a maximal ratio of 2.9 at 4 hr.

Rats: After a single oral dose of 30 mg/kg [14C]tiagabine to male rats (Long-Evans, non-albino), concentrations of radioactivity were maximal in most tissues at 0.5 hr and were highest in tissues of the gi tract, liver (41.1 ug equivalents/g), pituitary (14.4), thyroid (13.6), kidneys (12.2), adrenal glands

(5.5), pancreas (4.1), and heart (3.5). At this time, the concentration was 3.9 ug/ml in the plasma and 0.43 ug/g in the brain. At 24 hr the plasma concentration was 0.6 ug equivalents/ml and the highest tissue concentrations were in the liver (6.7 ug equivalents/g), kidneys (2.7), eyes (0.7), whole blood (0.5), and tissues of the gi tract (0.6-34.7). After 48 hr, radioactivity was detected only in the brain (0.09 ug/g), eyes (0.08), heart (0.08), kidneys (0.1), liver (0.09), lungs (0.07), pigmented and non-pigmented skin (0.05 and 0.06, respectively), and whole blood (0.05). Radioactivity was not detected in the plasma at this time.

In a repeat dose study in male and female CD rats (30 mg/kg, po, for 7 days) the highest concentration of radioactivity was found at 30 min in the liver (T/P = 9), with decreasing levels in the kidneys (3.2), lung (1.2), blood (0.78), muscle (0.7), and brain (0.24). The decline in tissue concentrations generally followed the fall in plasma levels, although the T/P ratio did show a tendency to increase with time. The same distribution profile was seen following the seventh dose, but the levels in both tissues and plasma were higher than the respective values from a single dose. The T/P ratios, however, did not increase with daily dosing, suggesting that there was no specific accumulation of radioactivity in any tissue examined.

Following oral administration of [14C]tiagabine (20 mg/kg) to pregnant CD rats on the seventh day of gestation, it was shown that radioactivity crossed the placenta, with fetal concentrations reaching 54% of those in the maternal plasma. In contrast to tissue distribution in the dams, the highest fetal tissue concentrations were found in the spleen, with lower levels in the kidneys and liver. After oral administration of a similar dose to lactating rats, the concentrations of radioactivity in milk were approximately equal to those in the maternal plasma (84-98%).

Dogs: In dogs given a single dose of 0.1 mg/kg [14C]tiagabine, levels of radioactivity in most tissues approximated the plasma concentrations; however, organs of elimination had concentrations greatly exceeding plasma levels, with 0.5 hr T/P ratios of 15 and 14 in the liver and kidneys, respectively. Brain levels were lower than those in plasma (T/P = 0.4) but the radioactivity appeared to be cleared from the brain more slowly than from the plasma so that the brain T/P ratio had increased to 1 by 4 hr. Metabolic patterns in brain tissue from pentobarbital anesthetized, bile duct cannulated dogs showed predominantly unchanged parent drug. Consistent with the high protein binding of tiagabine, only low levels were found in the cerebrospinal fluid of dogs. A similar observation has been made in patients with partial epilepsy, where tiagabine concentrations in CSF were 6-9% of those in plasma. Although levels of radioactivity in the eyes were generally low, the levels in the retina and uvea at 0.5 hr (similar to average plasma level of 0.08 ug eq/ml) and 4 hr (T/P ratio increased to 2.7) were markedly higher than those in the non-pigmented ocular tissues or ocular fluids (**Table II.2**). Levels of radioactivity in the pigmented ocular tissue had started to decline by 24 hr (0.03 ug/g) and had fallen to 0.01 and 0.002 ug/g by 5 and 21 days after dosing, respectively. Although tiagabine or its metabolites exhibited a greater affinity for the pigmented than non-pigmented ocular tissues, (in the opinion of the sponsor) it did not demonstrate the marked ability to concentrate and persist in the melanin-containing tissues of the eye reported for some cationic compound such as chloroquine and phenothiazines.

Species Comparison: Tissue to plasma ratios were similar for most tissues among the three experimental animal species (**Table II.1**), averaging 9-15 in the liver, 0.4-1 in muscle, and 0.2-0.3 in the testes (rat and dog). The brain/plasma ratio appeared higher in mice (1) than in rats or dogs (0.2-0.4). Another noticeable difference was that a considerably higher percentage of the [C14]dose was found in the liver of dogs (35%) than mice (13%) or rats (4%). Similar blood/plasma ratios were found in rats (0.8) and dogs (0.7) at 0.5 hr after oral dosing. *In vitro* studies demonstrated that the distribution of [14C]tiagabine (0.01-10 ug/ml) in human blood also favored the extracellular fraction, with a mean blood/plasma concentration ratio of 0.65, a cell/plasma ratio of 0.15, and a fraction bound to the blood cells of 0.09.

D) METABOLISM

Tiagabine is extensively metabolized, with very little unchanged parent drug eliminated in the urine or feces of any species studied. The biotransformation has not been completely elucidated, but the following pathways have been tentatively identified (Fig II.1):

- thiophene ring oxidation leading to formation of 5-oxo-tiagabine
- acyl glucuronidation of parent and 5-oxo metabolite
- hydroxylation of the methyl substituent on 5-oxo-tiagabine
- formation of glutathione conjugates of tiagabine and/or oxidized metabolite(s)
- formation of dihydroxytiagabine

Thiophene ring oxidation, leading to the formation of the (E) and (Z) isomers of 5-oxo-tiagabine, was a significant *in vivo* metabolic pathway in rats, dogs, and humans but appeared to be a minor pathway in mice. 5-oxo formation was also the major pathway seen following incubation of [14C]tiagabine with rat hepatic microsomes in the presence of an NADPH generating system. The rates of tiagabine disappearance and 5-oxo-tiagabine appearance in male rat liver microsomal incubation mixtures were 2- to 3-fold higher than those seen with female rat microsomes, 4- to 5-fold higher than those seen with male mouse microsomes, and 6- to 8-fold higher than those found with male dog or male and female human microsomes. Glucuronidation was a major pathway in dogs, with the acyl glucuronide being excreted in the bile and probably contributing to the enterohepatic recirculation seen in that species. Only trace amounts of glucuronide were found in humans; however, meal-related, secondary peaks seen in humans indicate that glucuronidation could also occur in humans. The rate of glucuronidation with hepatic microsomes from male dog was 2.5- and 14-fold higher than the respective rates with male rat and male human liver microsomes. There was some indication that secondary metabolism of 5-oxo-tiagabine could occur. Hydroxymethyl-5-oxo-tiagabine was tentatively identified in dog urine, and chromatographic evidence suggested its presence as a minor metabolite in mice and possibly humans. Enzymatic hydrolysis studies in dog bile suggested the possible presence of glucuronide conjugates of the 5-oxo isomers. Dihydroxytiagabine was isolated from mouse feces. It was found that this metabolite coeluted with one of the 5-oxo isomers in the HPLC analysis used for the rat, dog, and human metabolism studies, which means that the peaks previously identified as one of the 5-ox isomers may have contained some dihydroxytiagabine; however, mass spectral analyses provided no evidence for its presence. Keto-tiagabine might be anticipated to be a metabolite, since it can be formed by dehydration of dihydroxytiagabine, but has not been identified. Tiagabine and/or an oxidized metabolite appeared to be conjugated with glutathione in rats, although it is unclear whether these metabolites were formed *in vivo* or *ex vivo*. These have not been identified in other species.

When 5-oxo-tiagabine was evaluated as an *in vitro* inhibitor of 3H-GABA binding, its IC50 of >3000 nM indicated that it had no appreciable activity as a GABA uptake inhibitor.

Cytochrome P450 isozymes: *In vitro* studies indicated that the principle isoform(s) responsible for the metabolism of tiagabine belong to the CYP3A subfamily: 1) the disappearance of tiagabine and formation of 5-ox-tiagabine were significantly correlated with the CYP3A catalyzed erythromycin A N-demethylase activity in a panel of ten human hepatic microsome samples; 2) the disappearance of tiagabine and formation of 5-ox-tiagabine were inhibited by the CYP3A selective inhibitors ketoconazole, troleandomycin, and erythromycin A; and 3) tiagabine was metabolized, although at low rates, by a purified CYP3A4 fusion protein and by microsomes prepared from human B-lymphoblastoid cells transfected with CYP3A4 and NADPH-P450 reductase. Although CYP2C9/10 has been implicated in the thiophene ring hydroxylation of tienilic acid and tenoxicam, neither correlation studies with the tolbutamide methyl hydroxylase activity in the panel of microsome samples nor sulfaphenazole inhibition studies provided evidence for a significant role of that isoform in the metabolism of tiagabine. Additional studies failed to provide conclusive evidence for the involvement of other CYP isoforms in tiagabine metabolism, although a minor contribution from CYP1A2, CYP2D6,

or CYP2C19 could not be excluded. The effect of tiagabine on the CYP3A-mediated metabolism of terfenadine in human liver microsomes was studied in an attempt to predict a potential *in vivo* interaction between the 2 drugs. Tiagabine at maximal concentrations (200 uM) far exceeding those anticipated *in vivo* did not inhibit the metabolism of terfenadine.

Beaune et al (Proc Natl Acad Sci 84:551-555,1987) have shown that thiophene ring oxidation, presumably through a sulfoxide intermediate which covalently binds to CYP2C9/10, may be responsible for the immunologically-based hepatotoxicity caused by tienilic acid. To investigate the possibility of covalent binding with tiagabine, Beaune performed exploratory studies using techniques similar to those used in earlier studies with tienilic acid. Covalent binding was detected following incubation of [14C]tiagabine with microsomes prepared from 4 different human liver samples. Results from immunoblotting, immunoinhibition, and tienilic acid inhibition studies suggested the involvement of CYP2C9/10 in the covalent binding, and the apparent Km and Vmax values for that process were estimated to be 1 mM and 250 pmol/nmol P450/min. This apparent Km value was 4- to 10-fold higher than the apparent Km for metabolism (100-250 uM), and the estimate of intrinsic clearance based on tiagabine disappearance was >50-fold greater than that for covalent binding. Both Km values, but especially that for covalent binding, were higher than the estimated tiagabine concentrations in the plasma or liver of patients receiving the anticipated therapeutic doses. So it was thought to be unlikely that covalent binding seen following the *in vitro* incubation of [14C]tiagabine with human hepatic microsomes would be significant *in vivo* at clinically relevant doses. In contrast to tiagabine, the Km values for the metabolism and covalent binding of tienilic acid are similar and are within the range of the plasma and liver concentrations achieved with therapeutic doses.

Enzyme induction: Administration (ip) of tiagabine to mice and rats at doses of 0.02, 0.1, 1, or 10 mg/kg for 3 days had no effect on the cytochrome P450 and cytochrome b5 content of hepatic enzymes in rats, and had minimal effects on the hepatic post-mitochondrial 7-ethoxycoumarin-O-deethylase activity in mice, or on hepatic microsomal aniline-4-hydroxylase and aminopyrine-N-demethylase activities in rats. Since hepatocellular hypertrophy was seen in subchronic studies with orally administered drug, an additional study was conducted in male and female rats given daily oral doses of 30 or 200 mg/kg (doses used in rat carcinogenicity study) for 14 days. Increased liver weights, without concomitant changes in body weight, were seen in both males (40% increase) and females (24%) at the HD. Although the specific content of total liver microsomal cytochromes P450 was not increased in either sex, the CYP2B-mediated 7-pentoxyresorufin O-dealkylase activity was increased in HD males (4.5X) and females (15X). For comparison, oral administration of sodium phenobarbital (50 mg/kg) for 14 days caused similar increases in liver weight (M, 50%; F, 28%), but also increased microsomal total cytochrome P450 content (M, 123%; F, 86%) and caused pronounced elevations in 7-pentoxyresorufin O-dealkylase activity (M, 436X; F, 208X) in both sexes. Thus, tiagabine exhibited Pb-type enzyme induction, but was a much weaker inducer than Pb.

E) EXCRETION

After iv or oral administration of [14C]tiagabine to mice (1 mg/kg) or rats (30 mg/kg), 15-16% of the radiolabel was excreted in the urine and 77-81% was eliminated in the feces (Table II.1). During daily administration of labeled drug to rats (30 mg/kg), the excretion curves paralleled the administered dose, and by the end of the study the total urinary and fecal excretion values of 17.2% and 78.6% agreed well with the single dose data. Urinary excretion tended to be greater in female mice and rats than in male mice and rats. A slightly larger percentage the labeled dose was excreted in urine (23-30%) of dogs given a 0.1 mg/kg oral or iv dose, while the remaining 68-73% of radiolabel was eliminated in feces. Similar percentages of urinary (25%) and fecal (63%) excretion were found in adult male subjects given a single oral dose of [14C]tiagabine (4 mg).

Consistent with the recovery of 70-80% of a parentally administered dose in feces, both rats and dogs excreted large amounts of radioactivity in bile. Within 24 hr after intraduodenal administration of a 30 or 1 mg/kg dose, rats excreted 72-73% of the label in the bile and 12-16% in the urine. In dogs given

a 0.1 mg/kg iv or id dose, 51-53% of the dose was secreted in the bile within 6 hr and 16% was eliminated in the urine. Similar amounts of biliary (65%) and urinary (11%) excretion were obtained after id administration of a 1 mg/kg dose to bile duct cannulated dogs.

Tiagabine and metabolites: Tiagabine was extensively metabolized, with ≤1% of the dose excreted in urine as parent drug in all species studied. The urinary metabolite profiles were qualitatively similar but quantitatively different after oral and iv administration of [14C]tiagabine to each of the laboratory species. The 5-oxo-tiagabine isomers represented approximately 90% of the urinary radioactivity in rats (30 mg/kg), 35% in dogs (0.1 mg/kg), and 60% in humans (4 mg), but <10% in mice (1 mg/kg, po or iv; 100 mg/kg, po). These values corresponded to 9-16% of the 14C dose in rats, 8-9% in dogs, ≤14% in humans, and ≤1% in mice. The urinary excretion of 5-oxo-tiagabine did not change with daily dosing in rats (30 mg/kg for 7 days). Hydroxymethyl-5-oxo-tiagabine was tentatively identified in dog urine and represented 2-4% of the dose; trace amounts of this metabolite (≤1% of the dose) may also have been present in mouse and human urine. Urinary excretion of the unidentified U-4 metabolite accounted for about 2% of dose in dogs, and it may have been present in mice (1-2%) as well. Dihydroxytiagabine accounted for about 30% of the urinary radioactivity in mice given a high oral dose (100 mg/kg), corresponding to 2% of the 14C dose.

Due to technical difficulties with extraction and chromatography, metabolic patterns in rat feces could not be determined. Although the majority of radioactivity in mouse feces could not be characterized, dihydroxytiagabine accounted for about 30% of the fecal radioactivity or 16% of a 100 mg/kg oral dose. Fecal extraction of the 5-oxo-tiagabine isomers appeared to account for 28% of the dose in dogs, 8% in humans, and 4% in mice; however, these values could overestimate the contribution of this metabolite in dogs and humans if significant quantities of dihydroxytiagabine were present in the feces of those species. Other minor metabolites tentatively identified in feces included glucuronides of 5-oxo-tiagabine in dogs (≤9-13%), and hydroxymethyl-5-oxo-tiagabine in dogs (10-12%) and mice (5%). Fecal excretion of unchanged tiagabine accounted for about 7-10% of the dose in dogs, but ≤1% in humans and mice. Two unidentified metabolites in human feces comprised approximately 40% of the dose but were not detected in plasma samples from subjects given [14C]tiagabine.

In the bile of rats (30 or 1 mg/kg, id), the glutathione conjugates of tiagabine and a dioxidized metabolite accounted for 20% and 8% of the 14C dose, respectively. Thus, about 30% of an oral dose appeared to be metabolized via a glutathione pathway in rats. In contrast, the acyl glucuronide of tiagabine was the major metabolite in the bile of dogs, representing 21% of a 0.1 mg/kg id dose. Free tiagabine was found in the bile of both species, representing about 9% of the 14C dose in the rats and 10% in dogs. Small amounts of 5-oxo-tiagabine were also present in bile and accounted for about 5% of the dose in rats and ≤7% in dogs. Glucuronide conjugates of the 5-oxo-tiagabine isomers were tentatively identified in dog bile, and although they represented only 7% of the dose in anesthetized dogs, they appeared to be more prevalent in bile obtained from the gall bladder of an unanesthetized dog.

In the plasma of rats and dogs given tiagabine orally, the 5-oxo-tiagabine isomers appeared to be major metabolites but were generally present in lower concentrations than the parent drug. No circulating metabolites could be detected in plasma samples from mice give tiagabine orally. However, a significant portion of the radioactivity in plasma samples from both mice and rats was not recovered following acetonitrile precipitation of the plasma proteins. Recovery of radioactivity from dog plasma was good, but a considerable fraction of radioactivity was associated with an uncharacterized polar peak in that species. In humans given a single oral dose, the parent drug accounted for 70-80% of the circulating radioactivity between 1 and 16 hr. The 5-oxo-tiagabine isomers represented about 4-5% of the chromatographed radioactivity, and an uncharacterized polar peak comprised about 2-5% of the radioactivity. In vitro studies indicated that 5-oxo-tiagabine did not inhibit GABA binding, suggesting that it would not have appreciable activity as a GABA uptake inhibitor.

F) TOXICOKINETICS

In the oral toxicity studies, plasma concentrations generally increased with dose, and the plasma concentrations for each species appeared to be reasonably consistent across studies (Table II.3). The mean, steady-state, dose-adjusted $AUC_{0-\infty}$ in induced patients receiving tiagabine (10, 14, or 20 mg, qid) was 38.35 ng·hr/ml/mg, which corresponds to approximately 3.1 ug·hr/ml at the highest dose studied (ie, 80 mg/day). The mean steady state Cmax was 16.4 ng/ml/mg, which corresponds to a peak concentration of approximately 0.33 ug/ml at 20 mg, qid. Compared to induced patients, healthy subjects displayed a similar dose-adjusted Cmax value of 20 ng/ml/mg (0.24 ug/ml at 12 mg tid), but a 2- to 3-fold higher dose-adjusted AUC of approximately 100 ng·hr/ml/mg (3.6 ug hr/ml).

Mice: Mean concentrations in the 1-hr plasma samples from mice in the 3-month and 2-year oral toxicity studies did not exhibit sex-related differences and increased from about 1 ug/ml at 10 mg/kg to 7.5 ug/ml at 100 mg/kg/day and 21.3 ug/ml at 1000 mg/kg/day. The increases appeared to be dose-proportional between 10 and 100, but were less than dose-proportional at the higher doses (250-1000 mg/kg). It is possible that the lack of proportionality in 1-hr concentrations resulted from protracted absorption at the higher doses, but AUCs were not determined. No consistent changes in 1-hr levels were seen over the course of the 2-yr carcinogenicity study; however, levels were below or at the detection limit of 0.01 ug/ml in the LD group at 3 months but were above 1 ug/ml in this group at 12 months. The 1-hr tiagabine concentration in mice at the HD in the 2-yr study (~10 ug/ml at 250 mg/kg) was about 30-40 times the mean steady-state Cmax value found in patients (0.33 ug/ml at 20 mg quid) or healthy subjects (0.24 ug/ml at 12 mg tid).

Rats: Sex-related differences in plasma levels were seen in the rat oral toxicity studies. The overall mean AUC values from the 10, 30, 100, and 200 mg/kg dose groups in the 2-yr carcinogenicity study were up to 2.4 times higher in female (2.0, 16.0, 99.4, and 212.0 ug·h/ml, respectively) than in male rats (2.9, 8.9, 62.2, and 88.3 ug·h/m, respectively). Greater than dose-proportional increases in levels were seen, as dose-adjusted AUC values increased from 0.2-0.3 at a dose of 10 mg/kg to about 0.4-1 at 100-200 mg/kg. AUCs tended to increase with duration of dosing, particularly in males. Plasma levels following oral administration to pregnant rats were quite variable, and increases in the mean Cmax (0.17, 3,05, 10.8 ug/ml) and AUC (0.94, 12.9, and 81.5 ug·h/ml) were greater than proportional with dose (4, 20, 100 mg/kg); however, pharmacokinetics did not appear to be altered by pregnancy. Concentrations in 0.5 hr samples from immature rats were also consistent with those measured in adults. Compared to the estimated steady-state AUC values of 3.1 ug·h/ml in induced patients and 3.6 ug·h/ml in non-induced subjects receiving the maximum doses of tiagabine, mean AUC values in the 2-yr rat study were similar at 10 mg/kg, about 2.5 to 5-fold greater at 30 mg/kg, 15 to 30-fold greater at 100 mg/kg, and 25 to 70-fold greater at 200 mg/kg.

Dogs: In dogs given capsules (0.5, 2, and 10 mg/kg) during 6- and 12-month oral toxicity studies, plasma levels were slightly higher in females than in males at the LD, but overall sex differences were minimal at these doses. There was some indication that the increase in levels between the LD and HD was greater than dose-proportional in males. The mean t1/2 also appeared to be longer at 10 mg/kg (2-2.3 hr) than at 2 mg/kg (1.3-1.4 hr). Together, these suggest that one or more elimination pathways in dogs might become saturated at higher doses. As in rats, AUCs tended to increase with duration of dosing. At doses higher than 10 mg/kg, sex differences became more pronounced, with higher levels in females than in males. Exposure in dogs was lower than the estimated exposures in patients at doses of 0.5 (<10%), 2 (30-35%), and 5 mg/kg (65-75%), but was approximately 1.5-fold greater at 10 mg/kg and about 7-fold greater at 40 mg/kg.

Table II.1 Summary of the Pharmacokinetic, Metabolism and Excretion Data for Tiagabine in Animals

Parameter	Mouse	Rat	Dog	Human
Tiagabine Pharmacokinetics				
Single IV Dose				
Dose (mg/kg)	5	9.1-10	1	0.08
AUC (μg-h/mL)	1.70	M 2.98-3.67 F 8.67	0.92	0.96
Cl (L/h/kg)	2.94	M 2.24 F 0.81	1.1	0.09
$t_{1/2}$ (h)	0.8	M 0.5-1.8 F 1.0	0.75	10.1
V_{area} (L/kg)	3.42	1.41	1.28	1.32
Single Oral Dose				
Dose (mg/kg)	20	9.1-40	1	0.13
T_{max} (h)	0.17	M 0.5-0.6 F 0.5	0.75	0.73
C_{max} (μg/mL)	3.59	M 0.49-1.20 F 2.46	0.31	0.26
AUC (μg-h/mL)	6.25	M 0.9-3.43 F 9.26	0.46	1.32
$t_{1/2}$ (h)	4.8	M 1.6-2.8 F 4.5	0.82	9.3
F (%)	91.7	M 24.5-31.1 F 26.7	49.9	89.9
Tiagabine in Oral Tox Studies				
Dose (mg/kg)	10-1000	10-200	0.5-40	
T_{max} (h)	1	M 0.8-1.2 F 0.9-1.5	0.8-3.6	
C_{max} (μg/mL)	0.62-21.3[a]	M 0.25-10.5 F 0.41-22.2	0.06-6.8	
AUC (μg-h/mL)		M 2.9-88.3 F 2.0-212	0.23-23.8	
$t_{1/2}$ (h)			1.0-2.3	
Plasma Protein Binding (%)	89.3	92.6	91.6	96.2
Distribution (at 0.5 h)				
Oral Dose (mg/kg)	1	30	0.1	
Liver (T/P ratio)	9.5	9.0	15.1	
Kidney (T/P ratio)	3.9	3.2	4.4	
Muscle (T/P ratio)	1.0	0.70	0.39	
Brain	0.96	0.24	0.39	
Blood		0.78	0.66	0.65[b]

a: concentration at 1 hour; b: *in vitro* determination.

Table II.1 **Summary of the Pharmacokinetic, Metabolism and Excretion Data for Tiagabine in Animals (Cont.)**

Parameter	Mouse	Rat	Dog	Human
Excretion				
Oral Dose (mg/kg)	1	30	0.1	0.05
Urine (%)	15.4	15.6	28.9	25.4
Feces (%)	77.4	80.4	73.0	63.0
Bile (%)		73.1[c]	≤3.0[d]	
Metabolism				
Oral Dose (mg/kg)	100	30	0.1	0.05
Urine (%)				
Carbon-14	6.8	13.0	22.5	23.8
Tiagabine	0.5	n.d.	n.d.	0.9
5-Oxo-tiagabine	0.6	11.2	7.6	≤14.4[e]
OH-Methyl-5-oxo-tiagabine	0.4		2.0	1.3
U-4	1.1		2.5	
Dihydroxytiagabine	2.1			
Polar	1.0	0.6	8.6	
Feces (%)				
Carbon-14	54.5		70.1	61.7
Tiagabine	Trace		6.8	1.2
5-Oxo-tiagabine	3.8		≤28.4[e]	≤8.1[e]
5-Oxo-tiagabine glucuronide			≤12.6	
OH-Methyl-5-oxo-tiagabine	5.1		10.5	0.5
U-4[e]	5.9		5.6	
Dihydroxytiagabine	15.8			
Tiagabine glucuronide			1.8	
Bile (%)				
Carbon-14		73.1	53.0	
Tiagabine		9.0	9.8	
Tiagabine glucuronide		0.2	20.7	
5-Oxo-tiagabine		≤4.5[e]	≤6.8[e]	
Tiagabine glucuronide		0.2	20.7	
5-Oxo-tiagabine glucuronide			7.0	
OH-Methyl-5-oxo-tiagabine			1.7	
Tiagabine Glutathione		19.8		
Di-oxo-tiagabine glutathione		7.7		
Plasma Metabolites				
Oral dose (mg/kg)	1	30[f]	0.1	0.05
1 Hour/0.5 Hour for rat				
Carbon-14 (µg eq/mL)	0.22	7.2	0.064	0.059
Tiagabine (µg/mL)	0.18	3.8	0.045	0.052
5-Oxo-tiagabine (µg/mL)	n.d.	0.7	0.019[e]	0.003
Polar (µg eq/mL)	n.d.			0.001
Non-extractable (µg eq/mL)	0.04	2.4		0.003
2 Hours				
Carbon-14 (µg eq/mL)	0.19	5.7	0.034	0.049
Tiagabine (µg/mL)	0.13	3.0	0.016	0.041
5-Oxo-tiagabine (µg/mL)	n.d.	0.3	0.006	0.002
Polar (µg eq/mL)	n.d.		0.012	0.001
Non-extractable (µg eq/mL)	0.06	2.4		0.004
4 Hours				
Carbon-14 (µg eq/mL)	0.06		0.015	0.039
Tiagabine (µg/mL)	0.04		0.002	0.035
5-Oxo-tiagabine (µg/mL)	n.d.		0.001	0.002
Polar (µg eq/mL)	n.d.		0.012	0.001
Non-extractable (µg eq/mL)	0.02			<0.001

a: concentration at 1 hour; b: *in vitro* determination; c: 0-24 h, intraduodenal dose; d: 0-6 h, intraduodenal dose; e: peak at similar retention time as U-4 in urine.

f: Seventh daily dose
n.d.= not detected.

Table II.2 Levels of Radioactivity in the Ocular Tissues of Dogs after Oral Administration of a 0.1 Mg/Kg Dose of Abbott-70569-^{14}C as the Hydrochloride

		Micrograms Equivalents per Gram or Milliliter						
			0.5 Hr		4 Hrs	1 Day	5 Days	3 Weeks
Tissue	Eye	Dog-7	Dog-8	Mean	Dog-9	Dog-10	Dog-11	Dog-12
Aqueous Humor	R	0.004	0.002	0.003	0.003	0.000	0.000	0.000
	L	0.003	0.002	0.002	0.002	0.000	0.000	0.000
	Avg	0.004	0.002	0.003	0.002	0.000	0.000	0.000
Cornea	R	0.031	0.005	0.018	0.013	0.005	0.003	0.000
	L	0.033	0.006	0.020	0.013	0.007	0.002	0.000
	Avg	0.032	0.006	0.019	0.013	0.006	0.002	0.000
Lens	R	0.003	0.001	0.002	0.005	0.001	0.000	0.000
	L	0.003	0.001	0.002	0.003	0.001	0.000	0.000
	Avg	0.003	0.001	0.002	0.004	0.001	0.000	0.000
Vitreous Humor	R	0.003	0.002	0.003	0.003	0.000	0.000	0.000
	L	0.004	0.001	0.003	0.002	0.002	0.000	0.000
	Avg	0.004	0.002	0.003	0.002	0.001	0.000	0.000
Retina & Uvea	R	0.091	0.046	0.068	0.083	0.029	0.006	0.003
	L	0.080	0.039	0.060	0.076	0.040	0.014	0.002
	Avg	0.086	0.042	0.064	0.080	0.034	0.010	0.002
Sclera	R	0.024	0.014	0.019	0.020	0.003	0.003	0.000
	L	0.033	0.015	0.024	0.016	0.003	0.002	0.000
	Avg	0.028	0.014	0.021	0.018	0.003	0.002	0.000

All levels based on total radioactivity and expressed as microgram equivalents of Abbott-70569.

Figure II.1 Partial Metabolic Pathways for Tiagabine

Table II.3 **Summary of the Plasma Concentrations and Pharmacokinetic Parameters for Tiagabine in Mice, Rats and Dogs from Oral Toxicity Studies**

Species	salt mg/kg	Sex	T_{max} (h) Mean (Range)	C_{max} (µg/mL) Mean (Range)	AUC (µg-h/mL) Mean (Range)	Dose adj Value
Mouse	10	M+F	1.0	0.62 (0.00-1.38)		0.062•
	30	M+F	1.0	2.42 (0.01-4.06)		0.081•
	50	M+F	1.0	5.40 (4.35-6.45)		0.108•
	100	M+F	1.0	7.46 (6.50-8.74)		0.075•
	250	M+F	1.0	10.37 (7.89-13.83)		0.041•
	400	M+F	1.0	11.01 (8.80-16.15)		0.028•
	600	M+F	1.0	16.82 (16.07-17.58)		0.028•
	1000	M+F	1.0	21.28 (20.01-22.56)		0.021•
Rat	4.0‡	F	1.0	0.17 (0.08-0.28)	0.94	0.24†
	10	M	0.9	0.25 (0.10-0.60)	2.89	0.29†
		F	0.9	0.41 (0.32-0.51)	1.98	0.20†
	30	M	0.8	2.25 (0.70-4.38)	8.88	0.30†
		F	0.9	3.42 (1.65-5.29)	16.0	0.53†
	100	M	1.0	6.25 (1.19-13.9)	62.2	0.62†
		F	1.1	11.4 (4.95-21.5)	99.4	0.99†
	200	M	1.2	10.5 (7.67-13.3)	88.3	0.44†
		F	1.5	22.2 (15.5-29.5)	212.0	1.06†
Dog	0.5	M+F	3.6	0.06 (0.03-0.14)	0.23	0.45†
	2.0	M+F	3.5	0.30 (0.10-0.63)	1.09	0.54†
	5.0	M+F	2.0	0.93 (0.91-0.96)	2.31	0.46†
	10	M+F	2.9	1.33 (0.35-3.94)	5.26	0.53†
	15	M+F	2.7	2.61 (2.16-3.05)	7.46	0.50†
	20	M+F	1.6	3.27 (1.69-7.88)	10.9	0.54†
	30	M+F	0.8	2.93 (1.94-3.79)	10.8	0.36†
	40	M+F	3.5	6.75 (0.47-12.6)	23.8	0.60†

See Tables 2, 4, and 6 for references. Dose-adjusted • C_{max} or † AUC. Range of mean values from different studies except ‡ where range represents individual animals in group from a single study. All doses administered to mice and rats by gavage and to dogs via capsule.

III. TOXICOLOGY

A) ACUTE PO AND IV TOXICITY IN MICE AND RATS (conducted by , R&D/90/293, 90/291, 90/294, 90/292, Vol. 1.33)

Mice: When NMRI mice (5/sex/group) received oral (gavage) doses of 0 (vehicle= water), 4, 20, 100, 500; 1000 or 2000 mg/kg tiagabine, T-R signs were observed within 30 min of dosing at doses of 20 mg/kg or more. These included decreased activity, ataxia (abduced hindlimbs and unsteady gait), nystagmus, mydriasis, decreased rectal temperature, increased paw temperature, and an absent pain reaction. At 2 hr, clinical signs were noted in mice treated with 100 mg/kg or more, and included myoclonic convulsions, head drop, decreased activity, ataxia, tremor upon handling, ptosis, and decreased respiration. Most of these signs were seen until 5 hr after dosing over this dose range (myoclonus was seen in 9/10 animals at 500 mg/kg). Three males and 3 females died within the first 2 hr after dosing with 2000 mg/kg. The remaining HD animals and all mice in the 1000 mg/kg group died prior to the end of the 14-day observation period. Necropsy of animals that died revealed no abnormalities. The oral LD50 in mice was between 500 and 1000 mg/kg.

When NMRI mice (5/sex/group) received iv doses of 0 (vehicle= saline), 1, 10, 40, or 160 mg/kg tiagabine, 7 of 10 HD animals died within 2 hr of dosing. No other animals died during the remaining 14-day observation period. Administration of 10 mg/kg or more produced convulsions, myoclonus, decreased motor activity, decreased respiration, ptosis, ataxia, hematuria, cyanosis of the tail, and decreased rectal temperature were also noted. Granulomatous hepatitis and a non-specific focal interstitial nephritis found in 1 HD mouse at necropsy were not considered T-R by the sponsor. The iv LD50 was between 40 and 160 mg/kg.

Rats: Wistar rats (5/sex/group) were given oral (gavage) doses of 0 (water), 16, 80, 400, or 2000 mg/kg. Thirty min after dosing, rats given 80 mg/kg tiagabine po or higher exhibited decreased activity and ataxia. Myoclonus was noted at 400 and 2000 mg/kg. These motor disturbances were somewhat more pronounced in females than in males. Bleeding from the nose and rales were also reported at the two highest doses. One female died 1 hr after receiving 2000 mg/kg, and a male from this group was found dead on day 12, but there were no other deaths. BW gain was significantly reduced in males receiving the HD during the first week after treatment, while females receiving the two highest doses had significantly reduced BW gain during this period. No T-R changes were observed at necropsy. The oral LD50 in rats was greater than 2000 mg/kg.

Wistar rats (5/sex/group) were given iv doses of 0 (saline), 1, 10, 50, or 75 mg/kg. Rats dosed with 10 mg/kg tiagabine or greater exhibited decreased activity, ataxia, and hematuria at 0-30 min post dosing. Rats receiving 50 or 75 mg/kg (HD) showed cyanosis of the tail vein and an increased rate of respiration. Approximately 1/2 of the rats in these two highest dose groups had "myoclonic movements." One rat dosed with the HD died 2 hr after dosing, but there were no other deaths. There were no significant effects on BW or BW gain. Necropsies revealed only chronic obstructive phlebitis in the tail veins of rats administered tiagabine. The hematuria was thought to have been due to irritative and/or hemolytic properties of the drug given iv at high doses. The iv LD50 in rats was greater than 75 mg/kg.

Table III.1: Acute Toxicity Summary

Species	Route	Compound	Vehicle	LD_{50} (mg/kg)
NMRI Mice	po	Tiagabine HCl	water	$500 < LD_{50} < 1000$
NMRI Mice	iv	Tiagabine HCl	saline	$40 < LD_{50} < 160$
Wistar Rats	po	Tiagabine HCl	water	> 2000
Wistar Rats	iv	Tiagabine HCl	saline	> 75

po = dosed orally
iv = dosed intravenously

B) 3-MONTH ORAL TOXICITY IN MICE (R&D/91/632, conducted by , Vol. 1.34)

1. Treatment

CD-1 mice (10/sex/group) were given initial daily doses of 0 (vehicle = 0.2% hydroxypropyl methylcellulose), 50, 100, 250, or 400 mg/kg by oral gavage. Six mice/sex/grp were treated with the same doses for plasma level determinations. The 50 mg/kg dosage was increased to 600 mg/kg on Day 29 and the 100 mg/kg dosage was increased to 1000 mg/kg on Day 50 because of a lack of adverse effects at the lower doses.

Drug lot #: 45-080-AL

2. Mortality

One mouse in each of the groups treated with 0, 50, or 250 mg/kg and 7, 12, and 17 mice treated with 400, 600, or 1000 mg/kg, respectively, died or were euthanized during the treatment period.

3. Clinical Signs

Decreased activity was observed in all drug-treated mice during the first 3 days of treatment. Over the course of the study, a D-R increase in the incidence of decreased activity and labored breathing was noted in mice receiving 400, 600, and 1000 mg/kg.

4. Body Weight and Food Consumption

Significant decreases in BW gain were observed in female mice treated with 250 mg/kg and in males and females treated with 400 mg/kg. Reductions in food consumption relative to controls were seen during the first week at doses of 250 mg/kg (males) or greater (males and females).

5. Plasma Drug Levels

Blood samples were collected from satellite mice approximately 1 hr after dosing on days 1 and 87 and plasma concentrations were determined by HPLC (see table below). Due to a number of deaths some main study group animals were used on day 87 to obtain 3/sex/group.

Table III.2: Plasma Tiagabine Concentrations in Mice during a 3-Month Oral Toxicity Study

Dosage (mg/kg/day)	Day 0, Plasma Concentration (ng/ml)		Day 87, Plasma Concentration (ng/ml)	
	Males	Females	Males	Females
50	6453 ± 371	4347 ± 2223	ND[a]	ND
100	9741 ± 3056	6301 ± 1678	ND	ND
250	11269± 3803	10308 ± 1017	9019 ± 1727	10671 ± 6547
400	16149± 5607	9418 ± 1523	8801 ± 1774	9653 ± 2258
600	ND	ND	17578 ± 5169	16069 ± 1188
1000	ND	ND	20005 ± 3076	22558 ± 2789

[a] ND = Not determined

6. Hematology (all survivors at termination)

No D-R hematologic changes were observed.

7. Clinical Chemistry (all survivors at termination)

Increases in alkaline phosphatase values were observed in males treated with 250 or 400 mg/kg. Elevated glucose values were found in mice treated with 250, 400, or 600 mg/kg.

8. Organ weights (all animals except satellite mice received complete post mortem exam)

D-R increases in mean absolute and relative liver weights were observed in all drug-treated mice; these increases were statistically significant for most groups.

9. Histopathology (specified tissues (very limited) examined for all C and 100/1000 mg/kg mice; all gross lesions examined; kidney, liver, and adrenal cortex examined for all animals)

a) *Liver* - Hepatocellular hypertrophy was increased in a dose-related manner. Generalized hepatocyte cytoplasmic vacuolization was observed in 5 males and 2 females treated with 100/1000 mg/kg and in 1 male in each group treated with 250 or 400 mg/kg.

b) *Adrenal cortex* - Female mice treated with 400 or 100/1000 mg/kg had an increased incidence of brown degeneration of the adrenal cortex.

10. MTD

Based on the results of this study, the sponsor considered 250 mg/kg the oral MTD in mice.

C) 13 WEEK ORAL TOXICITY IN RATS (R&D/90/297, conducted by
. Vol. 1.45)

1. Treatment

Fifteen CD rats/sex/group were dosed with 0 (distilled water), 25, 100, or 400 mg/kg orally (gavage) for 13 weeks.

Drug batch #: K88/7

2. Clinical Signs

Decreased activity and lethargy were observed in HD animals, and salivation was increased in MD and HD animals. Other signs seen primarily in moribund HD and MD animals included firm and distended or swollen abdomen, rales, gasping, inanition, hunched posture, piloerection, pallor, loose feces, and perianal staining.

3. Mortality

One male and 1 female from the MD group died during treatment (Days 39 and 6, respectively). Another MD female was sacrificed moribund on Day 87. Five males and 5 females from the HD group were euthanized in moribund condition between Days 5 and 91. Six HD females were found dead on Days 3-91. All deaths were attributed to treatment.

4. Body Weights and Food Consumption

Food consumption and BW gain were decreased during Week 1 in HD rats, but subsequent values were comparable to C. A D-R increase in water consumption was seen in treated rats.

5. Neurological Exams (all animals in C and HD groups after 6 and 12 weeks)

Neurological exams revealed a slightly higher incidence of depression of the pupillary light reflex in the right eye and consensual light reflex in the left eye of treated (HD) rats (1 CM vs 3 HDM, 1 CF vs 2 HDF at 6 and 12 wks; same rats affected at each test). Since the effect was also seen in controls it was not considered T-R by the sponsor, but attributed to trauma resulting from blood collection from the retro-orbital sinus for hematology at 6 and 12 weeks ("trauma in the right eye affecting detection of the light stimuli may have resulted not only in depression of the pupillary reflex in that eye but also depression of the consensual reflex in the left"), and the higher incidence in treated animals was considered coincidental. No corresponding structural changes were detected by ophthalmoscopic or histopathologic exams. It is not clear when in relation to dosing the neurological exams were conducted.

6. Ophthalmoscopic Exam (all animals in C and HD groups after 12 weeks)

Ophthalmoscopy revealed no changes related to treatment.

7. Hematology (10/sex from each group after 6 and 12 weeks; blood collected by retro-orbital puncture prior to dosing)

Decreased values for packed cell volumes, HGB, and RBCs were seen in HD females after 6 and 12 weeks. Two HD males had increased prothrombin times. All male and 2 female rats in the HD group that were euthanized in moribund condition had low total leucocyte and lymphocyte counts. Reduced packed cell volume and HGB concentration were noted for 1 HD male and 1 HD female that were sacrificed prematurely. Erythrocyte counts were lower in the same female and an additional HD male. Other findings in HD rats that were euthanized moribund included elevated packed cell volumes in 2 males and 2 females, high HGB in the same 2 males, high erythrocyte values in 1 female, and slightly high reticulocyte counts in 1 male and 1 female.

8. Clinical chemistry (10/sex from each group at 6 and 12 weeks)

Elevated alkaline phosphatase activities, urea, cholesterol, and total protein and albumin concentrations and decreased glucose (males) were seen at 6 and/or 12 weeks primarily in HD group rats (occasionally in MD). Elevated alanine aminotransferase and aspartate aminotransferase activities along with elevated urea levels were seen in the majority of samples from rats euthanized in moribund condition. A few of these rats also had high alkaline phosphatase, creatinine, cholesterol, and total protein.

9. Urinalysis (10/sex from each group at 5 and 11 weeks)

Significantly higher urine volumes and a dark coloration were seen at 5 and 11 weeks in HD rats. Three MD males also showed dark colored urine after 11 weeks.

10. Gross Pathology (all groups)

Gaseous distention of the gi tract was observed primarily in HD animals, but was also seen in a few MD rats. This was accompanied by nonspecific staining of the face and perineum. Most of the animals with these changes had died or been sacrificed in moribund condition.

11. Organ Weights (all groups)

Increased mean relative liver, kidney, and thyroid weights were observed in HD rats. Mean relative liver weights were also slightly higher at the MD.

12. Microscopic Pathology (all specified tissues examined from 10/sex in C and HD, and from all rats that died or were sacrificed; livers examined in LD and MD; brain sectioned to include cerebellum, cerebral cortex, and medulla)

a) *Liver* - D-R periacinar hepatocyte hypertrophy was observed in all treated rats. Hepatocyte necrosis and hepatocytic eosinophilia were also seen in HD animals that died or were sacrificed prematurely.
b) *Lung* - Pulmonary congestion, hemorrhage, alveolar flooding, and alveolar macrophage aggregates were found in HD animals that died or were sacrificed during the study.
c) *GI tract* - Gastric lesions associated with ulceration were found in some HD males.
d) *Lymphoid tissue* - Lymphocytolysis in the mandibular lymph node and thymus and lymphocyte deficit in the thymus, spleen, and popliteal lymph node were seen in animals sacrificed moribund. These were thought to be agonal changes.

13. Plasma Drug Concentrations (blood samples collected from all rats 1 hr after dosing at 2 wks)

Mean levels were 527, 1308, and 3236 ng/ml in LD, MD, and HD males, respectively, and 823, 5444, and 11801 ng/ml in corresponding females; levels were highly variable, however (CV up to 120%).

D) SIX MONTH ORAL TOXICITY IN RATS (R&D/90/313, Abbott Study No. TA89-324, conducted by GLP, Vol. 1.47).

1. Treatment

CD rats (20/sex/group) were dosed with 0 (distilled water), 10, 30, or 100 mg/kg orally (gavage) for 6 months. An additional 5/sex were added to the C and HD groups for evaluation of recovery. Dose selection was based on the results of the 3-month rat study (above), where death, hypoactivity, decreased BW gain, increased liver, kidney, and thyroid weights, and hepatic hypertrophy occurred at 400 mg/kg; marginally elevated relative liver weight, hepatic hypertrophy, and deaths were seen at 100 mg/kg; and no effects were evident at 25 mg/kg.

Drug lot #: 109-842-AX

2. Clinical Signs

There were no T-R clinical signs.

3. Mortality

Mortality rates were not clearly affected by treatment. The numbers of rats that either died or were euthanized in moribund condition during the study were as follows:

Dose (mg/kg)	0	10	30	100
Male	1	1	2	3
Female	3	0	1	1

4. Body Weights and Food Consumption

There were no significant differences in BW gain or food consumption among groups.

5. Ophthalmological Exams (prior to treatment, after 6 mo and 1 mo recovery)

Higher incidences of conjunctivitis (2 MDM, 1 MDF, 1 HDF), focal retinopathy (1 CM, 3 MDM, 5 HDM, 1 HDF), and retinal degeneration (1 MDF) were found in MD and HD rats after 6 months. These were considered to be infectious in origin rather than T-R by the ophthalmologist. No ocular abnormalities were noted in the HD recovery group after 1 month.

6. Hematology (clinical pathology: 10/sex/group after 3 mo, all rats at 6 mo and 1 mo recovery)

WBC values were significantly increased in HD males. There were no T-R effects on total and differential leucocyte counts, erythrocyte morphology, or bone marrow differential counts.

7. Clinical chemistry

SGOT and SGPT values were somewhat decreased (up to 40%) in MD and HD rats at 3 and 6 months and after 1 month recovery. Serum K+ was significantly decreased (4.3 vs 4.6 mEq/L in C) in HD males at 3 months, and Ca++ was increased in HD males at 3 (10.4 vs 10 mEq/L) and 6 months (9.9 vs 9.7). Triglycerides, total protein, and albumin were increased in HD males at 3 and 6 months. Triglycerides remained elevated after recovery in HD males.

8. Urinalysis

No T-R effects observed.

9. Gross Pathology (gross exams on rats that died during the study; gross exam and organ wts on all rats at scheduled sacrifice)

Gaseous distension of the jejunum, cecum, and/or rectum was found in 1 HD female that died on Day 187 (also had tarry stomach contents) and 1 HD female that was euthanized at the end of treatment. Enlarged liver and dark red mottled lung were observed in the majority of MD and HD rats that died during the study. Edema in the lung and/or thoracic cavity filled with red fluid were noted in some of the control and LD rats that died during the study. All unscheduled deaths except the HDF with gastric distension were attributed to dosing accidents.

10. Organ Weights

Statistically significant increases in absolute and/or relative liver weights were seen at the HD. This effect was not seen in the recovery group.

11. Microscopic Pathology (histopathology exams on all C and HD rats and on LD and MD rats found dead or moribund)

a) *Liver* - Minimal centrilobular hepatocyte hypertrophy was found in 3 HD females. There were no changes in the recovery group. There was a small increase in hepatic congestion in HD males (3/20) compared to C (1/21).

b) *Brain* - 1 HD male that died accidentally after 3 wks of treatment had moderate vacuolization of the white matter of the brain and spinal cord. This was considered artifactual by the sponsor.

c) *Eye* - Retinal dystrophy was found in 1 HD male. Periorbital hemorrhage noted

similar numbers of control and treated animals was attributed to blood sampling.

d) *Kidney* - There was a slightly increased incidence of regenerative epithelium in HD males (14/20) compared to C (8/20). Incidences were similar at recovery (5/5 HD vs 3/4 C). The incidence of casts was slightly increased in HD males at terminal sacrifice (8/20 HD vs 6/21 C) and after recovery (5/5 vs 2/4 in C).

e) *Lung*- There were no differences in incidences of lung findings between HD and C groups.

f) *Thymus*- Congestion of the thymus (3/20 vs 0 C) and mediastinal lymph node (7/20 vs 2/20 C) was increased in treated (HD) males. There were no differences between recovery groups.

E) 14-WEEK ORAL TOXICITY IN DOGS (R&D/90/29 Vol. 1.61)

1. Treatment

Four groups of beagle dogs (4/sex/group) were given daily oral (capsule) doses of 0, 5, 10, or 20/15 mg/kg for 14 weeks (HD reduced after 7 days due to severe loss of appetite). In a preliminary 6-week range-finding study, beagles (1/sex/group) received doses of 10/40, 20, and 30 mg/kg. D-R signs occurred at all doses and included salivation, tremor, incoordination, apparent visual impairment, and marked sedation. Convulsions were seen at 20 and 40 mg/kg together with BW loss and decreased food consumption. Bradycardia was evident at all doses 2 hr after dosing and persisted for up to 24 hr at the HD. Increased packed cell volume, HGB concentrations and RBC counts seen at 40 mg/kg were attributed to dehydration. Elevated cholesterol was also seen at this dose.

Drug batch #: K88/7

2. Clinical Signs (animals observed daily for signs of toxicity and systemic effects)

a) Prostration, tremors, ataxia, and apparent visual impairment (lack of awareness of objects, failure to fix on and follow a moving object or absence of blink reaction) were observed in all treatment groups (D-R). The most notable sign was marked sedation which was seen in all treated groups from the first day. The onset of these signs was rapid, generally within 30 min, and a maximal effect consisting of almost complete unconsciousness was said to be quickly reached. Dogs in this state were said to be totally unresponsive. This state progressed to a state of stupor in which animals had open eyes, could be roused by sound or tactile stimulus but appeared unaware and were unable to stand or walk. This was followed by a state resembling sleep. A state of "dazed semi-awareness," during which animals wandered aimlessly and showed apparent visual impairment and ataxia sometimes preceded, followed, or alternated with the state of sleep. The apparent visual impairment was not associated with any structural abnormality and was considered to be of central origin.

b) Emesis and salivation were also seen in MD and HD animals.

c) Convulsions were seen on one occasion each in 1 HD male and in 1 MD female.

d) Episodes of anxiety and vocalization were observed in 1 HD and 1 MD male and in 2 MD females.

3. Mortality

There were no T-R deaths

4. Food Consumption and Body Weight (BWs recorded pretest and twice weekly during the treatment period; food consumption measured pretest and weekly during treatment)

   a) Loss of appetite was evident in 1 male and all females in the HD group during the first week. This improved following dose reduction to 15 mg/kg. Decreased food consumption was also seen in some MD females and in 1 MD male. There was no effect on water consumption.
   b) Decreased BW gain was seen in MD and HD groups, particularly in females, during the first 2 weeks of treatment; but at the end of the treatment period, the overall BW gains of treated animals was similar to that of controls.

5. Physical, Neurological, and Ophthalmoscopic exams (neurological exams, ECG, blood pressure measurements performed pretest and after 6 and 12 weeks of treatment; ophthalmoscopy conducted pretest and after 12 weeks.

   a) Excessive salivation and thin appearance were noted in 1 MD and 1 HD female.
   b) Slight exaggeration of the gag reflex was seen in a number of treated animals.
   c) There were no ophthalmologic changes ascribed to treatment.

6. Blood Pressure and ECG (all dogs pre-dosing and during weeks 6 and 12)

   There were no significant treatment-related changes.

7. Hematology and Bone Marrow (clinical pathology evaluations performed pretest and after 6 and 12 weeks of treatment for all animals)

   a) Platelet counts were significantly increased in HD males at 12 weeks. This was attributed to low control values.
   b) Activated partial thromboplastin times were decreased in some treatment groups but the effect was not D-R.
   c) There was no evidence of any T-R effect on bone marrow.

8. Clinical Chemistry (wks 6 and 12)

   There were no changes attributed to treatment.

9. Urinalysis (wks 6 and 12)

   No treatment-related changes.

10. Gross Pathology (all dogs examined for gross abnormalities, organs weighed, and tissues collected for histopathological examination)

    No TR changes were noted.

11. Organ Weights

    Absolute and relative adrenal weights were slightly decreased in HD males.

12. Histopathology (specified tissues from each dog examined microscopically; brain sectioned to allow examination of cerebellum, cerebral cortex, medulla, midbrain, and thalamic nuclei and spinal cord prepared in transverse section at the cervical, thoracic, and lumbar levels)

    No TR changes were noted.

13. Plasma concentrations

Blood samples were taken at 0.5, 1, 3, 5 and 7 hr after dosing on study Day 1 and during Weeks 12. Values were highly variable within groups, but generally showed an increase (about 2-fold) between Day 1 to Wk 12 and higher levels in females at the HD (2-fold). The increase with time was not attributed to accumulation, since the pre-dose levels were low, but was thought to have resulted from an (unexplained) increase in bioavailability with daily dosing. Mean plasma AUCs ± SD at week 12 were 2527 ± 1155, 6077 ± 2552, and 5483 ± 1968 ng·hr/ml for males and 2547 ± 403, 6478 ± 1310, and 10904 ± 3201 ng·hr/ml for females at the LD, MD, and HD, respectively.

F) 6-MONTH ORAL TOXICITY IN DOGS (R&D/90/379, Abbott Study No. TB89-323, conducted by completed 1/2/91, GLP, Vol. 1.62).

1. Treatment

Tiagabine was administered po (capsules) to beagle dogs (4/sex/group) at dosages of 0, 0.5, 2, or 10 mg/kg/day for 6 months. Additional 1-month recovery animals (2/sex/group) were included in the C and HD groups. Dose selection was based on the results of the 14-week dog study (above).

Drug lot #: 109-842-AX

2. Clinical Signs (observed twice daily)

T-R observations in HD animals included irregular gait, recumbency, lethargy, tremors, hyperactivity, spontaneous vocalization, visual impairment, salivation, changes in respiration, and lack of awareness/responsiveness. These signs were observed within 0.5-1 hr after dosing; animals recovered between doses. Signs were most frequent and severe during the first 4 weeks of treatment, but continued throughout. Irregular gait, salivation, tremors, and increased respiration were also seen in a few MD dogs during the first month of treatment.

3. Mortality

All study animals survived until termination.

4. Body Weight and Food Consumption (pretest, once weekly for Weeks 1-13, then monthly)

HD males and females lost weight during the first 2 weeks of treatment, but BW gain was comparable to C thereafter. Food consumption was also decreased in the HD group during the first week.

5. Ophthalmoscopic Examination (pretest, 6 months, and after recovery)

No treatment-related ophthalmoscopic abnormalities were detected.

6. Hematology (in all animals pre-study, at 3 and 6 months, and after 1-month recovery)

No clearly T-R changes in hematologic values (including total and differential leucocyte counts and erythrocyte morphology) were observed during treatment or after recovery. One HD male (4001) had slightly decreased erythrocyte parameters and increased WBCs, and WBCs were significantly increased in HD females after 6 months (+35% compared to C).

7. Clinical Chemistry

Serum alkaline phosphatase was increased in HD males at 3 (40%) and 6 months (35%) but not after recovery. SGOT and SGPT were increased in MD and HD females at 3 (30-40%) and 6 months (25-55%).

8. Urinalysis

No T-R effects were observed at 3 or 6 months.

9. Organ Weights

At the 6 month sacrifice, increases in adrenal (34%), brain (6%), and testes (18%) weights and decreases in prostate (30%) weight were observed in HD males; decreases in ovary (42%) and spleen (18%) weights were seen in HD females. however. There were no obvious differences in organ weights between C and HD recovery groups.

10. Pathology (gross and microscopic exams performed on all animals)

There were no obviously T-R findings after 6 months of treatment.

a) *Brain* - Focal gliosis was seen in 1/4 CM, 2/4 MDM, and 1/4 HDF at terminal sacrifice, and in 1/2 HDM recovery dogs; but no other brain histopathology was reported.
b) *Kidney* - Papillary mineral deposition appeared somewhat increased in treated animals, but was not D-R. Incidences were 1/4, 3/4, 3/4, and 1/4 in C, LD, MD, and HD males, respectively, and 2/4, 2/4, 4/4, and 3/4 in corresponding females. There were no differences between recovery groups.
c) *Lymphatic*- Congestion of mediastinal lymph nodes was seen only in treated dogs, but there was no D-R: 2/4 HD males, 3/4 LDF, 1/4 MDF, 1/4 HDF.
d) *Reproductive organs* - Prostatitis was seen in 1/2 HD recovery males. Sperm granuloma was found in the testis of 1/4 MD main study males and in the epididymis of 1/2 HD recovery males.

11. Plasma Drug Levels (blood collected from all animals on Day 180 at 0, 0.5, 1, 2, 4, 6, and 8 hr after dosing)

Tiagabine was fairly rapidly absorbed in most dogs; the Tmax ranged from 0.75 to 2.2 hr. Absorption was delayed in some subjects, however, with the Tmax as late as 4 hr. Delayed absorption has also been observed in some human subjects. Cmax and AUC values were dose-related and tended to be slightly higher in females than males (Table III.3). There was a statistically significant difference between dose-adjusted AUC values at 0.5 and 10 mg/kg in male dogs and a significantly lower elimination rate constant at the HD in both sexes, indicating that elimination pathways may become saturated with increasing doses. Mean t1/2 was 1.3 and 1.4 hr at the MD and 2.3 and 2 hr at the HD for males and females, respectively.

Table III.3. Tiagabine Pharmacokinetics in Dogs during 6-Month Oral Toxicity Study

Dose mg salt/kg	Sex	T_{max} h	C_{max} µg/mL	Adj C_{max} µg/mL	AUC_{0-8} µg·h/mL	Adj AUC_{0-8} µg·h/mL	$t_{1/2}$ h
0.5	Male	1.8±1.5	0.099±0.012	0.198±0.025	0.222±0.060	0.444±0.121	nd
	Female	1.9±1.5	0.141±0.060	0.283±0.121	0.363±0.074	0.727±0.147	nd
2.0	Male	0.75±0.29	0.579±0.186	0.290±0.093	1.37±0.28	0.687±0.142	1.3
	Female	1.0±0.0	0.632±0.116	0.316±0.058	1.54±0.38	0.770±0.191	1.4
10	Male	2.2±1.5	2.95±1.01	0.295±0.101	9.89±3.46	0.989±0.346	2.3
	Female	1.6±0.7	3.94±1.84	0.394±0.184	11.0±3.3	1.10±0.33	2.0

All values are means ± standard deviations. nd = not determined.
Doses equivalent to 0.45, 1.8 and 9 mg base/kg. Dose adjusted values based on salt.

G) 12-MONTH ORAL TOXICITY IN DOGS (R&D/93/425, Abbott Study No. TB91-467, conducted by GLP; Vol. 1.64).

1. Treatment

Tiagabine was administered po (capsules) to beagle dogs (5/sex/group) at dosages of 0, 0.5, 2, or 10 mg/kg/day for 12 months. Dose selection was based on the results of the 14-week and 6-month dog studies (above). Capsule drug content was below the acceptable range (92-107%) at two dose levels on one of the two analysis dates: at least 2 LD and 1 HD capsule contained only 89% of the nominal content.

Drug lot #: 62-210-AL

2. Clinical Signs (observed twice daily, weekly physical exams)

Treatment-related signs of CNS depression including frequent and severe ataxia and prostration (lasting between 4 and 24 hr) were observed throughout the study in HD males and females. Males were more often affected than females. These findings were also seen occasionally in MD dogs. Occasional hypoactivity, tremors, emesis, and salivation were also observed primarily in the MD and HD groups. Urination and defecation were decreased in HD males and females during the first month of dosing. Ocular discharge was frequently noted in treated animals.

3. Mortality

All study animals survived until termination.

4. Body Weight and Food Consumption (BW recorded weekly; food consumption recorded daily)

BW gain and food consumption were dose-dependently decreased in males and females during the first 2-3 weeks of treatment (75% at HD during 1-2 wks) but were comparable among groups (highly variable) thereafter. There were no significant group differences in final BWs.

5. Ophthalmoscopic Examination (pretest and during wks 12, 25, 38, and 51)

Apparent D-R increases in the incidence of cataracts (described as small fine opacities occurring on the anterior suture lines with normal lenticular tissues overlayed) were found during wks 25 and 38, but at the final ophthalmoscopic exam (wk 51), the incidence and severity of these opacities was similar across groups (Table III.4).

A 2nd (sponsor's) consultant ophthalmologist examined the same dogs after 40 weeks of treatment and the results again indicated a dose-related occurrence of cataracts or opacities (Table III.5). He concluded that, since similar opacities were seen in untreated dogs and have been seen in other recent studies at this facility with beagles from the same supplier, the abnormality is probably not a direct toxic drug effect but may be a condition that is exacerbated by treatment with tiagabine. The opacities were said to be very small, and none of the affected animals was expected to have measurable visual loss.

Table III.4: Incidence of Cataracts in Dogs Treated with Tiagabine during 1-Year Toxicity Study (sponsor's initial examination)

Sex	Male				Female			
Group (mg/kg)	0	0.5	2.0	10	0	0.5	2.0	10
No.	5	5	5	5	5	5	5	5
-1 weeks	1	0	0	0	0	0	0	0
12 weeks	1	0	0	0	0	0	0	0
25 weeks	2	1	3	4	2	0	2	3
38 weeks	1	0	4	5	3	1	4	5
51 weeks	4	4	5	5	3	5	4	4

Table III.5: Incidence of Cataracts in Dogs after 40 Weeks of Treatment with Tiagabine (2nd consultant's examination)

Dose	male	female	total
Control	1	2	3
Low dose	1	2	3
Mid dose	3	2	5
High dose	4	3	7

6. Electrocardiogram (pretest and during wks 14, 25, 38, and 51)

No T-R changes were observed.

7. Hematology (pretest and during wks 12, 24 or 25, 38, and 51)

Erythroid parameters tended to be slightly reduced in MD and HD males and females compared to C. WBC counts were increased in MD and HD females at some measurement times (up to 40% compared to C). Eosinophils (% and count) appeared to be increased in treated males compared to C. Mean bone marrow erythroid counts were decreased and myeloid counts increased in HD males. The myeloid to erythroid ratios were 1.01, 1.01, 1.03, and 1.11 in C, LD, MD, and HD males, respectively; the corresponding ratios in females were 0.97, 1.05, 1.08, and 1.03.

8. Clinical Chemistry (same as hematology)

Alkaline phosphatase was increased in treated females at some measurement times (up to 130% compared to C). Glucose was slightly increased at times in treated males (15 and 11% in MD and HD males, respectively, at 51 weeks). None of the changes were considered biologically significant by the sponsor.

9. Urinalysis (same as hematology)

No T-R effects were observed.

10. Organ Weights (brain, liver, kidneys, gonads, adrenals, thyroid/parathyroid, thymus, heart, spleen, prostate, uterus, and pituitary weighed for all dogs)

Ovarian weights were dose-dependently decreased in females (50% at HD), and uterine and thymic weights were decreased in HD females (60 and 30%, respectively).

11. Macroscopic Examinations (gross exams were performed on all animals)

No T-R findings.

12. Microscopic Examinations (microscopic exams were performed on all animals)

A variety of findings occurred more often in treated animals, but in most cases the effects were not clearly D-R. The incidence of lymphocyte infiltration of the prostate was higher in HD males. Changes in the tongue (nonsuppurative inflammation) and lungs (granulomatous or suppurative inflammation) were found more frequently in treatment groups. Capsular fibrosis of the spleen was increased in MD and HD females. Thyroid C-cell hyperplasia and lymphocyte infiltration were increased in treated animals. Uterine endometrial cystic hyperplasia was seen only in treated females and endometrial hyperplasia only in HD females. (See Table III.6).

13. Plasma Drug Levels (blood samples drawn on days 0, 30, 182, and 364 at 0, 1, 2, 4, 6, and 10 hr post-dosing from all dogs)

Approximately dose-proportional increases in mean plasma Cmax and AUC were seen (Table III.7). On day 364, Cmax values were 40.6, 257.1, and 827.9 ng/ml for LD, MD, and HD males, respectively; corresponding female values were 58.4, 99.5, and 992.4 ng/ml. AUC values were 192.3, 1308.3, and 7103.1 ng·hr/ml for males and 249.1, 591.2, and 6379.8 ng·hr/ml for females in the LD, MD, and HD groups, respectively. There was no evidence of sex-related PK differences in this study. Drug accumulation was evident, although Day 0 AUCs may have been underestimated somewhat compared to the remainder of the study because the 24 hr time point was assumed to be 0 ng/ml.

Table III.6: Microscopic Findings in 1-Year Toxicity Study of Tiagabine in Dogs

Finding	Male				Female			
	0	0.5	2.0	10	0	0.5	2.0	10
lung								
inflammation, suppurative	0/5	0/5	1/5	2/5	1/5	1/5	1/5	2/5
inflammation, granulomatous	0/5	1/5	1/5	1/5	0/5	1/5	4/5	1/5
prostate								
infiltrate cell, lymphocyte	2/5	1/5	1/5	4/5	-	-	-	-
spleen								
fibrosis, capsular	0/5	0/5	1/5	1/5	1/5	0/5	3/5	2/5
thyroid								
hyperplasia, C-cell	1/5	4/5	3/5	2/5	2/5	4/5	3/5	4/5
tongue								
inflammation, nonsuppurative	0/5	1/5	1/5	2/5	0/5	1/5	2/5	2/5
uterus								
hyperplasia, cystic endometrial	-	-	-	-	0/5	2/5	1/5	1/5
hyperplasia, endometrial	-	-	-	-	0/5	0/5	0/5	2/5

Table III.7: Tiagabine Pharmacokinetics in Dogs during 12-Month Oral Toxicity Study

Dose as salt mg/kg/day	Interval	Males C_{max} ng/mL	Males T_{max} h	Males AUC_{0-24} ng·h/mL	Males $t_{1/2}$ h	Females C_{max} ng/mL	Females T_{max} h	Females AUC_{0-24} ng·h/mL	Females $t_{1/2}$ h
0.5	Day 0	38.30	3.2	143.7	n.d.	68.89	3.2	204.3	0.98
	Day 30	36.65	3.2	152.9	2.6	52.76	3.4	280.6	n.d.
	Day 182	30.98	6.8	217.2	n.d.	71.97	2.4	249.9	1.2-1.8
	Day 365	40.55	4.4	192.3	n.d	58.36	5.2	249.1	n.d.
2.0	Day 0	216.21	3.4	966.4	1.5-2.9	306.94	2.2	1040.3	1.2-2.4
	Day 30	245.99	3.6	966.9	1.9-2.2	314.18	2.2	943.6	2.2
	Day 182	246.87	5.6	1247.4	0.8-2.3	100.55	6.0	893.5	n.d.
	Day 365	257.09	4.0	1308.3	1.2-2.5	99.51	6.0	591.2	1.5
10	Day 0	666.15	2.2	2493.9	2.8	727.69	2.2	3746.1	4.2-5.2
	Day 30	1048.31	4.0	5150.5	3.6-4.0	668.74	5.6	4759.1	1.9-3.7
	Day 182	1597.50	5.0	9505.0	1.6-6.0	1092.19	5.2	6309.2	2.4-4.2
	Day 365	827.90	4.0	7103.1	2.9-3.9	992.35	6.0	6379.8	1.6

* Calculated from means of males and females; n.d. = not determined.
C_{max}, T_{max} and AUC values are means; $t_{1/2}$ values are range in individual animals.

IV. CARCINOGENICITY

A) TWO YEAR CARCINOGENICITY STUDY IN MICE (Abbott Study No. TD91-214, conducted by [redacted] GLP, completed 8/25/94, Vols. 1.36-1.44)

1. Treatment

Mice (70/sex/group) were dosed with 0 (C A; vehicle= 0.2% hydroxypropylmethylcellulose), 0 (C B), 10, 30, 100, or 250 mg/kg, by gavage, for 2 years (99 weeks at HD due to high mortality). Satellite groups (25/sex/group) were dosed for plasma drug level analyses. The HD was based on the 13-week gavage dose range-finding study.

Strain: Crl: CD-1 BR

Drug Lot #s: 50-120-AL, 50-121-AL, 62-210-AL, 69-466-AL, 75-529-AL

2. Mortality

Study mortality rates ranged from 41 (LDM) - 84% (HDM). Survival was significantly decreased in HD males and females compared to controls. Trend analysis showed a significant, D-R increasing mortality rate for males and females. Deaths attributed to gavage error were increased at the 2 highest doses in both sexes.

Table IV.1: Mortality in 2-Year Mouse Carcinogenicity Study

Mortality, Week 106[a]

MALES

Dose Level (mg/kg/day)	Gavage Related Deaths*	Total Incidence of Mortality	% Mortality
Vehicle Control A	1	36/70	51
10	1	29/70	41
30	0	40/70	57
100	2	43/70	61
250	8	59/70	84
Vehicle Control B	-	39/70	56

FEMALES

	Gavage Related Deaths*	Total Incidence of Mortality	% Mortality
Vehicle Control A	1	38/70	54
10	0	37/70	53
30	0	38/70	54
100	5	45/70	64
250	11	52/70	74
Vehicle Control B	-	47/70	67

[a]Number dead/initial group number
-Not examined microscopically
*Determined by macroscopic/microscopic examination findings

3. Observed Signs

Hypoactivity was observed at 100 and 250 mg/kg, primarily during the first 2 weeks of treatment. There were no other treatment-related differences in clinical signs.

4. Body Weight

BW means were dose-dependently decreased in treatment groups compared to controls throughout the study, reaching statistical significance at the three highest doses.

Table IV.2: Group Mean Body Weights in 2-Year Mouse Carcinogenicity Study

Group Mean Body Weights in Grams*

Dose Level (mg/kg/day)	MALES Week 98	Week 104
Vehicle Control A	37	36
10	36 (-2.7)	35 (-2.8)
30	34 (-8.1)	33 (-8.3)
100	32 (-13.5)	31 (-13.9)
250	30 (-18.9)	30 (-16.6)
Vehicle Control B	36 (-2.7)	35 (-2.8)

	FEMALES Week 98	Week 104
Vehicle Control A	33	31
10	31 (-6.1)	31 (0.0)
30	30 (-9.1)	30 (-3.2)
100	29 (-12.1)	27 (-12.9)
250	28 (-15.2)	28 (-15.2)
Vehicle Control B	32 (-3.0)	32 (+3.2)

*(percent difference from Vehicle Control A)

5. Food Consumption

Food consumption was significantly lower than C A for males at the two highest doses and for females at all doses. There were no T-R differences in food efficiency values.

6. Tissue Mass Observations

There were no drug-related changes in palpable masses.

7. Hematology (conducted on 10/sex/group at 12, 18, and 24 months)

No toxicologically significant hematological changes were noted.

8. Gross Pathology (complete postmortems conducted on all animals in main study groups)

a) Increased incidences of gaseous distension of various segments of the intestinal tract were found in HD males and females. There were no microscopic correlates.

b) Increased incidences of congestion and/or red discoloration of the lung were found in males and females from the two highest dose groups, primarily in mice dying during the course of the study. These findings were not considered treatment-related by the sponsor but were attributed to postmortem vascular relaxation.

9. Microscopic Pathology (complete microscopic examinations were performed on all animals from all main study groups except C B)

a) Non-neoplastic

i. *Liver* - Hepatocellular hypertrophy, usually involving centrilobular areas but sometimes extending into the periphery, was observed in the livers of 27/70, 26/70, 40/70, 49/70, and 67/70 male and 4/70, 12/70, 11/70, 26/70, and 49/70 female mice administered 0, 10, 30, 100, and 250 mg/kg of the drug, respectively. Severity increased dose-dependently. Hepatocellular vacuolization was found in 1/70, 0/70, 2/70, 3/70, and 9/70 male and 3/70, 1/70, 1/70, 1/70, and 8/70 female mice receiving 0, 10, 30, 100, or 250 mg/kg, respectively.

ii. *Lung* - Congestion was increased at the two highest doses in both sexes. Incidences were 2/70, 2/70, 1/70, 5/70, and 8/70 in males and 0/70, 2/70, 3/70, 5/70, and 10/70 in females at 0, 10, 30, 100, and 250 mg/kg, respectively.

b) Neoplastic

There were no statistically significant increases in tumor incidence in the sponsor's analysis. The high mortality in the HD group often resulted in a lower tumor incidence for this group; therefore, an analysis which excluded this group was also performed. These results again indicated no significant increase in tumor incidence. However, the incidence of alveolar bronchiolar carcinoma of the lung appeared to increase with dose in males when the HD was excluded, and the unadjusted Peto p-value of the trend test for this tumor type was <0.05 (Table IV.3). The incidence of this tumor also appeared to be increased in treated females, but there was no dose relationship. Historical control incidences of alveolar bronchiolar carcinoma for CD mice in 2-year studies range from 1.9-20% in males and from 0-13.5% in females.

Table IV.3: Incidence of Lung Tumors in a 2-Year Mouse Carcinogenicity Study of Tiagabine

Tumor Incidence – Males

Body System Organ Tumor Type	Control (0 mg/kg/day) (C)	Low Dose (10 mg/kg/day) (L)	Mid-Low Dose (30 mg/kg/day) (ML)	Mid-High Dose (100 mg/kg/day) (MH)	High Dose (250 mg/kg/day) (H)	Test of Trend	Pairwise Comparison (H) vs (C)
RESPIRATORY SYSTEM (061)							
Lung (026)							
Alveolar bronchiolar adenoma (1010)	13/70 (18.6%)	21/70 (30.0%)	13/70 (18.6%)	12/70 (17.1%)	9/70 (12.9%)	NS	NS
Alveolar bronchiolar carcinoma (1011)	0/70 (0.0%)	2/70 (2.9%)	3/70 (4.3%)	4/70 (5.7%)	1/70 (1.4%)	↑ (p=0.0420) (p=0.0377) (p=0.1472)	NS
Alveolar bronchiolar adenoma and/or carcinoma (1010/1011)	13/70 (18.6%)	23/70 (32.9%)	15/70 (21.4%)	16/70 (22.9%)	10/70 (14.3%)	NS	NS

Table IV.3 (cont.):

Tumor Incidence – Females

Body System Organ Tumor Type	Control (0 mg/kg/day) (C)	Low Dose (10 mg/kg/day) (L)	Mid-Low Dose (30 mg/kg/day) (ML)	Mid-High Dose (100 mg/kg/day) (MH)	High Dose (250 mg/kg/day) (H)	Test of Trend	Pairwise Comparison (H) vs (C)
RESPIRATORY SYSTEM (0651)							
Lung (0326)							
Alveolar bronchiolar adenoma (10110)	13/70 (18.6%)	8/70 (11.4%)	13/70 (18.6%)	11/70 (15.7%)	5/70 (7.1%)	NS	NS
Alveolar bronchiolar carcinoma (10111)	1/70 (1.4%)	5/70 (7.1%)	3/70 (4.3%)	2/70 (2.9%)	4/70 (5.7%)	NS	NS
Alveolar bronchiolar adenoma and/or carcinoma (10110/10111)	14/70 (20.0%)	13/70 (18.6%)	16/70 (22.9%)	13/70 (18.6%)	9/70 (12.9%)	NS	NS

10. Drug levels - Plasma samples collected 1 hour after drug administration.

After 3 months of treatment, 1-hr plasma levels were below or near the detection limit (0.01 ug/ml) at 10 (both sexes) and 30 (males) mg/kg, but after 12-months, mean plasma levels were greater than 1 ug/ml for all groups. No explanation for this finding was given, but there were no apparent problems with drug stability or dosing formulations. No consistent sex-related differences in plasma levels were seen.

Table IV.4. Plasma Tiagabine Levels in Mice during 2-Year Oral Carcinogenicity Study

Dose	Mean Tiagabine Plasma Level (ug/ml)			
	3 - Month		12 - Month	
	Male	Female	Male	Female
10	0.00	0.01	1.38	1.11
30	0.01	2.40	4.06	3.22
100	7.40	8.07	6.47	6.82
250	9.34	13.83	10.60	7.89

B) TWO YEAR CARCINOGENICITY STUDY IN RATS (Abbott Study No. TD91-213, conducted by GLP, completed 9/2/94, Vols. 1.50 - 1.59)

1. Treatment

Rats (70/sex/group) were dosed with 0 (C A; vehicle= 0.2% hydroxypropyl methylcellulose), 0 (C B), 10, 30, 100, or 200 mg/kg, by gavage, for 2 years (101 weeks for HD). Satellite groups (25/sex/group) were dosed for plasma drug level determinations. The HD was based on the 3- and 6-month rat studies.

Strain: Charles River CD

Drug Lot #: 50-120-AL, 50-121-AL, 62-210-AL, 69-466-AL, 75-529-AL

2. Mortality

Study mortality rates ranged from 57 - 84%. Survival was significantly decreased in HD males and females compared to C A.

Table IV.5: Mortality in 2-Year Rat Carcinogenicity Study

Intercurrent Mortality – Males

Study Week	Controls (0 mg/kg/day) (C)	Low Dose (10 mg/kg/day) (L)	Mid-Low Dose (30 mg/kg/day) (ML)	Mid-High Dose (100 mg/kg/day) (MH)	High Dose (200 mg/kg/day) (H)	Trend Test	Pairwise Comparisons with Control
Week 1 to 52	7/70 (10.0%)	6/70 (8.6%)	17/70 (24.3%)	12/70 (17.1%)	14/70. (20.0%)	—	—
Week 53 to 78	13/63 (20.6%)	13/64 (20.3%)	15/53 (28.3%)	10/58 (17.2%)	17/56 (30.4%)	—	—
Week 79 to 106	27/50 (54.0%)	34/52 (65.4%)	20/38 (52.6%)	25/48 (52.1%)	20/39 (71.8%)	—	—
Week 1 to 106	47/70 (67.1%)	52/70 (74.3%)	53/70 (76.3%)	47/70 (67.1%)	59/70 (84.3%)	! (p=0.0330)	H>C (p=0.0093)

Intercurrent Mortality – Females

Study Week	Controls (0 mg/kg/day) (C)	Low Dose (10 mg/kg/day) (L)	Mid-Low Dose (30 mg/kg/day) (ML)	Mid-High Dose (100 mg/kg/day) (MH)	High Dose (200 mg/kg/day) (H)	Trend Test	Pairwise Comparisons with Control
Week 1 to 52	3/70 (3.9%)	1/70 (1.4%)	4/70 (5.7%)	0/70 (0.0%)	10/70. (14.3%)	—	—
Week 53 to 78	14/68 (20.6%)	14/69 (20.3%)	13/66 (19.3%)	20/64 (31.3%)	26/60 (46.7%)	—	—
Week 79 to 106	27/54 (50.0%)	25/55 (45.5%)	25/54 (46.3%)	25/44 (56.8%)	19/33 (59.4%)	—	—
Week 1 to 106	43/70 (61.4%)	40/70 (57.1%)	41/70 (58.6%)	51/70 (72.9%)	57/70 (81.4%)	! (p=0.0001)	H>C (p=0.0004)

Notes: 1. Number of deaths/Number of animals alive at the beginning of period.
2. Explanation of abbreviations used: NS = Not significant, ! = Positive trend, — = Not analyzed.
3. Statistical significance assessed at $p < 0.05/4 = 0.0125$.

3. Observed Signs

Most signs observed at 100 and 200 mg/kg, ie, rales, body surface staining, hair loss, and material around nose and mouth, were probably related to the increased mortality seen in those groups. Increased salivation after dosing was also noted in these 2 groups.

4. Body Weight

BWs were significantly lower in males from the 100 and 200 mg/kg groups and in HD females compared to C A.

Table IV.6: Group Mean Body Weights in 2-Year Rat Carcinogenicity Study

	Group Mean Body Weights at Week 104 in Grams*	
Dose (mg/kg)	Males	Female
0 (Control A)	790	523
0 (Control B)	728 (-7.8)	530 (+1.3)
10	768 (-2.8)	524 (+0.2)
30	764 (-3.3)	500 (-4.4)
100	661 (-16.3)	483 (-7.6)
200 ●	686 (-14.7)	434 (-16.7)

* (percent difference from Control A)
●week 100 body weights; compared to Control A week 100 body weights

5. Food Consumption

No consistent drug-related effects on food consumption or efficiency.

6. Tissue Mass Observations

No drug-related changes in number of palpable masses.

7. Hematology (conducted on 10/sex/group at 12, 18, and 24 months)

Slight decreases in erythrocyte parameters (RBCs, HCT, HGB) were seen in males at 30, 100, and 200 mg/kg (15% at HD). Reticulocytes were increased in the 30, 100, and 200 mg/kg group males and in HD females at the 18 and/or 24 mo sampling intervals. Mild anisocytosis, hypochromia, and polychromia were also noted in the 30, 100, and 200 mg/kg group males. MCV was significantly decreased in MDH and HD females at the 12 mo sampling interval. None of the changes were considered toxicologically significant by the sponsor.

8. Gross Pathology

Incidences of discolored lung foci were increased in HD males and females. These correlated microscopically with either alveolar macrophages or foci of inflammation. These types of changes are common in gavage studies, but the markedly increased incidences of lung findings in treated animals was unexplained (see Table IV.7).

9. Microscopic Pathology

Complete microscopic examinations were performed on all animals from all groups except C B, from which only livers and testes were examined.

a) Non-neoplastic

i. *Liver* - Hepatocellular hypertrophy was slightly increased in females at 100 (2/70) and 200 mg/kg (4/70) compared to C (0/140).

ii. *Lungs* - Pulmonary alveolar macrophages, inflammation, and edema were increased at 100 and 200 mg/kg, in both sexes (Table IV.7).

iii. *Eye* - Keratitis appeared to be slightly increased in males at 100 and 200 mg/kg; incidences were 8/70, 7/70, 8/70, 14/70, and 13/70 at 0, 10, 30, 100, and 200 mg/kg, respectively.

Table IV.7: Microscopic Lung Findings in 2-Year Carcinogenicity Study of Tiagabine in Rats

Finding	Male					Female				
	0	10	30	100	200	0	10	30	100	200
lung (examined)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)
alveolar macrophages	18	23	13	35	40	17	14	18	36	51
edema	1	0	3	6	4	0	0	0	2	14
inflammation	6	9	4	5	6	6	5	7	11	19

b) Neoplastic (Table IV.8)

Despite the high mortality rate at the HD, additional analyses excluding this group were not performed by the sponsor.

i. *Liver* - The incidence of liver tumors appeared to be increased by treatment in females and possibly males. Statistical analysis (sponsor's) showed a significantly increased incidence of hepatocellular adenomas in HD compared to C A females (10% vs 1.4%) and a significant positive D-R trend for adenomas in females. The combined incidence of adenoma/carcinoma was significantly increased in both HD males (5.7%) and females (11.4%) compared to C A males (0) and females (1.4%), and there was a significant positive trend for combined incidence in females. Incidences of hepatocellular adenoma and carcinoma in C B males (1.4 and 2.85%, respectively) were similar to those in HD males (2.85% for each). The historical control ranges provided by the study facility were 1.3-10% (male) and 0.8-3.3% (females) for adenoma, 1.7-6% (males) and 0-1.7% (females) for carcinoma, and 1.3-11.7% (male) and 0.8-3.3% (female) for adenoma/carcinoma.

ii. *Testes* - The incidence of Leydig cell tumors was increased in males at 100 (5.7%) and 200 mg/kg (10%) compared to C A (2.9%). There was a positive trend of increasing Leydig cell tumors with dose and the incidence was significantly increased (sponsor's analysis) and outside the historical control range (1.33 -6%) in the HD group.

Table IV.8: Tumor Incidence in a 2-Year Rat Carcinogenicity Study of Tiagabine

INCIDENCE OF HEPATOCELLULAR ADENOMA/CARCINOMA
DIED ON STUDY AND TERMINAL SACRIFICE - MALE

Dose	Vehicle Control A		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		200 mg/kg/day		Vehicle Control B	
	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS
Number Examined	(47)	(23)	(52)	(18)	(52)	(18)	(47)	(23)	(59)	(11)	(44)	(26)
Hepatocellular Adenoma	0	0	2	0	0	1	1	2	1	1	0	1
Hepatocellular Carcinoma	0	0	0	0	1	0	0	0	2	0	2	0

INCIDENCE OF HEPATOCELLULAR ADENOMA/CARCINOMA
DIED ON STUDY AND TERMINAL SACRIFICE - FEMALE

Dose	Vehicle Control A		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		200 mg/kg/day		Vehicle Control B	
	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS
Number Examined	(43)	(27)	(40)	(30)	(41)	(29)	(51)	(19)	(57)	(13)	(46)	(24)
Hepatocellular Adenoma	0	1	0	0	2	0	2	1	3	4	0	0
Hepatocellular Carcinoma	0	0	2	0	0	0	1	0	1	0	0	0

INCIDENCE OF BENIGN LEYDIG CELL TUMORS OF THE TESTES
DIED ON STUDY AND TERMINAL SACRIFICE - MALE

Dose	Vehicle Control A		10 mg/kg/day		30 mg/kg/day		100 mg/kg/day		200 mg/kg/day		Vehicle Control B	
	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS	DOS	TS
Number Examined	(47)	(23)	(52)	(18)	(52)	(18)	(47)	(23)	(59)	(11)	(44)	(26)
Benign Leydig Cell Tumors	1	1	0	1	1	1	2	2	3	4	0	2

10. Drug levels

Plasma samples were collected from up to 10 rats/sex from the satellite groups at 1, 4, and 24 hr post-dose after 3, 12, and 24 months of treatment. Exposures (AUC) increased with increasing dose and were generally higher in females than males. Levels increased with duration of dosing, markedly in some cases. (The typical clinical AUC is 2.5-3.0 ug·hr/ml in patients receiving 80 mg/day).

Table IV.9. Plasma Tiagabine Levels in Rats during 2-Year Oral Carcinogenicity Study

Sex/Dosage (mg salt/kg/day)	Time Interval	Mean AUC ± SD (μg·hr/ml)	Mean C_{max} ± SD (μg/ml)
Males			
10	3-month	--[a]	0.10 ± 0.09
	12-month	2.2 ± --[b]	0.14 ± 0.13
	24-month	3.6 ± 0.6[c]	0.60 ± 0.52
30	3-month	3.9 ± 1.8	1.00 ± 0.71
	12-month	5.1 ± 2.0	1.23 ± 0.46
	24-month	21.5 ± 22.4	4.32 ± 4.02
100	3-month	21.6 ± 16.0	3.92 ± 2.49
	12-month	46.0 ± 17.9	9.45 ± 3.82
	24-month	118.9 ± 84.8	13.86 ± 5.83
200	3-month	60.2 ± 34.2	7.67 ± 4.62
	12-month	93.7 ± 32.9	10.41 ± 2.13
	24-month	110.9 ± 81.3	13.34 ± 5.97
Females			
10	3-month	1.6 ± 0.6[c]	0.32 ± 0.18
	12-month	1.9 ± 0.7[c]	0.43 ± 0.20
	24-month	2.5 ± 0.3[c]	0.51 ± 0.25
30	3-month	6.1 ± 2.8	1.65 ± 0.76
	12-month	17.9 ± 14.4	4.10 ± 2.45
	24-month	24.1 ± 16.6	5.29 ± 3.92
100	3-month	71.4 ± 37.8	9.57 ± 2.85
	12-month	128.9 ± 48.7	21.50 ± 5.86
	24-month	115.7 ± 73.4	15.97 ± 8.82
200	3-month	163.7 ± 42.0	15.49 ± 2.97
	12-month	266.3 ± 104.4	29.47 ± 9.57
	24-month	206.0 ± 82.5	21.75 ± 4.40

a. Drug concentrations were measurable only at the one-hour interval for all animals in this group, and AUCs could not be estimated.
b. An AUC could be estimated for only one rat.
c. Plasma concentrations below the level of detection precluded estimations of AUCs for most rats in this group.

V. SPECIAL TOXICITY

A) ACUTE ORAL TOXICITY OF THE S(+) ENANTIOMER IN MICE (R&D/90/365, Vol. 1.68)

The S(+) enantiomer of is a potential low level component of the bulk drug, which is the R(-) enantiomer. CD-1 mice (5/sex/group) were given oral doses of 0.68, 0.82, 1.0, 1.22, and 1.48 g/kg of the S(+) enantiomer and were observed and weighed for 2 weeks before necropsy. Histopathological exams were performed only on organs with apparent T-R gross lesions. Clinical signs observed in males and females at all doses included hypoactivity, ataxia, squinting, dyspnea, and prostration. Tonic convulsions were observed in males at a dose of 0.82 g/kg and in both sexes at doses greater than 0.82 g/kg. Tremors were observed in some mice of both sexes in scattered dose groups. No signs of toxicity were observed in males beyond day 5 of the observation period and in females beyond day 2. In males, 1, 1, 3, 3, and 4 deaths occurred in the respective dose groups; 2, 3, 4, and 4 females died. No apparent effects on BW were seen in either sex. Fluid-filled intestinal tracts were observed in some mice in all treatment groups, and in the only two that were examined microscopically (males in the 1.0 and 1.22 g/kg groups), syncytial giant cells were noted in the large intestines of both and colitis was found in one of the mice. Pale, tan livers observed in 2 males from the 1.0 g/kg group correlated with moderate hepatocellular vacuolization microscopically. The combined sex oral LD50 of the S(+) enantiomer was determined to be 1.0 g/kg, which was similar to the combined oral LD50 value of the R(-) enantiomer of tiagabine (ie, 500<LD50<1000).

B) FOUR-WEEK ORAL TOXICITY IN IMMATURE (JUVENILE) RATS (R&D/92/239, Vol. 1.68).

Oral (gavage) doses of 0 (vehicle=0.2% HPMC),10, 30, and 100 mg/kg (lot 56-160-AL) were administered to 15 day old rat pups (10/sex/group) for 28-31 days. An additional 5/sex/group were dosed for plasma level determinations. No T-R deaths or changes in behavior or physical condition were observed. A number of rats in each group, including C, exhibited signs of sialodacryoadenitis, ie, cervical swelling, diffuse corneal opacities, and photophobia. This viral infection is said to be common in laboratory rats. Keratitis observed by ophthalmoscopic exam in 2 C, 2 LD, and 2 HD rats was also attributed to infection. BW gain was dose-dependently decreased by treatment, and mean BW for HDF was significantly lower on days 8 to 20. There were no toxicologically significant differences in hematology, clinical chemistry, urinalysis, organ weight, or histopathology data. Plasma concentrations approximately 0.5 hr after treatment near the end of the 4-week treatment period were 166, 698, and 2838 ng/ml for LD, MD, and HD males, respectively, and 367, 2650, and 5427 ng/ml for corresponding females.

C) DETECTION OF ANTIBODIES TO TIAGABINE IN MICE (R&D/94/723, Vol. 1.69).

The ability of tiagabine to induce specific antibodies was tested in male C3H/He mice (N=10/grp) given the drug as an aqueous solution, by gavage, for 14 days at doses of 1, 5, or 10 mg/kg. Additional animals (N=10) received tiagabine conjugated with bovine gamma globulin (tiagabine-BGG, 10 mg/kg), formulated as an aqueous solution with Freund's complete adjuvant; these animals were treated on two occasions a week apart, by the sc route. A positive control (N=7) received BGG in water with FCA (1 mg/kg), and two vehicle control groups received water, by gavage (N=10), or water with FCA, sc (N=14). Using an ELISA assay, specific antibodies to BGG were detected in sera from animals treated with this protein. No specific antibodies were detected in sera from animals dosed with tiagabine by gavage when these sera were assayed in wells coated with either tiagabine-BSA or BSA (bovine serum albumin). However, sera from animals treated with tiagabine-BGG were found to contain specific antibodies that bound to wells coated with tiagabine-BSA but not in those coated with BSA alone. Sera from the vehicle control animals contained no detectable specific antibodies to any of the coating materials. It was concluded that tiagabine given by the intended clinical route did not induce detectable antibodies in mice, but that a conjugated form (tiagabine-BGG) did induce specific antibodies (reactive with tiagabine-BSA) when administered sc with adjuvant.

VI. GENETIC TOXICOLOGY

A) AMES TEST (NO-050-0328, conducted by ',1/88, GLP, Vol. 1.74)

Tiagabine (batch #: K88/3-s5) was tested in Salmonella strains TA-98, TA-100, TA-1535, and TA-1537, with or without metabolic activation(S-9), using pour-plate assays, over concentrations ranging from 5 to 500 ug/plate. All tests included solvent (distilled water) controls. No increase in revertants occurred for any strain in these tests. Inhibition of growth was seen in all strains following exposure to 500 ug/plate. Appropriate positive control responses were obtained.

B) ASSESSMENT OF MUTAGENIC POTENTIAL IN E COLI (R&D/90/340, conducted by . 4/93, GLP, Vol. 1.74)

Tiagabine (batch #: 168-566-3) was tested, in the presence and absence of S-9, in a tryptophan-dependent auxotroph of E coli, strain WP2 uvrA, using pour-plate assays, over concentrations ranging from 25 to 2500 ug/plate. Tests included solvent (water) controls. No increases in reversion to prototrophy were obtained at the tiagabine levels tested. Inhibition of growth occurred at 2500 ug/plate. Appropriate positive control responses were seen.

C) CHROMOSOME ABERRATION IN HUMAN LYMPHOCYTES IN VITRO (R&D/90/301, conducted by 9/88, GLP, Vol. 1.74)

Tiagabine (batch #: K 88/3 -s5), at concentrations ranging from 25 to 200 ug/ml without activation and from 25 to 1000 ug/ml with metabolic activation (high concentrations chosen on the basis of dose range-finding assays showing toxicity and/or insolubility at higher concentrations), was tested for its ability to induce structural chromosome aberrations in cultured human lymphocytes. Solvent controls were treated with the maximum amount of solvent used in cultures (10 ul/ml DMSO).

In assays performed without S9 activation, there was a large and statistically significant increase in the percentage of cells with aberrations after exposure to 200 ug/ml (**Table VI.1a**). Most of the aberrations were chromatid breaks. This concentration was cytotoxic as evidenced by a reduction in the mitotic index and abnormal cell morphology. The positive control (mitomycin-c) also induced a statistically significant increase in aberrations.

In the presence of S9 (**Table VI.1b**), the positive control (cyclophosphamide) induced a significant increase in aberrations, but no increase in aberrant cells was seen in any of the cultures treated with tiagabine.

Tiagabine was considered positive in this chromosome aberration test without metabolic activation.

D) ASSESSMENT OF MUTAGENIC POTENTIAL IN CHINESE HAMSTER (V79) CELLS (R&D/90/341, conducted by 10/88; GLP, Vol. 1.74)

The ability of tiagabine (batch #: K88/7) to induce mutations at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus in Chinese hamster (V79) cells was tested in duplicate assays at concentrations ranging from 10 to 750 ug/ml in the presence and absence of metabolic activation (based on preliminary assays showing excessive toxicity at higher concentrations). Treatments took place for 3 hr. Solvent (DMSO) controls were included in all experiments.

In the first mutation assay without S-9 (**Table VI.2a**), increases in mutation frequencies were observed in cultures exposed to tiagabine at 50 ug/ml (15.2 per 10^5 survivors, average of 2 cultures) and 500 ug/ml (15.5 per 10^5 survivors) compared to the control value of 6.2 per 10^5

survivors. It was not possible to plate out one of the cultures exposed to 750 ug/ml because of low cell counts, and both cultures treated at 750 ug/ml gave plating efficiencies of zero when plated out for survival immediately post-treatment; therefore, the mutation frequencies for these cultures were considered invalid. In the second assay, the highest concentration was reduced to 600 ug/ml, and no increases in mutation frequency or mutant colonies were observed. Positive control frequencies were increased in both assays, but were much higher in the first than the second.

In the first assay with S-9 (Table VI.2b), increased mutation frequencies were observed in cultures exposed to 10 ug/ml (8.0 per 10^5 survivors, average of 2 cultures), 50 (8.3 per 10^5 survivors), and 250 ug/ml (9.7 per 10^5 survivors) compared to the solvent control value of 1.9 per 10 ^{5}survivors. In the second activation assay, only the colonies exposed to 10 ug/ml gave an increase in mutation frequency (9.5 per 10^5 survivors) compared to the solvent control frequency of 3.4 per 10^5 survivors.

A single culture exposed to 50 ug/ml in the absence of S-9 (27.5 per 10^5 survivors) and single cultures exposed to 10 (13.8 per 10^5 survivors) or 250 ug/ml (15.4 per 10 ^{5}survivors) in the presence of S-9 had mutation frequencies outside the range of DMSO solvent control data for the conducting laboratory. Solvent and positive control frequencies were appropriate for a valid assay in all cases. Due to the size and inconsistency of the effects observed, the sponsor concluded that tiagabine was not mutagenic under the conditions of the study.

E) MOUSE MICRONUCLEUS (R&D/90/300, conducted by 10/88, GLP, Vol. 1.74)

Micronucleus formation in bone marrow polychromatic erythrocytes was determined in mice (5/sex/group/time) after administration of single oral doses of 2, 10, or 50 mg/kg (batch: K88/7). The doses were chosen on the basis of an acute study using 25, 50, 100, 250 mg/kg, in which convulsions and death occurred at the two highest doses. In the main study, clinical signs (tremors) were seen at the MD and HD, but no deaths occurred. Among mice killed 24 hr after treatment, the mean incidence of micronucleated PCEs was 0.7 (per 1000) for the vehicle control, and 0.5, 0.6, and 0.4 at the LD, MD, and HD, respectively. Among mice killed after 48 or 72 hr, the mean incidences were 0.6 and 0.5 for controls and 1.0 and 0.6 for HD mice, respectively. There were no statistically significant differences between control and treatment groups at any time. Chlorambucil produced a significant increase in micronucleated PCEs (57.5). The ratio of polychromatic to mature erythrocytes was 0.8 in the control group and 0.9, 1, and 0.9 in the LD, MD, and HD groups, respectively. After 48 hr, the ratios were 0.8 for controls and 0.9 for the HD group. After 72 hr, the ratios were 0.8 for C and 0.9 for the HD group. It was concluded that tiagabine did not induce chromosomal or other damage leading to micronucleus formation in PCEs of treated mice.

F) UNSCHEDULED DNA SYNTHESIS IN RAT PRIMARY HEPATOCYTE CULTURE (R&D/95/002-Study No TX94-295, conducted by 3/95, GLP, Vol. 1.74)

Tiagabine (Lot no.: 87-728-AL) was tested for potential to induce DNA damage in the *in vivo/in vitro* assay for unscheduled DNA synthesis in rat primary hepatocytes. Primary hepatocyte cultures were prepared at two time points, 2-4 and 15-16 hr, after dosing of female Sprague-Dawley rats (3/group) with 60, 300, 600, and 1200 mg/kg of tiagabine (po). Clinical signs (increased salivation, rales, hunched posture, epistaxis, ataxia, hypoactivity) and hepatocyte toxicity (decreased cell attachment efficiencies, increased cellular vacuoles) were observed in rats treated with 600 and 1200 mg/kg. The vehicle controls were negative and the positive controls were pesitive. It was concluded that tiagabine did not induce DNA damage under the conditions of this study. When samples of dosing solutions were assayed, the values ranged from 86.6-95.2%, but since clinical signs and cytotoxicity were observed the assay was considered valid by the sponsor.

Table VI.1: Chromosome Aberrations in Purified Human Lymphocytes

a.

CHROMOSOME ABERRATIONS IN PURIFIED HUMAN LYMPHOCYTES
Pooled results from replicate cultures

Assay No.: E-9802 Trial No.: I Lab Code: 120188 Activation: With ☐ Without ☒
Compound: NO-05-0328 Dosing Date: January 13, 1988

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION																NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
		NOT COMPUTED			SIMPLE			COMPLEX								OTHER				
		TG	SG		TB	SB	DM	IO	TR	QR	CR	D	R	CT		PU	GT			
CONTROLS: [a] UNTREATED AND SOLVENT	200	6																0.03	0.0	0.0
POSITIVE: Mitomycin-C 100.0 ng/ml	25				2													0.08	8.0*	0.0
TEST COMPOUND:																				
25.0 µg/ml	200	7	1		2	1												0.015	1.5	0.0
50.0 µg/ml	200	6			1		3											0.020	1.5	0.5
[b] 100.0 µg/ml	200	4	1		5													0.025	2.5	0.0
[c] 200.0 µg/ml	200	18	1		14		5		1	2								0.410	19.5*	7.0

*Significantly greater than the pooled untreated and solvent controls, p<0.05
Mitotic index: a. 4.0%
b. 3.4%
c. 1.5%

TG: Chromatid gap
SG: Chromosome gap
TB: Chromatid break
SB: Chromosome break
DM: Double minute
TR: Triradial
QR: Quadriradial

b.

S9 Batch No.: I 0386

Assay No.: E-9802 Trial No.: I Lab Code: 120188 Activation: With ☒ Without ☐
Compound: NO-05-0328 Dosing Date: January 13, 1988

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION																NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
		NOT COMPUTED			SIMPLE			COMPLEX								OTHER				
		TG	SG		TB	SB	DM	IO	TR	QR	CR	D	R	CT		PU	GT			
CONTROLS: [a] UNTREATED AND SOLVENT	200	5				1												0.005	0.5	0.0
POSITIVE: Cyclophosphamide 25.0 µg/ml	25	2			8													0.320	20.0*	8.0
TEST COMPOUND:																				
25.0 µg/ml	200	10			2	1	1											0.020	2.0	0.0
50.0 µg/ml	200	5			6					1								0.035	3.0	0.5
[b] 100.0 µg/ml	200	13			2	1									T 1			0.020	2.0	0.0
[c] 250.0 µg/ml	200	4			3	3	1											0.035	3.0	0.5

*Significantly greater than the pooled untreated and solvent controls, p<0.05
Mitotic index: a. 5.4%
b. 5.3%
c. 4.9%

TG: Chromatid gap
TB: Chromatid break
SB: Chromosome break
DM: Double minute
QR: Quadriradial
T : Translocation

Table VI.2: Mutagenicity of Tiagabine in Chinese Hamster (V79) Cells

a. Main mutation assays - treatment mean in the absence of S-9 mix

Treatment (ug/ml)	First mutation assay Plating efficiency	First mutation assay Mutation frequency[a]	Second mutation assay Plating efficiency	Second mutation assay Mutation frequency[a]
DMSO (0)	111.5	6.2	94.1	8.2
No-05-0328 (10)	100.9	5.0	84.8	1.9
No-05-0328 (50)	87.2	15.2	87.6	0.9
No-05-0328 (250)	107.7	4.2	80.8	2.3
No-05-0328 (500)	93.5	15.5	102.1	4.1
No-05-0328 (600)		*	94.6	0.6
No-05-0328 (750)	62.0*	0.0*		
EMS (1000)	84.7	219.9	79.0	99.8
DMBA (10)	105.3	18.3	96.5	5.4

a - per 10^5 survivors
* - Data from one culture only; duplicate culture not plated out due to insufficient cell growth.

b. Main mutation assays - treatment mean in the presence of S-9 mix

Treatment (ug/ml)	First mutation assay Plating efficiency	First mutation assay Mutation frequency[a]	Second mutation assay Plating efficiency	Second mutation assay Mutation frequency[a]
DMSO (0)	92.4	1.9	93.5	3.4
No-05-0328 (10)	87.6	8.0	81.9	9.5
No-05-0328 (50)	68.4	8.3	87.6	2.3
No-05-0328 (250)	106.7	9.7	65.0	3.1
No-05-0328 (500)	98.0	2.7	72.4	0.0
No-05-0328 (600)			86.6	2.0
No-05-0328 (750)	103.0	0.6		
DMBA (10)	86.5	109.1	73.1	104.9

a - per 10^5 survivors

VII) REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

A) FERTILITY AND GENERAL REPRODUCTION STUDY IN RATS (Abbott Study No. TA89-351, conducted by GLP, Vol. 1.70)

1. Treatment

Male and female rats (42/sex/grp) received 0 (vehicle = water), 4, 20, or 100 mg/kg, by oral gavage. Males were dosed for 70 days prior to mating, throughout mating (1:1 cohabitation; 10 day maximum) and until females were sacrificed. Females were dosed for 28 days prior to mating, throughout mating (up to 15 days), and until sacrifice on Day 20 of gestation (22/group) or at weaning. Dose selections were based on the results of a 13-week toxicity study conducted with doses of 25, 100, and 400 and an unsubmitted 12-day range-finding study in pregnant rats with doses of 25, 100, 200, and 400 mg/kg. The 400 and 200 mg/kg doses were considered excessively toxic, since they resulted in significant mortality; 100 mg/kg decreased weight gain in pregnant rats and resulted in 10% mortality in the 13-week study.

Strain: Sprague Dawley (Crl:CD BR VAF/Plus)
Drug lot #: 109-842-AX

2. Fo Data

a) No T-R mortalities occurred during the study.
b) T-R clinical signs observed in HD males and females during dosing included rales, nasal discharge, and colored material around the nose. Rough coat and piloerection were also reported in HD females.
c) Significant decreases in BW (7%), BW gain (15%), and food consumption were observed in HD males during the dosing period. BW gain was significantly decreased (13%) in HD females during the gestational dosing period. BW gain was significantly increased in MD and HD females during the lactation period.
d) There were no group differences in mating or fertility indices.
e) No T-R gross internal abnormalities were observed at necropsy. Neither histopathologic evaluation of reproductive organs nor sperm analysis were performed.

3. Term Sacrifice Reproductive Parameters and Fetal Evaluations

All term fetuses were examined for external abnormalities. Approximately 1/2 were fixed in Bouin's solution, then examined for visceral defects using Wilson's method. The remaining fetuses were cleared and stained by the KOH-Alizarin red technique prior to skeletal evaluation.

a) Preimplantation loss was increased 2-fold at the HD (Table VII.1), primarily due to 1 litter with 16 corpora lutea but only 2 implants. Increases in postimplantation loss (mostly early resorptions; 1 late resorption at HD) were seen in MD (7% vs 3% in C) and HD (9%) group litters, but values were within the historical control range.
b) Fetal BWs were only slightly decreased at the MD and HD (effects could have been offset by differences in litter size).
c) Total incidences of fetal malformations and variations were comparable among groups (1, 2, 1, and 2 malformed fetuses in C, LD, MD, and HD groups). Craniofacial and CNS defects (cleft palate: 1 HD fetus; anophthalmia and/or

microphthalmia: 2, 1, and 1 affected fetuses at LD, MD, and HD; hydrocephaly: 1 MD fetus), and a single case of brachydactyly (HD) were seen in treated litters. The single malformed C fetus had an umbilical hernia. Incidences of unossified or malaligned sternebrae were increased in all treated groups, but not in a dose-related manner.

4. Delivery and Offspring Developmental Parameters

Viability, growth, development, and function of the F0 offspring (F1 generation) were evaluated during lactation and the during the postweaning period. Selected offspring were allowed to mature and deliver and rear their offspring (F2).

a) There were no significant treatment-related effects on reproductive parameters in dams allowed to deliver (Table VII.2). One LD female showed signs of dystocia and died during parturition. Single occurrences of litter cannibalization occurred at the MD (PND 0) and HD (PND 3); this resulted in decreases in pup viability percentages on Day 4 (significant at HD). There were no group differences in litter retrieval.

b) Mean pup weights during lactation were decreased in the HD group compared to C. Although not statistically significant, the differences were sufficient to indicate a drug-related effect, according to the sponsor. The HD group weight deficit persisted following weaning until study termination, and statistical significance was reached between weeks 15 and 21 in HD males.

c) There were slight delays in the acquisition of developmental landmarks at the MD and HD, usually limited to 1 day in 1 or 2 pups/group. An effect on open field behavior was observed when offspring were tested at between 35 and 45 days of age. Treated pups, primarily in the HD group, were generally less active in this test than controls, ie, center square latency was increased, squares entered decreased (significant for HD males), and rearing was decreased. The effect was seen in both sexes but was fairly small and the individual data were variable (the HD male effect was attributed to 1 pup that remained in the center square throughout the test). There were no effects on a multiple T-maze test of learning and memory.

d) There were no T-R differences in the fertility or reproductive capabilities of F1 animals. F2 parameters were comparable among groups.

Table VII.1. Segment I - Caesarean Section Data

Group (dose in mg/kg)	0	4	20	100
Number of Litters	19	22	21	21
Corpora Lutea[a]	13.9 ± 1.5	12.9 ± 3.1	13.6 ± 2.0	13.8 ± 1.8
Uterine Implants	12.9 ± 1.9	12.1 ± 3.7	12.9 ± 2.4	12.0 ± 2.7
Pre-Implantation Loss	0.9 ± 1.5	0.8 ± 2.0	0.7 ± 1.3	1.8 ± 3.3
Live Fetuses	12.6 ± 1.8	11.7 ± 3.5	12.0 ± 2.6	10.9 ± 2.8
Early Resorptions	0.4 ± 0.7	0.5 ± 0.6	0.9 ± 1.1	1.0 ± 1.4
Late Resorptions	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.2
Post-Implantation Loss	0.4 ± 0.7	0.5 ± 0.6	0.9 ± 1.1	1.1 ± 1.4
Fetal Body Weight (g)	3.5 ± 0.3	3.5 ± 0.4	3.4 ± 0.2	3.4 ± 0.2

[a] mean ± SD

Table VII.2. F0 Dam Delivery - Maternal and Litter Parameters

Treatment	Vehicle	Tiagabine (mg/kg)		
	0	4	20	100
Total no. females	20	20	20	19
No. gravid	20	19	18	17
No. nongravid	0	1	2	2
No. dying/sacrificed prior to delivery	0	1	0	0
No. delivering pups	20	18	18	17
No. with complete litter loss	0	0	1	0
No. with no or partial litter loss	20	18	17	17
Gestation duration (days)[a]	21.9 ± 0.4	21.9 ± 0.4	21.8 ± 0.4	21.9 ± 0.4
Liveborn	11.5 ± 2.7	11.2 ± 2.8	10.9 ± 2.2	12.0 ± 2.4
Stillborn/dead Day 0	0.3 ± 0.6	0.6 ± 1.2	0.3 ± 0.5	0.2 ± 0.4
Litter size	11.8 ± 2.7	11.8 ± 2.0	11.2 ± 2.3	12.2 ± 2.4
Pup weight Day 1 (gm)	6.5 ± 0.6	6.7 ± 0.6	6.9 ± 0.7	6.3 ± 0.6
Day 4 viability (%)	95.2	97.5	92.4	89.7

[a] Mean ± SD

B) TERATOLOGY STUDY OF TIAGABINE IN RATS (TA90-237, conducted by Abbott, GLP, Vol. 1.73).

1. Treatment

Mated female rats (25/group) were treated with 0 (vehicle = water), 4, 20, or 100 mg/kg, by oral gavage, on gestational Days 6 through 17; necropsies were performed on Day 20. Plasma drug level analyses were performed on 3 additional mated rats per group. Dose selection was based on the results of a 12-day range-finding study in pregnant rats (not submitted), in which, reportedly, maternal toxicity (deaths, decreased BW and food consumption) occurred at doses of 100, 200, and 400 mg/kg and fetal toxicity (reduced fetal weight on GD 20) was noted at 200 mg/kg or greater. Visceral and skeletal evaluations were not performed. The maternal and developmental NOAEL was said to be 25 mg/kg. This study has been requested.

Strain: Sprague Dawley (Crl:CD BR VAF/Plus)
Drug lot #: 109-842-AX

2. F0 Effects:

a) There were no maternal deaths. A low incidence of rales and ocular and nasal discharge was observed at the HD only.

b) Maternal BW gain during dosing (GDs 6-18) was decreased at the HD (~10% less than C, ns); however, corrected gestational BW gain (maternal BW less gravid uterine weight) was even lower in the HD group compared to C (28% over GDs 6-20, ss) due to increased gravid uterine weights in treated groups (ss at MD and HD) resulting from the larger litter sizes in these groups. Absolute maternal BWs at term were similar among groups (HD 4% below C). Maternal food consumption during the first 3 days of treatment was significantly decreased (-28%) in HD dams.

3. Maternal plasma levels

Blood samples were obtained from 3 rats/group approximately 0.5, 1, 2, 4, and 24 hr after dosing on the 17 day of gestation (last day of dosing), and plasma concentrations were determined using a validated HPLC (Table VI.3). Plasma levels were variable, especially in the MD group. Two of three rats in the MD group were excluded from the PK analysis because of the "unusual shape of the plasma concentration-time curves." AUC values must be interpreted cautiously because of the extended time between the last two samples.

Table VII.3: Maternal Plasma Drug Levels

Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
4 (n=3)	173.6 ± 98.4	1.0 ± 0.9	942.6 ± 182.4
20 (n=1)	3049.5	2.0	12932.6
100 (n=3)	10841.3 ± 4136.7	2.0 ± 1.7	81491.5 ± 54614.3

4. C-Section Data

Term fetuses were examined for external abnormalities. Approximately 1/2 were fixed in Bouin's solution, then examined for visceral defects using Barrow and Taylor's modification of Wilson's method. The remaining fetuses were cleared and stained by the KOH-Alizarin red technique prior to skeletal evaluation.

a) Implantation sites were significantly increased in MD and HD dams, probably reflecting a low number of implants in the C group. Early resorptions were very slightly increased and mean fetal BW was slightly decreased (5%) in HD litters (Table VII.4).

b) Total malformations were markedly increased in the HD group (Table VII.5a). Craniofacial malformations predominated, although no single defect was seen in more than 2 fetuses/litters (agnathia, open eye, situs inversus). All of the defects observed are known to occur spontaneously in rats, and there was no dose-relationship; however, the increased overall incidence of malformations in the HD group clearly indicates that development was perturbed by this dose, which also produced minimal maternal toxicity.

i. *External defects*: A variety of malformations were found only in HD fetuses (Table VII.5a): one litter (3014) contained a fetus (7) with microcephaly, anophthalmia, agnathia, astomia, and a proboscis and another fetus (8) with agnathia, aglossia, and an encephalocele. A second litter (3028) also had two malformed fetuses: one (4) with a threadlike vestigial tail, umbilical hernia, clubbed hindlimbs, and generalized edema and another (12) with a right open eye. Two additional HD litters contained single malformed fetuses: one (3036-13) with a missing tail (acaudia), and one (3050-11) with a right open eye. Findings of hematoma were distributed across groups without any obvious relationship to treatment, but an increased incidence of pale fetuses occurred at the HD (Table VII.5b).

ii. *Visceral defects*: Visceral malformations were found only in HD litters (Table VII.5a): hydrocephaly was observed in one of the fetuses previously noted with multiple external craniofacial anomalies (3014-7); situs inversus of thoracic and abdominal organs was detected in two fetuses from two litters (3030-6, 3038-4); and one fetus with a reduced pulmonary artery was found in another litter (3018-8). Hydronephrosis and aortic remnant occurred at similar frequencies across groups (Table VII.5b).

iii. *Skeletal defects*: There were no group differences in skeletal malformations (seen only in 1 LD fetus) or variations.

Table VII.4. Segment II - Caesarean Section Data

Group (dose in mg/kg)	0	4	20	100
Number of Litters	22	22	18	20
Corpora Lutea (no./litter)[a]	11.52 ± 0.53	13.09 ± 0.59	12.94 ± 0.40	13.10 ± 0.40
Uterine Implants	9.09 ± 0.82	10.68 ± 0.45	11.50 ± 0.69	11.60 ± 0.44
Live Fetuses	8.68 ± 0.75	10.23 ± 0.43	10.94 ± 0.73	11.05 ± 0.37
Early Resorptions	0.36 ± 0.32	0.36 ± 0.12	0.50 ± 0.20	0.55 ± 0.17
Late Resorptions	0.05 ± 0.05	0.09 ± 0.06	0.06 ± 0.06	0.00 ± 0.00
Fetal Body Weight (g)	3.71 ± 0.06	3.68 ± 0.04	3.78 ± 0.07	3.51 ± 0.06

[a] mean ± SE

Table VII.5a: SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

	FETUSES				LITTERS			
GROUP:	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
LEVEL (MG/KG/DAY):	0	4	20	100	0	4	20	100
NUMBERED EXAMINED EXTERNALLY	191	225	197	221	22	22	18	20
AGNATHIA	0	0	0	2	0	0	0	1
ANOPHTHALMIA	0	0	0	1	0	0	0	1
ASTOMIA	0	0	0	1	0	0	0	1
BLISTERS ON CRANIUM	0	0	0	1	0	0	0	1
CLUBBED PAW(S)	0	0	0	1	0	0	0	1
"ELEPHANT NOSE"	0	0	0	1	0	0	0	1
MICROCEPHALUS	0	0	0	1	0	0	0	1
MISSING TAIL	0	0	0	1	0	0	0	1
VESTIGIAL TAIL	0	0	0	1	0	0	0	1
NO TONGUE	0	0	0	1	0	0	0	1
OPEN EYE	0	0	0	2	0	0	0	2
UMBILICAL HERNIA	0	0	0	1	0	0	0	1
NUMBER EXAMINED VISCERALLY	88	106	91	107	20	22	17	20
HYDROCEPHALUS	0	0	0	1	0	0	0	1
PULMONARY ARTERY-REDUCED DIAMETER	0	0	0	1	0	0	0	1
SITUS INVERSUS-COMPLETE	0	0	0	2	0	0	0	2
NUMBER EXAMINED SKELETALLY	103	119	106	114	22	22	18	20
FUSED VERTEBRA(E) ARCHES	0	1	0	0	0	1	0	0
TOTAL MALFORMATIONS								
NUMBER WITH EXTERNAL MALFORMATIONS	0	0	0	6	0	0	0	4
NUMBER WITH VISCERAL MALFORMATIONS	0	0	0	4	0	0	0	4
NUMBER WITH SKELETAL MALFORMATIONS	0	1	0	0	0	1	0	0
TOTAL NUMBER WITH MALFORMATIONS	0	1	0	9	0	1	0	7

Table VII.5b: SUMMARY OF FETAL OBSERVATIONS - VARIATIONS AND PATHOLOGIES

GROUP:	FETUSES				LITTERS			
LEVEL (MG/KG/DAY):	T_0 0	T_1 4	T_2 20	T_3 100	T_0 0	T_1 4	T_2 20	T_3 100
NUMBERED EXAMINED EXTERNALLY	191	225	197	221	22	22	18	20
EDEMA	0	0	0	1	0	0	0	1
HEMATOMA	4	1	2	4	4	1	2	4
PALE	1	3	1	6	1	2	1	5
NUMBER EXAMINED VISCERALLY	88	106	91	107	20	22	17	20
EXTRA STRUCTURE-AORTIC ARCH	5	3	0	3	4	3	0	3
HYDRONEPHROSIS	2	2	1	3	1	2	1	2
NUMBER EXAMINED SKELETALLY	103	119	106	114	22	22	18	20
EXTRA RIB(S)/RUDIMENTS	1	0	0	0	1	0	0	0
MALALIGNED STERNEBRA(E)	2	3	1	5	1	2	1	4
RUDIMENTARY RIB(S)	1	4	4	2	1	4	3	2
THICKENED RIB(S)	0	1	0	1	0	1	0	1
WAVY RIB(S)	1	0	0	0	1	0	0	0
FUSED STERNEBRA(E)	0	0	0	1	0	0	0	1
MISSING RIB(S)	0	2	1	1	0	2	1	1
MISSING VERTEBRA(E)	0	0	0	1	0	0	0	1
INCOMP. OSS. HYOID BODY	4	7	10	10	2	4	6	6
INCOMP. OSS. INTERPARIETAL(S)	13	13	14	19	8	10	9	12
INCOMP. OSS. ISCHIUM	2	0	0	1	2	0	0	1
INCOMP. OSS. PARIETAL(S)	6	3	1	3	5	2	1	3
INCOMP. OSS. PHALANGE(S)	16	18	11	20	7	9	6	9
INCOMP. OSS. PUBIS	1	0	2	0	1	0	1	0
INCOMP. OSS. RIB(S)	1	0	1	0	1	0	1	0
INCOMP. OSS. STERNEBRA(E)	49	62	48	63	18	20	15	18
INCOMP. OSS. SUPRAOCCIPITAL(S)	16	13	8	15	10	8	6	10
INCOMP. OSS. VERTEBRA(E)	6	10	15	16	5	6	8	10
UNOSS. HYOID BODY	8	5	2	8	4	2	1	3
UNOSS. PHALANGE(S)	6	5	4	7	3	2	2	3
UNOSS. RIB(S)	0	0	0	1	0	0	0	1
UNOSS. STERNEBRA(E)	36	33	31	27	15	17	14	14
UNOSS. VERTEBRA(E)	0	0	0	1	0	0	0	1
TOTAL VARIATIONS AND PATHOLOGIES								
NUMBER EXTERNAL	5	4	2	11	4	3	2	9
NUMBER VISCERAL	7	5	1	5	5	5	1	4
NUMBER SKELETAL	77	91	77	82	22	22	18	20

C) TERATOLOGY STUDY IN RABBITS (TE90-253, conducted by Abbott, GLP, Vol. 1.73).

1. Treatment

Pregnant rabbits (16/group) were treated with 0 (vehicle), 1, 5, or 25 mg/kg, po, on gestation Days 6 through 19, and C-sections were performed on GD 29. An additional 4/group were treated for drug level determinations. In a dose range-finding study with doses of 5, 20, 80, and 300 mg/kg (not submitted, requested), maternal deaths reportedly occurred at the HD and maternal BW loss was observed at 80 mg/kg. Embryotoxicity, characterized by reduced fetal viability and BW, was observed at 20 and 80 mg/kg, and open eyelid was found in 6 fetuses from 1 of 4 litters dosed with 80 mg/kg.

Strain: New Zealand White
Drug lot #: 104-571-AX

2. F0 Effects:

a) One HD animal designated for drug level determination died on GD 19, but no other animal died in the study. No T-R clinical observations were reported.
b) No significant group differences were detected for any of the BW measurements, but the mean BW gain during dosing was 40% lower in HD animals compared to C. Food consumption was also decreased at the HD.
c) Blood samples were collected from 4 rabbits/group approximately 0.5, 1, 2, 4, and 24 hr after dosing on day 19 of gestation (last day of dosing), and plasma drug levels were determined(Table VII.6).

Table VII.6: Maternal Plasma Drug Levels

Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)
1	81.7 ± 30.4	1.5 ± 1.7	787.8 ± 274.6
5	631.4 ± 212.7	0.8 ± 0.3	4115.5 ± 1478.1
25	3630.9 ± 3721.7	0.5 ± 0.0	20436.7 ± 16118.2

3. Reproductive and Fetal Parameters

a) The numbers of corpora lutea, implantation sites, and live and dead fetuses were comparable across groups; however, early resorptions were increased at the HD (litter mean of 1.0 vs 0.15 in C; 1 total litter loss; Table VII.7).
b) Fetal weights were slightly decreased (3% less than C) in HD female offspring.

4. Fetal Evaluation (Table VII.8a and b)

a) There were no group differences in total malformations, but the incidence of gallbladder agenesis showed a significant increasing trend with dose (4/105 fetuses and 3/14 litters at HD). The historical control incidence of this defect (may be considered a variation rather than a malformation) in this NZW rabbits is about 0.125% of fetuses and 0.8% of litters.
b) Frequencies of some specific skeletal variations (incompletely or unossified phalanges, vertebrae, and sternebrae) appeared to be somewhat increased in HD litters.

Table VII.7 Rabbit Segment II - Caesarean Section Data

		Treatment Group T0	T1	T2	T3
Incidence of Uterine Findings.					
Corpora Lutea	No. of Corpora Lutea	123	140	128	149
	Mean (per litter)	9.46	10.00	9.14	9.93
	S.E. Mean	0.43	0.49	0.48	0.41
Implantation Sites	No. of Implantation Sites	97	115	94	120
	Mean (per litter)	7.46	8.21	6.71	8.00
	S.E. Mean	0.45	0.49	0.64	0.37
Viable Fetuses	No. of Viable Fetuses	92	109	91	105
	Mean (per litter)	7.08	7.79	6.50	7.00
	S.E. Mean	0.45	0.45	0.62	0.65
Dead Fetuses	No. of Dead Fetuses	0	1	0	0
	Mean (per litter)	0.00	0.07	0.00	0.00
	S.E. Mean	0.00	0.07	0.00	0.00
Early Resorptions	No. of Early Resorptions	2	3	0	15
	Mean (per litter)	0.15	0.14	0.00	1.00
	S.E. Mean	0.10	0.10	0.00	0.59
Late Resorptions	No. of Late Resorptions	3	3	3	0
	Mean (per litter)	0.23	0.21	0.21	0.00
	S.E. Mean	0.12	0.15	0.15	0.00
	No. of Litters	13	14	14	15

Table VII.8a SUMMARY OF FETAL OBSERVATIONS - MALFORMATIONS

GROUP:	FETUSES T_0	T_1	T_2	T_3	LITTERS T_0	T_1	T_2	T_3
LEVEL (MG/KG/DAY):	0	1	5	25	0	1	5	25
NUMBER EXAMINED EXTERNALLY	92	109	91	105	13	14	14	14
CLUBBED PAW(S)	0	0	0	1	0	0	0	1
CURLY TAIL	1	0	0	0	1	0	0	0
DOMED HEAD	0	0	1	0	0	0	1	0
GASTROSCHISIS	0	0	0	1	0	0	0	1
UMBILICAL HERNIA	1	0	0	0	1	0	0	0
NUMBER EXAMINED VISCERALLY	92	109	91	105	13	14	14	14
HYDROCEPHALUS	0	1	1	0	0	1	1	0
URETER-ABNORMAL COURSE	3	0	0	0	3	0	0	0
MULTIPLE HEART DEFECTS	1	0	0	0	1	0	0	0
MULTIPLE KIDNEY DEFECTS	0	0	1	0	0	0	1	0
GALL BLADDER AGENESIS	0	0	1	4	0	0	1	3
NUMBER EXAMINED SKELETALLY	92	109	91	105	13	14	14	14
BRANCHED RIB(S)	0	0	1	0	0	0	1	0
FUSED RIB(S)	1	0	0	0	1	0	0	0
EXTRA VERTEBRA(E)	0	0	1	1	0	0	1	1
MISSING VERTEBRA(E)	0	1	1	0	0	1	1	0
TOTAL MALFORMATIONS								
NUMBER WITH EXTERNAL MALFORMATIONS	2	0	1	2	2	0	1	2
NUMBER WITH VISCERAL MALFORMATIONS	4	1	3	4	4	1	2	2
NUMBER WITH SKELETAL MALFORMATIONS	1	1	2	1	1	1	2	1
TOTAL NUMBER WITH MALFORMATIONS	7	2	6	7	6	2	3	5

Table VII.8b SUMMARY OF FETAL OBSERVATIONS - VARIATIONS

GROUP:	FETUSES				LITTERS			
LEVEL (MG/KG/DAY):	T_0 0	T_1 1	T_2 5	T_3 25	T_0 0	T_1 1	T_2 5	T_3 25
NUMBERED EXAMINED EXTERNALLY	92	109	91	105	13	14	14	14
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	92	109	91	105	13	14	14	14
POSTERIOR LUNG LOBE AGENESIS	4	0	0	0	2	0	0	0
ELONGATED LIVER LOBE(S)	6	5	3	6	5	3	3	3
EXTRA STRUCTURE-AORTIC ARCH	10	14	14	14	6	7	7	6
SMALL GALL BLADDER	0	0	0	1	0	0	0	1
NUMBER EXAMINED SKELETALLY	92	109	91	105	13	14	14	14
EXTRA OSSIFICATION STERNUM	1	0	0	1	1	0	0	1
EXTRA/RUDIMENTARY RIB(S)	65	73	55	61	11	14	14	14
THICKENED RIB(S)	0	0	1	1	0	0	1	1
FUSED STERNEBRA(E)	1	0	0	1	1	0	0	1
INCOMP. OSS. HYOID BODY	0	0	1	0	0	0	1	0
INCOMP. OSS. PHALANGE(S)	1	0	1	7	1	0	1	1
INCOMP. OSS. STERNEBRA(E)	12	9	11	22	7	7	5	9
INCOMP. OSS. VERTEBRA(E)	0	2	2	8	0	2	1	3
MALALIGNED STERNEBRA(E)	1	0	0	0	1	0	0	0
UNOSS. OLECRANON	1	2	0	1	1	2	0	1
UNOSS. PATELLA	12	19	12	13	5	7	5	6
UNOSS. STERNEBRA(E)	2	3	4	11	2	1	3	6
TOTAL VARIATIONS								
NUMBER WITH EXTERNAL VARIATIONS	0	0	0	0	0	0	0	0
NUMBER WITH VISCERAL VARIATIONS	17	19	16	18	7	10	9	7
NUMBER WITH SKELETAL VARIATIONS	65	73	57	61	11	14	12	14

E) PERINATAL-POSTNATAL STUDY IN RATS (TA93-286, conducted by Abbott, GLP, Vol. 1.74).

1. Treatment

Female rats (24/group) were treated with 0 (vehicle), 4, 20, or 100 mg/kg, po, from Day 15 of pregnancy through Day 20 postpartum. Dams were observed for survival and overt changes in behavior and appearance throughout the study and litters were evaluated for postnatal survival and growth. The HD formulation sample assayed during the first week was only 86.6% of the intended concentration, but a HD sample assayed during week 2 was 96.2% of theoretical, and this deviation was not thought to have any effect on the study.

Strain: Sprague Dawley (Crl:CD®BR VAF/Plus)
Drug lot #: 77-558-AL

2. F0 Effects:

a) No dams died during the study. Decreased activity, rough coat, hunched posture, and lethargy were reportedly observed in HD females during treatment. These dams were also reported to show poor nursing behavior, to which an increase in pup mortality was attributed; however, neither this nor other clinical observations were documented in any way.

b) Maternal BW and BW gain during the gestational treatment period were significantly decreased at the HD, while weight gain during lactation was increased in this group. Food consumption was decreased during the gestational treatment period in HD dams.

3. Parturition

a) There were no T-R differences in gestation length. Where observation was possible, deliveries were noted to be unaffected by treatment history of the dam. Litter size was comparable between groups.

b) The number of stillborn pups was increased (D-R) in all treatment groups relative to C (Table VII.9). At the HD, this was largely due to 2 litters with 100 and 30% litter loss. An additional 3 HD dams had lost their entire litters by PND 4. Pup viability between birth and PND 4 was decreased (15%) in HD litters compared to C (Table VII.10). There appeared to be a correlation between maternal weight gain deficit during gestation and decreased pup viability.

c) Pup BW was significantly decreased (about 10% in both sexes) in HD litters at birth (Table VII.11). This deficit had been made up by weaning (means similar on PND 21), but some affected pups had died, and litters were culled on PND 4.

d) Pup necropsy findings were similar among C, LD, and MD groups; however, several findings occurred only in HD litters. These included opacity in one eye and mottled lungs in 2 HD pups from 2 litters.

Table VII.9. F0 Dam Delivery - Maternal and Litter Parameters

Treatment	Vehicle	Tiagabine Dose (mg/kg)		
	0	4	20	100
Total no. females	24	24	24	24
No. gravid	24	24	24	24
No. nongravid	0	0	0	0
No. dying/sacrificed prior to delivery	1	0	1	0
No. dying postpartum	0	0	0	0
No. delivering pups	23	24	23	24
No. with complete litter loss	0	0	0	4
No. with no or partial litter loss	23	24	23	20
Gestation duration (days)[a]	21.57 ± 0.51	21.46 ± 0.51	21.74 ± 0.45	21.83 ± 0.48
Liveborn	12.57 ± 0.51	12.38 ± 1.86	11.09 ± 2.52	11.83 ± 3.07
Stillborn/dead Day 0	0.09 ± 0.29	0.21 ± 0.51	0.35 ± 0.57	0.63 ± 1.79
Litter size	12.87 ± 1.69	12.38 ± 1.86	11.09 ± 2.52	11.83 ± 3.07

[a] Litter mean ± SD

Table VII.10. Summary of Offspring Viability

Lactation Day	Abbott-70569 HCl			
	0 mg/kg/day	4 mg/kg/day	20 mg/kg/day	100 mg/kg/day
0	99.3% 294/296	98.4% 297/302	97.0% 255/263	95.0% 284/299
4BR	96.9% 285/294	98.3% 292/297	98.8% 252/255	82.4% 234/284
7	100.0% 184/184	100.0% 192/192	100.0% 179/179	99.4% 158/159
14	100.0% 184/184	100.0% 192/192	100.0% 179/179	99.4% 157/158
21	100.0% 184/184	100.0% 192/192	100.0% 179/179	100.0% 157/157

Notes: (1) Summary statistics and results from the statistical analysis were obtained using the jackknife procedure.

(2) The symbol "BR" has been used to denote survival data collected on Lactation Day 4 prior to litter reduction.

(3) No statistically significant differences were detected between the Abbott-70569 HCl treatment groups and the control group at the 0.0167 (=0.05/3) level of significance.

Table VII.11. Summary of Offspring Growth

Treatment Group	Statistic	Males on Lactation Day					Females on Lactation Day				
		0	4BR	7	14	21	0	4BR	7	14	21
T0: Abbott-70569 HCl, 0 mg/kg/day	Mean	6.10	9.81	15.77	32.56	51.52	5.84	9.43	15.16	31.86	50.05
	SEM	0.11	0.27	0.38	0.74	1.25	0.11	0.20	0.35	0.60	0.95
T1: Abbott-70569 HCl, 4 mg/kg/day	Mean	6.20	10.10	16.15	32.86	53.90	5.83	9.61	15.24	31.65	50.65
	SEM	0.12	0.23	0.32	0.61	0.73	0.11	0.26	0.34	0.45	0.69
T2: Abbott-70569 HCl, 20 mg/kg/day	Mean	6.42	10.53	16.72	33.33	53.52	6.07	9.90	15.66	31.69	50.30
	SEM	0.13	0.17	0.25	0.60	0.95	0.10	0.18	0.29	0.69	1.12
T3: Abbott-70569 HCl, 100 mg/kg/day	Mean	5.42*	9.13	15.15	31.56	51.63	5.15*	8.83	14.60	30.86	49.35
	SEM	0.17	0.24	0.36	0.49	0.79	0.13	0.23	0.36	0.44	0.68

Notes: (1) Summary statistics and results from the statistical analysis were obtained using the jackknife procedure.

(2) The symbol "BR" has been used to denote body weight data collected on Lactation Day 4 prior to litter reduction.

(3) The symbol "*" has been used to denote Abbott-70569 HCl treatment groups that were found to be significantly different from the control group at the 0.0167 (=0.05/3) level of significance.

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VIII. SUMMARY

PHARMACODYNAMICS

Tiagabine hydrochloride was characterized as a potent, specific GABA uptake inhibitor in a variety of brain tissue preparations (IC50, 67 nM in synaptosomal membranes). It appeared to bind to a single class of high-affinity binding sites associated with the GABA uptake carrier (without preference for glial or neuronal uptake sites; carrier selectivity not defined) and lack significant affinity for other neurotransmitter receptor binding sites and/or uptake sites (weak binding to BDZ and H1 receptors). In rat hippocampal slices, tiagabine prolonged inhibitory postsynaptic potentials (IPSPs) and currents (IPSCs) produced by the addition of exogenous GABA, prolonged the duration of monosynaptic IPSPs elicited by excitatory AA antagonists, and increased the decay time constant of GABAA-mediated synaptic currents. Tiagabine was shown by *in vivo* microdialysis to increase extracellular GABA overflow in several brain regions (including the substantia nigra) of awake rats, and this effect appeared to correlate with the degree of anticonvulsant protection. Thus, it is thought that inhibition of GABA uptake increases synaptic concentrations of this inhibitory neurotransmitter and that the resultant facilitation of the action of GABA at its CNS receptors is responsible for the anticonvulsant effects of tiagabine.

The overall anticonvulsant profile of tiagabine in animal models is generally consistent with enhancement of GABA-mediated neurotransmission (**Table I.1**). In anticonvulsant screening studies, it was active against DMCM-induced clonic convulsions in mice (ED50: 0.8 mg/kg, ip; 3.4 mg/kg, po), sound-induced seizures in DBA/2 mice (ED50: 0.4 mg/kg, ip, for tonic component) and GEPR rats (ED50: 11 and 30 mg/kg, ip, for tonic and clonic components), and kindled seizures in rats (ED50: 3 mg/kg, ip) but was only partially effective against sc PTZ-induced clonic seizures in mice (maximum of 65% inhibition; ED50: 1.3 mg/kg, ip) and photic seizures in photosensitive baboons. Tiagabine produced a biphasic dose-response curve against DMCM- and PTZ-induced clonic convulsion, with attenuated effectiveness at higher doses. It had little efficacy against MES (ED50, 40 mg/kg, ip), and was not active in the iv PTZ seizure threshold test or against seizures induced by bicuculline. In rodents, tiagabine produced behavioral neurotoxic effects, including decreased spontaneous locomotor activity, impairment of motor function (**Table I.2**), sedation, tremor, and myoclonus, at multiples of the anticonvulsant dose comparable to those for other AEDs (PIs ranged from 4.6 -14; **Tables I.3** and **I.4**).

Tiagabine administration to mice for up to 21 days (15 or 30 mg/kg, po, bid) did not produce significant anticonvulsant tolerance (assessed against DMCM-induced seizures) but did result in some apparent tolerance to sedative/motor-impairing effects. There were no signs of withdrawal after discontinuation of treatment (no changes in PTZ seizure threshold, body weight, or general behavioral observations). In another tolerance study, repeated administration of tiagabine (2 mg/kg, ip, bid) to mice resulted in a significant reduction in the anticonvulsant effect against iv PTZ after just 2 doses; however, there was an apparent recovery of anticonvulsant efficacy during continued dosing, with full effectiveness returning after 3 days and being maintained for up to 8 days. The possible role of PK changes (ie, accumulation) in this effect was not investigated. Cross tolerance with BDZs was also demonstrated in this study. Tiagabine (up to 30 mg/kg,ip) did not substitute for direct or indirect BDZ agonists or amphetamine in drug discrimination testing in rats, however, and was not self-administered by rats. It did, however, show some ability to potentiate dopaminergic function, as reflected in enhancement of methylphenidate-induced gnawing in mice (ED50: 0.9 mg/kg, ip). Tiagabine exhibited analgesic and anxiolytic in rats and mice, and produced antiischemic effects in rats at high doses (50 mg/kg, ip). Doses up to 30 mg/kg, po, did not affect the rat electroencephalogram.

Administration of 10 mg/kg, iv, to rats produced a significant increase in blood pressure (maximal at 2-5 min, recovery by 15 min). The hypertension was associated with a decrease in heart rate, suggesting that it may have been due to increased peripheral vascular resistance. In anesthetized dogs on the other hand, decreased vascular resistance and increased cardiac output were seen at a dose of 0.3 mg/kg, intraduodenally (id). At a dose of 3 mg/kg, id, there were no significant CV changes, but respiratory rate was markedly depressed, which interfered with interpretation of the CV effects at that dose. The reduction in spontaneous respiration rate observed in isoflurane-anesthetized dogs was not seen in unanesthetized dogs, suggesting a possible drug-drug interaction, although behavioral effects interfered with data collection in the unanesthetized dogs. Tiagabine decreased gi motility in isolated guinea pig ileum *in vitro* (3 and 30 ug/ml) and in mice *in vivo* (3 and 30 mg/kg, iv) and produced significant diuresis in rats (0.5 - 50 mg/kg, po).

ADME (Table II.1)

Absorption and Pharmacokinetics

Orally administered tiagabine appeared to be well absorbed in mice, rats, dogs, and humans. Bioavailability was also high in mice (>85%) and humans (90%) but was lower in rats (25-30%) and dogs (50-55%). Absorption was rapid, with peak plasma concentrations usually attained within the first hr after oral administration of an aqueous solution (Tmax ~10 min in mice, 30 min in rats, and 1 hr in dogs). After iv administration, the clearance in mice, rats, and dogs ranged from 0.8 to 3 L/h/kg, but was at least 10-fold slower in humans. The iv half-lives exhibited similar species differences, averaging <1 hr in mice and dogs, <2 hr in rats, and 10 hr in humans. After oral administration, t1/2s averaged 3-6 hr in mice, 1-4.5 hr in rats, and 1-2 hr in dogs. The oral half-life in healthy volunteers is 7 hr but is shortened to approximately 3.5 hr in comedicated patients; therefore, frequent dosing intervals may be required. Small, meal-related secondary plasma level peaks, suggestive of enterohepatic recirculation, were seen in dogs and humans. Plasma concentrations in rats increased with daily dosing at a dose of 30 mg/kg, resulting in Cmax values of 1.2, 1.9, and 4.4 ug/ml and AUCs of 3.4, 5.9, and 8.1 ug·hr/ml after 1, 7, and 14 days, respectively. Apparent bioavailability increased from 31 to 73%, but t1/2s and 24 hr (trough) concentrations did not change appreciably with repeated dosing, suggesting that the increase in levels may have been due to increased absorption or saturation of first-pass metabolism rather than accumulation. Levels also tended to increase with duration of treatment in both the rat and dog toxicity studies.

Protein Binding

The *in vitro* plasma protein binding of [14C]tiagabine was independent of concentration over a wide range (0.01 to 10 ug/ml) and similar among species, averaging 89.3% in mice, 92.6% in rats, 91.6% in dogs, 90.8% in rabbits, and 96.2% in humans. Tiagabine was bound to both human serum albumin and alpha1-acid glycoprotein, although albumin was the more important binding protein. Binding to albumin was non-saturable at therapeutically or toxicologically relevant concentrations, but was decreased at lower albumin concentrations, in subjects with hepatic impairment, or by addition of salicylate, naproxen, or valproate, all drugs that are extensively bound to albumin. Tiagabine did not affect protein binding of phenytoin, carbamazepine, valproate, or phenobarbital.

Distribution, Metabolism, and Elimination

The apparent volume of distribution was 1.41 in rats and 1.28 L/kg in dogs. Thirty minutes after administration of a single oral dose of [14C]tiagabine to rats (30 mg/kg to albino and pigmented strains) or dogs (0.1 mg/kg), the highest concentrations were found in the excretory organs, with tissue to plasma ratios of 9 -15 in the liver and 3 - 4 in the kidney. Levels of radioactivity in most other tissues were similar to or lower than those in plasma. The brain/plasma ratio in rats remained relatively constant at 0.24, but in dogs, the ratio increased from 0.4 at 30 min after administration to 1.0 after 4 hr, indicating a slower elimination from the brain or slow equilibration between plasma and brain. Radioactivity also appeared to be cleared more slowly from the eyes (in pigmented rats and dogs), liver, and kidneys than from the plasma or other tissues. Levels of radioactivity in the retina and uvea of dogs were considerably higher than those in the non-pigmented ocular tissues (peak concentrations similar to those in plasma; maximum T/P ratio of about 3 at 4 hr), and radiolabel remained in these tissues at low levels for at least 3 weeks (last measurement; Table II.2). The sponsor concluded that tiagabine did not demonstrate the marked ability to concentrate and persist in the eye that has been reported for cationic amphiphilic compounds such as chloroquine and phenothiazines, but it is not clear that this could be established following a single low dose. With repeated dosing (30 mg/kg for 7 days) in rats (CD), tissue radioactivity levels were higher than the respective values after a single dose; however, there was no indication of specific accumulation in any of the limited number of tissues examined (eye not included), ie, T/P ratios did not change appreciably. Following oral administration of radiolabled tiagabine to pregnant or lactating rats, fetal to maternal plasma ratios of 0.2-0.5 and milk to maternal plasma ratios of 0.8-1 were found.

The routes of excretion were similar across species. About 15% of the dose was excreted in the urine in mice

and rats, compared to 25% in humans and dogs; the remainder was eliminated in the feces, presumably through biliary secretion. Tiagabine appears to be extensively metabolized in the mouse, rat, dog, and human, with no more than 1% of the parent drug being excreted in the urine of any species studied and relatively small amounts of unchanged drug found in feces. The metabolic pathways of tiagabine have been only partially elucidated (see Fig II.1). The major pathway is thought to involve thiophene ring oxidation to form geometric 5-oxo- isomers. In rats, a dioxidized metabolite conjugated with glutathione was identified in bile, while in dogs, the oxo-thiolene isomers were further metabolized, either by hydroxylation of the methyl group or by conjugation with glucuronic acid. 5-oxo-tiagabine was found to be the major metabolite in the plasma and urine of rats and dogs (the primary species for toxicity testing) as well as humans. It accounted for about 90% of the urinary radioactivity in rats, 35% in dogs, 60% in humans, corresponding to 8-16% of the dose in all three species, but was a minor metabolite in the mouse. This metabolite was shown have little *in vitro* activity as a GABA uptake inhibitor. Pronounced species differences have been observed in the biliary metabolites of the rat and dog. Tiagabine glucuronide is the major metabolite in the bile of the anesthetized dog, whereas glutathione conjugates of tiagabine (or an oxidized metabolite) appear to be the major components of rat bile.

Pretreatment of rats with oral doses of tiagabine (200 mg/kg for 14 days) led to increased liver weights, without concomitant changes in body weight, in both sexes, and produced up to a 15-fold increase in cytochrome P450 2B-mediated 7-pentoxyresorufin)-dealkylase activity. Thus, tiagabine appears to be a Pb-type inducer, although the responses were less marked than those elicited by Pb. *In vitro* studies with hepatic microsomes indicated that thiophene ring oxidation leading to the formation of 5-oxo-tiagabine was the major NADPH-dependent pathway in all species studied. The rate of metabolism was highest in hepatic microsomes from male rat, with decreasing rates in female rats, followed by mouse, dog, and human. In contrast to oxidative metabolism, the *in vitro* rate of glucuronidation was greatest with microsomes from dog liver, with decreasing rates in rats and humans; glucuronidation was not detected with mouse microsomes under similar incubation conditions. Studies with human hepatic microsomes indicated that members of the cytochrome P450 3A subfamily were primarily responsible for the oxidative metabolism of tiagabine, including the formation of 5-oxo-tiagabine.

Beaune et al (Proc Natl Acad Sci 84:551-555,1987) have shown that thiophene ring oxidation, presumably through a sulfoxide intermediate which covalently binds to CYP2C9/10, may be responsible for the immunologically-based hepatotoxicity caused by tienilic acid. To investigate the possibility of covalent binding with tiagabine, Beaune performed exploratory studies using techniques similar to those used in earlier studies with tienilic acid. Covalent binding involving CYP2C9/10 was detected following incubation of [14C]tiagabine with microsomes prepared from human liver samples, but since Km values were considerably higher than the tiagabine concentrations expected in the plasma or liver of patients receiving the anticipated therapeutic doses, it was thought unlikely that the covalent binding seen *in vitro* would be clinically relevant.

Toxicokinetics

Tiagabine concentrations increased with dose over a wide range of doses in all species studied (**Table II.3**). A trend for greater than dose-proportional increases was noted in rats, especially females, and possibly dogs. Rats and dogs displayed sex-related differences in plasma concentrations (M<F). AUCs tended to increase with duration of dosing in rats and dogs. For comparison with the tiagabine concentrations in the animal toxicity studies, the mean steady-state Cmax in induced patients receiving tiagabine was approximately 0.33 ug/ml and the AUC_{0-t} was approximately 3.1 ug·hr/ml at the highest dose studied (80 mg/day). Healthy subjects displayed a Cmax value of 0.24 ug/ml and an AUC of approximately 3.6 ug·hr/ml, at 12 mg tid.

Mice: In 3-month and 2-year oral (gavage) toxicity studies, the 1-hr plasma concentrations increased with increasing dosage and did not exhibit any consistent duration- or sex-related differences. Increases appeared to be dose-proportional between 10 (0-1.4 ug/ml, mean=0.62 ug/ml) and 100 (6.3-9.7 ug/ml, mean=7.5 ug/ml), but were less than dose-proportional at the higher doses (eg, 7.9-13.8 ug/ml, mean=10.4 at 250 mg/kg and 20-22 ug/ml at 1000 mg/kg). It is possible that the lack of proportionality in 1-hr concentrations resulted from protracted absorption at the higher doses, but a complete plasma concentration vs time profile was not determined. The 1-hr tiagabine concentrations in the 2-year carcinogenicity study ranged from about 2-fold to 30-fold the mean steady-state Cmax in patients getting 20 mg qid (0.33 ug/ml).

Rats: Sex-related differences in plasma levels were seen in the rat oral toxicity studies. The overall mean AUC values from the 10, 30, 100, and 200 mg/kg dose groups in the 2-year carcinogenicity study were up to 2.4 times higher in female (2.0, 16.0, 99.4, and 212.0 ug·h/ml, respectively) than in male rats (2.9, 8.9, 62.2, and 88.3 ug·h/m, respectively). Greater than dose-proportional increases in levels were seen, as dose-adjusted AUC values increased from 0.2-0.3 at a dose of 10 mg/kg to about 0.4-1 at 100-200 mg/kg. AUCs tended to increase with duration of dosing, particularly in males. Plasma levels following oral administration to pregnant rats were variable, but the mean Cmax (0.17, 3.05, 10.8 ug/ml) and AUC (0.94, 12.9, and 81.5 ug·h/ml) values were comparable to those in non-pregnant rats. Concentrations in 0.5 hr samples from immature rats were also consistent with those measured in adults. Compared to the estimated steady-state AUC values of 3.1 ug·h/ml in induced patients and 3.6 ug h/ml in non-induced subjects receiving the maximum doses of tiagabine, mean AUC values in the 2-yr rat study were similar at 10 mg/kg, about 2.5 to 5-fold greater at 30 mg/kg, 15 to 30-fold greater at 100 mg/kg, and 25 to 70-fold greater at 200 mg/kg.

Dogs: In dogs given capsules (0.5, 2, and 10 mg/kg) during 6- and 12-month oral toxicity studies, plasma levels were slightly higher in females than in males at the LD, but overall sex differences were minimal at these doses. There was some indication that the increase in levels between the LD and HD was greater than dose-proportional in males. The mean t1/2 also appeared to be longer at 10 mg/kg (2-2.3 hr) than at 2 mg/kg (1.3-1.4 hr). Together, these suggest that one or more elimination pathways in dogs might become saturated at higher doses. As in rats, AUCs tended to increase with duration of dosing. At doses higher than 10 mg/kg, sex differences became more pronounced, with higher levels in females than in males. Exposure in dogs was lower than the estimated exposures in patients at doses of 0.5 (<10%), 2 (30-35%), and 5 mg/kg (65-75%), but was about 1.5-fold greater at 10 mg/kg and about 7-fold greater at 40 mg/kg.

TOXICOLOGY

Acute toxicity

Mice: Clinical signs following oral administration to mice included decreased activity, ataxia, nystagmus, mydriasis, decreased rectal temperature, increased paw temperature, and an absent pain reaction at doses of 20 mg/kg or more, with myoclonic convulsions, head drop, tremor upon handling, ptosis, and decreased respiration seen at 100 mg/kg or more. 100% mortality was observed at doses of 1000 mg/kg or more (500 mg/kg<LD50<1000 mg/kg). The decreased motor activity and ataxia are expected consequences of increased GABA activity in the CNS. Convulsions are a paradoxical reaction seen with high doses of GABA agonists or other anticonvulsants. Hypothalamic temperature regulation also appears to be modulated by GABA.

Intravenous administration of 10 mg/kg or more of tiagabine to mice produced convulsions followed by myoclonus. Decreased motor activity, ataxia, opisthotonos, ptosis, hematuria, cyanosis of the tail, and decreased rectal temperature were also seen. Seven/10 animals (4 males, 3 females) given the HD of 160 mg/kg died within 2 hr of dosing. No other animals died during the subsequent 14-day observation period. Necropsy showed no gross changes attributed to treatment. The hematuria seen primarily at 40 mg/kg or greater was thought to be due to hemolytic properties of the drug at high intravenous doses, which would not be expected to be a problem at therapeutic, oral doses.

Rats: Rats were less sensitive to the toxic effects of tiagabine than mice. Clinical signs consisting of decreased activity and ataxia were seen in rats given oral doses of 80 mg/kg or higher, while myoclonus was evident at 400 and 2000 (HD) mg/kg. These motor disturbances were somewhat more pronounced in females than males. Bleeding from the nose and rales were also reported at the two highest doses, possibly due to local irritation following reflux or aspiration. Clinical signs were seen only during the first 2 days, but decreased BW gain occurred over the course of the 14-day observation period. Mortality was only observed at the HD.

Rats dosed iv with 10 to 75 mg/kg tiagabine exhibited decreased activity, ataxia, myoclonus, hematuria and cyanosis of the tail vein. Myoclonus was seen in approximately half of the rats in the two highest dose groups (50 and 75 mg/kg). One HD rat died 2 hr after dosing, but there were no other deaths. There were no significant effects on BW or BW gain. Necropsies revealed only chronic obstructive phlebitis in the tail veins of rats administered tiagabine.

Multidose toxicity

Mice: A 13-week dose range-finding study was conducted in CD-1 mice (10/sex/group) with oral (gavage) doses of 0 (HPMC vehicle), 50, 100, 250, and 400 mg/kg of tiagabine. The LD was increased to 600 mg/kg on Day 29 and the 100 mg/kg dose was increased to 1000 mg/kg on Day 50 because no toxicity was observed at the lower doses. All mice were necropsied at the same time, approximately 3 months after initiation of treatment. Increased mortality was observed at the 3 highest doses: 1, 1, 1, 7, 12, and 17 mice died at 0, 50, 250, 400, 600, or 1000 mg/kg, respectively. T-R clinical signs were seen primarily at 400, 600, and 1000 mg/kg and consisted of ataxia, hypoactivity, and dyspnea. BW gain and food consumption were decreased at 250 mg/kg or greater compared to controls. Serum alkaline phosphatase values were increased in males treated with 250 or 400 mg/kg, and elevated glucose values were seen in the 250, 400, and 600 mg/kg groups. Increased liver weights and hepatocellular hypertrophy were observed at all doses, and hepatocyte vacuolization was seen in mice from the 250, 400, and 100/1000 mg/kg groups. Brown degeneration of the adrenal cortex was observed with an increased incidence in females in the 400 and 100/1000 mg/kg groups. The apparent lack of dose response relationships was probably due to the dose adjustments and high mortality. Increased liver weights and enzyme activities presumably reflected the induction of hepatic microsomal drug metabolizing enzymes, which was demonstrated in a 2 week (ADME) study in mice. Mean plasma concentrations on day 87 were 9019, 8801, 17578, and 20005 ng/ml for males and 10671, 9653, 16069, and 22558 ng/ml for females at doses of 250, 400, 600, and 1000 mg/kg, respectively.

Rats: A 13 week study was conducted in CD rats (15/sex/group) with oral (gavage) doses of 0 (water vehicle), 25, 100, and 400 mg/kg. Hypoactivity was observed at the HD and salivation was observed in MD and HD groups. Swollen abdomen, rales, hunched posture, and other agonal signs were also noted in animals from the these groups. Deaths attributed to treatment occurred in the MD (1 M, 2 F) and HD groups (5 M, 11 F). Food consumption and BW gain were decreased only during Week 1 in HD rats. Water consumption was increased in all treated animals (D-R). Depression of the pupillary light reflex in the right eye and consensual light reflex in the left eye (cranial nerve function) was noted in an increased number of treated rats (only C and HD tested); however, interpretation of this finding was confounded by the fact that blood was collected for hematological analysis by retro-orbital puncture from the right eye prior to neurological testing. The sponsor attributed the effects to trauma from blood collection and the higher incidence in treated mice was considered coincidental. Ophthalmoscopic and histopathological exams revealed no structural changes in the eye or nervous system (but it is not clear that histopathology exams included all relevant structures, such as those in the midbrain). The changes may have represented an acute nervous system effect (affecting afferent pathways, since the consensual reflex was also affected), but the time of testing with respect to dosing was not given, and this finding was not further investigated. Decreased erythrocyte parameters (females) and increased prothrombin times (2 males) were seen in rats from the HD group. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, cholesterol, total protein, and albumin levels were somewhat elevated in treated animals, primarily in HD males and females, consistent with effects on the liver. Increased urine volume and a dark coloration of the urine were seen in HD rats (tiagabine was shown to have diuretic effects in pharmacology studies). Liver weights were increased in MD animals, and liver, kidney, and thyroid weights were increased in the HD group. Gaseous distention of the gi tract was observed in MD and HD rats; the majority of animals with this finding had died or were killed prematurely. Pulmonary congestion, hemorrhage, alveolar flooding, and alveolar macrophage aggregates were found in HD animals that died or were sacrificed during the study (it was suggested that noisy respiration and pulmonary histopathological changes may have resulted from distended gut pushing against diaphragm). 2/5 HD males that died during the study had gastric lesions associated with ulceration. D-R hepatocyte hypertrophy was seen in all treatment groups, and hepatocyte necrosis and eosinophilia were found in some HD animals. Plasma levels 1 hr after dosing during the second week were 3-4 times higher in females than in males; mean concentrations were 527, 1308, and 3236 ng/ml in LD, MD, and HD males, respectively, and 823, 5444, and 11801 ng/ml in corresponding females. The NOAEL in this study was 25 mg/kg.

A 6-month study was conducted in CD rats (20/sex/group) with oral (gavage) doses of 0 (water vehicle), 10, 30, and 100 mg/kg. There were no T-R clinical signs, effects on mortality, or differences in BW gain, indicating that dose selection may have been inappropriate (HD of 200 mg/kg used in carcinogenicity study). There were no toxicologically significant hematological or urinalysis changes. Triglycerides, total protein, and albumin were

increased in HD rats, as seen in the 3-month study, but liver enzymes were decreased somewhat. Slightly decreased serum K^+ seen in HD males could have been due to the diuretic effect observed in other studies. Gaseous distention, increased liver weights, and minimal hepatocellular hypertrophy were seen in some HD females. Conjunctivitis (2 MDM, 1 MDF, 1 HDF), focal retinopathy (1 CM, 3 MDM, 5 HDM, 1 HDF), and retinal degeneration (1 MDF) were found primarily in MD and HD rats during ophthalmoscopic exams at 6 months. These were considered to be infectious in origin rather than T-R by the ophthalmologist. No ocular abnormalities were noted in the HD recovery group, and the only microscopic finding was retinal dystrophy in 1 HD male. In addition, no T-R effects on the retina were seen in the 2-year rat study at doses up to 200 mg/kg. 30 mg/kg was the NOAEL in this study.

Dogs: In a dose range finding study in dogs (1/sex/group) in which tiagabine was administered for up to 6 weeks at doses of 10-40 mg/kg, clinical signs consisting of salivation, tremor, incoordination, convulsions, apparent visual impairment, and marked sedation were observed at all doses. Bradycardia was evident in most dogs 2 hr after dosing and for up to 24 hr after treatment in dogs given 30 mg/kg. Increased packed cell volumes, Hb concentrations, and erythrocyte counts (thought by sponsor to be due to hemoconcentration resulting from prolonged sedation) and a D-R decrease in activated partial thromboplastin time were seen at the HD. Elevated serum cholesterol (up to 200% of C), glucose (slight), and potassium and decreased urine volume were also noted in treated dogs.

Four groups of beagle dogs (4/sex/group) were given oral (capsule) doses of 0, 5, 10, or 20/15 mg/kg for 14 weeks (HD reduced after 7 days due to severe loss of appetite). Clinical signs were observed with a D-R incidence in treated dogs and consisted of insensibility, prostration, stupor, tremors, ataxia, and apparent visual impairment. The latter was described as a lack of awareness of objects, failure to fix on and follow a moving object, or absence of a blink reaction. The most notable sign was said to be marked and persistent sedation, which was seen in all treated groups from the first day, with rapid onset, generally within 30 min, and a maximal effect consisting of almost complete unconsciousness. Dogs in this state were said to be totally unresponsive. This progressed to a state of stupor in which animals had open eyes, could be roused by sound or tactile stimulus but appeared unaware and were unable to stand or walk. This was followed by a state resembling sleep. A state of "dazed semi-awareness," during which animals wandered aimlessly and showed apparent visual impairment and ataxia sometimes preceded, followed, or alternated with the state of sleep. The apparent visual impairment was not associated with any structural abnormality and was considered to be of central origin. Emesis and salivation were also seen in MD and HD animals, convulsions were seen on one occasion each in 1 HD male and 1 MD female, and episodes of anxiety and vocalization were observed in 1 HD and 1 MD male and in 2 MD females. No T-R deaths occurred. There were effects on weight gain and food consumption (decreased) in MD and HD dogs during the first 2 weeks, but overall BW gain was similar among groups. Neurological exams revealed what was described as an exaggerated gag reflex in some treated dogs. There were no cardiovascular changes attributed to treatment. An increase in platelets in treated males (significant at HD after 12 wks) was attributed to low control values. Activated partial thromboplastin times were decreased in some treatment groups but the effect was not D-R. Adrenal weights were slightly decreased in HD males, but there were no apparent histological correlates. Plasma tiagabine levels showed an increase (about 2-fold) between Day 1 to Wk 12 and were higher in females at the HD (2-fold). The increase with time was not attributed to accumulation, since the pre-dose levels were low, but was thought to have resulted from an increase in bioavailability with daily dosing (unexplained). Mean plasma AUCs ± SD at week 12 were 2527, 6077, and 5483 ng·hr/ml for males and 2547, 6478, and 10904 ng·hr/ml for females at the LD, MD, and HD, respectively.

Tiagabine was administered po (capsules) to beagle dogs (4/sex/group) at dose levels of 0, 0.5, 2, and 10 mg/kg/day for 6 months. An additional 2/sex were included in the C and HD groups for evaluation of recovery. Recumbency, sedation, irregular gait, tremors, salivation, increased respiration, spontaneous vocalizations, lack of awareness/responsiveness, and absence of the blinking and pinch reflexes were noted in treated animals, primarily at the HD. Signs were most frequent and severe during the first 4 weeks of treatment but continued to be observed throughout the study. All animals survived to termination. BW gain and food consumption were decreased in HD dogs only during the first week. No changes in hematologic, clinical chemistry, or urinalysis values were noted. Ophthalmoscopic exams revealed no T-R ocular abnormalities. At terminal sacrifice, adrenal and testes weights were increased (fairly uniformly) in treated males (30% and

20%, respectively, at HD). Weights of both organs were similar between the treated (HD) and C recovery animals. Prostate weights appeared to be dose-dependently decreased in treated males (30% at HD) after 6 months but were higher in treated recovery males compared to C. Ovarian weights were decreased in treated females compared to C at 6 months (40% at HD) but were similar between the recovery animals. There were no correlating gross or microscopic observations. Microscopic examinations of the brain and spinal cord revealed no remarkable findings. Cmax and AUC values were dose-related and tended to be slightly higher in females than males. There was a statistically significant difference between dose-adjusted AUC values at 0.5 and 10 mg/kg in male dogs and a significantly lower elimination rate constant at the HD in both sexes, suggesting that one or more elimination pathways for tiagabine may become saturated with increasing doses. The NOAEL was considered by the sponsor to be 2 mg/kg, although some clinical signs were transiently observed at this dose.

Groups of 5 beagle dogs/sex received doses of 0, 0.5, 2, or 10 mg/kg for 12 months. There were no deaths in the study. T-R clinical signs consisted of hypoactivity, ataxia and prostration (described as frequent and severe at HD), as well as tremors, emesis, salivation, decreased defecation and urination, and ocular discharge, all seen primarily in MD and HD dogs. BW gain and food consumption were decreased in HD animals during the first few weeks of treatment, but final BWs were similar among groups. No T-R ECG abnormalities were observed, but ophthalmological examination revealed what appeared to be a D-R increase in cataracts (anterior suture opacities). Since similar opacities were seen in controls and have been reported in other recent studies in the same facility, one consultant ophthalmologist concluded that the condition was probably not directly caused by the drug but could have been exacerbated by treatment (final incidences were similar among groups, but onset appeared to be accelerated by treatment; **Table III.A-5**). Erythroid parameters tended to be reduced and WBCs increased in MD and HD dogs. Myeloid/erythroid ratios in the bone marrow smear performed *post mortem* were slightly elevated in some treatment groups, indicating possible effects on erythrogenesis. Alkaline phosphatase was slightly increased in treated females at some measurement times and glucose appeared to be increased somewhat in treated males. Decreases in ovarian (50% at HD) and uterine (60% at HD) weights were seen in treated females. Microscopic findings that occurred with increased, but not clearly D-R, frequencies in treated dogs included inflammatory changes in the lungs, thyroid C-cell hyperplasia, lymphocyte infiltration of the prostate, and uterine hyperplasia. On day 364, Cmax values were 40.6, 257.1, and 827.9 ng/ml for LD, MD, and HD males, respectively; corresponding female values were 58.4, 99.5, and 992.4 ng/ml. AUC values were 192.3, 1308.3, and 7103.1 ng·hr/ml for males and 249.1, 591.2, and 6379.8 ng·hr/ml for females in the LD, MD, and HD groups, respectively. There was no evidence of sex-related PK differences in this study.

CARCINOGENICITY

Mice: CD-1 mice (70/sex/group) were dosed with 0 (C A), 0 (C B), 10, 30, 100, or 250 mg/kg, by gavage, for 2 years (99 weeks at HD due to high mortality). Satellite groups (25/sex/group) were dosed for plasma drug level analysis. The HD was based on the results of the 3-month oral range-finding study (decreased BW gain and hepatotoxicity at 250 mg/kg). In the 2-year study, survival was significantly decreased in HD males (84% mortality) and females (74%) compared to C (51-67%), and trend analysis showed significant, D-R increases in mortality for both sexes. Although HD mortality may have been excessive, inclusion of the MHD, which met criteria for a MTD (BW and toxicity), provided adequate numbers for assessment of carcinogenicity. Deaths attributed to gavage error were increased at the 2 highest doses, which also produced clinical signs of neurotoxicity. BWs were dose-dependently decreased in treatment groups compared to C (13 and 12% below C A in 100 mg/kg group males and females, respectively). There were no D-R changes in palpable masses. At necropsy, gaseous distention of the gi tract was noted in HD males and females, and congestion and/or red discoloration of the lung were found in mice of both sexes from the 2 highest dose groups, primarily those that died during the study. Microscopic examination revealed increased incidences of hepatocellular hypertrophy (27/70, 26/70, 40/70, 49/70, and 67/70 males and 4/70, 12/70, 11/70, 26/70, and 49/70 females administered 0, 10, 30, 100, and 250 mg/kg, respectively) and vacuolization (1/70, 0/70, 2/70, 3/70, and 9/70 males and 3/70, 1/70, 1/70, 1/70, and 8/70 females receiving 0, 10, 30, 100, or 250 mg/kg, respectively) at the 2 highest doses.

There were no statistically significant increases in tumor incidence in the sponsor's or FDA (HFD-710)

statistical reviewer's analysis of all groups. Since high mortality and the slightly shorter treatment period could have affected tumor incidence rates in the HD group, separate analyses were also performed without this group. There were again no statistically significant tumor trends in the sponsor's analysis, but the more conservative FDA analysis found a significant trend for alveolar bronchiolar carcinoma of the lung in males when the HD was excluded (0, 2.9, 4.3, 5.7, and 1.4 % at 0, 10, 30, 100, and 250 mg/kg, respectively; **Table IV.3**). There was no significant difference in the pairwise comparison between MHD (4/70) and C (0/70) males for this tumor, however, and no other significant trends were found among males in the FDA analysis. The incidence of alveolar bronchiolar carcinoma also appeared to be increased in treated females, but there was no dose relationship (1.4, 7.1, 4.3, 2.9, and 5.7 % at 0, 10, 30, 100, and 250 mg/kg, respectively; **Table IV.3**); and no significant trend was observed for this or other tumor types in females. Incidences of alveolar bronchiolar carcinoma in tiagabine-treated mice were all within historical control ranges for CD mice in 2-year studies(M:1.9-20%, F:0-13.5%). At 12 months, the mean plasma tiagabine levels were 1382, 4055, 6467, and 10598 ng/ml in males and 1113, 3219, 6821, and 7887 ng/ml in females from the 10, 30, 100, and 250 mg/kg dose groups, respectively.

Rats: CD rats (70/sex/group) received oral (gavage) doses of 0 (C A), 0 (C B), 10, 30, 100, or 200 mg/kg for 2 years (101 weeks at HD). Satellite groups (25/sex/group) were treated with the same doses for drug level determinations only. Dose selection was based on the 3- and 6-month toxicity studies, in which clinical signs and gi (gaseous distention, ulceration) and liver (increased weights, hepatocellular hypertrophy) toxicity were observed at 100 mg/kg or greater and mortality was increased at 400 mg/kg. In the carcinogenicity study, increased salivation was noted at the 2 highest doses, survival was significantly decreased in HD males and females (84 and 81% mortality, respectively) and in 100 mg/kg females (73%) compared to C A (67 and 61% in males and females, respectively), and BWs were significantly lower in males from the 2 highest dose groups (16 and 15% below C A at 100 and 200 mg/kg, respectively) and in HD females (17% below C A). There were no T-R changes in palpable masses. Hematological findings suggestive of hemolytic effects (decreased erythrocyte parameters, reticulocytosis, and changes in erythrocyte appearance) were variably observed at the 3 highest doses, but these were not pronounced. Non-neoplastic findings at necropsy included increased incidences of pulmonary alveolar macrophages, pulmonary inflammation, and edema at 100 and 200 mg/kg in both sexes, inflammation of the trachea in HD animals, slight increases in keratitis in some treated males, and a small increase in hepatocellular hypertrophy in females receiving the 2 highest doses (2/70 and 4/70 at 100 and 200 mg/kg, respectively, vs 0/140 in C).

Liver tumors appeared to be increased by tiagabine treatment in female rats (**Table IV.8**). The sponsor's statistical analysis showed a significant increase in the incidence of hepatocellular adenoma in HD females (7/70), together with a positive D-R trend for adenomas in females, and a significantly increased combined incidence of hepatocellular adenoma and carcinoma in both HD males (4/70) and females (8/70) compared to C A (M: 0/70 combined, F: 1/70 combined - adenoma). However, incidences of hepatocellular adenoma and carcinoma in HD males (2/70 for each) were similar to those in C B males (1/70 adenoma, 2/70 carcinoma; not included in statistical analysis) and were within the study facility's historical control ranges for males (adenoma: 1.3-10%; carcinoma: 1.7-6%). The incidence of adenoma in HD females was outside the historical control range (0.77 - 3.33%), but the rate of carcinoma (1/70) was not increased (control range: 0-1.7%). The incidence of Leydig cell tumors of the testes was increased in tiagabine-treated males (**Table IV.8**). In the sponsor's analysis, there was a significant trend for Leydig cell tumors to increase with dose, and the incidence of this tumor in the HD group (7/70) was significantly increased and outside the historical control range (1.67 -6%). The FDA statistical reviewer also found significant positive trends for Leydig cell tumors in males and for hepatocellular adenomas in females (no paired comparisons were performed). At 12 months, the mean AUC values were 2.2, 5.1, 46, and 93.7 ug·hr/ml for males and 1.9, 17.9, 128.9, and 266.3 ug·hr/ml for females in the 10, 30, 100, and 200 mg/kg groups, respectively. (In patients with epilepsy, the 24-hr systemic plasma exposure (AUC) of tiagabine has been determined to be approximately 3.1 ug·hr/ml at the highest recommended clinical dosage of 80 mg/day).

GENETIC TOXICITY

In vitro, no significant increases in revertant colonies occurred following exposure of Salmonella typhimurium strains TA 98, TA 100, TA 1535, and TA 1537 or E coli strain WP2 uvrA to tiagabine; however, equivocal

effects on mutation frequency at the HGPRT locus in V79 Chinese hamster lung cells were seen, and tiagabine was positive in a chromosome aberration test: structural chromosome aberration frequency (primarily chromatid breaks) in human lymphocytes was increased by exposure to tiagabine concentrations of 200 ug/ml (cytotoxic concentration) in the absence of metabolic activation (**Table VI.1 and VI.2**). No significant differences in micronucleus formation were detected in mice given single po doses of up to 250 mg/kg, and there were no increases in unscheduled DNA synthesis in rat primary hepatocytes cultured *in vitro* following *in vivo* administration of tiagabine doses up to 1200 mg/kg. All genotoxicity assays appeared valid according to established criteria.

REPRODUCTIVE TOXICITY

Segment I (male and female)

CD rats (42/sex/grp) were dosed with 0, 4, 20, or 100 mg/kg, by oral gavage, for 70 (males) or 28 days (females) prior to mating, throughout mating, and until sacrifice. Females were either sacrificed on Day 20 of gestation (22 dams) or following weaning on postnatal Day 21, and males were sacrificed after the last female had been sacrificed. No T-R mortalities occurred during the study. T-R clinical signs observed in HD animals during dosing included rales, nasal discharge, rough coat and piloerection. Significant decreases in BW (7%), BW gain (15%), and food consumption were observed in HD males during the dosing period. BW gain was significantly decreased (13%) in HD females during the gestational dosing period. There were no group differences in mating or fertility indices.

At C-section, small increases in postimplantation loss were seen in MD (7% vs 3% in C) and HD (9%) group dams, but values were within the historical control range. Fetal BWs were only slightly decreased at the MD and HD. Total incidences of fetal malformations and variations were comparable among groups (1, 2, 1, and 2 malformed fetuses in C, LD, MD, and HD groups). The malformations were: cleft palate (1 HD fetus), anophthalmia and/or microphthalmia (2, 1, and 1 affected fetuses at LD, MD, and HD), hydrocephaly (1 MD), brachydactyly (1 HD), and umbilical hernia (1 C). Incidences of unossified or malaligned sternebrae were increased in all treated groups, but not in a dose-related manner (possibly due to intrauterine deaths at MD and HD).

There were no significant treatment-related effects on reproductive parameters in dams allowed to deliver, but pup viability on Day 4 was significantly decreased at the HD, and pup weights during lactation were decreased in the HD group compared to C. There were no group differences in litter retrieval. The HD group weight deficit persisted following weaning until study termination, and statistical significance was reached between weeks 15 and 21 in HD males. There were slight delays in the acquisition of developmental landmarks at the MD and HD, usually limited to 1 day in 1 or 2 pups/group. An effect on open field behavior was observed when offspring were tested at between 35 and 45 days of age. Treated pups, primarily in the HD group, were generally less active in this test than controls, ie, center square latency was increased, squares entered decreased (significant for HD males), and rearing was decreased. The effect was seen in both sexes but was fairly small and the individual data were variable (the HD male effect was attributed to 1 pup that remained in the center square throughout the test). There were no effects on a multiple T-maze test of learning and memory. There were no T-R differences in the fertility or reproductive capabilities of F1 animals. F2 parameters were comparable among groups.

Segment II (rat)

Mated CD rats (25/group) were treated with 0, 4, 20, or 100 mg/kg, by gavage, on gestation Days 6 through 17, and C-sections were performed on gestation Day 20. There were no maternal deaths. A low incidence of rales and ocular and nasal discharge was observed at the HD only. Maternal BW gain during dosing (GDs 6-18) was decreased at the HD (10%) and corrected gestational BW gain (maternal BW less gravid uterine weight) was even lower in this group compared to C (28% over GDs 6-20). Gravid uterine weights were increased in the MD and HD groups due to larger litter sizes. Maternal Cmax values on gestation day 15 were

174, 3050, and 10841 ng/ml in LD, MD, and HD dams, respectively.

At C-section, implantation sites were significantly increased in MD and HD dams, probably reflecting an unusually low number of implants in the C group. Resorptions were slightly increased and mean fetal BWs slightly decreased (5%) in HD litters. The overall malformation rate was markedly increased in the HD group (0/191 (0/22), 1/225 (1/22), 0/197 (0/18), 9/221 (7/20) affected fetuses (litters) in C, LD, MD, and HD groups, respectively; about 3 -10-fold historical control frequencies for total malformations during the same period; **Table VII.5a**), with a variety of external and visceral malformations (craniofacial, limb, CNS and cardiovascular) found only in HD litters: one litter contained a fetus with microcephaly, hydrocephalus, agnathia, astomia, anophthalmia, and a proboscis (rhinocephalus) and another with agnathia, aglossia, and an encephalocele; a second litter had one fetus with a threadlike vestigial tail, umbilical hernia, clubbed hindlimbs, and generalized edema and another with open eye; and fetuses with missing tail (acaudia), open eye, situs inversus (2), and pulmonary stenosis were each found in separate litters. An increased number of pale fetuses were found in HD litters, but there were no other apparent group differences in incidences of variations. Despite the HD malformations, the sponsor concluded that because of their "diverse nature" and the failure to find an increase in malformations in the segment I or a dose range-finding study, "there is no compelling evidence to conclude that they were directly related to treatment with tiagabine." However, exposure to teratogens in a segment II study often results in a spectrum of malformations, reflecting treatment throughout organogenesis; higher doses are often needed in segment I than in segment II studies because of the longer treatment period; and the dose range-finding study was apparently conducted with 2-4 dams/group and limited fetal examinations.

Segment II (rabbit)

Mated NZW rabbits (20/group) were dosed orally (gavage) with 0, 1, 5, or 25 mg/kg on gestation days 6 to 19. An additional 4/group were treated for drug level determination. C-sections were performed on Day 29 of gestation. No T-R clinical signs were observed. Reduced BW gain during dosing was seen in HD animals compared to C. Food consumption was also decreased at the HD. Mean maternal plasma levels on Day 19 were 82, 631, and 3631 ng/ml in LD, MD, and HD does.

The numbers of corpora lutea, implantation sites, and live and dead fetuses were comparable across groups; however, resorptions were increased at the HD (litter mean of 1.0 vs 0.15 in C) and fetal weights were slightly decreased (3% less than C) in HD female offspring. Incidences of specific visceral abnormalities (gallbladder agenesis - often considered a variation - in 4/105 fetuses and 3/14 litters; control range: 0-3.7% fetuses, 0-14% litters) and skeletal variations (incompletely or unossified phalanges, vertebrae, and sternebrae) were increased in HD litters (**Tables VII.8**).

Segment III

Female rats (24/group) were treated with 0 (vehicle), 4, 20, or 100 mg/kg, po, from Day 15 of pregnancy through Day 20 postpartum. Dams were observed for survival and overt changes in behavior and appearance throughout the study and litters were evaluated for postnatal survival and growth.

Maternal BW and BW gain during the gestational treatment period were significantly decreased at the HD. There were no differences in gestation length, and litter sizes were comparable between groups. There was a D-R increase in stillborn pups in treatment groups relative to C (**Table VII.9**). At the HD, this was largely due to 2 litters with 100 and 30% litter loss. An additional 3 HD dams had lost their entire litters by PND 4. Pup viability between birth and PND 4 was decreased (15%) in HD litters compared to C. There appeared to be a correlation between maternal weight gain deficit during gestation and decreased pup viability. Pup BW was significantly decreased (about 10% in both sexes) in HD litters at birth. This deficit had been made up by weaning (means similar on PND 21), but some affected pups had died, and litters were culled on PND 4. Pup necropsy findings were similar among C, LD, and MD groups; however, several findings occurred only in HD litters. These included ocular opacity and mottled lungs in 2 HD pups from 2 litters.

IX. EVALUATION

Pharmacology: Drugs that enhance central inhibitory (primarily GABAergic) neurotransmission should, theoretically, be useful in the treatment of epilepsy. One strategy for facilitating GABA-mediated function in the CNS is to increase synaptic concentrations of GABA by blocking its active reuptake into nerve terminals via a high-affinity, sodium dependent transport system. This process can be inhibited by cyclic amino acids such as nipecotic acid and guvacine, but these compounds do not readily cross the blood-brain barrier. Tiagabine was the most promising in a series of lipophilic nipecotic acid derivatives developed by Novo Nordisk, and pharmacological data suggest that it is a potent, specific, and bioavailable inhibitor of GABA uptake into neurons and glia in the CNS. Such a mechanism has the potential advantage, relative to direct-acting agonists, of enhancing only the effects of endogenously released GABA, which might be expected to preserve physiological specificity and limit side effects; and unlike the GABA aminotransferase inhibitor vigabatrin, tiagabine's action is reversible (there was no evidence of intramyelinic edema in animal toxicology studies of tiagabine). In addition, tiagabine appears to be better tolerated clinically than previously assessed GABA uptake inhibitors such as CI-966 (Parke-Davis), a guvacine derivative. In the initial single dose trial in healthy volunteers, this compound caused severe psychiatric and neurological side effects, including psychotic symptoms, mania, memory impairment, tremor, myoclonus, and parkinsonian-like symptoms, that forced the termination of clinical studies (Sedman et al, Drug Dev Res 21:235-242,'90).

The anticonvulsant profile of tiagabine in animal models is generally consistent with enhancement of GABA-mediated neurotransmission and would predict therapeutic potential for partial and generalized tonic-clonic seizures. Despite its higher *in vitro* affinity for the GABA uptake site than CI-966 (IC50: 67 nM vs 0.3 uM), tiagabine was somewhat less potent in blocking PTZ-induced clonic seizures in mice (po ED50: 6.8 mg/kg vs 0.4-1 mg/kg), suggesting that it may less effectively penetrate the BBB (Rogawski and Porter, Pharmacol Rev 42:223-286,'90). In addition to its (partial) activity against sc PTZ-induced clonic seizures (ip ED50:1.3 mg/kg in mice, but maximum of 65% protection), tiagabine was active against clonic seizures induced by the BDZ inverse agonist DMCM in mice (0.8 mg/kg), blocked audiogenic seizures in DBA/2 mice (0.4 mg/kg) and GEPR rats (11 mg/kg), and was effective against amygdala-kindled seizures in rats (3 mg/kg). Tiagabine had little efficacy against MES in rats (ED50: 40 mg/kg, ip) and was not active in the iv PTZ seizure threshold test or against seizures induced by bicuculline, which is unlike BDZs but like some other agents that act by enhancing GABAergic neurotransmission (eg, VGB, THIP, progabide, CI-966). Tiagabine only partially suppressed photic seizures in baboons at doses (1 mg/kg, iv) associated with neurological side effects (impaired motor coordination, diffuse tremor, and slow abnormal movements of the limbs). The behavioral effects of tiagabine in animals were similar to those of other GABA-enhancing agents. In rats and mice, tiagabine produced sedation, hypothermia, impairment of motor function, tremor, and, at higher doses (100-400 mg/kg, po), myoclonus. In dogs, neurotoxicity was observed at oral doses as low as 0.5 mg/kg, with convulsions at doses of 10 mg/kg or greater. Myoclonus was a prominent effect of CI-966 in several species, including humans, and has also been seen with GABAA agonists (Taylor et al, Drug Dev Res 21:195-215,'90).

Tiagabine produced a U-shaped dose response curve against PTZ- and DMCM-induced seizures, with loss of efficacy at higher doses. A similar biphasic response has been reported with other GABA uptake inhibitors (eg, NNC-711, SKF 100330A, and CI-966) as well as other GABAergic agents such as vigabatrin. The mechanism for this effect is unknown, but a possible explanation is the phenomenon of double inhibition. When GABA neurons are connected in series, the net effect of an increase in GABA activity in one brain area can be disinhibition of target neurons in other areas, so the specific neuronal connections determine whether GABA will be pro- or anticonvulsant in any given brain region; eg, increasing GABA transmission in the substantia nigra is anticonvulsant, while enhancing GABA transmission in parts of the thalamus has been shown to potentiate seizures (Gale, Epilepsia 33(Suppl 5):S3-12,'92). Therefore, with increasing occupancy of the GABA uptake carrier, the net effect of augmenting GABA activity at synapses throughout the brain could shift from anticonvulsant to proconvulsant. A paradoxical electrographic response to tiagabine has been reported in an experimental model of status epilepticus (Walton et al, Epilepsy Res 19:237-44,'94). When tiagabine was administered to cobalt-lesioned rats in which status epilepticus was induced by injection of homocysteine thiolactone, it was potent in controlling generalized tonic-clonic seizures (ED50, 8.3 mg/kg, ip) but produced an abnormal, hyporeactive behavioral state accompanied by an EEG pattern of high-amplitude,

rhythmic, 3-5 Hz spike-wave activity. This EEG and behavioral syndrome could be reproduced in normal, non-epileptic rats by administration of a high dose of tiagabine (100 mg/kg). The EEG change was seen within 2-3 min of tiagabine injection and remained unchanged for about 2 hr before gradually reverting to baseline over another 2 hr. The EEG pattern and behavioral hyporeactivity produced appeared to be an ictal phenomenon and was thought to resemble human non-convulsive status epilepticus. No dose-response relationship was determined for production of these EEG changes in normal rats (only a single high dose was tested); however, in a study conducted by the sponsor, spontaneous EEGs were unaffected in normal rats treated with oral doses up to 30 mg/kg. Tiagabine also exacerbated absence-like spike-wave discharges in rat models of non-convulsive epilepsy (WAG/Rij, GAERS). This effect has been seen with other GABAmimetics such as SKF 100330 and vigabatrin, leading to speculation that non-convulsive epilepsy may be associated with GABA hyperfunction (Coenen et al, Epilepsy Res 21:89-94,'95); however, non-GABAergic drugs such as phenytoin and carbamazepine also aggravate non-convulsive seizures in these models, while BDZs inhibit them, so the picture is not entirely consistent. There is some evidence that the paradoxical effects of GABAergic drugs on brain excitability might be more pronounced in the developing, aging, or pathologic brain (Monaco, Neurology 47 (Suppl 1): S9, '96). For example, studies in developing rats indicate that the role of GABA changes during ontogeny, with GABAA receptors mediating primarily excitatory activity in the neonatal hippocampus (Cherubini et al, Trends Neurosci 14:515-519,'91). The possible relationship of EEG changes (rhythmic, bisynchronous, spike and wave discharges) and impaired mentation in several patients receiving tiagabine to the effects observed experimentally is reportedly being investigated by the sponsor (Annual Report, '96).

Toxicology: The toxicology of tiagabine has been adequately studied in mice, rats, and dogs. GABA and its receptors are ubiquitous in the brain and are also found in a wide range of peripheral tissues, including parts of the peripheral nervous system, endocrine system, smooth muscle, and reproductive system. Many of the effects of tiagabine in the toxicity studies appear to represent exaggerated physiological actions of GABA, as would be predicted by the drug's mechanism of action. The following toxicities were prominently or consistently observed:

CNS: Clinical signs in all species tested were primarily related to CNS effects, ie, depression (reduced motor activity, ataxia) and/or stimulation (tremor, convulsions). In acute studies in mice, decreased activity and ataxia were seen at oral doses of 20 mg/kg (Cmax, 3.6 ug/ml) or more, with tremor and myoclonic convulsions at 100 mg/kg (Cmax, 7.5 ug/ml) or more. During the 3-month mouse study, CNS effects (decreased activity) were primarily seen at doses of 400-1000 mg/kg (Cmax, 10-20 ug/ml). Following acute administration to rats, hypoactivity and ataxia were seen at oral doses of 80 mg/kg or higher, while myoclonus was reported at 400 mg/kg (Cmax, 3.2-11.8 ug/ml) or greater. Hypoactivity was reported at 400 mg/kg in the 3-month rat study. In repeated dosing studies in dogs, severe CNS effects (including insensibility, prostration, stupor, tremors, convulsions, and visual impairment - see below) were observed at oral doses of 5-40 mg/kg (Cmax, 0.9-6.8 ug/ml; AUC, 2.3-33.8 ug·hr/ml). Thus, plasma exposures at the lowest neurotoxic dose in dogs were similar to or lower than human exposures at the highest clinical doses (2.5 ug·hr/ml at 64 mg/day, 3.1 ug·hr/ml at 80 mg/day). The neurological toxicity of tiagabine is not unexpected: CI 966 caused ataxia and other neurotoxic effects including tremors and myoclonus at low doses in dogs and monkeys and produced severe psychiatric and neurological side effects in humans; catalepsy has been reported following administration of SKF 89976A (nipecotic acid ester) and SKF 100330A (guvacine ester) to rodents; and when muscimol is given to humans at relatively high doses, it causes an intoxication characterized by difficulties in concentration, palinopia (endless repetitions of visions seen minutes before), ataxia, catalepsy, and hallucinations. As with other AEDs, CNS-related side effects should be an important clinical concern with tiagabine.

Visual system: Clinical signs noted in dogs receiving oral doses ≥5 mg/kg included an apparent visual impairment, characterized by a lack of awareness of objects, failure to fix on and follow moving objects, or absence of a blink reaction. No structural changes in the eye were associated with the visual disturbance, so the effect was assumed to be central in origin. This seems likely, particularly in view of the known GABAergic regulation of visual centers in the CNS. For example, so-called fixation cells in the rostral superior colliculus that inhibit the generation of saccadic eye movements and form part of a system of oculomotor control, ie, that of visual fixation, have been shown to be under GABAergic control. Injection of muscimol into the rostral SC of monkeys, which would increase normal GABA inhibition and decreases activity of these neurons, reduced

the latency for saccades to visual targets so that the monkeys had difficulty maintaining visual fixation and suppressing unwanted saccades (Munoz and Wurtz, J Neurophysiol 70:576-89,'93). The behavioral effects in tiagabine-treated dogs were reversible and no neuropathology was found. In a possibly related finding, depression of the pupillary light reflex was noted in rats treated with 400 mg/kg in the 3-month toxicology study, with no corresponding structural changes in the eye or nervous system. Visual complaints have also occurred with tiagabine in clinical trials, but frequencies were similar to those in placebo groups.

Radiolabel distribution studies in dogs showed localization and persistence of tiagabine in pigmented tissues of the eye (retina and uvea), making ocular toxicity a potential concern. (Tiagabine may have certain structural similarities to melanin-binding drugs such as chloroquine or, alternatively, tiagabine binding sites could act as a sink, since GABA receptors are abundant in the retina. Retinopathy has been observed in rats and patients following administration of vigabatrin, but the involvement of GABA is unclear.) No pronounced morphological alterations in the eye were reported in animals receiving tiagabine, however, although incidences of various ocular findings appeared to be increased somewhat by treatment in long-term studies, including keratitis (at 100 and 200 mg/kg in 2-year study) and focal retinopathy (30 and 100 mg/kg in 6-month study) in rats and cataracts in dogs (2 and 10 mg/kg in 1-year study). These could have resulted from direct effects on the eye or from a pathophysiological mechanism involving effects on sensory innervation, blinking, or tear formation, as has been seen, for example, with narcotic analgesics. Spontaneous inflammation of the cornea and conjunctiva with or without subsequent scarring and opacity is common in laboratory animals (rats are particularly susceptible to the development of keratitis as a result of infection with the sialodacryoadenitis virus) and may be exacerbated when ocular defense mechanisms are depressed following high dose drug administration (Greaves). Focal retinopathy is also a common finding in rats, and its increased incidence in the 6-month tiagabine study was thought to indicate the presence of an infectious process in a closely housed group of animals; no T-R retinal changes were noted in the 2-year rat study at the same or higher doses. (Toxicity testing in albino rats would clearly not assess effects due to melanin binding; however, vigabatrin-induced retinopathy was seen in albino but not pigmented rats). The apparent D-R development of cataracts (small anterior suture opacities) in dogs during the 1-year toxicity study was also thought to represent an exacerbation of a spontaneously occurring condition, since similar opacities were seen in the study controls (incidences were similar across groups at the end of the study) and had been reported in other recent studies in the same facility.

GI: Gaseous distension of the gi tract was reported in oral toxicity studies of tiagabine in both mice (250 mg/kg) and rats (≥100 mg/kg), and gastric ulceration was also seen in rats. In pharmacology studies, tiagabine antagonized histamine- (EC50: 1.5 ug/ml) and acetylcholine-induced contractions (EC50: 20 ug/ml) and reduced spontaneous motility (3 and 30 ug/ml) in isolated guinea pig ileum and produced a significant D-R increase in gi transit time in mice (3 and 30 mg/kg, iv), indicating an inhibitory effect on gi motility that could explain the findings in the toxicity studies. These effects were seen at higher than therapeutic doses and plasma concentrations in rodents and were not seen in dogs, so the risk of serious gi side effects in humans is probably small. However, some irritant effects might be anticipated on the basis of structural features of tiagabine; (according to Chemistry) compounds that are structurally related to the 3-methyl-2-thienyl portion of the molecule can cause gi irritation.

Liver: Liver changes indicative of microsomal enzyme induction were observed in mice and rats administered tiagabine doses of 10 -1000 mg/kg in the subchronic and chronic toxicology studies. These included increases in liver weight, hepatocellular hypertrophy and vacuolization, and increases in smooth endoplasmic reticulum. The occurrence of liver tumors was also increased somewhat in tiagabine-treated rats during the 2-year carcinogenicity study. There was a positive D-R trend for hepatocellular adenoma in female rats, and the incidence of adenomas was significantly increased in females at the HD of 200 mg/kg. The combined incidence of hepatocellular adenoma and carcinoma was significantly increased at the HD in both males and females compared to their respective controls. However, since incidences of adenoma and carcinoma in HD males were within historical control ranges and similar to those in a second control group and the incidence of carcinoma in treated females was low and not D-R, it could be concluded that the increase in adenomas in females was the only meaningful treatment effect. The 10% incidence of this tumor in HD females was outside the study facility's historical control range of 0.8-3.3%, although published data on spontaneous

tumors in female CD rats give a range of 0-22% for adenomas. The significant HD effects occurred at levels of exposure (mean AUCs of 88 ug·hr/ml in males and 212 ug·hr/ml in females at 200 mg/kg) that were considerably higher than the highest anticipated human exposure (2.5 - 3.1 ug·hr/ml). No increase in liver tumors was seen in mice at doses up to 250 mg/kg in the 2-year study, although increased incidences of hepatocellular hypertrophy and vacuolization were observed. Since tiagabine was negative for genotoxicity in most tests (it was clastogenic in human lymphocytes at a cytotoxic concentration of 200 ug/ml), a nongenotoxic mechanism related to hepatic enzyme induction or induction of liver growth was assumed. Oral administration of 200 mg/kg for 2 weeks was shown to produce a significant increase in hepatic microsomal enzyme metabolizing activity in female but not male rats. Thus, higher AUC values were measured in female rats following tiagabine administration, and females showed greater sensitivity to the induction of hepatic enzymes and liver tumors by tiagabine. The liver effects seen with tiagabine are common in long-term rodent toxicity studies with enzyme-inducing drugs and their relevance to human risk assessment is questionable.

Testis: An increased prevalence of Leydig cell tumors was observed in treated male rats during the 2-year carcinogenicity study: incidences of 2.9, 1.4, 2.9, 5.7, and 10% were found in the 0, 10, 30, 100, and 200 mg/kg dose groups, respectively, reaching statistical significance at the HD (study facility's historical control range: 0-6%; published range: 0-20%). A possible mechanism (proposed by sponsor) for the induction of Leydig cell tumors in rats by tiagabine could involve effects on LH secretion, since GABA is known to play an important role in the regulation of LHRH release from the hypothalamus (both stimulatory and inhibitory effects reported), and increased LH levels have been associated with Leydig cell tumors in rats. In addition, tiagabine was shown to potentiate dopaminergic function (enhanced methylphenidate-induced stereotypic gnawing in mice), which could affect prolactin levels. Although no investigation of the endocrine effects of tiagabine was undertaken by the sponsor, such an explanation is plausible. Rats are known to be particularly prone to the development of Leydig cell tumors with age, and this tendency is enhanced by administration of agents that modulate age-related changes in hypothalamic-pituitary function; eg, Leydig cell tumors have been reported following chronic administration of the LHRH analog buserelin, the antiandrogen flutamide, or the H2 blocker cimetidine, which also has antiandrogenic properties (Greaves). Because of the unusual sensitivity of rats to such changes, these findings probably have little clinical relevance. The mean plasma tiagabine exposure in male rats receiving 200 mg/kg in the carcinogenicity study was approximately 35-fold that expected at the highest clinical dose. No T-R changes in Leydig cell tumors were seen in the 2-year mouse study of tiagabine.

Respiratory tract: Treatment-related increases in the incidences of pulmonary congestion, inflammation, edema, and alveolar macrophages were observed in toxicity studies of tiagabine in mice and rats. These findings are common in gavage studies, but their sometimes markedly increased occurrence in tiagabine-treated animals was unexplained. Such changes are often associated with general ill health or agonal states and can have a variety of causes. It was suggested that pulmonary changes in tiagabine-treated rodents may have been produced when gaseous distention of the gi tract interfered with diaphragmatic movement. However, GABA and GABA agonists have been shown to inhibit a number of airway responses, including neuronally induced cholinergic and tachykinin-mediated smooth muscle contraction, anaphylactic bronchospasm, and cough (Chapman et al, Trends Pharmacol Sci 14:26-9,'93); so it is possible that tiagabine treatment, in addition to producing generalized sedation and respiratory depression, could have a specific inhibitory effect on the airway (including effects on pharyngeal or laryngeal reflexes) that would increase susceptibility to aspiration or infection. In a possibly related finding, incidences of alveolar bronchiolar carcinoma were increased somewhat in treated mice during the 2-year carcinogenicity study, although frequencies were not clearly dose-related and were within historical control ranges. No effect on lung tumor incidence was observed in the rat carcinogenicity study. Although the amphiphilic structure of tiagabine might predict it, no histological evidence of phospholipidosis (could be suspected as a common mechanism for lung and eye effects) was reported. Pulmonary changes generally occurred at levels of exposure considerably above those expected clinically, and it seems unlikely that any of these findings are of importance for humans.

Development: The effects seen at the high doses in the rat (increased malformations, decreased growth and viability at 100 mg/kg) and rabbit (increase in resorptions, gallbladder agenesis, and skeletal variations at 25 mg/kg) reproductive toxicity studies indicate that tiagabine is a developmental toxicant in both species at maternally toxic doses. GABA is thought to play an important role as a morphogenetic signal during

development, so a drug that alters GABA levels might be expected to adversely affect development. In the segment II study in rats, an increased malformation frequency was found in litters exposed to the HD of 100 mg/kg (fetal incidence of 9/221 vs 0/191 in C; about 3 -10 times historical control frequencies in Sprague-Dawley rats). There was no clear pattern of malformations or dose-response (ie, HD effect), and all of the defects observed occur spontaneously at low incidences in rats. However, the markedly increased combined incidence of malformations in the HD group indicates that development was perturbed by this dose, which also produced minimal maternal toxicity. Exposure to teratogens in a segment II study can result in a spectrum of malformations, since animals are treated throughout organogenesis, and often produces only an increased frequency of the malformations that occur spontaneously in a particular species and strain (Manson and Kang, Principles and Methods of Toxicology, Hayes, ed, '94). But it is difficult to conclusively attribute such an increase to treatment without a clear dose-response relationship. The dose range-finding study (not submitted) reportedly showed that 200 mg/kg was too materno- and embryotoxic, but the next lower dose was 100 mg/kg. The dose-response for teratogenic effects may be very steep before embryolethality is seen; therefore, another study with additional doses around the HD used in the definitive study (ie,100 mg/kg) might help define a dose-response pattern for developmental toxicity. When effects are observed only at maternally toxic dose levels, it is always hard to know if they are indicative of selective developmental toxicity or reflect nonspecific alterations in maternal homeostasis; however, while there is general agreement that increased embryolethality, decreased fetal BW, or increased incidences of variations may occur secondary to maternal toxicity, the role of maternal toxicity in the production of malformations is much less clear (Manson and Kang; Khera, Teratology 31:129-136,'85). The sponsor argued that the failure to find an increase in malformations in the segment I study or the dose range-finding study meant that the segment II findings were probably not treatment-related. However, higher doses are often needed in segment I than in segment II studies, since the longer treatment period permits maternal detoxification mechanisms to be established prior to gestation, and the dose range-finding study was apparently conducted with only 4 dams/group and limited fetal examinations. The teratogenic HD in the rat studies was associated with maternal plasma Cmax and AUC values approximately 30 times those expected clinically, but since species differences in sensitivity to teratogens may be considerable, the developmental findings warrant caution.

LABELING

PRECAUTIONS

Binding to Melanin-Containing Tissues

When dogs received a single dose of radiolabeled tiagabine, levels of radioactivity in the retina and uvea were considerably higher than those in non-pigmented ocular tissues, and radiolabel remained in these tissues for at least 3 weeks (the latest time point measured). Although not directly measured, melanin binding is suggested. This raises the possibility that tiagabine could accumulate in melanin-rich tissues such as the eye over time and that it could cause toxicity in these tissues after extended use.

Carcinogenesis

In rats, a study of the potential carcinogenicity associated with tiagabine HCl showed that 200 mg/kg/day (plasma exposure [AUC] 35 - 85 times that at the maximum human dosage [MRHD] of 64 mg/day) for 2 years resulted in statistically significant increases in the incidences of hepatocellular adenomas in females and Leydig cell tumors of the testis in males. The no effect dose for induction tumors in this study was 100 mg/kg (54 25 - 40 times the exposure at the MRHD). No statistically significant increases in tumor formation were noted in mice at doses up to 250 mg/kg (36 times the peak plasma levels at the MRHD.

The implications of the rat tumor findings for human health are unknown.

Mutagenesis

No mutagenic activity was found in the *in vitro* Ames test, *in vitro Escherichia coli* WP2 uvr A assay, and the *in vitro* HGPRT forward mutation assay in Chinese hamster lung cells, either with or without metabolic activation.

Tiagabine produced an increase in structural chromosome aberration frequency in human lymphocytes *in vitro* in the absence of metabolic activation. No chromosomal aberrations were demonstrated in the presence of a metabolic activating system. The mouse micronucleus test showed no mutagenic activity. An *in vivo/in vitro* unscheduled DNA synthesis assay in primary rat hepatocytes showed no genetic damage in the liver.

Impairment of Fertility

Studies of male and female rats at administered dosages of tiagabine HCl up to 100 mg/kg/day (plasma exposure [AUC] 32 times that at the maximum recommended human dosage [MRHD] of 64 mg/day) prior to and during mating, gestation, and lactation have shown no impairment of fertility. Lowered maternal weight gain and decreased viability and growth in the rat pups were found at 100 mg/kg, but not at 20 mg/kg/day (5 times the exposure at the MRHD).

Pregnancy: Pregnancy Category B C

Tiagabine has shown teratogenic effects when given to pregnant rats. An increased incidence of fetal malformations (primarily craniofacial and visceral defects) was observed following administration of 100 mg/kg/day (plasma exposure [AUC] 32 times that at the maximum recommended human dosage [MRHD] of 64 mg/day) during the period of organogenesis. This dosage also reduced maternal weight gain. No adverse maternal or embryofetal effects were seen at a dosage of 20 mg/kg/day (5 times the exposure at the MRHD). In a perinatal/postnatal study using the same dosages in rats, decreased maternal weight gain during gestation and decreased offspring viability and growth were found at 100 mg/kg/day.

Decreased maternal weight gain, increased resorption of embryos, and increased incidences of fetal variations were observed when pregnant rabbits were given 25 mg/kg/day (8 times the exposure at the MRHD) during organogenesis.

th There are no adequate and well-controlled studies of tiagabine in pregnant women.

Use in Nursing Mothers

Studies in rats have shown that tiagabine HCl and/or its metabolites are excreted in the milk of that species. Following oral administration of radiolabeled tiagabine to lactating rats, concentrations of radiolabel in milk approached those in the maternal plasma (84-98%). Levels of excretion of tiagabine and/or its metabolites in human milk have not been determined and effects on the nursing infant are unknown.

X. RECOMMENDATIONS

The NDA is approvable with respect to the pharmacology/toxicology portion. Recommendations concerning the proposed labeling are made in the Evaluation section of the review.

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cc:
NDA (20-646)
Div File
HFD-120/GFitzgerald/EFisher/JWare

J.E. Fisher, Ph.D.

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