c). Perforant Pathway Excitation

Electrical stimulation of the perforant pathway causes cell death in pyramidal cells of hippocampal areas in rats (62 on page 5-1085, v1.14). Pretreatment with vigabatrin (500 mg/kg) 24 hours prior to stimulation diminished the degree of damage in the pyramidal layer and weakened seizure manifestations.

d). Spinal Cord Seizures

Electrical stimulation of the spinal cord induces tonic flexion and extension of the hindlimbs in rats (63 on page 5-1087, v1.15). Vigabatrin (1500 mg/kg, IP) administration to rats facilitated seizure manifestations induced 2 hours later by reducing the duration of tonic flexion. GABA levels in spinal cord were elevated 2-fold at this time point, suggesting that activation of GABAergic neurons does not play a key regulatory role in spinal cord convulsions.

vi. Chemically-Induced Seizures

a). Bicuculline

Intravenous bicuculline administration to mice induces pronounced myoclonic seizures and vocalization (64 on page 5-1096, v1.15). Toxic doses of bicuculline result in tonic seizures and thrusts and death usually ensues (64 on page 5-1096, v1.15). Pharmacologically, bicuculline acts as a competitive antagonist at GABA postsynaptic sites.

Vigabatrin in doses ranging from 700 to 1500 mg/kg was administered IP to mice, 4 hours prior to bicuculline administration (65 on page 5-1105, v1.15). A dose of 3 mg/kg bicuculline SC produced tonic seizures in 100% of the mice tested. Vigabatrin had no effect on seizure parameters induced by 1.5 to 3.0 mg/kg SC bicuculline. In other studies with mice given 3 mg/kg bicuculline, SC, the times to clonus, tonus and death were increased at the 4-hour time point with a 1000 mg/kg dose of vigabatrin only (66 on page 5-1111, v1.15, 67 on page 5-1117, v1.15). Administration of vigabatrin (1500 mg/kg, IP) 24 hours prior to bicuculline also resulted in significant increases in the time to clonus, tonus and death.

More consistent results were obtained with lower IV doses of bicuculline (0.55 mg/kg), suggesting that a maximal seizure induction may not respond to vigabatrin therapy. The lower doses were chosen to produce a reproducible clonic seizure syndrome (64 on page 5-1096, v1.15). The ED_{50} value of vigabatrin for antagonism of myoclonic seizures induced by bicuculline in mice was 54 mg/kg (IP), 5 hours postdose. In a study in rats (54 on page 5-1032, v1.14), complete protection against clonic convulsions in bicuculline-induced (0.25 mg/kg, IV) seizures was observed 60 hours after vigabatrin treatment (900 mg/kg, IP). The rat midbrain and prepiriform cortex seem to play a key role in seizure development. Vigabatrin (5 μg) microinjected into the ventral midbrain tegmentum completely blocked tonic and clonic components of seizures induced with bicuculline (0.30 mg/kg, IV) after 6 hours (56 on page 5-1043, v1.14).

The same dose of vigabatrin microinjected into the area tempestas of the deep prepiriform cortex of the rat caused a significant decrease ($P < 0.05$) in mean seizure score and a decreased incidence

of major clonic and/or tonic seizures at the 6-hour and 24-hour time points (59 on page 5-1062, v1.14).

b). Picrotoxin

The convulsant properties of picrotoxin, like bicuculline, are thought to result from reversible antagonism of postsynaptic GABA sites. A dose of 6 mg/kg picrotoxin given IV produced tonic seizures in 100% of mice tested (65 on page 5-1105, v1.15). Vigabatrin pretreatment (1000 to 2000 mg/kg, IP) did not affect seizure frequency or mortality induced by 6 mg/kg picrotoxin in mice observed for 1 hour. However, vigabatrin caused a dose-dependent prolongation of the CT_{50} (time required to produce seizures in 50% of animals) and in the MT_{50} (time required for 50% mortality). In a more detailed study of the effect of vigabatrin (1000 mg/kg, IP) on picrotoxin-induced seizures (2.5 mg/kg, IV) as a function of time after injection, mice were tested at multiple time points from 2 to 96 hours postdose (68 on page 5-1128, v1.15). The mean latency of seizures was increased from 4.47 minutes to >8 minutes, 2 and 4 hours postdose. Increases in seizure latency were present up to 72 hours after treatment but not at the 96-hour time point.

Additional studies in mice suggested that the time course of vigabatrin efficacy varied with the mode of picrotoxin administration. A high protection rate (70%) was observed after 1 hour pretreatment with vigabatrin (1500 mg/kg, IP) in mice given 7.5 mg/kg picrotoxin, IP (53 on page 5-1010, v1.14). Anticonvulsant activity was weak 2, 4 and 24 hours after vigabatrin administration. When vigabatrin (1500 mg/kg, IP) was administered 4 or 24 hours prior to picrotoxin (10 mg/kg, SC), there was a prolongation of the times to clonus, tonus and death as well as a 25% decrease in mortality at the 4-hour time point (66 on page 5-1111, v1.15, 67 on page 5-1117, v1.15). No significant seizure protection was noted at the 24-hour time point.

c). GAD Inhibitors

(i). Mercaptopropionic Acid and Hydrazone

Vigabatrin (100 to 300 mg/kg, IP) given 5 hours prior to mercaptopropionic acid (MPA) (40 mg/kg, IP) antagonized the total number of seizure episodes in mice as well as the number of convulsing mice in a dose-dependent manner (69 on page 5-1131, v1.15, 70 on page 5-1139, v1.15). The ED_{50} of vigabatrin for decreasing the number of mice with convulsions was between 150 and 200 mg/kg. Doses of vigabatrin >750 mg/kg were less protective (15 on page 5-607, v1.13, 70 on page 5-1139, v1.15). The effect of vigabatrin in MPA-induced seizures was smaller in rats than in mice (71 on page 5-1160, v1.15). Mice treated chronically for 18 days (via the drinking water) with 95 mg/kg vigabatrin had fewer convulsions than controls without pretreatment in the MPA (40 mg/kg, IP) model (72 on page 5-1194, v1.15). Administration of IP doses of vigabatrin ranging from 100 to 400 mg/kg given on the 18th day enhanced the protective effect of vigabatrin against MPA-induced seizures in a dose-dependent manner. Paradoxically, lower doses of vigabatrin (25 and 50 mg/kg) increased the number of tonic seizures.

Vigabatrin was also effective in reducing the number of convulsing mice induced with glutamyl-γ-hydrazide plus pyridoxal phosphate (hydrazone) (205 μmol/kg, IP). The optimal dose of vigabatrin (750 mg/kg, IP) reduced the number of convulsing animals by 50%, 4 hours

postdose (15 on page 5-607, v1.13). The anti-seizure activity of vigabatrin against MPA and hydrazone-induced seizures occurred at a time when there was a greater increase in brain GABA in the synaptosomal pool than in the nonsynaptosomal pool.

(ii). Isoniazid

Isoniazid has been shown to inhibit GAD (53 on page 5-1010, v1.14, 194 on page 5-2703, v1.19). Convulsions were induced in mice with isoniazid (250 mg/kg, SC) 4 or 24 hours after administration of 1500 mg/kg vigabatrin, IP (66 on page 5-1111, v1.15). Seizures within the isoniazid group differed qualitatively from other chemically-induced seizures in that control animals developed tonic seizures but no clonic seizures. Only 10% of mice had tonic seizures and no animals died in the isoniazid group 4 hours after vigabatrin treatment. The protective effects of vigabatrin were markedly reduced at the 24-hour time point.

Additional studies have examined the relationship of increased GABA induced by vigabatrin and protection of isoniazid-induced seizures. Studies have shown (53 on page 5-1010, v1.14, 73 on page 5-1210, v1.15) that vigabatrin pretreatment (200 to 1500 mg/kg, IP) dose-dependently prolonged the time to seizure or death 1 hour before administration of isoniazid (250 mg/kg, IP). Up to 6 hours after vigabatrin treatment (1500 mg/kg, IP), latencies to clonus and tonus were increased and mortality decreased. However, the protective effects of vigabatrin were absent 24 hours after treatment even though cortical GABA levels were still elevated 8-fold. Elevations in cortical brain GABA are therefore insufficient to explain the anticonvulsive effect of vigabatrin in this model. An additional study (205 on page 5-2871, v1.19) showed that isoniazid (600 mg/kg, IP) caused a net decrease in GABA 45 minutes after treatment whether or not rats were pretreated with vigabatrin 12 hours before. However, microinjection of vigabatrin into the superior colliculus or the substantia nigra attenuated the decrease in GABA caused by isoniazid, suggesting that specific GABA pools may be more relevant than a cortical GABA increase.

d). Strychnine

Strychnine, a CNS stimulant, acts as a competitive antagonist of glycine thereby selectively blocking inhibition of nerve impulses (206 on page 5-2875, v1.19). In a study of neuronal activity in the cat cuneate nucleus, strychnine fully antagonized glycine-induced depression, but had a very weak antagonistic effect on GABA-mediated depression.

Convulsions were induced in mice with strychnine (2.0 to 2.5 mg/kg, IP) 4 or 24 hours after administration of 1500 mg/kg vigabatrin, IP (53 on page 5-1010, v1.14, 66 on page 5-1111, v1.15, 67 on page 5-1117, v1.15). At the 4-hour time point, times to clonus, tonus and death were prolonged and mortality was reduced by 70 to 85%. The protective effect of vigabatrin was greatly reduced at the 24-hour time point.

e). Pentylenetetrazol

Pentylenetetrazol-induced seizures are thought to result from GABA antagonism which leads to cellular disinhibition and altered properties of nervous cell membranes (58 on page 5-1057, v1.14, 77 on page 5-1253, v1.15). In this model, vigabatrin caused a dose-dependent protection against generalized seizures (ED_{50} = 940 mg/kg) and a dose-dependent seizure threshold elevation in mice

(49 on page 5-973, v1.14, 50 on page 5-985, v1.14, 52 on page 5-1000, v1.14, 53 on page 5-1010, v1.14). Protective effects of vigabatrin in the pentylenetetrazol model decreased with doses > 1500 mg/kg (49 on page 5-973, v1.14). Seizure suppressive effects were more modest in the rat (54 on page 5-1032, v1.14). Microinjection of vigabatrin into rat brain regions gave variable results (56 on page 5-1043, v1.14, 58 on page 5-1057, v1.14, 77 on page 5-1253, v1.15). In one study (58 on page 5-1057, v1.14), microinjection of vigabatrin into the anterior thalamus was effective whereas microinjection into the substantia nigra was not. In another study (56 on page 5-1043, v1.14), microinjection of a lower dose of vigabatrin into the ventral midbrain tegmentum adjacent to the substantia nigra completely blocked seizure manifestations.

In other miscellaneous studies with pentylenetetrazol, vigabatrin (> 500 mg/kg, IP) caused a marked decrease in seizure scores obtained with pentylenetetrazol kindling in the rat (74 on page 5-1216, v1.15, 75 on page 5-1218, v1.15). Vigabatrin (100 mg/kg) also enhanced chlordiazepoxide, diazepam and phenobarbital anti-pentylenetetrazol activity (76 on page 5-1220, v1.15).

f). Muscimol

Muscimol has been categorized as a specific GABA receptor agonist (79 on page 5-1275, v1.15, 194 on page 5-2703, v1.19). Myoclonic jerks can be reproducibly induced by muscimol administration at relatively high doses (3 mg/kg, IP) in a susceptible mouse strain and are thought to mimic postanoxic action myoclonus or Lance-Adams syndrome. Results of drug interaction studies suggest that a complex interaction between serotonin, norepinephrine and GABA is involved in this response to muscimol (79 on page 5-1275, v1.15).

Myoclonic jerks were elicited in mice with a dose of 3 mg/kg muscimol administered IP (79 on page 5-1275, v1.15). Pretreatment with 100 mg/kg vigabatrin, IP, caused inhibition of the muscimol response 4 and 24 hours after treatment. A high dose of vigabatrin (1500 mg/kg, IP) caused a more pronounced inhibition of myoclonic jerks, 1 to 72 hours after vigabatrin treatment.

vii. Miscellaneous

a). Decahydroquinoline-5-carboxylic acid epimers

Decahydroquinoline-5-carboxylic acid epimers elicit seizure activity in mice characterized as tonic-clonic convulsions (80 on page 5-1281, v1.15). Some of the analogs are thought to antagonize GABAergic transmission. The protective effect of 1500 mg/kg vigabatrin, administered IP, was tested in mice prior to intraventricular injection of the cis-1, cis-2, trans-3 and trans-4 epimers. Vigabatrin was generally ineffective in improving seizure manifestations except for causing a reduced duration of seizures elicited by the trans-4 epimer. This epimer also inhibited [^{3}H] binding to $GABA_A$ and $GABA_B$ receptors *in vitro,* albeit weakly.

b). Methionine Sulfoximine

Methionine sulfoximine elicits tonic-clonic seizures and, in some cases, status epilepticus in mice and rats by an unknown mechanism (83 on page 5-1317, v1.15). This compound is known to inhibit the enzyme glutamine synthetase but does not seem to affect GABA levels. Seizures were elicited in rats with 200 mg/kg methionine sulfoximine, IP, and rated on a scale of 0 (no motor manifestations) to 4 (generalized tonic seizure with opisthotonus). Vigabatrin (10 μg) was injected bilaterally into the substantia nigra, 24 hours prior to seizure induction. The mean control seizure score (2.79) was significantly ($P < 0.05$) lowered by 81% after vigabatrin treatment. Microinjection of vigabatrin into the striatum lowered scores by 50%.

c). Flurothyl

The activity of GABAmimetic agents in suppressing flurothyl-induced seizures in rats appears to vary with age (84 on page 5-1322, v1.15). In rat pups, the $GABA_B$ receptor agonist, baclofen, injected into the substantia nigra suppresses these seizures whereas muscimol, a $GABA_A$ agonist, is effective in adult rats. Microinjection of vigabatrin (20 μg) into the substantia nigra suppressed the latency of flurothyl-induced seizures in 15-day-old rat pups.

d). Isosolafloridine

In mice, 10-30 mg/kg, IP, of an extract from *Solanum pseudo-quina* bark identified as (25S)-isosolafloridine produced tremors, rigid tail, clonic convulsions and salivation (81 on page 5-1294, v1.15). Pretreatment with vigabatrin (1000 mg/kg, IP), 4 hours prior to (25S)-isosolafloridine resulted in a complete block of convulsions and tremors.

e). Benzodiazepine receptor inverse agonists

Methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) and methyl-β-carboline-3-carboxylate (β-CCM) are inverse agonists of the benzodiazepine receptors which can induce clonic seizures in adult DBA/2 mice at doses of 1.2-2 mg/kg, IP and 0.5-2 mg/kg, IP, respectively (82 on page 5-1306, v1.15). Vigabatrin (1500 mg/kg, IP) administered 3.25 hours before DMCM and β-CCM had protective effects against seizures induced by both agents although it is not known to bind to the benzodiazepine receptor (82 on page 5-1306, v1.15). The ED_{50} value for DMCM-induced convulsions increased 2.7-fold from a control value of 1.3 mg/kg. A more pronounced increase of 5.9-fold from a control value of 0.8 mg/kg occurred in the β-CCM model. Forebrain GABA levels were measured 2.25 hours after vigabatrin treatment and were increased by 280%. This increase was not affected by β-CCM administration.

viii. Synergism

The synergistic anticonvulsant effect of vigabatrin plus glycine, L-proline or atropine was tested in various chemically-induced seizure models, as described below.

a). Vigabatrin plus glycine

(i). MPA model

Seizures induced with MPA (40 mg/kg, IP) in mice were protected by vigabatrin alone with an ED_{50} of 150 to 200 mg/kg when vigabatrin was given 5 hours prior to seizure induction (70 on page 5-1139, v1.15, 71 on page 5-1160, v1.15). Doses of vigabatrin > 750 mg/kg were less protective. Glycine (2250 mg/kg, SC) protected 40% of the animals. The addition of a lower dose of glycine (1500 mg/kg, SC) to an ineffective dose of vigabatrin (50 mg/kg, IP) administered 1 hour and 5 hours, respectively, prior to seizure induction resulted in full protection against MPA-induced seizures. The synergistic effect of glycine was short-lasting; no protection was observed 2 hours after glycine administration.

In a similar study in rats (71 on page 5-1160, v1.15), the effect of vigabatrin was generally smaller. At an optimal dose of 200 mg/kg vigabatrin IP, only 40% of the rats were seizure-free when treated with MPA (40 mg/kg, IP). With glycine alone (750 mg/kg, SC), 50% of the rats had repeated convulsions and only 1 out of 10 rats were seizure-free. Administration of vigabatrin (300 or 500 mg/kg, IP) plus glycine (750 mg/kg, SC) protected more than 60% of the rats. In a similar study by Liu et al. (48 on page 5-965, v1.14), vigabatrin (200 mg/kg, IP) alone was more protective against MPA-induced (30 mg/kg, IP) seizures in that 50% of the rats did not show convulsive seizures. The addition of glycine (750 mg/kg, IP) increased protection to 78%, lowered mortality and markedly suppressed the appearance of isolated EEG spikes.

The synergistic effect of vigabatrin and glycine in protecting against MPA-induced seizures was examined in mice after long-term treatment (71 on page 5-1160, v1.15). Oral solutions of vigabatrin (41 mg/kg) and glycine (4.1 g/kg) were given daily for up to 16 days. A dose of glycine (750 mg/kg, SC) was administered 1 hour before induction of seizures with MPA (40 mg/kg, IP). Although vigabatrin alone had marginal effects, 40 to 60% of the mice were protected from seizures with the combined vigabatrin plus glycine chronic treatment, indicating that tolerance did not develop over the time period tested.

(ii). Bicuculline (1.8 mg/kg, SC) model

The protective effect of vigabatrin in mice (200 mg/kg, IP) was further improved with glycine (750 mg/kg, IP), but this test lacked reproducibility due to a steep dose response curve (70 on page 5-1139, v1.15).

(iii). Pentylenetetrazol (75 mg/kg, IP) model

Vigabatrin (750 mg/kg, IP) plus glycine (750 to 2250 mg/kg, SC) decreased the number of convulsing mice and prolonged the time to onset of first seizures. Vigabatrin or glycine alone had no effect (70 on page 5-1139, v1.15).

(iv). Picrotoxin (5 mg/kg, SC) model

Vigabatrin (up to 750 mg/kg, IP) alone, glycine (750 mg/kg, IP) alone or a combination of vigabatrin plus glycine were ineffective in this model when tested in mice (70 on page 5-1139, v1.15).

(v). Strychnine (2 mg/kg, IP) model

Vigabatrin (750 mg/kg, IP) alone had no effect. The time to onset of first seizures in mice was prolonged by glycine (2250 mg/kg, SC) and further prolonged by administering vigabatrin (750 mg/kg, IP) (70 on page 5-1139, v1.15).

The short duration of action of glycine prompted the investigation of glycine esters as potential prodrugs (207 on page 5-2887, v1.19). The most promising glycine ester was identified as glycine tert.butylester. MPA-induced convulsions (40 mg/kg, IP) were protected by 80 to 90% after coadministration of vigabatrin (100 mg/kg, IP) and glycine tert.butylester (200 mg/kg, SC) given 5 hours and 1 hour, respectively, prior to seizure induction. A 50% protection level was still present 4 hours after glycine tert.butylester administration (with vigabatrin pretreatment).

b). Vigabatrin plus proline

Synergism between L-proline (as well as derivatives of L-proline) and vigabatrin was tested in the MPA, pentylenetetrazol and strychnine seizure models (85 on page 5-1324, v1.15, 86 on page 5-1344, v1.15). L-proline and trans-4-hydroxy-L-proline were effective in the MPA and pentylenetetrazol models but did not have any advantages over glycine.

c). Vigabatrin plus atropine

The synergism between GABAergic and cholinergic systems was evaluated in the bicuculline seizure model by administering vigabatrin (1000 mg/kg, IP) and atropine (5 mg/kg, IP) to mice 2 hours and 10 minutes, respectively prior to bicuculline (3 mg/kg, SC) (67 on page 5-1117, v1.15). Vigabatrin and atropine alone had no protective effect; vigabatrin plus atropine doubled the time to tonus, clonus and death, suggesting that the anticonvulsant effect of vigabatrin may be attenuated by cholinergic activation.

ix. Hyperbaric Oxygen-Induced Seizures

Exposure to oxygen at high pressure causes convulsions which have been classified as generalized tonic-clonic (grand mal) seizures (87 on page 5-1364, v1.15). The role of GABA in hyperoxic seizures has not been well established but could be linked to decreases in brain GABA due to inhibition of GAD prior to seizure manifestations or to oxygen-induced changes in GABAergic systems.

In a study of oxygen-induced seizures in mice (2 on page 5-288, v1.13, 197 on page 5-2764, v1.19), oxygen pressure was maintained at 5.44 atmosphere until generalized convulsions occurred. Mice were treated with vigabatrin (800 mg/kg, IM) 4 or 24 hours before exposure to high pressure oxygen. Most of the mice (90%) were protected against convulsions due to hyperbaric oxygen at the 4-hour time point. However, no protection occurred at the

24-hour time point. Vigabatrin (50 to 1000 mg/kg, IP) was also highly protective in rats when they were exposed to 5 atmospheres oxygen, 4 hours after treatment (87 on page 5-1364, v1.15). An increase of the latent period preceding electrical discharge was dose-dependent. Complete seizure protection (for as long as 50 minutes) was obtained at a dose of 180 mg/kg vigabatrin. This protective effect lasted for 24 hours, but was no longer present after 72 hours. Background EEG changes included a dose-related increase in the low frequency bands and a decrease in the faster activity. Signs of behavioral toxicity (unsteady gait, antinociception) were seen at a dose of 500 mg/kg vigabatrin.

x. *Generalized Absence Seizures*

a). Seizure-Prone Rats

Clinical and EEG signs of spontaneous seizures in the [redacted] rat resemble those of petit-mal-epileptic absences in humans (88 on page 5-1370, v1.15, 89 on page 5-1375, v1.15). Vigabatrin (50 to 1000 mg/kg, IP) increased paroxysmal EEG discharges in a dose-dependent manner, 4 hours after injection, thus aggravating seizure manifestations. Seizure discharges were reduced or abolished by administration of vigabatrin (1000 mg/kg, IP) followed by diazepam (1 to 4 mg/kg, IP), suggesting that activation of neurotransmitter systems other than GABA is efficacious in this model. b(4)

In an effort to examine potential brain regions responsible for the generation of spike and wave discharges in generalized absence seizures, vigabatrin was microinjected into regions of rat thalamus (90 on page 5-1382, v1.15). Spike and wave discharges increased 24 to 48 hours after unilateral injection of vigabatrin (20 μg) into midline thalamus and increased 6 to 36 hours after bilateral injection (10 μg/side) into mediolateral thalamus. However, bilateral injection of vigabatrin (10 μg/side) into lateral thalamus caused a biphasic effect. Spike and wave discharges were suppressed from 6 to 12 hours and increased from 36 to 48 hours after injection. These results suggest that vigabatrin may be capable of both suppressing initiation of absence seizures while promoting the spread of convulsant activity.

b). Synergism

The [redacted] rats used in this model of absence epilepsy exhibited spontaneous spike and wave discharges (48 on page 5-965, v1.14). Glycine (750 mg/kg, IP) was administered to rats 4 hours after vigabatrin (200 mg/kg, IP). After an additional 20 minutes, the duration of spontaneous spike and wave discharges was recorded on an EEG. Although vigabatrin and glycine alone were without effect, the combined treatment aggravated seizure symptoms by increasing spike and wave discharges. b(4)

b. Secondary Activity

i. Entry of Vigabatrin Into the CNS

Vigabatrin entered the brain rapidly following IP administration (400 and 1500 mg/kg) to mice (Figure 5-15 on page 5-74, v1.12) (10 on page 5-563, v1.13, 91 on page 5-1390, v1.15). Peak concentrations of free vigabatrin occurred within 30 minutes to one hour, then declined exponentially. The half-life of the drug in the brain was estimated to be approximately 16-18 hours. Vigabatrin was still detectable three days after acute administration of 1500 mg/kg. Thus, the prolonged presence of free vigabatrin in the brain, coupled with an estimated half-life for GABA-T of 3.5 days, accounts for the long-lasting inhibition of GABA-T by vigabatrin.

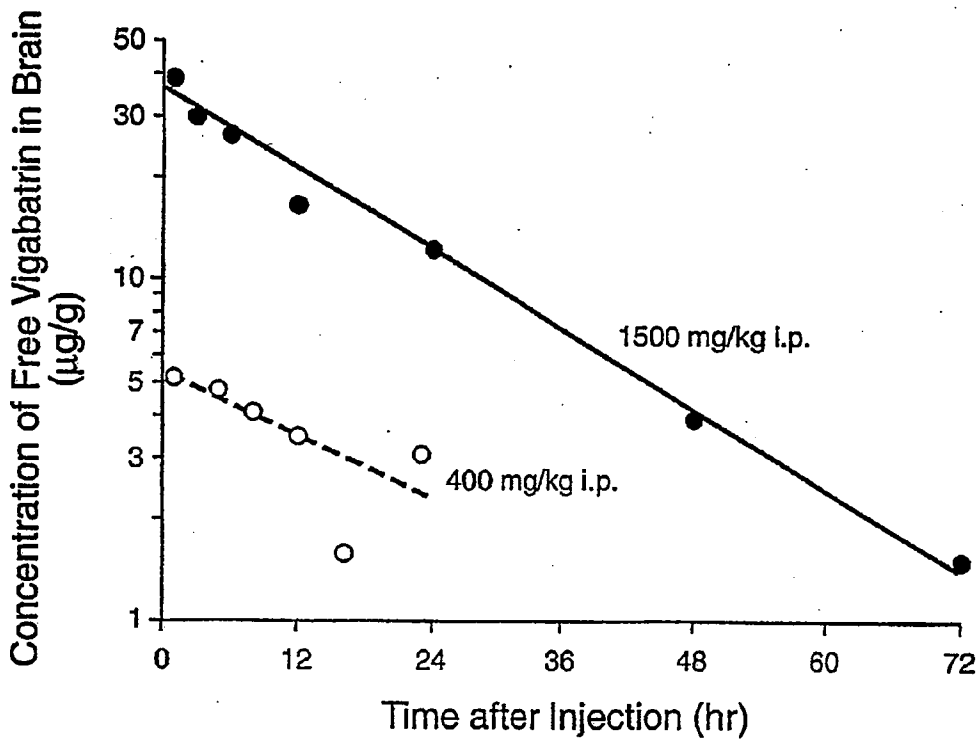

Figure 5-15. Concentration of free vigabatrin in mouse brain after IP administration of vigabatrin. Male mice were injected IP with a single dose of either 400 mg/kg (o) or 1500 mg/kg (•) vigabatrin. At various times after injection, free vigabatrin levels were determined. Each point represents the mean of 3-5 mice.

Attempts to measure central levels of vigabatrin have also been made in cats and rats. In cats (92 on page 5-1407, v1.15), CSF levels of vigabatrin have been demonstrated to increase linearly over the 2-3 hour period following a 1000 mg/kg IP dose of the drug. Levels of vigabatrin in the CSF then plateaued at about 1 μmol/ml, and remained constant out to six hours. In rats (13 on page 5-591, v1.13), vigabatrin was not detectable in whole brain homogenates 24 hours after

either a single SC injection or 11 daily SC doses of 100 mg/kg (assay detection limit of 0.05 μmol/g wet weight).

ii. Effects on Other Brain Enzymes, Amino Acids and Neurotransmitters In Vivo

Vigabatrin, at a single dose of 1500 mg/kg IP to mice, had no significant effect on the activity of succinic semialdehyde dehydrogenase, aspartate transaminase or ornithine transaminase (10 on page 5-563, v1.13, 93 on page 5-1420, v1.15). This dose of vigabatrin did produce a 20% reduction in alanine transaminase activity at 24 hours after dosing, but not at 6 hours after dosing. Alanine transaminase (SGPT, also designated ALT) activity was also slightly inhibited by high concentrations of vigabatrin *in vitro* (see Section 3.a.i.b. on page 5-42, v1.12). Studies of the modification of brain amino acids and neurotransmitter levels by vigabatrin treatment are summarized in Table 5-4 and Table 5-5, on pages 5-75, v1.12 through 5-78, v1.12.

Table 5-4 (Page 1 of 2). Summary of the Effects of Vigabatrin on Brain Amino Acids Other than GABA

Species (reference)	Duration of Treatment	Dose of Vigabatrin (mg/kg/day)	Mode of Administration	Effects and Comments
Rat (13 on page 5-591, v1.13)	11 days[a]	100	SC	Vigabatrin caused expected increases in whole brain levels of homocarnosine (the GABA-histidine dipeptide) and β-alanine (an alternate substrate for GABA-T) of 160% and 300%, respectively. There were also increases in hypotaurine levels (280%) and decreases in threonine (11%) and glutamine (13%) levels.
Rat (94 on page 5-1431, v1.15)	12 days[a]	100 or 250	IP	Brain levels of the excitatory amino acids, aspartate and glutamate, as well as metabolically related asparagine and glutamine were decreased in cortex, hippocampus and cerebellum by 8 to 24%.
Rat (95 on page 5-1433, v1.16)	4 hours[b]	1200	IP	Decreased levels of glutamate, aspartate, glutamine, and alanine were measured in different brain regions.
Rat (96 on page 5-1435, v1.16)	3 months[a]	300	PO	Treatment with vigabatrin caused a large decrease (90%) in whole brain putreanine. The functional role of putreanine in the brain has not been clearly established.
Mouse (97 on page 5-1451, v1.16)	24 hours[b]	250 to 1500	IP	Increasing doses of vigabatrin caused elevations in whole brain ornithine (14 to 100%), homocarnosine (60 to 153%) and carnosine (40 to 140%). Ornithine may be utilized as a precursor of L-glutamate and GABA. Although vigabatrin does not inhibit ornithine transaminase either *in vitro* or *in vivo*, feedback inhibition may occur with high GABA concentrations.

a Daily dosing

b Time elapsed after an acute dose

Table 5-4 (Page 2 of 2). Summary of the Effects of Vigabatrin on Brain Amino Acids Other than GABA

Species (reference)	Duration of Treatment	Dose of Vigabatrin (mg/kg/day)	Mode of Administration	Effects and Comments
Mouse (98 on page 5-1459, v1.16)	16 hours[b] or 1-3 days	750 mg/kg.	IP????	Single dose VGB produced increase in ornithine concentration in various brain regions. Three doses produced only a small additional increase in ornithine levels.

a Daily dosing
b Time elapsed after an acute dose

Table 5-5 (Page 1 of 2). Summary of the Effects of Vigabatrin on Brain Neurotransmitters

Species (reference)	Duration of Treatment	Dose of Vigabatrin (mg/kg/day)	Mode of Administration	Effects and Comments
Rat (99 on page 5-1467, v1.16)	21 days[a]	250	IP	Vigabatrin had no effect on the concentration of norepinephrine, dopamine, or serotonin and some of their major metabolites in the striatum, olfactory tubercles, or frontal cortex. Treatment did not affect apomorphine-induced locomotor activity, [^{3}H]-spiperone binding or dopamine-stimulated adenylate cyclase in the corpus striatum.
Rat (2 on page 5-288, v1.13)	4 hours[b]	1500	IP	Vigabatrin had no effect on endogenous dopamine levels in a number of different brain regions. Norepinephrine levels were reduced by 27% in the hypothalmus.
Rat (100 on page 5-1476, v1.16)	10 days[a]	50 to 150	IP	Small increases in 5-HIAA and trytophan concentrations and a small decrease in dopamine concentrations were found in the amygdaloid cortex after olfactory bulbectomy.
Baboon (101 on page 5-1484, v1.16)	3.5 hours[b]	300	IV	Endogenous dopamine levels were decreased in the striatum as indicated by a 25% increase in the distribution volume of ^{11}C-raclopride using positron emission tomography.
Rat (102 on page 5-1489, v1.16)	3 minutes[b]	100 μg	Intradorsal Raphe Infusion	Treatment resulted in a decreased release of extracellular 5-HIAA from striatum.
Rat (103 on page 5-1500, v1.16)	3 hours[b]	1000	IP	Treatment increased the level of serotonin by 40% and decreased the level of norepinephrine by 11% in the frontal cortex.

a Daily dosing
b Time elapsed after an acute dose
ICV intracerebroventricular
5-HIAA 5-hydroxyindoleacetic acid

Table 5-5 (Page 2 of 2). Summary of the Effects of Vigabatrin on Brain Neurotransmitters

Species (reference)	Duration of Treatment	Dose of Vigabatrin (mg/kg/day)	Mode of Administration	Effects and Comments
Rat (104 on page 5-1503, v1.16)	4 hours[b]	1500	IP	An increase in serotonin content was measured in one of the raphe nuclei (30%), in the subcommisural organ (25%), and in the habenula complex (60%). 5-HIAA levels were increased slightly (13 to 25%) in raphe nuclei and the habenula complex.
Rat (104 on page 5-1503, v1.16)	4 hours[b]	125 μg	ICV	A decrease in the serotonin content (20 to 30%) was observed in the raphe nuclei.

a Daily dosing
b Time elapsed after an acute dose
ICV intracerebroventricular
5-HIAA 5-hydroxyindoleacetic acid

Definite conclusions from these studies are not easily reached due to considerable variations in methodology. Nevertheless, certain trends emerge suggesting that vigabatrin causes a large increase in brain homocarnosine (a GABA-histidine dipeptide metabolite of GABA), lowers levels of excitatory amino acids, and may modulate monoamine neurotransmission, particularly serotoninergic and dopaminergic pathways.

4. Effects Related to Possible Adverse Reactions

a. CNS Effects

i. Body Temperature

Vigabatrin (1500 mg/kg, IP) significantly reduced rectal temperature in mice kept at 22°C, 6 hours postdose. The same dose of vigabatrin had no effect on rectal temperature in mice kept at 34°C (105 on page 5-1529, v1.16). Hypothermia can also be induced by vigabatrin in rats after 500 mg/kg PO doses or 250 to 1000 mg/kg IP doses (106 on page 5-1534, v1.16, 107 on page 5-1562, v1.16, 108 on page 5-1568, v1.16). Oral doses of 170 and 300 mg/kg vigabatrin had no effect on temperature in rats 3 to 4 hours postdose (106 on page 5-1534, v1.16).

ii. Antipyretic Activity

Vigabatrin antagonized hyperthermia due to Brewers' yeast in rats 3 to 4 hours after PO doses of 100 to 300 mg/kg (106 on page 5-1534, v1.16).

iii. Analgesic Activity and Muscle Relaxation

a). Analgesia

In mice, vigabatrin (500 to 2000 mg/kg, SC) pretreatment prevented acetic-acid-induced writhing in a dose-dependent manner after 1 hour (106 on page 5-1534, v1.16). Oral doses of 320 mg/kg and SC doses of 500 mg/kg vigabatrin were ineffective 3 to 4 hours postdose (106 on page 5-1534, v1.16).

Latencies in the hot plate-induced pain model were increased in mice by vigabatrin in a dose-dependent manner (ED_{50} = 770 mg/kg, IP) with a maximal effect 4 to 6 hours postdose (110 on page 5-1583, v1.16, 109 on page 5-1575, v1.16). This latter effect only occurred with the S-enantiomer (active as a GABA-T inhibitor) and not with the R-enantiomer (inactive).

In the rat, analgesia induced by vigabatrin was dose-dependent (ED_{50} = 1100, IP) as shown by the tail-stimulation test (110 on page 5-1583, v1.16).

The tail-immersion test in rodents is often used to distinguish between antinociceptive and sedative effects (109 on page 5-1575, v1.16). Vigabatrin (1500 mg/kg, IP) was effective in prolonging reaction time in the mouse tail-immersion test, indicating that its analgesic properties occur independently of sedation. The analgesia produced by vigabatrin (900 mg/kg, IP) in the mouse hot-plate test was more protracted than that observed with muscimol, baclofen or morphine (111 on page 5-1592, v1.16). As described below (see drug interactions), vigabatrin enhanced the analgesic activity of morphine in mice (110 on page 5-1583, v1.16).

The antinociceptive action of vigabatrin was reversed by atropine administration in the mouse tail-immersion test (109 on page 5-1575, v1.16) but was not reversed by naloxone in the mouse hot-plate test (112 on page 5-1600, v1.16). These results suggest that vigabatrin-mediated analgesia may activate components of the cholinergic system rather than the opiate system.

b). Muscle Relaxation

Vigabatrin (200 to 1600 mg/kg, IP) had muscle relaxant properties in mice as shown by a dose-dependent blockade of morphine-induced Straub tail (113 on page 5-1617, v1.16). Maximal effect was observed at 4 hours postdose (ED_{50} = 796 mg/kg), with effects diminished at 16 hours. Treatment with 200 mg/kg vigabatrin b.i.d. for 3 days had no muscle relaxant effects in the Straub tail test. The action of vigabatrin on muscle tone was studied in the genetically spastic rat (114 on page 5-1638, v1.16). Microinjection of 0.4 μmoles vigabatrin into the substantia nigra caused a depressant effect on the electromyogram, indicating a decrease in muscle tone. This effect was maximal 24 to 26 hours postdose and lasted for at least 50 hours.

iv. Dyskinetic Activity

Following unilateral intrastriatal injection to rats, vigabatrin (1 to 10 μmoles; 129-1290 μg) produced a characteristic dose-related dyskinetic syndrome (115 on page 5-1647, v1.16, 116 on page 5-1674, v1.16, 117 on page 5-1683, v1.16, 118 on page 5-1694, v1.16, 119 on page 5-1704, v1.16). This dyskinesia was blocked by IP pretreatment with vigabatrin (1000 mg/kg) and by intrastriatal injection of either GABA, muscimol, or a low (0.5 μmole) dose of vigabatrin prior to a subsequent high dose of vigabatrin. These observations suggest that vigabatrin may have two opposite actions on the GABAergic system. First, vigabatrin may, at high doses, block GABAergic function by an action on GABA receptors. Second, the drug may increase brain GABA concentrations by inhibition of GABA-T activity. Since excitation has not been reported with peripheral administration of vigabatrin, dyskinesia may only occur when the dyskinesia threshold is surpassed by high doses of vigabatrin in critical brain regions.

Topical cortical application of vigabatrin to rats produced a similar incidence and intensity of dyskinetic movements to those observed after intrastriatal injections (116 on page 5-1674, v1.16, 118 on page 5-1694, v1.16). The abnormal movements were produced without evidence of the drug reaching the underlying striatum (117 on page 5-1683, v1.16, 118 on page 5-1694, v1.16). Subsequent experiments in rats showed that both picrotoxin and homocarnosine applied topically to the cortex induced an incidence of dyskinesia similar to that seen with vigabatrin (117 on page 5-1683, v1.16, 118 on page 5-1694, v1.16, 120 on page 5-1713, v1.16, 121 on page 5-1723, v1.16). These results indicate that the cortex, over a relatively large area, is a locus for dyskinetic induction, and that this dyskinetic induction is not limited to GABA-T inhibitors.

v. Stereotypy

Amphetamine-induced stereotypy in rats was used as an animal model of tardive dyskinesia. Vigabatrin (1000 mg/kg, IP) facilitated the development of stereotypy 16-18 hours postdose as indicated by a greater frequency and intensity of licking-gnawing behavior although locomotor activity was decreased (107 on page 5-1562, v1.16). In contrast with these results, pretreatment

with vigabatrin (1200 mg/kg, IP) caused an increase in the duration of the anesthetic phase and less rotational effects in a study of ketamine-induced rotation and stereotypic head swinging in rats (208 on page 5-2893, v1.19). Vigabatrin (200 mg/kg, IM) also decreased dyskinesia scores in haloperidol-treated monkeys 2 hours postdose with effects lasting for 4 to 5 days (122 on page 5-1729, v1.16).

vi. Kainic Acid Neurotoxicity

Peripheral administration of kainic acid to rats caused a characteristic syndrome consisting of stereotypies, convulsions, and behavioral effects (123 on page 5-1734, v1.16, 124 on page 5-1739, v1.16, 125 on page 5-1748, v1.16). Pretreatment with vigabatrin (900 mg/kg, IP) protected against kainic acid-induced aphagia, aggressiveness, and convulsions. Vigabatrin pretreatment also reversed the brain GABA deficit caused by kainic acid in various brain regions (124 on page 5-1739, v1.16, 125 on page 5-1748, v1.16). However, behavioral symptoms induced by intrastriatal kainic acid administration were unaffected by vigabatrin (1000 mg/kg, IP) pretreatment (126 on page 5-1763, v1.16).

vii. Morphine Dependence and Withdrawal

Vigabatrin (200 to 400 mg/kg) potentiated the analgesic effects of morphine (1 to 5 mg/kg, SC) in the mouse hot-plate test (127 on page 5-1777, v1.16) and in the mouse tail-immersion test (128 on page 5-1784, v1.16). In addition, vigabatrin (60 to 240 mg/kg, IP) attenuated morphine consumption in morphine-dependent rats (129 on page 5-1788, v1.16). The effects of vigabatrin on morphine withdrawal symptoms were inconsistent in that vigabatrin (400 mg/kg) reduced the intensity of withdrawal behavior induced by naloxone in the mouse hot-plate test (127 on page 5-1777, v1.16), but vigabatrin (up to 1000 mg/kg) did not affect symptoms in rats withdrawn from continuous morphine infusion (130 on page 5-1793, v1.16). In other studies, dipropylacetate-induced body shakes in rats were unaffected by 500 mg/kg, IP, vigabatrin 2.5 to 5 hours postdose, but were stimulated by 1000 mg/kg vigabatrin, 36 to 60 hours postdose (131 on page 5-1799, v1.16). Ethylenediamine, a GABA receptor agonist, aggravated naloxone-induced withdrawal symptoms in morphine-dependent mice. Ethylenediamine effects were suppressed by vigabatrin (400 mg/kg) (132 on page 5-1804, v1.17).

viii. Motor Activity

a). Spontaneous Motor Activity

In mice, vigabatrin did not affect spontaneous motor activity following single PO doses of 160 to 640 mg/kg (106 on page 5-1534, v1.16). Similarly, following multiple PO doses of 25 to 100 mg/kg/day for five days, spontaneous motor activity was not altered. However, higher IP doses of 500 to 2500 mg/kg vigabatrin in mice produced a dose-related decrease in spontaneous motor activity (2 on page 5-288, v1.13). This effect was apparent within 1 hour and peaked in intensity at 3 to 4 hours. In rats, vigabatrin (250 to 1500 mg/kg, IP) caused a decrease in exploration and spontaneous motor activity, but this effect was not dose-related (2 on page 5-288, v1.13, 134 on page 5-1816, v1.17).

b). Phencyclidine (PCP)-induced Hyperactivity

PCP-induced hyperactivity was partially antagonized in mice treated with 200 to 700 mg/kg vigabatrin IP, 5 hours prior to PCP (135 on page 5-1823, v1.17). The same doses of vigabatrin administered alone had no significant effect on motor activity.

ix. Food Consumption

Vigabatrin (250 mg/kg, IP) inhibited food and water consumption by rats, while at 125 to 500 mg/kg, IP, vigabatrin markedly depressed the rate at which rats performed a food reward task (136 on page 5-1839, v1.17). Further studies showed the reduction in food consumption in rats was dose-related (125 to 1000 mg/kg, IP). Vigabatrin-induced anorexia was unaffected by the mode of administration in that IP or PO doses of 500 mg/kg gave identical results (137 on page 5-1850, v1.17). No tolerance to these effects was observed with chronic IP administration of vigabatrin (250 mg/kg/day for 13 days). The anorectic effects of vigabatrin were enhanced with amphetamine, whereas amphetamine antagonized effects on water consumption and locomotor activity (136 on page 5-1839, v1.17, 137 on page 5-1850, v1.17). In a microinjection study in sheep, vigabatrin (up to 10 μmoles) had no effect on feed intake after cerebroventricular administration (138 on page 5-1860, v1.17). The threshold of vigabatrin for inducing changes in feeding behavior in rats was examined by intracisternal injection of increasing doses of vigabatrin (139 on page 5-1865, v1.17). Feeding suppression became significant at the 50 μg-dose and was dose-dependent. With higher doses of vigabatrin, water intake was suppressed and other behavioral changes occurred including decreased activity, catalepsy, nociception, and hypothermia. Changes in brain GABA levels were correlated with all of the responses except for analgesia.

x. Visual Function

Vigabatrin (300 mg/kg, PO) administration had no effect on pupillary size in rats up to 4 hours postdose (140 on page 5-1879, v1.17).

xi. Polyamine Metabolism

In mouse and rat CNS, polyamine metabolic pathways were modified by acute or chronic treatment with vigabatrin. Increases in brain putrescine levels and in ornithine decarboxylase activity were observed 16 hours posttreatment with vigabatrin (1500 mg/kg, IP) (141 on page 5-1889, v1.17, 142 on page 5-1902, v1.17). The activity of S-adenosylmethionine decarboxylase was decreased 6 to 8 hours postdose. The elevation in brain putrescine by vigabatrin was confirmed in another study with acute dosing (750 mg/kg, IP) and chronic dosing for 9 days (750 mg/kg/day, IP) (143 on page 5-1922, v1.17).

xii. Other CNS Effects

a). Anxiolytic Activity

In an anxiogenic test where rats were trained to discriminate pentylenetetrazol from saline, vigabatrin (1000 mg/kg, IP) did not block the discriminative stimulus (144 on page 5-1939, v1.17). Vigabatrin had no anti-conflict activity in a water-lick test (145 on page 5-1948, v1.17) and in a conflict test using electroshock (146 on page 5-1958, v1.17) at IP doses of 900 mg/kg and 62.5 to 1000 mg/kg, respectively.

b). Aggression

The effects of vigabatrin were studied in two models of aggression in rats. In a study of shock-induced defensive fighting, vigabatrin (100 and 200 mg/kg, IP) caused a dose-related reduction in fighting (147 on page 5-1968, v1.17). Vigabatrin (200 - 800 mg/kg, IP) also reduced killing in experienced killer rats (133 on page 5-1808, v1.17, 148 on page 5-1975, v1.17).

c). Antipsychotic Activity

Vigabatrin (125 and 250 mg/kg, IP) had no effect on conditioned avoidance in the rat (136 on page 5-1839, v1.17).

d). Antidepressant Activity

The olfactory bulbectomized rat is used in an animal model of depression (149 on page 5-1979, v1.17). Vigabatrin (75 mg/kg/day, IP) administered chronically for 14 days decreased hypermotility induced in a stressful environment in the bulbectomized rat, indicating an antidepressant effect. The antidepressant activity of vigabatrin was also shown in a forced swimming test in mice (150 on page 5-1989, v1.17). Vigabatrin (200 to 800 mg/kg, IP) caused a dose-dependent reduction in immobility. Chronic treatment with vigabatrin doses < 300 mg/kg/day decreased immobility on day 10 but not on day 20.

e). EEG

Vigabatrin (1200 mg/kg, IP) caused EEG hypersynchronization in rats 4 hours after pretreatment (151 on page 5-1995, v1.17). The EEG effects were accompanied by behavioral changes such as passivity and loss in activity, indicating that vigabatrin had sedative effects at this dose.

f). Memory and Learning

Subchronic pretreatment with vigabatrin (50 to 200 mg/kg/day, IP) for 2 to 5 days did not affect the performance of rats in a passive avoidance test and did not markedly affect performance in water maze tasks (152 on page 5-2007, v1.17). However, the performance of spatial navigation tasks was aggravated dose-dependently in nucleus basalis-lesioned rats treated with acute doses of 50 to 200 mg/kg, IP, vigabatrin (153 on page 5-2014, v1.17).

b. Effect on the Cardiovascular System

i. Effect on Heart Rate and Blood Pressure

a). Anesthetized Dogs

No significant effect on heart rate or blood pressure was obtained with IV doses of 3, 10, or 30 mg/kg vigabatrin (154 on page 5-2019, v1.17). There was no effect of vigabatrin on blood pressure responses to bilateral carotid artery occlusion at the 3 mg/kg and 30 mg/kg doses. Blood pressure responses to IV administered norepinephrine, acetylcholine, isoproterenol, or tyramine were unaffected by increasing doses of vigabatrin (3, 10, and 30 or 10, 20, 40, and 80 mg/kg, IV) (154 on page 5-2019, v1.17, 155 on page 5-2184, v1.17). In another study (156 on page 5-2206, v1.17), vigabatrin administered IV at a dose of 60 mg/kg to one dog caused a prolonged blood pressure fall of 20 mm Hg.

b). Anesthetized Rats

A slight increase in blood pressure was observed following IV administration of 50 mg/kg vigabatrin (155 on page 5-2184, v1.17). Additional IV doses of 100 and 200 mg/kg produced no further increase in blood pressure. Pressor responses to IV noradrenaline and tyramine were reduced, while the hypotensive response to acetylcholine was increased. These effects, however, were not dose-related. In another study using higher doses, vigabatrin (120 to 1000 mg/kg, IV) produced a dose-related sustained hypotension and bradycardia, and a reduction of central venous pressure (156 on page 5-2206, v1.17).

ii. Hemodynamic Effects in Anesthetized Dogs

No effect of increasing doses of vigabatrin (3, 10, and 30 or 10, 20, 40, and 80 mg/kg, IV) was observed on blood pressure, cardiac output, heart rate, ventricular pressure, or dP/dt (154 on page 5-2019, v1.17, 155 on page 5-2184, v1.17).

iii. Electrocardiographic Effects in Beagle Dogs

Oral treatment with vigabatrin (150 mg/kg twice daily for 5 days) showed no evidence of drug-related electrocardiographic changes (154 on page 5-2019, v1.17).

iv. Miscellaneous Cardiovascular Effects

Vigabatrin (up to 20 μg/ml), unlike other anticonvulsants such as phenytoin, did not have antifibrillatory activity in the perfused isolated rabbit heart (157 on page 5-2235, v1.17).

Localized injection of vigabatrin (1 to 100 nmoles) into the nucleus tractus solitarius of the rat elicited a dose-dependent increase in blood pressure, probably via an action on $GABA_B$ receptors (158 on page 5-2241, v1.17).

c. Hepatic Encephalopathy

Changes in the pattern of visual evoked responses (VERs) occurred in rabbits 18 hours after vigabatrin treatment (750 mg/kg, IV) (159 on page 5-2248, v1.18, 160 on page 5-2258, v1.18). The changes in VERs brought about by vigabatrin treatment resembled VER patterns observed in hepatic encephalopathy due to fulminant hepatic failure. These results suggest that hepatic encephalopathy may be linked to increased GABA neurotransmission although the contribution of other neurotransmitters cannot be excluded.

d. Hormonal Effects

Vigabatrin (1000 mg/kg, IP) reduced plasma corticotrophin-releasing factor (CRF) and stress-induced plasma corticotropin (ACTH) secretion, but increased plasma corticosterone levels 18 hours postdose in rats (161 on page 5-2267, v1.18, 162 on page 5-2275, v1.18). In addition to its modulatory role on components of the hypothalamo-pituitary adrenal axis, vigabatrin treatment altered levels of reproductive hormones. The latter effect occurred predominantly under conditions of amplified secretion. A dose of 500 mg/kg vigabatrin IP in rats blocked the luteinizing hormone (LH) proestrus surge and prevented ovulation in pregnant mare serum gonadotropin (PMSG)-treated animals (163 on page 5-2290, v1.18, 164 on page 5-2298, v1.18, 165 on page 5-2308, v1.18, 166 on page 5-2317, v1.18). Microinjection of vigabatrin (10 to 20 μg) into brain areas related to gonadotropic regulation inhibited the post-castration LH release in male rats 24 to 48 hours postdose; in female rats, plasma LH levels were decreased 7 to 9 hours later and ovulation was prevented. Vigabatrin (800 mg/kg, IP) administered to rats every 2nd day for 4 days caused a decrease in anterior pituitary prolactin mRNA levels (167 on page 5-2325, v1.18, 168 on page 5-2338, v1.18). In other miscellaneous hormonal effects in rodents, vigabatrin decreased plasma growth hormone levels (169 on page 5-2346, v1.18), inhibited TSH-stimulated thyroid hormone secretion (170 on page 5-2351, v1.18) and lowered a polyethylene glycol (PEG)-induced increase in plasma vasopressin (171 on page 5-2356, v1.18) at IP doses of 500 mg/kg, 50 mg/kg, and 1500 mg/kg, respectively. The decrease in vasopressin by vigabatrin suggests that it may have a hypotensive effect in the rat. *In vitro* studies in rat cortical slices showed that the IC_{50} for inhibiting somatostatin release was 240 μM (171 on page 5-2356, v1.18).

5. Interactions With Other Drugs

a. Benzodiazepines

i. Benzodiazepine-Induced Anxiolytic Activity

Vigabatrin (62.5 mg/kg, IP) did not potentiate the anti-conflict effect of diazepam in rats (146 on page 5-1958, v1.17).

ii. Benzodiazepine-Induced Muscle Relaxation

The potency of diazepam in blocking morphine-induced Straub tail in mice was decreased by vigabatrin (100 mg/kg, IP) (113 on page 5-1617, v1.16).

b. Amphetamines

The effects of 250 mg/kg vigabatrin and 4 mg/kg dexamphetamine were additive on food intake but antagonistic on water consumption and locomotor activity in rats (136 on page 5-1839, v1.17). Vigabatrin (250 mg/kg, IP) and dexamphetamine (2.5 mg/kg, IP) given for 8 days produced profound anorexia which showed less rapid tolerance than treatment with dexamphetamine alone. This combination induced a marked fall in rat body weight. Activity counts of rats treated with the combination were similar to controls (137 on page 5-1850, v1.17).

c. Barbiturates

i. Thiopental-Induced Sleep in Mice

Vigabatrin (320 mg/kg, PO) produced a significant decrease in the time of onset and a significant increase in the duration of sleep induced by thiopental. Potentiation of thiopental activity by vigabatrin was not significant with oral doses of 40 to 160 mg/kg (106 on page 5-1534, v1.16).

d. Opioids

i. Morphine-Induced Analgesia

As described above, (see section 4.a.vii., page 5-80, v1.12) vigabatrin (200 to 400 mg/kg, IP) potentiated the effects of morphine in the mouse hot-plate test and in the mouse tail-immersion test (127 on page 5-1777, v1.16, 128 on page 5-1784, v1.16).

6. Miscellaneous Pharmacology

a. Receptor binding activity

Vigabatrin was tested in a receptor selectivity screen at concentrations of 1, 10, and 100 μM in 38 different receptor-radioligand assays. No significant inhibitory activity was detected at any concentration, for any receptor (209 on page 5-2899, v1.19). Table 5-6 shows the results obtained in the screen.

Table 5-6 (Page 1 of 2). Receptor Screen Results for Vigabatrin

Receptor/Selectivity	Radioligand	Percent Inhibition (Average; N=2) (Molar)		
		1E-6	1E-5	1E-4
Neurotransmitters				
Adenosine 1	[^{3}H]-CPX	19.2	11.3	31.8
Adenosine 2	[^{3}H]-CGS 21680	-5.0	-0.5	41.5
Alpha 1	[^{3}H]-Prazosin	6.9	8.4	5.6
Alpha 2	[^{3}H]-RX 781094	5.8	6.8	1.8
Beta	[^{3}H]-DHA	8.3	4.9	15.2
Dopamine 1	[^{3}H]-SCH 23390	9.6	1.7	-4.3
Dopamine 2	[^{3}H]-Sulpiride	-4.3	-2.6	5.6
GABA^A	[^{3}H]-GABA	3.3	5.4	30.3
GABA^B	[^{3}H]-GABA + Isoguvacine*	-3.9	6.4	34.0
Histamine 1	[^{3}H]-Pyrilamine	2.3	2.4	9.2
Serotonin 1	[^{3}H]-5HT	2.9	23.5	27.7
Serotonin 2	[^{3}H]-Ketanserin	-4.1	-7.9	4.0
Muscarinic 1	[^{3}H]-Pirenzepin	0.3	-0.4	18.0
Muscarinic 2	[^{3}H]-AFDX384	-0.3	-5.9	-8.0
Nicotinic	[^{3}H]-NMCl	4.4	5.3	5.7
NMDA	[^{3}H]-CGS 19755	-0.3	-5.8	-2.8
Kainate	[^{3}H]-Kainic Acid	-4.6	-8.6	-31.8
Quisqualate	[^{3}H]-AMPA	-5.8	-6.2	-3.1
Glycine (stry sens.)	[^{3}H]-Strychnine	17.6	-4.5	-2.8
Regulatory Sites				
Benzodiazepine (centrl)	[^{3}H]-Flunitrazepam	3.6	1.4	2.2
Glycine (stry insens.)	[^{3}H]-Glycine	13.4	5.3	25.2
PCP	[^{3}H]-TCP	0.0	-22.4	-11.0
sigma	[^{3}H]-DTG	5.0	7.3	12.6

Table 5-6 (Page 2 of 2). Receptor Screen Results for Vigabatrin

Receptor/Selectivity	Radioligand	Percent Inhibition (Average; N=2) (Molar)		
		1E-6	1E-5	1E-4
Opioids				
mu	[^{3}H]-DAGO	2.7	2.3	-2.3
delta	[^{3}H]-DPDPEN	12.2	12.8	14.7
kappa	[^{3}H]-U69593	1.3	-8.7	-10.5
Ion Channels				
Calcium (Type N)	[^{125}I]-Omegaconotoxin	7.6	-2.5	5.1
Calcium (Type T & L)	[^{3}H]-Nitrendipine	-1.2	-6.7	-9.1
Chloride	[^{3}H]-TBOB	2.7	15.4	34.2
Potassium (low conduct)	[^{125}I]-Apamin	-1.1	5.6	-0.9
Prostaglandins				
Leukotriene B^4	[^{3}H]-LTB^4	18.2	4.4	-5.6
Leukotriene D^4	[^{3}H]-LTD^4	0.3	-2.8	-1.5
Thromboxane A^2	[^{3}H-SQ 29548	-3.6	-12.1	-7.2
Second Messengers				
Forskolin	[^{3}H]-Forskolin	-1.9	0.2	-7.4
Phorbol Ester	[^{3}H]-PDBU	8.6	-3.3	-8.3
Uptake Sites				
Dopamine Reuptake	[^{3}H]-VIN 35428	6.9	6.1	-0.7
Norepinephrine Uptake	[^{3}H]-DMI	2.8	-3.9	15.6
Serotonin Uptake	[^{3}H]-Citalopram	-0.9	-7.5	-1.7

b. Agonist-induced contraction in guinea pig intestinal smooth muscle

Strips of guinea pig ileum were suspended in organ baths to investigate the effects of vigabatrin on agonist-induced contractions. Vigabatrin (1-100 μM) had no effect on resting tension of the ileum. Vigabatrin also did not alter histamine-, acetylcholine-, 5-HT and Ba^{2+}-induced contractions at concentrations up to 100 μM (210 on page 5-2947, v1.19).

2. Table of Pharmacology Studies

Table 5-1 (Page 1 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	GABA-T inhibition	In vitro	0.05 to 1 mM	25 min incubation	GABA-T is rapidly and irreversibly inhibited. The pyridoxal form of the enzyme is inhibited.	1 on page 5-281, v1.13
Rat	Specificity of GABA-T inhibition	In vitro	0.5 to 10 mM	50 min incubation	GABA-T inhibition is selective.	
Mouse	GABA-T inhibition	In vitro	IC_{50} =350 μM	60 min incubation	Pronounced inhibitory effect on GABA-T.	4 on page 5-489, v1.13
Rat	Brain GABA-receptor binding	In vitro	IC_{50} = 4mM	10 min incubation	Weak inhibition of Sodium-independent GABA-receptor binding.	
Rat	Brain GABA uptake	In vitro	IC_{50} = 110 μM	10 min incubation	Moderate inhibition of Sodium-dependent GABA uptake.	
Rat	Brain GABA release	In vitro	0.25-5 mM	10 minutes	Increased release of pre-loaded [^{14}-C]-GABA; similar effect on release of endogenous GABA.	5 on page 5-497, v1.13
Rat	Brain GABA release	IP pretreatment	1000 mg/kg	14 hours	Control release from synaptosomes increased; tissue content of GABA also increased.	
Rat	Brain GABA release	IP	1000-1500 mg/kg	190 minutes	Increase in GABA release from sensorimotor cortex after contralateral brachial plexus stimulation.	6 on page 5-526, v1.13
Rat	Brain GABA release	IP treatment	1000-1500 mg/kg	1 - 3 hours	Increased release of pre-loaded [^{14}-C]-GABA. Direct comparison to other anticonvulsant with superior results.	7 on page 5-539, v1.13
Rat	Brain GABA release	Cortical superfusion	100 μM	continuous superfusion	Increased release of pre-loaded [^{14}-C]-GABA to a lower extent than did IP injection.	
Rat	Synaptosome GABA release	In vitro	100 μM - 1 mM	continuous superfusion	Increased release of pre-loaded [^{14}-C]-GABA 6-fold.	
Mouse	Cortex neuronal GABA release	In vitro	10 - 300 μM	24 hour incubation	Increased stimulated release of [^{14}-C]-GABA by 100% at 25 μM concentrations. Increased both cellular and releasable GABA pools.	8 on page 5-547, v1.13

Table 5-1 (Page 2 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	GABA concentration in hippocampal slices or pancreatic islets	In vitro	1 μM - 1 mM	120 minute incubation	100 μM increased GABA concentration in both hippocampal and pancreatic homogenates. 1 mM decreased same, suggesting high concentrations may inhibit GAD as well as GABA-T.	9 on page 5-557, v1.13
Mouse	GABA brain concentration	IP pretreatment	1500 mg/kg	1 - 72 hours	GABA-T inhibition half-life is 3.5 days (resynthesis) with peak GABA concentrations reached in 3 - 4 hours.	10 on page 5-563, v1.13
Mouse	GABA brain concentration	IP pretreatment	100 - 3200 mg/kg	8 hours	Dose response occurs both in GABA-T activity and GABA concentrations.	
Mouse	GABA brain concentration	IP pretreatment	100 mg/kg	q 12 h for 6 doses	Repeated treatment has cumulative effects.	
Mouse	GABA brain concentration	IV, IP, IM, SC, oral	1500 mg/kg	4 hour	GABA-T inhibition is dependent on route of administration. GAD inhibition is slight and does not differ with route.	
Mouse	GABA brain concentration	IP	10 - 1250 mg/kg/day	10 days	Dose response over 10 - 750 mg/kg/day. LD_{50} approx. 1000-1250 mg/kg/day for 10 days. All doses inhibit GABA-T and GAD.	11 on page 5-571, v1.13
Mouse	GABA metabolism in brain	IP	100 mg/kg/day or 500 mg/kg/day	1 - 12 days	GAD activity reaches steady state at 8 days and 72% of controls. GABA concentration reaches steady state at 4 days and 20% or 42% of controls (dose-dependent). Inconsistent effects on aspartic acid and glutamine concentrations.	12 on page 5-580, v1.13
Rat	GABA metabolism in brain	SC	100 mg/kg/day	11 days	Increased β-alanine, homocarnosine and GABA. Decreased glutamine and threonine. Suggests non-specific action. No VGB detected in brain 24 hours after last dose.	13 on page 5-591, v1.13
Mouse	GABA concentration in synaptosomal fractions	IP	1900 mg/kg	6 hours	Increased GABA concentration 300% equally in both whole brain and synaptosomal fractions.	14 on page 5-598, v1.13

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Mouse	GABA compartmentalization in brain cortex	IP	750 mg/kg	2 - 6 hours	Specific increase in GABA at synaptosome is more important to anticonvulsant activity than a large GABA increase in whole brain.	15 on page 5-607, v1.13
Mouse	GABA compartmentalization in brain cortex	IP	100 - 2500 mg/kg	4 hour pretreatment	Anticonvulsant activity maximum at 750 mg/kg and decreases with increased dose. Doses less than 250 mg/kg offer no protection against 3-mercaptopropionic acid induced seizures.	
Rat	Seizure control in denervated substantia nigra	IP	900 mg/kg	60 hours	Anticonvulsant activity in denervated substantia nigra occurs readily and persists approx. 5 days. Nerve terminal dependent GABA protection is delayed.	16 on page 5-632, v1.13
Rat	Synaptosomal superfusion	IP	750 mg/kg	18 hour pretreatment	Increased whole brain and synaptosomal potassium stimulated GABA release. [$^3H^+$]-GABA is not a quantitative tool.	17 on page 5-642, v1.13
Rat	Compartmental brain GABA concentrations	IP	750 mg/kg	0.5 - 18 hours	Evidence for multiple pools of GABA and multiple pathways of increasing GABA concentrations at the nerve terminal exits.	18 on page 5-665, v1.13
Rat	K^+ induced GABA release from cortex spinal cord and retina	IP	250 mg/kg	18 hours - 17 days	Tolerance to chronic administration occurs in brain and spinal cord but not in retina and is attributed to increased feedback inhibition of GAD with resulting decrease in presynaptic GABA stores.	19 on page 5-703, v1.13
Rat	GABA-T and GAD activity in brain regions	SC	0.2 - 50 mg/kg	4 hours	Both GAD and GABA-T are differentially reduced in various brain regions at these low doses. Retinal GABA-T inactivation is greatest, then frontal lobe. VGB may selectively enter the retina via aqueous humor.	20 on page 5-709, v1.13
Rat	GABA immunoreactivity in isolated retina	IP	250 mg/kg	18 hours	VGB induced GABA immunoreactivity in Muller fibers. Untreated, reactivity occurs only in amacrine cells.	21 on page 5-717, v1.13

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat, cat	GABA concentrations in brain and CSF	IP	125 - 2000 mg/kg	8 hours	Free GABA in CSF is exponentially correlated with brain GABA. Conjugated GABA is linearly correlated with brain GABA.	22 on page 5-722, v1.13
Rat	GABA and homocarnosine in brain	IP	125 - 2000 mg/kg	8 hours	Homocarnosine identified as primary GABA conjugate in CSF. Whole brain GABA is correlated with CSF conjugated GABA + homocarnosine.	23 on page 5-734, v1.13
Rat	GABA-T inhibition in brain and platelets	IP	50 - 1600 mg/kg/day	1, 8, 28 days	Platelet GABA-T is completely inhibited by low doses and does not correlate with brain inhibition. Chronic dosing increases inhibition in brain. Unclear if difference is due to access, or either VGB or GABA accumulation patterns in different tissues. 400 mg/kg max. tolerated dose.	24 on page 5-740, v1.13
Rat	CSF amino acid concentrations	IP	1000 mg/kg	5 - 96 hour pretreatment	Single dose elevates GABA, homocarnosine et al. Increases in glycine and taurine parallel VGB plasma concentrations, while glutamate and aspartate follow. May explain hyperexcitability associated with rapid VGB withdrawal.	25 on page 5-749, v1.13
Baboon	CSF amino acid concentrations	IM	500 mg/kg	2 - 24 hour pretreatment	GABA was only increase noted. Glutamate decreased but not correlated to VGB levels. Aspartate decrease at 2 and 24 hours; asparagine decrease at 6 hours; alanine decreased at 2 and 6 hours. Glycine, glutamine and taurine unchanged.	26 on page 5-755, v1.13
Rat	Control of Metrazol activated seizure	IP	1200 mg/kg	4 hour pretreatment	Seizure severity increased with most animals proceeding to status epilepticus and death.	27 on page 5-765, v1.14
Rat	Control of kindled seizure	IP	800 mg/kg	4 - 5 hour pretreatment	Pronounced decrease in severity of kindled seizures.	
Rat	Kindled seizure prevention	IP	100 mg/kg/d	4 days	No protection from kindled seizures.	28 on page 5-774, v1.14

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Kindled seizure prevention	IP	900, 1200, 1500 mg/kg	8 hour pretreatment	Seizure suppression followed time course of increased GABA with complete suppression at 32 hours and marked suppression remaining at 56 hours.	29 on page 5-784, v1.14
Rat	Kindled seizure prevention	IP	1200, 1500 mg/kg	16 hour pretreatment	100 - 150% increase in number of stimulations required to establish kindling.	
Rat	Kindled seizure development prevention	Microinjection into substantia nigra	5 μg	16 hour pretreatment	Substantia nigra controls kindling development rate. VGB direct injection suppresses kindling as nerve terminal GABA concentrations increase.	30 on page 5-790, v1.14
Rat	Kindled amygdala seizure suppression	Prepiriform cortex infusion	2.5 μg	3, 6, 24 hour pretreatment	VGB suppresses kindled seizures when injected at the junction of the anterior pole of the endopiriform nucleus (ventral claustrum) and the prepiriform cortex.	31 on page 5-798, v1.14
Rat	Kindled amygdala seizure suppression	IP	800, 1200 mg	4 hours - 3 days	VGB prolonged seizures at 4 hour and reduced seizure severity at 1 - 3 days. Withdrawal limbic seizures were noted.	32 on page 5-807, v1.14
Rat	Amygdala kindling	IP	800 - 1200 mg/kg	4 - 24 hours pretreatment	Anticonvulsant effects are preceded by a proconvulsant period at 4 hours post dose in the amygdala kindled rat model. Withdrawal hyperexcitability may also occur.	
Rat	Kindled amygdala seizure suppression	IP	100, 200 mg/kg/day	17 days	Initial (3 day) protection from kindled seizures was lost at 15 days. Seizure severity and spread from the focus was reduced. No consistent withdrawal hyperexcitability was noted.	33 on page 5-822, v1.14
Rat	Kindled amygdala seizure suppression	IP	600 mg/kg	48 - 60 hour pretreatment	Pronounced elevation of afterdischarge threshold at both 48 and 60 hours post dose.	34 on page 5-839, v1.14
Rat	Kindled amygdala seizure suppression	Microinjection into substantia nigra	20 μg	24 - 48 hour pretreatment	Duration of seizures reduced with no change in motor manifestations of seizures.	35 on page 5-846, v1.14

Table 5-1 (Page 6 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Kindled amygdala seizure suppression	Microinjection into substantia nigra	40 μg	6 - 96 hour pretreatment	Generalized convulsive seizure duration was reduced at 24 and 48 hours, but not at 6 hours post injection. Return to baseline at 96 hours.	36 on page 5-853, v1.14
Rat	Hippocampal kindling seizure suppression	Microinjection into amygdala	20 μg	6 - 72 hour pretreatment	Hippocampal seizures blocked or delayed motor convulsive latency.	37 on page 5-857, v1.14
Rat	Kindled amygdala seizure suppression	Microinjection into endopiriform	2.5 μg	2 hour - 15 days pretreatment	Total seizure suppression in 50% of rats for 2 - 15 days. Spontaneous seizures followed VGB injection in 35% of rats.	38 on page 5-867, v1.14
Rat	Kindled amygdala seizure suppression	Microinjection into amygdala	20 μg	0.5 - 48 hour pretreatment	Intra-amygdala VGB selectively attenuated motor seizure expression without altering afterdischarge duration.	39 on page 5-892, v1.14
Rat	Kindled amygdala seizure suppression	Microinjection into ventral midbrain tegmentum	N/A	N/A	VGB suppressed motor seizures only when injected into this brain area, not in other regions.	40 on page 5-898, v1.14
Rat	Pilocarpine induced seizure suppression	Microinjection into substantia nigra pars reticula	5 μg	6 hour pretreatment	Electrographic display shows minor or no alterations and is indistinguishable from pre-drug control activity at 6 - 8 hours post injection.	41 on page 5-901, v1.14
Mouse	Audiogenic seizure suppression	IP	1500 mg/kg	0 - 120 hour pretreatment	Complete protection at 4 hours. Declines to 56% at 72 hours. Smaller, repeated doses accumulate to same effectiveness. 50% decrease in seizure frequency achieved with 324% increase in whole brain GABA concentration.	42 on page 5-918, v1.14
Mouse	Audiogenic seizure suppression	IP	200 - 3000 mg/kg	4 - 6 hour pretreatment	Complete seizure protection at 4-6 hours with (S)-isomer, less so with racemate and no protection from (R)-isomer. At 72 hours, 1200 mg/kg (S) retained protection from myoclonus but (R,S) at 1200 mg/kg and (R) at 3000 mg/kg were ineffective.	43 on page 5-930, v1.14

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Audiogenic seizure suppression during alcohol withdrawal	IP	450 - 900mg/kg	90 minutes pretreatment	No reduction in seizures or forelimb tremors over time period studied.	44 on page 5-936, v1.14
Baboon	Photic epilepsy	IV	450 - 950 mg/kg	1 - 24 hour pretreatment	Complete protection from photic stimulated generalized myoclonus. Maximum protection at 1 - 3 hours which persisted for 7 - 24 hours.	45 on page 5-943, v1.14
Chicken	Febrite convulsions	IP	100 mg/kg	3 - 8 hour pretreatment	Significant reduction in seizures at 5 - 8 hours with increased latency at 3 - 6 hours. Febrile seizures occurred at higher temperatures than in untreated chicks and were associated with increased GABA (but not other transmitters) concentration in many brain areas.	46 on page 5-949, v1.14
Gerbil	Air blast-induced seizures	PO / IP	20 - 100 mg/kg/day 100 mg/kg q 2 days 100 mg/kg q 3 days	2.5 weeks	ED_{50} 50 mg/kg on acute dosing. Daily dosing led to tolerance attributed to feedback inhibition of GABA synthesis. No tolerance on alternate day dosing, but no persistent anticonvulsant effects were seen on days between doses.	47 on page 5-956, v1.14
Rat	Glycine potentiation in MPA, audiogenic induced seizures	IP	200 mg/kg	9 hour pretreatment	Coadministration of 750 mg/kg IP glycine 5 hours before seizure inducement gave 78% protection from MPA induced seizures and 63% protection from audiogenic seizures. Both seizure types responded more favorably to combined treatment than to either drug alone.	48 on page 5-965, v1.14
Mouse	Electroconvulsive threshold	IP	1000 - 2000 mg/kg	0.5 - 24 hour pretreatment	Threshold maximum elevation occurred at 6 hours and persisted for 24 hours.	49 on page 5-973, v1.14
Mouse	Pentylenetetrazol induced seizures	IP	720 - 1220 mg/kg	0.5 - 24 hour pretreatment	Dose dependent reduction in seizures ED_{50} at 940 mg/kg and doses exceeding 1500 mg/kg reduced protection.	

Table 5-1 (Page 8 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Mouse	Electroconvulsive threshold/ pentylenetetrazol induced seizures	IP	1900 mg/kg	6 hour pretreatment	GABA levels in brain tissue correlate to chemical-induced, but not electroconvulsive seizure control.	50 on page 5-985, v1.14
Mouse	Electroconvulsive threshold/ pentylenetetrazol induced seizures	IP	2000 mg/kg	1 - 24 hour pretreatment	GAD activity decreased following increase in GABA concentration. Seizure threshold paralleled GAD activity. Suggests feedback inhibition of GAD activity due to increasing GABA may limit effectiveness of GABA-T inhibitors.	51 on page 5-991, v1.14
Mouse	Electroconvulsive threshold/ pentylenetetrazol induced seizures	IP	490 - 1900 mg/kg	6 hour pretreatment	490 mg/kg increases pentylenetetrazol induced seizure threshold by 20%. 1900 mg/kg increases electroconvulsive threshold by same. VGB demonstrated to be one of least potent (mg basis) and one of least toxic (therapeutic ratio) of GABA-mimetic drugs tested.	52 on page 5-1000, v1.14
Mouse	Maximum electroshock, chemical induced seizures	IP	1500 mg/kg	1 - 24 hour pretreatment	Protection against chemical induced seizures at 1, 2 & 4 hours, but not at 24 hours. No protection from MES.	53 on page 5-1010, v1.14
Rat	Maximum electroshock	IP	600 - 1600 mg/kg	60 hour pretreatment	ED_{50} 900 mg/kg MES protection peaks at 60 hours post dosing and correlates with GABA concentration increases in nerve terminus (but not whole brain).	54 on page 5-1032, v1.14
Rat	Maximum electroshock	Intracerebral microinjection	20 μg	6 - 24 hour pretreatment	Microinjection into reticular formation of midbrain afforded 80% protection from MES induced seizures at 6 & 24 hours. No other brain areas provided any protection.	55 on page 5-1038, v1.14
Rat	Maximum electroshock, chemical induced seizures	Intracerebral microinjection	10 μg	6 - 96 hour pretreatment	Substantia nigra identified as the primary site of activity. Activity persists 72 hours and parallels GABA activity in the SN. No activity at 96 hours.	56 on page 5-1043, v1.14

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Species	Test/Model	Route of Admin-istration	Doses	Duration	Results/Comments	Reference Number
Rat	Maximum electro-shock, chemical induced seizures	Microinjection	1 - 100 μg	6 hour pretreatment	Only injection of 3 - 30 μg into substantia nigra protected (83%) from MES. Neither 1 nor 100 μg were effective. Chemical induced seizures were prevented by microin-jection into various brain areas, but NOT the SN.	57 on page 5-1049, v1.14
Rat	Maximum electro-shock, Pentylenetetrazole induced seizures	Microinjection	30 μg	6 hour pretreatment	Anterior thalamus injection protected from chemical, but not from MES, induced seizures. Substantia nigra injection protected from either.	58 on page 5-1057, v1.14
Rat	Maximum electro-shock, bicuculline induced seizures	Microinjection	5 - 10 μg	6 - 96 hour pretreatment	Deep prepiriform injection protects from chemical but not MES induced seizures.	59 on page 5-1062, v1.14
Rat	Maximum electro-shock	Microinjection into substantia nigra	5 μg	24 hour pretreatment	Direct injection into substantia nigra reduced seizures 38%. Idoxazin, a specific α_2 antagonist, abolished VGB effects. No other evidence of norepinephrine mediated anticonvulsant neuronal activity.	61 on page 5-1080, v1.14
Rat	Perforant pathway stimulation	N/A	100 - 500 mg/kg	24 hour pretreatment	500 mg/kg prevented the loss of hilar somatostatin-containing neurons and diminished damage in pyramidal layer. Seizure severity during stimulation was reduced.	62 on page 5-1085, v1.14
Rat	Spinal cord electroconvulsive seizures	IP	1500 mg/kg	2 - 24 hour pretreatment	Only slight reductions in electroconvulsive spinal cord sei-zures at 2 hours. GABA concen-tration is elevated but not correlated to effect.	63 on page 5-1087, v1.15
Mouse	Bicuculline, picrotoxin induced seizures	IP	47 - 63 mg/kg	5 hour pretreatment	ED_{50} 54 mg/kg. Much higher potency than expected based on whole brain GABA concentration suggesting regional distribution of GABA may be critical.	64 on page 5-1098, v1.15
Mouse	Chemically induced seizures	IV	200 mg/kg	2 minute pretreatment	Not active under these conditions.	

Table 5-1 (Page 10 of 26). Table of All Pharmacology Studies.

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Mouse	Chemically induced seizures	IP	700 - 2000 mg/kg	4 hour pretreatment	No effect on latency or frequency of seizures or death from bicuculline. No change in frequency or mortality with picrotoxin, but dose-dependent prolongation of time to onset was noted.	65 on page 5-1105, v1.15
Mouse	Chemically induced seizures	IP	1500 mg/kg	4 - 24 hour pretreatment	Protection from seizures follows a different time course for each chemical inductant. Isoniazid protection is complete at 4 hours and diminished at 24 hours. Bicuculline protection is essentially absent at 4 hours but approaches 75% at 24 hours.	66 on page 5-1111, v1.15
Mouse	Chemically induced seizures	IP	1000-1500 mg/kg	2 - 24 hour pretreatment	Low dose results in earlier onset of antipicrotoxin activity. Suggests a central cholinergic activity by VGB which must diminish before anticonvulsant effects can be measured.	67 on page 5-1117, v1.15
Mouse	Picrotoxin induced seizures	IP	1000 mg/kg	2 - 96 hour pretreatment	Significant increased latency at 2 & 4 hours in 100% of mice. 80% at 16, 24 hours. 25% at 65 hours and 11% at 72 hours. No protection remained at 96 hours.	68 on page 5-1128, v1.15
Mouse	Mercaptopropionic acid induced seizures	IP	25 - 300 mg/kg	5 hour pretreatment	(S) isomer exhibits twice the activity of racemate suggesting (R)-VGB is inactive.	69 on page 5-1131, v1.15
Mouse	Chemically induced seizures	IP	100 - 1500 mg/kg	5 hour pretreatment	ED_{50} is 150 - 200 mg/kg for MPA and bicuculline and diminishes above 750 mg/kg for MPA. VGB ineffective for metrazole, picrotoxin or strychnine. Addition of glycine improved protection from all but picrotoxin induced seizures.	70 on page 5-1139, v1.15

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Chemically induced seizures	IP	100 - 1500 mg/kg	5 hour pretreatment	Optimum VGB dose in rats is 200 mg/kg but only 60% effective in protecting from MPA seizures.	71 on page 5-1160, v1.15
Mouse	Glycine protection from chemically induced seizures	PO	41 mg/kg/day	16 days	Prolonged coadministration of glycine did not alter brain GABA levels. No changes in pharmacokinetics of either VGB or glycine, but synergism of anticonvulsant effect was noted. No counter regulation of GABA synthesis is observed.	
Mouse	MPA induced seizures	PO	95 mg/kg/day	19 days	Protection from MPA induced seizures was diminished by IP injection of 25 or 50 mg/kg VGB in addition to oral dose. Injection of 100 - 400 mg again produced a dose-related anticonvulsant effect.	72 on page 5-1194, v1.15
Mouse	Isoniazid induced seizures	IP	1500 mg/kg	1 - 24 hour pretreatment	Protection from isoniazid induced seizures was evident at 1 - 6 hours, disappeared by 24 hours. Brain GABA levels were 4 - 12 times controls at 1 - 6 hours and 8 times controls at 24 hours.	73 on page 5-1210, v1.15
Rat	Pentylenetetrazol induced kindling	N/A	N/A	N/A	VGB reduced mean scores of pentylenetetrazol kindling.	74 on page 5-1216, v1.15
Mouse, rat, rabbit	Pentylenetetrazol induced kindling	IP, intraventricular	> 500 mg/kg	N/A	VGB reduced mean scores of pentylenetetrazol kindling, but no dose dependency was observed. VGB potentiated anticonvulsive effects of phenobarbital,sodium valproate and diazepam.	75 on page 5-1218, v1.15
Mouse	Anti-pentylenetetrazol induced seizure enhancement by other anticonvulsants	IP	100 - 800 mg/kg	0.5 - 4 hour pretreatment	Potentiated effects of chlordiazepoxide 3.4 X, phenobarbital 3 X and diazepam 1.5 X.	76 on page 5-1220, v1.15

Table 5-1 (Page 12 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Guinea pig	Pentylenetetrazol induced seizures	Intracerebral microinjection	30 μg	1 - 24 hour pretreatment	Injection into anterior thalamic nucleus resulted in time dependent protection for pentylenetetrazol, peaking at 6 hours and dissipating by 24 hours.	77 on page 5-1253, v1.15
Rat	Pentylenetetrazol induced seizure threshold	Intracerebral microinjection	15 μg	24 hours pretreatment	Injection into medial anterior thalamus or other medial regions of caudal hypothalamus, midbrain tegmentum, pons and rostral medulla reduced pentylenetetrazol induced seizures.	78 on page 5-1266, v1.15
Mouse	Muscimol induced jerks	IP	100 - 1500 mg/kg	30 min - 96 hour pretreatment	VGB pretreatment blocks myoclonic jerks induced by muscimol in dose dependent fashion. Effects peak at 4 hours.	79 on page 5-1275, v1.15
Mouse	Decahydroquinoline carboxylic acid induced seizures	IP	1500 mg/kg	4 hour pretreatment	Ineffective in preventing seizures stimulated by intraventricular injection of this agent.	80 on page 5-1281, v1.15
Mouse, rat	(25S)-isosofloridine induced seizures	IP	1000 mg/kg	4 hour pretreatment	Complete protection from induced seizures and in some animals protects from tremor as well.	81 on page 5-1294, v1.15
Mouse	β-carboline induced seizures	IP	1500 mg/kg	3.25 hour pretreatment	GABA concentration in whole brain increased 280% and was unaffected by administration of β-carbolines. Convulsive doses were increased 3.5 - 4.5 fold.	82 on page 5-1308, v1.15
Rat	Methionine induced seizures	Substantia nigra microinjection	10 μg	24 hour pretreatment	Injection into the substantia nigra reduced seizure scores by 80%. Injection dorsal to SN or into striatum reduces scores 60% and 50% respectively.	83 on page 5-1317, v1.15
Rat	Flurothyl induced seizures	Perinigral microinjection	20 μg	24 hour pretreatment	Suppressed latency of flurothyl induced seizures.	84 on page 5-1322, v1.15
Mouse	Proline enhanced protection from chemical induced seizures	IP	100 - 1500 mg/kg	5 hour pretreatment	Combinations of proline and VGB protected against seizures from pentylenetetrazol and MPA but not from strychnine. Proline enhancement of VGB effects lasts about 2 hours.	85 on page 5-1324, v1.15

Table 5-1 (Page 13 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Mouse	Proline enhanced protection from chemical induced seizures	IP	100 mg/kg	5 hour pretreatment	Trans-4-hydroxy proline enhanced VGB effects when administered orally (262 mg/kg) 60 minutes prior to chemical inductant. Maximum protection in combination exists at 30 minutes and is observable for up to 2 hours vs. MPA. HO-proline is less protective than proline in combination against pentylenetetrazol induced seizures.	86 on page 5-1344, v1.15
Rat	Hyperbaric oxygen induced seizures	IP	50 - 500 mg/kg	4 hour pretreatment	Duration of latency increases with dose to 100% at 180 mg/kg. Protection lasts 24 hours and slowly erodes to no effect at 72 hours.	87 on page 5-1364, v1.15
Rat	Petit mal epilepsy	IP	800 mg/kg	4 hour pretreatment	VGB and other GABAergic transmitter potentiators increased duration of petit mal-like seizures in dose-related fashion.	88 on page 5-1370, v1.15
Rat	Petit mal epilepsy	IP	1000 mg/kg	4 hour pretreatment	VGB aggravated petit mal seizures and persisted for >2 hours. Diazepam 4 mg/kg abolished the seizures.	89 on page 5-1375, v1.15
Rat	Absence seizures	Microinjection into thalamus	10 - 25 μg	1 - 72 hour pretreatment	Midline thalamus injection had no effect for 24 hours then increased spike and wave discharge (SWD) up to 48 hours. Medial lateral thalamic injection increased SWD from 6 - 36 hours. Lateral thalamic injection suppressed SWD through 6 - 12 hours, then increased from 36 - 48 hours.	90 on page 5-1382, v1.15
Mouse	GABA-T inhibition in brain	IP	11.6 mmol/kg	single dose	Half-life of GABA-T in mouse brain is 3.5 days.	91 on page 5-1390, v1.15
Mouse	VGB concentration in brain	IP	400 - 1500 mg/kg	single dose	Half life of VGB is 18 hours in brain.	
Cat	GABA concentration in brain	IP	1000 mg/kg	1 - 10 hour pretreatment	VGB increases GABA (both free and total) in cat brain beginning 1 - 2 hours after injection and continuing through 10 hours. VGB detectable in plasma for 8 hours.	92 on page 5-1407, v1.15

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat, mouse	Ornithine aminotransferase inhibition	IP	100 mg/kg	1 - 24 hour pretreatment	VGB is not inhibitory for ornithine aminotransferase but effectively inhibits 4-aminobutyrate aminotransferase.	93 on page 5-1420, v1.15
Rat	Amino acid concentration in brain	IP	100 - 250 mg/kg/day	12 days	Brain concentration of numerous excitatory amino acids increased in a dose-related fashion following subchronic VGB administration.	94 on page 5-1431, v1.15
Rat	Amino acid concentration in brain	IP	1200 mg/kg	4 hour pretreatment	VGB reduced glutamate, aspartate and alanine levels in 12 regions of brain and glycine and taurine levels in a few.	95 on page 5-1433, v1.16
Rat, mouse	Putreanine	PO	150 mg/kg/day (S) 300mg/kg/day (R,S)	3 months	Putreanine levels reduced to 10% of control values.	96 on page 5-1435, v1.16
Mouse	Feedback inhibition of ornithine aminotransferase	IP	250 - 1500 mg/kg	24 hour pretreatment	Ornithine, carnosine & homocarnosine levels increased in brain whereas ornithine aminotransferase did not.	97 on page 5-1451, v1.16
Mouse	Feedback inhibition of ornithine aminotransferase	IP	750 mg/kg/day	1 - 3 days	Ornithine is suggested to be a precursor of glutamate or GABA. Feedback inhibition of OAT by increased GABA is of major importance in the GABAergic nerve terminal.	98 on page 5-1459, v1.16
Rat	GABAergic receptor sensitivity	IP	250 mg/kg/day	21 days	No withdrawal effects on neurotransmitter concentration were noted after 1, 7 or 21 days.	99 on page 5-1467, v1.16
Rat	GABA brain concentration	IP	50 - 150 mg/kg/day	10 days	Following olfactory bulbectomy, turnover of GABA and tryptophan were increased in the amygdaloid cortex relative to controls. Other neurotransmitters were not consistently affected.	100 on page 5-1476, v1.16
Baboon	Dopamine distribution	IV	3000 mg/kg	3.5 hour pretreatment	V_d of radiolabeled raclopride increased in the corpus striatum consistent with hypothesis that increased GABA would decrease dopamine.	101 on page 5-1484, v1.16

Table 5-1 (Page 15 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Striatal serotonergic transmission	Intradorsal raphe infusion	10 - 100 μg	4 hour pretreatment	VGB decreased striatal voltammetric signal and decreased extracellular 5-HIAA release.	102 on page 5-1489, v1.16
Rat	5HT levels in brain	IP	1000 mg/kg	3 hour pretreatment	VGB increased 5HT concentration by 40% and decreased norepinephrine by 11% in frontal cortex.	103 on page 5-1500, v1.16
Rat	Serotonin metabolism	IP	1500 mg/kg	4 hour pretreatment	Increased 5HT in the nuclei raphe magmus (30%), subcommissural organ (25%) and the habenula complex (60%).	104 on page 5-1503, v1.16
Rat	Serotonin metabolism	Microinjection into cerebral lateral ventricles	125 μg	3 hour pretreatment	Decreased 5HT (20 - 30 %) in raphe nuclei.	
Mouse	Thermoregulation	IP	1500 mg/kg	6 - 24 hour pretreatment	Mean rectal temperature reduced by 8°C (to 30°C) 6 hours after dose and returned to normal at 24 hours. Increasing ambient temperature to 34°C prevented hypothermia. VGB may influence thermoregulatory mechanisms.	105 on page 5-1529, v1.16
Mouse, Rat	Psychotropic activity	PO	30 - 1000 mg/kg/day	5 days	No change in mouse actography. Potentiated barbiturate activity; antagonized pentylenetetrazol seizures, but not electroshock, isoniazid, strychnine or thiosemicarbazole induced seizures. In rats, no catalepsy eliciting or antiapomorphine activity.	106 on page 5-1534, v1.16
Mouse	Analgesic activity	PO	30 - 1000 mg/kg/day	5 days	None	
Rat	Anti-inflammatory activity	PO	30 - 1000 mg/kg/day	5 days	None	
Rat	Hypothermia	PO	30 - 1000 mg/kg/day	5 days	Doses of 170 to 300 mg/kg--no effect. At 500 mg/kg hypothermia was significant at 3 & 4 hours.	
Rat	Antipyretic	PO	30 - 1000 mg/kg/day	5 days	Antagonized hyperthermia due to brewer's yeast at 1000 mg/kg.	

Table 5-1 (Page 16 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Amphetamine antagonism	IP	1000 mg/kg/day	16 hour pretreatment	VGB inhibited response to 2 mg amphetamine and potentiated effects of 4 mg amphetamine.	107 on page 5-1562, v1.16
Rat	Amphetamine, apomorphine stereotypy	N/A	1000 mg/kg	N/A	VGB suppressed amphetamine, but not apomorphine stereotypy in rats.	
Rat	Amphetamine stereotypy	IP	1000 mg/kg	18 hour pretreatment	Locomotor activity diminished at all doses of amphetamine. At threshold doses to induce stereotypical gnawing and licking, VGB facilitated development of that response.	
Rat	Amphetamine stereotypy	IP	1000 mg/kg	18 hour pretreatment	Dual action of VGB on locomotor diminishing and stereotypy facilitating effects of amphetamine suggests differing interactions between GABA and dopamine systems in neostriatum and mesolimbic striatum.	
Rat	Hypothermia, hole board exploration	IP	250 - 500 mg/kg	1 - 5 hour pretreatment	Hypothermia present at 1 hour and persists through 5 hours. VGB reduced hole board exploration only at 2.5 hours.	108 on page 5-1568, v1.16
Mouse	Antinociception	IP	1500 mg/kg	0 - 24 hours pretreatment	Antinociception noted at 2 and 4 hours not reversible by naloxone but was reversible by atropine. This effect is unrelated to sedative effects.	109 on page 5-1575, v1.16
Rat	Antinociception (tail stimulation)	IP	600 - 800 mg/kg	6 hour pretreatment	Antinociceptive effects are of a strong analgesic type. VGB does not antagonize withdrawal syndrome induced with naloxone in morphine addicted rats. VGB potentiates morphine analgesia.	110 on page 5-1583, v1.16
Mouse	Analgesic activity	IP	800 - 900 mg/kg	1 - 5 hour pretreatment	VGB increases both peak and duration of baclofen-induced analgesia. This effect is limited to the stereoisomer that inhibits GABA-T.	111 on page 5-1592, v1.16

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Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Mouse	Muscle relaxant activity	IP	50 - 200 mg/kg BID	3 days	Muscle relaxant effects maximized at 4 - 16 hours and not present 16 - 60 hours following first dose. No relaxant effect on multiple dose days. Pretreatment with atropine or MFDM did not block Straub tail reversal. VGB decreased potency of diazepam.	113 on page 5-1617, v1.16
Rat	Electromyelogram muscle relaxant	Microinjection into substantia nigra	0.4 μMol	0 - 50 hour pretreatment	Muscle relaxant effect measured 4 - 5 hours post injection. Maximum effect measured 24 - 26 hours and slowly abated. Effects still measurable at 48 - 50 hours.	114 on page 5-1638, v1.16
Rat	Dyskinesia	Intra striatal microinjection	129 - 1120 μg	0 - 48 hour pretreatment	VGB produces dyskinesias with dose-dependent extent when locally injection. Dyskinesias can be reversed for 1 min to 2 hours by striatal injection of GABA or muscimol. VGB itself protects from dyskinesias when injected IP (1000 mg/kg) 5 - 16 hours prior to striatal injection. VGB exhibits some reversible GABAergic blocking potential at high doses.	115 on page 5-1647, v1.16
Rat	Dyskinesia	Cortical superfusion	3 μMol	Single application	Cortical superfusion of VGB produces dyskinesias with involvement of the striatum.	116 on page 5-1674, v1.16
Rat	Dyskinesia	Microinjection into cortex or striatum	2 - 6 μMol	Single application	Dyskinesias are produced by cortical injection or superfusion without evidence of VGB diffusing into striatum.	117 on page 5-1693, v1.16
Rat	Dyskinesia	Microinjection into cortex or striatum	2 - 6 μMol	Single application	Cortical topography of dyskinesias is presented.	118 on page 5-1694, v1.16
Rat	Dyskinesia	Microinjection into cortex or striatum	770 μg	Single application	Dyskinesias produced by highly concentrated local injection are reversed by any means of increasing local GABA concentration.	119 on page 5-1704, v1.16

Table 5-1 (Page 18 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Dyskinesia	Cortical topical application	3 μMol	Single application	VGB induced dyskinesias are not mediated through homocarnosine but are a direct GABA receptor interaction.	120 on page 5-1713, v1.16
Rat	Dyskinesia	Cortical topical application	3 μMol	Single application	Homocarnosine direct application to cortex induces identical dyskinesia to GABA-T inhibitors. VGB induces dyskinesias without increasing homocarnosine levels.	121 on page 5-1723, v1.16
Monkey	Haloperidol induced dyskinesia	IM	100 - 200 mg/kg	2 hour - 5 day pretreatment	VGB reduced dyskinesias for 2 hours to 5 days after IM injection. Some reductions in quantity of coordinated motor activity was noted.	122 on page 5-1729, v1.16
Rat	Kainic acid neurotoxicity	IP	900 mg/kg	16 hour pretreatment	Kainic acid induced behavior similar to amphetamine but without aggression. VGB significantly reduced neurotoxic response.	123 on page 5-1734, v1.16
Rat	Kainic acid neurotoxicity	IP	900 mg/kg	18 hour pretreatment	VGB prevented most abnormal behavior recurrence. Only 6% exhibited convulsions and none hyperactivity or aggressiveness for 24 hours. Behavior returned to normal in 4 days.	124 on page 5-1739, v1.16
Rat	Kainic acid neurotoxicity	IP	600 mg/kg	18 hour, 4 day pretreatment	Kainic acid induced convulsions are due to decreased GABA in hypothalamus, striatum and olfactory bulbs. Aggression is a combination of GABA and 5-HT alterations. Both behaviors are prevented by increasing GABA.	125 on page 5-1748, v1.16
Rat	Intrastriatal kainic acid toxicity	IP	1000 mg/kg	0, 16 hour pretreatment	Pretreatment simultaneous IP or injection of VGB does not diminish toxicity of KA injected directly into striatum. VGB does not enhance toxicity of KA.	126 on page 5-1763, v1.16
Mouse	Morphine analgesia	N/A	200 - 400 mg/kg	4 hour pretreatment	VGB increased analgesia induced by morphine and reduced naloxone-induced abstinence signs in morphine tolerant mice.	127 on page 5-1777, v1.16

Table 5-1 (Page 19 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Mouse	Morphine, pentazocine analgesia	IP	400 mg/kg	4 hour pretreatment	VGB increased morphine analgesia and had no effect on pentazocine analgesia in tail stimulation but antagonized its effects in hot plate test. Kappa opiate system appears to be unrelated to GABA functions.	128 on page 5-1784, v1.16
Rat	Morphine dependence	IP	60-240 mg/kg/day	27 days	VGB caused dose dependent reduction in voluntary morphine intake in dependent rats. Elevated GABA may reduce reinforcing properties of morphine.	129 on page 5-1788, v1.16
Rat	Morphine dependence	IP	500 - 1000 mg/kg	4 hour pretreatment	VGB failed to significantly effect withdrawal body shakes in morphine dependent rats.	130 on page 5-1793, v1.16
Rat	Dipropylacetate induced body shakes	IP	250 - 1000 mg/kg	2.5 - 60 hour pretreatment	VGB had no effect on body shakes when DPA was administered 2.5 - 5 hours after VGB. At 12 - 60 hours, 1000 mg/kg VGB dose increased body shakes. Delayed onset suggests nerve terminal GABA concentration, rather than whole brain GABA, is important to this activity.	131 on page 5-1799, v1.16
Mouse	Ethylenediamine/ morphine dependence	N/A	400 mg/kg	4 hour pretreatment	VGB suppressed effects of ethylenediamine on morphine withdrawal.	132 on page 5-1804, v1.17
Rat	Aggression	IP	100 - 400 mg/kg	0 - 9 hour pretreatment	VGB reduced mouse-killing behavior in dose dependent manner 2 - 9 hours following dose.	133 on page 5-1808, v1.17
Rat	Motor activity	IP	250 - 1500 mg/kg	4 hour pretreatment	VGB decreases exploratory and motor activity in rats at doses of 250 mg/kg or greater.	134 on page 5-1816, v1.17
Mouse	PCP hyperactivity	IP	100 - 800 mg/kg	5 hour pretreatment	Decreased locomotor activity at VGB doses of 200 - 700 mg/kg related to PCP controls. Maximum reduction in activity at 400 mg/kg.	135 on page 5-1823, v1.17
Rat	Food reward, conditional avoidance	IP	125 - 500 mg/kg	4 hour pretreatment	VGB reduced food intake in normal feeding and in food reward situations but had no impact on electric shock avoidance.	136 on page 5-1839, v1.17

Table 5-1 (Page 20 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Feeding behavior	IP, PO	125 - 1000 mg/kg/day	13 days	VGB reduced food intake in dose dependent manner regardless of route of administration. No tolerance developed on repeated dosing.	137 on page 5-1850, v1.17
Sheep	Feeding behavior	Intracerebral microinjection	10 nMol - 10 μMol	30 minutes - multiple days (unspecified)	VGB failed to provoke any feeding response.	138 on page 5-1860, v1.17
Rat	Feeding behavior	Intracisternal injection	25 - 60 μg	6 hour pretreatment	VGB induced dose-dependent food intake decrease at doses > 50 μg, decreased water intake at doses > 150 μg. Higher doses decreased pain sensitivity, locomotor activity and body temperature.	139 on page 5-1865, v1.17
Rat	Vision	PO	300 mg/kg	4 hour pretreatment	No effect on rat pupil size.	140 on page 5-1879, v1.17
Rat, mouse	Brain polyamine concentration	IP	1500 mg/kg	0 - 24 hours	GABA concentration increases rapidly. Putrescine concentration is unchanged for 6 hours then increases to maximum at 16 hours. S-adenosylmethionine decarboxylase activity rapidly decreases and reaches a minimum (35-40% of controls) at 6 - 8 hours after injection.	141 on page 5-1889, v1.17
Rat, mouse	Brain polyamine concentration	IP	1500 mg/kg	0 - 24 hours	Ornithine decarboxylase activity increased to peak at 16 hours then declined. Putrescine concentrations rose steadily through 24 hours following a 6 hour delay.	142 on page 5-1902, v1.17
Mouse	Polyamine reutilization	IP	750 mg/kg/day	9 days	N-acetyl spermidine and spermine levels accumulate steadily over 8 hours following a single dose, and following the last of a series of doses.	143 on page 5-1922, v1.17
Rat	Anxiolytic	IP	1000 mg/kg	4 - 72 hour pretreatment	VGB failed to block pentylenetetrazol discriminatory stimulus at all doses and times tested. Only anxiolytic agents were capable of blocking this effect.	144 on page 5-1939, v1.17
Rat	Anxiolytic	IP	900 mg/kg	4 hour pretreatment	VGB had no anxiolytic activity in water lick paradigm.	145 on page 5-1948, v1.17

Table 5-1 (Page 21 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Reward/conflict behavior	IP	62.5 - 250 mg/kg	3 hour pretreatment	VGB decreased performance during reward but had no effect during conflict periods. Reversed anticonflict effects of diazepam and markedly depressed performance during reward periods in combination with diazepam. Highest dose completely abolished conditioned responses for one week.	146 on page 5-1958, v1.17
Rat	Shock induced aggression	IP	100 - 200 mg/kg	3 hour pretreatment	VGB resulted in selective inhibition of shock induced fighting (results not statistically significant).	147 on page 5-1968, v1.17
Rat	Aggression	IP	200 - 800 mg/kg	4 - 24 hour pretreatment	Maximum reduction in aggressive behavior occurred at 4 hours and decreased over 24 hours.	148 on page 5-1975, v1.17
Rat	Depression	IP	75 mg/kg/day	14 days	VGB attenuates hypermotility in olfactory bulbectomized rats.	149 on page 5-1979, v1.17
Rat	Depression	IP	75 mg/kg/day	5 - 20 days	VGB reduced immobility response at 10 days, but not at 5 or 20 days, and may possess antidepressant properties on subchronic administration.	150 on page 5-1989, v1.17
Mouse	Depression	IP	400 - 1200 mg/kg	4 - 24 hour pretreatment	Low doses produced no EEG effects. High dose resulted in hypersynchronization in all animals after 4-6 hours resembling absence seizures. Suggests somnolence associated with GABA-T inhibitors may be due to toxic seizures rather than simple sedation.	151 on page 5-1995, v1.17
Rat	Learning and memory	IP	50-200 mg/kg/day	8 - 9 days	VGB did not affect learning and memory in passive avoidance training.	152 on page 5-2007, v1.17
Rat	Learning in nucleus basalis lesioned rats	IP	50-200 mg/kg/day	8 days	VGB dose dependently aggravated the spatial navigation deficit in NB lesional rats but not in controls.	153 on page 5-2014, v1.17

Table 5-1 (Page 22 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Dog	Cardiovascular activity	IV	3 - 30 mg/kg	single dose	No effect on blood pressure, intraventricular pressure, heart rate, cardiac output , ECG or other measured parameters.	154 on page 5-2019, v1.17
Dog	Cardiovascular activity	PO	140 mg/kg BID	5 days	No effect on any measured parameter.	
Dog, rat	Cardiovascular activity	IV	10-150 mg/kg (rat) 50-200 mg/kg (dog)	single dose	No cardiovascular effects alone in dog or rat, nor in combination with any drug in dogs. In rats, VGB diminished the response to noradrenaline & tyramine and enhanced hypotension due to acetylcholine, but no dose-dependency was noted.	155 on page 5-2184, v1.17
Dog, rat	Cardiovascular activity	IV	120-1000 mg/kg (rat) 100 mg/kg (dog)	single dose	One dog had prolonged 20 mm drop in BP. Rats demonstrated prolonged hypotension, bradycardia and decreased central venous pressure. Effects were antagonized by picrotoxin, bicuculline and other GABA blockers.	156 on page 5-2206, v1.17
Rabbit	Cardiovascular activity	Coronary perfusion	5 - 20 μg	single dose	No effect on electrical stimulation threshold or ventricular fibrillation threshold.	157 on page 5-2235, v1.17
Rat	Central blood pressure control	Microinjection into nucleus tractus solitarius	1 - 100 nmol	single dose	VGB increased arterial pressure in a dose-dependent manner, with little effect on heart rate. Pressor response is a result of direct stimulation of GABA receptors by VGB.	158 on page 5-2241, v1.17
Rabbit	Hepatic encephalopathy	IV	750 mg/kg	18 hour pretreatment	VGB induces a pattern of visual evoked responses resembling those of hepatic encephalopathy. Benzodiazepines and other GABA blockers reduce this response.	159 on page 5-2248, v1.18

Table 5-1 (Page 23 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rabbit	Hepatic encephalopathy	IV	750 mg/kg	18 hour pretreatment	Similarity in response to hepatic encephalopathy induced by VGB suggests GABAergic tone increase contributes to the encephalopathy but does not necessarily require benzodiazepine receptor involvement.	160 on page 5-2258, v1.18
Rat	Hypothalamo-pituitary adrenal axis	IP	250 - 1000 mg/kg	18 hour pretreatment	Pituitary ACTH concentration reduced. Basal and CRF or AVP-stimulated ACTH release was inhibited. Hypothalamus concentration of CRF reduced at highest dose.	161 on page 5-2267, v1.18
Rat	Corticotropin releasing factor	IP	250 - 1000 mg/kg	18 hour pretreatment	GABA concentration doubles without increase in CRF in hypothalamus. VGB reduced plasma ACTH concentration in response to stress relative to controls. Cortisone levels were elevated under stress or non-stress conditions.	162 on page 5-2275, v1.18
Rat	Ovulation, luteinizing hormone release	IP	500 mg/kg	4 hour pretreatment	VGB blunted LH release and subsequent ovulation when administered on the preovulatory day.	163 on page 5-2290, v1.18
Rat	Luteinizing hormone release	Intraventricular microinjection	10 - 20 μg	48 hour pretreatment	VGB injected into locus coeruleus area blunted LH release over 48 hours. In orchidectomized males, when injected into anterior hypothalamic suprachiasmatic area, also decreased LH release.	164 on page 5-2298, v1.18
Rat	Luteinizing hormone release	Intracerebral microinjection	10 - 20 μg	0 - 48 hour pretreatment	Variable responses to local VGB injection on LH secretion in different parts of brain following orchidectomy.	165 on page 5-2308, v1.18
Rat	Luteinizing hormone release	Intracerebral microinjection	20 μg	20 hour pretreatment	VGB injection into locus coeruleus reduced LH plasma concentration and prevented ovulation in 13/18 animals.	166 on page 5-2317, v1.18
Rat	Prolactin mRNA biosynthesis	IP	800 mg/kg	48 hour pretreatment	Prolactin mRNA levels in adenohypophyseal area decreased 35% two days following injection.	167 on page 5-2325, v1.18

Table 5-1 (Page 24 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	Prolactin mRNA biosynthesis	IP	100 - 800 mg/kg q 2nd day	8 days	VGB induced decrease in prolactin mRNA is dose and time dependent. Effects are antagonized by bicuculline and mimicked by $GABA_A$ receptor agonists.	168 on page 5-2338, v1.18
Rat	Growth hormone secretion	IP	25 - 500 mg/kg	3 hour pretreatment	VGB decreases plasma growth hormone levels indicating GABA inhibits growth hormone secretion.	169 on page 5-2346, v1.18
Mouse	Thyroid hormone secretion	IP	50 mg/kg	2 hour pretreatment	VGB decreased the stimulatory effects of TSH, suggesting that GABA inhibits TSH-stimulated thyroid hormone secretion.	170 on page 5-2351, v1.18
Rat	Vasopressin release	IP	1500 mg/kg	6 hour pretreatment	In PEG-induced hypovolemia, VGB diminished vasopressin release without altering plasma renin concentration.	171 on page 5-2356, v1.18
Rat	Somatostatin release	In vitro	1 - 1000 μM	28 minutes	VGB reduced somatostatin release by 69% (IC_{50} = 240 μM)	172 on page 5-2364, v1.18
Rat	Ganglionic surface potential	In vitro	1 - 100 μM	N/A	VGB is neither agonist nor antagonist at the $GABA_A$ receptor in isolated superior cervical sympathetic ganglia.	186 on page 5-2646, v1.19
Rat	GABA accumulation	In vitro	100μM	5 - 90 minute incubation	GABA accumulation 0.2 - 0.6 nmol/mg protein/min and comparable to those following 600 mg/kg IP dose in vivo.	187 on page 5-2654, v1.19
Rat	GABA release	IP	250 mg/kg	18 hour pretreatment	Cortical slices depolarized by K in solution released twice the GABA as did controls. Retinal and spinal cord slices did not respond to VGB. Feedback inhibition may occur via $GABA_B$ inhibitory autoregulation.	189 on page 5-2663, v1.19
Rat	GABA brain concentration	IP	1900 mg/kg	6 - 24 hour pretreatment	Highest GABA concentration found in caudoputamen, cerebellum and frontal cortex at 6 hours. After 24 hours, highest concentration found in superior colliculus, hippocampus and caudoputamen.	190 on page 5-2672, v1.19

Table 5-1 (Page 25 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Rat	GABA concentration in CSF	IP	500 mg/kg	4 - 96 hour pretreatment	CSF total GABA concentration (free and conjugated) correlates linearly with brain GABA concentration.	191 on page 5-2689, v1.19
Rat	CSF/Brain GABA concentrations	IP	250 - 1000 mg/kg	18 hour pretreatment	Brain GABA concentration is linearly correlated with CSF homocarnosine levels but the two do not follow the same time course.	192 on page 5-2697, v1.19
Mouse	Electroconvulsive threshold	PO	100 - 200 mg/kg/day	1 - 12 days	At high dose, electroconvulsive threshold was increased through day 8, but fell below baseline at day 12. Low dose yielded a consistent increase at all times tested.	204 on page 5-2863, v1.19
Rat	Isoniazid-induced GABA decrease	IP	1800 mg/kg	12 - 18 hour pretreatment	INH induced seizures in VGB pretreated rats 45 minutes post injection corresponding to a decrease in whole brain GABA concentrations (equivalent to controls receiving INH only). Rate of GABA decline was highest in superior colliculus and substantia nigra.	205 on page 5-2871, v1.19
Rat	Glycine ester potentiation	IP	100 mg/kg	4 hour pretreatment	Glycine tert-butyl ester potentiates anticonvulsant activity of VGB vs. MPA-induced seizures. Similar to effects seen with glycine.	207 on page 5-2887, v1.19
Rat	Ketamine induced rotation	IP	1200 mg/kg	4 hour pretreatment	VGB shortened the first rotation phase, increased the anesthetic phase and changed the pattern of post-anesthesia rotation, reducing the total and net rotation scores.	208 on page 5-2893, v1.19
Rat (27) Guinea Pig (6) Mouse (1) Bovine (4)	Receptor selectivity screen	In vitro	1 - 100 μM	10 minute - 2 hour incubation, per individual assay protocol	No significant activity at any of 38 sites tested.	209 on page 5-2899, v1.19

Table 5-1 (Page 26 of 26). Table of All Pharmacology Studies

Species	Test/Model	Route of Administration	Doses	Duration	Results/Comments	Reference Number
Guinea pig	Agonist-Induced contraction/ Ileum	In vitro	1 - 100μM	10 minute incubation	No effect on resting tension or on subsequent contraction induced by histamine, ACh, 5-HT and Ba^{+2}	210 on page 5-2947, v1.19

3. Studies Related to ADME

a. Absorption

The fraction of the dose absorbed, based on the urinary recovery of ^{14}C following intravenous and oral administration of ^{14}C-vigabatrin to the rat, dog, and monkey, is shown in Table 5-42.

Table 5-42. Absorption of Vigabatrin Following Oral Administration of ^{14}C-Vigabatrin to Rat, Dog, and Monkey. (Data from References 279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32)

Species	50 mg/kg		300 mg/kg	
	% Absorbed[a]	Calculated Absorbed Dose (mg/kg)[b]	% Absorbed	Calculated Absorbed Dose (mg/kg)
Rat (N=4)	93.0[c]	47	92.4[c]	273
Dog (N=4)	96±7	48	101±3	303
Monkey (N=4)	44±4	22	25±7	75

[a] Determined by comparing the urinary recovery of radioactivity over the 120 hour period following a single oral dose of ^{14}C-vigabatrin to the urinary recovery of radioactivity over the same time period following a single IV dose of ^{14}C-vigabatrin. The percent of dose absorbed is expressed as the mean±SD.

[b] Determined by multiplying together the mean percent of dose absorbed and the administered dose.

[c] Determined by comparing the urinary recovery of radioactivity over the 120 hour period following a single oral dose of ^{14}C-vigabatrin to one group of rats to the urinary recovery of radioactivity over the same time period following a single IV dose of ^{14}C-vigabatrin given to another group of rats. Means of the two groups were compared.

Oral absorption of vigabatrin at both the 50 mg/kg and the 300 mg/kg doses was essentially complete in the rat and the dog. In contrast, the urinary recovery data from the monkey indicate that both incomplete and dose-dependent absorption of vigabatrin occurred in this species. Liquid scintillation analysis of feces excreted by monkeys given ^{14}C-vigabatrin confirmed that oral absorption of the drug in this species is incomplete (see below). Evidence for incomplete oral absorption of vigabatrin in the monkey was also obtained in a preliminary pharmacokinetic analysis in this species (284 on page 5-8049, v1.32). Assuming an inverse linear relationship between the percent of vigabatrin absorbed and the amount administered, it can be estimated that the oral

doses of 50, 100, and 300 mg/kg/day of vigabatrin given to monkeys in the chronic toxicity studies (232 on page 5-4476, v1.24, 233 on page 5-4756, v1.24) resulted in absorbed doses of 22, 40, and 75 mg/kg/day of the drug, respectively (281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32).

The mean peak concentration, time to peak, and the area under the plasma concentration time curve have been determined using an HPLC validated method (283 on page 5-7907, v1.32) for parent compound in rat, dog, and monkey following a single IV or PO dose of ^{14}C-vigabatrin. These results are summarized in Table 5-43.

Table 5-43. Summary of Pharmacokinetic Parameters for Vigabatrin (Parent Compound) in Rat, Dog, and Monkey Following Single PO and IV Doses of ^{14}C-Vigabatrin. (Data from References 279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32)

Species	Dose (mg/kg)	Route	C_{max} (μg/mL)	T_{max}(hr)	$AUC_{(0-\infty)}$ (μg hr/mL)
Rat (N=4)	50	IV	N/A[a]	N/A	53.3±9.6[b]
	50	PO[c]	–	–	–
	300	IV	N/A	N/A	341±48
	300	PO	250±36[b]	0.5	363±43
Dog (N=4)	50	IV	N/A	N/A	204±18
	50	PO	103±15	0.25-1.0[d]	192±32
	300	IV	N/A	N/A	1305±168
	300	PO	458±60	0.5-1.0	1178±282
Monkey (N=4)	50	IV	N/A	N/A	253±20.7
	50	PO	15.8±7	2-3	–[e]
	300	IV	N/A	N/A	1707±421
	300	PO	38±4	2-3	–[e]

a N/A - Not applicable

b Values for C_{max} and $AUC_{0-\infty}$ are expressed as the mean±SD.

c Insufficient data was obtained from this group to allow calculations of pharmacokinetic parameters. This was due to instrument difficulties that occurred during the analysis.

d T_{max} values for dog and monkey following oral dosing with vigabatrin are expressed as a range.

e Elimination phase for vigabatrin was not well defined in the monkey after oral dosing. Consequently, $AUC_{0-\infty}$ values could not be calculated.

These data clearly show that in both the rat and the dog, vigabatrin was rapidly absorbed. The $AUC_{0-\infty}$ values in both species increased proportionally with dose, indicating the existence of linear pharmacokinetics over the dosage range studied.

In contrast, in the monkey, the time to peak plasma concentration following a single oral dose of vigabatrin (2-3 hours) was slower than in the rat and the dog. Peak plasma concentrations of vigabatrin were also lower in the monkey than in the other two species studied. This lower peak plasma concentration is due to the apparent poor oral absorption of vigabatrin and the slower rate of absorption of the drug in the monkey. Plasma elimination half-lives following oral dosing

with 50 or 300 mg/kg ^{14}C-vigabatrin were not well defined in the monkey from the data generated in this study, therefore, $AUC_{0-\infty}$ values could not accurately be calculated following PO dosing in this species. However, in an earlier study in monkeys (284 on page 5-8049, v1.32), $AUC_{0-24\ hr}$ values were calculated following single oral doses of 50 and 260 mg/kg vigabatrin, and were found to be 54.6±14.5 and 203±66 μg hr/mL, respectively. These $AUC_{0-24\ hr}$ values are not proportional to the doses that were administered. As the rate of elimination for vigabatrin in the monkey is constant over the dosage range of 50-260 mg/kg (232 on page 5-4476, v1.24), the lack of dose-proportionality in $AUC_{0-24\ hr}$ values confirms that the percent absorption of vigabatrin is inversely related to dose in this species.

Further evidence for the existence of dose-limited absorption of vigabatrin in monkeys was provided from analysis of plasma samples obtained at the end of a four week dose-ranging toxicology study (285 on page 5-8070, v1.33). In this study, four monkeys (two/sex) were dosed daily with 0, 500, 750, or 1000 mg/kg vigabatrin. Plasma samples for analysis of vigabatrin were taken 2 and 24 hours after the last dose of drug. At two hours, there were no statistically significant differences between any of the plasma concentrations measured, either among doses or between the sexes. At 24 hours, only the two female monkeys receiving the low dose of vigabatrin (500 mg/kg) had plasma concentrations significantly less than the other dose groups. This finding, combined with the data discussed above, strongly suggests that the absorption of vigabatrin from the GI tract of the monkey is both dose-dependent and saturable.

In conclusion, over a dose range of 50-300 mg/kg, vigabatrin appears to be both rapidly and essentially completely absorbed from the GI tract of the rat and the dog. In the monkey, however, GI absorption of vigabatrin occurs more slowly, and is both incomplete and dose-limited.

b. Distribution

i. Volume of Distribution

The ionizable properties of the carboxyl and amine groups on vigabatrin would produce a net neutral charge resulting in vigabatrin being in a zwitterion form. The small molecular size and neutrality at physiological pH would increase the propensity of vigabatrin to cross the blood-brain-barrier. Vigabatrin is a hydrophilic compound and as such would be expected to distribute into the total body water. As shown in Table 5-44 on page 5-233, v1.12, this was found to be true in both the dog and the monkey. In the rat, however, the volume of distribution for vigabatrin exceeded the total body water of this species by 33-34% for the 50 and 300 mg/kg doses, respectively (Table 5-44 on page 5-233, v1.12). This increased volume of distribution in the rat implies either tissue binding or an increased compartmentalization of unbound drug in tissues relative to plasma.

Table 5-44. Volume of Distribution of Vigabatrin in the Rat, Dog, and Monkey Following A Single IV Dose of Either 50 or 300 mg/kg. (Data from References 279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32)

Species	V_{SS} (l/kg)[a]		Theoretical Body Water For This Species (l/kg)[b]
	50 mg/kg	300 mg/kg	
Rat	0.95±0.04[c]	0.97±0.16	0.64
Dog	0.59±0.06	0.52±0.09	0.60
Monkey	0.45±0.02	0.55±0.12	0.56

[a] V_{SS} - volume of distribution at steady state.

[b] Values for theoretical body water for rat, dog, and monkey can be found in references 297 on page 5-8282, v1.34, 298 on page 5-8298, v1.34, 299 on page 5-8306, v1.34 and 300 on page 5-8328, v1.34.

[c] Data are expressed as the mean±SD of four determinations.

II. Tissue Distribution

The tissue distribution of ^{14}C-vigabatrin has been examined in detail in rats. In addition, a number of studies have been conducted examining the concentration of vigabatrin and GABA in brain tissue and CSF after single and repeated doses of the test compound. This was done as the beneficial clinical effects of vigabatrin are due to actions of the drug in the brain.

a). Tissue Distribution and Elimination of Orally Administered ^{14}C-Vigabatrin in Sprague-Dawley and Long-Evans Rats

The distribution of radioactivity following a single 60 mg/kg dose of ^{14}C-vigabatrin has been evaluated in both Sprague-Dawley (albino) and Long-Evans (pigmented) rats (286 on page 5-8093, v1.33). In the first part of this study, 33 male Sprague-Dawley rats received a single 60 mg/kg oral dose of ^{14}C-vigabatrin prepared as an aqueous solution in distilled water. The amount of radioactivity administered to each rat was about 14 μCi/kg. Animals (3 at each time point) were killed pre-dose (controls) and 0.25, 0.5, 1, 3, 6, 12, 18, 24, 48, and 72 hours post dose. Blood and samples of 17 other tissues were taken from each rat. Tissues were rinsed with 0.9% saline (where applicable), weighed and frozen. The ^{14}C content of each tissue was determined by liquid scintillation counting. Urine and feces were collected from animals assigned to the 72 hour sacrifice group, and were analyzed for radioactivity. The residual radioactivity was also determined in the carcasses of rats killed at this latest time point.

^{14}C-vigabatrin was well absorbed by Sprague-Dawley rats in this study. Within the first 24 hours following drug administration an average of 94% of the radioactivity had been excreted in the urine of Sprague-Dawley rats assigned to the final sacrifice group, with 2% appearing in the feces. By the end of the 72 hour collection period, a total of 97% of the radioactivity in the adminis-

tered dose had appeared in the excreta. Correspondingly, less than 0.3% of the administered radioactivity was present in the carcass of rats sacrificed 72 hours after drug dosing.

In addition to being well absorbed, ^{14}C from labeled vigabatrin was rapidly distributed in Sprague-Dawley rats. In most tissues examined (including plasma and erythrocytes), maximum concentrations of radioactivity occurred within 0.5 hours of drug administration. The tissue with the longest T_{max} was the eye of Sprague-Dawley rats, where maximum concentrations occurred one hour after dosing. The highest ^{14}C concentrations were found in the stomach (1022 μg-eq/g, excluding contents), followed by the kidney (257 μg-eq/g), small intestine (154 μg-eq/g, excluding contents), and liver (143 μg-eq/g). All other tissues had maximum ^{14}C levels < 100 μg-eg/g. Tissues containing $> 1\%$ of the total amount of administered radioactivity were the skeletal muscle (12%), stomach (11% excluding contents), skin (10%), liver (8.6%), small intestine (5.9% excluding contents) and kidneys (3.4%).

Using the data generated in this study, standard pharmacokinetic parameters for radioactivity were calculated for each of the tissues examined. As noted above, T_{max} for radioactivity was ≤ 1 hour for all tissues. Radioactivity was eliminated from all tissues in either a monoexponential or a biexponential fashion. Terminal elimination half-lives were shortest for the adrenal glands (1.2 hours), perirenal fat (1.5 hours) and bone marrow (1.6 hours), and longest for the erythrocytes (95 hours) and spleen (93 hours). The prolonged terminal elimination half-lives for ^{14}C in the erythrocytes and spleen suggests that either ^{14}C-vigabatrin or its radiolabelled metabolites have an affinity for one or more constituents of blood. The terminal elimination half-life for radioactivity from the plasma was 25 hours. $AUC_{0-\infty}$ values, which are a measure of total radioactivity exposure, were highest for the erythrocytes (1382 μg-eq hr/g), stomach (752 μg-eq hr/g, excluding contents) and kidney (706 μg-eq hr/g), while the lowest exposure to ^{14}C was found in lipophilic organs such as brain and perirenal fat.

In the second part of this study, Long-Evans rats were administered a single 60 mg/kg oral dose of ^{14}C-vigabatrin as described above. Radioactivity was determined in the plasma, skin, eyes and excreta. This was done in an attempt to determine whether ^{14}C-vigabatrin or its radiolabelled metabolites bind to pigmented elements of the eye or skin. Based on urinary radioactivity levels, the 60 mg/kg oral dose of ^{14}C-vigabatrin was at least 90% absorbed by Long-Evans rats. A summary of the pharmacokinetic parameters for radioactivity in the plasma, eyes, and skin of Sprague-Dawley and Long-Evans rats following a single 60 mg/kg dose of ^{14}C-vigabatrin can be found in Table 5-45 on page 5-235, v1.12. In plasma, the C_{max}, T_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ values for the two strains of rats were similar. In contrast, the $AUC_{0-\infty}$ and terminal $t_{1/2}$ values for the eyes and the skin were significantly higher in the Long-Evans rat than in the Sprague-Dawley rat (286 on page 5-8093, v1.33). These results suggest that ^{14}C-vigabatrin or its metabolites may bind to pigmented elements in the eye or the skin.

Table 5-45. Pharmacokinetic Parameters for Radioactivity in the Plasma, Eye, and Skin of Male Sprague-Dawley and Male Long-Evans Rats Following A Single 60 mg/kg Oral Dose of ^{14}C-Vigabatrin. (Data from Reference 286 on page 5-8093, v1.33)

Parameter	Plasma		Eyes		Skin	
	S/D[a]	L/E[b]	S/D	L/E	S/D	L/E
C_{max} (μg-eq/g)	65.1	60.2	9.5	8.3	33.3	29.8
T_{max} (hours)	0.5	0.5	1	0.5	0.5	0.5
$AUC_{0-\infty}$ (μg-eq hr/g)	118	158	77	236	81	170
Terminal elimination $t_{1/2}$(hours)	25	30	12	66	3.5	62

a S/D - Sprague-Dawley

b L/E - Long-Evans

b). Concentration of Vigabatrin in the Brain and Plasma of Rats Given Daily 50 or 300 mg/kg Oral Doses for One Week

Rats were administered daily oral doses of either 50 mg/kg or 300 mg/kg ^{3}H-vigabatrin (287 on page 5-8126, v1.33). Animals were killed 24 hours after dosing 1, 3, 5, and 6, and 4, 8, 12, 24 or 48 hours after dose 7 of the drug. Plasma, CSF, urine, and brain samples were collected. Vigabatrin levels in the urine of rats given 300 mg/kg of the drug were determined using an HPLC method (283 on page 5-7907, v1.32). A capillary gas chromatography/mass spectroscopy method, capable of distinguishing the enantiomers of vigabatrin (288 on page 5-8152, v1.33), was used for all other samples.

Plasma and brain concentrations of vigabatrin determined at various time points in this study are summarized in Table 5-46 on page 5-236, v1.12. As can be seen, vigabatrin enters the brain, and appears to reach steady state in this organ by the third daily dose. Steady state trough brain concentrations of vigabatrin were 0.8-1.2 nmoles/g for the 50 mg/kg dose and 16-19 nmoles/g for the 300 mg/kg dose, values that are clearly not proportional to the amount of drug that was administered. However, peak brain concentrations increased in an almost linear manner. Because of variability in the plasma data, steady-state plasma concentrations could not be accurately determined. Peak plasma concentrations of vigabatrin were proportional to dose.

Table 5-46. Plasma and Brain Concentrations of Racemic Vigabatrin Following Single and Multiple Dosing to the Rat. (Data from Reference 287 on page 5-8126, v1.33).

Dose (mg/kg/day)	Number of Doses	Time After Previous Dose (hours)	Concentration Of Vigabatrin (nmole/g)	
			Plasma	Brain
50	1	24	–[a]	0.452±0.074
	3	24	–[a]	0.806±0.237
	6	24	1.14±0.26	0.845±0.279
	7	4	49.5±15.1	5.36±1.19
		12	2.95±1.00	2.71±0.527
		24	–[a]	1.20±0.13
		48	–[a]	–[b]
		72	–[a]	–[b]
300	1	24	38.0±41.1	6.3±3.6
	3	24	10.9±17.1	16±4.7
	5	24	23.3±30.2	19±2.4
	6	24	36.4±32.6	18±0
	7	4	283±104	37±12
		8	101±36.4	34±9
		12	34.3±29.2	27±3
		24	32.3±51.9	16±0.5
		48	0.8[c]	9.8±1.6

Data are expressed as the mean±SD of three determinations

[a] Not determined

[b] Not detectable

[c] Vigabatrin detected in plasma of two out of three animals

Levels of S- and R-vigabatrin were also measured in both plasma and brain. In plasma, concentrations of the two enantiomers were approximately equal at all time points. In contrast, brain levels of the active S-enantiomer tended to be higher than those of the inactive R-enantiomer after multiple dosing with the racemate (Figure 5-19 on page 5-237, v1.12). This difference appears to be due to a more rapid elimination of the R-enantiomer of vigabatrin from the rat brain (287 on page 5-8126, v1.33).

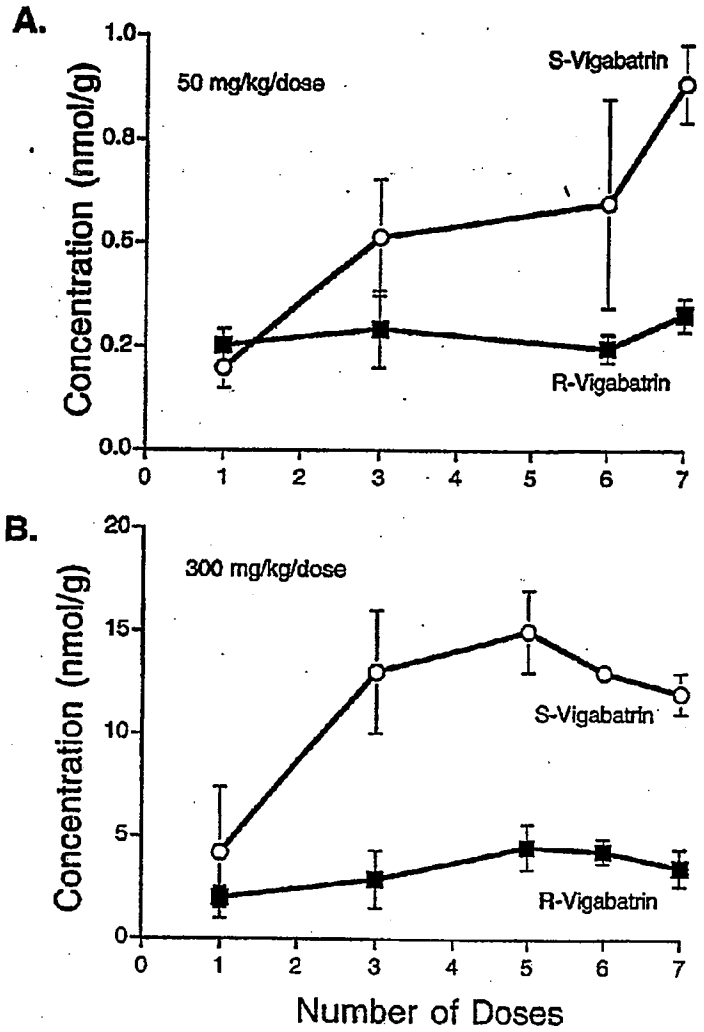


Figure 5-19. Levels of the R- and S-enantiomers of vigabatrin in rat brain following oral administration of either 50 mg/kg (A) or 300 mg/kg (B) racemic vigabatrin. Rats were given 1-7 daily oral doses of racemic vigabatrin. Twenty-four hours after the last dose the animals were killed and levels of the two enantiomers were measured by gas chromatography/mass spectroscopy. Data are expressed as the mean±SD of three determinations (Data from Reference 287 on page 5-8126, v1.33).

c). Concentration of Vigabatrin and Total GABA in the CSF of Male Rats and Dogs Following Repeated Dosing with Vigabatrin

Rats and dogs were administered daily oral doses of 0, 50, 100, and 200 (dog) or 300 (rat) mg/kg vigabatrin (289 on page 5-8170, v1.33, 290 on page 5-8189, v1.33). On study days 15, 29, and 112 (i.e., after 2, 4, and 16 weeks of dosing), CSF was collected. On study days 15 and 29, sampling occurred 2 and 24 hours after dosing; on study day 112, sampling occurred 0.5, 2, and 24 hours after dosing. CSF samples were assayed for levels of vigabatrin. In addition, most samples were also assayed for levels of total GABA.

In the rat and the dog, the concentration of vigabatrin in the CSF did not increase between study weeks 2 and 16. This confirms that steady state CSF levels of the drug are achieved in both species within two weeks of daily oral dosing. Representative data showing CSF levels of vigabatrin in the rat and dog 2 and 24 hours after administration of the drug for 2, 4, or 16 weeks can be found in Table 5-47 on page 5-239, v1.12. As can be seen, concentrations of vigabatrin in the CSF of the dog were considerably higher than those in the rat at all collection intervals. This suggests that vigabatrin may be cleared from the CSF of the rat more rapidly than from the CSF of the dog. As discussed below, vigabatrin is also cleared from the plasma of the rat more rapidly than from the plasma of the dog. Alternatively, it is possible that vigabatrin enters the CSF of the dog more readily than it does the CSF of the rat.

The 2 and 24 hour mean concentrations of total GABA in the CSF of the rat did not increase between weeks 2 and 16 at any of the three doses of vigabatrin studied. This indicates that, in this species, vigabatrin-induced changes in CSF levels of total GABA also reach steady state within two weeks of daily oral dosing with the drug. Within each dose level of vigabatrin that was evaluated, the mean concentration of total GABA remained constant over the entire 24 hour dosing interval. Representative CSF levels of total GABA in the rat determined two hours after administration of vigabatrin for 2, 4, or 16 weeks can be found in Table 5-48 on page 5-240, v1.12.

Table 5-47. Levels of Vigabatrin in Rat and Dog CSF Measured Two Hours and 24 Hours After Daily Administration of Vigabatrin for 2, 4, or 16 Weeks. (Data from References 289 on page 5-8170, v1.33 and 290 on page 5-8189, v1.33)

A. Rat

Dose (mg/kg/day)	Mean (±SD) Vigabatrin Concentration (nmole/g)					
	2 Weeks (n)		4 Weeks (n)		16 Weeks (n)	
	2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
50	4.75±2.27 (3)	0.39±0.16 (3)	3.42±1.63 (3)	ND	1.4±2.4 (3)	0.07±0.1 (2)
100	8.53±4.07 (3)	0.43±0.08 (3)	6.30±2.24 (3)	0.26±0.11 (3)	6.3±8.7 (4)	1.0±1.4 (3)
300	16.95±4.39 (3)	3.84±1.46 (3)	20.54±9.41 (3)	1.82±1.27 (3)	30.2±18.9 (3)	0.6±0.8 (6)

B. Dog

Dose (mg/kg/day)	Mean (±SD) Vigabatrin Concentration (nmole/g)					
	2 Weeks (n)		4 Weeks (n)		16 Weeks (n)	
	2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
50	74±9 (4)	29±8 (4)	66±17 (4)	13±10 (4)	76±6 (3)	12.5±11.5 (3)
100	147±75 (4)	94±19 (4)	165±94 (4)	91±5 (3)	273±99 (2)	92.8±57.7 (3)
200	393±120 (4)	386±203 (4)	209±74 (4)	372±288 (4)	380±95 (3)	204±56 (3)

ND = not detected (detection limit = 0.1 nmole/g)

In the dog, total GABA levels in the CSF were determined only in samples taken after 16 weeks of dosing with vigabatrin. Therefore, a comparison of total GABA concentrations across weeks cannot be done for this species. However, at the 16 week sampling interval (Table 5-48 on page 5-240, v1.12), the total GABA concentration remained fairly constant within each dose level of vigabatrin over the entire 24 hour sampling interval.

Table 5-48. Levels of Total GABA in CSF Measured Two Hours and 24 Hours After Daily Administration of Vigabatrin to Rats for 2, 4, or 16 Weeks and to Dogs for 16 Weeks. (Data from Reference 290 on page 5-8189, v1.33)

Dose Vigabatrin (mg/kg/day)	Mean (±SD) GABA Concentration (nmole/g)							
	Rat						Dog	
	2 Weeks (n)		4 Weeks (n)		16 Weeks (n)		16 Weeks (n)	
	2 hr	24 hr	2 hr	24 hr	2 hr	24 hr	2 hr	24 hr
0	4.4±0.2 (3)	4.3±0.8 (3)	4.1±0.9 (2)	2.7±0.6 (3)	4.0±1.6 (2)	3.2±0.5 (3)	0.6±0.3 (2)	0.4 (1)
50	11.4±1.9 (3)	11.7±1.8 (3)	9.6±0.9 (3)	11.1±3.1 (3)	13.3±0.9 (3)	12.0±1.0 (2)	4.3±2.1 (3)	1.8±0.8 (3)
100	12.5±1.3 (3)	13.2±2.3 (3)	13.1±0.4 (3)	14.1±4.4 (3)	15.8±1.4 (4)	14.3±1.8 (3)	7.0±0.9 (2)	6.7±2.0 (3)
200	ND	ND	ND	ND	ND	ND	13.4±(2.6) (3)	10.5±4.6 (3)
300	19.4±3.0 (3)	18.2±3.1 (3)	18.6±1.9 (3)	22.3±8.5 (3)	19.1±4.7 (3)	12.8±1.9 (6)	ND	ND

ND = Not Determined

d). Effect of Chronic Administration of Vigabatrin to Monkeys on Vigabatrin Levels and GABA Metabolism in the CNS

In a chronic toxicity study with vigabatrin, monkeys received doses of vigabatrin of 0, 50, 100, or 300 mg/kg/day (233 on page 5-4756, v1.24, 291 on page 5-8211, v1.33, 292 on page 5-8220, v1.33, 293 on page 5-8226, v1.34). The animals given 50 or 100 mg/kg/day were dosed for 6 years, while the animals given 300 mg/kg/day were dosed for 16 months. Six hours following the final dose of drug, a sample of the CSF was removed from the cisterna magna and was analyzed for levels of vigabatrin and total GABA. Animals were then immediately sacrificed, and a piece of cortex from the left temporal lobe was removed from each animal for biochemical studies. Although it would have been preferable to conduct all assays simultaneously, this was not practical due to the different lengths of treatment employed in this study.

CSF levels of vigabatrin in monkeys used in this study are summarized in Table 5-49 on page 5-241, v1.12. In both males and females, the level of vigabatrin in the CSF increased as the dosage of the drug increased. At the lower two doses, levels of vigabatrin in the CSF of females were significantly higher than those in males. This was not observed in the high dose group.

Table 5-49. CSF Concentrations of Vigabatrin Measured Six Hours After Chronic Dosing with the Compound in the Monkey. (Data from References 233 on page 5-4756, v1.24 and 291 on page 5-8211, v1.33)

Dose of Vigabatrin (mg/kg/day)	CSF Vigabatrin Levels (nmoles/mL)	
	Males	Females
50[a]	2.39±0.65 (3)	6.06±2.32 (3)
100[a]	7.04±2.48 (3)	11.41±4.48 (3)
300[b]	15.15±0.92* (2)	13.78±3.28 (3)

Data are expressed as the mean±SD.

The number of observations are shown in parentheses. Vigabatrin levels were determined using a gas chromatography/mass spectrometry method.

[a] Monkeys were dosed for six years

[b] Monkeys were dosed for 16 months

* Excludes data from one animal where CSF levels of vigabatrin were extraordinarily high due apparently to the presence of an interfering substance.

The effect of chronic vigabatrin administration on GABA metabolism in the CNS of monkeys is summarized in Table 5-50 on page 5-242, v1.12. In all three treatment groups, brain GABA-T activity was reduced by 30-37%. This produced an elevation in CSF levels of GABA of 20-34%. The significance of this small change in CSF levels of total GABA is unclear (rat, dog, man = 300-1000%), as free brain GABA levels were not consistently altered by the test compound and no dose-response was observed. Studies in mice and rats have indicated that a 50% or greater inhibition of brain GABA-T is required to produce significant elevations in brain GABA (301 on page 5-8334, v1.34, 302 on page 5-8339, v1.34). Brain GAD activity was essentially unchanged in the low-dose group, while being somewhat reduced in the upper two dose groups.

Table 5-50. Effect of Chronic Treatment with Vigabatrin in the Monkey on Brain GABA-T and GAD Activities, Free Brain GABA Levels, and Total CSF GABA Levels Measured Six Hours Postdose. (Data from References 233 on page 5-4756, v1.24 and 293 on page 5-8226, v1.34)

Dose of Vigabatrin (mg/kg/day)	Brain GABA-T Activity (% change)	Free Brain GABA Levels (% change)	Total CSF GABA Levels (% change)	Brain GAD Activity (% change)
50[a]	-30.5	+10.1	+32.8	+4.0
100[a]	-32.2	-4.4	+34.2	-21.0
300[b]	-37.0	+1.4	+20.5	-10.4

Results are expressed as the percent change from findings in corresponding control animals.

[a] Monkeys were dosed for six years

[b] Monkeys were dosed for 16 months

Levels of vigabatrin in the cerebral cortex were determined in monkeys dosed with 300 mg/kg of the compound for 16 months (292 on page 5-8220, v1.33). In these animals, tissue levels of vigabatrin ranged from 2.4-7.2 nmole/g. Cerebral cortical levels of vigabatrin were not determined in animals dosed with 50 or 100 mg/kg/day of the compound for 6 years.

iii. Summary of Distribution of Vigabatrin

Vigabatrin is widely distributed throughout the body, as would be expected given its hydrophilic nature and the fact that at physiological pH it is a zwitterion. The tissues that receive the greatest drug exposure following a single dose of ^{14}C-vigabatrin to the rat are the erythrocytes, stomach, and kidney. In the rat, the ^{3}H drug penetrates the brain, reaching steady state concentration after three days of daily oral dosing. In both the rat and the dog, steady state concentrations of ^{14}C-vigabatrin in the CSF are achieved within two weeks of daily oral dosing. At equivalent oral doses, the concentrations of vigabatrin found in the CSF of dogs after repeated drug administration were significantly higher than those of either the rat or the monkey. Vigabatrin-induced increases in CSF levels of GABA in the rat reach steady state within two weeks of daily oral dosing with the drug. In addition, in both rat and dog, GABA concentrations at each dose level of vigabatrin studied remained nearly constant (300-1000% over baseline) over the entire 24 hour dosing interval when the drug was administered once a day. In monkeys dosed chronically with vigabatrin, the level of the drug in the CSF increased as the dose of the drug increased. Vigabatrin (50-300 mg/kg) produced only 20-34% increase in CSF levels of total GABA, and only small and inconsistent changes in free brain GABA, which are of questionable significance.

c. Metabolism

Vigabatrin undergoes minimal biotransformation in all three species (279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32). This is evidenced by a comparison of the urinary recoveries of ^{14}C and unchanged vigabatrin over the 48 hour period following a single dose of 50 or 300 mg/kg ^{14}C-vigabatrin (Table 5-51 on page 5-243, v1.12). As can be seen, in all cases, the majority of the radioactivity excreted was in the form of parent compound.

Table 5-51. Urinary Recovery of Unmetabolized Vigabatrin and Radioactivity Over the 48 Hour Period Following A Single Dose of 50 or 300 mg/kg Radiolabeled-Vigabatrin to Rats, Dogs, and Monkeys. (Data from References 279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32)

Species	Dose (mg/kg)	Route	Mean Percent Urinary Recovery (0-48 Hours)	
			Parent Compound	Radioactivity (^{14}C)
Rat	50	PO	89.7±15.54[a]	85.1±4.3[a]
	50	IV	93.3±10.6	90.3±3.0
	300	PO	86.0±7.0	87.3±2.6
	300	IV	88.1±7.5	93.5±5.6
Dog	50	PO	67.6±7.7	81.8±4.4
	50	IV	72.5±4.0	85.2±2.6
	300	PO	85.0±6.7	85.0±4.5
	300	IV	77.2±9.5	84.3±2.0
Monkey	50	PO	28.4±4.2	37.0±3.1
	50	IV	81.5±6.4	91.4±1.9
	300	PO	13.7±4.4	18.8±3.2
	300	IV	89.1±3.0	93.0±1.1

[a] N=3; in all other cases, N=4

Data are expressed as the mean±SD.

Urinary metabolites have been evaluated by radiochromatographic profile (Figure 5-20 on page 5-244, v1.12). Small amounts of 1 or 2 metabolites have been detected in the rat, dog, and monkey. Metabolite #1 has not been identified. Metabolite #2, seen in both dog and human, has been identified as the vigabatrin lactam (5-vinyl-2-pyrrolidinone), which is also known to be a

b(4)

on page 3-40, v1.2). The two metabolites comprise less than 5% of the radioactivity found in human urine (C-92-0115-D).

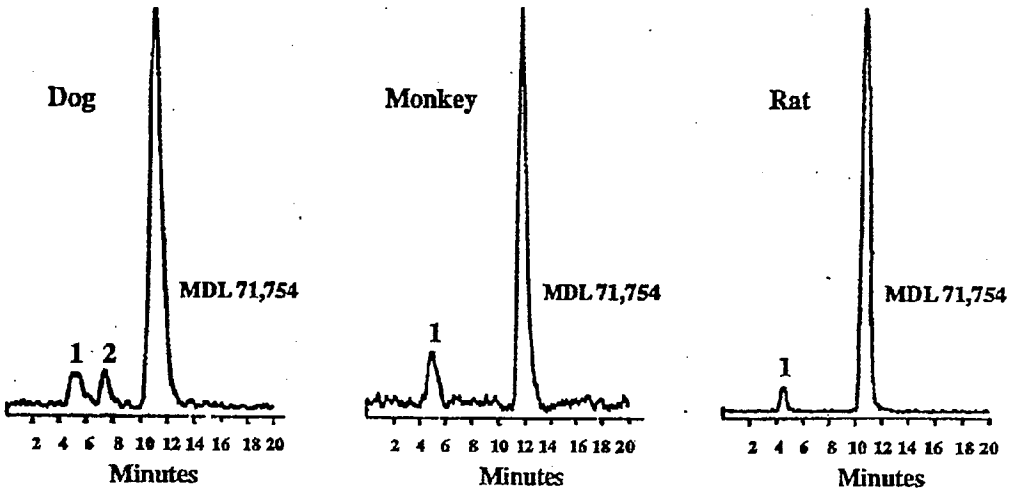


Figure 5-20. Typical radiochromatographic profiles of dog, monkey and rat urine collected 0-12 hours after a 50 mg/kg oral dose of (^{14}C) vigabatrin. Analyses were done by cation exchange HPLC with gradient elution.

d. Excretion

The majority of vigabatrin is excreted by the kidney via glomerular filtration. The terminal half-life, total plasma clearance (CLT), and renal clearance (CLR) of vigabatrin following single oral and IV doses of 50 and 300 mg/kg ^{14}C-vigabatrin to the rat, dog, and monkey have been determined (279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32) and are shown in Table 5-52 on page 5-245, v1.12.

Table 5-52. Half-Life, Plasma Clearance (CLT), and Renal Clearance (CLR) Values for Vigabatrin (Parent Compound) Calculated After a Single PO or IV Dose of 50 or 300 mg/kg. (Data from References 279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32)

Species	Dose (mg/kg)	Route	Terminal Half-Life (hours)	CLT (l/hr/kg)	CLR (l/hr/kg)
Rat	50	IV	1.26±0.21	0.99±0.14	0.92±0.18
	300	IV	1.43±0.92	1.00±0.18	0.88±0.09
	50	PO[a]	–	–	–
	300	PO	2.60±3.29	0.78±0.11	0.68±0.12
Dog	50	IV	2.40±0.43	0.24±0.03	0.19±0.01
	300	IV	3.36±1.73	0.23±0.03	0.18±0.02
	50	PO	2.23±0.23	0.27±0.05	0.18±0.04
	300	PO	3.36±0.75	0.28±0.08	0.23±0.05
Monkey[b]	50	IV	2.78±0.61	0.20±0.01	0.16±0.01
	300	IV	4.44±1.33	0.18±0.04	0.17±0.04

[a] Inadequate data to calculate parameters due to instrument difficulties during analysis.

[b] Kinetic parameters were not determined from the oral data due to inconsistencies in the elimination phase. Results are expressed as the mean±SD of four determinations

In the rat, the terminal half-life of vigabatrin in plasma was 1.3-1.4 hours after IV administration of 50 or 300 mg/kg of the drug. When given orally, the average half-life following a single 300 mg/kg dose of vigabatrin appeared to be somewhat longer than that determined after IV administration of the drug (Table 5-52). However, examination of data from individual animals revealed that this was due to an extremely long half-life for vigabatrin determined in one of the four rats used in this study. Excluding this one possibly aberrant value, the mean plasma half-life of vigabatrin after an oral dose of 300 mg/kg to the rat was calculated to be about 0.95 hours. Terminal elimination half-lives for ^{14}C in plasma were not calculated, but appeared to be significantly longer than those of the parent compound. As noted above, the terminal elimination half-life for ^{14}C following a 60 mg/kg oral dose of ^{14}C-vigabatrin in the tissue distribution study in Sprague-Dawley rats was calculated to be 25 hours (286 on page 5-8093, v1.33). In the dog, the terminal half-life of vigabatrin was about two hours after the 50 mg/kg dose, and three hours after the 300 mg/kg dose. As was seen in the rat, the terminal elimination half-life for radioactivity in the plasma of the dog appeared to be greater than that of the parent compound. In the monkey, the terminal half-life for vigabatrin could be determined only after IV dosing, and was found to be 2.8 hours after the low dose, and 4.4 hours after the high dose. However, in an

earlier study in monkeys (284 on page 5-8049, v1.32), the terminal half-life following a single 50 mg/kg PO dose of vigabatrin was calculated to be about seven hours, while the corresponding value after a dose of 260 mg/kg PO was about 12 hours.

Plasma and renal clearance values for vigabatrin following oral and IV dosing were calculated for each of the three species, and are also shown in Table 5-52 on page 5-245, v1.12. As can be seen, the rat clears vigabatrin from plasma more rapidly than do the other two species. Clearance of vigabatrin from the CSF also appears to occur more rapidly in the rat than in the dog (see above). The data in Table 5-52 on page 5-245, v1.12 also show that renal clearance is the main mechanism for eliminating vigabatrin from the circulation of the rat, dog, and monkey, comprising at least 80% of total plasma clearance. Values for renal clearance of vigabatrin in the rat were not significantly different from inulin renal clearance values in the same substrain (279 on page 5-7789, v1.32). Similarly, renal clearance of vigabatrin in dogs and monkeys are not significantly different from the estimated glomerular filtration rate in each of these species (297 on page 5-8282, v1.34, 300 on page 5-8328, v1.34). Consequently, it appears that renal elimination of vigabatrin occurs almost exclusively via glomerular filtration, with tubular secretion contributing little, if any, to the renal elimination of the drug.

Excretion of radioactivity in the feces was also monitored in rat, dog and monkey (279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32, 282 on page 5-7884, v1.32) Over the 120 hour period following IV dosing, mean fecal elimination of radioactivity at both 50 and 300 mg/kg ^{14}C-vigabatrin ranged from 0.7-1.1% of total dose in the rat and the monkey, and 3.9-4.2% of total dose in the dog. Following oral dosing, fecal elimination of radioactivity in the rat and the dog at both doses of ^{14}C-vigabatrin studied ranged from 3.7-7.0% of total dose. In monkey, however, 50% of the radioactivity in the low dose and 70% of the radioactivity in the high dose of ^{14}C-vigabatrin was excreted in the feces. These findings support the conclusion that orally administered vigabatrin is well absorbed in the rat and the dog, but poorly absorbed in the monkey.

4. Protein Binding

The protein binding of vigabatrin was determined in human serum using equilibrium dialysis (294 on page 5-8238, v1.34). At both concentrations of drug tested (20 and 100 μM), no binding to serum proteins was detected.

5. Enzyme Induction

a. Effect on Cytochrome P-450

Rats (six per group) were administered oral doses of 30, 100, or 300 mg/kg/day vigabatrin for four days (295 on page 5-8243, v1.34). Twenty-four hours after the last dose, livers were collected and the following were measured: cytochrome P-450, ethylmorphine N-demethylase, aniline hydroxylase, recoverable microsomal protein, and relative liver weights. The protocol was repeated three times.

In the first experiment, hepatic cytochrome P-450 was slightly decreased at all doses, to values ranging from 72-81% of control. At the top dose (300 mg/kg/day), the activities of ethylmorphine N-demethylase and aniline hydroxylase were decreased to 64.0% and 71.9% of control, respectively. In the second experiment, the relative liver weights were decreased at 300 mg/kg/day (to 90.2% of control) and aniline hydroxylase activities were increased at 100 and 300 mg/kg/day (to 124.8% and 142.5% of control, respectively). In the third experiment, a similar decrease in relative liver weight was observed, but this time aniline hydroxylase activity was decreased at both 100 mg/kg/day (to 77.2% of control) and 300 mg/kg/day (to 73.2% of control). In addition, cytochrome P-450 concentrations were less than controls at 100 mg/kg/day (83.3%) and 300 mg/kg/day (85.6%).

Finally, using four rats per group, the effects of single 30 and 100 mg/kg oral doses of vigabatrin on cytochrome P-450 drug metabolizing enzymes and recoverable microsomal protein were assessed four hours after drug administration. In this study, no differences from controls were noted.

These results suggest that vigabatrin does not induce hepatic drug metabolizing enzymes. There were some decreases in enzymatic activities, but these were variable, and the mechanism responsible for this effect is not known.

2. Table of Metabolism Studies

Table 5-41 (Page 1 of 4). Table of Metabolism Studies.

Species/Strain/Sex (n)	Dose (mg/kg)	Route	Comment	Reference
ABSORPTION AND DISPOSITION OF [^{14}C] VIGABATRIN				
Rat/Sprague-Dawley/Male (4/group)	50, 300	Oral, I.V.	Based on the ratio of oral to I.V. urinary ^{14}C recoveries, absorption was 92-93%. Elimination was independent of dose and was primarily by renal excretion of unchanged drug. Elimination half-lives averaged 1.4 hours in the I.V. studies. Biotransformation of only a small fraction of the dose was evident from HPLC profiles of urinary radioactivity. The steady-state volume of distribution following I.V. administration averaged 0.95 L/kg, which exceeds the total body water of the rat.	279 on page 5-7789, v1.32
Dog/Beagle/Male (1/group/route)	50, 300	Oral, I.V.	Based on the ratio of oral to I.V. urinary ^{14}C recoveries, absorption was 96% for the low dose and 101% for the high dose. Absorption and elimination were independent of dose and renal excretion of unchanged drug was the major route of elimination. Mean elimination half-lives were 2.8 and 2.9 hours following oral and I.V. administration. Biotransformation of approximately 2-15% of the dose was estimated based upon differences between urinary ^{14}C and VGB excreted in 120 hours. The steady-state volume of distribution calculated from I.V. data averaged 0.55 L/kg, similar to the total body water of the dog.	280 on page 5-7825, v1.32
Monkey/Cynomolgus/Male (1/group/route)	50, 300	Oral, I.V.	Based on the ratio of oral to I.V. urinary ^{14}C recoveries, absorption means (±SD) were 44±4% and 25±7% of the 50 and 300 mg/kg doses, respectively. Absorption appeared dose-dependent since there was a significant decrease in urinary recoveries of ^{14}C after oral but not I.V. doses. Elimination after I.V. administration was dose-independent with a mean elimination half-life of 3.6±1.3 hours. Oral elimination half-lives were not adequately defined in this study. Mean steady-state volume of distribution was 0.50±0.10, suggesting that VGB is distributed to total body water.	281 on page 5-7853, v1.32
Rat/Sprague-Dawley/Male Dog/Beagle/Male Monkey/Cynomolgus/Male	50, 300	Oral, I.V.	Report of interim results from the studies cited above (Reference Nos. 279 on page 5-7789, v1.32, 280 on page 5-7825, v1.32, 281 on page 5-7853, v1.32).	282 on page 5-7884, v1.32

Table 5-41 (Page 2 of 4). Table of Metabolism Studies.

Species/Strain/Sex (n)	Dose (mg/kg)	Route	Comment	Reference
ANALYTICAL METHOD DEVELOPMENT IN PLASMA AND URINE, AND PRELIMINARY PHARMACOKINETIC STUDIES IN DOGS AND HUMANS				
Dog/Beagle/Female (1/route) Human/Male (2/group)	50 500, 1000, 3000 (total dose)	Oral, I.V. Oral	An HPLC method for the quantitative measurement of VGB in plasma and urine was developed and applied to preliminary PK studies in dogs and humans. Five minutes after I.V. dosing in dogs, the plasma concentration was 138 μg/mL. Oral dose peak concentration of 77 μg/mL occurred at 1 hour. A β-phase half-life of about 2 hours was calculated for the IV data. In humans, mean AUCs for the 500, 1000 and 3000 mg doses were 59.5, 132 and 378 μg-hr/mL, respectively. The kinetics appeared linear over this dose range. Absorption was rapid. The disposition half-life was 4.9 hours. Average urinary recovery was 75% of the dose for all doses. Renal clearance results suggested glomerular filtration as the most likely mode of excretion.	283 on page 5-7907, v1.32
Monkey/Cynomolgus/Male (2/group)	50, 260	Oral	Renal excretion accounted for 20% and 15% of the 50 and 260 mg/kg doses, respectively. Absorption was dose-dependent, but plasma elimination was not affected by dose. Terminal disposition half-lives indicated that elimination pathways were not saturated.	284 on page 5-8049, v1.32
Monkey/Cynomolgus/Male and Female (5/sex/group)	0, 50, 100	Oral	Refer to Toxicology Reference Number 232 on page 5-4476, v1.24.	232 on page 5-4476, v1.24
Monkey/Cynomolgus/Male and Female (3/sex/group)	0, 50, 100	Oral	Refer to Toxicology Reference Number 233 on page 5-4756, v1.24	233 on page 5-4756, v1.24
Monkey/Cynomolgus/Male and Female (2/sex/group)	0, 500, 750, 1000	Oral	Refer to Toxicology Reference Number 227 on page 5-3925, v1.22	285 on page 5-8070, v1.33
Rat/Sprague-Dawley/Male (33) Rat/Long-Evans/Male (33)	60	Oral	Approximately 93% of the dose was absorbed and excreted in the urine of Sprague-Dawley (SD) and Long-Evans (LE) rats; renal elimination half-lives were 17 hours in SD and 21 hours in LE. Highest tissue concentrations were in RBCs, stomach, kidney, liver, spleen and lung. Concentrations in skin and eyes were 2 - 3-fold greater in pigmented LE rats. Half-life elimination from lipophilic organs was 1.2 - 15 hours; elimination from remaining tissues was biexponential with terminal half-lives between 16 and 95 hours. Plasma terminal elimination half-lives averaged 25 hours and 30 hours for SD and LE rats, respectively.	286 on page 5-8093, v1.33

Table 5-41 (Page 3 of 4). Table of Metabolism Studies.

Species/Strain/Sex (n)	Dose (mg/kg)	Route	Comment	Reference
Rat/Sprague-Dawley/Male (3/group)	50, 300	Oral	Renal excretion of unchanged drug was 83% of the low dose and 92% of the high dose. Recovery of R- and S-enantiomers from urine was equal at the low dose. Apparent steady-state was observed in brain after 3 daily doses and brain concentrations of the active S-enantiomer exceeded the inactive R-enantiomer. The elimination rate constant of R in brain was almost twice that of S. Brain concentrations increased linearly 4 hours after the last dose, but a greater than proportional increase was observed 24 hours after the last dose.	287 on page 5-8126, v1.33
In Vitro	NA	NA	This report describes development of an analytical procedure (GC/MS) to determine and quantitate R- and S-enantiomers of VGB in human urine, plasma and cerebrospinal fluid (CSF).	288 on page 5-8152, v1.33
Rat/Sprague-Dawley/Male (128 in 4 groups) Dog/Beagle/Male (28 in 4 groups)	0, 50, 100, 300 0, 50, 100, 200	Oral Oral	This study was done to determine if steady-state concentrations of VGB in rat and dog CSF would be reached within two weeks following oral administration. Steady-state concentrations were achieved in both species within two weeks, but concentrations were greater in the dog. Mean steady-state concentrations two hours after administration increased linearly with dose. As dose increased, a greater than proportional increase was evident in the dog 24 hours after treatment. Clearance from CSF was greater in the rat than in the dog.	289 on page 5-8170, v1.33
Rat/Sprague-Dawley/Male (128 in 4 groups) Dog/Beagle/Male (28 in 4 groups)	0, 50, 100, 300 0, 50, 100, 200	Oral Oral	This report is a continuation through 2, 4 and 16 weeks of the study cited above (Reference No. 289 on page 5-8170, v1.33). Its purpose was to measure GABA concentrations in rat CSF at 2, 4 and 16 weeks and in dog CSF at 16 weeks. Steady-state concentrations of GABA were achieved in CSF sometime during the first two weeks of dosing. In both species, within each dose level, GABA concentrations remained relatively constant over the 24-hour dosing interval. Mean concentrations of VGB in CSF at 16 weeks were the same as at 2 and 4 weeks.	290 on page 5-8189, v1.33
Monkey/Cynomolgus/Male and Female (3/sex)	0, 300	Oral	Mean VGB concentration in CSF following 16 months of oral treatment with 300 mg/kg/day (Toxicology Study Ref. No. 229 on page 5-4101, v1.23) was 20.3, 22.2 and 24.8 nmol/mL, respectively, as determined by HPLC, ion exchange and GC/MS.	291 on page 5-8211, v1.33

Table 5-41 (Page 4 of 4). Table of Metabolism Studies.

Species/Strain/Sex (n)	Dose (mg/kg)	Route	Comment	Reference
Monkey/Cynomolgus/Male and Female (1 male, 2 female) (3/sex)	0 300	Oral	VGB concentrations in brain cortex and CSF were determined following 16 months of oral treatment with 300 mg/kg/day (Toxicology Study Ref. No. 230 on page 5-4233, v1.23). Mean CFS concentration was 13.17 nmol/mL; mean concentration in cortex was 4.2 nmol/g.	292 on page 5-8220, v1.33
Monkey/Cynomolgus/Male and Female (1 male, 2 female) (3/sex)	0 300	Oral	GABA-transaminase activity in cerebral cortex was decreased following 16 months oral treatment with 300 mg/kg/day, whereas glutamate decarboxylase activity was not (Toxicology Study Ref. No. 230 on page 5-4233, v1.23). Brain and CSF GABA concentrations were not elevated.	293 on page 5-8226, v1.34
In Vitro	NA	NA	VGB did not bind to any components of human serum.	294 on page 5-8238, v1.34
N/A	0, 30, 100, 300	Oral	This study evaluated the effects of VGB on cytochrome P-450 dependent drug metabolizing enzymes in male Sprague-Dawley rat liver. No stimulation of the enzymes occurred.	295 on page 5-8243, v1.34
NA	NA	NA	Human exposure to [^{14}C] VGB was estimated from rat, dog and monkey data. Predicted whole body and tissue exposures in a 50 μCi oral dose of [^{14}C] VGB are less than 0.4% of the 3-5 rem limits specified by the Code of Federal Regulations.	296 on page 5-8260, v1.34

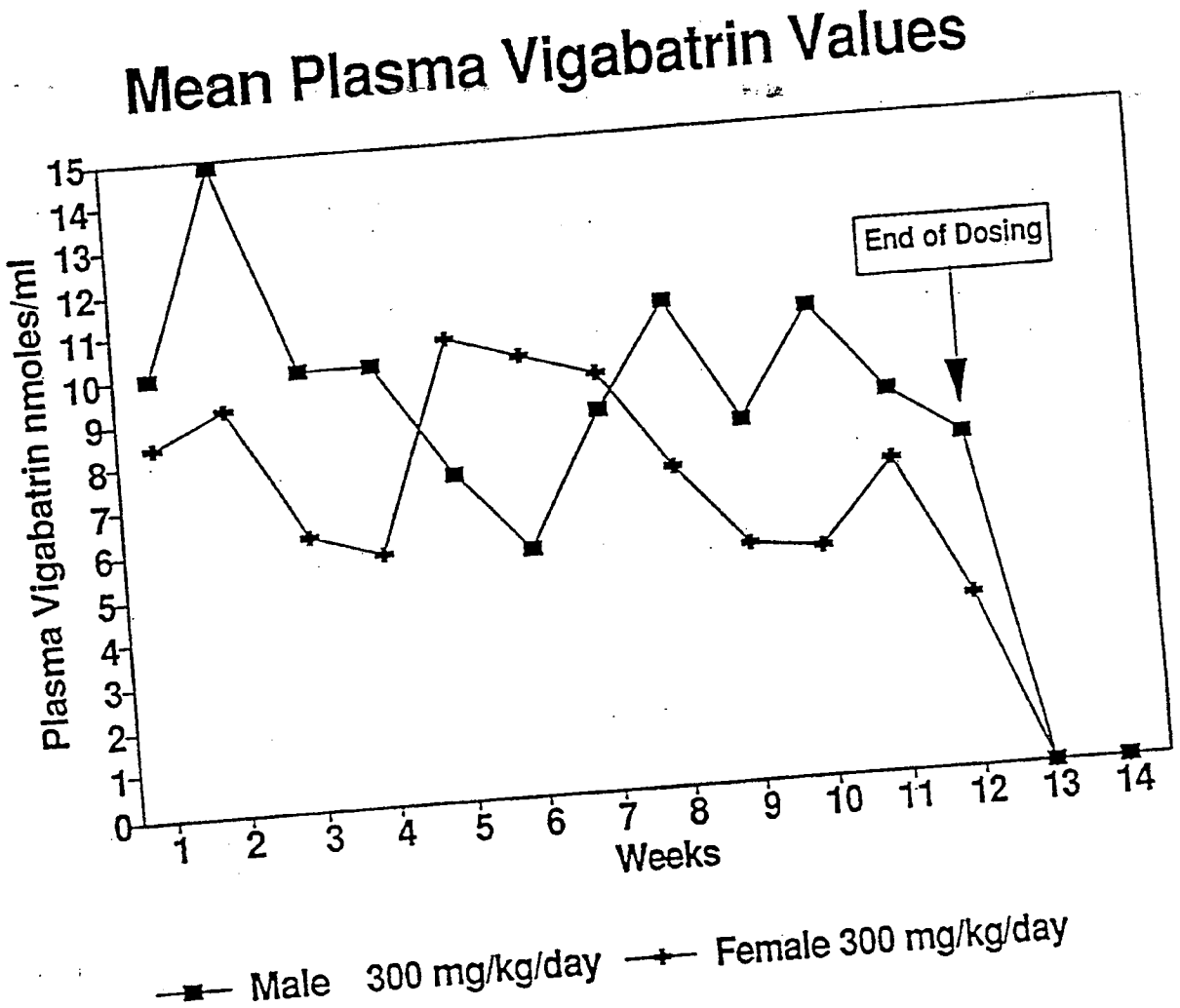
3a

Dog

Figure 7

Vigabatrin (MDL 71,754): Sequential Neuropathology Study In Beagle Dogs During Twelve Weeks of Treatment With 300 mg/kg/day and Sixteen Weeks of Recovery

24 hr. post-dose



36

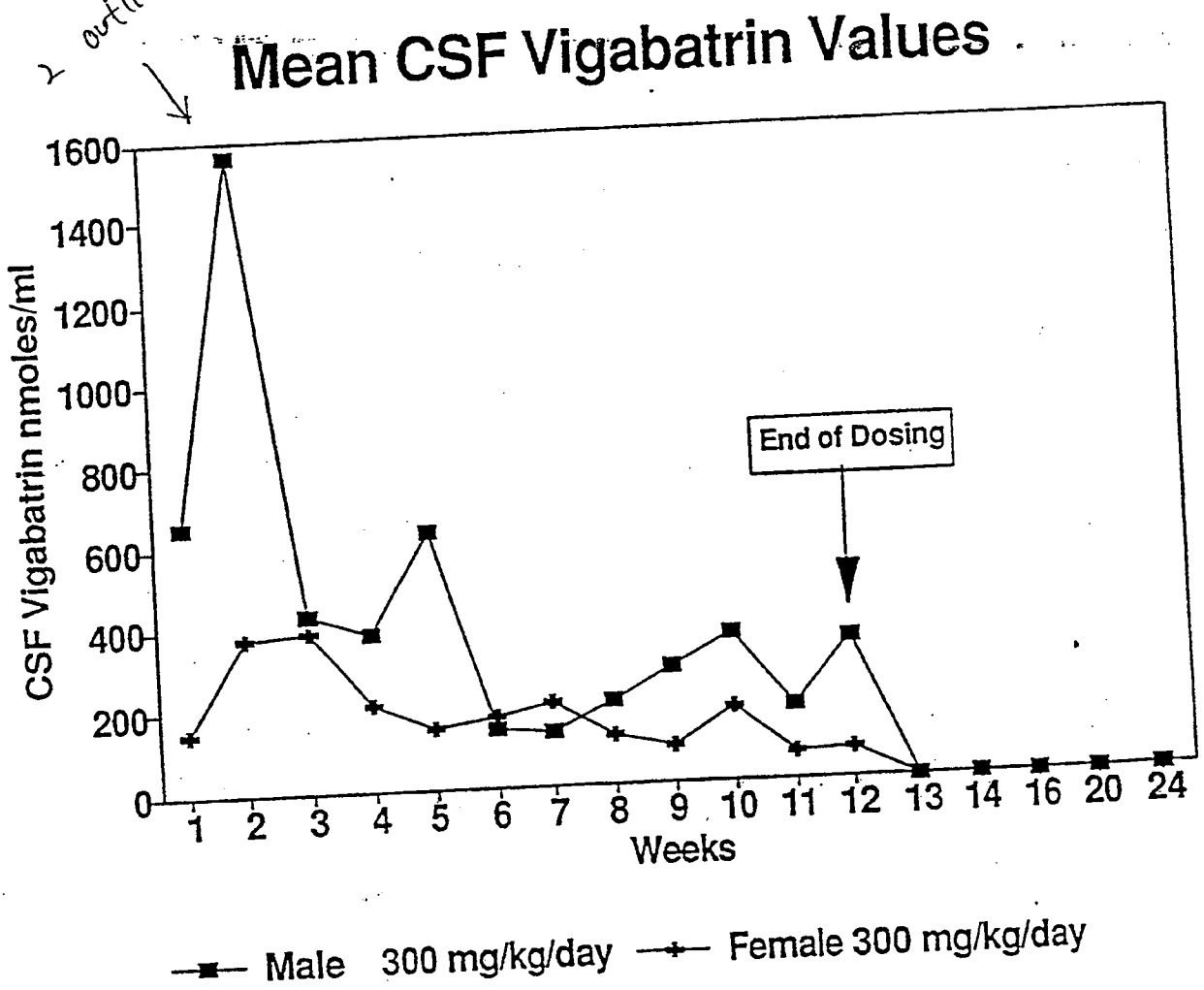
DOG

Figure 8

Vigabatrin (MDL 71,754): Sequential Neuropathology Study In Beagle Dogs During Twelve Weeks of Treatment With 300 mg/kg/day and Sixteen Weeks of Recovery

-24 hr. post-dose

2 outliers ?



DOG

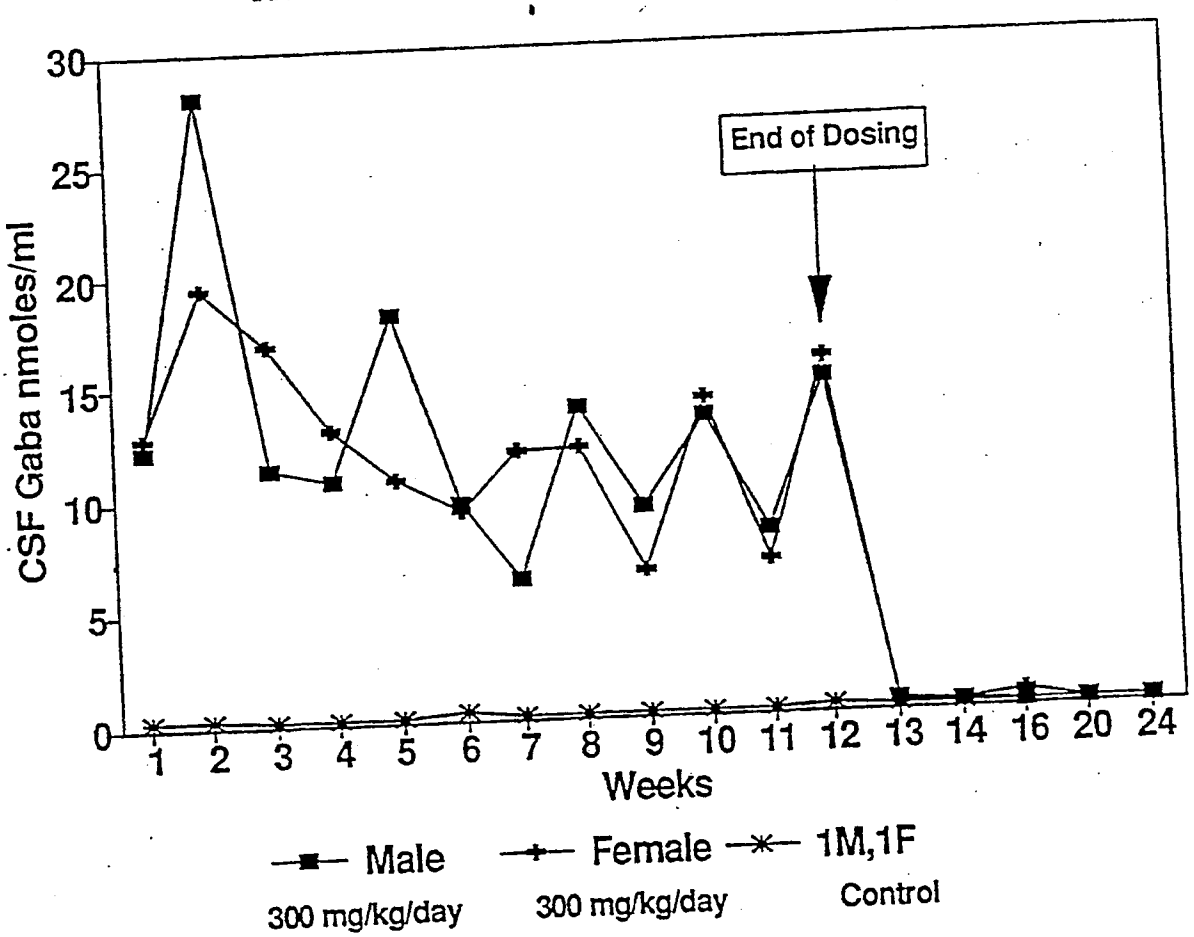
3c

Figure 9

Vigabatrin (MDL 71,754): Sequential Neuropathology Study In Beagle Dogs During Twelve Weeks of Treatment With 300 mg/kg/day and Sixteen Weeks of Recovery

TOTAL

24 hr. post dose



DOG

3d

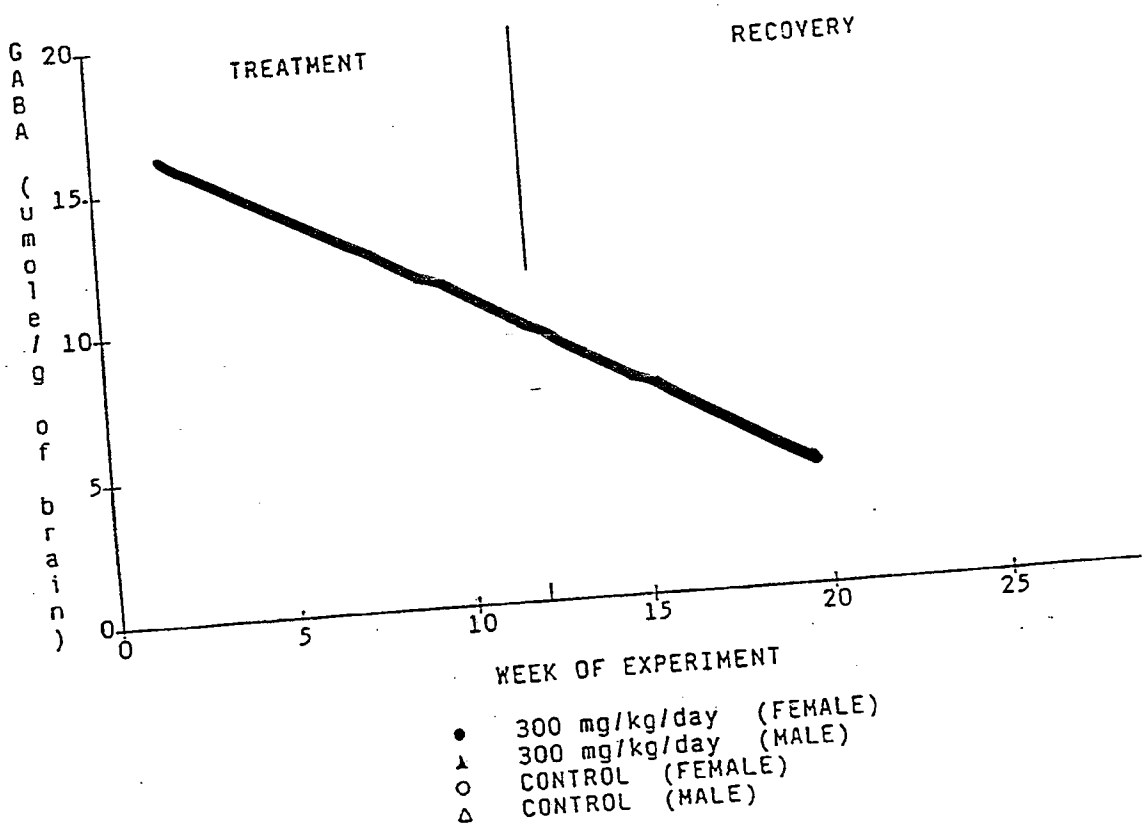
Figure 10

Vigabatrin (MDL 71,754): Sequential Neuropathology Study In Beagle Dogs During Twelve Weeks of Treatment With 300 mg/kg/day and Sixteen Weeks of Recovery

TOTAL

24 hr post dose ?

INDIVIDUAL DOG BRAIN GABA LEVELS



b(4)

3e

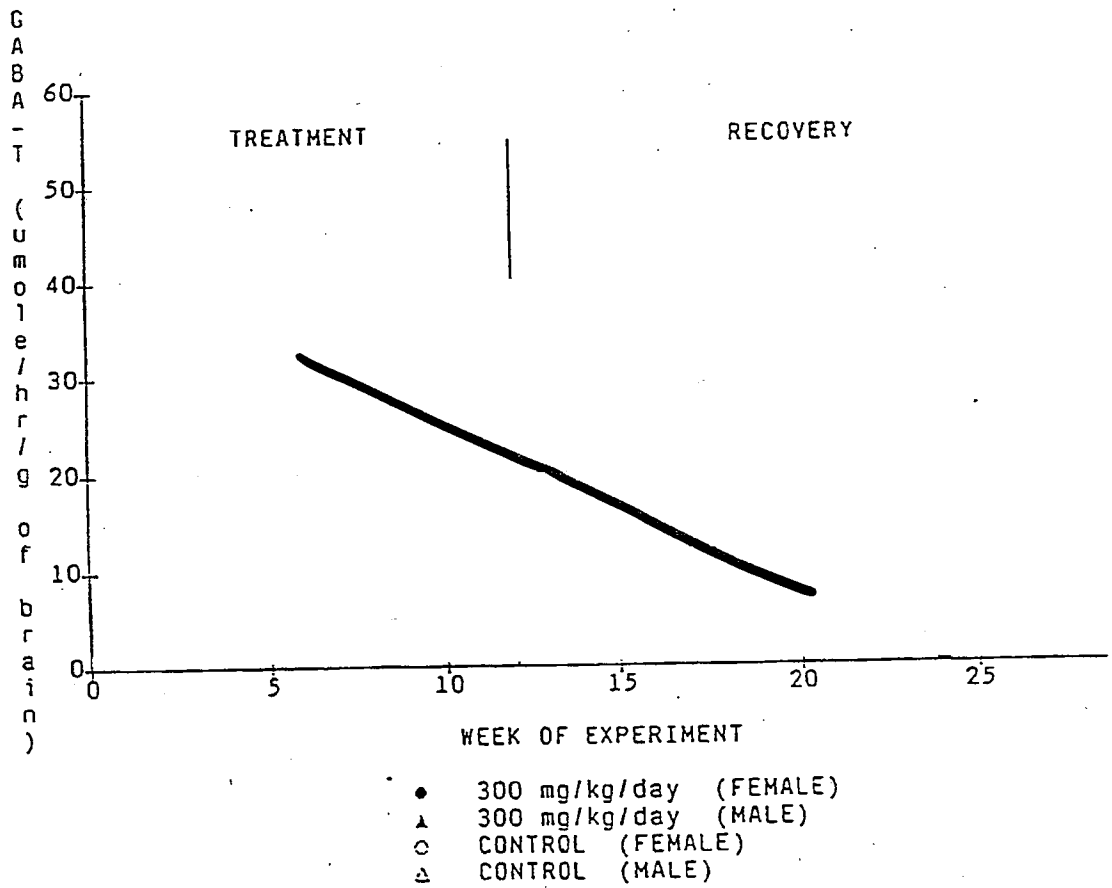
DOG

Figure 11

Vigabatrin (MDL 71,754): Sequential Neuropathology Study In Beagle Dogs During Twelve Weeks of Treatment With 300 mg/kg/day and Sixteen Weeks of Recovery

INDIVIDUAL DOG BRAIN GABA-T LEVELS

24 hr. post-dose ?



b(4)

DOG

3f

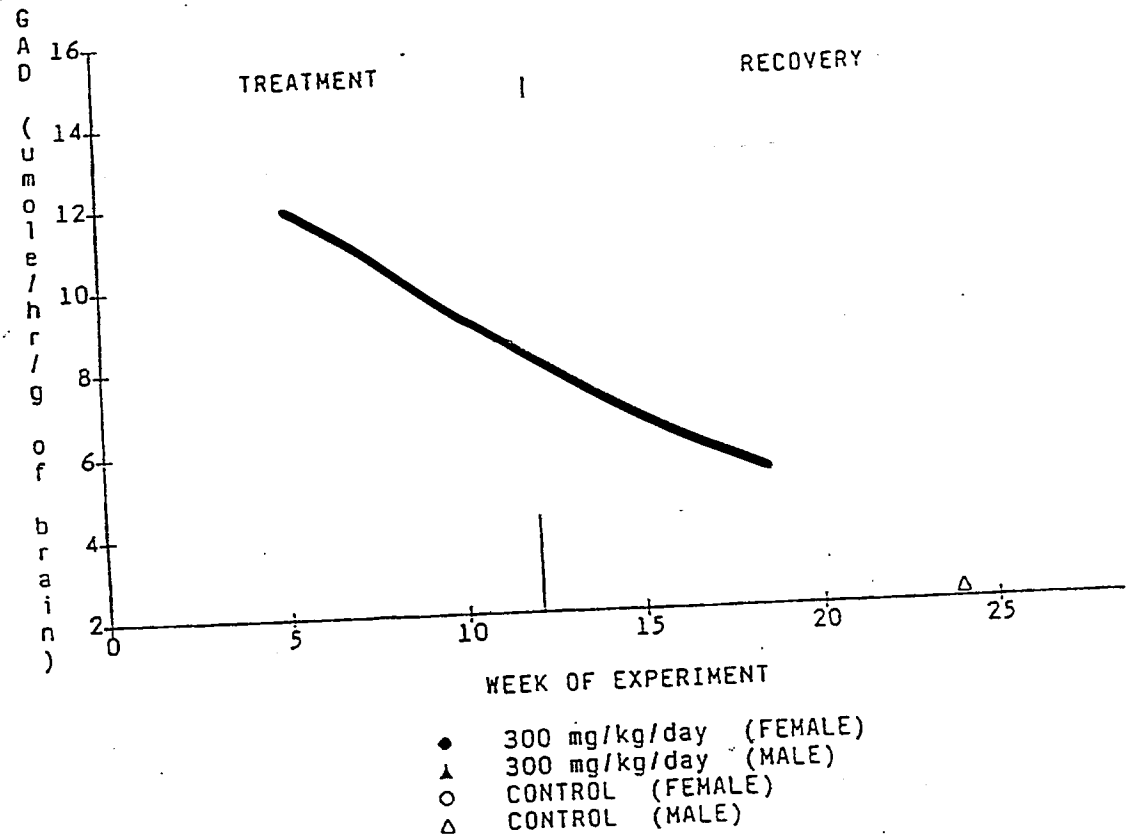
Figure 12

Vigabatrin (MDL 71,754): Sequential Neuropathology Study In Beagle Dogs During Twelve Weeks of Treatment With 300 mg/kg/day and Sixteen Weeks of Recovery

24 hr post dose ?

INDIVIDUAL DOG BRAIN GAD LEVELS

b(4)



Page 4

ACUTE TOXICITY:

1) LD 50 values

SPECIES	STRAIN/SEX/WEIGHT (g)	ROUTE	LD 50 (mg/kg)
RAT (FASTED)	SD/M+F/64-95	P.O.	3100
		I.P.	1473
MOUSE (FASTED)	CD-1/M+F/12-20	P.O.	2830
		I.P.	1098
MOUSE (NON-FASTED)	CD-1/M/16-23	I.P.	3000
MOUSE (NON-FASTED)	CD-1/M+F/15-25	I.P.	2447

2) Observed signs

Dose-related sedation occurred at all doses used in both species by both routes (lowest doses used = 2500 mg/kg p.o. and 100 mg/kg I.P.). The sedation was quite long-lasting (3-4 days in survivors, or to time of death). In one I.P. study in non-fasted mice, catatonia and hunched posture with splayed paws was seen at the higher doses; no excitation or convulsions were noted. In the other I.P. study in non-fasted mice, however, convulsions were seen on day 2 in 2/10 at 3000 mg/kg and in 1/10 at 3500 mg/kg. Piloerection was seen in rats only, at all doses. Deaths were generally delayed with most occurring within 4 days post-dosing. No specific gross pathological effects attributable to drug were noted. No sex differences in susceptibility to drug toxicity were apparent. The drug is apparently significantly less toxic when given to non-fasted animals, at least by the I.P. route. (It should be noted however, that the non-fasted mice were slightly older, suggesting a possible age factor). In non-fasted mice, no deaths occurred at 1500 mg/kg and below after I.P. administration, whereas the I.P. LD 50 in fasted mice was 1098 mg/kg.

18 MONTH CARCINOGENICITY STUDY IN MICE

A) Dosage

50/sex at 0, 50, 100, or 150 mg/kg/day, in diet

Strain: CD-1 (ICR) BR, [redacted] b(4)

Lot #: 71,754-49 (days 1-455)
71,754-79 (DAYS 456-554)

B) Results

1) Observed signs

a) Convulsions (not further described) seen in 2 MD M and 9 HD M. Not seen until 10th month. Seen on 1-2 days each in all animals except 1 HD (6 days). (No convulsions were seen in a one month dietary rangefinding study which used doses up to 500 mg/kg).

b) Nothing was stated regarding routine palpation for masses.

2) Mortality

There were slightly more deaths in all treated groups compared to controls; this was not D-R, and not statistically significant by the sponsor's analysis. The excess deaths in F was most apparent early in the study. Overall survival was about 80-90% in M and 65-75% in F. (See attached table and figures).

3) Bodyweight

According to the text, weight gain was decreased at MD and HD with final weight in HD M and HD F 86% and 94% of control, resp.; no data shown aside from attached graphs.

4) Food Consumption

It was stated that food consumption was "similarly but less noticeably affected" [than bodyweight]; presumably implying a slight decrease at MD and HD; no data shown aside from attached graphs (in which it is difficult to clearly discern any drug effects). (In a one month dietary rangefinding study, no effects on food consumption were seen up to 500 mg/kg despite decreases in weight gain).

5) Lab tests

Not performed

6) Organ weights

Not performed

7) Gross pathology

No drug effects

8) Microscopic pathology

(Routine exam done at all doses)

a) Non-neoplastic lesions

1) Brain

Increase in incidence of vacuolation in sites as shown in attached table, in males only, in some cases at all doses but generally not clearly D-R. The severity of the vacuolation in males (not shown in table) was generally minimal or very slight and was not clearly drug-related. There was no clear drug effect on vacuolation in F (note that female control incidence values were greater than male treated values); however several MD and HD F (as well as several MD and HD M) had foci of mineralization in the area of the cerebellar roof nuclei, which was considered to be evidence of residual damage. (In a 1 month dietary rangefinding study at 100, 200, 300, and 500 mg/kg, cerebellar and/or midbrain vacuolation were seen at 200 mg/kg and above in F and at all doses in M; none in controls.)

There were no drug effects on sciatic nerve either in this study (see table) or in the rangefinding study.

2) Eye

As shown in the attached table, the incidence of "retinal degeneration" appeared to be increased in F (1/47, 3/38. 4/39, 8/39 in C, LD, MD, and HD, resp.). It was described in the text as "mild", involving "focal loss of rods and rod nuclei from outer retinal layers". It was stated that "this lesion was somewhat similar to that previously reported in albino rats treated with Vigabatrin". (Note that the finding of "outer nuclear layer dysplasia" appeared to be <u>decreased</u> in treated groups of both sexes; this was not discussed in the text). (There were no findings in eye in the 1 month dietary rangefinding study [HD = 500 mg/kg]).

b) Neoplastic lesions

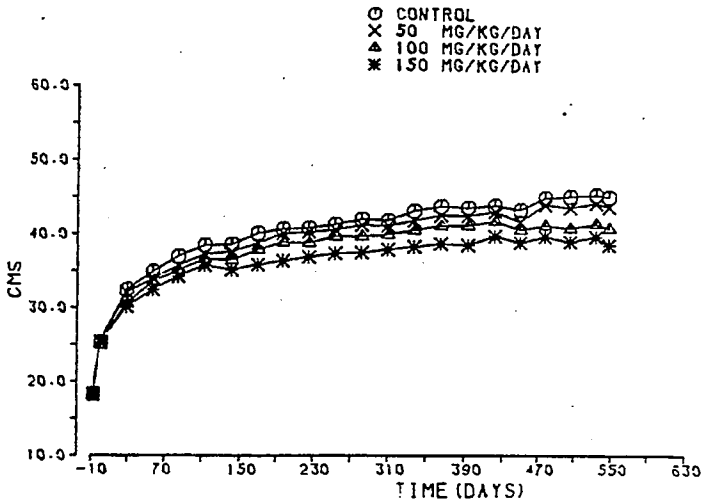
No drug related increases. (The incidence of total primary and total benign tumors appeared to be decreased in the drug groups. [See attached table]).

CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

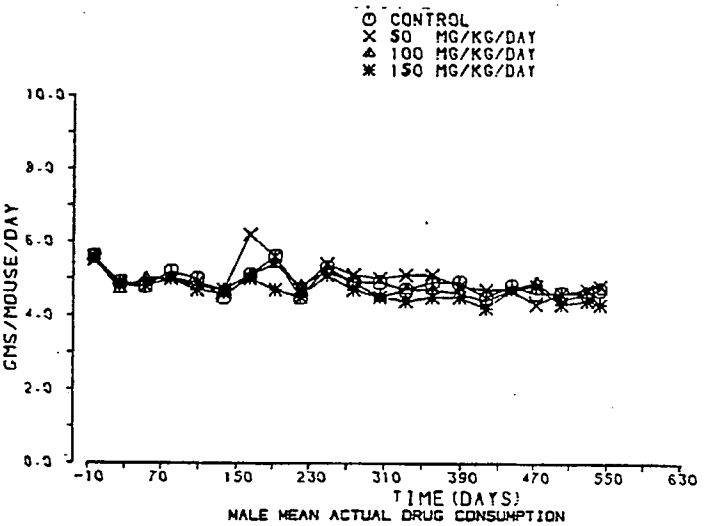
~~300C-11~~
~~7~~

Plate 1

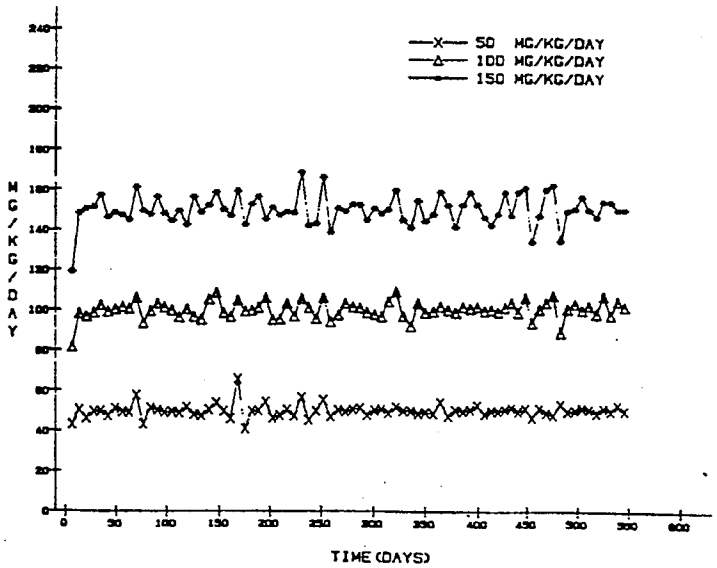
MALE MEAN BODY WEIGHTS (GMS)



MALE MEAN FOOD CONSUMPTION



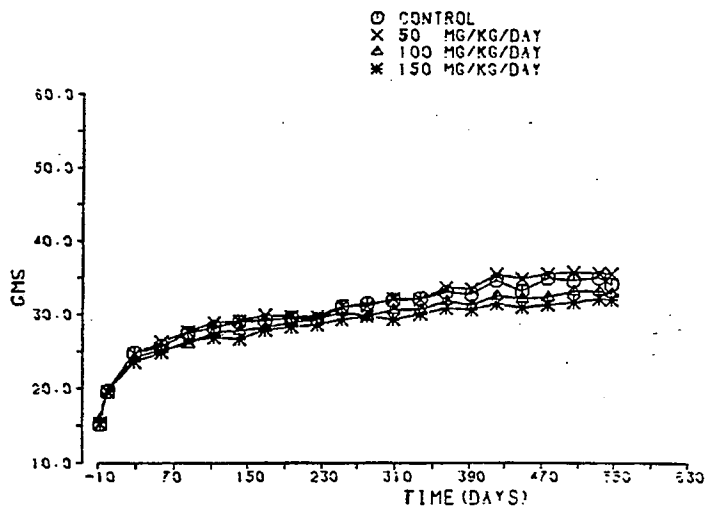
MALE MEAN ACTUAL DRUG CONSUMPTION



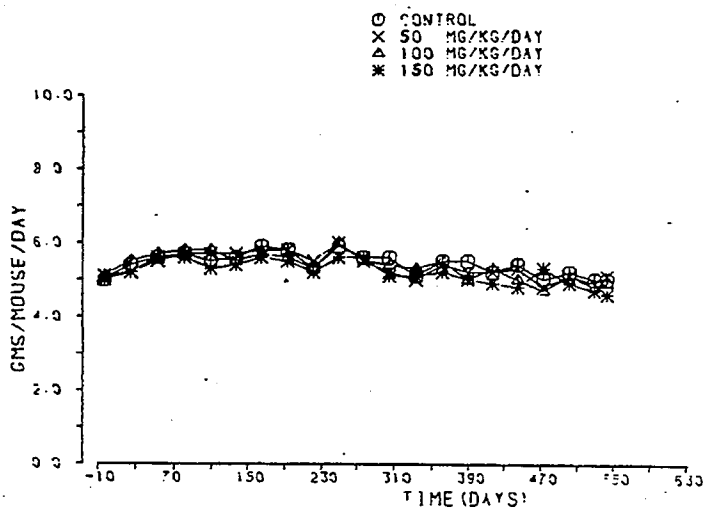
CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Plate 2

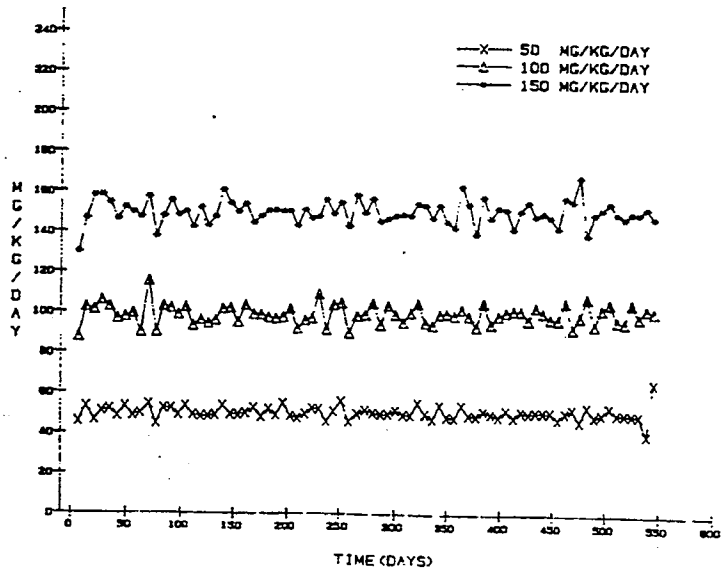
FEMALE MEAN BODY WEIGHTS (GMS)



FEMALE MEAN FOOD CONSUMPTION



FEMALE MEAN ACTUAL DRUG CONSUMPTION



CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 2

Deaths by Months*

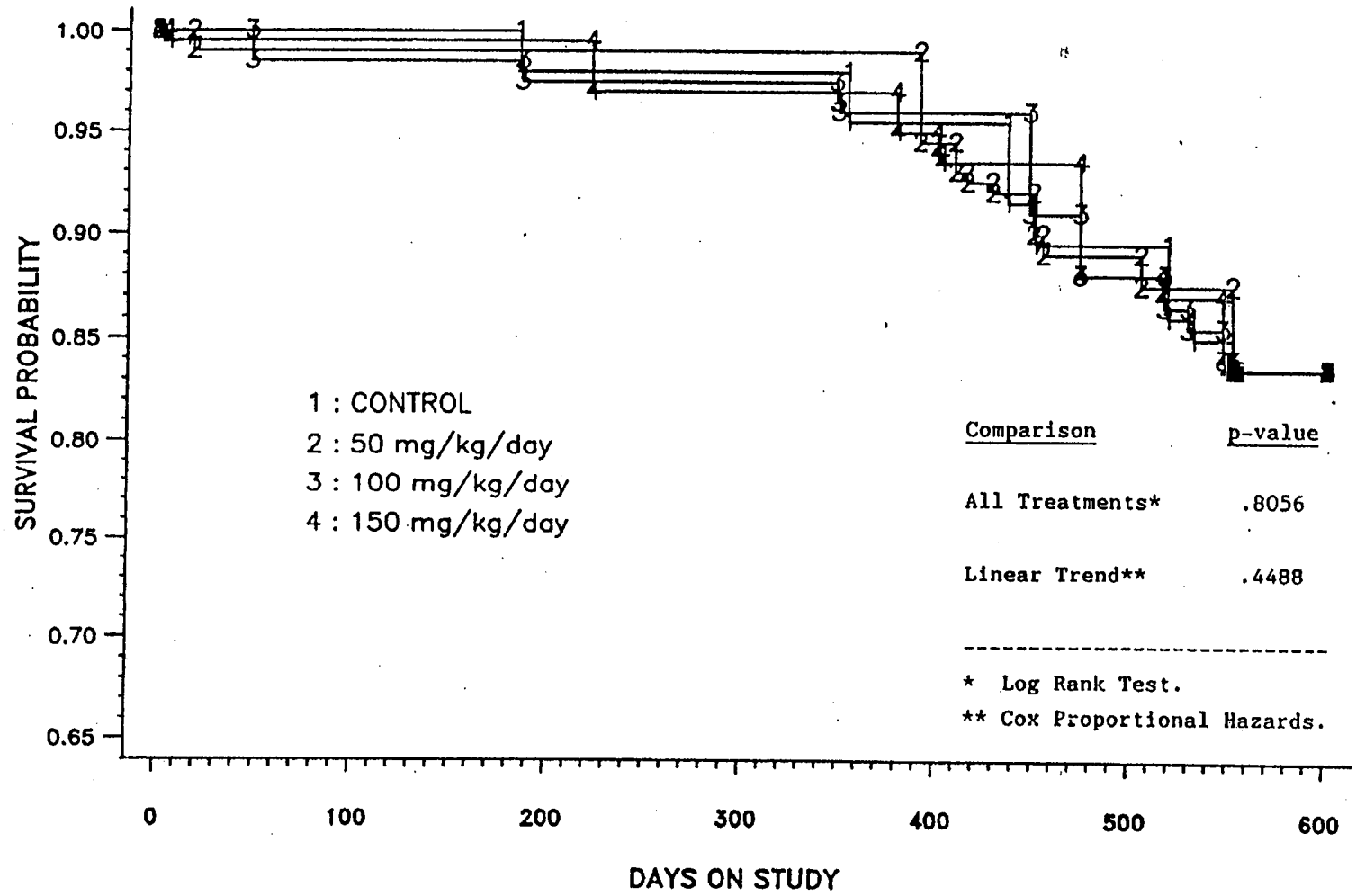
		Dose Groups (mg/kg/day)							
		Control		50		100		150	
Months	(Days)	50M	50F	50M	50F	50M	50F	50M	50F
1	(1-30)	-	-	1	1	-	2	1	1
2	(31-60)	-	-	-	1	1	-	-	1
3	(61-90)	-	-	-	2	-	1	-	1
4	(91-120)	-	-	-	-	-	1	-	1
5	(121-150)	-	-	-	1	-	1	-	1
6	(151-182)	-	2	-	2	-	1	-	-
7	(183-212)	1	2	-	-	1	-	-	2
8	(213-242)	-	-	-	1	-	1	1	-
9	(243-273)	-	-	-	2	-	-	-	-
10	(274-304)	-	3	-	2	-	2	-	1
11	(305-335)	-	-	-	-	-	-	-	-
12	(336-365)	1	1	-	-	2	-	-	1
13	(366-396)	-	1	1	1	-	-	1	-
14	(397-426)	-	-	2	-	-	1	2	1
15	(427-456)	2	-	4	1	1	1	-	-
16	(457-486)	-	1	-	-	1	1	1	5
17	(487-516)	-	-	1	-	-	2	1	2
18	(517-547)	2	1	-	2	3	1	1	1
19	(548-554)	-	1	-	1	-	-	1	-
Total deaths		6	12	9	17	9	15	9	18
Number remaining at Terminal Sacrifice		44	38	41	33	41	35	41	32

* Includes those sacrificed moribund and accidentally killed

FIGURE 1A

EIGHTEEN MONTH CARCINOGENICITY STUDY WITH GVG IN MICE

KAPLAN–MEIER SURVIVAL PROBABILITY DISTRIBUTIONS FOR MALE MICE



Comparison	p-value
All Treatments*	.8056
Linear Trend**	.4488

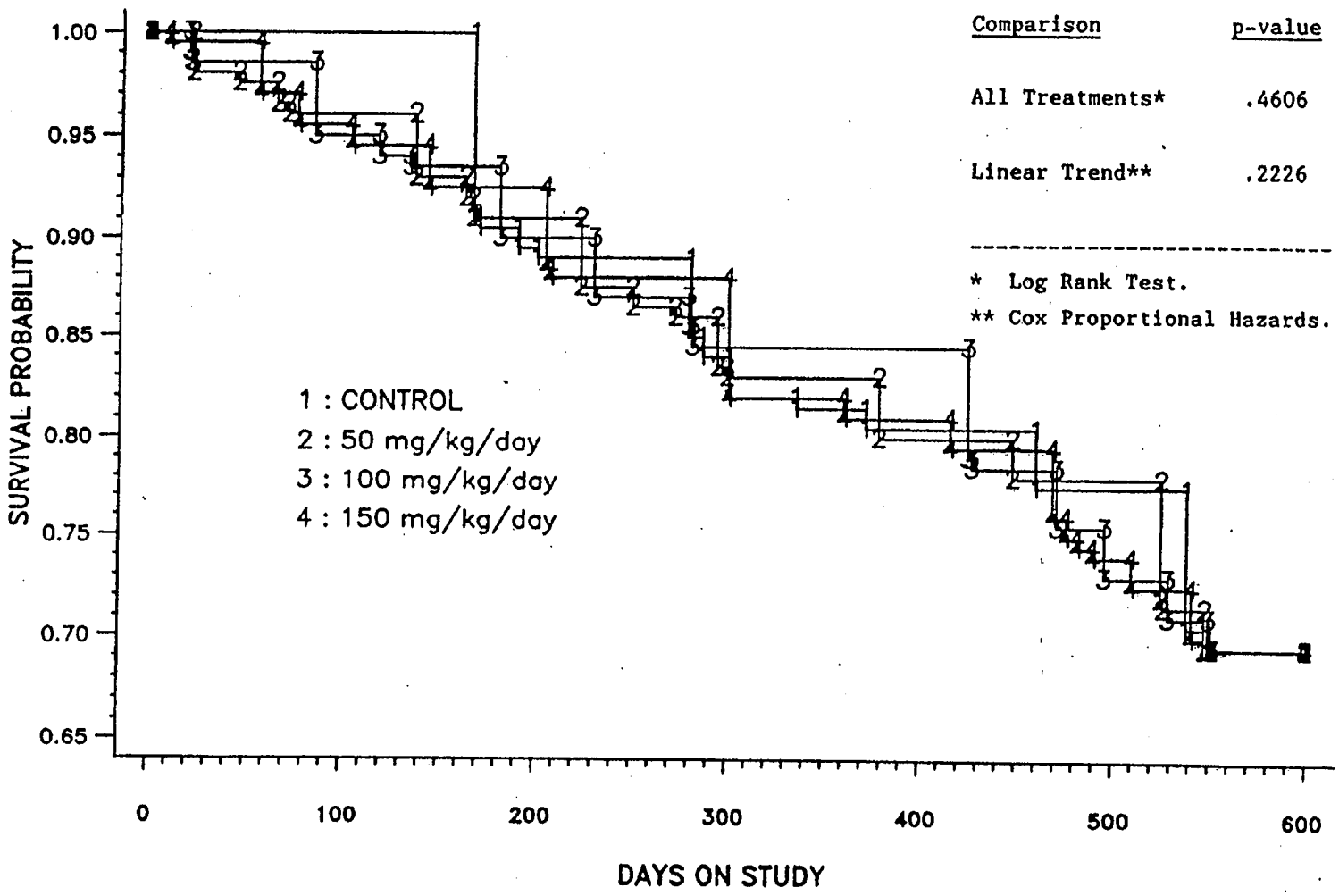
* Log Rank Test.

** Cox Proportional Hazards.

FIGURE 1B

EIGHTEEN MONTH CARCINOGENICITY STUDY WITH GVG IN MICE

KAPLAN–MEIER SURVIVAL PROBABILITY DISTRIBUTIONS FOR FEMALE MICE



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Best Possible Copy

CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 5

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY
CINCINNATI, OHIO 45215

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 300C-110

PRINTED: 06-AUG-86
PAGE: 1

ALL ANIMALS * NON NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES --				-- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
BRAIN	NUMBER EXAMINED:	49	49	50	50	50	50	50	49
-CEREBELLAR VACUOLATION		8	10	20	18	25	21	27	26
-CEREBELLAR MINERALIZATION		0	0	7	9	0	0	4	5
-RETICULAR FORMATION VACUOLATION		6	16	16	17	22	21	16	17
-INFERIOR COLLICULUS VACUOLATION		0	1	1	4	9	11	6	9
-INTERNAL CAPSULE VACUOLATION		1	0	2	5	6	2	6	8
-THALAMIC VASCULAR MINERAL DEPOSITS		16	11	19	18	3	4	6	9
-THALAMIC VACUOLATION		6	4	9	11	24	19	14	16
-PIA MATER MINERALIZATION		0	1	0	0	0	0	0	1
-OPTIC NERVE VACUOLATION		0	0	0	0	1	0	0	3
-ANTERIOR COMMISSURE VACUOLATION		0	0	0	0	0	0	0	1
-CEREBRAL CORTEX VACUOLATION		1	4	2	0	2	11	6	3
-CORPUS CALLOSUM VACUOLATION		0	0	2	0	0	0	1	2
-CEREBELLAR GRANULAR LAYER DYSPLASIA		1	0	0	0	0	0	0	0
-RETICULAR FORMATION MINERALIZATION		0	0	0	1	0	0	0	0
-HIPPOCAMPAL VACUOLATION		0	0	0	0	0	1	0	0
PERIPHERAL NERVE	NUMBER EXAMINED:	50	50	49	48	47	50	49	49
-VACUOLATION		0	0	0	0	2	0	1	1
EYE	NUMBER EXAMINED:	46	45	44	42	47	38	39	39
-RETINAL DEGENERATION		1	0	1	1	1	3	4	8
-OUTER NUCLEAR LAYER DYSPLASIA		8	1	0	1	9	0	1	0
-FIBRINOPURULENT ENDOPHTHALMITIS		0	0	0	0	1	0	0	0
PITUITARY	NUMBER EXAMINED:	48	38	44	44	47	43	47	40
THYROID	NUMBER EXAMINED:	48	48	49	47	46	47	45	43
ADRENAL(S)	NUMBER EXAMINED:	49	49	48	50	49	49	48	48
-HEMORRHAGIC CYST		0	0	0	1	0	0	0	0
-CORTICAL VACUOLIZATION		0	0	1	0	0	0	0	1
-MEDULLARY HYPERPLASIA		1	0	0	0	0	1	0	0
-CORTICAL MINERALIZATION		0	0	0	0	0	0	1	0
LACRIMAL GL. EX	NUMBER EXAMINED:	45	48	48	50	37	42	42	40
-LYMPHOCYTIC DACROADENITIS		22	19	13	13	15	8	12	12

5 ~~[illegible]~~ Page 13 #

CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 6

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY
CINCINNATI, OHIO 45215

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STUDY NUMBER 300C-110

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PAGE: 1

ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES				-- FEMALES			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
BRAIN	NUMBER EXAMINED:	49	49	50	50	50	50	50	49
-N- LYMPHOCYTIC LEUKEMIA		0	0	1	0	1	0	2	0
PERIPHERAL NERVE	NUMBER EXAMINED:	50	50	49	48	47	50	49	49
EYE	NUMBER EXAMINED:	46	45	44	42	47	38	39	39
-N- LYMPHOSARCOMA		0	0	1	0	0	1	0	0
PITUITARY	NUMBER EXAMINED:	48	38	44	44	47	43	47	40
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
THYROID	NUMBER EXAMINED:	48	48	49	47	46	47	45	43
-N- LYMPHOSARCOMA		0	0	1	0	0	0	0	0
ADRENAL(S)	NUMBER EXAMINED:	49	49	48	50	49	49	48	48
-B- CORTICAL NODULAR HYPERPLASIA		2	0	3	3	0	0	0	0
LACRIMAL GL. EX	NUMBER EXAMINED:	45	48	48	50	37	42	42	40
-N- LYMPHOSARCOMA		0	1	1	0	0	1	0	0
LACRIMAL GL. POS	NUMBER EXAMINED:	49	48	49	50	49	48	49	48
-B- PAPILLARY ADENOMA		1	2	0	1	0	0	1	1
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
SALIVARY GLAND	NUMBER EXAMINED:	49	48	50	50	49	49	49	49
-N- LYMPHOSARCOMA		0	0	1	0	1	0	0	0
LYMPH N., MAND.	NUMBER EXAMINED:	47	42	47	38	42	42	43	41
-N- LYMPHOSARCOMA		0	1	1	1	3	3	3	1
SPLEEN	NUMBER EXAMINED:	49	48	49	50	49	45	46	47
-N- LYMPHOSARCOMA		0	2	1	1	4	4	2	0
-B- HEMANGIOMA		0	0	0	0	0	0	1	0
THYMUS	NUMBER EXAMINED:	47	42	46	44	45	39	41	48
-N- LYMPHOSARCOMA		0	2	1	2	5	3	2	1

M = [illegible] gnant
B = [illegible] nign
N = metastatic

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CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 6 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 300C-110
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

PRINTED: 06-AUG-86
PAGE: 2

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES --				-- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
LUNG	NUMBER EXAMINED:	50	49	50	50	49	49	49	50
-N- LYMPHOSARCOMA		0	0	1	1	1	2	3	0
-B- PULMONARY ADENOMA		4	8	3	3	4	5	4	0
-M- PULMONARY CARCINOMA		1	0	0	1	1	0	0	0
-N- CARCINOMA		0	0	0	0	0	0	0	1
-N- SARCOMA		0	0	0	0	0	0	1	0
-N- FIBROUS HISTIOCYTOMA		0	1	0	0	0	0	0	0
ESOPHAGUS	NUMBER EXAMINED:	50	48	50	50	49	50	49	49
-N- LYMPHOSARCOMA		0	0	0	0	1	0	0	0
DIAPHRAGM	NUMBER EXAMINED:	50	50	50	50	49	48	47	50
-N- LYMPHOSACOMA		0	0	0	0	1	1	1	0
HEART	NUMBER EXAMINED:	50	49	50	50	50	50	50	50
-N- LYMPHOSARCOMA		0	1	0	0	2	2	0	0
AORTA	NUMBER EXAMINED:	49	49	50	50	48	49	50	50
-N- LYMPHOSARCOMA		0	1	1	1	0	2	0	0
TONGUE	NUMBER EXAMINED:	50	49	50	50	50	50	50	50
LIVER	NUMBER EXAMINED:	49	49	50	50	49	49	47	48
-B- HEPATOCELLULAR ADENOMA		5	2	5	2	1	0	0	0
-N- LYMPHOSARCOMA		0	2	1	1	3	3	4	0
-B- BASOPHILIC FOCI		1	0	0	1	0	0	0	0
-M- HEPATOCELLULAR CARCINOMA		6	1	4	2	0	1	0	0
-N- FIBROUS HISTIOCYTOMA		0	0	0	0	0	0	0	1
-B- HEMANGIOMA		1	0	0	0	0	0	0	0
-B- HYPERPLASTIC NODULE		1	0	0	0	0	0	0	0
-M- HEMANGIOSARCOMA*		1	0	0	0	0	0	0	0
-B- CLEAR CELL FOCI		1	0	1	0	0	0	0	0
-B- ANGIOMATOUS FOCI		0	1	0	0	0	0	0	0
STOMACH	NUMBER EXAMINED:	50	48	50	49	49	46	44	49
-N- LYMPHOSARCOMA		0	1	1	0	1	1	0	0

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CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 6 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 300C-110
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

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PAGE: 3

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES				-- FEMALES			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
PANCREAS	NUMBER EXAMINED:	49	49	49	50	49	47	42	47
-N- LYMPHOSARCOMA		0	1	0	1	1	0	2	0
SMALL INTESTINE	NUMBER EXAMINED:	48	45	45	44	44	39	39	35
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
LARGE INTESTINE	NUMBER EXAMINED:	49	46	48	49	48	47	40	43
-B- POLYPS		0	0	1	0	0	0	0	0
LYMPH N., MESEN.	NUMBER EXAMINED:	49	45	44	46	46	42	40	40
-N- LYMPHOSARCOMA		0	2	1	1	5	4	3	0
-N- FIBROUS HISTIOCYTOMA		0	1	0	0	0	0	0	0
KIDNEY(S)	NUMBER EXAMINED:	49	50	50	50	49	49	50	49
-N- LYMPHOSARCOMA		0	1	1	2	2	3	1	1
URINARY BLADDER	NUMBER EXAMINED:	49	48	49	49	47	44	42	45
-N- LYMPHOSARCOMA		0	0	0	0	1	1	1	0
-M- LEIOMYOSARCOMA		0	0	1	0	0	0	0	0
MAMMARY GLAND	NUMBER EXAMINED:	49	50	50	50	50	48	50	50
-N- LYMPHOSARCOMA		0	0	0	0	1	1	1	0
-M- ADENOCARCINOMA		0	0	0	0	1	1	0	0
SKELETAL MUSCLE	NUMBER EXAMINED:	50	50	50	50	50	50	50	50
-N- LYMPHOSARCOMA		0	0	0	0	2	1	0	0
SKIN/SUBCUTIS	NUMBER EXAMINED:	50	50	50	50	50	49	50	49
-M- SUDORIFEROUS ADENOCARCINOMA		0	0	0	0	0	2	0	1
-N- LYMPHOSARCOMA		0	0	0	0	1	1	0	0
-M- FIBROSARCOMA		0	0	0	0	0	1	0	0
TESTIS	NUMBER EXAMINED:	49	50	50	50				
-B- INTERSTITIAL CELL TUMOR		0	0	0	1				
-B- SERTOLI CELL TUMOR		0	0	1	0				

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CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 6 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 300C-110
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

PRINTED: 06-AUG-86
PAGE: 4

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS -- MALES --				AFFECTED -- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
EPIDIDYMIS	NUMBER EXAMINED:	49	50	50	50				
PROSTATE	NUMBER EXAMINED:	49	50	50	50				
-N- LYMPHOSARCOMA		0	1	1	0				
SEM.VES.	NUMBER EXAMINED:	49	49	50	49				
-N- LYMPHOSARCOMA		0	1	0	0				
OVARY(S)	NUMBER EXAMINED:					48	45	45	47
-N- FIBROUS HISTIOCYTOMA						0	0	0	1
-N- LYMPHOSARCOMA						2	1	3	0
UTERUS	NUMBER EXAMINED:					48	47	46	48
-B- HEMANGIOMA						2	0	0	0
-B- ENDOMETRIAL STROMAL POLYP						4	0	1	0
-M- LEIOMYOSARCOMA						0	0	1	0
-B- LEIOMYOMA						0	0	1	0
-N- LYMPHOSARCOMA						0	1	1	0
-M- ENDOMETRIAL STROMAL SARCOMA						0	2	0	0
VAGINA	NUMBER EXAMINED:					46	48	45	46
-N- LYMPHOSARCOMA						0	1	0	0
BONE MARROW	NUMBER EXAMINED:	49	49	50	50	50	49	46	50
-N- LYMPHOSARCOMA		0	0	0	0	2	1	1	0
LYM.-HEMOPOIETIC	NUMBER EXAMINED:	0	5	1	2	5	5	4	2
-M- LYMPHOSARCOMA/LEUKEMIA		0	4	1	2	5	5	4	1
-M- FIBROUS HISTIOCYTOMA		0	1	0	0	0	0	0	1
GALL BLADDER	NUMBER EXAMINED:	0	0	0	0	0	0	0	1
PAW/FOOT	NUMBER EXAMINED:	0	0	0	0	0	0	0	1
-M- FIBROSARCOMA		0	0	0	0	0	0	0	1

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CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 6 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 300C-110
ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES --				-- FEMALES --			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
EAR	NUMBER EXAMINED:	1	0	0	0	0	0	0	0
-M- SQUAMOUS CELL CARCINOMA		1	0	0	0	0	0	0	0

~~5~~ ~~1986 VIES~~
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CARCINOGENICITY STUDY OF MICE TREATED WITH VIGABATRIN

Table 6 (continued)

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY
CINCINNATI, OHIO 45215

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 300C-110

PRINTED: 06-AUG-86
PAGE: 1

ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

SUMMARY REPORT	-- ANIMALS AFFECTED --							
ANIMAL SEX:	-- MALES --				-- FEMALES --			
DOSAGE GROUP:	1	2	3	4	1	2	3	4
NO. IN GROUP:	50	50	50	50	50	50	50	50
TOTAL PRIMARY NEOPLASMS	25	19	20	16	18	17	13	5
ANIMALS WITH ONE OR MORE	21	18	18	16	17	14	11	5
PERCENT WITH ONE OR MORE	42	36	36	32	34	28	22	10
TOTAL BENIGN NEOPLASMS	16	13	14	11	11	5	8	1
ANIMALS WITH ONE OR MORE	15	12	13	11	11	5	7	1
PERCENT WITH ONE OR MORE	30	24	26	22	22	10	14	2
TOTAL MALIGNANT NEOPLASMS	9	6	6	5	7	12	5	4
ANIMALS WITH ONE OR MORE	9	6	6	5	7	12	4	4
PERCENT WITH ONE OR MORE	18	12	12	10	14	24	8	8
METASTATIC NEOPLASMS								
ANIMALS WITH ONE OR MORE	0	5	1	2	6	5	6	3
PERCENT WITH ONE OR MORE	0	10	2	4	12	10	12	6

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2 WEEK ORAL RANGEFINDING STUDY IN RATS:

A. Dosage

1. 10 M + 10 F at 0 , 10, 25, 50, 100, or 200 mg/kg/day, by gavage. (Strain: Sprague Dawley, [redacted]

2. 5 M + 5 F at 0, 500, or 1000 mg/kg/day, by gavage. (Strain: COBS CD (SD) BR, [redacted]

b(4)

B. Results

1. Observed signs

Sedation and emaciation seen at 1000 mg/kg only.

2. Mortality

1 F at 1000 mg/kg died day 10; no unusual histopathology.

3. Bodyweight

In both M and F, decreased gain at 200 mg/kg; at 500 and 1000 mg/kg, weight loss first week followed by slight gain (but still below pre-study value) second week.

4. Food consumption

Decreased at 200 mg/kg in M and F first week only. Larger decrease at 500 and 1000 mg/kg in M and F both weeks, but greater effect at first week. (HD value about 1/6 control).

5. Laboratory studies

(Performed post-study in 5/sex/group. Mean values only were presented, plus ranges for hematology results).

a. Hematology

1. WBC

Very slight decreases at most doses in M and at 100 mg/kg + in F; moderate effect at 1000 mg/kg (mean value 48% and 78% of control in F and M, resp.). Report states that the decrease at 1000 mg/kg was related to the emaciated condition of this group.

2. Differential

2 WEEK ORAL RANGEFINDING STUDY IN RATS:

A. Dosage

1. 10 M + 10 F at 0 , 10, 25, 50, 100, or 200 mg/kg/day, by gavage. b(4)
(Strain: Sprague Dawley, [redacted]

2. 5 M + 5 F at 0, 500, or 1000 mg/kg/day, by gavage.
(Strain: COBS CD (SD) BR, [redacted].

B. Results

1. Observed signs

Sedation and emaciation seen at 1000 mg/kg only.

2. Mortality

1 F at 1000 mg/kg died day 10; no unusual histopathology.

3. Bodyweight

In both M and F, decreased gain at 200 mg/kg; at 500 and 1000 mg/kg, weight loss first week followed by slight gain (but still below pre-study value) second week.

4. Food consumption

Decreased at 200 mg/kg in M and F first week only. Larger decrease at 500 and 1000 mg/kg in M and F both weeks, but greater effect at first week. (HD value about 1/6 control).

5. Laboratory studies

(Performed post-study in 5/sex/group. Mean values only were presented, plus ranges for hematology results).

a. Hematology

1. WBC

Very slight decreases at most doses in M and at 100 mg/kg + in F; moderate effect at 1000 mg/kg (mean value 48% and 78% of control in F and M, resp.). Report states that the decrease at 1000 mg/kg was related to the emaciated condition of this group.

2. Differential

Decrease in % PMN at 500 and 1000 mg/kg in M; however, most F groups had a slight increase. Changes in numbers of lymphocytes roughly paralleled changes in total WBC. Monocytes decreased in all M groups (¼-½ of control, not dose-related) and decreased in HD F (½ of control).

3. % reticulocytes

   Slight decrease at 1000 mg/kg in M and F (mean value 41% and 50% of control, resp.).

4. No effects on rest of differential, RBC, Hct, Hb, Heinz bodies, and platelets.

b. Blood chemistry

1. Ca

   Very slight decrease in HD M and F

2. BUN

   Slight increase in HD F (mean value 47% above control)

3. Glucose

   Very slight increase in most M groups, partly dose-related (mean value 29% above control at HD)

4. AP

   Very slight increase in F at 500 and 1000 mg/kg (mean value about 38% above control)

5. ALT and AST

   ALT decreased at 200 mg/kg + in M and F; AST decreased at 500 mg/kg + in M. Attributed to pharmacological effect of the drug.

6. No effects on total protein, albumin, A/G, total bilirubin, Cl.

c. Urinalysis

   No drug effects (pH, SG, occult blood, bilirubin, ketones, glucose, protein, sediment

6. Organ weights

a. Males

1. Seminal vesicle

Dose-related decrease in absolute and relative weights in all groups (rel. wt. 48% below control at HD).

2. Ventral prostate

Partly dose-related decrease in absolute and relative weights in all groups (rel. wt. 70% below control at HD).

3. Thymus

Decrease in absolute and relative weight at 500 and 1000 mg/kg (rel. wt. 58% below C at HD).

b. Females

1. Uterus

Decreased abs. and rel. wt. at 500 and 1000 mg/kg (rel. wt. 38% below control at HD).

2. Thymus

Decreased abs. and rel. wt. at 1000 mg/kg (rel. wt. 43% below control)

7. Gross pathology

(Performed on 5/sex/group)

No drug effects

8. Microscopic pathology

(Complete exam on 5/sex/group at 500 and 1000 mg/kg and corresponding controls; brain only in 5/sex/group at 200 mg/kg and corresponding controls).

No drug effects

3 MONTH ORAL TOXICITY IN RATS

A. Dosage

20 M + 20 F at 0, 30, 100, or 300 mg/kg/day, by gavage. 5 M + 5 F per group sacrificed day 38 for hematology and blood chemistry. Strain: CRL: COBS CD (SD) BR from [redacted] b(4)

B. Results

1. Observed signs

a. Alopecia in 1 MD and 6 HD
b. Tonic convulsions upon handling, days 85-99, in 2 HD
c. Emaciation in 1 HD, day 85

2. Mortality

1 HD F, day 51. Gross and microscopic pathology showed no changes not seen in other rats of same group.

3. Bodyweight

a. Males

Dose-related decreased weight gain in all groups. Effect slight at LD and MD; moderate at HD (initial weight loss followed by rate of gain slightly less than controls at HD; final weight 68% of controls).

b. Females

Very slight decreased gain at MD; at HD, slight initial weight loss followed by rate of gain approx. equal to controls. Final weight at HD 83% of controls.

4. Food consumption

a. Males

Very slight decrease at LD and MD; larger decrease at HD (about 75% of controls; effect most marked first 3 wks.)

b. Females

Very slight decrease in all groups, most marked first 3 wks; effect less than in M.

5. Ophthalmoscopic exam

(Performed pre- and post-study)

No drug effect

6. Galton whistle test

(measure of auditory capability - performed at termination)

No drug effect

7. Laboratory studies

(Performed at termination in 10/sex/group; also hematology and blood chemistry on day 38 in 5/sex/group. Mean values only were presented, plus ranges for hematology results).

a. Hematology

1. Differential

Slight increase in % PMN in HDF; effect more pronounced (relative to control) at day 38 than at termination. HDM also had borderline increases in % PMN, in contrast to rangefinding study where a decrease was seen in M at 500 and 100 mg/kg. No consistent effects on other parameters.

2. No consistent effects on total WBC, RBC, Hct, Hb, platelets.

b. Blood chemistry

1. Very slight increase in albumin at day 38 and termination, and in total protein at termination, in HD M.

2. Very slight increase in fasting glucose at termination (mean value = 182 mg/dl vs. 156 in control) in HD M; sponsor states a moderate increase occurred in a few rats but no individual values were given.

3. No effects on A/G ratio, Ca, Pi, BUN, total bilirubin, AP, AST, ALT, Cl, Na, K.

c. Urinalysis

No drug effects (pH, SG, occult blood, bilirubin, ketones, glucose, protein, sediment)

8. Organ weights

a. Males

1. Seminal vesicle

Dose-related decrease in abs. wt. in all groups; rel. wt. decreased at HD (21% below controls)

2. Ventral prostate

Dose-related decrease in abs. wt. in all groups; rel. wt. decreased at HD (24% below controls).

b. Females

Decreased abs. and rel. wt. of uterus at HD (rel. wt. 23% below controls)

9. Gross pathology

(Performed on all rats except for the 5/sex/group sacrificed day 38)

No drug effects.

10. Microscopic pathology

(Complete exam in 15/sex in controls and HD; brain only in 15/sex at MD.)

The only drug effect originally noted was vacuolization of the white matter and/or roof nuclei of the cerebellum, which was seen in all rats at HD:

	Control		HD	
	15 M	15 F	15 M	15 F
vacuolization of white matter	0	0	12	13
vacuolization of roof nuclei	0	0	6	2
total # affected	0	0	15	15

Based on retinal findings in other rat studies, eyes from control and HD rats were re-evaluated; the incidence of "retinal degeneration" was as follows (See "Summary" Section for further discussion):

	C	HD
M	0/15	1/15
F	0/15	6/15

1 YEAR DIETARY TOXICITY IN RATS:

A) Dosage

20-40/sex at 0, 30, 100, 200 or 300 mg/kg/day, in diet.

Interim sacrifices performed at 6 months, 6 months + 3 and 6 month recovery period, 12 months, and 12 months + 3 month recovery period. (See table)

Strain: [redacted] b(4)

Lot #: 71,754-40 (days 1-183)
71,754-46 (days 183-400)

B) Results

1) Observed signs (see tables [separate table for first and second 6 month periods])

a) Convulsions - Seen at 2 highest doses after about 3 months of treatment; also seen in 4 at 100 mg/kg near the end of the study. Convulsions could be induced by noises, handling, or by rattling the animal's cage; many rats at the 2 highest doses convulsed whenever they were handled and "probably convulsed several times a day." Ataxia was often seen just before or after convulsions. Many rats also developed lesions about the head, eyes, and teeth (see table) "apparently from trauma associated with the convulsions". Convulsions slowly regressed following cessation of treatment (see separate table); no convulsions were seen by 4 months off drug following 6 months' treatment; however, after 12 months' treatment some rats were still convulsing after a 3 month recovery period (at which time all remaining rats were sacrificed).

b) Alopecia - mainly at 2 highest doses

c) A few HD rats became moribund and were eventually sacrificed. Prior to becoming moribund they often exhibited wet belly and chromodacryorrhea. By the end of the 1 year treatment period many HD "were in rather poor condition;" all HD were terminated at this time (i.e., no recovery period).

2) Mortality

Increased at 2 highest doses (died or sacrificed moribund: 3, 1, 0, 7, 16 in C, LD, MD, M-HD, and HD, resp.)

1 YEAR DIETARY TOXICITY IN RATS:

A) Dosage

20-40/sex at 0, 30, 100, 200 or 300 mg/kg/day, in diet.

Interim sacrifices performed at 6 months, 6 months + 3 and 6 month recovery period, 12 months, and 12 months + 3 month recovery period. (See table)

Strain: [redacted] b(4)

Lot #: 71,754-40 (days 1-183)
71,754-46 (days 183-400)

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a) Convulsions - Seen at 2 highest doses after about 3 months of treatment; also seen in 4 at 100 mg/kg near the end of the study. Convulsions could be induced by noises, handling, or by rattling the animal's cage; many rats at the 2 highest doses convulsed whenever they were handled and "probably convulsed several times a day." Ataxia was often seen just before or after convulsions. Many rats also developed lesions about the head, eyes, and teeth (see table) "apparently from trauma associated with the convulsions". Convulsions slowly regressed following cessation of treatment (see separate table); no convulsions were seen by 4 months off drug following 6 months' treatment; however, after 12 months' treatment some rats were still convulsing after a 3 month recovery period (at which time all remaining rats were sacrificed).

b) Alopecia - mainly at 2 highest doses

c) A few HD rats became moribund and were eventually sacrificed. Prior to becoming moribund they often exhibited wet belly and chromodacryorrhea. By the end of the 1 year treatment period many HD "were in rather poor condition;" all HD were terminated at this time (i.e., no recovery period).

2) Mortality

Increased at 2 highest doses (died or sacrificed moribund: 3, 1, 0, 7, 16 in C, LD, MD, M-HD, and HD, resp.)

3) Bodyweight gain and food consumption

See attached graphs; text states decreases seen at all doses but LD. After 12 months, weights at MD, M-HD, and HD were said to be approx. 88, 70, and 64% of control, resp.

4) Ophthalmoscopic exam

(Said to have been done pre-study and after 6 and 12 months; however only results at 12 months were shown).

Approximately 20-40% of M-HD and HD rats had inflammatory and/or traumatic lesions which were thought to be possibly secondary to convulsion - induced trauma.

5) Hematology

(Done at 6 months in 10/sex in controls, M-HD, and HD and in 5/sex in remaining groups; done at 12 months in 5/sex/group)

a) Slight decrease in total WBC and numbers of lymphocytes in all M groups at 6 months only; greatest effect at HD.

b) Increases in PT and/or PTT in a very small number of animals across all drug groups.

c) Other parameters measured: RBC, Hb, Hct, rest of differential, platelets.

6) Blood chemistry

(Done in same animals as hematology, above)

a) Numerous trends toward slight changes in mean values, primarily (but not always) at the higher doses. No very aberrant individual values.

These changes include:

1) Decreases in total protein and albumin (A/G ratio not clearly affected except for possible decrease in M-HD F and HD F at 6 months).

2) Decreased calcium

3) Increased Pi

4) Decreased fasting glucose

5) Decreased Na and Cl (at one year only)

6) Increased K

b) Slight increases seen in AP in a very small number of animals across all doses; largest individual value ~ 2x highest concurrent control.

c) Other parameters measured: ALT, AST, BUN, total bilirubin.

7) Urinalysis

(Done in same numbers of animals as hematology, above, at 6 months but in 9-10/sex/group at 1 year)

a) Occult blood and RBC slightly increased in M-HD and HD F at 1 year.

b) Protein slightly increased in HD F at 6 months and in M-HD and HD F in 1 year.

c) Specific gravity equivocally slightly increased in all F groups but LD at 6 and 12 months. (Not D-R).

8) Organ weights

No clear drug effects (aside from changes likely secondary to decreased bodyweight).

9) Macroscopic organ exam

There was an increased incidence of pale/granular/pitted surface of kidney at HD at the 1 year sacrifice and among unscheduled deaths; however the significance of this is unclear in the absence of any clearly drug-related histopathology in kidney.

10) Microscopic organ exam

(After recovery periods, only brain and gross lesions were examined. In addition to routine H and E exams, brain sections from the following animals were stained with luxol fast blue for myelin: 12/20 controls and 1/sex at LD, MD, and M-HD after 6 months, and 5/5 M and 5/5 F at M-HD and HD in the 6 month + 3 month recovery animals. Spinal cord was examined [H & E] only at the 1 year sacrifice (in most animals sacrificed at this time) and in 2 HD premature deaths. EM exam was performed in brains of selected animals to help characterize the light microscopic findings. EM exam was also done in tibial nerve in 4 HD [3 which died at 11 months; 1 sacrificed after 6 months Tx + 6 month's recovery] and in sural nerve in 1 M-HD [sacrificed after 6 month's treatment + 6 month's recovery] and 1 HD [which died at 11 months]).

The only drug-related finding was in brain. With light microscopy, numerous vacuoles were seen, primarily in white matter, in several areas of brain. EM exam showed that the vacuoles occurred in between layers of myelin sheath. Results of the light microscopic exams are shown in the attached tables. The results (regarding vacuoles) indicate that:

a) The primary areas affected were the areas around the roof nuclei of cerebellum and cerebellar folia, reticular formation, optic tract, anterior commissure, columns of fornix, colliculus, hippocampus, thalamus, and cerebral peduncle.

b) At the 6 month sacrifice, all doses but LD were affected; at the 12 month sacrifice all doses were affected.

c) The vacuoles were reversible after a 3 month recovery period. (But note that recovery was not studied at HD following 12 month's treatment.)

Although the vacuoles appear to be reversible, other lesions were seen (in cerebellum) which were not reversible (and in fact appeared to become more pronounced during the recovery period): "eosinophilic spheroids" (said to be suggestive of swollen or degenerated axons) and "mineralized microbodies" (shown to contain calcium by Von Kossa's stain). The incidence of these findings is shown in the attached table. They were seen at M-HD and HD at 6 months and in all doses but LD at 12 months. Their incidence (and size) was greater in animals sacrificed after the recovery periods than in animals sacrificed immediately after treatment. (In a published review of this study [Gibson, et.al., Tox. Pathol. 18: 225, 1990] the finding of "occasional shrunken or ghost-like neurons" in cerebellar roof nuclei following 12 months' treatment + 3 months' recovery at 100 mg/kg and above was noted. It was also noted that although there was no indication of gliosis by light microscopy, EM exam of rats given 300 mg/kg showed that "astroglial processes were sometimes more prominent and contained more glial filaments". Also note that gliosis was demonstrated by GFAP staining as discussed later in this review. In addition, one of the consultant's reports to this study noted a decreased number of neuronal cell bodies of the deep cerebellar nuclei with astrocytic gliosis and abundant mineral deposits in 1 rat each at 100 and 200 mg/kg).

Luxol fast blue staining of brain (done only after 6 month's treatment and corresponding 3 month recovery period) was said to show "apparent normal myelination". (One consultant's report to this study did state that some HD rats had slightly less intense luxol staining). Also, EM exam of brain done at HD after 6 month's treatment was said to show "occasional macrophages containing lamellar inclusions"...which "tended to regress during the recovery period;" it was stated that this "may be indicative of some slight myelin degradation, however, there was no evidence of any segmental demyelination as seen in classical demyelinating disease (e.g., multiple sclerosis)."

No drug effects were seen in spinal cord or peripheral nerve.

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 1

Disposition of Animals

Dosage mg/kg/day	Start		6 month Sacrifice		6 month treatment 3 month Recovery		6 month treatment 6 month Recovery		12 month Sacrifice		12 month treatment 3 month Recovery		Died or sacrificed moribund	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	30	30	10	10	-	-	-	-	7	10	10	10	3	-
30	20	20	5	5	-	-	-	-	10	10	4	5	1	-
100	20	20	5	5	-	-	-	-	5	5	10	10	-	-
200	40	40	10	10	5	5	5	4	7	8	9	10	4	3
300	40	40	10	10	5	5	5	5	9	15	0	0	11	5

Table 3a

Clinical Observations During First 6 Months[a] (Days 1-185)

	Dose Groups (mg/kg/day)									
	Control		30		100		200		300	
	30M	30F	20M	20F	20M	20F	40M	40F	40M	40F
Convulsive behavior	-	-	-	-	-	-	13	18	37	34
Tremors	-	-	-	-	-	-	-	-	2	-
Ataxia	-	-	1	-	-	-	-	-	2	-
Eyes - opacity	1	-	-	-	-	-	1	-	-	-
- chromodacryorrhea/ocular discharge	1	-	-	-	1	-	4	2	7	2
- missing	-	-	-	-	-	1	-	-	-	2
Teeth - pulled loose/clipped/out of alignment	1	-	-	-	1	1	6	1	11	5
Mouth-lacerations	-	-	-	-	-	-	1	-	-	-
Skin - alopecia	-	-	1(1)	1(1)	2	-	4(2)	9(2)	10(5)	6(1)
- rough hair coat	2	-	1	-	-	-	1	1	-	-
- reddish fur	1	-	-	-	1	-	-	-	1	1
- abscess	-	-	-	-	-	-	-	-	2	-
- raw area	-	-	-	1	-	-	-	-	-	-
- lesion, ventral neck	-	-	-	-	-	-	1	-	-	-
- mass on throat (lesion on mass)	1	-	-	-	-	-	-	-	-	-
Snout - reddish nasal discharge	1	-	-	-	-	-	1	2	1	-
- swollen	-	-	-	-	-	-	-	-	2	-
Ears - swollen	-	1	-	-	-	1	-	1	-	6
Tail - lesion (at base of tail)	-	-	1	-	-	-	-	-	-	-
- tip missing/mutilated	1	2	-	-	-	1	1	-	2	2
Toes/toenails - missing	-	-	-	-	4	-	4	2	3	1
Penis - reddened	-	-	-	-	-	-	-	-	1	-
- reddish fluid	-	-	-	-	-	-	-	-	1	-
Wet belly - yellow	1	-	1	-	-	-	4	1	9	4
- red	-	-	-	-	-	-	1	1	1	-
- colorless	-	-	-	-	-	-	-	-	2	-
- brown	-	-	-	-	-	-	-	-	1	-

(continued)

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 3a(continued)

Clinical Observations During First 6 Months[a] (Days 1-185)

	Dose Groups (mg/kg/day)									
	Control		30		100		200		300	
	30M	30F	20M	20F	20M	20F	40M	40F	40M	40F
Weight loss (water nipple malfunction)	-	1	-	-	-	-	-	-	-	-
Thin	-	-	-	-	-	-	1	1	1	3
Weak	1	-	-	-	-	-	-	1	-	1
Abdomen swollen, delivered pups	-	1	-	-	-	-	-	-	-	-
Sacrificed moribund	-	-	-	-	-	-	-	-	1	-
Found dead	2	-	-	-	-	-	1	-	3	1
Nothing significant	26	25	19	19	13	16	13	14	2	-

( ) number of animals that recovered

[a] Necropsies were started on day 183 and completed on day 185

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 3b

Clinical Observations During Second 6 Months (Days 183-395)

	Dose Groups (mg/kg/day)									
	Control		30		100		200		300	
	18M	20F	15M	15F	15M	15F	19M	20F	16M	19F
Convulsive behavior	-	-	-	-	3	1	18	16	16	19
Ataxia	-	-	-	-	2	-	-	-	1	-
Eyes - opacity/cloudy cornea	-	-	-	-	-	-	1	-	-	2
- chromodacryorrhea/ocular discharge	-	1	-	-	-	3	1	4	5	4
- traumatized	-	-	-	-	-	-	-	-	1	-
- appears to contain reddish material	-	-	-	-	-	-	1	-	-	-
- reddish fluid around eyeball	-	-	-	-	-	1	-	-	-	-
- appears ruptured	-	-	-	-	-	-	-	-	-	1
- exophthalmia	-	-	-	-	-	-	-	2	-	4
Teeth - pulled loose/clipped/out of alignment/ missing/caught in cage	1	1	-	-	1	2	4	5	7	5
Mouth - palate - abscess/swollen	-	-	-	-	-	-	-	-	2	1
- palate - hole	-	-	-	-	-	-	-	1	-	-
- reddish discolored fur around mouth	-	-	-	-	-	-	1	-	-	-
- area around mouth swollen	-	-	-	-	-	-	-	-	-	1
Skin - alopecia	-	-	-	-	1	-	-	4	3	3
- rough hair coat	-	-	-	-	-	-	1	1	6	3
- lesion - ventral neck	-	-	-	-	-	-	1	-	-	-
Mass/nodule - mid abdomen	-	-	1	-	-	-	-	-	-	-
- ventral abdomen	-	2	-	-	-	-	-	-	-	-
- inguinal area	-	1	-	-	-	-	-	-	-	-
- axillary area	-	2	-	-	1	-	1	-	-	-
- rear hip	-	1	-	-	-	-	-	-	-	-
Snout - swollen or broken	-	-	-	-	-	-	-	1	3	2
- reddish discolored fur around snout	-	-	-	-	-	-	1	-	-	-
Throat - swollen	-	-	1	-	-	-	-	-	-	-
Ears - swollen	-	1	-	-	-	1	-	-	-	4

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 3b (continued)

Clinical Observations During Second 6 Months (Days 183-395)

	Dose Groups (mg/kg/day)									
	Control		30		100		200		300	
	18M	20F	15M	15F	15M	15F	19M	20F	16M	19F
Tail - tip missing	1	1	-	-	-	1	1	1	-	-
- black nodule	-	-	-	-	-	-	4	-	-	-
- necrotic	-	-	-	-	-	-	-	-	-	1
Toes/toenails - missing	-	-	-	-	1	-	1	1	2	-
Foot - lesion	-	-	-	-	1	-	-	-	-	-
- swollen	-	-	-	-	2	-	-	-	-	-
Penis - reddish fluid	1	-	-	-	-	-	-	-	1	-
- reddened	-	-	-	-	-	-	-	-	1	-
Gonads (area) - swollen	-	-	-	-	-	-	1	-	-	-
Vagina - reddish fluid/reddish discharge	-	-	-	-	-	-	-	1	-	4
- dark fluid	-	-	-	-	-	-	-	1	-	-
Inguinal area - reddish material matted to fur	1	-	-	-	-	-	-	-	-	-
Ventral thorax - reddish fur	-	-	-	-	-	-	-	1	-	-
Wet belly - yellow	-	-	-	-	-	-	5	1	5	6
- red	-	-	-	-	-	-	-	1	-	-
- colorless	-	-	-	-	-	-	-	-	1	-
Weak/emaciated	-	-	-	-	-	-	-	1	-	-
Prostrate in cage	-	-	-	-	-	-	-	-	-	1
Moribund	-	-	-	-	-	-	-	-	1	2
Found dead	1	-	-	-	-	-	1	2	4	3
Sacrificed in moribund condition	-	-	-	-	-	-	1	-	3	1
Nothing significant	15	13	13	15	8	10	-	3	-	-

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ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 4

No. of Rats Exhibiting Convulsions During Recovery Phases

	100 mg/kg/day		200 mg/kg/day		300 mg/kg/day	
	M	F	M	F	M	F
6 mo. treatment[a]	-	-	4/10	6/10	9/10	9/10
1 mo. recovery[b]	-	-	7/10	4/9	9/10	9/10
2 mo. recovery[b]	-	-	2/10	1/9	9/10	7/10
3 mo. recovery[b]	-	-	0/10	0/9	3/10	4/10
Number remaining	-	-	0/5	0/4	3/5	1/5
4 mo. recovery[b]	-	-	0/5	0/4	0/5	0/5
12 mo. treatment[a]	3/10	1/10	10/10	10/10	-	-
3 mo. recovery[a]	2/10	1/10	4/9	9/10	-	-

[a] Number based on routine observations
[b] Number obtained following single daily stimulation over a one week period at each recovery interval.

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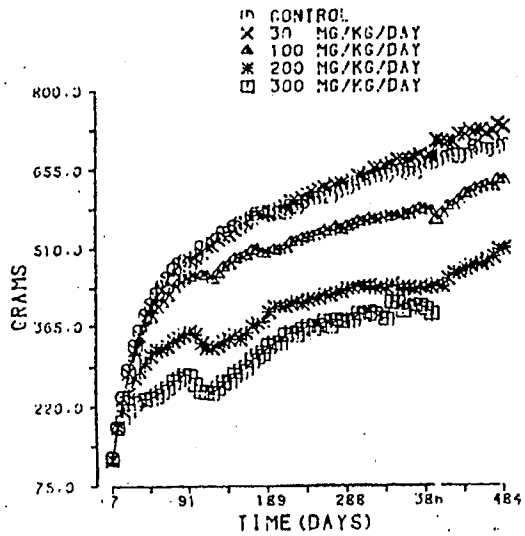
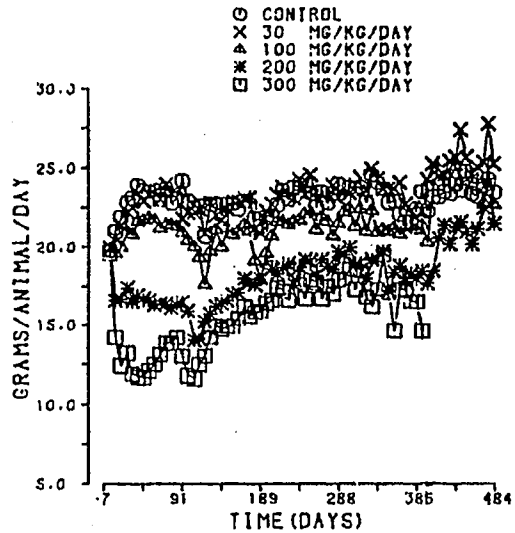
ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Plate I

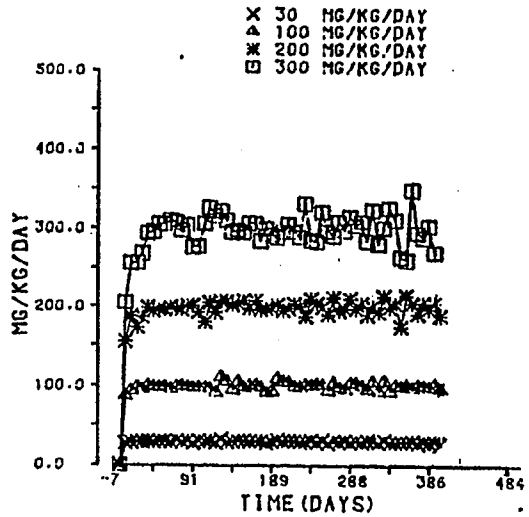
MALE MEAN BODY WEIGHT



MALE MEAN FOOD CONSUMPTION

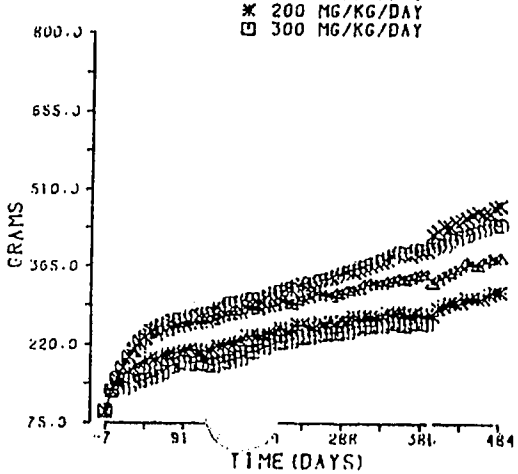


MALE MEAN ACTUAL DRUG CONSUMPTION

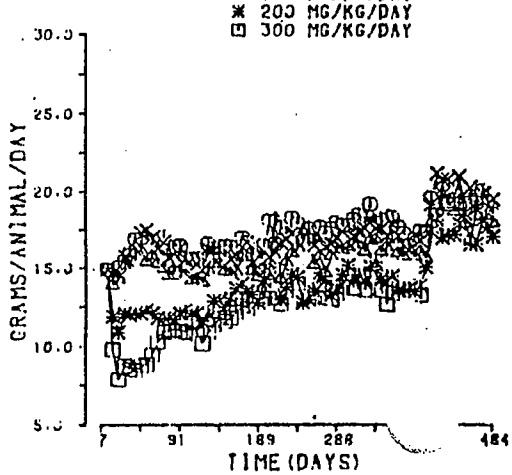


Best Possible Copy

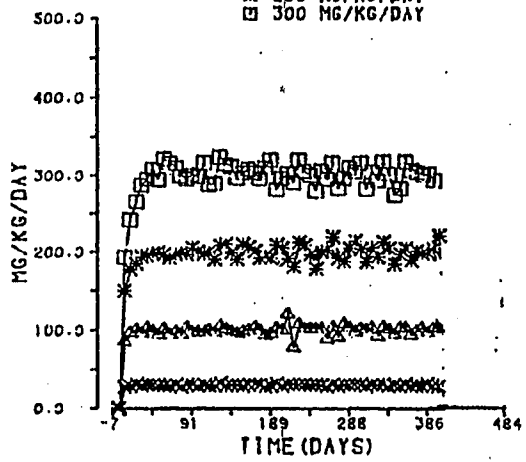
FEMALE MEAN BODY WEIGHT



FEMALE MEAN FOOD CONSUMPTION



FEMALE MEAN ACTUAL DRUG CONSUMPTION



36

Table A

Numerical Summaries of Histopathologic Findings *

Location	Dose Groups (mg/kg/day)	Evaluation Periods: Six Months		Six Months Plus Three Months Recovery		Twelve Months		Twelve Months Plus Three Months Recovery	
		No. with Microvacuoles / No. Examined	Average ** Severity	No. with Microvacuoles / No. Examined	Average Severity	No. with Microvacuoles / No. Examined	Average Severity	No. with Microvacuoles / No. Examined	Average Severity
Cerebellum	0	0/20	0	-	-	4/17	1.0	5/20	1.2
	30	0/10	0	-	-	12/20	1.42	0/9	0
	100	10/10	1.6	-	-	10/10	1.9	2/20	1.0
	200	18/20	1.56	0/10	0	8/15	1.0	0/19	0
	300	20/20	1.4	0/10	0	6/24	1.0	-	-
Reticular Formation	0	0/20	0	-	-	2/17	1.0	0/20	0
	30	0/10	0	-	-	2/20	1.0	0/9	0
	100	4/10	1.5	-	-	8/10	1.88	0/20	0
	200	12/20	1.42	0/10	0	14/15	1.29	1/19	1.0
	300	10/20	1.40	0/10	0	20/24	1.15	-	-
Optic Tract	0	0/20	0	-	-	0/17	0	0/20	0
	30	0/10	0	-	-	0/20	0	0/9	0
	100	0/10	0	-	-	0/10	0	0/20	0
	200	8/20	1.0	0/10	0	2/15	1.0	0/19	0
	300	12/20	1.66	0/10	0	10/24	1.10	-	-
Hippocampus	0	0/20	0	-	-	0/17	0	0/20	0
	30	0/10	0	-	-	11/20	1.27	0/9	0
	100	1/10	1.0	-	-	5/10	1.2	0/20	0
	200	1/20	1.0	0/10	0	2/15	1.0	0/19	0
	300	0/20	0	0/10	0	2/24	1.0	-	-

* See Table B, Appendix A for the raw data listings used to calculate these summaries. Table B also provides raw data listings for the other six areas of the brain on which histopathologic evaluations were made.

** Average severity of observed lesions was calculated using data only from animals with lesions (i.e., zero values were not included in the mean).

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table II

Incidence and Severity of Brain Vacuolation

	Grade	6 Months treatment										6 mo. treatment + 3 mo. recovery				6 mo. treatment + 6 mo. recovery			
		Control		30		100		200		300		200		300		200		300	
		10M	10F	5M	5F	5M	5F	10M	10F	10M	10F	5M	5F	5M	5F	5M	4F*	5M	5F
Cerebellar vacuolation	0	10	10	5	5	-	-	2	-	-	-	5	5	5	5	5	4	4	5
	1	-	-	-	-	3	2	3	6	5	7	-	-	-	-	-	-	1	-
	2	-	-	-	-	1	3	5	3	5	3	-	-	-	-	-	-	-	-
	3	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-
Reticular formation vacuolation	0	10	10	5	5	2	4	3	5	4	6	5	5	5	5	5	4	5	5
	1	-	-	-	-	3	-	4	3	5	2	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	3	2	1	1	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
Optic tract vacuolation	0	10	10	5	5	5	5	6	6	3	5	5	5	5	5	5	4	5	5
	1	-	-	-	-	-	-	4	4	4	3	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-
Thalamic vacuolation	0	10	10	5	5	5	5	7	9	5	8	5	5	5	5	5	4	5	5
	1	-	-	-	-	-	-	2	1	4	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-
Hypothalamic vacuolation	0	10	10	5	5	5	5	7	10	6	8	5	5	5	5	5	4	5	5
	1	-	-	-	-	-	-	3	-	4	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Collicular vacuolation	0	10	10	5	5	3	5	8	9	10	7	5	5	5	5	5	4	5	5
	1	-	-	-	-	2	-	2	1	-	3	-	-	-	-	-	-	-	-
Anterior commissure vacuolation	0	10	10	5	5	5	4	8	7	6	6	5	5	5	5	5	4	5	5
	1	-	-	-	-	-	1	1	3	3	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	1	-	1	3	-	-	-	-	-	-	-	-
Columns of fornix vacuolation	0	10	10	5	5	5	5	8	10	8	8	5	5	5	5	5	4	5	5
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	2	-	1	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Hippocampal vacuolation	0	10	10	5	5	5	4	9	10	10	10	5	5	5	5	5	4	5	5
	1	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Cerebral peduncle vacuolation	0	10	10	5	5	5	5	10	10	10	10	5	5	5	5	5	4	5	5

* An additional rat died on study, not included in count

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 11 (continued)

Incidence and Severity of Brain Vacuolation

		12 Month Treatment										12 mo. treatment + 3 mo. recovery							
		Control		30		100		200		300		Control		30		100		200	
	Grade	7M	10F	10M	10F	5M	5F	7M	8F	9M	15F	10M	10F	4M	5F	10M	10F	9M	10F
Cerebellar vacuolation	0	7	6	5	3	-	-	4	3	8	10	8	7	4	5	10	8	9	10
	1	-	4	4	4	2	3	3	5	1	5	1	3	-	-	-	2	-	-
	2	-	-	-	3	1	-	-	-	-	-	1	-	-	-	-	-	-	-
	3	-	-	1	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-
Reticular formation vacuolation	0	6	9	9	9	1	1	-	1	1	3	10	10	4	5	10	10	8	10
	1	1	1	1	1	2	2	5	6	6	11	-	-	-	-	-	-	1	-
	2	-	-	-	-	2	-	1	1	2	1	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Optic tract vacuolation	0	7	10	10	10	5	5	5	8	6	8	10	10	4	5	10	10	9	10
	1	-	-	-	-	-	-	2	-	2	7	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Thalamic vacuolation	0	7	10	10	10	5	5	7	8	9	15	10	10	4	5	10	10	9	10
Hypothalamic vacuolation	0	7	10	10	10	5	5	7	8	9	15	10	10	4	5	10	10	9	10
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Collicular vacuolation	0	7	10	10	10	5	4	7	8	9	15	10	10	4	5	10	10	9	10
	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Anterior commissure vacuolation	0	7	10	10	10	3	5	7	8	9	15	10	10	4	5	10	10	9	10
	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Columns of fornix vacuolation	0	7	10	10	10	5	5	7	8	8	14	10	10	4	5	10	10	9	10
	1	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Hippocampal vacuolation	0	7	10	5	4	3	2	7	6	7	15	10	10	4	5	10	10	9	10
	1	-	-	3	5	1	3	-	2	2	-	-	-	-	-	-	-	-	-
	2	-	-	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Cerebral peduncle vacuolation	0	7	10	10	10	5	5	4	8	6	14	10	10	4	5	10	10	9	10
	1	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	2	-	2	1	-	-	-	-	-	-	-	-

ONE YEAR TOXICITY STUDY WITH MDL 71,754 IN RATS

Table 12

Incidence of Eosinophilic Spheroids and Mineralized Bodies[a]

	100 mg/kg		200 mg/kg		300 mg/kg	
	ES	MB	ES	MB	ES	MB
MALES						
6 mo.	0/5	0/5	2/10	0/10	1/10	0/10
6 mo. + 3 & 6 mo. rec.	-	-	5/10	7/10	9/10	2/10
12 mo.	2/5	2/5	4/7	1/7	4/9	0/9
12 mo. + 3 mo. rec.	6/10	7/10	8/9	2/9	-	-
FEMALES						
6 mo.	0/5	0/5	2/10	2/10	1/10	0/10
6 mo. + 3 & 6 mo. rec.	-	-	5/9	1/9	4/10	1/10
12 mo.	1/5	0/5	3/8	0/8	9/15	1/15
12 mo. + 3 mo. rec.	2/10	1/10	5/10	3/10	-	-
TOTALS						
6 mo.	0/10	0/10	4/20	2/20	2/20	0/20
6 mo. + 3 & 6 mo. rec.	-	-	10/19	8/19	13/20	3/20
12 mo.	3/10	2/10	7/15	1/15	13/24	1/24
12 mo. + 3 mo. rec.	8/20	8/20	13/19	5/19	-	-

ES = eosinophilic spheroids
MB = mineralized bodies

[a] does not include animals dying on study

DOW CONFIDENTIAL

TWO YEAR CARCINOGENICITY STUDY IN RATS

A) Dosage

50/sex at 0, 50, 100, or 150 mg/kg/day, in diet

Strain: Long Evans

Lot #: 71,754-49 (days 1-679)
71,754-79 (days 673-736)

B) Results

1) Observed signs

a) Convulsions (not further described) seen at all doses (See attached figure). Incidence and time of onset were D-R. Overall incidence 3, 26, and 60% at LD, MD, and HD, resp. Not seen until 6 months at MD and HD; only seen near the end of the study at LD.

b) Although apparently not considered drug-related by the sponsor, 5 LD and 6 HD rats were "unable to move hind quarters". Earliest occurrence day 167. Duration per animal ranged from 1 to 21 days. Most affected animals died or were sacrificed in poor condition; thus reversibility was not established. Sections of lumbar spinal cord from some of these rats (3 LD and 4 HD) were examined histologically; all were said to be negative except for 2 HD; one of these showed "occasional vacuole present appears to be the result of artifactual leaching of myelin during processing" and the other showed "considerable postmortem autolytic changes including multifocal vacuolar changes of the white matter." It is difficult to evaluate these findings in the absence of similarly examined controls. Sciatic nerve, which was routinely examined histologically in most animals in the study, showed no drug effect.

c) Nothing was stated regarding routine palpation for masses.

2) Mortality

No drug effects. (See attached figure). Overall mortality rate was about 30%.

3) Bodyweight

D-R decrease at all doses. See attached figures. Terminal weights, as % of control, were said to be as follows (aside from attached graphs, no group or individual data shown):

	M	F
LD	94	92
MD	85	91
HD	70	80

4) Food consumption

D-R decrease at all doses; see attached figure. (No other data shown).

5) Hematology

(Done in 10/sex/group at termination)

a) Slight, partly D-R increase in RBC, Hb, and Hct in all M groups. No very high individual values.

b) Other parameters measured: WBC, differential, platelets

6) Blood chemistry

(Done in 10/sex/group at termination)

a) Fasting glucose-equivocal , slight non D-R increase in all F groups. No very high individual values

b) Ca slightly decreased in HD M. No very low individual values.

c) Other parameters measured: ALT, AST, AP, total bilirubin, total protein, albumin, A/G, Pi, Cl, BUN.

7) Organ weights

Apparently not done

8) Gross pathology

a) The incidence of infarctions/scars in kidney was slightly increased at MD and HD at termination; this was not seen among premature deaths and was not associated with any drug-induced histopathology in kidney.

b) The incidence of large and hemorrhagic pituitary was decreased in all F groups, D-R. There was also a trend toward a slight decrease in skin/subcutis nodules/masses in all groups of both sexes, D-R.

9) Microscopic pathology

(Routine exam done at all doses)

a) Non-neoplastic lesions.

1) The only clearly drug-related increase was in brain, consisting of vacuolation, eosinophilic spheroids, and mineralized bodies, as seen in previous studies. All doses were affected. The distribution and incidence of these findings is shown in the attached table. (Note that the table does not indicate severity, and thus might obscure some of the drug- and dose-relationships. For example, a spot check of the severity of cerebellar vacuolation in F indicates the least severity in the control group, thus indicating a drug effect which is not apparent from the table. The sponsor did present figures [attached] showing mean severity grades at termination for cerebellum and "all sites"; these figures confound incidence and severity in that animals without vacuoles were apparently given a grade "0" which was averaged in with severity grades in affected animals; however, taken as some overall measure of drug effect it does indicate an effect at all doses although not dose-related; however the lesser effect at HD is associated with the appearance of other lesions [mineralized bodies and eosinophilic spheroids], which did occur in a D-R fashion and are probably indicative of a residual, permanent change.)

The sponsor stated that "compared to the results of the previous one-year study, the combined severity grades of vacuolation for "all sites'...appeared to be slightly greater after two years of treatment" and that "this appeared to be due to the slightly more widespread distribution of the vacuolation after two years as there was no noticeable difference in severity at any individual site". The sponsor also noted that, as in the one year study, the cerebellum was the most severely affected area of brain. Regarding the mineralized bodies, it was stated that, in comparison to the one year study, the incidence after 2 years was "somewhat higher", and that "the impression was that they were somewhat larger and more widespread, at least at the highest dose".

2) As also shown in the attached table, there was no drug effect in sciatic nerve.

3) The incidence of pituitary hemorrhage was decreased at all doses, D-R.

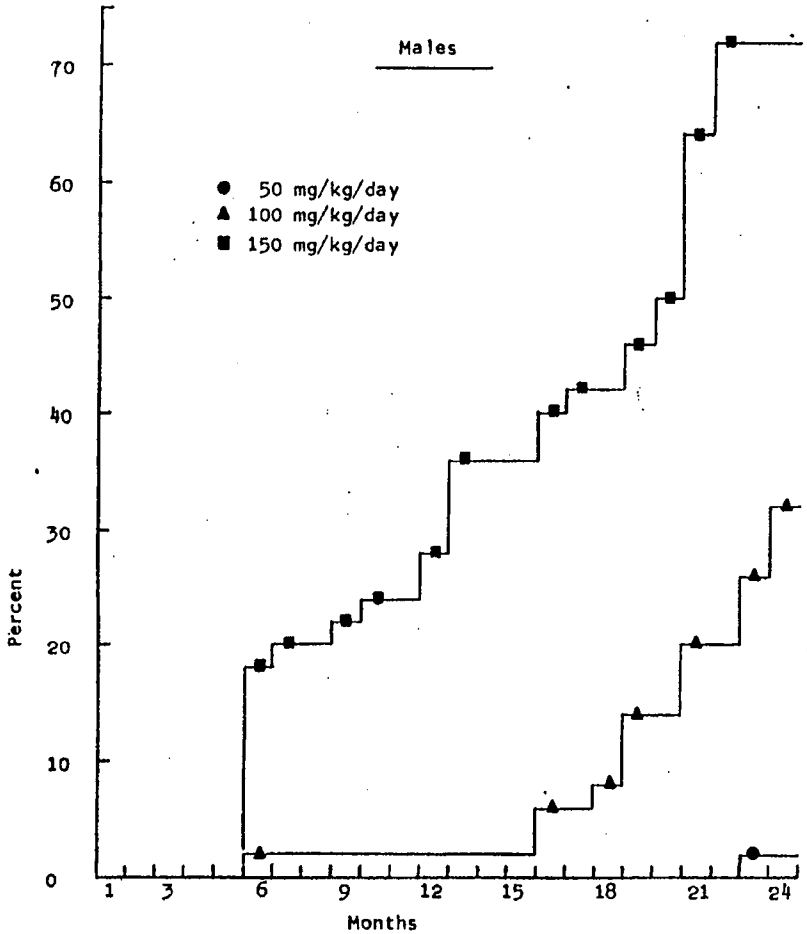
b) Neoplastic lesions

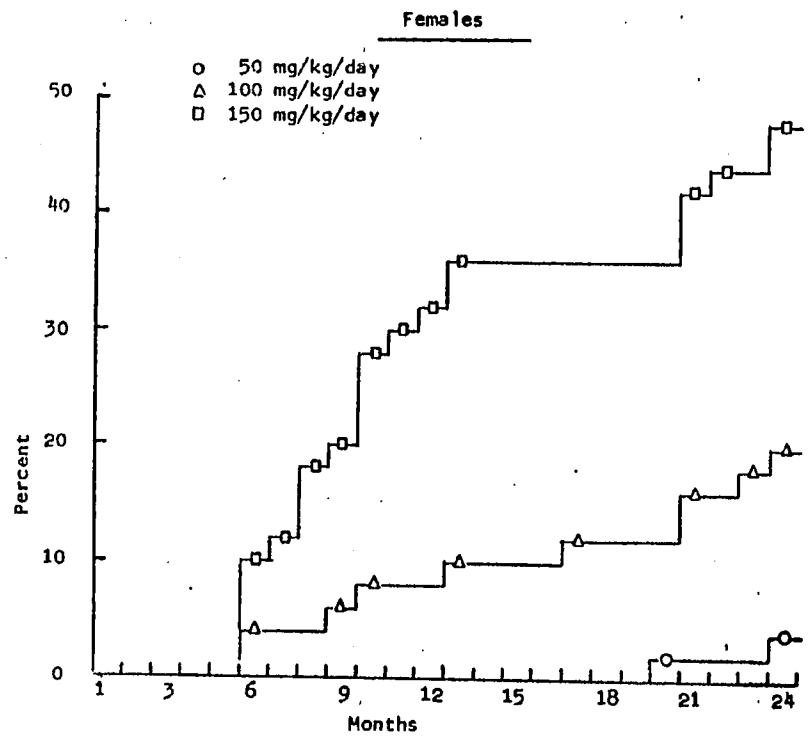
No drug-related increases. The incidence of pituitary adenomas was decreased at all doses, D-R. The incidence of total benign and total malignant tumors was also decreased, but not always D-R. (See attached table).

CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Plate 3

Percent of Rats Developing Convulsions



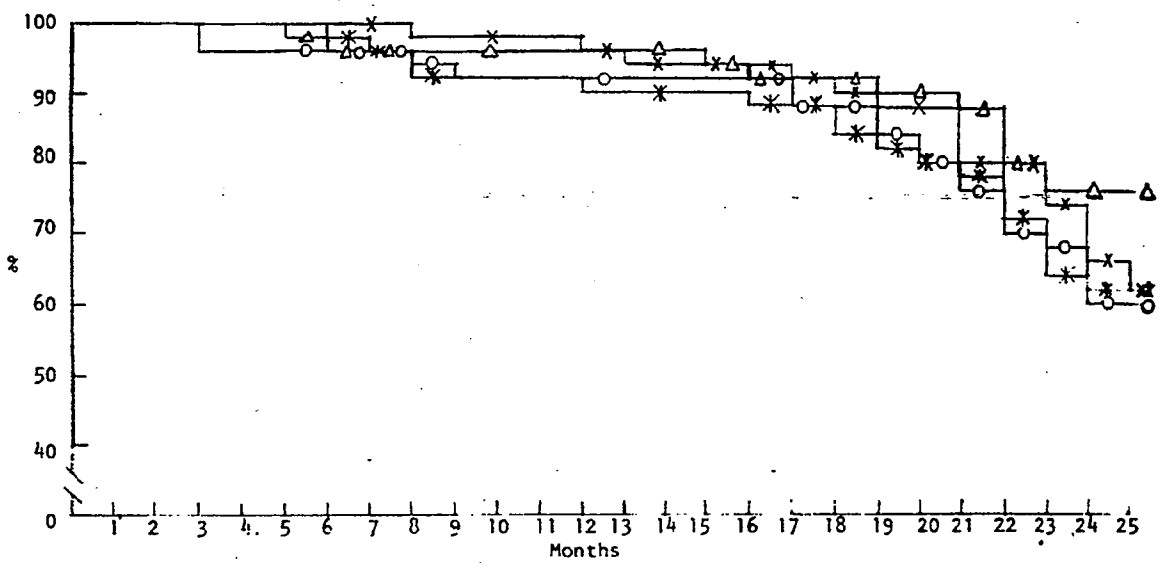


CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

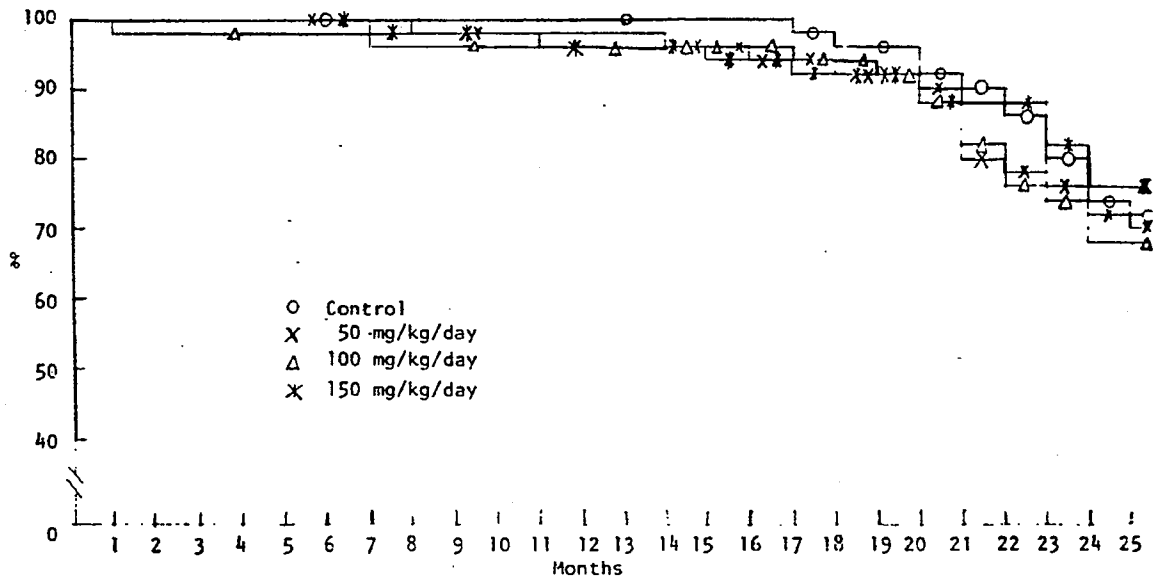
Plate 4

Survival Rate

Males



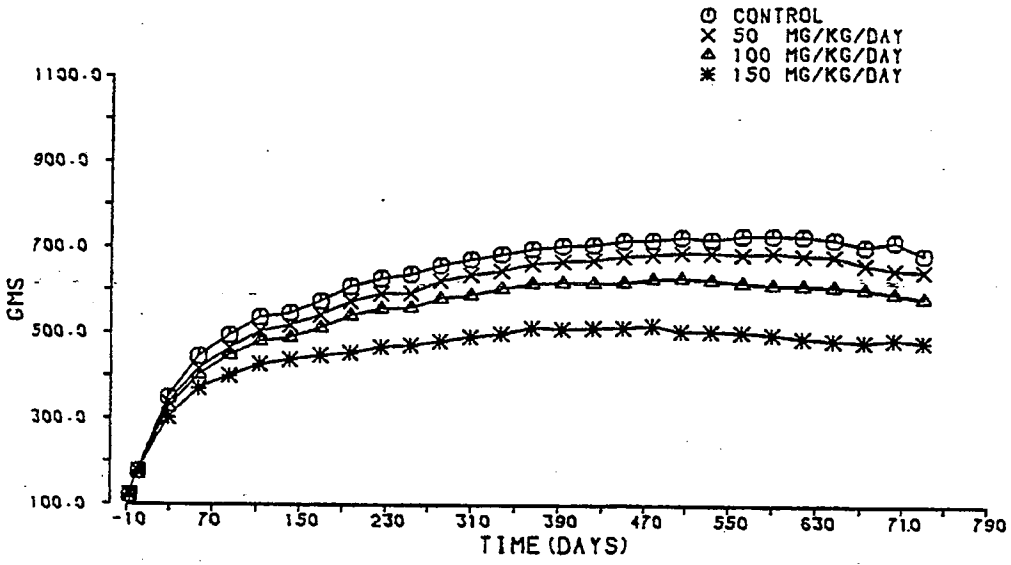
Females



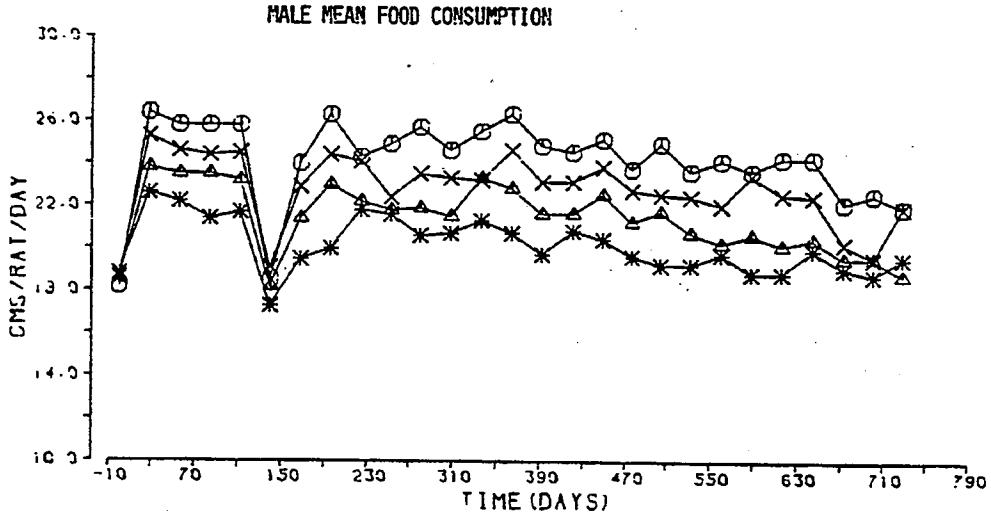
CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Plate 1

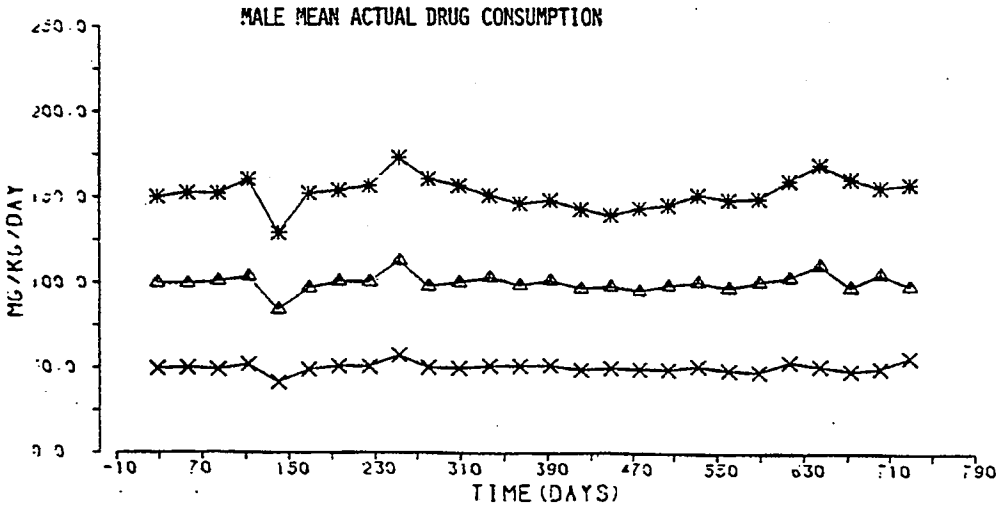
MALE MEAN BODY WEIGHTS (GMS)



MALE MEAN FOOD CONSUMPTION



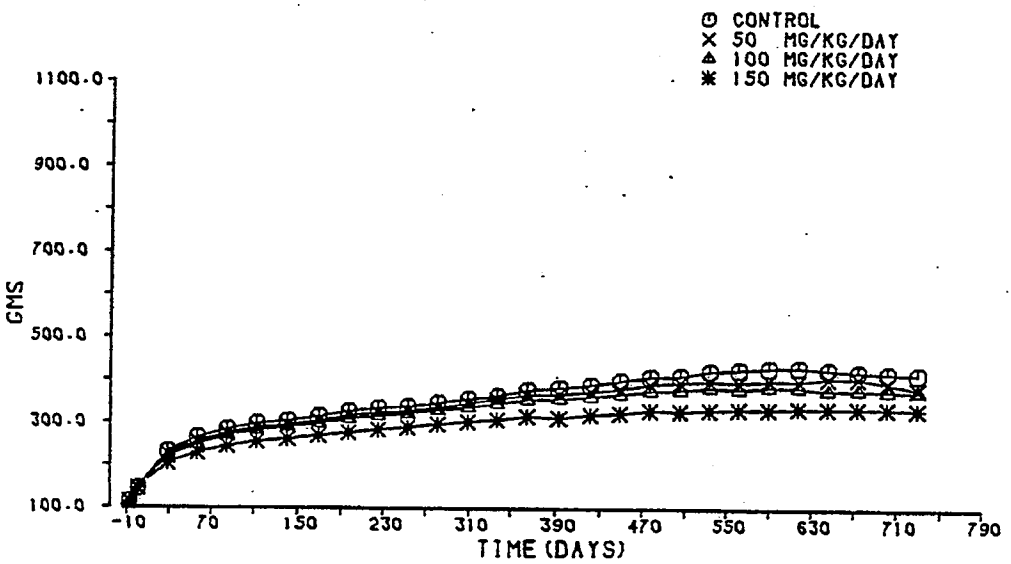
MALE MEAN ACTUAL DRUG CONSUMPTION

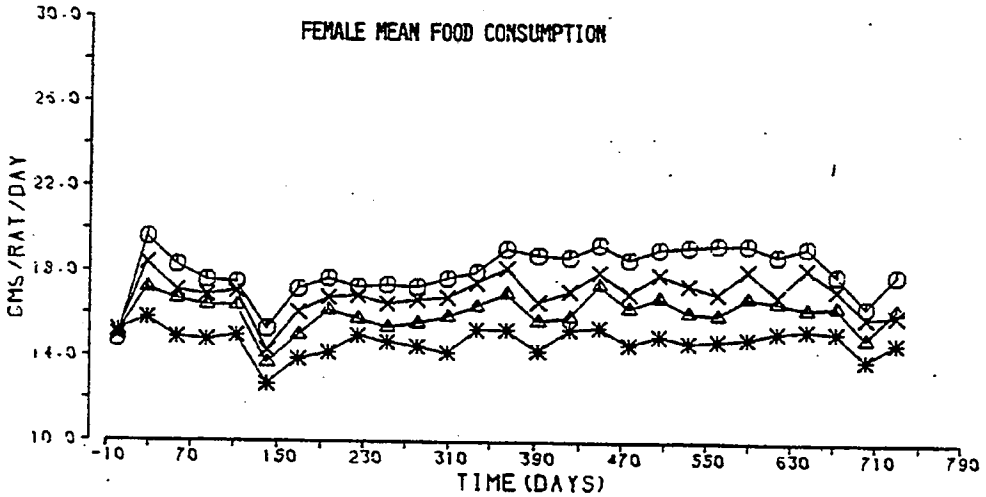


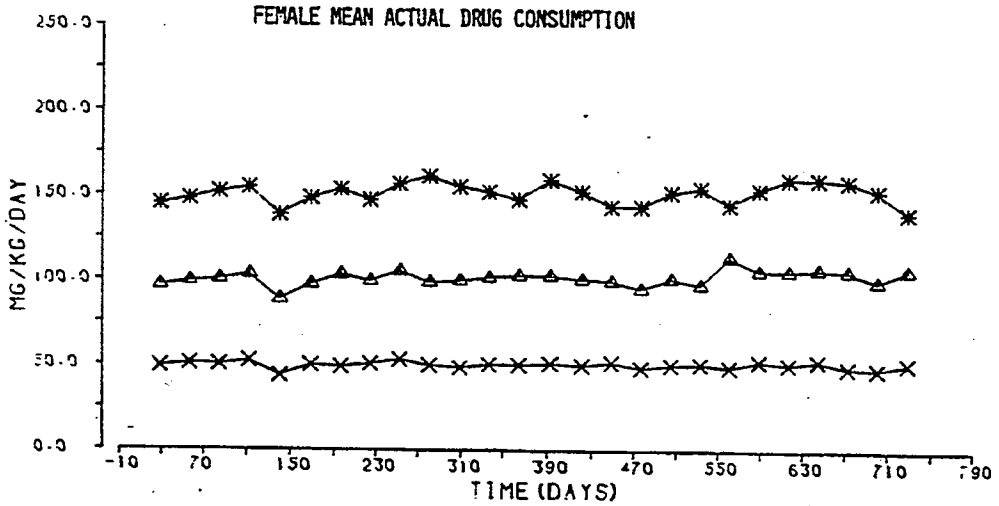
CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Plate 2

FEMALE MEAN BODY WEIGHTS (GMS)







CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABARTIN

Table 7

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY
CINCINNATI, OHIO 45215

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4

PRINTED: 25-AUG-86
PAGE: 1

ALL ANIMALS * NON NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES				-- FEMALES			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
BRAIN	NUMBER EXAMINED:	49	48	50	50	50	50	50	50
-CEREBELLAR VACUOLATION		15	39	41	33	36	42	44	38
-THALAMIC MINERALIZATION		2	1	0	9	0	0	1	6
-THALAMIC GLIOSIS		1	0	0	0	0	0	0	0
-MENINGEAL VASCULARIZATION		1	0	0	0	0	0	0	0
-RETICULAR FORMATION VACUOLATION		2	33	36	34	7	33	38	40
-CEREBELLAR MINERALIZED BODIES		0	13	36	44	0	5	21	37
-HIPPOCAMPAL VACUOLATION		0	14	15	16	0	17	16	11
-OPTIC TRACT VACUOLATION		0	4	20	15	1	9	19	23
-ANTERIOR COMMISSURE VACUOLATION		0	2	7	8	0	7	9	0
-THALAMIC VACUOLATION		0	20	21	10	0	25	31	19
-INFERIOR COLLICULUS VACUOLATION		0	22	16	10	0	21	27	18
-CEREBRAL CORTEX VACUOLATION		0	14	16	18	1	22	26	22
-CEREBRAL PEDUNCLE VACUOLATION		0	0	6	6	0	0	3	16
-INTERNAL CAPSULE MINERALIZATION		0	0	2	4	2	2	0	0
-CEREBELLAR EOSINOPHILIC SPHEROIDS		0	11	24	31	1	12	16	21
-INFERIOR COLLICULUS MINERALIZATION		0	0	0	3	0	0	0	1
-COLUMN OF FORNIX VACUOLATION		0	0	1	1	0	2	7	4
-MICROABSCESS		0	1	1	1	0	0	0	0
-INTERNAL CAPSULE VACUOLATION		0	1	5	1	0	2	8	8
-HIPPOCAMPAL MINERALIZATION		0	0	0	1	0	0	0	0
-CHOROID PLEXUS MINERALIZATION		0	1	0	1	0	0	0	0
-CORPUS CALLOSUM VACUOLATION		0	1	7	5	0	5	14	10
-RETICULAR FORMATION MINERALIZATION		0	0	1	0	0	0	0	2
-CEREBRAL CORTEX MINERALIZATION		0	0	0	0	0	0	0	1
-HEMORRHAGE		0	0	0	0	1	1	1	1
SCIATIC NERVE	NUMBER EXAMINED:	48	48	49	48	50	50	49	47
-MINERALIZED BLOOD VESSELS		3	1	1	0	0	0	0	0
-SEGMENTAL DEGENERATION WITH FRAGMENTED EOSINOPHILIC SPHEROIDS		32	22	30	25	24	28	26	20
EYE	NUMBER EXAMINED:	34	40	43	40	44	38	43	42
-OUTER NUCLEAR LAYER DYSPLASIA		0	3	0	1	1	0	1	2
-RETINAL DEGENERATION		0	0	0	0	1	0	0	1
-SUPPURATIVE KERATITIS		0	0	0	0	0	1	0	0
-CORNEAL NEOVASCULARIZATION		0	0	0	0	0	1	0	0

49

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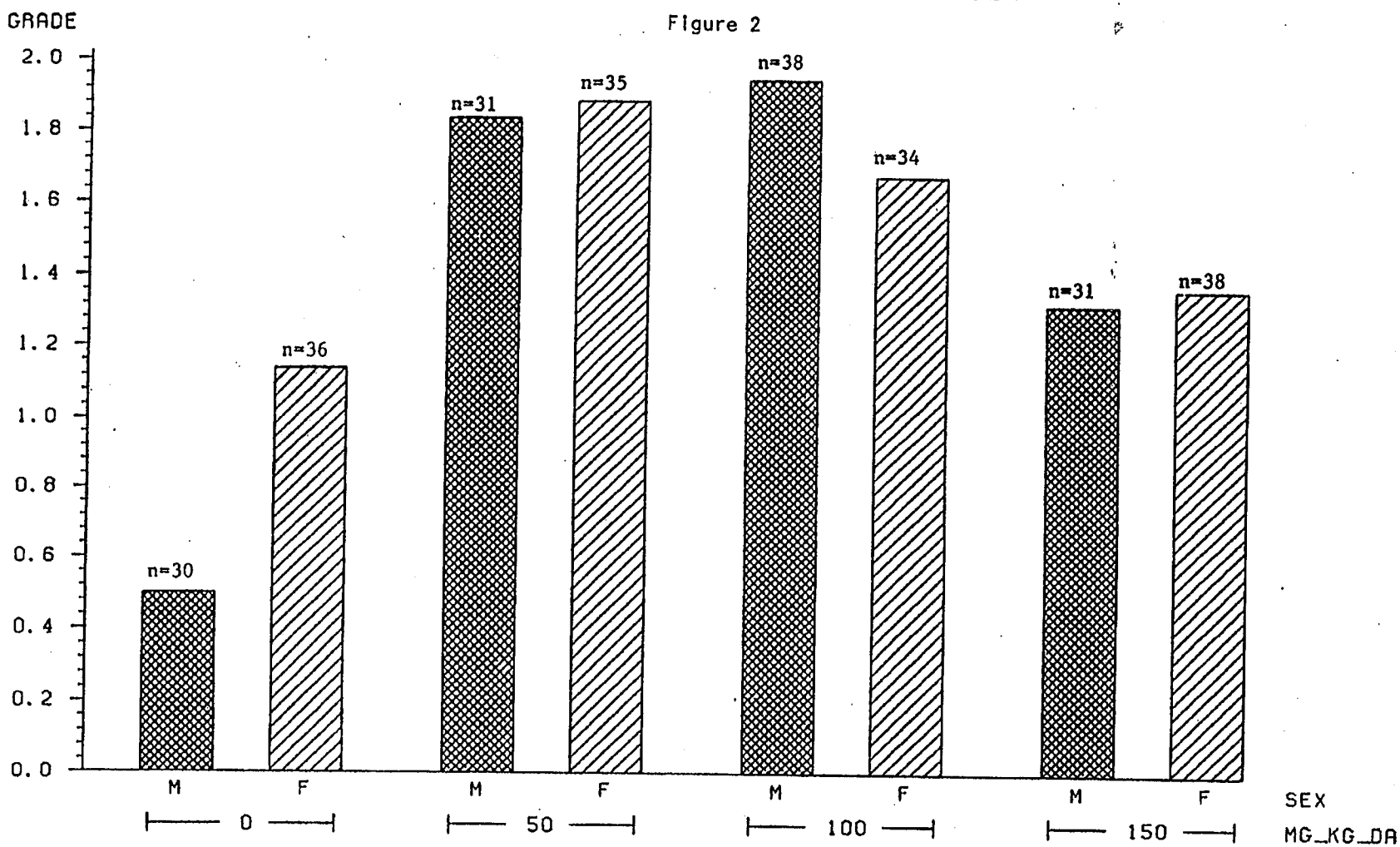
VINYL GABA CARCINOGENICITY RAT STUDY

CEREBELLAR VACUOLATION
MEAN GRADE AT 24 MONTHS OF STUDY

Figure 2

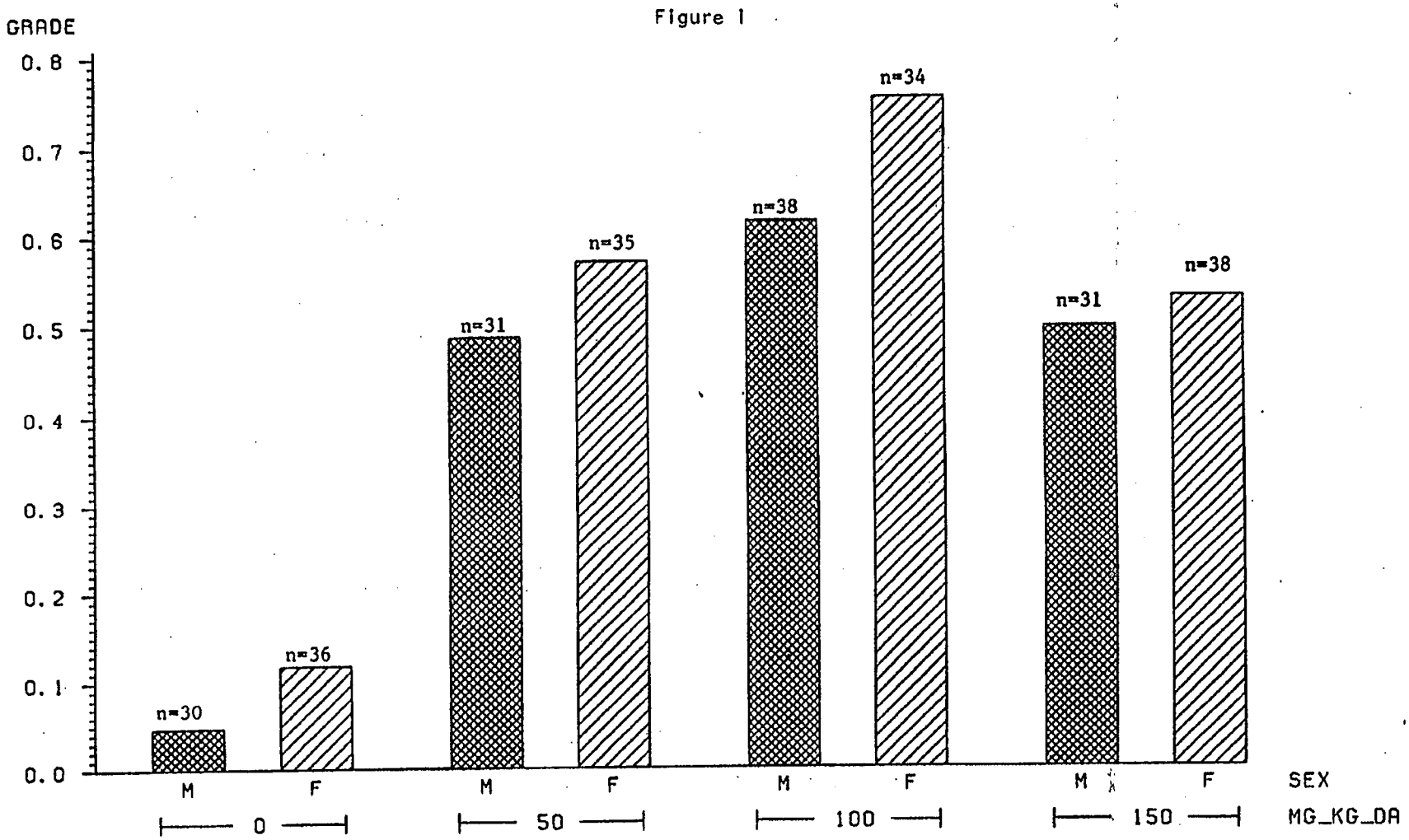


0=ABSENT 1=VERY SLIGHT
2=MILD 3=MODERATE
4=MARKED 5=SF RE

VINYL GABA CARCINOGENICITY RAT STUDY

BRAIN VACUOLATION (ALL SITES)
MEAN GRADE AT 24 MONTHS OF STUDY

Figure 1



0=ABSENT 1=VERY SLIGHT
2=MILD 3=MODERATE
4=MARKED 5=SEVERE

CARCINOGENICITY STUDY OF RATS TREATED WITH VIGABATRIN

Table 8

MERRELL DOW RESEARCH INSTITUTE
PATHOLOGY TOXICOLOGY
CINCINNATI, OHIO 45215

SUMMARY INCIDENCE OF MICROSCOPIC OBSERVATIONS
STUDY NUMBER 800C-4

PRINTED: 25-AUG-86
PAGE: 1

ALL ANIMALS * ALL NEOPLASTIC FINDINGS *

TISSUES WITH FINDINGS	ANIMAL SEX:	-- ANIMALS AFFECTED -- MALES				FEMALES			
	DOSAGE GROUP:	1	2	3	4	1	2	3	4
	NO. IN GROUP:	50	50	50	50	50	50	50	50
BRAIN	NUMBER EXAMINED:	49	48	50	50	50	50	50	50
-M- ASTROCYTOMA		1	0	1	0	0	0	0	0
-N- LYMPHOSARCOMA		0	0	1	0	0	2	0	0
SCIATIC NERVE	NUMBER EXAMINED:	48	48	49	48	50	50	49	47
EYE	NUMBER EXAMINED:	34	40	43	40	44	38	43	42
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
-N- FIBROSARCOMA		0	1	0	0	0	0	0	0
PITUITARY	NUMBER EXAMINED:	49	46	49	50	49	48	49	48
-B- ADENOMA		19	13	10	9	31	21	17	16
-N- ASTROCYTOMA		1	0	0	0	0	0	0	0
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
-M- CARCINOMA		0	0	0	0	0	1	0	0
THYROID	NUMBER EXAMINED:	46	46	49	48	48	48	49	48
-B- C-CELL ADENOMA		4	5	3	3	2	2	3	0
-B- FOLLICULAR ADENOMA		0	0	0	0	0	0	0	1
ADRENAL	NUMBER EXAMINED:	49	50	50	49	50	50	49	49
-B- PHEOCHROMOCYTOMA		2	6	1	1	1	0	0	0
-B- MEDULLARY NODULAR HYPERPLASIA		1	4	2	2	0	1	0	0
-M- CORTICAL CARCINOMA		0	0	0	1	0	0	0	0
-B- CORTICAL ADENOMA		0	0	0	1	0	0	0	1
-N- LYMPHOSARCOMA		0	0	0	0	0	1	1	0
-N- ADENOCARCINOMA		0	0	0	0	0	1	1	0
LACRIMAL GL. EX	NUMBER EXAMINED:	44	43	48	48	49	48	50	49
-N- LYMPHOSARCOMA		0	0	0	1	0	1	0	0
LACRIMAL GL. POS	NUMBER EXAMINED:	50	49	50	49	50	48	50	50
-N- LYMPHOSARCOMA		0	1	0	1	0	0	0	0
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	0
SALIVARY GLAND	NUMBER EXAMINED:	50	48	50	50	50	49	50	50
-N- LYMPHOSARCOMA		0	0	0	0	0	1	0	1
-M- FIBROSARCOMA		1	0	1	0	1	0	0	0
-M- FIBROUS HISTIOCYTOMA		0	0	0	0	0	0	0	1

M = lignant
B = benign
N = metastatic