

**CENTER FOR DRUG EVALUATION AND RESEARCH**

*APPLICATION NUMBER:*

**22-006**

**PHARMACOLOGY REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology  
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**NDA:** 20-427 and 22-006

**Submission date:** December 28, 2007 (Complete response)

**Drug:** vigabatrin

**Sponsor:** Ovation Pharmaceuticals

**Indication:** 20-427: refractory complex partial seizures in adults  
22-006: infantile spasms

**Reviewing Division:** Division of Neurology Products

**Introductory Comments:** The regulatory history of these two NDAs is summarized in the supervisory pharm/tox review. The pharm/tox review of NDA 20-427 found the nonclinical information adequate to support approval. The pharm/tox reviewer for NDA 22-006 did not find the nonclinical information adequate to support approval primarily based on evidence that juvenile animals were sensitive to neurotoxic effects of vigabatrin. The pharm/tox supervisor recognized this concern but did not object to the approval of NDA 22-006 based on the clinical benefit of vigabatrin in infantile spasms which is a serious indication with no other approved therapy. The supervisor recommended that additional studies on the retinal damage and neurotoxicity induced by vigabatrin be conducted as postmarketing requirements. This includes the following studies:

1. A toxicology study in the juvenile rat examining the potential of vigabatrin exposure during development to produce neuronal damage.
2. A juvenile animal toxicology study of vigabatrin in a non-rodent species.
3. A study examining the effect of taurine on vigabatrin-induced retinal damage in rodent.

### **Conclusions:**

I have discussed these NDAs with the division pharm/tox supervisor and agree that they may be approved from a pharm/tox perspective. It is my understanding that the postmarketing requirements have already been discussed with the sponsor. The requirement for juvenile studies in two species is somewhat unusual; however, the potential seriousness of the brain lesions observed in the rat may warrant further investigation in an additional species to help inform on the nature of the lesions and the likelihood of the lesions occurring in multiple species.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 20427	ORIG 1		SABRIL (VIGABATRIN) TABLET 500MG
NDA 20427	ORIG 1		SABRIL (VIGABATRIN) TABLET 500MG
NDA 22006	ORIG 1		SABRIL (VIGABATRIN)

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PAUL C BROWN  
08/07/2009

**MEMORANDUM**

**DEPARTMENT OF HEALTH & HUMAN SERVICES  
Public Health Service  
Food and Drug Administration**

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**Division of Neurology Products (HFD-120)  
Center for Drug Evaluation and Research**

Date: July 21, 2009

From: Lois M. Freed, Ph.D.  
Supervisory Pharmacologist

Subject: Sabril<sup>®</sup> (vigabatrin), NDAs 20-427 (refractory complex partial seizures in adults) and NDA 22-006 (infantile spasms)

Re: Complete Responses submitted December 28, 2007.

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**Regulatory Background**

**NDA 20-427**

NDA 20-427 for Sabril (vigabatrin), an irreversible GABA transaminase inhibitor, for treatment of complex partial seizures in adults was originally submitted to the Agency on April 29, 1994 by Aventis, Inc. The nonclinical studies submitted at that time were reviewed by Barry N. Rosloff, Ph.D., and consisted of a full battery of nonclinical studies, including oral chronic toxicology ( $\leq 1$  year in rat and dog and 6-year in monkey), oral reproductive toxicology (mating and fertility in male and female rat [separate studies], embryo-fetal development in rat [2 studies] and rabbit [2 studies], peri/postnatal development in rat), dietary carcinogenicity (18-month mouse, 2-year rat), and genetic toxicology (*in vitro* Ames, CHO/HGPRT forward mutation, and chromosomal aberration assay in rat lymphocytes; *in vivo* oral micronucleus assay in rat) studies.

Based on review of these data, Dr. Rosloff concluded that the nonclinical data supported approval of the NDA (Pharmacologist Review of NDA 20-427 Original Summary, 2/28/95). In the NDA review, Dr. Rosloff noted the following nonclinical findings of relevance to humans:

- Brain intramyelinic edema, detected in mouse, rat, and dog (and considered an equivocal finding in monkey).
- Retinal lesions, characterized as “retinal degeneration”, detected in mouse and rat and considered possibly related to the intramyelinic edema noted in these species.

- Malformations (cleft palate) reproducibly demonstrated in embryo-fetal studies in rabbit
- Decreases in transaminases (particularly ALT), of concern when monitoring potential toxicity of vigabatrin or other drugs in humans.

[The malformations and decreases in transaminases are being addressed in labeling, and require no further discussion.]

In a Supervisory Overview (March 23, 1995), Glenna G. Fitzgerald, Ph.D. concurred on the adequacy of the nonclinical data, and recommended that findings of intramyelinic edema and retinal degeneration be included in labeling. Dr. Fitzgerald noted that the intramyelinic edema findings had been presented to a PCNS Advisory Committee on November 20, 1989 and that it was the Committee's recommendation that "...a noninvasive method for clinical monitoring of the onset of neurotoxicity be developed". In response, the Sponsor (at that time, Marion Merrill Dow Inc.) conducted studies in dogs to develop methods (MRI, somatosensory, visual, and auditory evoked potentials) which were then used to monitor humans (a discussion of the nonclinical data is included in Dr. Rosloff's NDA review).

In subsequent memos, Drs. Rosloff (October 14, 1997 addendum to the NDA review) and Fitzgerald (Memorandum, October 17, 1997) made additional labeling recommendations; these included a recommendation to incorporate findings from two published studies assessing potential adverse effects of vigabatrin on pre- and post-natal development (Abdulrazzaq YM *et al. Teratology* 55:165-176, 1997; Sidhu *et al. Exp Neurol* 144:400-405, 1997).

The Agency issued an Approvable letter on November 26, 1997. On October 27, 1998, the Agency issued a Not Approvable (NA) letter due to post-marketing reports from Europe of visual field defects (VFD) in patients treated with vigabatrin. (VFDs had not be observed in clinical trials in the U.S.) The Agency requested additional information on the visual field defects in adults and children.

In a December 1, 2004 meeting, the sponsor (now Ovation; meeting minutes dated 2/18/05) asked for comments on the clinical plan for development of vigabatrin for refractory complex partial seizures in adults and infantile spasms. The Division stated that "Juvenile animal toxicity studies will be required for approval of vigabatrin use in a pediatric population. In addition to possible effects on the retina and possible general systemic effects, the possible effect on myelination in the developing brain is of specific concern".

The sponsor submitted (December 23, 2005) a response to the Agency's October 27, 1998 NA letter. Included in this submission were nonclinical studies conducted since October 27, 1998 to investigate the "...potential for vigabatrin to induce retinal toxicity". These consisted of the following:

- Reassessment of "representative eye section" from the following studies:

- 6-12 month + 6 months recovery in dog
- 6-year (3-6-16-month interim sacrifice) oral toxicity in cynomolgus monkey
- 18-month carcinogenicity in CD-1 (albino) mice
- 2-week oral exploratory study in rat
- 2-week oral dose range-finding study in juvenile rat
- 4-week oral ocular toxicity study in juvenile rat
- 1-day to 3-month oral exploratory ocular study in non-epileptic or pilocarpine-induced epileptic rat
- 13-week (4-week interim sacrifice) + 4-week recovery ocular toxicity study in rat
  - Amendment 1: TK analysis of retina
- 3-month oral exploratory study of ocular effects in albino and pigmented rats (with or without exposure to excessive light during the first week of dosing)
- 3-month oral exploratory study of ocular effects in pigmented rat

The December 23, 2005 submission was considered an Incomplete Response (Agency letter of 2/15/06) due to deficiencies in reports of clinical (including serious adverse events) and CMC (dissolution) data. Subsequently, the sponsor re-submitted the NDA on October 10, 2006 and March 2, 2007; both of these submissions were also considered Incomplete Responses (Agency letters of 11/9/2006 and 4/3/07). Only the October 10, 2006 submission contained new nonclinical reports:

- In vitro hERG assay in HEK cells
- In vitro isolated rabbit cardiac Purkinje fiber assay
- Oral (gavage) repeated-dose toxicity in juvenile rat (Study No. OV-1007)
- Pathology Working Group Peer Review Report of potential treatment-related brain lesions from Study No. OV-1007

The sponsor submitted a Complete Response on December 28, 2007; this submission was filed (Agency letter of 2/26/08). The December 28, 2007 contained no new nonclinical studies.

## **NDA 22-006**

NDA 22-006 for Sabril (vigabatrin) for treatment of infantile spasms was original submitted to the Agency on October 17, 2006, but was determined to be incomplete. The NDA was re-submitted on March 9, 2007; a refuse-to-file action was taken on April 5, 2007 due to the lack of sufficient data to evaluate MRI findings reported in children. The NDA was resubmitted on December 28, 2007. The nonclinical studies submitted at that time were reviewed by J. Edward Fisher, Ph.D., and consisted of the following:

- Oral dose-range finding studies in adult and juvenile rats
- Four-week oral study in juvenile rat (OV-1007)
- Pathology Working Group review of the four-week oral study in juvenile rat
- Nine-week oral study in juvenile rat (OVNC-9004)

- Published literature

Based on the review of these data, and taking into consideration the nonclinical data submitted under NDA 20-427, Dr. Fisher has concluded that from a pharmacology/toxicology standpoint, the data do not support approval.

## **Nonclinical Issues**

There are two primary issues that need to be addressed. The following discussion is based on reviews/memo prepared by Drs. Rosloff, Fitzgerald, Fisher, and Dr. Larry Schmued (for neurotoxicity; NCTR Division of Neurotoxicity), and, in some cases, on examination of nonclinical data submitted by the sponsor and published literature.

### **1. Neurohistopathology**

The neurohistopathology findings in adult animals have been discussed in detail in reviews by Drs. Rosloff and Fisher. The neurohistopathological findings in the juvenile rat studies were reviewed by Dr. Fisher and Dr. Larry Schmued. Brain lesions, referred to as intramyelinic edema (IME), were detected in adult animals (mouse, rat, dog; equivocal in monkey). These lesions were characterized by microvacuoles in white matter, resulting from splitting of the interperiod line of the myelin sheath, and were observed after 3 months or more of dosing. Evidence of vacuoles in monkey was considered equivocal; however, brain GABA levels were not elevated at the doses tested. This is in contrast to the notable increases in brain or csf GABA levels detected in the other animal species tested, and in human.

In adult animals, the effect-dose tended to decrease and the affected areas become more widespread with increased duration of dosing. Vacuoles were found to be reversible in rat by 3 months after 6 or 12 months of treatment; however, reversibility was not assessed at after 12 months of dosing at the highest dose tested. Reversibility was also observed in dog by 6 months after 1 year of dosing. However, in rat, additional findings (eosinophilic spheroids and mineralized bodies) considered suggestive of residual damage were observed at the end of the 12-month dosing period that appeared to increase, at least in incidence, during the recovery period. As noted by the sponsor (*Toxicology Written Summary*), “While the vacuolar changes caused by the edema subsided with withdrawal of vigabatrin treatment, rats continued to have swollen axons and mineralized bodies in the cerebellum, indicative of increased myelin turnover, axonal damage, and mild astrocytic gliosis.” Similarly, at the end of the dosing period in the 18-month mouse study, findings considered evidence of residual damage (“foci of mineralization in the area of the cerebellar roof nuclei”) were detected in addition to the characteristic vacuolation.

No clear behavioral correlates were observed in adult animals. Delayed onset of convulsions (generally after three months of dosing in rat and 9-10 months of dosing in the mouse), often apparent upon handling, was observed in mouse and rat. (Convulsions were not observed in dog or monkey.) The effect-dose lowered with increased duration of

dosing, and in the 6- and 12-month studies, convulsions continued into the recovery period at all doses tested for recovery. In the adult, it is unlikely that these convulsions are related to IME since dog was equally affected, but did not develop convulsions. It is of note, however, that evidence of residual brain lesions was detected only in mouse and rat.

Based on the IME findings in mouse, rat and dog, the sponsor conducted investigative studies in animals that demonstrated that IME could be monitored in animals using MRI and measurement of evoked potentials. IME was not detected in older children and adults using this monitoring strategy. Although the negative MRI findings certainly reduce the safety concern, at least for adults, it is difficult to completely dismiss the concern regarding this potentially serious finding since (1) IME was detected in multiple species, i.e., in those species in which vigabatrin increases central GABA levels (mouse, rat, dog), as it does in humans and (2) although the vacuoles appeared reversible, prolonged administration of vigabatrin in mouse and rat resulted in additional microscopic changes reflective of irreversible injury.

In the more recently conducted juvenile toxicology studies in rat, neurohistopathology findings were also detected. Findings in the initial study (Study OV-1007), according to the sponsor, were consistent with IME and, therefore, based on the (mainly) adult human experience were not clinically relevant. However, Drs. Fisher and Schmued have reviewed these data and have concluded that the neurohistopathology findings in juvenile animals are distinctly different than that observed in adult animals. That was also the conclusion of the Pathology Working Group (PWG) that conducted a review of brain sections from Study OV-1007. The PWG specifically stated that:

“Vacuolar changes in white matter and gray matter are morphologically similar via light microscopy, and have a morphologic appearance that is not characteristic of intramyelinic edema.”

The sponsor references the results of a second juvenile study (Study OVNC-9004) as confirmation of the sponsor’s position that the neuropathological findings in juvenile animals are consistent with IME. Dr. Fisher notes that, while findings consistent with IME were detected in Study OVNC-9004, additional effects on myelin (i.e., demyelination, hypomyelination) were detected that indicate “a unique effect seen with VGB [vigabatrin] administration during periods of active myelination.” Dr. Fisher also notes that “A prominent [adverse] effect on myelination...has been reported previously...” in a published study by Qiao *et al.* [Qiao M *et al. Epilepsia* 41(6):655-66, 2000].)

As in the adult, no clear behavioral correlates were observed. However, convulsions with delayed onset were observed in both juvenile animal studies. The data indicate that the juvenile animal is notably more sensitive to the convulsive properties of vigabatrin than the adult, as evidenced by convulsions appearing earlier (beginning after 2-3 weeks of dosing) and at lower doses in the juvenile. A relationship, if any, between convulsions and the neurohistopathology detected in the juvenile animal is unknown. The fact that

delayed convulsions were observed in both adult and juvenile animals might suggest that the convulsions are not related to the neurohistopathology findings; however, it is possible that the apparently unique findings in the juvenile may be related to the increased sensitivity in the juvenile.

Dr. Schmued reviewed the two juvenile animal studies, focusing on the neurohistopathological findings. Dr. Schmued concluded that the results of Study OV-1007 and the conclusions of the “associated PWG constituted the more credible study by identifying the grey matter lesions as unique and distinct from the reversible IME seen in the adults.” [It is of note that MRI findings in infants treated with vigabatrin for infantile spasms indicate gray matter involvement.] Dr. Schmued identified potential deficiencies in the conduct of Study OVNC-9004 and suggested an experimental design to more definitively assess vigabatrin-induced neurotoxicity in juvenile animals.

Based on the available data, I agree that the neurohistopathological findings detected in juvenile animals appear distinct from those detected in the adult brain and that they are clinically relevant; the findings in juvenile animals occur at plasma exposures lower than those at the proposed therapeutic doses in infants. Therefore, I recommend that the sponsor be required to conduct the study suggested by Dr. Schmued post approval. In addition to further investigating the brain lesions detected in the juvenile animal studies, the study as designed by Dr. Schmued is also capable of providing data on the potential of vigabatrin to induce apoptotic neuronal degeneration characteristic of GABA-mimetic compounds (cf. Gascon E. *et al. Eur J Anaesthesiology* 24:213-224, 2007; Jevtovic-Todorovic V, Olney JW. *Anesth Analgesia* 106(6):1659-1663, 2008). I would also recommend a juvenile study in a non-rodent species (dog) in order to determine if the developmental neurotoxicity (especially gray matter involvement) of vigabatrin can be confirmed in a second species.

## **2. Retinal lesions**

Vigabatrin-induced retinal degeneration was observed in albino mouse and rat, but not in pigmented rat, dog, or monkey. Due to the lack of histologic effects in pigmented animals, the retinal findings were attributed to vigabatrin-induced exacerbation of light-induced retinal toxicity in an “overly sensitive albino retina...” The sponsor originally concluded that the nonclinical “...studies clearly demonstrate that pigmentation of ocular structures protects the retina from the degeneration associated with vigabatrin administration in albino rats. Such findings are quite consistent with the absence of ophthalmologic pathology reported to date in vigabatrin-treated patients.”

Renewed interest in the retinal findings in rodent resulted from a report to the EMEA of visual field defects (VFD), sometimes severe, in 40% of patients treated with vigabatrin versus 0% in non-medicated patients or patients treated with carbamazepine (cf. *Opinion of the Committee for Proprietary Medicinal Products Pursuant to Article 12 of Council Directive 75/319/EEC as Amended, for Vigabatrin*; CPMP/1357/99-EN). The EMEA recommended additional animal studies “...to investigate the mechanisms of vigabatrin induced retinotoxicity and to provide information regarding the possible differences in

sensitivity to retinotoxicity in young and adult animals.” (As previously noted, the report of VFDs in humans was the basis for the Agency’s non-approvable action on October 27, 1998.) In response, the sponsor re-evaluated the original tissue (eye) sections from previously completed animal studies and conducted investigational studies to further assess the retinal toxicity induced by vigabatrin.

These studies provided better characterization of the retinal lesions in albino rodents and confirmed their absence in pigmented animals. The studies in juvenile albino animals were equivocal for retinal injury. Retinal findings were observed in a 2-wk oral range finding study in juvenile rats, but not in a 4-wk study at the same doses. In the pivotal juvenile rat study, focal retinal dysplasia was detected at the two highest doses, in both males and females. The sponsor concluded that findings were possibly related to reduced body weight gain resulting in delayed retinal maturation. Regardless of whether or not the retinal findings in these studies were a direct or indirect effect of treatment, the sponsor argued that they were not relevant to humans due to differences in the timing of retinal development between species. In rat, retinal maturation occurs in the early postnatal period, whereas in humans, the retina is fully mature at birth.

The additional studies did not provide conclusive evidence to document the mechanism(s) responsible for the lesions. It would appear that increased levels of GABA in retina, resulting from vigabatrin-induced inhibition of GABA-T, are not solely responsible for the retinal injury. GABA levels in retina are significantly increased by vigabatrin in both pigmented and non-pigmented retina (Cubella JF *et al. J Pharm Exp Therap* 238(2):508-514, 1986; Cubella JF *et al. Brain Res* 419:208-215, 1987; sponsor’s studies #DSE 2001-0465, DSE 2001-0466), but only non-pigmented retina appears affected. Izumi *et al.* (Izumi Y *et al. Epilepsia* 45(9):1043-1048, 2004) reported that in *ex vivo* studies in retinal preparations from Sprague-Dawley rat, neither vigabatrin nor GABA alone in the absence of excessive light (20,000 lux white light) or excessive light alone induced retinal toxicity. The effects of vigabatrin in the presence of excessive light were both concentration and light-exposure duration dependent. In the *in vivo* studies conducted by Izumi *et al.* (2004), a high acute dose of vigabatrin (1000 mg/kg/i.p.) did not induce retinal toxicity when animals were maintained under standard light condition (12 hours light [300-500 lux]/12 hours dark [<10 lux]) for 24 hours; however, animals administered the same dose of vigabatrin followed by 24-hour exposure to white light (6000-8000 lux) experienced retinal damage. Twenty-four hour exposure to white light alone had no effect on the retina. Unfortunately, the pigmented retina was not tested in these studies. These data indicate that, at least under acute conditions, vigabatrin and light together, but not each alone, induce retinal toxicity. How relevant these data are to the retinal toxicity detected in albino animals administered multiple daily doses of vigabatrin and maintained under “standard” lighting conditions is unclear. However, the lack of retinal findings in control animals in the sponsor’s studies (e.g., no retinal findings in any control animal in the 18-month carcinogenicity study in albino mouse) is consistent with Izumi *et al.* (2004), i.e., lighting conditions alone produced no notable retinal toxicity, even in “sensitive” animals.

In a recently published study, Jammoul *et al.* (Jammoul F *et al. Ann Neurol* 65:98-107, 2009) report prevention of light-induced retinal toxicity in rats and mice by administration of taurine. In this study, vigabatrin was administered to Wistar rats or BALB/c mice (6-7 weeks of age) by daily intraperitoneal (i.p.) injection at a dose of 40 mg ( $\approx$ 200 mg/kg) for 45-65 days (rat) or of 3 mg ( $\approx$ 150 mg/kg) for 29 days (mouse). Taurine was provided in drinking water (0.1 M) and light intensities in home cages were 120-130 lux (rat) or 70-85 lux (mice). Treatment of controls was not, with one exception, clearly stated in the publication; however, it is presumed that control and treated rats and mice were maintained in 12/12-hr light/dark cycles, except for the study assessing the effects of vigabatrin in rats maintained in the dark. In rats, vigabatrin administered for 45 days produced retinal toxicity (disorganization of the photoreceptor layer [decreased cone density, increased length of displaced photoreceptors], reduced photopic ERG amplitude, and increased GFAP immunolabeling) in animals maintained under 12/12-hr light/dark cycles, but not in animals maintained in the dark. Vigabatrin administered to rats for 65 days produced decreases in circulating levels of glutamic acid (56%), taurine (67%), and methionine (22%); plasma levels of taurine were reported to be highly correlated with changes in ERG amplitude and cone density. Taurine supplementation partially prevented vigabatrin-induced retinal toxicity; however, photopic ERG and cone density remained lower and GFAP staining remained higher in vigabatrin-treated animals compared to controls. Plasma taurine levels in taurine-supplemented animals were higher than in non-aurine supplemented and control animals. In mice, vigabatrin administered for 29 days produced retinal toxicity similar to that observed in rat; taurine supplementation partially or completely prevented vigabatrin-induced effects on ERG, organization of the outer nuclear layer, cone density, and bipolar cell plasticity.

Jammoul *et al.* (2009) propose that vigabatrin induces a taurine deficiency, which then produces retinal toxicity in animals exposed to light. They conclude that “Patients taking vigabatrin could gain immediate benefit from reduced light exposures and dietetic advice on taurine-rich foods.” The authors note that the doses used in rat and mouse are “in line” with therapeutic doses in adults.

A thorough literature review is beyond the scope of this memo. However, it is clear that taurine (purported to be the most abundant amino acid in the retina) is considered to have an important role in the development and function of the visual system in animals and humans (cf. Lima L. *Neurochem Res* 24(11):1333-1338, 1999; Militante JD, Lombardini JB. *Nutri Neurosci* 5(2):75-90, 2002). In the taurine transporter knock-out (*taut*<sup>-/-</sup>) mouse, tissue levels of taurine are markedly reduced, and “The most prominent morphological feature of (*taut*<sup>-/-</sup>) mice [is] severe and progressive retinal degeneration” (Heller-Stilbe *et al FASEB J* 16(2):231-233, 2002). Heller-Stilbe *et al.* (2002) state that “The importance of taurine for retinal function is underlined by the fact that ERGs are abnormal in cases of human taurine deficiency due to long-term parenteral nutrition”, but provide no reference for this statement. Yu *et al.* (Yu X *et al. Brit J Nutri* 98:711-719, 2007) report a decrease in light-induced retinal damage in Sprague-Dawley rats administered taurine (4 gm/100 gm diet) for 15 days; retinal taurine levels were decreased in rats exposed to light, but increased in those exposed to light and supplemented with taurine.

Reports on the effects of vigabatrin on taurine levels in plasma and retina are inconsistent. Jammoul *et al* (2009) reported, as previously noted, that vigabatrin administration ( $\approx 200$  mg/kg i.p. for 65 days) to rats results in a significant decrease in circulating levels of taurine (and glutamic acid). However, in the sponsor's investigative study (Study DSE-2001-1068) in Sprague-Dawley rat, oral doses of vigabatrin (0, 100 or 300 mg/kg) for 4- or 13-weeks resulted in slightly higher plasma taurine levels in treated animals. Loscher and Horstermann (Loscher W, Horstermann D. *Naunyn-Schmiede Arch Pharmacology* 349(3):270-278, 1994) reported a 25% decrease in plasma taurine (but increased taurine levels in the hippocampus) 4 hours after a single 1200-mg/kg i.p. dose of vigabatrin to Wistar rats. Abdulrazzaq *et al.* (Abdulrazzaq YM *et al. Reprod Toxicol* 20:549-560, 2005) reported that a single dose of vigabatrin (450 mg/kg i.p.) administered to dams on gestation day 15 had no significant effect on plasma taurine levels in maternal plasma, placenta, or fetal tissue. Retinal taurine levels in vigabatrin-treated albino rats have been reported to be decreased (Wasowicz M *et al. Invest Ophthalmol Vis Sci* 43(4):813-820, 2002; in light-exposed animals) or unchanged (Neal MJ, Shah MA. *Br J Pharmacol* 100:324-328, 1990). In the sponsor's studies, retinal levels of taurine were relatively unchanged in albino and pigmented rats (Study DSE-2001-0465; Study DSE-2001-0467). Retina changes may be difficult to interpret since decreases in taurine levels in light-exposed albino animals treated with vigabatrin may reflect damage to the retina, rather than an effect of vigabatrin on taurine status. In the sponsor's Study DSE-2001-0465, light exposure alone resulted in decreases in retinal taurine levels in albino and, to a lesser extent, pigmented rat.

It is unclear how the retinal toxicity detected in rodent compares to that observed in humans. It would appear that there is no consensus on the exact nature of the retinal toxicity induced in humans by vigabatrin, except that once produced it is irreversible. In rat, it has been demonstrated that vigabatrin exacerbates light-induced retinal degeneration. The fact that pigmented rats appear less sensitive to vigabatrin-induced retinal toxicity is consistent with this mechanism, since pigment is considered to protect the retina from light-induced damage. However, the high incidence of retinal toxicity (i.e., visual field defect) in humans ( $\approx 30$ -40% of patients treated) suggests that in humans, pigmented retina is not particularly protective and that, therefore, light may not have as significant a role in humans as it does in the rodent. It is also of note that a similar pattern of retinal degeneration in animals (i.e., only albino animals affected) has been observed with other pharmaceuticals (e.g., Mirapex<sup>®</sup>, Abilify<sup>®</sup>, Lyrica<sup>®</sup>), with no clinical correlate in humans. This would suggest that the mechanisms underlying the retinal toxicity in albino rodents and the VFDs in humans are intrinsically different. This would also suggest that taurine may not prevent retinal toxicity in humans. However, further investigation into the potential for taurine to prevent or mitigate retina toxicity induced by vigabatrin is warranted, considering the availability of taurine (as a dietary supplement) and the seriousness of the finding in humans.

I would recommend that the sponsor be asked to conduct a study to further investigate the potential protective effect of taurine as a post-approval requirement. Since the relevance of the albino rodent to human is unclear, I would suggest that the sponsor continue attempts to induce the retinal lesion in pigmented rodent. Light-induced retinal damage is

intensity and duration of exposure dependent. Therefore, it might be possible to find illumination parameters that would successfully induce retinal degeneration in the pigmented rodent, perhaps in conjunction with dilatation of the pupil (Rapp LM, Williams TP. *Visual Res* 20:1127-1131, 1980).

### Conclusions/Recommendations

A comprehensive body of nonclinical data is available for vigabatrin. These data clearly indicate that, in animals, vigabatrin is neurotoxic in the adult and during pre- and post-natal development, with the developing animal demonstrating greater sensitivity to vigabatrin-induced toxicity than the adult.

Based on his original NDA review [1995], Dr. Rosloff recommended approval of NDA 20-427 for complex partial seizures in adults. I agree that the nonclinical data support approval of vigabatrin in adults with refractory complex partial seizures, with appropriate labeling.

Dr. Fisher has recommended that Sabril not be approved for use in Infantile Spasms (IS) due to “a risk for significant neurotoxicity at clinically relevant exposures”. I understand Dr. Fisher’s concern, based on the increased sensitivity of the developing animal (pre- and post-natal) to the neurotoxic effects of vigabatrin. However, considering vigabatrin’s demonstrated clinical benefit in IS, a serious indication with no currently approved therapy, I have no objection to Sabril being approved for IS. I do, however, recommend that the sponsor be required to conduct the following additional nonclinical studies post approval, for the reasons discussed in this memo:

- A toxicology study in the juvenile rat examining the potential of vigabatrin exposure during development to produce neuronal damage.
- A juvenile animal toxicology study of vigabatrin in a non-rodent species.
- A study examining the effect of taurine on vigabatrin-induced retinal damage in rodent.

Wording for the Post Marketing Requirements and the approval letter have been provided in separate documents.

### **Recommended labeling for NDA 20-427 (Refractory Complex Partial Seizures)**



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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-006
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/28/07
PRODUCT:	Sabril (vigabatrin) for Oral Solution
INTENDED CLINICAL POPULATION:	pediatric epilepsy (infantile spasms)
SPONSOR:	Ovation Pharmaceuticals
REVIEW DIVISION:	Division of Neurology Products (HFD-120)
PHARM/TOX REVIEWER:	Ed Fisher
PHARM/TOX SUPERVISOR:	Lois Freed
DIVISION DIRECTOR:	Russell Katz
PROJECT MANAGER:	Tamy Kim

## I. TOXICOLOGY

1. Oral (Gavage) Repeated-Dose Toxicity Study of Vigabatrin in Rats (Report No. OV-1007, dated 6/7/06, conducted by Charles River, Horsham, PA, GLP)

- a. Methods

Young rats (80/sex/dose main; whole litter design) were given 0 (water), 5, 15, or 50 mg/kg VGB by oral gavage (5 ml/kg) once daily from postnatal day (PND) 4 through PND 65. Subsets of approximately 20/sex/group were sacrificed on PND 67 or following a recovery period (on day 13 of presumed gestation in subset of females mated or PND 196 through 201 for males). TK blood samples were collected from 2-3/sex/group/timepoint after dosing on PNDs 17 and 65. The following parameters were evaluated for all rats: viability, clinical observations, body weight and body weight changes, feed consumption, and gross necropsy observations for the treatment and recovery periods. Subsets of rats (at least 10/sex/group) were evaluated for alterations in the eye (electroretinograms [5/sex/group] and ophthalmoscopic examination) on 3 occasions (after approximately 3 weeks, end of dosing, and end of recovery); other parameters (sexual maturation, and for motor activity, functional observation battery, acoustic startle habituation and prepulse inhibition, hematology, clinical chemistry, urinalysis, organ weights, and histopathology) were assessed either during dosing or at the end of the treatment period, and either during or at the end of the recovery period. During the recovery period, subsets of rats (at least 10/sex/group) were evaluated for estrus cycle, mating and fertility, sperm motility and count, and learning and memory (simple spatial discrimination water maze and a Morris water maze).

Strain: Cri:CD(SD)

Drug Lot #: 152972

Dose selection was based on a dose range-finding study in which oral doses of 30, 50, 100, 300, 500 and 1000 mg/kg/day were given to rats (SD) from PND 4 for 2 weeks. Severe and early toxic effects were seen at  $\geq 300$  mg/kg (all animals were euthanized within 5 days). Deaths, marked clinical signs (absence of milk in the stomach and decrease/absence of motor activity), and a marked decrease in body weight gain were seen at 100 mg/kg. Effects at 50 mg/kg/day were less severe and included transient absence of milk intake and/or decreased motor activity in some animals and reduced body weight gain. Changes at 30 mg/kg/day were limited to a minimal decrease in body weight gain. Scattered nuclei in the photoreceptor layer and an increased number of apoptotic bodies in the inner nuclear layer of the retina were observed at 50 or 100 mg/kg (see **Table II.2**). 30 mg/kg/day was considered to be close to a NOAEL.

- b. Results

- i. Mortality, clinical signs

A total of 3, 5, 2, and 7 rats in the C, LD, MD, and HD groups, respectively, died or were sacrificed prior to scheduled termination. Three (1 male, 2 females) of the seven HD deaths were considered treatment-related (T-R), based on clinical signs (spasms or tremors) and/or body weight losses; the other deaths were due to either dosing or anesthesia accidents. Whole body spasm occurred in a significantly increased number of male (28) and female (23) rats at the HD. No other T-R clinical signs were noted.

- ii. Body weight

Significant reductions in BW gain were seen at the MD and HD in males and at the HD in females (**Table I.1.1**). BWs in the respective dose groups were 3, 10, and 27% below C in males and +3.0, -2, and 20% above/below C in females on

PND 65. Post-treatment BW gains continued to be reduced in males (8, 13, and 24%) but not females. Terminal body weights in the respective groups were 5, 11, and 27 below C in males and +2, -1, and -18% above/below C in females.

iii. Developmental Landmarks

The days on which preputial separation or vaginal patency were observed significantly increased at the HD (**Table I.1.2**). These delays in sexual maturation correlated with reduction in BW, although BW at maturation was significantly reduced only in males.

iv. Ophthalmologic and ERG Examinations

According to the consultant's report, there were no indications of T-R ocular damage in the ophthalmoscopic examinations. The ERG measurements (onset latency of the b-wave [1 Hz], baseline-to-peak amplitude of the b-wave [1, 10, 15 Hz], and peak-to-peak amplitude of the oscillatory responses [1 Hz]) were generally comparable among the dosage groups in all testing sessions, and according to the report, there was no evidence that drug exposure was associated with altered ERG responses at the doses and time points evaluated.

v. Developmental Neurotoxicity Testing

In males, the average startle response during the habituation phase was attenuated somewhat (not statistically significant [NS]) at the HD on PNDs 59-63 and increased (NS) at all doses on PND 89-91 (**Tables I.1.3**). There was also an increased response to prepulse inhibition at the MD in the recovery period. There were no apparent changes in females at either evaluation time.

No clear effects on motor activity (number of movements and time spent in movement) were seen during the dosing period (PND 56 ±1 day) or during the recovery period (PND 86 ±1 day).

In the FOB, landing foot splay and forelimb and hindlimb grip strength (maximum and average values) were dose-dependently (D-D) decreased (statistically significant [SS] in MD and/or HD) in treated males and females during the dosing (PNDs 56, 57, 58 or 59) and post-dosing periods (PNDs 87, 88 or 89). BW on day of testing was decreased (SS) in MD and HD males and in HD females. (**Tables I.1.4-5**)

In a simple water (M) maze conducted during the recovery phase (beginning on PND 105), there was an increase in the number of HD females (3/18: 6707, 7107 and 7307) that failed to meet the criterion during the reversal learning session (**Table I.1.6**).

Although there was some tendency (NS) for increased latencies in treated groups in the Morris maze, particularly during the initial trials, there was no clear effect on learning or memory in this test (**Table I.1.7**). It should be noted that this was performed very late (PNDs 153-177) due to the test being added to the protocol after the study had started.

vi. Reproductive Performance (13th week of age, 1:1 non-sibling mating)

Estrous cyclicity was D-D reduced in treated females (**Table I.1.8**). The mating and fertility data are anomalous, and do not allow a valid assessment of drug

effects. Only 50-60% of C, LD, and MD rats mated, while 90% of HD animals mated; thus, while there is the suggestion of an effect on fertility, the D-R is non-monotonic (fertility index: 100.0, 45.4, 66.7, and 93.8% at C, LD < MD, and HD).

Reproductive outcome parameters at laparotomy were generally similar among groups; however, due to the poor pregnancy rate only 9, 6, 8, and 15 litters were available in the C, LD, MD, and HD groups, respectively.

There were no T-R differences in sperm parameters. Absolute testicular and epididymal weights were decreased in HD males, but relative weights were increased.

vii. Pathology

No T-R gross lesions were observed. Terminal body weights were decreased in MD and HD males and in HD females. Absolute brain weights were significantly reduced at the MD and HD in males and females (**Table I.1.9**), and epididymis and testis weights were decreased at the HD. However, relative brain (**Table I.1.10**) and testis weights were increased at the HD. Terminal body weights remained significantly reduced after the recovery period at the HD. The brain weight reductions also persisted into the recovery period (SS in HD males and females after 19 weeks).

Microscopic Findings:

Of the 20/sex/group necropsied on PND 67, 1/2 were perfusion-fixed and the brains and retinas of all of these animals from all groups were microscopically examined by the study neuropathologist. The other 1/2 had tissues immersion-fixed and a general histopathology examination involving all tissues from the C and HD groups was performed by the study pathologist. Another subset was necropsied during PND 196-210 after an approximately 19-week recovery period and 10/sex/group were perfusion-fixed for neuropathology examination by the same pathologist.

With the exception of the CNS, all tissues appeared normal on histological evaluation. For the immersion-fixed tissues, three sections of brain were examined from each C and HD rat. The report noted that “vacuolations in white matter and/or gray matter of minimal or mild severity involving one or more brain regions of posterior thalamus, hypothalamus, hippocampus and/or brainstem (midbrain and hindbrain) were evident in 8/9 males and 7/9 females administered 50 mg/kg/day of Vigabatrin from Group 4 and terminated on PND 67.”

All perfusion-fixed HD male and female brains examined by the neuropathologist had increased levels (over background) of vacuolation within the neuropil of selected brain regions on PND 67 (**Tables I.1.11-12**). The following is verbatim from the neuropathologist’s (Robert Garman) report:

“The most commonly affected brain regions included the following (listed in the approximate order of frequency affected): central midbrain (tegmentum), substantia nigra, dorsal subiculum, medulla oblongata, hippocampal CA1 region, thalamus, deep cerebellar nuclei and basal forebrain (particularly the medial forebrain bundle). In most of these regions, vacuolation was graded as minimal to mild. Although some white matter tracts had increased numbers of vacuoles (*viz.* the medial longitudinal fasciculus and medial forebrain bundle), most foci of vacuolation were present in subcortical grey matter or mixed grey and

white matter regions. The specific cellular/subcellular locations of the vacuoles could not be determined at the light microscopic level. Although vacuolation in brain sections often represents a nonspecific finding (relative to pathogenesis) and may sometimes be the result of artifact, the consistent inter-animal pattern of vacuolation in the high dose group rats and the absence of this degree of vacuolation within the other treatment groups in this study indicates that the vacuolation represents a treatment-related effect... Within the substantia nigra (which was not present on section for all rats), vacuolation always involved the most lateral portion of the pars compacta. Within the midbrain, the most consistently vacuolated region was within the medial longitudinal fasciculus and the adjacent neuropil present just inferior to the midline raphe nuclei. In the cerebella, vacuolation was typically restricted to the deep cerebellar nuclei. [Note that only a minority of the rats had the deep cerebellar nuclei represented within the sections.] Although the CA1 sector of the hippocampus was occasionally characterized by minimal to mild vacuolation, it was the dorsal subicular region (immediately medial to the CA1 pyramidal layer of the hippocampus) that was most consistently vacuolated."

Two MD males also had single foci of minimal neuropil vacuolation ("in the dorsal subicular region of one rat and in the deep cerebellar nuclear region of the other"). Although one HD female rat had a unilateral focus of gliosis within the hippocampus, this was considered by the neuropathologist to represent a background lesion. Dr. Garman also considered several instances of Fluoro-Jade B staining to represent background degenerative processes even though these were also only seen in MD and HD animals. Other microscopic findings within the nervous system other than for the eye were considered to be incidental; this conclusion seems questionable in some cases.

With the exception of a minimal finding in 1 C female, retinal dysplasia (minimal to mild, "characterized by the formation of rosettes within the outer nuclear layer of the retina that typically protruded inwards into overlying inner nuclear layer") was detected only in MD and HD males and females.

Although frequencies of both neuropil vacuolation and retinal dysplasia were decreased in the post-recovery period rats, minimal to mild vacuolation ("highly restrictive in location") was present in 2 HD males and 1 HD female. In all three rats, the foci of vacuolation were restricted to the deep cerebellar nuclei and/or pons. One focus of vacuolation within the deep cerebellar nucleus of the HD female had two small-sized foci of mineralization. There was no evidence of gliosis (based on GFAP staining) in any of the foci of vacuolation. The Fluoro-Jade B-stained sections from recovery rats were considered to indicate only background degenerative processes not related to treatment. One HD male and 5 females (2 each in LD and MD groups and 1 HD) had retinal dysplasia (all minimal in degree).

viii. Plasma drug levels

TK data are summarized in **Table I.1.13** below.

c. Conclusions

Administration of VGB to young rats from PND 4 through PND 65 at doses of 0, 5, 15, or 50 mg/kg increased mortality (HD), decreased BW gain (MD, HD), produced clinical signs of neurotoxicity (spasms or tremors at HD), delayed sexual maturation (HD), altered

estrus cyclicity (all doses), decreased absolute brain weights (D-D), and produced neurobehavioral (altered FOB performance, deficits in maze learning) and neurohistopathological (brain vacuolation and retinal dysplasia) changes (MD, HD) in treated animals.

**Table I.1.1** Body Weight Gain in Juvenile Rats  
Males

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
<u>DOSAGE PERIOD:</u>					
RATS TESTED	N	80	80	80	80
INCLUDED IN ANALYSES	N	79b	80	80	80
BODY WEIGHT CHANGE (G)					
<u>ALL SUBSETS:</u>					
DAYS 4 - 11	MEAN±S.D.	+13.0 ± 2.6	+11.9 ± 2.9*	+12.1 ± 3.0	+8.7 ± 2.3**
DAYS 11 - 17	MEAN±S.D.	+14.1 ± 2.7	+14.3 ± 2.7 [ 79]b	+13.3 ± 3.3	+11.3 ± 3.2**
<u>SUBSETS 2, 3 AND 4:</u>					
DAYS 17 - 24	MEAN±S.D.	+26.8 ± 4.1 [ 59]b	+27.5 ± 4.1 [ 58]b	+24.7 ± 4.4** [ 60]	+14.5 ± 4.0** [ 59]b
DAYS 24 - 29	MEAN±S.D.	+30.1 ± 4.6 [ 59]b	+31.2 ± 4.0 [ 58]b	+28.8 ± 3.8 [ 60]	+21.4 ± 4.0** [ 57]b
<u>SUBSETS 2 AND 3:</u>					
DAYS 29 - 36	MEAN±S.D.	+56.0 ± 8.2 [ 40]	+55.8 ± 7.2 [ 40]	+51.0 ± 7.0** [ 40]	+40.3 ± 6.7** [ 40]
DAYS 36 - 42	MEAN±S.D.	+58.4 ± 5.9 [ 40]	+56.2 ± 5.2 [ 40]	+54.4 ± 6.4** [ 40]	+43.8 ± 6.0** [ 40]
DAYS 42 - 49	MEAN±S.D.	+65.7 ± 8.2 [ 40]	+62.6 ± 7.6 [ 40]	+58.7 ± 5.7** [ 40]	+47.9 ± 6.8** [ 40]
DAYS 49 - 56	MEAN±S.D.	+64.9 ± 10.0 [ 40]	+61.9 ± 8.2 [ 40]	+58.3 ± 7.6** [ 40]	+47.1 ± 9.7** [ 39]b
DAYS 56 - 63	MEAN±S.D.	+51.5 ± 9.4 [ 40]	+48.3 ± 9.1 [ 40]	+44.6 ± 7.7** [ 40]	+39.4 ± 9.3** [ 39]b
DAYS 63 - 65	MEAN±S.D.	+16.7 ± 5.8 [ 40]	+15.0 ± 3.8 [ 40]	+12.6 ± 5.6** [ 40]	+9.7 ± 4.8** [ 39]b
DAYS 4 - 65	MEAN±S.D.	+398.0 ± 38.4 [ 40]	+383.8 ± 38.1 [ 40]	+357.5 ± 32.9** [ 40]	+286.2 ± 32.5** [ 39]b
<u>RECOVERY PERIOD:</u>					
RATS TESTED	N	80	80	80	80
INCLUDED IN ANALYSES	N	20	20	20	20
BODY WEIGHT CHANGE (G)					
<u>SUBSET 3:</u>					
DAYS 67 - 70	MEAN±S.D.	+17.3 ± 5.3	+19.5 ± 5.5	+17.6 ± 3.2	+15.2 ± 4.9
DAYS 70 - 77	MEAN±S.D.	+41.7 ± 10.1	+42.0 ± 9.0	+37.8 ± 8.6	+31.4 ± 8.7**
DAYS 77 - 84	MEAN±S.D.	+32.4 ± 8.3	+29.8 ± 6.7	+31.6 ± 8.6	+28.2 ± 5.6
DAYS 84 - 91	MEAN±S.D.	+29.7 ± 8.8	+24.8 ± 13.2	+25.7 ± 7.4	+22.8 ± 8.1
DAYS 91 - 98	MEAN±S.D.	+22.8 ± 11.4	+23.3 ± 10.6	+17.9 ± 9.3	+19.6 ± 7.8
DAYS 67 - 98	MEAN±S.D.	+144.0 ± 27.0	+139.4 ± 24.1	+130.6 ± 16.8	+117.2 ± 20.2**
DAYS 98 -125	MEAN±S.D.	+82.5 ± 19.0	+73.1 ± 15.9	+63.9 ± 35.6	+61.2 ± 14.0**
DAYS 125 -152	MEAN±S.D.	+52.2 ± 18.1	+46.2 ± 15.7	+46.5 ± 15.0	+36.2 ± 12.3**
DAYS 152 -181	MEAN±S.D.	+19.3 ± 20.9	+17.7 ± 16.0	+22.2 ± 13.0	+15.9 ± 12.8
DAYS 181 -195	MEAN±S.D.	+12.5 ± 14.6	+10.2 ± 11.2	+6.9 ± 12.3	+7.1 ± 9.0
DAYS 67 -195	MEAN±S.D.	+310.4 ± 75.6	+286.6 ± 65.2	+270.0 ± 54.4*	+237.6 ± 36.6**
DAYS 4 -195	MEAN±S.D.	+723.7 ± 108.7	+688.2 ± 95.7	+644.0 ± 72.9**	+527.4 ± 59.5**
NOTE TERMINATIONS: SUBSET 1 - POSTNATAL DAY 17 OR 18; SUBSET 2 - POSTNATAL DAY 67; SUBSET 3 - POSTNATAL DAYS 195 - 201; SUBSET 4 - POSTNATAL DAY 29 DAYS = POSTNATAL DAYS a. Dosage occurred on postnatal days 4 through 65. * Significantly different from the control group value (p<0.05). ** Significantly different from the control group value (p<0.01).					

Females

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
<u>DOSAGE PERIOD:</u>					
RATS TESTED	N	80	80	80	80
INCLUDED IN ANALYSES	N	80	79b	80	80
BODY WEIGHT CHANGE (G)					
<u>ALL SUBSETS:</u>					
DAYS 4 - 11	MEAN±S.D.	+12.7 ± 2.4	+11.8 ± 2.8*	+11.5 ± 3.2**	+8.8 ± 2.3**
DAYS 11 - 17	MEAN±S.D.	+13.4 ± 2.5	+13.8 ± 2.7	+13.2 ± 3.0	+11.5 ± 3.0**
<u>SUBSETS 2, 3 AND 4:</u>					
DAYS 17 - 24	MEAN±S.D.	+24.8 ± 3.0 [ 60]	+25.2 ± 4.0 [ 58]b	+22.4 ± 3.2** [ 58]b	+13.1 ± 4.7** [ 60]
DAYS 24 - 29	MEAN±S.D.	+26.5 ± 3.6 [ 60]	+27.0 ± 3.9 [ 58]b	+25.7 ± 2.8 [ 58]b	+20.4 ± 3.5** [ 59]b
<u>SUBSETS 2 AND 3:</u>					
DAYS 29 - 36	MEAN±S.D.	+41.6 ± 5.3 [ 40]	+43.5 ± 4.6 [ 40]	+40.6 ± 4.8 [ 40]	+31.8 ± 4.4** [ 40]
DAYS 36 - 42	MEAN±S.D.	+31.8 ± 4.2 [ 40]	+32.3 ± 4.3 [ 40]	+32.7 ± 4.8 [ 40]	+30.8 ± 5.1 [ 40]
DAYS 42 - 49	MEAN±S.D.	+27.7 ± 5.6 [ 40]	+29.2 ± 6.0 [ 40]	+26.2 ± 5.5 [ 40]	+23.7 ± 5.9** [ 40]
DAYS 49 - 56	MEAN±S.D.	+27.8 ± 6.3 [ 40]	+28.0 ± 4.8 [ 40]	+26.5 ± 7.3 [ 40]	+20.7 ± 5.8** [ 40]
DAYS 56 - 63	MEAN±S.D.	+19.2 ± 7.4 [ 40]	+20.0 ± 6.3 [ 40]	+20.3 ± 6.6 [ 40]	+18.4 ± 6.7 [ 39]b
DAYS 63 - 65	MEAN±S.D.	+6.3 ± 5.8 [ 40]	+6.8 ± 6.0 [ 40]	+7.2 ± 5.4 [ 40]	+5.0 ± 5.1 [ 39]b
DAYS 4 - 65	MEAN±S.D.	+231.4 ± 20.0 [ 40]	+237.8 ± 21.0 [ 40]	+226.2 ± 21.3 [ 40]	+183.8 ± 20.6** [ 39]b
<u>RECOVERY PERIOD:</u>					
RATS TESTED	N	20	20	20	19
BODY WEIGHT CHANGE (G)					
<u>SUBSET 3:</u>					
DAYS 67 - 70	MEAN±S.D.	+9.1 ± 4.5	+8.1 ± 6.2	+12.5 ± 8.8	+6.3 ± 4.2
DAYS 70 - 77	MEAN±S.D.	+16.0 ± 6.7	+14.2 ± 9.4	+12.8 ± 6.8	+11.8 ± 5.5
DAYS 77 - 84	MEAN±S.D.	+11.6 ± 6.1	+10.3 ± 7.8	+12.2 ± 8.3	+10.8 ± 6.3
DAYS 84 - 91	MEAN±S.D.	+8.0 ± 6.4	+8.4 ± 7.2	+8.2 ± 7.3	+9.8 ± 5.2
DAYS 91 - 98	MEAN±S.D.	+11.5 ± 12.8	+7.6 ± 7.0 [ 19]b	+6.8 ± 6.0	+10.4 ± 6.5 [ 18]b
DAYS 67 - 98	MEAN±S.D.	+56.2 ± 18.9	+49.2 ± 10.4 [ 19]b	+52.5 ± 15.3	+49.4 ± 11.0 [ 18]b
DAYS 98 -125	MEAN±S.D.	+23.4 ± 10.2 [ 19]b	+27.0 ± 11.3 [ 19]b	+23.1 ± 11.8	+16.8 ± 8.7 [ 18]b
DAYS 125 -152	MEAN±S.D.	+19.5 ± 7.4 [ 19]b	+19.7 ± 17.6 [ 19]b	+21.4 ± 12.5	+15.9 ± 8.4 [ 18]b
DAYS 152 -170	MEAN±S.D.	+7.5 ± 9.7 [ 19]b	+10.0 ± 9.2 [ 19]b	+10.6 ± 14.4	+7.5 ± 7.0 [ 18]b
DAYS 67 -170	MEAN±S.D.	+106.0 ± 26.9 [ 19]b	+105.9 ± 26.3 [ 19]b	+107.7 ± 33.0	+89.6 ± 18.2 [ 18]b
DAYS 4 -170	MEAN±S.D.	+343.2 ± 43.2 [ 19]b	+349.7 ± 38.5 [ 19]b	+338.7 ± 46.1	+277.8 ± 35.1** [ 18]b

NOTE TERMINATIONS: SUBSET 1 - POSTNATAL DAY 17 OR 18; SUBSET 2 - POSTNATAL DAY 67; SUBSET 3 - DAY 13 OF PRESUMED GESTATION; SUBSET 4 - POSTNATAL DAY 29  
 DAYS = POSTNATAL DAYS [ ] = NUMBER OF VALUES AVERAGED  
 a. Dosage occurred on postnatal days 4 through 65.  
 b. Excludes values for rats that were found dead or sacrificed due to moribund condition.  
 \*\* Significantly different from the control group value (p<0.01).

**Table I.1.2 Morphological Landmarks in Juvenile Rats**

DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) a		0	5	15	50	
SUBSET 2						
MALE RATS		N	20	20	20	19b
PREPUTIAL SEPARATION c	MEAN±S.D.	46.0 ± 2.3	48.4 ± 3.2	46.4 ± 2.8	48.1 ± 2.5*	
BODY WEIGHT AT MATURATION (G)	MEAN±S.D.	243.8 ± 23.8	234.8 ± 27.6	226.0 ± 24.4	195.8 ± 21.3**	

a. Dosage occurred on postnatal days 4 through 65.  
b. Excludes rat 7704, which was found dead on postnatal day 54.  
c. Average day postnatal that the prepuce was observed to be separated.  
\* Significantly different from the control group value (p<0.05).  
\*\* Significantly different from the control group value (p<0.01).

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DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) a		0	5	15	50	
SUBSET 2						
FEMALE RATS		N	20	20	20	20
VAGINAL PATENCY b	MEAN±S.D.	34.0 ± 2.1	32.6 ± 1.7	34.0 ± 2.0	38.4 ± 2.3**	
BODY WEIGHT AT MATURATION (G)	MEAN±S.D.	113.8 ± 14.9	114.2 ± 10.3	109.7 ± 13.3	105.8 ± 9.2	

a. Dosage occurred on postnatal days 4 through 65.  
b. Average day postnatal that the vagina was observed to be patent.  
\*\* Significantly different from the control group value (p<0.01).

**Table I.1.3 Acoustic startle response in males during the dosing and post-dosing periods**

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
SUBSET 2					
POSTNATAL DAY 59, 60, 62 OR 63					
NUMBER OF RATS		20	20	20	19
<b>ACOUSTIC STARTLE HABITUATION</b>					
INCLUDED IN ANALYSES b		17	17	19	17
BLOCK 1	MEAN ± S.D.	101.76 ± 43.46	103.18 ± 53.37	111.62 ± 44.29	88.47 ± 39.01
BLOCK 2	MEAN ± S.D.	80.04 ± 50.40	83.86 ± 46.82	83.66 ± 42.51	71.91 ± 41.99
BLOCK 3	MEAN ± S.D.	68.30 ± 36.18	72.14 ± 34.80	87.69 ± 49.48	69.08 ± 46.19
BLOCK 4	MEAN ± S.D.	68.19 ± 35.46	67.29 ± 44.81	88.44 ± 46.78	59.49 ± 40.96
BLOCK 5	MEAN ± S.D.	70.83 ± 30.09	59.39 ± 27.58	71.95 ± 36.98	57.55 ± 32.45
AVERAGE	MEAN ± S.D.	77.82 ± 34.07	77.17 ± 35.07	88.67 ± 39.82	69.30 ± 36.40
<b>FREPULSE INHIBITION c</b>					
INCLUDED IN ANALYSES d		15	14	16	14
CONTROL	MEAN ± S.D.	4443 ± 1104	4448 ± 880	4610 ± 525	4076 ± 538
FREPULSE (70dB)	MEAN ± S.D.	3381 ± 718	3337 ± 485	3473 ± 624	3360 ± 316
FREPULSE (90dB)	MEAN ± S.D.	2176 ± 748	2214 ± 768	1917 ± 740	2564 ± 568

BLOCK = AVERAGE OF 10 TRIAL SESSIONS MINUS THE BODY WEIGHT  
CONTROL = AVERAGE OF RESPONSES IN 10 TRIAL SESSIONS MINUS THE BODY WEIGHT.  
FREPULSE = AVERAGE OF RESPONSES IN THE 70dB OR 90dB FREPULSE CONDITION MINUS THE BODY WEIGHT.  
a. Dosage occurred on postnatal days 4 through 65.  
b. Excludes rats with extremely low responses or whose responses to the stimulus were apparently affected by a mechanical mal  
c. Normalized (standardized) response values.  
d. Excludes rats with extremely low responses to the stimulus or whose responses were apparently affected by a mechanical or malfunction.

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
SUBSET 3					
POSTNATAL DAY 89, 90 OR 91					
NUMBER OF RATS		20	20	20	20
<u>ACOUSTIC STARTLE HABITUATION</u>					
INCLUDED IN ANALYSES b		15	17	18	18
BLOCK 1	MEAN ± S.D.	118.71 ± 56.96	132.00 ± 49.43	222.45 ± 126.38	145.82 ± 65.61
BLOCK 2	MEAN ± S.D.	108.64 ± 56.41	106.06 ± 60.83	209.94 ± 170.59	116.93 ± 52.40
BLOCK 3	MEAN ± S.D.	88.38 ± 58.62	85.98 ± 53.18	178.17 ± 137.79	88.30 ± 39.21
BLOCK 4	MEAN ± S.D.	79.00 ± 43.63	74.31 ± 47.30	156.59 ± 108.45	73.36 ± 33.10
BLOCK 5	MEAN ± S.D.	83.71 ± 59.75	70.00 ± 46.71	157.53 ± 105.70	64.64 ± 31.43
AVERAGE	MEAN ± S.D.	95.69 ± 48.51	93.67 ± 48.73	184.94 ± 120.24**	97.81 ± 40.40
<u>PREPULSE INHIBITION c</u>					
INCLUDED IN ANALYSES b		15	17	18	18
CONTROL	MEAN ± S.D.	4036 ± 639	4228 ± 761	4811 ± 954*	4244 ± 554
PREPULSE (70dB)	MEAN ± S.D.	3828 ± 553	3542 ± 565	3546 ± 886	3434 ± 709
PREPULSE (90dB)	MEAN ± S.D.	2125 ± 675	2130 ± 516	1643 ± 382*	2322 ± 552
BLOCK = AVERAGE OF 10 TRIAL SESSIONS MINUS THE BODY WEIGHT					
CONTROL = AVERAGE OF RESPONSES IN 10 TRIAL SESSIONS MINUS THE BODY WEIGHT.					
PREPULSE = AVERAGE OF RESPONSES IN THE 70dB OR 90dB PREPULSE CONDITION MINUS THE BODY WEIGHT.					
a. Dosage occurred on postnatal days 4 through 65.					
b. Excludes rats with extremely low responses to the stimulus, rats that escaped from the holding cage during the testing or any squads that included such a rat or rats that were apparently affected by an acoustical malfunction.					
c. Normalized (standardized) response values.					
* Significantly different from the control group value (p<0.05).					
** Significantly different from the control group value (p<0.01).					

**Table I.1.4** Functional Observational Battery scores in males during the dosing and post-dosing periods

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0	5	15	50
SUBSET 2					
POSTNATAL DAY 56, 57, 58 OR 59					
MALE RATS		N	20	20	19b
<u>AIR RIGHTING RESPONSE</u>					
(1) All feet land on ground	N	20	20	20	18
(2) Lands on side	N	0	0	0	1
(3) Lands on back	N	0	0	0	0
	MEAN SCORE	1.0	1.0	1.0	1.0
<u>PUPIL RESPONSE TO LIGHT</u>					
N		20	20	20	19
<u>FORELIMB GRIP TEST</u>					
Maximum (G)	MEAN±S.D.	358.0 ± 108.0	339.8 ± 112.1	322.2 ± 144.3	283.9 ± 84.3
Average (G)	MEAN±S.D.	322.6 ± 100.0	290.4 ± 98.6	276.9 ± 125.6	240.0 ± 69.9
<u>HINDLIMB GRIP TEST</u>					
Maximum (G)	MEAN±S.D.	417.8 ± 115.1	402.5 ± 91.5	399.8 ± 94.8	330.0 ± 100.6*
Average (G)	MEAN±S.D.	374.8 ± 103.7	371.0 ± 77.5	355.8 ± 70.1	293.7 ± 72.6**
<u>LANDING FOOT SPREAD</u>					
Average (CM)	MEAN±S.D.	7.4 ± 1.1	7.2 ± 1.3	6.5 ± 1.2*	6.0 ± 1.0**
<u>BODY TEMPERATURE (°C)</u>					
	MEAN±S.D.	37.7 ± 0.6	37.7 ± 0.4	37.7 ± 0.6	37.5 ± 0.5
<u>BODY WEIGHT (G)</u>					
	MEAN±S.D.	348.7 ± 40.6	333.1 ± 35.2	316.6 ± 30.4**	258.0 ± 36.5**
n = Category number for descriptive test item.					
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats					
a. Dosage occurred on postnatal days 4 through 65.					
b. Excludes rat 7704, which was found dead on postnatal day 54.					
* Significantly different from the control group value (p<0.05).					
** Significantly different from the control group value (p<0.01).					

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	5	15	50
SUBSET 3					
POSTNATAL DAY 55 ± 1					
MALE RATS	N	20	20	20	20
AIR RIGHTING RESPONSE					
(1) All feet land on ground	N	19	20	19	20
(2) Lands on side	N	1	0	1	0
(3) Lands on back	N	0	0	0	0
	MEAN SCORE	1.0	1.0	1.0	1.0
FUPIL RESPONSE TO LIGHT	N	20	20	20	20
FORELIMB GRIP TEST					
Maximum (G)	MEAN±S.D.	637.5 ± 230.0	568.8 ± 212.0	559.2 ± 226.6	344.0 ± 135.3**
Average (G)	MEAN±S.D.	550.0 ± 179.7	508.8 ± 184.8	502.4 ± 208.0	309.8 ± 120.4**
HINDLIMB GRIP TEST					
Maximum (G)	MEAN±S.D.	478.8 ± 128.4	436.8 ± 116.2	400.5 ± 79.0*	280.5 ± 72.5**
Average (G)	MEAN±S.D.	418.9 ± 102.7	384.2 ± 82.8	360.0 ± 77.4*	258.6 ± 68.2**
LANDING FOOT SPREAD					
Average (CM)	MEAN±S.D.	7.18 ± 1.31	6.86 ± 1.31	6.11 ± 1.52*	5.02 ± 1.00**
BODY TEMPERATURE (°C)	MEAN±S.D.	37.4 ± 0.5	37.5 ± 0.5	37.2 ± 0.6	37.5 ± 0.7
BODY WEIGHT (G)	MEAN±S.D.	536.2 ± 56.9	507.0 ± 70.3	488.8 ± 46.1*	389.4 ± 43.8**

n: = Category number for descriptive test item.  
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats  
a. Dosage occurred on postnatal days 4 through 65.  
\* Significantly different from the control group value (p<0.05).  
\*\* Significantly different from the control group value (p<0.01).

**Table I.1.5** Functional Observational Battery scores in females during the dosing and post-dosing periods

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	5	15	50
SUBSET 2					
POSTNATAL DAY 56, 57, 58 OR 59					
FEMALE RATS	N	20	20	20	19 <sup>b</sup>
AIR RIGHTING RESPONSE					
(1) All feet land on ground	N	20	19	20	19
(2) Lands on side	N	0	1	0	0
(3) Lands on back	N	0	0	0	0
	MEAN SCORE	1.0	1.0	1.0	1.0
FUPIL RESPONSE TO LIGHT	N	20	19 <sup>c</sup>	20	19
FORELIMB GRIP TEST					
Maximum (G)	MEAN±S.D.	288.0 ± 88.2	283.0 ± 94.5	256.5 ± 88.2	200.8 ± 67.3**
Average (G)	MEAN±S.D.	247.0 ± 72.6	241.2 ± 86.3	222.9 ± 85.0	176.4 ± 54.8**
HINDLIMB GRIP TEST					
Maximum (G)	MEAN±S.D.	370.8 ± 78.8	339.5 ± 114.7	334.5 ± 83.6	259.2 ± 49.2**
Average (G)	MEAN±S.D.	331.5 ± 62.4	303.4 ± 94.8	300.2 ± 71.9	239.6 ± 50.4**
LANDING FOOT SPREAD					
Average (CM)	MEAN±S.D.	6.4 ± 0.8	6.4 ± 1.0	6.1 ± 0.9	5.0 ± 0.8**
BODY TEMPERATURE (°C)	MEAN±S.D.	38.4 ± 0.5	38.4 ± 0.5	38.2 ± 0.5	38.5 ± 0.6
BODY WEIGHT (G)	MEAN±S.D.	216.8 ± 18.5	222.8 ± 22.0	210.1 ± 15.7	174.4 ± 18.5**

n: = Category number for descriptive test item.  
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats  
a. Dosage occurred on postnatal days 4 through 65.  
b. Excludes rat 7708, which was not tested for functional observation battery.  
c. Excludes rat 2106, which was not tested due to corneal opacity.  
\*\* Significantly different from the control group value (p<0.01).

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	5	15	50
SUBSET 3					
POSTNATAL DAY 88 ± 1					
FEMALE RATS	N	20	20	20	19 <sup>b</sup>
AIR RIGHTING RESPONSE					
(1) All feet land on ground	N	20	20	20	18
(2) Lands on side	N	0	0	0	1
(3) Lands on back	N	0	0	0	0
	MEAN SCORE	1.0	1.0	1.0	1.0
PUPIL RESPONSE TO LIGHT	N	20	20	20	19
FORELIMB GRIP TEST					
Maximum (G)	MEAN±S.D.	375.2 ± 125.0	388.5 ± 100.2	380.5 ± 111.2	266.6 ± 92.1**
Average (G)	MEAN±S.D.	329.5 ± 110.5	351.1 ± 95.5	320.4 ± 93.0	228.7 ± 71.4**
HINDLIMB GRIP TEST					
Maximum (G)	MEAN±S.D.	295.5 ± 86.1	266.5 ± 67.0	241.8 ± 75.4*	203.9 ± 65.5**
Average (G)	MEAN±S.D.	262.0 ± 63.3	242.9 ± 68.6	217.1 ± 63.2*	180.4 ± 54.2**
LANDING FOOT SPLAY					
Average (CM)	MEAN±S.D.	5.47 ± 1.11	5.61 ± 1.17	5.16 ± 0.95	4.19 ± 0.86**
BODY TEMPERATURE (°C)	MEAN±S.D.	37.9 ± 0.4	38.0 ± 0.4	37.9 ± 0.6	38.1 ± 0.5
BODY WEIGHT (G)	MEAN±S.D.	290.1 ± 30.0	296.4 ± 21.2	284.4 ± 26.5	226.6 ± 31.1**

n = Category number for descriptive test item.  
(n) = Score assigned to graded test items; mean score was calculated by multiplying each score by the number of rats with that score and then dividing the sum of the products by the total number of rats

a. Dosage occurred on postnatal days 4 through 65.  
b. Excludes rat 7207, which was found dead on postnatal day 59.  
\* Significantly different from the control group value (p<0.05).  
\*\* Significantly different from the control group value (p<0.01).

**Table I.1.6** M-maze performance in female rats during the post-dosing period (PND 105)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	5	15	50
SUBSET 3					
SESSION 1 <sup>b</sup>	N	19 <sup>c</sup>	19 <sup>c</sup>	20	18 <sup>c</sup>
TRIALS TO CRITERION	MEAN±S.D.	7.9 ± 2.2	7.9 ± 1.8	8.0 ± 2.1	7.6 ± 1.5
ERRORS PER TRIAL	MEAN±S.D.	0.30 ± 0.14	0.34 ± 0.12	0.35 ± 0.23	0.42 ± 0.30
LATENCY TRIAL 2 <sup>d</sup>	MEAN±S.D.	14.9 ± 10.2	13.9 ± 9.5	11.5 ± 3.8	13.9 ± 7.9
FAILED TO LEARN <sup>e</sup>	N(%)	0( 0.0)	0( 0.0)	0( 0.0)	0( 0.0)
SESSION 2 <sup>b</sup>	N	19 <sup>c</sup>	19 <sup>c</sup>	20	18 <sup>c</sup>
TRIALS TO CRITERION	MEAN±S.D.	6.9 ± 2.4	6.9 ± 3.1	7.2 ± 2.5	6.7 ± 2.6
ERRORS PER TRIAL	MEAN±S.D.	0.17 ± 0.23	0.16 ± 0.20	0.18 ± 0.16	0.10 ± 0.15
LATENCY TRIAL 1 <sup>d</sup>	MEAN±S.D.	12.5 ± 10.5	11.1 ± 8.0	12.6 ± 11.8	11.1 ± 9.3
REVERSAL SESSION	N	19 <sup>c</sup>	19 <sup>c</sup>	20	18 <sup>c</sup>
TRIALS TO CRITERION	MEAN±S.D.	9.2 ± 2.5	8.8 ± 2.3	8.3 ± 2.7	9.9 ± 3.0
ERRORS PER TRIAL	MEAN±S.D.	0.63 ± 0.33	0.72 ± 0.44	0.62 ± 0.35	0.62 ± 0.35
LATENCY TRIAL 1 <sup>d</sup>	MEAN±S.D.	27.6 ± 19.6	25.4 ± 19.9	27.8 ± 14.3	23.9 ± 15.5
FAILED TO LEARN	N(%)	0( 0.0)	0( 0.0)	0( 0.0)	3( 16.7)**

a. Dosage occurred on postnatal days 4 through 65.  
b. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval; Reversal Session occurred immediately after the rat had met the criterion for the initial phase of the second test session.  
c. Excludes values for rats that were found dead or unscheduled sacrificed.  
d. The latency was recorded in seconds.  
e. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.  
\*\* Significantly different from the control group value (p<0.01).

**Table I.1.7** Morris Maze performance in juvenile rats  
Males

DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	5	15	50	
SUBSET 3						
POSTNATAL DAYS 153 THROUGH 177 <sup>b</sup>						
RATS TESTED		N	10	10	10	10
SESSION 1						
AVERAGE TRIALS 1-3	MEAN ± S.D.	36.7 ± 10.2	47.9 ± 12.4	39.0 ± 10.6	42.0 ± 10.6	
AVERAGE TRIALS 4-6	MEAN ± S.D.	24.2 ± 17.3	23.8 ± 9.1	23.5 ± 13.3	24.4 ± 11.0	
AVERAGE TRIALS 7-11	MEAN ± S.D.	17.8 ± 9.8	15.4 ± 7.2	17.2 ± 16.2	15.7 ± 9.1	
PROBE TRIAL (%) <sup>c</sup>	MEAN ± S.D.	45.7 ± 15.9	45.0 ± 13.9	47.0 ± 21.7	51.7 ± 19.4	
SESSION 2						
AVERAGE TRIALS 1-3	MEAN ± S.D.	16.0 ± 10.2	14.5 ± 8.1	17.3 ± 13.9	18.5 ± 11.1	
AVERAGE TRIALS 4-6	MEAN ± S.D.	7.6 ± 2.9	6.8 ± 2.3	9.5 ± 6.3	11.8 ± 7.6	
AVERAGE TRIALS 7-11	MEAN ± S.D.	6.9 ± 4.8	8.4 ± 6.6	8.1 ± 2.6	10.3 ± 4.7	
PROBE TRIAL (%) <sup>c</sup>	MEAN ± S.D.	55.3 ± 21.2	43.0 ± 11.1	48.3 ± 14.7	60.3 ± 16.4	
SESSION 3						
AVERAGE TRIALS 1-3	MEAN ± S.D.	13.1 ± 12.8	15.2 ± 13.2	14.4 ± 8.5	10.0 ± 5.2	
AVERAGE TRIALS 4-6	MEAN ± S.D.	6.7 ± 5.5	8.4 ± 6.2	9.2 ± 4.1	7.8 ± 3.7	
AVERAGE TRIALS 7-11	MEAN ± S.D.	5.7 ± 2.2	7.9 ± 3.8	7.0 ± 2.6	6.9 ± 3.0	
PROBE TRIAL (%) <sup>c</sup>	MEAN ± S.D.	47.7 ± 17.2	48.7 ± 15.1	55.3 ± 10.6	55.7 ± 21.4	

AVERAGE TRIALS = AVERAGE TIME (SECONDS) TO REACH THE PLATFORM FOR ALL RATS IN A GROUP FOR THE SPECIFIED TRIALS  
a. Dosage occurred on postnatal days 4 through 65.  
b. Age of the rats ranged from postnatal day 153 through 177; testing occurred over a three day period for each rat.  
c. Probe trial was recorded in seconds and reported as a percentage of time the rat spent in the goal quadrant.

**Females**

DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) <sup>a</sup>		0	5	15	50	
SUBSET 3						
POSTNATAL DAYS 153 THROUGH 177 <sup>b</sup>						
RATS TESTED		N	10	10	10	10
SESSION 1						
AVERAGE TRIALS 1-3	MEAN ± S.D.	29.9 ± 13.2	42.0 ± 11.2	40.8 ± 14.7	34.7 ± 10.5	
AVERAGE TRIALS 4-6	MEAN ± S.D.	19.0 ± 11.4	20.3 ± 12.6	21.8 ± 11.6	30.2 ± 13.4	
AVERAGE TRIALS 7-11	MEAN ± S.D.	13.8 ± 7.5	12.4 ± 8.4	18.2 ± 9.0	20.1 ± 12.2	
PROBE TRIAL (%) <sup>c</sup>	MEAN ± S.D.	50.0 ± 11.6	48.7 ± 13.1	51.0 ± 9.4	54.7 ± 21.2	
SESSION 2						
AVERAGE TRIALS 1-3	MEAN ± S.D.	17.0 ± 8.6	13.9 ± 9.8	24.0 ± 10.8	27.0 ± 11.5	
AVERAGE TRIALS 4-6	MEAN ± S.D.	12.0 ± 9.0	10.9 ± 8.4	9.4 ± 3.6	12.4 ± 4.5	
AVERAGE TRIALS 7-11	MEAN ± S.D.	9.8 ± 6.8	8.5 ± 5.0	9.8 ± 5.8	10.1 ± 6.3	
PROBE TRIAL (%) <sup>c</sup>	MEAN ± S.D.	44.3 ± 14.1	44.3 ± 14.0	51.3 ± 13.8	45.0 ± 13.1	
SESSION 3						
AVERAGE TRIALS 1-3	MEAN ± S.D.	13.2 ± 9.9 [ 9]d	13.2 ± 11.3	8.8 ± 5.0	16.8 ± 11.5 [ 9]d	
AVERAGE TRIALS 4-6	MEAN ± S.D.	7.7 ± 3.2 [ 9]d	5.7 ± 3.7	7.9 ± 3.7	8.7 ± 3.0 [ 9]d	
AVERAGE TRIALS 7-11	MEAN ± S.D.	8.8 ± 6.5 [ 9]d	5.4 ± 1.9	11.0 ± 8.2	8.5 ± 3.2 [ 9]d	
PROBE TRIAL (%) <sup>c</sup>	MEAN ± S.D.	57.4 ± 13.5 [ 9]d	51.3 ± 11.8	55.0 ± 15.3	58.5 ± 21.6 [ 9]d	

AVERAGE TRIALS = AVERAGE TIME (SECONDS) TO REACH THE PLATFORM FOR ALL RATS IN A GROUP FOR THE SPECIFIED TRIALS  
[ ] = NUMBER OF VALUES AVERAGED  
a. Dosage occurred on postnatal days 4 through 65.  
b. Age of the rats ranged from postnatal day 153 through 177; testing occurred over a three day period for each rat.  
c. Probe trial was recorded in seconds and reported as a percentage of time the rat spent in the goal quadrant.  
d. Excludes values for rats that had an error during testing in session 3.

**Table I.1.8** Reproductive performance in juvenile rats

DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) a		0	5	15	50	
RATS IN COHABITATION		N	20	19b	20	18b
DAYS IN COHABITATION c,d MEAN±S.D.		5.0 ± 2.4	4.5 ± 2.4	4.7 ± 2.3	3.0 ± 1.9	
RATS THAT MATED d		N (%)	10 ( 50.0)	11 ( 57.9)	12 ( 60.0)	16 ( 88.9)
FERTILITY INDEX e,f		N/N (%)	10/ 10 (100.0)	5/ 11** ( 45.4)	8/ 12* ( 66.7)	15/ 16 ( 93.8)
RATS WITH CONFIRMED MATING DATES		N	10	11	12	16
MATED WITH FEMALE g DAYS 1-7		N (%)	10(100.0)	11(100.0)	12(100.0)	16(100.0)
RATS PREGNANT/RATS IN COHABITATION f		N/N (%)	10/ 20 ( 50.0)	5/ 19 ( 26.3)	8/ 20 ( 40.0)	15/ 18** ( 83.3)

- a. Dosage occurred on postnatal days 4 through 65.  
 b. Excludes values for rats that were not assigned to cohabitation because there were no available female rats.  
 c. Restricted to rats with a confirmed mating date and rats that did not mate.  
 d. Includes only one mating for each male rat.  
 e. Number of pregnancies/number of rats that mated.  
 f. Includes only one pregnancy for each rat that impregnated more than one female rat.  
 g. Restricted to rats with a confirmed mating date.  
 \*\* Significantly different from the control group value (p<0.01).

DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) a		0	5	15	50	
ESTROUS CYCLING OBSERVATIONS						
RATS EVALUATED		N	20	20	20	19b
PRECOHABITATION ESTROUS CYCLING						
ESTROUS STAGES/ 72 DAYS		MEAN±S.D.	15.6 ± 2.4 [ 19]b	15.2 ± 3.0 [ 19]b	14.7 ± 2.6	14.5 ± 3.2 [ 18]b
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS		N	4	7	6	7
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS		N	0	2	1	1

- [ ] = NUMBER OF VALUES AVERAGED  
 a. Dosage occurred on postnatal days 4 through 65.  
 b. Excludes values for rats that were found dead or sacrificed due to moribund condition.

**Table I.1.9** Mean brain weight and brain/bw ratios in juvenile rats at the end of treatment

	Absolute (gm)		Brain/BW Ratio (%)	
	Male	Female	Male	Female
<b>Control</b>	<b>2.18</b>	<b>2.05</b>	<b>0.563</b>	<b>0.925</b>
<b>5 mg/kg</b>	<b>2.09</b>	<b>2.04</b>	<b>0.589</b>	<b>0.925</b>
<b>15 mg/kg</b>	<b>2.02**</b>	<b>1.95*</b>	<b>0.600</b>	<b>0.910</b>
<b>50 mg/kg</b>	<b>1.94**</b>	<b>1.79**</b>	<b>0.636</b>	<b>1.026*</b>

- \* p<0.05  
 \*\* p<0.01

**Table I.1.10** Mean brain weight and brain/bw ratios in juvenile rats at the end of the recovery period

	Absolute (gm)		Brain/BW Ratio (%)	
	Male	Female	Male	Female
<b>Control</b>	<b>2.43</b>	<b>2.16</b>	<b>0.349</b>	<b>0.591</b>
<b>5 mg/kg</b>	<b>2.40</b>	<b>2.13</b>	<b>0.348</b>	<b>0.582</b>
<b>15 mg/kg</b>	<b>2.37</b>	<b>2.06</b>	<b>0.371</b>	<b>0.575</b>
<b>50 mg/kg</b>	<b>2.23**</b>	<b>1.96**</b>	<b>0.439**</b>	<b>0.683**</b>

- \* p<0.05  
 \*\* p<0.01

Table I.1.11

## SUMMARY OF MICROSCOPIC DIAGNOSES – PND 67 MALE RATS

	GROUP:			
	1	2	3	4
Number of animals included	10	10	10	10
<b>Piriform Cortex</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	9
No. With Microscopic Diagnoses	0	0	0	1
NEUROFIL VACUOLATION		0	0	0
mild	-	-	-	1
NEURON DEGENERATION (FLUORO-JADE)		0	0	0
minimal	-	-	-	1
<b>Frontal Cortex</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Cingulate Cortex</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Septal Nuclei</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	9
No. With Microscopic Diagnoses	0	0	0	1
NEUROFIL VACUOLATION		0	0	0
minimal	-	-	-	1
<b>Anterior Commissure</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Parietal Cortex</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Caudate Nucleus/Putamen</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Globus Pallidus</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Basal Forebrain</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	6
No. With Microscopic Diagnoses	0	0	0	4
NEUROFIL VACUOLATION		0	0	0
minimal	-	-	-	3
mild	-	-	-	1
<b>Corpus Callosum</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>External Capsule</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Internal Capsule</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Amygdala</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
<b>Retrosplenial Cortex</b>				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10

Subiculum				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	9	5
No. With Microscopic Diagnoses	0	0	1	5
NEUROPIIL VACUOLATION				
	0	0	1	5a
minimal	-	-	1	1
mild	-	-	-	4
Hippocampus, CA1				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	8
No. With Microscopic Diagnoses	0	0	0	2
NEUROPIIL VACUOLATION				
	0	0	0	2
mild	-	-	-	2
Hippocampus, CA2				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Hippocampus, CA3				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Hippocampus, CA4				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Dentate Gyrus				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Thalamus				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	6
No. With Microscopic Diagnoses	0	0	0	4
NEUROPIIL VACUOLATION				
	0	0	0	4a
minimal	-	-	-	3
mild	-	-	-	1
Hypothalamus				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	8
No. With Microscopic Diagnoses	0	0	0	2
NEUROPIIL VACUOLATION				
	0	0	0	2
minimal	-	-	-	1
mild	-	-	-	1
Temporal Cortex				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Occipital Cortex				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Fornix				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Mamillary Bodies				
Number of Tissues Examined	0	0	0	1
No. With Microscopic Diagnoses	-	-	-	1
NEUROPIIL VACUOLATION				
	-	-	-	1
mild	-	-	-	1

Midbrain				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	0
No. With Microscopic Diagnoses	0	0	0	10
NEUROFIL VACUOLATION	0	0	0	10b
minimal	-	-	-	5
mild	-	-	-	5
Entorhinal Cortex				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Substantia Nigra, Comp				
Number of Tissues Examined	0	0	0	5
No. With Microscopic Diagnoses	-	-	-	5
NEUROFIL VACUOLATION	-	-	-	5
minimal	-	-	-	1
mild	-	-	-	4
Substantia Nigra, Retic				
Number of Tissues Examined	0	0	0	2
No. With Microscopic Diagnoses	-	-	-	2
NEUROFIL VACUOLATION	-	-	-	2
minimal	-	-	-	1
mild	-	-	-	1
Cerebral Peduncle				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Cerebellar Cortex				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Cerebellar nuclei				
Number of Tissues Examined	0	0	1	3
No. With Microscopic Diagnoses	-	-	1	3
NEUROFIL VACUOLATION	-	-	1	3
minimal	-	-	1	1
mild	-	-	-	1
moderate	-	-	-	1
Cerebellar White Matter				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Pons				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	9
No. With Microscopic Diagnoses	0	0	0	1
NEUROFIL VACUOLATION	0	0	0	1
mild	-	-	-	1
Trapezoid Body				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Vestibular Nucleus				
Number of Tissues Examined	0	0	0	1
No. With Microscopic Diagnoses	-	-	-	1
NEUROFIL VACUOLATION	-	-	-	1
mild	-	-	-	1

Reticular Formation				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Medulla Oblongata				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	5
No. With Microscopic Diagnoses	0	0	0	5
NEUROFIL VACUOLATION	0	0	0	5a
minimal	-	-	-	3
mild	-	-	-	2
Pyramids				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Trigeminal Tract				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	10
Spinal Cord, Cervical				
Number of Tissues Examined	10	0	0	10
Microscopically Normal	10	-	-	9
No. With Microscopic Diagnoses	0	-	-	1
AXON DEGENERATION	0	-	-	1
minimal	-	-	-	1
Spinal Cord, Lumbar				
Number of Tissues Examined	10	0	0	10
Microscopically Normal	6	-	-	10
No. With Microscopic Diagnoses	4	-	-	0
AXON DEGENERATION	4	-	-	0a
minimal	4	-	-	-
Spinal Nerve Roots				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	9	10	9	8
No. With Microscopic Diagnoses	1	0	1	2
NERVE FIBER DEGENERATION	1	0	1	2
minimal	1	-	-	1
mild	-	-	1	1
Dorsal Root Ganglia				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	10	10	10	9
No. With Microscopic Diagnoses	0	0	0	1
GANGLION CELL PALLOR & VACUOLATION	0	0	0	1
mild	-	-	-	1
Gasserian Ganglia				
Number of Tissues Examined	10	0	0	10
Microscopically Normal	10	-	-	9
No. With Microscopic Diagnoses	0	-	-	1
GANGLION CELL PALLOR & VACUOLATION	0	-	-	1
mild	-	-	-	1
Sciatic Nerve				
Number of Tissues Examined	10	10	10	10
Microscopically Normal	9	10	9	7
No. With Microscopic Diagnoses	1	0	1	3
NERVE FIBER DEGENERATION	1	0	1	3
minimal	1	-	1	3

Tibial Nerve					
Number of Tissues Examined		10	10	10	10
Microscopically Normal		10	10	10	9
No. With Microscopic Diagnoses		0	0	0	1
NERVE FIBER DEGENERATION					
	minimal	0	0	0	1
		-	-	-	1
Brain, NOS					
Number of Tissues Examined		0	0	1	0
No. With Microscopic Diagnoses		-	-	1	-
HYDROCEPHALUS					
	minimal	-	-	1	-
		-	-	1	-
Eye					
Number of Tissues Examined		10	10	10	10
Microscopically Normal		9	10	7	7
No. With Microscopic Diagnoses		1	0	3	3
RETINAL DYSPLASIA					
	minimal	0	0	3	2
		-	-	2	1
	mild	-	-	1	1
CORNEAL OSSIFICATION					
	mild	1	0	0	1
		-	-	-	1
	moderate	1	-	-	-
Skeletal Muscle					
Number of Tissues Examined		10	0	0	10
Microscopically Normal		10	-	-	10

Group Legend: 1 is 0 mg/kg/day, 2 is 5 mg/kg/day, 3 is 15 mg/kg/day,  
4 is 50 mg/kg/day

Statistics performed using Fisher's exact (1-tail)  
a = Significantly different from GROUP 1 at P<=0.05  
b = Significantly different from GROUP 1 at P<=0.01

**Table I.1.12**

**SUMMARY OF MICROSCOPIC DIAGNOSES - PND 67 FEMALE RATS**

	GROUP:			
	1	2	3	4
Number of animals included	10	10	10	11
Piriform Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Frontal Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Cingulate Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Septal Nuclei				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Anterior Commissure				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Parietal Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	10
No. With Microscopic Diagnoses	0	0	0	1
NEUROPIIL VACUOLATION	0	0	0	1
minimal	-	-	-	1
Caudate Nucleus/Putamen				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Globus Pallidus				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Basal Forebrain				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	8
No. With Microscopic Diagnoses	0	0	0	3
NEUROPIIL VACUOLATION	0	0	0	3
minimal	-	-	-	1
mild	-	-	-	2
Corpus Callosum				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
External Capsule				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Internal Capsule				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Amygdala				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Retrosplenial Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Subiculum				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	3
No. With Microscopic Diagnoses	0	0	0	8
NEUROPIIL VACUOLATION	0	0	0	7b
minimal	-	-	-	2
mild	-	-	-	5

NEURON DEGENERATION (FLUORO-JADE)		0	0	0	1
	minimal	-	-	-	1
Hippocampus, CA1					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	3
No. With Microscopic Diagnoses		0	0	0	8
NEUROFIL VACUOLATION		0	0	0	8b
	minimal	-	-	-	6
	mild	-	-	-	2
Hippocampus, CA2					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	11
Hippocampus, CA3					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	10
No. With Microscopic Diagnoses		0	0	0	1
FOCAL GLIOSIS		0	0	0	1
	mild	-	-	-	1
Hippocampus, CA4					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	11
Dentate Gyrus					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	9	11
No. With Microscopic Diagnoses		0	0	1	0
NEURON DEGENERATION (FLUORO-JADE)		0	0	1	0
	mild	-	-	1	-
Thalamus					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	6
No. With Microscopic Diagnoses		0	0	0	5
NEUROFIL VACUOLATION		0	0	0	5a
	minimal	-	-	-	3
	mild	-	-	-	2
Hypothalamus					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	11
Temporal Cortex					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	9	11
No. With Microscopic Diagnoses		0	0	1	0
NEURON DEGENERATION (FLUORO-JADE)		0	0	1	0
	minimal	-	-	1	-
Occipital Cortex					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	11
Fornix					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		10	10	10	11
Mamillary Bodies					
Number of Tissues Examined		0	0	0	3
No. With Microscopic Diagnoses		-	-	-	3
NEUROFIL VACUOLATION		-	-	-	3
	minimal	-	-	-	2
	mild	-	-	-	1

Midbrain				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	0
No. With Microscopic Diagnoses	0	0	0	11
NEUROFIL VACUOLATION	0	0	0	11b
minimal	-	-	-	5
mild	-	-	-	5
moderate	-	-	-	1
Entorhinal Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Substantia Nigra, Comp				
Number of Tissues Examined	0	0	0	7
No. With Microscopic Diagnoses	-	-	-	7
NEUROFIL VACUOLATION	-	-	-	7
minimal	-	-	-	6
mild	-	-	-	1
Substantia Nigra, Retic				
Number of Tissues Examined	0	0	0	6
No. With Microscopic Diagnoses	-	-	-	6
NEUROFIL VACUOLATION	-	-	-	6
minimal	-	-	-	5
mild	-	-	-	1
Cerebral Peduncle				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Cerebellar Cortex				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Cerebellar nuclei				
Number of Tissues Examined	0	0	0	5
No. With Microscopic Diagnoses	-	-	-	5
NEUROFIL VACUOLATION	-	-	-	5
minimal	-	-	-	2
mild	-	-	-	3
Cerebellar White Matter				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	9	10	11
No. With Microscopic Diagnoses	0	1	0	0
CYSTIC FOCUS	0	1	0	0
mild	-	1	-	-
Pons				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	9
No. With Microscopic Diagnoses	0	0	0	2
NEUROFIL VACUOLATION	0	0	0	2
minimal	-	-	-	2
Trapezoid Body				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11

Reticular Formation				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Medulla Oblongata				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	5
No. With Microscopic Diagnoses	0	0	0	6
NEUROFIL VACUOLATION				
	0	0	0	6b
minimal	-	-	-	4
mild	-	-	-	2
Pyramids				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Trigeminal Tract				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Spinal Cord, Cervical				
Number of Tissues Examined	10	0	0	11
Microscopically Normal	10	-	-	10
No. With Microscopic Diagnoses	0	-	-	1
AXON DEGENERATION				
	0	-	-	1
minimal	-	-	-	1
Spinal Cord, Lumbar				
Number of Tissues Examined	10	0	0	11
Microscopically Normal	10	-	-	11
Spinal Nerve Roots				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	9	9	10	11
No. With Microscopic Diagnoses	1	1	0	0
Spinal Nerve Roots(continued)				
NERVE FIBER DEGENERATION				
	1	1	0	0
minimal	1	1	-	-
Dorsal Root Ganglia				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	10	11
Gasserian Ganglia				
Number of Tissues Examined	10	0	0	11
Microscopically Normal	10	-	-	11
Sciatic Nerve				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	9	11
No. With Microscopic Diagnoses	0	0	1	0
NERVE FIBER DEGENERATION				
	0	0	1	0
minimal	-	-	1	-
Tibial Nerve				
Number of Tissues Examined	10	10	10	11
Microscopically Normal	10	10	9	11
No. With Microscopic Diagnoses	0	0	1	0
NERVE FIBER DEGENERATION				
	0	0	1	0
minimal	-	-	1	-
Brain, NOS				
Number of Tissues Examined	0	0	1	0
No. With Microscopic Diagnoses	-	-	1	-
HYDROCEPHALUS				
	-	-	1	-
minimal	-	-	1	-

Eye					
Number of Tissues Examined		10	10	10	11
Microscopically Normal		9	9	9	7
No. With Microscopic Diagnoses		1	1	1	4
RETINAL DYSPLASIA		1	0	1	4
	minimal	1	-	-	3
	mild	-	-	1	1
CORNEAL MINERALIZATION		0	1	0	0
	minimal	-	1	-	-
CORNEAL VASCULARIZATION		0	1	0	0
	mild	-	1	-	-
Skeletal Muscle					
Number of Tissues Examined		10	0	0	11
Microscopically Normal		10	-	-	11

Group Legend: 1 is 0 mg/kg/day, 2 is 5 mg/kg/day, 3 is 15 mg/kg/day,  
4 is 50 mg/kg/day

Statistics performed using Fisher's exact (1-tail)  
a = Significantly different from GROUP 1 at P<=0.05  
b = Significantly different from GROUP 1 at P<=0.01

**Table I.1.13.** Summary of pharmacokinetic data in juvenile rats (Study OV-1007)

Gender	Dose (mg/kg/day)	C <sub>max</sub> µg/mL PND 17	C <sub>max</sub> µg/mL PND 65	AUC <sub>0-∞</sub> µg·h/mL PND 17	AUC <sub>0-∞</sub> µg·h/mL PND 65
Males	5	2.88	2.95	8.18	6.45
	15	10.69	10.42	28.26	17.33
	50	35.20	27.17	125.25	89.78
Females	5	3.55	3.22	9.40	5.58
	15	8.27	7.44	25.04	13.61
	50	31.03	21.60	110.68	49.53

2. Pathology working group (PWG) review of potential treatment-related lesions in the brain of rats from an oral (gavage) repeated-dose toxicity study of vigabatrin (Ovation Pharmaceuticals, Inc.'s Study Number OV-1007) (EPL Project No. 794-001, dated 5/11/06, Conducted By Experimental Pathology Laboratories, Inc., Research Triangle Park, NC; GLP)

a. Methods

In Study No. OV-1007, microscopic examination of multiple brain sections from perfusion fixed rats fixed showed that all HD (50 mg/kg/day) group rats had neuropil vacuolation in various brain regions at the PND 67 necropsy. Two MD (15 mg/kg/day) rats were also considered to have neuropil vacuolation, although the neuropathologist considered the vacuolar changes limited and not conclusively T-R. The neuropil vacuolation was attenuated in VGB-dosed animals after the 19-week recovery period but was still present in 2 males and 1 female from the HD group.

A Pathology Working Group (PWG) was convened to have a panel of experts confirm the presence of neuropil vacuolation in the brains of rats in Study OV-1007 and to provide expert advice on questions pertinent to the observed morphologic changes and their pathogenesis. The PWG (Henry Wall, Marc Del Bigio, Robert Garman, Georg Krinke, Reid Patterson, Karen Regan, and Stephen Collins) reviewed all H&E stained brain sections from all perfusion-fixed rats that were diagnosed by the study neuropathologist to have microscopic neuropil vacuolation in one or more brain regions and from all control rats that were killed at the end of the dosing period.

b. Results

Overall, the PWG panel agreed with the original study neuropathologist's characterization of the presence or absence of neuropil vacuolation in the different brain regions of the 62 animals examined (**Appendix A**). The exceptions were said to reflect "minor differences in diagnoses for alterations that were usually graded as minimal by the study neuropathologist or graded as minimal by the PWG." It should be noted, however, that the number of recovery animals with neuropil vacuolation present was increased in the PWG evaluation (from 2 males and 1 female to 2 males and 3 females). The differences did not influence the original interpretation of treatment-related effects and no changes in original diagnoses were considered warranted.

Based on the results of their peer review of slides and data available in published and unpublished reports, the Panel provided the following responses to specific questions regarding the morphologic characterization of vacuolation in the brain of rats and the relevance to humans (verbatim):

*1. Is there general agreement that the primary neurohistologic change is multifocal vacuolation in the central midbrain (tegmentum), lateral portion of the pars compacta of the substantia nigra, dorsal subiculum, deep cerebellar nuclei, posterior thalamus, basal forebrain (particularly the medial forebrain bundle) and pons/medulla?*

*Yes, the PWG confirmed these changes as reported by the Study Neuropathologist. The PWG also noted recovery in rats receiving 50 mg/kg/day as demonstrated by the absence of neuropil vacuolation in all but two rats held untreated after PND 67 and necropsied on PND 196-210. (Note: there were actually 5 recovery rats with vacuolation).*

*2. Does the histologic presentation of these vacuoles provide sufficient information to determine the affected cell(s) in the neuropil?*

- No, the specific characterization of the nature of the vacuoles or specific cells affected cannot be determined. It was noted that the vacuoles were in neuroanatomic areas where GABA is known to be a predominant neurotransmitter.
- However, the H&E-stained slides do allow the observer to exclude the involvement of some structures, namely neuron cell bodies, blood vessels, and astrocytic end processes around blood vessels as suggested by light microscopy.
- There is no light microscopic evidence of cell degeneration, cell loss, or gliosis. However, morphometric or stereologic procedures would need to be performed to quantify cell loss.
- Some of the possibilities for the potential location of the vacuoles included preterminal or perisynaptic vesicles, and terminals of GABAergic axons, as in some areas the vacuoles appear close to nerve cell bodies. The possibility that vacuoles are associated with multiple targets (e.g., axons or dendrites, microglia) could not be excluded. The effect was not considered to be an anti-glucose effect.

3. Are there methods available with these tissues to determine or confirm the cells that are vacuolated?

Morphologic methods to explore this possibility using existing paraffin-embedded tissues may include the use of additional special stains:

- Neurofilament staining might help to localize the effect and characterize the structure of the vacuoles. The neurofilament stain may also be useful for comparing the diameters of axons in controls to the diameters of axons in VGB-treated rats.
- Staining for GABAergic neurons (e.g. with parvalbumin) may help determine if the vacuoles are associated with terminals of these neurons.
- Lectin staining (e.g., Griffonia simplicifolia) will demonstrate blood vessels and microglial proliferation. If the vacuoles are not associated with microglia, it would suggest that the vacuolation is more likely to be indicative of a fluid distribution problem rather than a more destructive process.
- Amyloid precursor protein (APP) was considered to be more specific than Fluro Jade® for staining degenerating neurons. Chromogranin A immunostaining was considered to be a useful alternate marker for damaged axons.
- Sections stained with the selected stains should be evaluated using at least five controls and five animals at 50 mg/kg with vacuolated brain areas.
- Luxol Fast Blue/Periodic Acid-Schiff (LFB/PAS) was considered advantageous for evaluating axons and myelin together. The PAS-stained axon could help to distinguish effects in axons from those that may be limited to myelin.
- Review of the available LFB-stained sections may help to determine if the flocculent material observed in some vacuoles contain lipoproteins that may have become a cellular inclusion.

4. Are the vacuolar changes in the white matter identical to those in the gray matter or different?

As viewed by light microscopy within this study, vacuolar changes in the gray matter and white matter are morphologically similar. They vary in size and sometimes contain granular or flocculent material, and borders of the vacuoles are indistinct.

5. Are the white-matter changes consistent with or different from what is termed intramyelinic edema as was described in older rats given VGB?

- The white matter findings by light microscopy are not characteristic of intramyelinic edema.
- Some differences that may be observed in older rats as compared to younger rats could be attributable to differences in bioavailability due to different routes of dosing, differences in dose, differences in length of exposure, age at start of exposure or at the

time of neurohistopathologic examination, and differences in the GABA system (e.g., the GABA excitatory/inhibitory switch).

- A better understanding of the morphology and pathogenesis of white matter lesions may clarify differences in lesion appearance.

6. If these changes are different from intramyelinic edema, are there hypotheses to explain why these rats failed to develop the lesion (seen in rats of equivalent age and treatment duration to these rats) toward the end of the study?

If the drug is present during the early stage of myelination, then the myelin may be more resistant to the development of IME as seen in rats exposed much later after myelination has commenced (i.e., adaptation may play a role). There may have been differences in other pertinent factors, such as, design, dose level, and duration of dosing.

7. Do you concur that there is little evidence to indicate gliosis, increased neuronal apoptosis or hypocellularity that would support loss of neuropil?

The PWG concluded that there is no evidence via light microscopy of H&E-stained sections to indicate gliosis, increased neuronal apoptosis or hypocellularity that would support loss of neuropil. Morphometric analysis may be necessary to quantify any potential indication of a morphologic effect on these structures.

8. Do these microscopic changes indicate or suggest probable pathogenetic mechanisms?

The morphologic information obtained via this study is insufficient to elucidate the pathogenesis of the vacuolar change.

9. Do you concur that the few rats with similar but more focal lesions in the mid-dose group are not conclusively related to VGB treatment?

The PWG concluded that the brains of animals in the mid-dose group did not differ from the controls and were, therefore not affected by treatment with VGB.

10. Do you concur that the recovery brains indicated some reduction in the incidence and severity of the vacuolar changes and no evidence for other neuronal pathology.

Yes, the PWG noted the absence of vacuoles in the brain of most VGB-treated rats following a recovery period after dosing was discontinued. Three rats in the recovery 50mg/kg/day group had focal vacuolar changes in the brain.

11. Would these lesions likely lead to the "spasms" described in association with handling or with the reduced neuromuscular performance in these rats, or is the latter more likely to be due to the marked growth suppression by the high dose of VGB?

- Until the affected cell or possible biochemical and/or molecular changes are better understood, it is not possible to make a definitive statement concerning the relationship of the vacuolar lesions and the clinically described "spasms."

- The "spasms" may or may not be related to the vacuolar change.

- The "spasms" could be related to earlier changes (physiological or pharmacological) that led to the vacuole formation.

- Marked growth suppression may have influenced the neuromuscular performance of these rats, as measured by landing foot splay and fore/hindleg grip strength.

(Note: long-term neuromotor impairment in FOB was also observed in MD females in the absence of significant growth deficit. Similar findings have been reported with triethyltin and hexachlorophene).

12. What studies would you suggest to better understand the pathogenesis and targeted cells within the neuropil that become vacuolated?

*The Panel suggested further morphologic characterization via light microscopy using histochemistry, with transmission electron microscopy (EM) follow-up to determine the precise anatomic location of the vacuoles. The additional morphologic characterization should sample animals at multiple time points after exposure beginning on postnatal day 4 (PND 4). Sampling is suggested at PND 25, PND 46 and PND 67. Five animals per group (controls and 50 mg/kg groups) per time point dosed PND4-65, as in the current study, and using males only were suggested. If feasible, an additional five animals per group per time point should be designated for freezing of half of the brain for potential frozen section immunohistochemistry and immersion fixation the other half of the brain in formalin followed by storage in absolute alcohol (95%) for potential paraffin-embedded section immunohistochemistry. Areas for evaluation should include brain (sites where vacuolation was seen via light microscopy), optic nerve, and cervical spinal cord, and the light microscopy should incorporate evaluation of 1um-thick plastic sections, where practical. These additional morphologic evaluations are expected to provide some direction for subsequent investigations of the pathogenesis of neuropil vacuoles in the brains of VGB-treated juvenile rats.*

c. Conclusions

*The PWG Panel concluded:*

- *The location of the vacuolar changes in the brain include the central midbrain (tegmentum), pars compacta of the substantia nigra, dorsal subiculum, pons/medulla, hippocampal CA1 region, thalamus, deep cerebellar nuclei, and basal forebrain.*
- *Vacuolar effects may be attributable to biochemical perturbations in GABAergic neurons or nerve terminals.*
- *The microscopic evaluation of paraffin-embedded brain suggests areas that are not vacuolated (e.g., neuron cell bodies, blood vessels, and astrocytic end processes around blood vessels).*
- *Vacuolar changes in white matter and gray matter are morphologically similar via light microscopy, and have a morphologic appearance that is not characteristic of intramyelinic edema.*
- *There is no morphologic evidence of gliosis, neuronal apoptosis or hypocellularity in the H&E stained material.*
- *The vacuolar changes are dose-related, occurring only at 50 mg/kg, and are reversible in most animals after a 19-week recovery period.*
- *Light microscopic observations alone are insufficient to explain the pathogenesis of vacuoles observed in the brains of rats treated with VGB.*
- *Specific light and electronic microscopic methods may be applied to better characterize the vacuolar changes and gain direction for investigation of the pathogenesis of the brain vacuoles.*
- *Based on available human clinical, imaging and pathology literature, there is no evidence that the vacuolar changes in rats are relevant in humans receiving vigabatrin.*

The scientific basis for the last statement is unclear, but it was presumably made without knowledge of the recent MRI data from children taking vigabatrin.

**APPENDIX A  
PWG Consensus Diagnoses for Individual Animals**

**Chemical Name: Vigabatrin  
Oral (Gavage) Repeated-Dose Toxicity Study of Vigabatrin in Rats  
Study No.: OV-1007**

Animal No.	Necropsy Day	No. of Slides	Study Pathologist's Diagnoses	PWG Consensus Diagnoses
<b>Group 0 mg/kg/day – Male Rats</b>				
202	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
302	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
502	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
1102	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
1202	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
1502	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
1702	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
1802	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
1902	PND 67	2	No Neuropil Vacuolation Present	Cerebrum Neuropil Vacuolation, Present
2002	PND 67	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
<b>Group 15 mg/kg/day – Male Rats</b>				
4202	PND 67	2	Cerebellum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present	No Neuropil Vacuolation Present
5702	PND 67	2	Cerebrum Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present
<b>Group 50 mg/kg/day – Male Rats</b>				
6402	PND 67	2	Cerebrum Neuropil Vacuolation, Present Cerebellum No Neuropil Vacuolation Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons No Neuropil Vacuolation Present	Cerebrum Neuropil Vacuolation, Present Cerebellum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present
6502	PND 67	2	Mid-Brain Neuropil Vacuolation, Present	Mid-Brain Neuropil Vacuolation, Present Cerebrum Neuropil Vacuolation, Present
6602	PND 67	2	Cerebellum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present	Cerebellum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present
6702	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present
6902	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present
7302	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present
7502	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present
7602	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present Cerebellum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present
7702	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present Cerebellum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present
8002	PND 67	2	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebrum Neuropil Vacuolation, Present Mid-Brain Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present
<b>Group 50 mg/kg/day Post-Recovery Period – Male Rats</b>				
6203	PND 196-210	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
6303	PND 196-210	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
6603	PND 196-210	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
6703	PND 196-210	2	No Neuropil Vacuolation Present	No Neuropil Vacuolation Present
6903	PND 196-210	2	Cerebellum Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present	Cerebellum Neuropil Vacuolation, Present Medulla/Pons Neuropil Vacuolation, Present



3. Nine-Week Oral (Gavage) Repeat-Dose Toxicity Study of Vigabatrin in Neonatal Rats (Study No. No. OVNC-9004, report dated 7/25/06, conducted by Charles River, Horsham, PA, GLP)

a. Methods

Forty male rat pups were assigned to 8 dose groups (Groups I through VIII, **Table I.3.1**), 5/group, to receive VGB (50 mg/kg, 5 mL/kg) or vehicle (water) by oral gavage once daily on postnatal days (PNDs) 4 through 25 (Groups I and V), 4 - 46 (Groups II and VI), 4 - 65 (Groups III and VII), and 12 - 26 (Groups IV and VIII). Pups were examined for viability, clinical observations, and body weights. All surviving rats were sacrificed on the last day of dose administration. Rats were administered a combination of heparin and sodium pentobarbital and perfused *in situ* with paraformaldehyde plus gluteraldehyde. Immediately after perfusion, the calvaria were removed, and the entire brain was weighed and dissected along the mid-sagittal plane. The right half was placed in chilled neutral buffered 10% formalin, while the left half was immersed in chilled perfusate solution. All samples were shipped to the College of Veterinary Medicine, Virginia Tech, for histological evaluation. Initially, multiple levels of the brains stained with H&E were examined (1-caudate nucleus, thalamus; 2-midbrain; 3-cerebellum, medulla). This led to study of selected blocks of epoxy resin embedded brain for light microscopic study. Based upon the latter, thin sections were selected for electron microscopic examination of the cerebellar nuclei and adjacent white matter.

Strain: CrI:CD(SD)  
Drug Lot #: 160207

The single daily dose of 50 mg/kg was selected on the basis of the previous study (OV-1007) in which this dose produced brain lesions evident by light microscopic examination.

**Table I.3.1**

Dosage Group	Dosage <sup>a</sup> (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Days of Dosage	Number of Male Pups/Rats	Assigned Pup/Rat Numbers
I	50	10	5	PNDs 4 to 25	5	101 - 105
II	50	10	5	PNDs 4 to 46	5	201 - 205
III	50	10	5	PNDs 4 to 65	5	301 - 305
IV	50	10	5	PNDs 12 to 26	5	901 - 905
V	0 (Vehicle)	0	5	PNDs 4 to 25	5	401 - 405
VI	0 (Vehicle) <sup>b</sup>	0 <sup>b</sup>	5	PNDs 4 to 46	5	501 - 505
VII	0 (Vehicle)	0	5	PNDs 4 to 65	5	601 - 605
VIII	0 (Vehicle)	0	5	PNDs 12 to 26	5	1001 - 1005

b. Results

i. Mortality, clinical signs

A total of 3 rats were found dead (1/5 in each of Groups II, III and IV on PNDs 46, 30, and 25). These deaths were considered treatment-related (T-R), based on clinical signs (tremors or convulsions) in 2 (Grps II and III) and the reduced body weight gains observed in all treated groups.

Clinical signs observed in Groups II and III included decreased motor activity, impaired righting reflex, ptosis, tremors, and chromorhinorrhea. Additionally, a clonic convulsion was observed in the Group II rat that was found dead on PND 46 and 2 rats in Group III were hyperreactive to touch. These observations often occurred as the rat was handled for observation as noted in the previously

conducted study. No clinical signs were noted in the rats in Groups I and IV, indicating that duration of dosing is important in the development of these signs.

ii. Body weight

Body weight gains were reduced in all treatment groups compared to C (55, 13, 18, and 29% in Groups I-IV over the respective treatment periods). On the day of sacrifice, mean body weights were 52 (Group I), 88 (Group II), 83 (Group III), and 85% (Group IV) of the respective control values.

iii. Necropsy observations

No gross lesions related to treatment were observed.

iv. Brain weights

Brain weights were reduced (SS in Grps I and II) in all treated groups compared to the respective controls, but the ratios of brain to terminal body weights were increased (also SS in Grps I and II).

v. Histopathology

According to the histopathology report, based on examination of the H&E stained sections, neuropil vacuoles were particularly prominent in Group I rats where there was said to be “marked involvement of the cerebellar white matter, including the folial and subcortical regions. The latter extended into the cerebellar nuclei. In addition, lesions were prominent in the medulla, in particular involving regions such as the dorsomedial trigeminal, spinal trigeminal tract and reticular nuclei.” According to the report, “lesser involvement was seen in midbrain regions, including the substantia nigra.” In Group II, lesions were seen in the cerebellum and medulla, but the white matter vacuolization was said to be diminished; and vacuoles were also noted in the midbrain, involving the substantia nigra and other nuclei, thalamus and region of the basal forebrain. In Group III, in addition to the medullary and cerebellar lesions, there was said to be involvement of more rostral regions such as the midbrain, thalamus and basal forebrain. Group IV showed vacuoles with a similar appearance and distribution, but which were said to be somewhat less extensive than those in rats with treatment beginning on PND 4.

Because of prominent involvement of the cerebellum and medulla found in the examination of H&E stained sections, these were the regions of focus in studies using 1 micron thick sections stained with toluidine blue and safranin, although in some cases more rostral levels of the brain were also examined (see **Table I.3.2**, below). The cerebellar sections included the nuclei and surrounding white matter, and the medullary sections included several levels of that part of the brain. In the cerebellum of Group I rats, there was demyelination and markedly diminished residual myelination of fibers. Vacuoles were said to be related to this myelinopathy. Thinly myelinated cerebellar white matter fibers were also seen in Group II rats. According to the report “vacuoles were found in the neuropil of nuclei and in myelinated fiber regions of the cerebellum, the medullary nuclei, in particular the trigeminal and reticular regions noted above, and midbrain (where examined) in Groups I, II, III and IV. They varied in shape and contain sparse stainable material. The latter often presented as wisps of fine membranous material, sometimes in contact with margin of the vacuole. In addition, more densely stained small bodies were seen, which occasionally were noted to

protrude from myelin sheaths on the margin of the vacuole. The vacuoles often were in contact with myelin sheaths, glia and neurons.” The association of vacuoles with myelin was said to be particularly prominent in the regions examined. Some vacuoles were observed to have “a complete or partial thin myelin-like limiting membrane.” Direct microscopic counts of cerebellum (nuclei and adjacent white matter) and medullary nuclear regions in rats of Groups III and VII indicated that a large proportion of the vacuoles had direct contact with myelin sheaths (Table I.3.3).

**Table I.3.2** Blocks sectioned at 1 micron and stained with toluidine blue and safranin

	Treatment			Rat Number	Block Number (replicates)	Tissue Identification
	Group	Duration	Total Days			
TEST ARTICLE	Group I	PND 4-25	22	102	4E (3)	cerebellum
					5E (2)	medulla oblongata
				103	4E (3)	cerebellum
					5E (2)	medulla oblongata
					104	4E (3)
	105	5E (2)	medulla oblongata			
	Group II	PND 4-46	43	202	2E (3)	dorsal hippocampus
					3E (3)	midbrain
					5E (3)	medulla oblongata
				204	1E (3)	basal forebrain
					3E (3)	midbrain
					4E (3)	cerebellum
					5E (2)	medulla oblongata
				205	2E (3)	dorsal hippocampus
					3E (3)	midbrain
	5E (3)	medulla oblongata				
	Group III	PND 4-65	62	301	3E (3)	midbrain
					4E (3)	cerebellum
					5E (1)	medulla oblongata
				302	4E (1)	cerebellum
5E (1)					medulla oblongata	
304				4E (3)	cerebellum	
				5E (1)	medulla oblongata	
305	4E (3)	cerebellum				
	5E (3)	medulla oblongata				
Group IV	PND 12-26	15	903	4E (3)	cerebellum	
			904	5E (3)	medulla oblongata	

<b>VEHICLE</b>	<b>Group V</b>	<b>PND 4-25</b>	<b>22</b>	<b>401</b>	4E (3)	cerebellum	
					5E (2)	medulla oblongata	
				<b>402</b>	4E (3)	cerebellum	
				<b>403</b>	5E (2)	medulla oblongata	
					<b>404</b>	4E (2)	cerebellum
	<b>Group VI</b>	<b>PND 4-46</b>	<b>43</b>	<b>502</b>	2E (3)	dorsal hippocampus	
					3E (3)	midbrain	
					5E (3)	medulla oblongata	
				<b>503</b>	1E (3)	basal forebrain	
					3E (3)	midbrain	
					4E (3)	cerebellum	
					5E (3)	medulla oblongata	
				<b>505</b>	2E (3)	dorsal hippocampus	
					3E (3)	midbrain	
					5E (3)	medulla oblongata	
	<b>Group VII</b>	<b>PND 4-65</b>	<b>62</b>	<b>601</b>	3E (3)	midbrain	
					4E (1)	cerebellum	
					5E (3)	medulla oblongata	
				<b>602</b>	4E (2)	cerebellum	
					5E (1)	medulla oblongata	
				<b>603</b>	4E (3)	cerebellum	
					5E (1)	medulla oblongata	
				<b>604</b>	4E (5)	cerebellum	
5E (1)					medulla oblongata		
4E (1)					cerebellum		
<b>605</b>				4E (1)	cerebellum		
	5E (1)	medulla oblongata					
<b>Group VIII</b>	<b>PND 12-26</b>	<b>15</b>	<b>1003</b>	4E (3)	cerebellum		
			<b>1005</b>	5E (3)	medulla oblongata		

Table I.3.3

**MICROSCOPIC COUNTS OF MYELIN-RELATED VACUOLES AFTER  
PND 4-64 EXPOSURE**

	Cerebellum (slide 4E)		Medulla (slide 5E)	
	<i>Myelin (+)</i>	<i>Myelin (-)</i>	<i>Myelin (+)</i>	<i>Myelin (-)</i>
<b>Group III*</b> (test compound)	26	2	40	6
<b>Group VII*</b> (vehicle)	0	0	1	0

Myelin (+) - vacuole contacts adjacent myelin sheath

Myelin (-) - vacuole does not contact adjacent myelin sheath

\*Direct counts of vacuoles in 4 microscopic fields from animals in these groups – see

Based on the examination of the toluidine blue and safranin stained sections, transmission electron microscopy was focused on sections of the cerebellum in the region of the (roof) nuclei and adjacent white matter, and regions of the medullary nucleus of the spinal tract of cranial nerve V and reticular nuclear regions (**Table I.3.4**). Relative to controls, there were said to be many non-myelinated or thinly myelinated fibers in Group I brains, particularly in cerebellar white matter. The report stated that, "Splits were noted in myelin sheaths, which appeared to expand along the intraperiod line in a fashion consistent with intramyelinic edema, giving rise to a vacuole bordered by a compressed lamellae of the sheath. Another form of vacuole was seen adjacent to demyelinated fibers, where the wall was made up of laminae of myelin debris. A striking feature at this stage was the presence of swollen glial cells adjacent to thinly or non-myelinated fibers. The nature of these glial cells was often difficult to discern, but some clearly were astrocytes."

Observations in Group II were consistent with retardation of myelination, although the active demyelination seen in Group I was not observed. Oligodendrocytes were prominent in the white matter in Group II, consistent with active myelination, and vacuoles were said to sometimes be associated with such cells. Most vacuoles in Groups II and III were said to be associated with myelin sheaths, and varied in ultrastructural appearance. According to the report, "Some were small, irregular and clearly related to splits of myelin sheaths. Others were expanded, with a more regular outline, contained granular and membranous material and had a thin membranous limiting membrane. At times lamellae were recognized in these membranous borders, consistent with attenuated myelin."

The report further stated that "medullary vacuoles derived from myelin splits, consistent with those described above, were present in a test article dosed animal from the same period (Group III). These demonstrated a series of evolutionary events as progressively larger vacuoles were examined. There was attenuation and multifocal interruption of the myelin limiting membrane. The membranous content of the vacuoles progressively increased, with protrusions from the altered limiting membrane being a source for them. These appeared to give rise to the frequently observed large vacuoles with prominent membranous material, but without a limiting membrane. Some of these membranes appeared to arise from adjacent compressed neuropil. However, significant degeneration of adjacent fibers was absent."

**Table I.3.4** Blocks thin sectioned for ultrastructural examination by transmission electron microscopy

	TREATMENT			Rat Number	Block Number	Tissue ID
	Group	Duration	Total Days			
Test article	Group I	PND 4-25	22	103	4E	cerebellum
					5E	medulla oblongata
				104	4E	cerebellum
				105*	5E	medulla oblongata
	Group II	PND 4-46	43	204	4E	cerebellum
				205	5E	medulla oblongata
	Group III	PND 4-65	62	304	4E	cerebellum
				305	5E	medulla oblongata
Group IV	PND 12-26	15	904	5E**	medulla oblongata	
Vehicle	Group V	PND 4-25	22	402	4E	cerebellum
				403	5E	medulla oblongata
	Group VI	PND 4-46	43	503	4E	cerebellum
					5E	medulla oblongata
	Group VII	PND 4-65	62	601	5E	medulla oblongata
				603	4E	cerebellum

\* Fixation was suboptimal as determined by electron microscopy, and limited observations were made from this grid.

\*\* Sample could not be observed due to malfunction of TEM; see Procedural Outline Deviations for details.

c. Conclusions

Administration of vigabatrin (50 mg/kg) to male rats during the neonatal/juvenile period of development (PNDs 4-25, 4-46, 4-65, or 12-26) resulted in increased mortality, clinical signs (including convulsions), decreased BW and brain weights, and brain vacuolation and demyelination/hypomyelination. Ultrastructural examination of vacuoles focused on 2 brain regions (cerebellum and medulla) in a small number of treated rats (1-2/grp). At least some of these vacuoles appeared to originate as splits in the myelin sheaths along

the intraperiod line, and there was progressive expansion of the vacuoles with continuing exposure to vigabatrin by a process thought to be consistent with intramyelinic edema described in the adult rat and dog following chronic administration of vigabatrin (Gibson et al. Toxicol Pathol 18: 225-238, 1990). According to the histopathology report,

“... the expansion of the vacuoles led to attenuation of myelin segments that formed their wall, although lamellae consistent with compressed intraperiod and major dense lines could be determined well into the process. These myelin derived vacuoles had fine granular contents, sometimes associated with membranous material. The latter were derived from the attenuating myelin limiting membrane, or from focal protrusions of adjacent myelin sheaths. Transitional stages between these clearly myelin-derived vacuoles and the larger ones noted below were seen. These had markedly thinned, focally interrupted myelin remnants forming the limiting membrane, and increase in intravacuolar membranous material. With progression, as seen with the longest exposure to the test compound (Group III - PND 4-65 dosing), the vacuoles increased in size, contained prominent membranous material, and often demonstrated loss of a definitive lining membrane. There was compression of adjacent neuropil by the expanding vacuoles, without significant associated neuronal injury. The ultrastructurally determined origin of vacuoles from split myelin sheaths is supported by light microscopic evidence of an association of vacuoles and myelin sheaths in the cerebellum and medulla in animals from Group III (PND 4-65 dosing with test compound).”

A prominent effect on myelination (demyelination, hypomyelination) was also noted in this study. This has been reported previously by Qiao et al. (*Epilepsia* 41:655-665, 2000) who examined the effect of vigabatrin during periods of active myelination in Wistar rats (25 mg/kg sc during PND 12-20 period, 40 mg/kg during PND 21-26). In animals sacrificed on PND 26 there was microvacuolation, oligodendroglial cell death, axonal swelling, and pallor of myelin staining in white matter of the brain.

## II. SUMMARY AND EVALUATION

Juvenile rat studies of vigabatrin (VGB) were conducted, with oral (gavage) administration beginning on postnatal day (PND) 4 and extending for up to 8.5 weeks, in order to characterize the toxicity of VGB in young, developing animals and compare any effects to those seen in adults. These studies employed standard toxicological endpoints as well as specific assessments of neurotoxicity and retinal toxicity.

The data indicate increased sensitivity of juvenile rats to the systemic, retinal, and neurotoxic effects of VGB. Lethality occurred in young rats at a dose of 100 mg/kg given for 2 weeks, but was not seen in adults at <1,000 mg/kg after 2 weeks or <300 mg/kg after 3 and 12 months. Systemic toxicity, as manifested by decreased activity, food consumption, and body weight gain, was only seen at  $\geq 200$  mg/kg in adult rats, but was seen in young rats at doses as low as 30 mg/kg. Convulsions were reported in young rats after 23 days at doses as low as 30 mg/kg (**Table II.1**, from dose range-finding study), but were not reported in adult rats until they had received 200 mg/kg for at least 3 months or 100 mg/kg for at least 1 year. Young rats given 50 or 100 mg/kg for 2 weeks had retinal lesions characteristic in distribution and morphology to those described in adult rats given 300 mg/kg po for 13 weeks or 250 mg/kg ip for 45 days (**Table II.2**, dose range-finding study). Young rats were also more sensitive to the development of vacuolar changes in the brain following VGB administration. It is not clear whether these studies identified a unique neurotoxic effect in young animals or only an enhanced susceptibility to and different location of the characteristic brain lesion seen in adult animals. This is particularly relevant in light of the recent reports of brain MRI abnormalities associated with VGB exposure in patients treated for infantile spasms (IS).

Adult rats have been shown to consistently develop intramyelinic edema (IME) in the white matter (cerebellum, reticular formation, optic tracts) within 3 months of oral dosing with 300 mg/kg/day VGB, within 6 months with 100 mg/kg/day, and within 12 months with 30 mg/kg/day. This edema appears on light microscopy as vacuoles in the white matter and by electron microscopy has been shown to represent splitting of the myelin sheath at the intraperiod line, the membrane formed from fusion of the surface membranes of encircling oligodendrocyte cytoplasm. There seemed to be a plateau effect in rats, with lesions not becoming more severe or more advanced with continued dosing and not progressing to segmental demyelination, as noted with other agents (triethyltin, hexachlorophene, isoniazid) causing intramyelinic edema. While the vacuolar changes subsided with withdrawal of VGB treatment, rats continued to have swollen axons and mineralized bodies in the cerebellum, indicative of increased myelin turnover, axonal damage, and astrocytic gliosis. Rats also developed convulsions after 3 months at 200 mg/kg/day or 1 year at 100 mg/kg/day. The relationship of convulsions to intramyelinic edema is unclear.

In Study # OV-1007, in which neonatal/juvenile rats were treated with daily doses of 5, 15 or 50 mg/kg/day VGB from PND 4 through 65, light microscopic evaluation of perfusion fixed brain tissues revealed vacuolar changes within the neuropil of various brain regions in rats treated with 50 mg/kg/day (**Tables I.1.11-12** above). Most affected was the gray matter in the central midbrain (tegmentum), substantia nigra, dorsal subiculum, medulla oblongata, hippocampal CA1 region, thalamus, deep cerebellar nuclei, and basal forebrain (listed in the approximate order of frequency of effect). While vacuoles were found in some white matter tracks, such as the medial longitudinal fasciculus and the medial forebrain bundle, most vacuoles were in or near the gray matter. The specific cell type containing these vacuoles could not be verified by light microscopy. Although there was no clear evidence of cell degeneration, cell loss, or gliosis in this study, morphometric or stereologic procedures would have been necessary to quantify cell loss. The vacuolar changes were temporally associated with spasms in high-dose rats. In addition, long-term neuromotor impairment (decreased grip strength and hindlimb splay) was observed at both the mid- and high-dose (in the absence of body weight reduction in females), and an apparent learning deficit was seen at the high dose. Similar neurobehavioral findings have been reported in rodents treated with triethyltin or hexachlorophene. Although neuropil vacuolation was reduced in

rats necropsied after an approximately 19-week recovery period, it was still evident in several animals. Plasma exposures associated with a 50 mg/kg dose in juvenile rats are lower than those in infants and children given the same oral dose (**Tables I.1.13** and **II.3**). (Sponsor is proposing 150 mg/kg as MRHD.)

A pathology working group (PWG) assembled to review these neuropathological findings (Study # 794001) agreed with the principal study neuropathologist on the regional location of the vacuolar changes in both gray and white matter of the brain of neonatal/juvenile rats given 50 mg/kg/day, the highest dose. Similar lesions were not confirmed at lower doses. While the panel could not determine by light microscopy which specific cells were vacuolated, they concluded that neuronal cell bodies, blood vessel endothelium, and perivascular astrocytic end processes were not affected; ultrastructural investigations would be necessary to confirm the cell type vulnerable to this vacuolation. Some of the suggested possibilities for the potential location of the vacuoles included preterminal or perisynaptic vesicles, and terminals of GABAergic axons, as in some areas the vacuoles appear close to nerve cell bodies. The possibility that vacuoles are associated with multiple targets (e.g., axons or dendrites, microglia) could not be excluded. These experts did not find morphologic evidence of neuronal cell death, hypocellularity, or reactive gliosis, but noted that the methods were not adequate to rule out cell loss. However, the panel stated that the morphologic appearance of the vacuoles was not characteristic of IME, which has been confined to white matter tracts in adult rats given VGB (“Vacuolar changes in white matter and gray matter are morphologically similar via light microscopy, and have a morphologic appearance that is not characteristic of intramyelinic edema”).

Based on the recommendation of the expert panel, a neurohistopathology study was performed in juvenile rats using both light microscopy and ultrastructural examination to characterize the microvacuolation associated with VGB treatment. In this study (Study # OVNC-9004), VGB (50 mg/kg) or vehicle were administered by oral gavage to rats starting on PND 4 for varying periods up to 8.5 weeks. The single dose of 50 mg/kg was selected on the basis of the previous study in which it produced vacuolar lesions. Light microscopic examination of H&E stained sections confirmed the previous finding of neuropil vacuoles in the brains of treated rats. In rats dosed on PND 4-25 there was also marked involvement of the cerebellar white matter, which had not been reported in the previous study, with vacuoles extending into the cerebellar nuclei. In addition, lesions were prominent in the medulla in this group, with lesser involvement seen in midbrain regions. With longer dosing (PND 4-46 and PND 4-65), medullary and cerebellar lesions persisted, although the white matter vacuolation was diminished, and there was also involvement of more rostral regions such as the midbrain, thalamus and basal forebrain. When toluidine blue and safranin stained sections of cerebellum and medulla were examined by light microscope, hypomyelination, demyelination and gliopathy were noted, and vacuoles were thought to be related to this myelinopathy. Vacuoles were found in the neuropil of nuclei and in adjacent myelinated fiber regions of the cerebellum, the medullary nuclei, and midbrain (where examined) of treated rats. The vacuoles were said to often be found in contact with myelin sheaths, glia, and neurons. Direct microscopic counts of cerebellar and medullary nuclear regions showed that a large proportion of the vacuoles had demonstrable direct contact with myelin sheaths. Electron microscopic examination of sections of the cerebellum and medulla from rats in the PND 4-25 treatment group showed vacuoles that reportedly appeared to form from splits in the myelin sheath along the intraperiod line, consistent with descriptions of IME in adult rats and dogs. Examination of the other groups indicated a progressive expansion of vacuoles with continued exposure to VGB resulting in compression of the adjacent neuropil. The ultrastructurally determined origin of vacuoles from split myelin sheaths combined with the light microscopic evidence of an association of vacuoles and myelin sheaths in the cerebellum and medulla in animals treated from PND 4-65 (as in the previous study) led to the conclusion that the neuropil vacuoles observed in juvenile rats are consistent with IME pathology previously described in the white matter of adult animals. A prominent effect on myelination (demyelination, hypomyelination) was also noted in this study, however, which is clearly a unique effect seen with VGB administration during periods of active myelination.

The discrepancies between the neurohistopathology results and conclusions in the two juvenile rat studies have not been satisfactorily resolved. For example, it is not clear whether the vacuoles examined by EM were the only types present or if they occurred in addition to those described by the PWG as having a morphologic appearance not characteristic of IME; the ultrastructural study focused on only two affected brain areas, cerebellum and medulla, so the vacuoles examined may not have been representative of those in other areas. However, even if the pathogenesis is the same for neuropil vacuolation in juvenile rats and IME in adults, as concluded by the sponsor, the increased sensitivity of young animals and the different location of the vacuoles (ie, gray vs white matter) could be clinically significant. Discussion of the juvenile rat findings and suggestions for additional investigation are found in the memo from the FDA neuropathology expert consultant, Larry Schmued.

**Table II.1** Incidence of tonic convulsions in juvenile rats treated with VGB

Gender	Dose per Period (mg/kg/day) <sup>1</sup>	Incidence (%)
Males	30-30	2/9 (22%)
	30-50	6/9 (67%)
	30-100	7/9 (78%)
Females	30-30	1/9 (11%)
	30-50	4/9 (44%)
	30-100	9/9 (100%)

<sup>1</sup> 30 mg/kg/day given to all rats for first 10 days, then 30, 50 or 100 mg/kg/day given on Study Days 11-28

**Table II.2** Incidence of retinal changes in VGB-treated juvenile rats

Dose (mg/kg/day)	0		30		50		100	
	M	F	M	F	M	F	M	F
N	8	8	6	6	6	6	5	6
Retinal changes	0	0	0	0	3	2	4	4

**Table II.3** Plasma VGB exposures in pediatric subjects given 50 mg/kg oral dose

Treated Subjects	C <sub>max</sub> (µg/mL)	AUC (µg·h/mL)
Human Infants (N=6) <sup>1</sup>		
Active S(+) VGB	13.90 ± 4.53	90.90 ± 27.90
Inactive R(-) VGB	21.00 ± 6.60	106.00 ± 28.50
Total VGB	34.90	196.90
Human Children (N=6) <sup>1</sup>		
Active S(+) VGB	23.80 ± 12.20	117.00 ± 26.00
Inactive R(-) VGB	41.30 ± 13.90	147.00 ± 34.00
Total VGB	65.10	264.00

### **III. RECOMMENDATIONS**

Because the juvenile rat studies of vigabatrin indicate a risk for significant neurotoxicity at clinically relevant exposures, the application should not be approved from a pharmacology/toxicology standpoint.

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/s/

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Edward Fisher  
11/22/2008 11:36:19 AM  
PHARMACOLOGIST

Lois Freed  
11/22/2008 12:23:38 PM  
PHARMACOLOGIST

**MEMORANDUM**  
**DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
**FOOD AND DRUG ADMINISTRATION**  
**CENTER FOR DRUG EVALUATION AND RESEARCH**  
**CONTROLLED SUBSTANCE STAFF**

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**Date:** August 1, 2008

**To:** Russell Katz, M.D., Director  
Division of Neurology Products

**Through:** Michael Klein, Ph.D., Director  
Controlled Substance Staff

**From:** Katherine Bonson, Ph.D., Pharmacologist  
Controlled Substance Staff

**Subject:** Evaluation of Abuse Potential of Vigabatrin  
Labeling Recommendations  
NDAs 22-006 and 20-427  
Indications: Treatment of Infantile Spasms (100-150 mg/day)  
and Adult Refractory Complex Partial Seizures (1 gm/day)  
Sponsor: Ovation Pharmaceuticals

**Summary:**

This CSS consult evaluates the abuse potential of vigabatrin (Sabril), as requested by the Division of Neurology Products, to help determine appropriate labeling of the drug and to assess whether the drug should be recommended for scheduling. Vigabatrin is proposed for the treatment of infantile spasms (IS) at an oral daily dose of 100-150 mg (NDA 22-006) and for adult refractory complex partial seizures (CPS) at an oral daily dose of 1 gm (NDA 20-427). CSS has evaluated the abuse-related data submitted in the NDA and concludes that, at this time, the data do not show that vigabatrin has abuse potential.

**Background:**

Vigabatrin is a selective irreversible inhibitor of gamma-amino-butyric-acid (GABA) transaminase, the enzyme responsible for metabolism of the neurotransmitter GABA. It is a racemate that is structurally similar to the neurotransmitter GABA and has no active metabolites. Serious safety concerns exist with vigabatrin that are related to visual field constriction, reduced visual acuity and abnormal color vision. These vigabatrin-induced visual changes appear to be irreversible and progressive with continued drug treatment, but symptoms do not appear to worsen upon drug discontinuation.

Vigabatrin is approved for use (in tablet and oral solution preparations) in 60 countries as a noncontrolled drug for the treatment of epilepsy-related conditions. In the present

NDA, the Sponsor proposes that vigabatrin not be controlled under the Controlled Substances Act, citing “its low intrinsic abuse and dependence potential”.

**Conclusions:**

For the abuse potential assessment of vigabatrin, CSS relied upon published preclinical abuse-related studies, preclinical studies conducted by the Sponsor as well as clinical and epidemiological AEs that were submitted in the NDA.

Although it is impossible to determine conclusively from the information submitted whether vigabatrin produces abuse potential, the preponderance of evidence at this time does not suggest that vigabatrin produces either abuse potential or physical dependence. These conclusions are based on the following materials:

1. *Seventy-three papers published in the scientific and medical literature submitted by the Sponsor that report on preclinical studies with vigabatrin.*

After reviewing the published material, CSS concludes that:

- a. Of the 73 published papers that were submitted, only 14 of them were directly related to the assessment of the abuse potential of vigabatrin. As published studies, none of them contain full protocols and primary data. Most critically, no information is provided in these papers regarding the plasma levels of vigabatrin that are produced by the animal doses used in the studies and how they relate to the plasma levels of vigabatrin produced in humans following administration of proposed therapeutic doses. Thus, it is not possible for CSS to adequately review the published studies for scientific validity and merit.
- b. The published studies that address abuse potential appear to show that vigabatrin increases GABA levels through inhibition of GABA transaminase, reduces dopamine levels, does not produce self-administration, does not produce conditioned place preference, does not generalize in drug discrimination to the GABAergic drugs pentobarbital or muscimol, reduces performance in the rotorod test of motor behavior, does not produce physical dependence and does not block the signs of withdrawal observed following discontinuation of chronic morphine or diazepam.
- c. Although it is not possible to make definitive conclusions from the published preclinical studies, it appears unlikely from these preclinical studies that vigabatrin has abuse potential.

2. *Two biochemical studies conducted by a previous Sponsor of vigabatrin.*

The data in these studies show that a) the mechanism of action of vigabatrin is inhibition of GABA transaminase, the enzyme that metabolizes the neurotransmitter GABA, and b) vigabatrin does not have direct binding activity at any receptors known to be associated with abuse potential (e.g., GABA, dopamine, serotonin, opioid, cannabinoid, sigma, monoamine transporters).

3. *A functional observational battery conducted during toxicity testing.*

In this test, vigabatrin does not produce overt animal behaviors that are indicative of abuse potential.

4. *The abuse-related adverse events (AE) profile in Phase 2/3 placebo-controlled clinical studies with vigabatrin.*

In patients with adult refractory CPS, vigabatrin produced a low incidence of the abuse-related AEs “feeling abnormal” (0.8%) and “feeling drunk” (0.2%) compared to placebo (0%), there was a similar incidence of “hallucination” between patients treated with vigabatrin and placebo (1.5% vs. 1.2%), and the rate of “euphoria” was greater in the placebo group than in the vigabatrin group (1.0% vs. 0.6%). In the infantile population with IS, there was a similar low incidence of abuse-related AEs following vigabatrin treatment (1.4% rate of “abnormal behavior” from vigabatrin vs. 0% placebo). In the patients treated with vigabatrin for any other psychiatric indication, there was also a low incidence of euphoria compared to placebo (0.4% vs. 0.1%), “feeling abnormal” (2.3% vs. 0.9%), “abnormal behavior” (1.4% vs. 0%), “thinking abnormal” (0.6% vs. 0.3%), “feeling drunk” (0.4% vs. 0%), “hallucination” (0.4% vs. 0.1%).

5. *AEs observed in Phase 2/3 placebo-controlled clinical studies that may suggest vigabatrin has similarity to CNS depressants.*

In IS patients, the incidence of “somnolence” following vigabatrin was 15.9% compared to 5.0% from placebo and “sedation” was 11.9% compared to 0% from placebo. In patients treated with vigabatrin for any other psychiatric indication, the incidence of “somnolence” was 18.4% compared to 8.5% from placebo and “sedation” was 2.8% compared to 0.1% from placebo. However, these sedative-like AEs are contrasted with a high incidence of “insomnia” in IS patients (7.8% vs. 0% from placebo) and in patients treated with vigabatrin for any other psychiatric indication (4.9% vs. 3.6% from placebo). Additionally, the incidence of “somnolence” in CPS patients after vigabatrin treatment (6.7%) was lower than that observed after placebo treatment (8.0%).

6. *Epidemiological databases that monitor European countries where vigabatrin is marketed.*

There were no mentions of AEs related to abuse potential in any of these epidemiological databases.

7. *Case reports in the scientific and medical literature regarding abuse-related signs following vigabatrin administration.*

There are no papers or other citations that suggest vigabatrin is associated with abuse, misuse, dependence, diversion or overdose.

8. *Studies that evaluate the development of physical dependence following chronic vigabatrin administration.*

An animal physical dependence study conducted during toxicity testing does not show that vigabatrin produces a withdrawal syndrome following drug discontinuation.

## **Recommendations:**

The label text proposed by the Sponsor for Section 9.0 (Drug Abuse and Dependence) should be revised and expanded. CSS proposes the following text wording:

### 9.0 Drug Abuse and Dependence

#### 9.1 Controlled Substance Class

Vigabatrin is not a controlled substance.

#### 9.2 Abuse

Vigabatrin did not produce adverse events or overt behaviors associated with abuse when administered to humans or animals. It is not possible to predict the extent to which a CNS active drug will be misused, diverted, and/or abused once marketed. Consequently, physicians should carefully evaluate patients for history of drug abuse and follow such patients closely, observing them for signs of misuse or abuse of vigabatrin (e.g., incrementation of dose, drug-seeking behavior).

#### 9.3 Dependence

Following chronic administration of vigabatrin to animals, there were no apparent withdrawal signs upon drug discontinuation. However, as with all AEDs, vigabatrin should be withdrawn gradually to minimize increased seizure frequency [see Section 5.2 Withdrawal of Antiepileptic Drugs (AEDs)].

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## **Discussion of Submitted Material Related to the Abuse Potential Assessment of Vigabatrin**

This section provides summaries of the abuse potential-related material on vigabatrin submitted in NDAs 22-006 and 20-447, followed by CSS discussion of the materials.

### **I. Summary of Preclinical Information Related to Abuse Potential of Vigabatrin**

#### **A. Published Preclinical Behavioral Studies with Vigabatrin**

##### *Summary of Submitted Information*

The Sponsor acknowledged in an April 10, 2008 letter to the Division of Neurology Products that “the nonclinical studies discussed in [the Abuse Potential section of the NDA] are all literature based and as such, primary data is (*sic*) not available to Ovation.” In Module 4.0 (Nonclinical Study Reports: Nonclinical Pharmacology and Toxicology)

of the NDA, the Sponsor submitted 73 papers published in the scientific and medical literature involving preclinical behavioral studies with vigabatrin. Of these, 14 had information regarding administration of vigabatrin alone that is relevant to the evaluation of its abuse potential. Brief summaries of the 14 abuse-related preclinical studies are presented below.

#### *Effect on GABA Levels*

##### Daune and Seiler (Neurochem. Res. 13:69, 1988)

Vigabatrin produces elevations of GABA. Typically GABA acts as a competitive inhibitor of L-ornithine:2-oxoacid aminotransferase (OAT), which induces a concentration-dependent increase in L-ornithine. However, vigabatrin does not act as an inactivator or an inhibitor of OAT, despite its ability to increase GABA.

##### Halonen et al. (J. Neurochem., 55:1870, 1990)

Vigabatrin (1000 mg/kg) produced elevated free GABA in rat cerebrospinal fluid. This effect peaked at 24 hours after drug administration.

##### Loscher (J. Neurochem. 36:1521, 1981)

Drugs that inhibit GABA transaminase, including vigabatrin, produced increases in whole brain and synaptosomal GABA concentrations and activated glutamate decarboxylase (GAD), the enzyme responsible for synthesizing GABA from glutamate.

##### Loscher and Frey (Eur. J. Pharmacol. 143:335, 1987)

In gerbils, vigabatrin produced an increase in regional levels of GABA compared to non-treated animals at any of the dose regimens administered (50 or 100 mg/kg/day or 100 mg/kg/every second or third day)

##### Perry et al. (J. Neurochem. 32:1641, 1979)

In rats, vigabatrin (100 mg/kg/day for 11 days) produced a 150% increase in brain GABA content and a 26% reduction in GABA transaminase activity, as well as a 22% reduction in GAD activity.

##### Seiler et al. (Neurochem Int. 10:391, 1987)

In mice, vigabatrin produces a dose-dependent increase in GABA levels in the brain, at doses ranging from 250-1500 mg/kg. However, it did not alter the activity of OAT.

#### *Effect on Dopamine*

##### Dewey et al. (J. Neurosci., 12:373, 1992)

Vigabatrin (300 mg/kg) induced an increase in [11C] raclopride binding in the striatum of baboons, indicating a reduction in dopamine release. Since vigabatrin is known to cause an increase in GABA, this indicates that GABA induces the observed reduction in dopamine release. The benzodiazepine lorazepam (0.75-1.25 mg/animal)

also produced an increase in [<sup>11</sup>C] raclopride binding, but this is thought to occur through direct activation of the benzodiazepine site of the GABA receptor.

### *Self-Administration*

#### Takeda and Yanagita (Drug Res. 47:1087, 1997)

Monkeys were trained to self-administer pentobarbital (1 mg/kg/infusion). Forced administration of vigabatrin (16 mg/kg/infusion) was initiated every 3 hours until self-administration was initiated. Vigabatrin was not self-administered at rates above saline at doses of 16, 32 and 64 mg/kg/infusion.

### *Conditioned Place Preference*

#### Dewey et al. (Synapse, 30:119, 1998)

Vigabatrin (150 and 300 mg/kg) does not produce a conditioned place preference (CPP) when paired with food reward. In contrast, cocaine (20 mg/kg) produces CPP when it serves as the reward.

### *Drug Discrimination*

#### Grech and Balster (Pharmacol. Biochem. Behav., 47:5, 1994)

In rats trained to discriminate pentobarbital, vigabatrin (10-1000 mg/kg) did not generalize to the pentobarbital cue. These responses at all doses were statistically indistinguishable from saline (~0% generalization at each dose). In contrast, other drugs that act through the GABA system (pentobarbital, midazolam, valproic acid) produced full generalization (≥80%) to the pentobarbital cue.

#### Jones and Balster (Pharmacol. Biochem., Behav. 59:319, 1998)

In rats trained to discriminate the GABA-A agonist muscimol, vigabatrin produced a maximum of 46% generalization to the muscimol cue. This result was interpreted as showing that the ability of vigabatrin to increase GABA levels is insufficient to produce the effects of a GABA agonist. In contrast, gaboxadol (a drug with structural similarity to muscimol) produced full generalization (≥80%) to the muscimol cue.

### *Effect on Motor Behavior*

#### Buckett et al. (Neuropharmacology 19:715, 1980)

Vigabatrin (800 mg/kg) did not produce a significant reduction in the ability of mice to perform in the rotorod test. In contrast, diazepam (1 mg/kg) produced a significant (79%) reduction in performance in the rotorod test.

Dewey et al. (Synapse, 30:119, 1998)

Vigabatrin (150 and 300 mg/kg) did not produce any alterations in locomotor behavior compared to saline.

### *Physical Dependence*

Takeda and Yanagita (Drug Res. 47:1087, 1997)

Monkeys were administered morphine (12 mg/kg/day) for 8 weeks until they were physically dependent. When animals were discontinued from the drug, vigabatrin (256 and 1000 mg/kg) was not able to suppress signs of opioid withdrawal, but codeine (8 and 16 mg/kg) was able to suppress withdrawal signs. This demonstrates that vigabatrin does not have opioid activity in animals that are opioid-dependent.

In a separate study, monkeys were administered barbital (75 mg/kg/day) for 8 weeks until they were physically dependent. Vigabatrin (up to 1000 mg/kg) was unable to suppress withdrawal signs, while diazepam (4 mg/kg) was able to suppress withdrawal signs. This demonstrates the vigabatrin does not have barbiturate activity in animals that are barbiturate-dependent.

In a separate study, rats received vigabatrin in food at a “low” dose (0.25 mg/kg/day for 2 weeks, followed by 0.5 mg/kg/day for 2 weeks) and a “high” dose (1 mg/kg for 2 weeks, followed by 2 mg/kg for 2 weeks). A positive control group was fed a diet that was admixed with diazepam (1 week each at 2, 4, 6 and 8 mg/kg/day) while a control group received food without drug. During drug administration, there was a reduction in food intake and reduced body weight in rats treated with vigabatrin but not in those rats treated with diazepam. Vigabatrin-treated rats also developed hyperreactivity to external stimuli, while diazepam-treated rats developed hyporeactivity, decreased locomotion and muscle relaxation. When the drugs were abruptly discontinued after 4 weeks, the vigabatrin-treated rats showed no behavioral abnormalities compared to control rats. In contrast, the diazepam-treated rats showed hyperreactivity during the discontinuation period. Thus, vigabatrin does not appear to induce physical dependence at the doses studied while diazepam produced withdrawal-like behaviors.

### *CSS Discussion*

Based on the information in these preclinical study reports, it does not appear at this time that vigabatrin has abuse potential. However, the limitations in reviewing these papers are that primary data and full protocols were not provided. More significantly, no information was provided regarding the plasma levels of vigabatrin produced by the doses used in the animal behavioral studies and how they relate to the plasma levels of vigabatrin produced by proposed human therapeutic doses. Thus, it is not possible to conclude that a lack of a signal in any of these tests is conclusively predictive of a lack of abuse potential of vigabatrin in humans.

## **B. Biochemical Studies Conducted by Sponsor**

### *Studies*

Two biochemical studies were conducted with vigabatrin by a former Sponsor (Marion Merrell Dow):

- \* A receptor binding study in which vigabatrin was tested at 38 receptor sites “including the primary CNS targets associated with known drugs of abuse”. These include the following sites associated abuse potential: dopamine D1 and D2, GABA-A, benzodiazepine, serotonin 5HT-1 and 5HT-2, N-methyl-D-aspartate (NMDA), phencyclidine (PCP), sigma, opioids (mu, kappa, delta), and monoamine transporters (dopamine, norepinephrine, serotonin).
- \* A study on the ability of vigabatrin to inhibit GABA transaminase, the enzyme responsible for metabolism of the neurotransmitter GABA.

### *Results*

The results of the receptor binding study show that there was no significant inhibitory activity at any of the sites tested at concentrations up to 100 micromolar.

In the study of GABA-transaminase, vigabatrin was shown to increase the amount and duration of GABA in the synapse. It also produced a time-dependent decrease in GABA transaminase activity, suggesting that vigabatrin was acting as an irreversible inhibitor.

### *CSS Discussion*

These results suggest that vigabatrin is a GABA transaminase inhibitor that does not have significant activity at any receptor known to be associated with abuse potential.

## **C. Preclinical Abuse-Related Behavioral Studies Conducted by Sponsor**

### ***Functional Observational Battery***

#### *Study Design*

In a rat toxicity study conducted by the Sponsor with vigabatrin, a functional observational battery was included.

#### *Results*

Compared to the placebo group, there was a significant ( $p < 0.05$  to  $0.01$ ) reduction in the gripping ability of rats (at 50 mg/kg), in landing foot splay (15 and 50 mg/kg). No other

behaviors resulting from vigabatrin administration were shown to be significantly different from those resulting from placebo treatment.

#### *CSS Discussion*

The alterations in muscle tone are not indicative of abuse potential in the absence of other behaviors associated with abuse potential.

### ***Animal Physical Dependence Studies***

#### *Study Design*

The Sponsor conducted physical dependence studies in both rats and dogs. In the rat study, animals received 30 to 300 mg/kg/day of vigabatrin for 6 or 12 months. In the first dog study, vigabatrin was administered at a dose of 300 mg/kg/day for 12 weeks and then discontinued. A second dog study was conducted in which animals received 50, 100 or 200 mg/kg/day for 7 or 12 months prior to drug discontinuation.

#### *Results*

Although convulsions were observed during treatment of rats with the higher doses of vigabatrin, no withdrawal signs were observed upon drug discontinuation.

In the first dog study, some of the animals exhibited weight loss so severe that they received euthanasia. No other signs of withdrawal were observed. During the second dog study, withdrawal signs were not observed in any animal.

#### *CSS Discussion*

Since no information was provided regarding the plasma levels produced in animals by the drug doses used and their relation to human plasma levels after proposed therapeutic doses, it is difficult to know how to interpret the severe anorexia observed in rats after the 300 mg/kg dose. However, since no withdrawal-associated signs were observed in rats or dogs after vigabatrin discontinuation, it does not appear that vigabatrin produces physical dependence.

## **II. Summary of Data Related to Abuse Potential of Vigabatrin in Humans**

### ***Human Abuse Potential Study***

A human abuse potential study was not conducted with vigabatrin because of safety issues related to ophthalmological toxicities, including visual field constriction, reduced visual acuity and abnormal color vision. Typically, abuse potential studies are conducted in individuals who are otherwise healthy except for their history of drug abuse and at

doses that are greater than therapeutic doses. Given that vigabatrin-induced visual changes appear to be irreversible and progressive with continued drug treatment, it was not considered ethical to expose non-patient subjects to vigabatrin in order to conduct a human abuse potential study.

### ***Adverse Events in Clinical Efficacy Studies***

#### *Evaluation of Adverse Events*

Clinical studies with vigabatrin have been conducted since 1994 under several Sponsors (Marion Merrill Dow, Hoechst Marion Roussel and Ovation Pharmaceuticals). According to the Abuse Potential section of the NDA, AEs in all clinical studies were re-coded to current MedDRA coding dictionaries. Data from a total of 6599 patients treated with vigabatrin (500 mg/day to 10 grams/day) and 1363 patients treated with placebo are represented in the database. Although patients with CPS and IS make up the majority of those studied in these clinical trials, other indications were also investigated, including other forms of epilepsy, tardive dyskinesia, “psychiatric disorder”, ataxia and tremor, Huntington’s Disease, spasticity, Parkinson’s Disease, dystonia and torticollis, (b) (4) . The analysis of AEs conducted on behalf of the Sponsor eliminated 47 studies because “vigabatrin exposure was short” with “exposure of less than 15 days”.

#### *Results*

In the CPS database, 7.2% of vigabatrin-treated patients had “euphoria-related” AEs compared to a greater incidence in placebo-treated patients of 8.0%. The majority of these euphoria-related AEs is accounted for by the AE of “dizziness” (5.5% for vigabatrin and 7.4% for placebo). Dizziness under MedDRA can include not only direct dizziness-related AEs, but also the euphoric-like AEs of “giddy” and “giddiness”. However, the incidence of these giddy-related terms was negligible.

The incidence of non-dizziness euphoria-related AEs in the CPS database was 1.7% from vigabatrin and 0.6% from placebo. For those reports where the incidence from vigabatrin was greater than that from placebo, the AEs include “feeling abnormal” (0.8% vs. 0%), “feeling drunk” (0.2% vs. 0%) and “hallucination” (1.5% vs. 1.2%). Of those patients who experienced a hallucination, 0.2% of those treated with vigabatrin had a “severe” incident compared to 0.6% of those treated with placebo. No other AE of interest was reported to be severe.

Notably, the incidence of “euphoric mood” in the CPS database was greater in placebo-treated patients (0.6%) than in patients treated with vigabatrin (0.1%). The incidence of “somnolence” was less in vigabatrin-treated patients (6.7%) than in placebo-treated patients (8.0%).

In the IS database, the only “euphoria-related” AE was “agitation”, reported at an incidence of 3.7% in vigabatrin-treated patients and 0% in placebo-treated patients. Other psychiatric AEs included “sedation” (11.9% vs. 0% from placebo), “somnolence” (15.9% vs. 5.0% from placebo), “insomnia” (7.8% vs. 0% from placebo) and “abnormal behavior” (1.4% vs. 0% from placebo).

In the database of patients treated for all other indications, “euphoria-related” AEs from vigabatrin reported at an incidence greater than placebo included (respectively): “euphoric mood” (0.4% vs. 0.1%), “feeling abnormal” (2.3% vs. 0.9%), “feeling drunk” (0.4% vs. 0%), “hallucination” (0.4% vs. 0.1%) and “thinking abnormal” (0.6% vs. 0.3%). The incidence of other psychiatric AEs from vigabatrin (compared to placebo) included “somnolence” (18.4% vs. 8.5%), “memory impairment” (6.1% vs. 1.5%), “insomnia” (4.9% vs. 3.6%), “confusional state” (4.1% vs. 0.6%), “anxiety” (3.2% vs. 1.7%), “sedation” (2.8% vs. 0.1%), and “abnormal behavior” (1.9% vs. 0.8%).

### *CSS Discussion*

The psychiatric and neurological AEs reported during vigabatrin administration do not suggest an abuse potential profile. There was a very low incidence of “euphoria-related” AEs in both the CPS and IS patient databases. Other psychiatric AEs observed are not indicative of abuse potential in the absence of “euphoria-related” AEs.

### ***Adverse Events Reported in Epidemiological and Other Databases***

#### *Evaluation of Vigabatrin Reports*

A Sponsor-commissioned study evaluated reports of vigabatrin related to abuse potential in epidemiological databases and case report sites. These sites included:

- \* Epidemiological databases in Australia, Canada, the European Union, Norway, France and the Netherlands
- \* Eighteen Periodic Safety Update Reports (PSURs) that were submitted by prior Sponsors for the years 1989 to 2006, representing (b) (4) total patients exposed to vigabatrin
- \* The WHO Collaborating Centre for International Drug Monitoring in Uppsala, Sweden that reports on spontaneous reports of adverse drug reactions related to vigabatrin abuse
- \* A search of six Internet databases where drug users are allowed to report on their experiences with drugs to identify vigabatrin mentions
- \* A PubMed search for terms related to drug abuse (including “abuse”, “misuse”,

“dependence”, “diversion”, “overdose” and “suicide”) in conjunction with either the term “vigabatrin” or its trade name “Sabril”.

### *Results*

According to the Sponsor-commissioned report:

- \* There were no reports of vigabatrin related to abuse potential found in the epidemiological databases from Australia, Canada, the European Union, Norway, France and the Netherlands.
- \* The analysis of the 18 PSURs showed that “scant detail was available” to evaluate the euphoria-related AEs that appear in this database. Although there were 79 reports of “hallucination”, all of the individuals in these case reports were taking vigabatrin in combination with other drugs. The narrative for this section suggests that the PSUR reports of hallucination may represent the same individuals as those reported in the WHO database.
- \* In the WHO database, there were 3513 AE reports for vigabatrin for the years 1987 to 2006. Three of these were for “euphoria”, 1 was for “illusion”, 8 were for “thinking abnormal”, 31 were for “dizziness” and 57 were for “hallucination”. Of the hallucinations, 14 were reported in 1992 and 16 were reported in 1994, with no more than 2 reported for any year since 1996. Other psychiatric AEs observed include 122 for “psychosis” and 80 for “somnolence”. As with hallucination, few reports of psychosis and somnolence are reported since 1996. However, these AEs were not systematically collected or reported.
- \* In the Internet databases, reports in which vigabatrin was mentioned were identified, but the majority of these discussed the pharmacology of vigabatrin or its potential use in the treatment of drug abuse. Ten mentions involved reports of vigabatrin use or its alleged positive beneficial effects, but eight of these were from the same individual. Of these reports, all of them discussed other GABAergic drugs, including non-scheduled drugs and drugs that have no known abuse potential (such as tiagabine, gabapentin, and baclofen).
- \* In the PubMed search, numerous papers are identified that report on the preclinical and clinical investigation of vigabatrin for the treatment of various forms of drug abuse (e.g., opioids, stimulants, etc). However, there are no papers or other citations responsive to these term combinations that directly relate to the abuse potential of vigabatrin itself.

### *CSS Discussion*

Given the brevity of the reports submitted in the Sponsor-commissioned report, it is not possible for CSS to independently assess whether the epidemiological databases

(reflecting European experience, PSURs, and WHO data) show an abuse signal in relation to vigabatrin.

CSS is also not able to verify the information from the Internet databases, since this information is inherently transient based on spontaneously-submitted reports from drug users.

However, CSS was able to conduct an independent PubMed search that verified a lack of published papers in the scientific and medical literature reporting on the abuse potential or dependence of vigabatrin. CSS additionally identified 4 papers reporting on “psychotic”-like behavioral responses following administration of vigabatrin to epilepsy patients, an AE which may be indicative of hallucinogen-like effects. However, these reports are difficult to interpret in the absence of a similar signal in the clinical study database and in the absence of primary data or full case reports on the patients.

Thus, from the information submitted, it does not appear that vigabatrin produces a strong signal in epidemiological databases that is indicative of abuse potential.

#### ***Clinical Assessment of Physical Dependence***

A clinical study to assess the development of physical dependence following administration of vigabatrin was not conducted. Thus, there is no information regarding whether vigabatrin produces physical dependence or withdrawal in humans.

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