CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022345Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

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

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

Date: November 30, 2010

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

Subject: NDA 22-345, Potiga (ezogabine; retigabine) Tablets, dated October 30, 2009

NDA 22-345 has been submitted by Valeant Pharmaceuticals to support marketing of Potiga (ezogabine), a neuronal potassium channel opener, as adjunctive treatment for patients 18 years of age or older with partial onset seizures with or without secondary generalization. A full battery of nonclinical studies was submitted to the NDA, and reviewed by Dr. Fisher (*Pharmacology/Toxicology Review and Evaluation N22-345, Ed Fisher, Ph.D., 11/30/2010*). Based on his review, Dr. Fisher has recommended that the application not be approved. The basis for this recommendation is the presence of a genotoxic impurity, (b) (4) at a level in the drug product that would exceed, at all proposed therapeutic doses, the acceptable daily dose (b) (4) for a genotoxic impurity.

A number of reverse bacterial mutation (Ames) assays were conducted on the drug substance. Positive results in initial assays led an investigation into the presence of potential impurities. The three of greatest concern (based on structural alert and/or *in vitro* Ames assay data) were (b) (4). The sponsor was able to lower the specification limit of (b) (4) to a combined level that would result in a daily intake of (b) (4) however, the sponsor set a specification limit for (b) (4) at NMT (b) (4), based on some indication that a lower limit was not achievable, an individual TTC (Threshold of Toxicological Concern) approach, and a negative *in vivo* combined micronucleus/Comet assay. The nonclinical data were reviewed by Dr. Fisher and found to be inadequate to support the proposed specification limit. In his initial NDA review, Dr. Sapru, the primary CMC reviewer, recommended that "…this NDA…cannot be recommended for approval unless the resolved deficiency regarding the high levels of potentially genotoxic impurity (b) (4) in the active pharmaceutical ingredient (API) is satisfactorily addressed", noting "…the applicant's apparent inability or unwillingness to tighten the acceptance limits for the (b) (4) impurity…" (*NDA 22-345 Quality Review #1, Mohan K. Sapru, Ph.D., 8/30/2010*). However, Dr. Sapru noted that the final Pharmacology/Toxicology recommendation regarding this impurity had not been made at that time.

The nonclinical assessment of (b) (4) has been a difficult one, primarily due to two factors:

- The lack of consensus (both internal and external) on the validity and reliability of the *in vivo* Comet assay and the ability of a negative response in the *in vivo* Comet assay to "de-risk" or minimize concern regarding the clear and reproducible positive response in the *in vitro* Ames assay.
- The apparent lack of effort on the sponsor's part to provide sufficient documentation that a lower specification limit (b) (4) is not achievable.

I will attempt to address the nonclinical issues regarding (b) (4) in this memo.

Nonclinical data on (b) (4) Four Ames assays (in two studies) were conducted on (b) (4) The data from these assays were reviewed in detail by Dr. Fisher. Briefly, (b) (4) was consistently positive in the presence of metabolic activation, producing up to 2-8 fold increases in revertants with TA 98 and/or TA 1537. In the absence of metabolic activation, (b) (4) was less consistently positive; however, in those assay in which (b) (4) was negative, excessive cytotoxicity (at concentrations as low as (b) (4) plate) prevented testing at concentrations demonstrated to be positive (up to 2-3 fold increases in revertants) in other assays. In all but one assay (under one condition), excessive cytotoxicity precluded testing of (b) (4) at concentrations (b) (4); it has been recommended that potentially genotoxic impurities be tested up to (b) (4) in the Ames assay, "as the majority of mutagens (>85%) are Ames positive at. (b) (4) plate or less" (b) (4)). (b) (4) was not tested directly in an *in vitro* cytogenetic assay in mammalian cells, and was negative in an *in vivo* combined micronucleus/Comet assay in male rats. Dr. Fisher notes that this *in vivo* study "…appeared to be adequately conducted…"

Evaluation: Based on review of published literature and internal discussions, Dr. Fisher has concluded that the information currently available do not support dismissing concerns regarding the genotoxic potential of (b) (4) based on the results of the *in vivo* Comet assay. In his discussion of the literature, Dr. Fisher makes the following points:

- Although the Comet assay is widely used to assess the potential for DNA damage (single and double strand breaks and alkali-labile sites), there is "some debate about whether the assay is adequately validated for routine regulatory use". Dr. Fisher cites a recent publication (Dearfield *et al*. *Environ Mole Mutagen*, 2010, online in advance of print) that classifies the *in vivo* Comet as a Category 2 assay, and indicates that limitations of the assay include the possibility that it "May be unable to detect mutagens that do not produce strand breaks or alkali-labile lesions" and "Interlaboratory variability and lack of guidelines." Dr. Fisher cites other published articles that also suggest that more data are needed before the *in vivo* Comet assay is "adequately validated for regulatory use".
- It is unclear that a negative *in vivo* Comet assay can override a clear positive response in the bacterial mutagenicity (Ames) assay. Dr. Fisher notes that, according to Dearfield *et al.* (2010), a positive Ames assay "represents a significant hurdle to overcome…[since]…the predictivity of the Ames test for

rodent carcinogenicity is high" and, according to Witte *et al*. (*Toxicol Sci* 97:21-26, 2007), "positive results from a bacterial test system cannot be overruled by negative results from mammalian systems".

Regarding the *in vivo* Comet assay, there has been much discussion in the literature of the most appropriate approach to following up on positive *in vitro* genotoxicity findings (Dearfield *et al*., 2010; Kirkland D, Speit G *Mutat Res* 654:114-132, 2008; Witte I *et al*., *Toxicol Sci* 97(1):21-26, 2007) and the potential role of the *in vivo* Comet assay in this approach. As Dr. Fisher states, the *in vivo* Comet assay is "widely used to assess the potential for DNA damage" (i.e., single and double strand breaks, alkali-labile sites). However, it has been recognized for a number of years, probably due, at least in part, to the growing use of this assay for chemicals and pharmaceuticals, that additional standardization and validation are needed (Burlinson B *et al*. *Mutat Res* 627:31-35, 2007; Hartmann A, Speit G. In: Dhawan A, Anderson D. Eds, *The Comet Assay in Toxicology* Royal Society of Chemistry, UK, 2009; Kirkland & Speit, 2008; Tice RR *et al*. *Environ Mol Mutagen* 35:206-221, 2000).

A recent presentation to CDER by Dr. Wei Ding of NCTR highlighted how a variety of factors (e.g., choice of sampling times, tissue processing, "small technical changes") can affect the results of the *in vivo* Comet assay, and concluded that "Sensitivity of the assay to various class of compounds is to be determined". Kirkland & Speit (2008) emphasize that "There is a need to check the accuracy and reproducibility of the results so far obtained, as many of the published *in vivo* comet-assay results have all been generated by one research group…"

Brendler-Schwaab *et al*. (Brendler-Schwaab S *et al. Mutagenesis* 20(4):245-254, 2005) point to *in vivo* Comet assay results for ortho-phenylphenol as "An example of a compound that revealed conflicting data"; one research group reported positive results in several organs, whereas a separate group, using the same dose and route of administration, obtained negative results. Brendler-Schwaab *et al*. (2005) note that "In such cases, differences in the comet assay protocol used as well as technical differences in the whole study performance may account for a conflicting comet assay result". Also of interest is a study conducted by (b) (4)) comparing the results of an "extremely potent direct-acting bacterial mutagen" and two metabolites. All three compounds were shown to be potent mutagens in the Ames assay and were positive in the *in vivo* Comet assay; however, one of the three compounds was positive in the *in vivo* Comet assay only in spleen (not in liver, kidney, lung, or bone marrow). (For (b) (4) the *in vivo* Comet assay only evaluated liver.) The authors note that differences in PK/ADME and/or sampling times may have affected the response.

An international validation "exercise", involving the Japanese Center for the Validation of Alternative Methods, the U.S. National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, the Interagency Coordinating Committee on the Validation of Alternative Methods, the European Center for the Validation of Alternative Methods, and the Japanese Environmental Mutagen Society, is

currently ongoing to "…evaluate the ability of the *in vivo* Comet assay to identify genotoxic chemicals as a potential predictor of rodent carcinogenicity" (Hartmann & Speit, 2009).

Therefore, it would seem premature at this time to use a negative *in vivo* Comet assay to dismiss an Ames-positive impurity. As Dr. Fisher noted, according to Witte *et al.* (2007), "…positive results from a bacterial test system cannot be overruled by negative results from mammalian systems". (Witte *et al*. (2007) actually evaluate use of the *in vivo* Comet assay as a follow up to *in vitro* clastogenicity assays, not the Ames assay.) Dearfield *et al*. (2010) also acknowledge that a positive response in the Ames assay is a "significant hurdle to overcome" since the Ames assay has been shown to have high predictivity (60-80%; for rodent carcinogenicity. Benigni *et al*. (Benigni R *et al*. *Expert Opin Drug Metab Toxicol* 6(7):809-819, 2010) conclude that "The Ames test and the structural alerts coding for DNA-reactivity predict efficiently the genotoxic carcinogens".

Another consideration is the available literature and guidance on the evaluation of genotoxic impurities. Although a full literature search (for this and the other issues addressed) was beyond the scope of this memo, it would appear that there is reasonable consensus on the overall strategy for assessing an impurity for genotoxic potential (Dobo KL *et al*. *Regul Toxicol Pharmacol* 44:282-293, 2006; Elder DP, Harvey JS *Organ Proc Res Dev* 14:1037-1045, 2010; EMEA Guideline on the Limits of Genotoxic Impurities 28 June 2006; EMEA Questions and answers on the 'Guideline on the limits of genotoxic impurities' 23 September 2010; Muller L *et al*. *Regul Toxicol Pharmacol* 44:198-211, 2006); Pierson DA *et al*. *Organ Proc Res Dev* 13:285-291, 2009; Robinson DI *Organ Proc Res Dev* 14:946-959, 2010; Snodin DJ *Organ Proc Res Dev* 14:960-976, 2010). Basically, the impurity is evaluated for a structural alert for genotoxicity. If the impurity is positive by SAR, it is to be evaluated in genotoxicity studies, "…typically in a bacterial reverse mutation assay.." (EMEA June 2006). If positive in that assay, it should be determined if there is sufficient evidence for a threshold-related mechanism. In the absence of evidence for a threshold, the level of the impurity should be lowered according to the ALARP ("as low as reasonably practicable") principle (according to the EMEA June 2006 guideline) or to the TTC (staged for the duration of exposure, to 1.5 µg/person/day for chronic use); the TTC may be lower for highly potent genotoxic impurities. (Topics of some debate include the actual TTC level of (b) (4)/person/day and whether or not genotoxic impurities with similar structural alerts need to be lowered to a combined level of (b) (4)/person/day.) Our recommended approach has been consistent with this strategy. (CDER has issued a draft guidance (*Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches Draft Guidance December 2008*) that recommends the same basic strategy, but proposes additional flexibility, e.g., setting specification limits "supported by compound-specific risk assessment…")

In addition to conducting an *in vivo* micronucleus/Comet assay, the sponsor also provided (under IND 53,950, SN616) a rationale for establishing a higher Threshold of Toxicological Concern (TTC) for (b) (4) based on extrapolation of published carcinogenicity data from several structurally similar molecules. Based on review of

those data under the IND, it was concluded that the approach proposed by the sponsor was inadequate to support a higher specification limit. Dr. Fisher discusses the basis for this conclusion in his NDA review. Although discussed in a different context (i.e., potential additive effects of genotoxic impurities), recent publications caution against extrapolating data from structurally related compounds when considering the genotoxic or carcinogenic potential of an untested molecule (Elder DP, Harvey JS *Organ Proc Res Dev* 14:1037-1045, 2010; Snodin DJ *Organ Proc Res Dev* 14:960-976, 2010). In discussing the setting of "logical" specification limits based on toxicity of structurally related genotoxic impurities, Elder & Harvey (2010) state that "…even in…well-documented cases the relative toxicity of the different members of [a] class is often different. The authors note, for example, that simple compounds, such as methyl and ethyl chloride, which have similar Ames-positive findings and are carcinogenic, have "quite distinct" tumor profiles; methyl chloride produces renal tumors in male mice and ethyl chloride produces uterine tumors in female mice. Elder & Harvey (2010) reference Wilkinson *et al*. (Wilkinson CF *et al*. *Regul Toxicol Pharmacol* 31:30-43, 2000), who addressed the difficulty in establishing relative toxicity and concluded (according to Elder & Harvey, 2010) that the process "could not be applied to pesticide regulation in food", which informs the conclusion by Elder & Harvey (2010) that "…if this is the case in the well-studied field of pesticide regulation, will it not also be the case in genotoxic impurity regulation in medicinal products?" Even in cases in which data are available for the compound itself, it has proved challenging to set a higher TTC. According to Robinson (Robinson DI *Organ Proc Res Dev* 14:946-959, 2010), "The experimental investigation of the toxicology of impurities, with a view to determining an acceptable intake specific for each compound, is likely to be a very expensive and time-consuming exercise- hence something which would only be contemplated in extreme circumstances". The author discussed several examples in which the exercise was successful, in order to document the effort needed. Based on the available information and internal discussions, I would agree with Dr. Fisher that the sponsor has not provided sufficient data to justify, based on data from structurally similar compounds, a specification limit higher than one consistent with a daily intake of (b) (4) of (b) (4). In fact, one might argue that data from structurally related compounds indicating *in vivo* carcinogenic potential, while not sufficient to set a quantitative specification limit, might increase the concern for the *in vivo* carcinogenic potential of (b) (4) and question the relevance of a negative *in vivo* Comet assay. (It is not known if *in vivo* Comet assay data are available for the structurally related compounds identified by the sponsor.)

Another issue that needs to be addressed is the extent to which (b) (4) was tested in the *in vivo* carcinogenicity studies of ezogabine (neonatal mouse assay, 2-year carcinogenicity study in rat). Ezogabine was negative in the 2-year rat study. Two drug batches were used in that study; batch 0206102, containing (b) (4) of (b) (4) was used for ≈41 weeks, while batch 0005005, containing (b) (4) of (b) (4) was used for the remainder of the study (≈63 weeks). At the specification limit of NMT (b) (4) the levels of (b) (4) at the highest dose tested (50 mg/kg) were well below (b) (4) basis) that allowed in the to-be-marketed drug product (at the maximum recommended human dose [MRHD] of 1200 mg/day). In the neonatal mouse study, in which only two daily doses (≤96 mg/kg on PND 8 and 15) were administered, the drug batch used (batch 0005005) contained (b) (4)

which resulted in two days of exposure to a level of (b) (4) well below (b) (4) that allowed for humans at the MRHD. Therefore, as Dr. Fisher points out, (b) (4) was clearly not adequately tested in either study. In recent submissions to the NDA, however, the sponsor has proposed lowering the specification limit from (b) (4) to (b) (4) (Amendment 0036, dated 10/20/2010) and then from (b) (4) to (b) (4) (Amendment 0038, dated 11/15/2010), with no change in the manufacturing process. Regarding the lowest proposed specification limit of NMT (b) (4) the daily dose of (b) (4) at the highest doses administered in the neonatal mouse and the 2-year rat study are approximately equivalent to that at the MRHD. While the nonclinical studies provide greater support for the lower specification limit, testing an Ames-positive impurity at maximum doses of (b) (4) cannot, in my opinion, be considered an adequate assessment of the genotoxic potential of (b) (4) As Dr. Fisher notes, the EMEA Guideline on the Limits of Genotoxic Impurities (2006) considers a negative carcinogenicity test of drug substance containing low levels of an impurity insufficient for setting limits, due to the insensitivity of this approach. The same arguments have been made for testing low levels of an impurity in genetic toxicology assays, but unlike for (at least) the Ames assay, there are no data to establish how much impurity would need to be present in a carcinogenicity study in order for it to be an adequate test. The only possibly relevant guidance that I am aware of is the ICH S1C guidance on dose selection for carcinogenicity studies (*Guidance for Industry S1C(R2), Revision 1, September 2008*) which allows a 25-fold (based on plasma AUC) margin to justify high dose selection in lifetime carcinogenicity bioassays. Although application of that criterion is arguably relevant to an impurity, clearly the 2-year rat study conducted for ezogabine do not provide an adequate test of (b) (4) on that basis. Therefore, it is my opinion that the neonatal mouse assay and the 2-year carcinogenicity studies of ezogabine do not provide support for even the lowest specification limit proposed by the sponsor for (b) (4)

One final issue that warrants consideration is the genotoxic and carcinogenic potential of the API (ezogabine). The genotoxic potential of ezogabine as measured in the numerous Ames assays is unclear because of, as discussed previously, the varying amounts of a number of impurities in the drug batches tested. However, ezogabine was concentration-dependently and reproducibly positive in the *in vitro* chromosomal aberration assay in human lymphocytes. Therefore, the question arises as to the possible implications of this finding in terms of the concern for the presence of an Ames positive impurity. In my opinion, the positive clastogenicity finding for ezogabine does not alter that concern. The fact that ezogabine was adequately tested and negative in the 2-year carcinogenicity study in rat might somewhat allay concerns regarding ezogabine's signal for clastogenicity. (It is true that the maximum plasma (AUC) exposures achievable (limited by toxicity) in rat were less than that in humans at the MRHD of 1200 mg/day, but they were similar to that in humans at the dose of 900 mg/day, which may be the recommended therapeutic dose.) The positive finding in the neonatal mouse, which is reported to be sensitive to genotoxic carcinogens (b) (4) may reflect genotoxic effects of ezogabine and/or (b) (4) by potentially two different modes of action. As Dr. Fisher notes, the positive findings may "strengthen the concern arising from the genotoxicity data", since they occurred at a plasma ezogabine AUC $\approx 1/6^{th}$ that in humans at the MRHD and a dose of (b) (4) similar to that in humans (at the newly proposed

specification limit of (b) (4) given for only two days. The negative findings in the 2-year rat study might suggest that (b) (4) is responsible for the positive findings in the neonatal mouse study.

Recent sponsor submissions

The sponsor has submitted two proposals: first, to lower the specification from (b) (4) (12 October 2010) and, second, to lower the specification from (b) (4) (15 November 2010). In the November, 2010 submission, in addition to lower the specification limit to (b) (4) "The sponsor has demonstrated that…alternative methods of manufacture under investigation are capable of further reducing the levels of (b) (4) to NMT (b) (4) which would result in a maximum dose of (b) (4)/person/day. With the current route of synthesis, that level is not possible. The sponsor further "…commits to provide a post-approval supplement with supporting modifications necessary to control (b) (4) to NMT (b) (4) for FDA review by mid-2011".

From a safety standpoint, a specification limit of NMT (b) (4) in conjunction with a commitment to lower the specification limit to NMT (b) (4) is acceptable. As the sponsor notes, that approach will result in a daily dose of (b) (4) consistent with the staged TTC (i.e., (b) (4)/person/day for 6-12 months of dosing.) However, it would be important for the sponsor to meet that commitment so that clinical exposure at the higher dose does not continue substantially beyond the acceptable period.

Recommendation

Based on the points discussed above, it is my opinion that the data are sufficient to characterize (b) (4) as a genotoxic impurity and to support a requirement for the sponsor to reduce the specification limit to NMT (b) (4) This may be done either prior to approval or, if considered a reasonable approach, post approval with a PMR to lower the specification limit from NMT (b) (4) ppm by mid-2011.

Recommended labeling

(b) (4)

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/s/

LOIS M FREED
11/30/2010

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO
NDA: 22-345
Submission date: October 30, 2009
Drug: Potiga (ezogabine; retigabine)
Applicant: Valeant Pharmaceuticals
Indication: Adjunctive therapy in epilepsy patients with partial-onset seizures
Reviewing Division: Division of Neurology Products

Introductory Comments:
The pharmacology/toxicology reviewer recommended that this NDA not be approved from a pharm/tox perspective based primarily on the presence of a genotoxic impurity. The supervisor considered this concern in a secondary review and concluded that the impurity should be lowered to a level that results in a daily dose of the impurity of not more than (b) (4)/day ((b) (4) The supervisor suggested that this could be achieved either prior to approval or in a step-wise manner with a specification of (b) (4) at approval followed by a postmarketing requirement to lower the specification to (b) (4)

Discussion:
The genotoxicity of the impurity ((b) (4) has been thoroughly discussed in the primary and secondary reviews. Briefly, the impurity was positive in *in vitro* bacterial reverse mutation assays and was negative in an *in vivo* combined micronucleus/Comet assay in male rats. The primary and secondary reviews discuss the strengths and weaknesses of these studies and conclude that the Comet assay results do not negate the positive bacterial mutation data. There is not universal agreement among the pharm/tox community on the utility of the Comet assay. However, this particular assay appears to have been adequately conducted and so even if the negative outcome of this assay does not entirely negate the positive bacterial assay, it does reduce the overall concern for genotoxicity.

The original specification for (b) (4) of (b) (4) could result in a daily dose of the impurity of approximately (b) (4)/day at the maximum human dose of 1200 mg. Lowering the specification to (b) (4) could result in a daily dose of the impurity of approximately (b) (4)/day. This exceeds the suggested threshold for genotoxic impurities of (b) (4)/day; however, the risk from this level of impurity is still likely to be relatively low. Reducing the specification to (b) (4) could result in a daily dose of the impurity of approximately (b) (4)/day which is below the threshold generally considered acceptable for genotoxic impurities.

Carcinogenicity studies conducted with ezogabine apparently did not contain sufficient levels of (b) (4) to qualify it at the (b) (4) level. The level of (b) (4) in the

carcinogenicity studies may have achieved doses similar to that if the specification were set at (b) (4) A two year rat study did not show any drug-related neoplasms. A neonatal mouse study in which mice were dosed with ezogabine twice on postnatal days 8 and 15 resulted in two male animals with lung carcinomas at the high dose. Although these were not statistically significantly elevated, the Executive Carcinogenicity Assessment Committee could not rule out that they might be drug-related based on very low background rates for these tumors in concurrent and historical control data. These studies may not be definitive for the impurity because high doses of the impurity were not tested; however, the results do not raise any particular concern for the lower levels of the impurity (i.e (b) (4)).

Conclusions:
The pharm/tox reviewer and supervisor have conducted a thorough evaluation of the nonclinical information submitted in support of this NDA and have carefully considered the safety of the product with particular emphasis on the impurity, (b) (4) A conservative approach from a safety perspective would be to lower the specification of the impurity to (b) (4) based on the positive bacterial mutagenicity assay. I believe that the risk at slightly higher levels of impurity (e.g., (b) (4) is also likely to be low when all the data are considered. It appears that the applicant can lower the level of this impurity to these levels. If the applicant lowers the limit to (b) (4) then I agree with the supervisor's conclusion that further lowering it (b) (4) can be done as a post marketing requirement if it is not done prior to approval.

There appear to be no other pharm/tox issues at this time.

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/s/

PAUL C BROWN
11/30/2010

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-345
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	10/30/09
PRODUCT:	Potiga (retigabine [ezogabine]) Tablets
INTENDED CLINICAL POPULATION:	epilepsy
SPONSOR:	Valeant
REVIEW DIVISION:	Division of Neurology Products (HFD-120)
PHARM/TOX REVIEWER:	Ed Fisher
PHARM/TOX SUPERVISOR:	Lois Freed
DIVISION DIRECTOR:	Russell Katz
PROJECT MANAGER:	Stephanie Keefe

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Note: All figures and tables in this review were excerpted from the sponsor's submission or literature.

I. INTRODUCTION AND DRUG HISTORY

NDA number: 22-345

Date of submission: 10/30/09

Sponsor: Valeant

Drug:

Trade name: Potiga

Generic name: retigabine (ezogabine)

Code names: D-23129; GKE-841

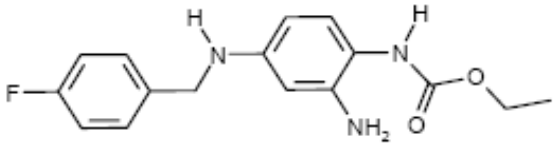
Chemical name: N-[2-amino-4-(4-fluorobenzylamino)-phenyl]-carbamic acid ethyl ester

CAS registry number: 150812-12-7

Molecular formula: $C_{16}H_{18}FN_3O_2$

Molecular weight: 303.3

Structure:



Relevant IND: 53,950

Drug class: potassium channel activator

Indication: epilepsy; adjunctive treatment of partial onset seizures in patients (b) (4) years of age or older. The recommended clinical starting dose is 300 mg/day, which can be increased by 150 mg/day every week to achieve an effective maintenance dose of between 600 mg/day and 1200 mg/day. The maximum dose is 1200 mg/day.

For comparison purposes, human AUC(0-24) retigabine (RTG) values at 600 mg and 1200 mg RTG are ~14400 and ~30590 ng.h/ml respectively, and human AUC(0-24) NAMR values at 600 mg and 1200 mg RTG are ~12700 and ~22970 ng.h/ml, respectively

Route of administration: oral (tablets)

II. PHARMACOLOGY

A. BRIEF SUMMARY (see initial IND review dated 9/11/97)

Retigabine (RTG), a structural analog of the marketed (in Europe) analgesic flupirtine, has shown activity in a wide range of animal models of epilepsy (chemically and electrically induced convulsions, kindling models of partial epilepsy, models of absence, genetically seizure-prone rodents) thought to indicate a broad spectrum of antiseizure activity (**Table IIA.1-2**). An anti-epileptogenic response was also shown in a rat amygdala kindling model in which rats treated during kindling acquisition showed reduced seizure severity, seizure duration and afterdischarge duration that persisted after discontinuation of treatment. The oral effective dose (5 mg/kg) in this model was associated with plasma concentrations that are considered clinically relevant (1500-2100 ng/ml). RTG has also shown activity in several animal pain models, and has demonstrated anxiolytic properties and neuroprotective potential in various test systems.

Results from a number of in vitro and in vivo mechanism of action studies conducted by the sponsor and others indicated that the pharmacological effects of RTG in the clinically-relevant concentration range of 0.1-10 µM (30.3-3033 ng/ml compared to ~1520 ng/ml Cmax following dosing at the MRHD of 1200 mg RTG in patients) may involve enhancement of transmembrane K^+ currents mediated by the KCNQ family of ion channels (KCNQ2-5 but not KCNQ1 isoforms; **Table IIA.3**). By activating central KCNQ channels, RTG is thought to produce a concentration-dependent enhancement of the M-current resulting in membrane stabilization and reduction in brain excitability. A role for KCNQ channels in some forms of epilepsy has been proposed based on the findings that mutations in KCNQ2 and KCNQ3 are associated with benign neonatal epilepsies and KCNQ blockers linopirdine and XE991 induce seizures *in vivo* and epileptiform cell activity *in vitro*.

Early studies in various in vitro neuronal cell preparations showed agonist effects of RTG on K^+ channels, but the particular type of channel was unknown. After the KCNQ (Kv7) family of K^+ ion channels was successfully cloned, several different investigators independently reported that RTG acts as a selective opener of KCNQ2-5 channels. Studies conducted by (b) (4) found that RTG (0.1 to 10M) induced a potassium current and hyperpolarized CHO cells expressing KCNQ2/Q3 receptors, and RTG-induced currents in KCNQ2/Q3-expressing CHO cells were inhibited by the KCNQ2/Q3 blocker linopirdine. The mechanism by which RTG enhanced KCNQ2/Q3 currents involved leftward shifts in the voltage dependence of channel activation. RTG shifted the voltage dependence of channel activation and also slowed the rate of channel deactivation, predominantly by increasing the contribution of a slowly deactivating tail current component. These results were thought to identify KCNQ2/Q3 channels as a molecular target for RTG and suggested that activation of KCNQ2/Q3 channels might be responsible for at least some of its anticonvulsant activity. Additional studies showed RTG to be a relatively non-selective KCNQ channel modulator, capable of activating all members of the KCNQ channel family except KCNQ1. RTG also enhanced native M-currents in PC-12 cells, rat sympathetic neurons and rat dorsal root ganglion cells. The structurally-related compound flupirtine was also shown to activate KCNQ2/3 channels and native M-currents.

However, other data indicated that additional actions, particularly on GABA pathways, may also contribute to the antiepileptic activity of RTG. RTG significantly augmented the GABA-induced current in cultured cortical neurons at concentrations from 0.1-100 µM, was shown to be a positive modulator of the $GABA_A$ receptor at concentrations of 10 µM or higher, and stimulated de novo synthesis of GABA in hippocampal slices. RTG ion channel activities were also noted on Na^+ and Ca^{2+} channels at higher concentrations (30 and 100 µM). No affinity for the benzodiazepine and GABA binding sites on the $GABA_A$ receptor complex or to the GABA re-uptake site or the glutamate and channel binding sites on the NMDA receptor complex was observed at RTG concentrations of up to 100 µM. In fact, in the full screen, RTG did not significantly interact with any of the CNS-related receptors, ion channels or second messengers tested. However, a literature study (van Rijn & Williems-van Bree 2004)) indicated that RTG acts on the $GABA_A$ receptor at a unique binding site which is independent from but interacts with the binding sites of GABA and the neuroactive steroid, Org 20549. RTG did not block NMDA-induced

currents in rat cortical neurons at concentrations of 1 and 10 μM. Although the initial NIH ADD report suggested kainite antagonism as a mechanism of action, RTG and flupirtine exerted only weak antagonistic effects on kainate-induced currents at high concentrations: approximately 17 and 10%, respectively, at a concentration of 100 μM.

In a literature study by Hansen et al (JPET, 318:1006-19, 2006), the effect of KCNQ channel modulators including RTG on the activity of dopaminergic neurons *in vitro* (0.5-600 μM) and *in vivo* (2.5 mg/kg iv) was investigated in the rat ventral mesencephalon. RTG (≥1 μM) inhibited the firing of DA neurons in a concentration-dependent manner even in the presence of tetrodotoxin and simultaneous blockade of $GABA_A$ receptors, small conductance calcium-activated K^+(SK) channels, and hyperpolarization-activated cation channels. Effects were reversed by the KCNQ channel blocker 4-pyridinylmethyl-9(10H)-anthracenone (XE991), indicating a direct effect of RTG on KCNQ channels. Similarly, *in vivo*, recordings from DA neurons showed that 2.5 mg/kg RTG reduced spike activity from 160 spikes/10s to 28 spikes/10s. RTG also inhibited DA synthesis and expression of c-Fos in the striatum and blocked the excitatory effect of DA D2 auto-receptor antagonists, which was reversed by XE991. These data indicate that RTG can have a negative modulatory effect on DA neurotransmission likely through stimulation of mesencephalic KCNQ4 channels (immunocytochemistry showed that KCNQ4 is the major KCNQ channel subunit expressed in all DA neurons in the mesolimbic and nigrostriatal pathways).

Table IIA.1 Profile of Anticonvulsant Activity and Minimal Toxicity of Retigabine HCl (RHCl) and Retigabine (RTG) in Mice and Rats

Species, Route of Administration	Test	Time of Test (h)	RHCl			Time of Test (h)	RTG		
			ED_{50} (mg/kg)	95% C.I.	P.I.[a]		ED_{50} (mg/kg)	95% C.I.	P.I.[a]
Mice, i.p.	Rotorod	0.25	26.7[b]	21.3-34.2		0.25	15.9[b]	9.5-24.6	
	Frings AGS	0.25	2.11	1.29-3.8	12.6[c]	0.25	2.1	1.43-2.96	7.6
	MES	0.25	8.39	6.67-10.2	3.2	0.25	9.31	6.32-13.1	1.7
	sc Met	0.25	11.0	9.37-13.0	2.4	0.25	13.5	9.21-18.0	1.2
	sc Bic	0.25	> 30	N.D	N.D.	N.D.			
	sc Pic	0.25	18.6	14.1-26.2	1.4	N.D.			
Rats, p.o.	MMI[d]	0.5	53.5[b]	32.6-76.2		2.0	83.5[b]	60.6-115	
	MES	1.0	3.03	2.6-3.76	18	0.5	2.9	1.93-4.14	29
	sc Met	0.5	73.9	44.6-119	0.7	0.5	68	53.1-87.6	1.2

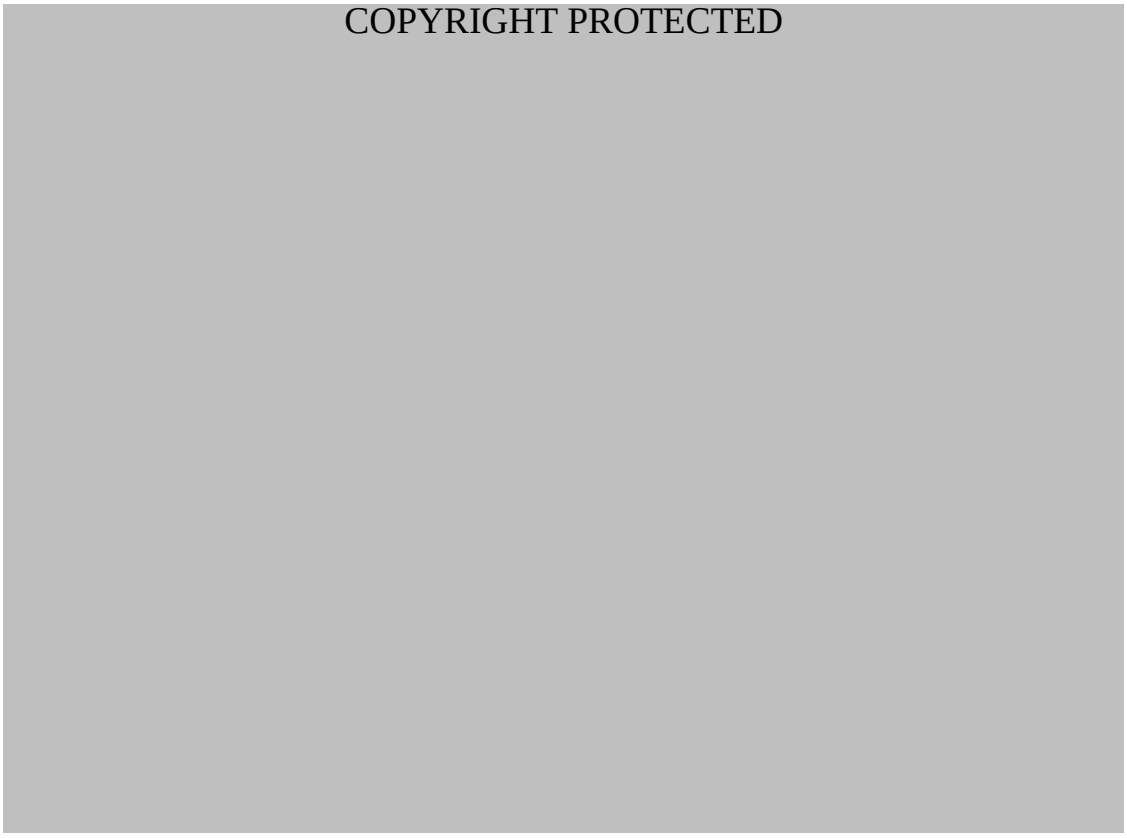
a Protective Index = TD_{50}/ED_{50}
b Median toxic dose TD_{50}
c P.I. calculated with TD_{50} obtained in CF#1 mice and ED_{50} in Frings AGS mice
d Minimal motor impairment
N.D. Not determined

Table IIA.2 Comparative Anticonvulsant Profile of Retigabine HCl (RHCL) and Retigabine (RTG) and Selected Prototype Anticonvulsants in Mice and Rats

Test Substance	Mice					Rats		
	MES	sc Met	sc Bic	sc Pic	AGS	MES	sc Met	Kindled Seizures
RTG	±	±	N.D.	N.D.	+	+	±	+
RHCl	+	+	-	±	+	+	±	+
VPA	+	+	±	±	+	+	±	+
Felbamate	+	+	-	+	+	+	+	+
PHT	+	-	-	-	+	+	-	±
LTG	+	-	-	-	N.D.	+	-	N.D.
CBZ	+	-	-	±	+	+	-	±
Gabapentin	+	+	-	-	N.D.	+	-	N.D.
Ethosuximide	-	+	±	+	+	-	+	-
Clonazepam	-	+	+	+	+	±	+	+

\+ Protection at doses producing no behavioral toxicity
± Protection at doses producing some behavioral toxicity
\- <50% protection at highest dose tested
N.D. Not determined

Table IIA.3 Pharmacological actions of retigabine on K_V7 (KCNQ) channels expressed in CHO and HEK 293 cells (Table 2 in Blackburn-Munro et al, CNS Drug Reviews, 11: 1-20, 2005)



B. SAFETY PHARMACOLOGY

In safety pharmacology studies in mice and rats, RTG (10, 30 and 100 mg/kg po and 3, 10, 30 mg/kg ip in both species) produced a range of transient CNS effects including decreased exploratory behavior and locomotor activity, ataxia, flat body posture, muscle relaxation, depressed breathing, hyperexcitability, and clonic convulsions. In addition to its effects on neuronal KCNQ channel activity, RTG has been shown to affect other organs systems including the urinary bladder and gallbladder as a result of its effects on KCNQ channels in those organs. Pathological effects on the urinary bladder have been observed in animal toxicity studies as a result of RTG enhancement of KCNQ activity leading to inhibition of contraction of the urinary bladder smooth muscle and associated inhibition of micturition. Inhibition of urinary bladder voiding leads to bladder distension with secondary effects observed in the kidneys as a result of the increase in urinary pressure. RTG was also shown to activate KCNQ channels in guinea pig gallbladder tissue, leading to relaxation of histamine-induced contractions in gallbladder smooth muscle. The relaxing effect of RTG on gallbladder contraction was thought to provide a mechanism for the hepatotoxicity seen in dog toxicity studies.

RTG had a concentration-dependent inhibitory effect on IKr (hERG) currents (inward tail-currents reduced by approximately 10, 25, and 49% at 1, 10 and 100 µM, respectively). The metabolite NAMR had a similar but less potent effect (0, 2, 14 and 46% inhibition at 10, 30, 300 and 1000 µM). RTG (1, 10, 100 µM) also reduced the amplitude of outward currents through KCNQ1/KCNE1 ion channels expressed in CHO cells in vitro (3, 14, and 58% at 1, 10 and 100 µM). Thus, the IC50 of RTG was approximately 100 µM for both of these cardiac ion channels.

The effects of RTG and NAMR (10 and 100 µM for both) on cardiac action potentials and ionic currents were tested *in vitro* on cat ventricular myocytes and dog Purkinje fibers. RTG (10 µM) decreased IKr by 14% and produced a 15% shortening of the action potential duration at 95% of full repolarization (APD95). This shortening of the APD95 was thought to be caused by a reduction of the L-type Ca2+ (ICa-L) current, which was blocked by 18% at 10 µM. NAMR (10 µM) produced a 13% shortening of the APD95 through a similar mechanism (reduction of ICa-L). At the higher concentration (100 µM), RTG and NAMR produced similar, but more pronounced effects: decreased APD95 (29 and 20%, respectively), and block of IKr (51 and 36%, respectively), IKs (29 and 22%, respectively), ICa-L (74 and 39%, respectively) and IKI (24 and 34%, respectively). In Purkinje fibers, RTG shortened the APD50 at 100 µM.

In Langendorff-perfused guinea pig hearts, RTG produced concentration–dependent decreases in heart rate (19 and 47% at 30 and 100 µM, respectively) and prolonged PQ interval (16 and 29% at 30 and 100 µM) were observed with RTG. An increase in QT interval (17%) was seen at 100 µM (**Table IIB.1**).

When hemodynamic function was measured in anaesthetized pigs, dose-dependent decreases in blood pressure, cardiac output, and left ventricular contractility were observed following iv bolus doses of 0.3, 1, 3 and 10 mg/kg. No reflex tachycardia was noted, however. The effects occurred rapidly during bolus application and were of short duration. IV infusion of 6 mg/kg over 5 minutes produced less marked effects. Intraduodenal administration of 30 mg/kg produced effects comparable to those after iv dosing, with a rapid onset but longer duration. The sponsor concluded that rapid iv injection should be avoided. The hemodynamic effects were thought to resemble those of Ca2+-channel blockers.

In contrast to the effects seen in pigs, intraduodenal administration of RTG to spontaneously breathing beagle dogs at doses of 10 and 30 mg/kg (cumulative administration, n=4) and 100 mg/kg (single administration, n=4) produced no clearly treatment-related (T-R) cardiovascular effects.

RTG was administered to 2 conscious male mongrel shepherd dogs (dog 1: 2+2+4 mg/kg, dog 2: 1+2+4 mg/kg,) using a short term infusion over 25 min for each dose to examine RTG's effects on behavior and ECG parameters. Vomiting was first observed in both dogs after a cumulative dose as low as 0.3 mg; hyperexcitability was observed in dog 1 after a cumulative dose of 5.6 mg/kg and in dog 2 after 2.4 mg/kg.

In the first dog, the total cumulative dose of 8 mg/kg was administered and no arrhythmias or changes in QTc intervals were observed. In the second dog, the infusion was stopped after administration of 3 mg/kg due to the pronounced CNS effects. No arrhythmias or prolongation in QTc interval were observed in the second animal. The plasma concentrations of RTG following short-term (25 min) iv infusions in dog 1 (cumulative doses of 2, 4 and 8 mg/kg) were 1084, 1247 and 2489 ng/ml, respectively and in dog 2 (cumulative doses of 1 and 3 mg/kg) were 881 and 1871 ng/ml, respectively.

When RTG (oral capsule doses of 5, 18, or 38 mg/kg/day) was administered to conscious male and female beagle dogs daily for 7 days, statistically significant decreases in mean heart rate (ranges of 25-31%, 18-41% and 21-38%, respectively) and mean arterial blood pressure (ranges of 10-18%, 9-21% and 9-19%, respectively) were seen in all treated dogs compared to controls (vehicle). However, there were no effects on the ECG at these relatively low doses (Detweiler report).

RTG was shown to have an effect on vascular smooth muscle in vitro, producing concentration dependent vasorelaxation of both mouse mesenteric and rat gracilis arteries. The RTG effect on gracilis arterial rings was reversed by XE991 indicating that the RTG effect was due to its activity on KCNQ channels. In addition, using the whole cell patch clamp technique, RTG (10 uM) was shown to stimulate outward potassium currents in arterial smooth muscle cells isolated from gracilis arteries of rats.

In preparations of isolated rat urinary bladder, RTG inhibited both spontaneous and stimulated (KCl- or carbachol-induced) bladder smooth muscle contraction, and it appeared that this effect was at least partly due to its effects on KCNQ channels. RTG inhibited contraction of bladder smooth muscle elicited by the addition of either 25 or 60 mM KCl (IC50 = 1.4 or 22 µM, respectively) or 200 nM carbachol (IC50 = 4 nM) in a concentration-dependent manner. The RTG effects were not inhibited by glyburide (an inhibitor of ATP-sensitive potassium channels), but were markedly reduced by the KCNQ inhibitor linopirdine.

RTG did not appear to have direct cytotoxic or mitogenic effects on male CD-1 mouse urinary bladder explants in organ culture at concentrations in the range found in vivo in urine (120 – 330 µg/ml) from RTG treated mice.

RTG (0.1 µM, 1 µM, 10 µM and 100 µM) concentration-dependently relaxed pre-contracted segments of guinea pig gallbladder smooth muscle. At the highest concentration relaxation was about 75% compared to the maximal histamine stimulated contractions (**Figure IIB.1**). The effect was only partially reversed by the KCNQ channel blocker linopirdine. This study also found that pretreatment with RTG (30 uM) reduced the sensitivity of the gallbladder to a contractile stimulus (histamine). These findings were considered to provide evidence for the existence of KCNQ channels in gallbladder tissue and show that activation of these channels may contribute to the hypomotility of the gallbladder induced by retigabine.

Table IIB.1

Effects of retigabine (10, 30 and 100 µmol/l) on cardiac impulse generation and conduction i
Langendorff perfused guinea pig hearts (n=4, mean ± sd, percent changes versus baseline).

	animal number	heart rate [n/min]	PQ-interval [ms]	QRS-interval [ms]	QT-interval [ms]
baseline	1	268	45	25	150
	2	249	50	27	156
	3	224	51	30	163
	4	219	50	30	170
	$\bar{x}$ ±sd	**240±23**	**49±2.7**	**28±2.4**	**160±8.7**
	%	100	100	100	100
retigabine 10 µmol/l (20 min)	1	259	-	30	150
	2	208	55	28	154
	3	203	50	30	161
	4	193	47	34	173
	$\bar{x}$ ±sd	**216±30***	**51±4.0**	**31±2.5**	**160±10**
	%	90	103	109	100
retigabine 30 µmol/l (40 min)	1	247	-	27	135
	2	182	58	28	157
	3	183	63	28	171
	4	169	49	33	175
	$\bar{x}$ ±sd	**195±35***	**57±7.1**	**29±2.7**	**160±18**
	%	81	116	104	100
retigabine 100 µmol/l (60 min)	1	174	71	34	162
	2	115	61	28	181
	3	110	70	26	196
	4	105	51	34	210
	$\bar{x}$ ±sd	**126±32***	**63±9.3***	**31±4.1**	**187±21***
	%	53	129	109	117

Figure IIB.1

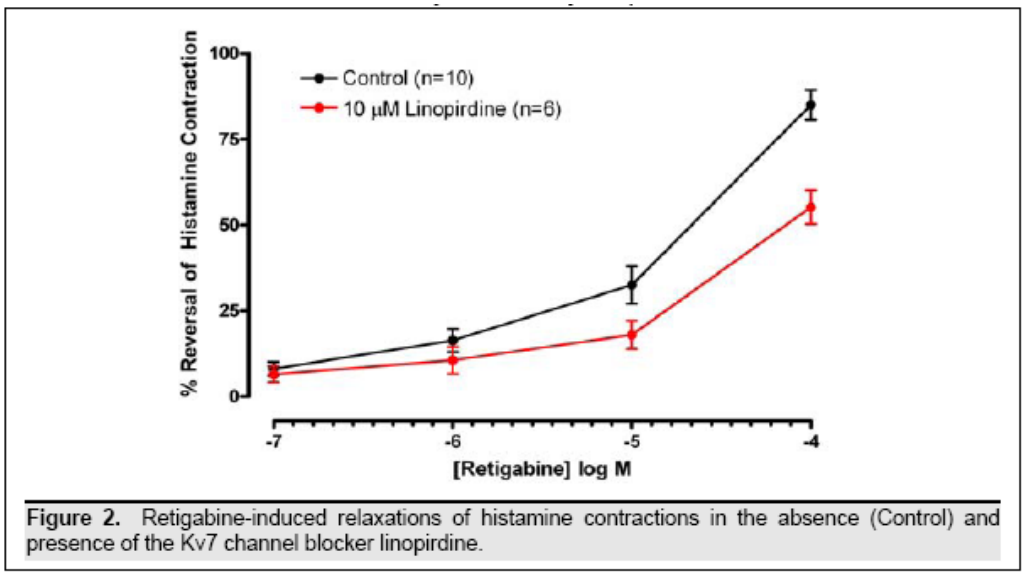


Figure 2. Retigabine-induced relaxations of histamine contractions in the absence (Control) and presence of the Kv7 channel blocker linopirdine.

III. **PHARMACOKINETICS/TOXICOKINETICS**

A. BRIEF SUMMARY

PK was investigated in mice, rats, rabbits, dogs, minipigs, and monkeys (**Table IIIA.1**). TK data were also generated in toxicology studies conducted in these species, including pregnant rats and rabbits (shown in the Toxicology section). Retigabine (RTG) was relatively well-absorbed orally in the toxicology species; absolute bioavailability values were generally around 60-70% in rats and dogs, but much lower in monkeys and minipigs. Peak concentrations were usually observed within 0.25-3 hours of oral dosing in rodents, rabbits, and dogs. RTG half-life values ranged from approximately 2 hr in rats up to 6-10 hours in dogs (8 hr in humans). Drug concentrations normally decreased to below the limit of quantification within 24 hrs post-dose. The N-acetyl metabolite of RTG (NAMR), an active circulating human metabolite, was also identified as a circulating metabolite in rodents but was not found or was present at very low levels in dogs and monkeys (**Table IIIA.2**). The anticonvulsant activity and neurotoxicity of NAMR were lower than that of RTG in rodents (1/4 and 1/3 as potent in the mouse and rat MES tests). Following single-dose administration to mice, RTG exposure tended to be greater (2 to 10-fold) in males than females over a range of dose levels. There were no notable sex differences in PK in rats or dogs. RTG did not significantly accumulate with repeated daily dosing in rats, dogs, or monkeys. Although total systemic exposure (AUC) to RTG increased with increasing dose level in all species across a wide range of dose levels, the increase tended to be less than dose-proportional, particularly at TK dose levels. TK data are provided with the individual studies.

The PK of (b) (4) (b) (4)) was investigated following RTG administration and following administration of (b) (4) itself in rats. (b) (4) was not quantifiable (<1.0 ng/ml) following administration of RTG (20 mg/kg po); however, absorption, high clearance and hepatic extraction of (b) (4) were confirmed following administration of (b) (4) itself (375 or 750 mg/kg po).

When tissue distribution of [14C]-RTG was investigated in Wistar rats after a single oral gavage dose of 8.25 mg/kg, most of the radiolabel was recovered in the GI tract, muscle, fat, liver, and blood, with rapid elimination from all tissues over a 48- to 72-hour period following dosing. There were no apparent sex differences in distribution. Unchanged RTG concentration was approximately 2X greater in brain than in plasma and there was a lower relative exposure of the brain to RTG metabolites than observed in plasma. In pregnant (GD18) rats, radioactivity in fetal tissues after single oral dosing at 8.25 mg/kg [14C]-RTG rapidly equilibrated with maternal tissues within 2 to 8 hours, with comparable exposures in dams and fetuses. In lactating rats orally dosed with 8.25 mg/kg [14C]-RTG, radioactivity in milk exceeded that in maternal plasma but most of the radioactivity extracted from milk was represented by the metabolite characterized as 5-acetamido-2-oxo-2,3-dihydro-1*H*-benzimidazole (M7; 61-85% of total radioactivity), which accounted for only very low amounts in plasma. This metabolite is apparently highly concentrated in milk. It is estimated that the milk/plasma ratio is about 35:1. In contrast, major radioactive fractions detected in plasma, such as N-glucuronide, acetyl metabolite, and the unchanged parent drug showed only very low milk levels. This major milk metabolite (also found in rat urine but to a much lower extent) was thought by the sponsor to be rat-specific, since there was no evidence for its formation in humans or other species, but milk was not tested in other species. RTG protein binding averaged 77% to 87% in mouse, rat, rabbit, dog and humans, and was reversible in rats, with no covalent binding observed. NAMR protein binding was low (39-49%) in all species. Free to bound ratios for RTG and NAMR were similar among the toxicology animal species and humans.

Metabolism data were obtained from in vitro and in vivo studies using mice, rats, rabbits, dogs, and monkeys. The primary routes of RTG metabolism were N-acetylation to NAMR and N-glucuronidation of RTG and NAMR (**Figure IIIA.1**, **Table IIIA.3**). All evaluated species formed N-glucuronides of RTG, although there was some evidence of species specificity for the specific structural location for glucuronidation. Across evaluated species, plasma metabolite profiles showed that NAMR was present at concentrations (AUCs) similar to (human) or less than (rat>rabbit>mouse) those of parent in species capable

of forming it, with the remaining plasma metabolites demonstrated or thought to be glucuronides (4-fluorobenzyl-amino-benzyl imidazolone (FBI) also identified in rat plasma). NAMR was not detected in dogs and monkeys, species deficient in N-acetyl transferase. Urine metabolite profiles varied across species, with rats demonstrating a complex profile of over 20 metabolites/degradation products with dogs excreting only RTG and few metabolites. In contrast, the fecal metabolite profile in rats showed only one distinct metabolite. In dog feces parent compound and two metabolites distinct from those in urine were observed.

Studies evaluating the excretion of RTG and NAMR following administration to rats and dogs showed that RTG and its metabolites are excreted in both feces and urine, with the feces generally accounting for approximately 2/3 of the eliminated dose and urine accounting for approximately 1/3. NAMR radioequivalents were excreted to a greater extent in the urine (approximately 1/2 to 2/3 of dose) than were RTG radioequivalents. Elimination patterns were similar following both oral and iv dosing. The extensive elimination of drug via the feces following iv dosing is consistent with the demonstration that large fractions of an RTG dose are excreted in bile.

Table IIIA.1 Comparative Pharmacokinetic Data and Systemic Exposure to RTG following Single Oral Administration to Mice, Rats, Dogs and Monkeys

Species [Formulation]	Gender [M/F]	Dose [mg/kg]	Pharmacokinetic Parameters						Report No.
			C_{max} [ng/mL]	t_{max} [h]	AUC_{0-24} [ng*h/mL]	$AUC_{0-\infty}$ [ng*h/mL]	$t_{1/2}$ [h]	abs. BA [%][b]	
Mouse (solution)	M	100	3,823	1.0	20,038	ND	ND	ND	D23129/9321020016
	F	200	3,638	1.0	22,521	ND	ND	ND	
Rat (solution)	M	8.25	1,887.3	1.50	ND	8,965.9	2.05	ND	D-23129/FBDD0895
	F	8.25	2,180.3	0.25	ND	8,692.0	1.94	ND	
Rat (solution)	M	50	1,770	1.0	ND	18,873	8.4	ND	D-23129/9321020044
Rat (suspension)	M	2.5	568.6	0.63	1,654.9	1,686.0	1.579	61.66	D-23129/FBDD0795
	F	2.5	649.7	0.59	2,058.1	2,098.4	1.952	70.06	
Rat (solution)	M	8.25	1,194.18	0.5	6,276.56	7,265.57	3.55	ND	D-23129/7099010030
Rat (suspension)	M	12.0	3,051	0.63	12,836	13,845	2.54	ND	D-23129/FBDD0394
	F	14.7[b]	2,010	0.69	7,447	7,940	2.20	ND	
Rat (suspension)	M	5	1,020[c] 547[d]	4.00[c] 0.25[d]	ND	10,700[c] 3,470[d]	2.93[c] 3.49[d]	ND[c] 144[d]	PR2007-004
Dog (capsule)	M	12.0	607	1.0	6,839	7,199	6.1	ND	D-23129/FBDD0394
	F	12.0	570	3.0	8,713	10,851	10.45	ND	
	M	12.0	842	1.0	9,136	15,918	20.1	ND	
	F	12.0	770	3.0	9,057	10,698	8.68	ND	
Dog (capsule)	M	8.25	232.2	1.83	ND	1,218	4.65	ND	D-23129/FBDD0995a
	F	8.25	315.3	1.33	ND	1,659	6.19	ND	
Dog (capsule)	M: Dog #1 M: Dog #2	8.25	365 231	3.0 2.0	ND ND	2,753 2,461	5.7 10.2	ND	D-23129/FBDD1295
Dog (gavage)	M: Dog #1 M: Dog #2	8.25	502 240	1.0 0.5	ND ND	1,910 886	6.2 6.5	ND	
Dog (capsule)[e]	M	5	2,050[c] 357[d]	1.5[c] 1.0[d]	ND ND	13,400[c] 1,060[d]	5.33[c] 3.33[d]	ND[c] 60[d]	PR2007-005
Monkey (gavage)	M	200	4,920	3.0	ND	31,290	5.4	58.3	D-23129/7099079042
Monkey (gavage)	M	200	1,314	5.50	ND	22,936	5.30	22.43	D-23129/7000029051

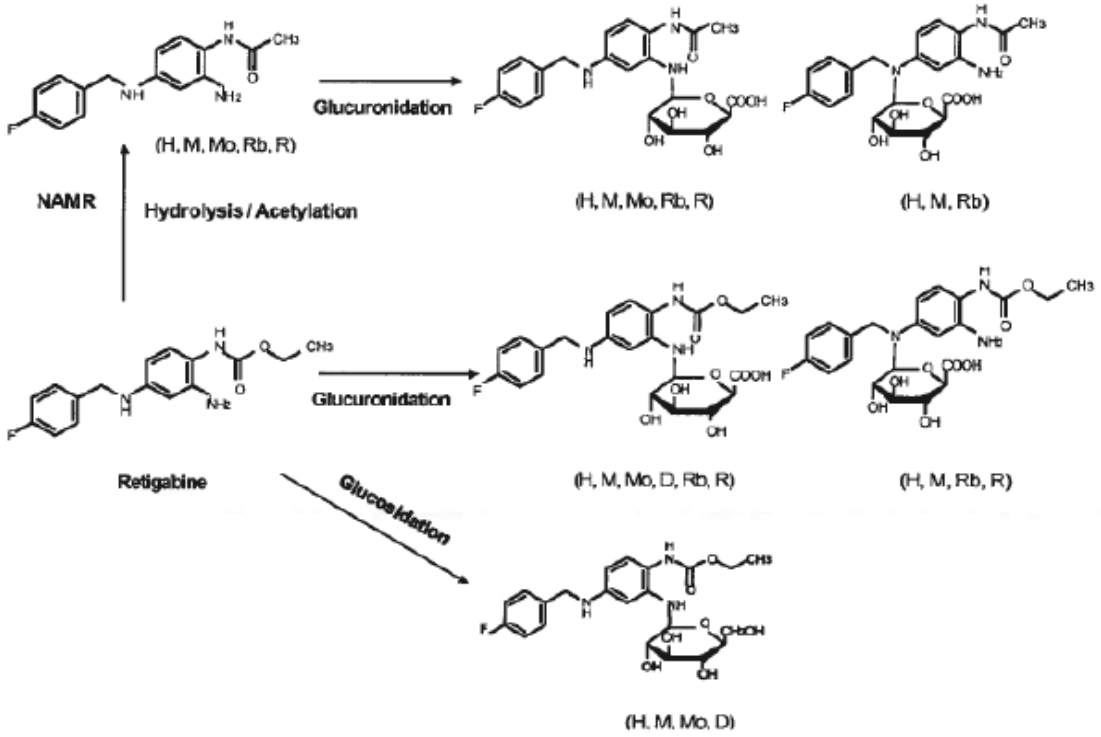
a: Comparative TK data from repeat-dose studies are summarized in 2.6.7.3
b: RHCl normalized for RTG content.
c: Value for radioactivity
d: Value for RTG
e: One animal had been excluded due to aberrant results.

Table IIIA.2 Comparative Pharmacokinetic Data and Systemic Exposure to NAMR following Single Oral Administration to Rats and Dogs

Species [Formulation]	Gender [M/F]	Dose [mg/kg]	Pharmacokinetic Parameters						Report No.
			C_{max} [ng/mL]	t_{max} [h]	AUC_{0-24} [ng*h/mL]	$AUC_{0-\infty}$ [ng*h/mL]	$t_{1/2}$ [h]	abs. BA [%][b]	
Rat (solution)	M	5	1,590[a] 695[b]	0.25[a] 0.25[b]	ND	15,900[a] 5,190[b]	8.04[a] 3.78[b]	ND[a] 157[b]	PR2007-006
Dog	M	5	5,380[a] 1,507[b]	1.5[a] 0.5[b]	ND	26,600[a] 4,525[b]	2.98[a] 2.64[b]	ND[a] 88.5[b]	PR2007-007

a: Value for radioactivity
b: Value for NAMR

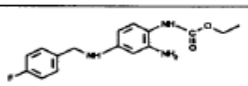
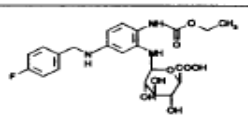
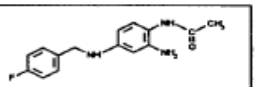
Figure IIIA.1 Major Metabolic Pathways of RTG in Different Species Based on *in Vitro* Studies



D=dog; H=human; M=monkey; Mo=mouse; Rb=rabbit; R=rat

a Human, monkey, dog, rabbit, rat and mouse liver microsomes and hepatocytes were employed.

Table IIIA.3 Species comparison in the metabolism of retigabine (D-23129) following oral dosing

Species/ Compartment		 D-23129	 Major N-Glucuronide	 Acetyl Metabolite
Man	P	+	+	+
	U	+	+	+
	F[2)]	+	-	-
Rat	P	+[1)]	+[1)]	+[1)]
	U	+	-	+
	F[2)]	-	+	-
	B	+	+	-
Dog	P	+[1)]	+[1)]	-[1)]
	U	+	+	-
	F[2)]	+	-	-
Mouse	P	+	+	+
	U	+	+	+
	F[2)]	+	-	-

Notes:
P plasma, U urine, F faeces, B bile
+ identified by LC/MS, - not detectable
[1)] HPLC peak was enlarged by spiking with the authentic substance or an isolated sample which was characterized by LC/MS/MS
[2)] With the exception of dog who excretes exclusively D-23129, the major part of the drug-related radioactivity in faeces remains to be unknown so far.

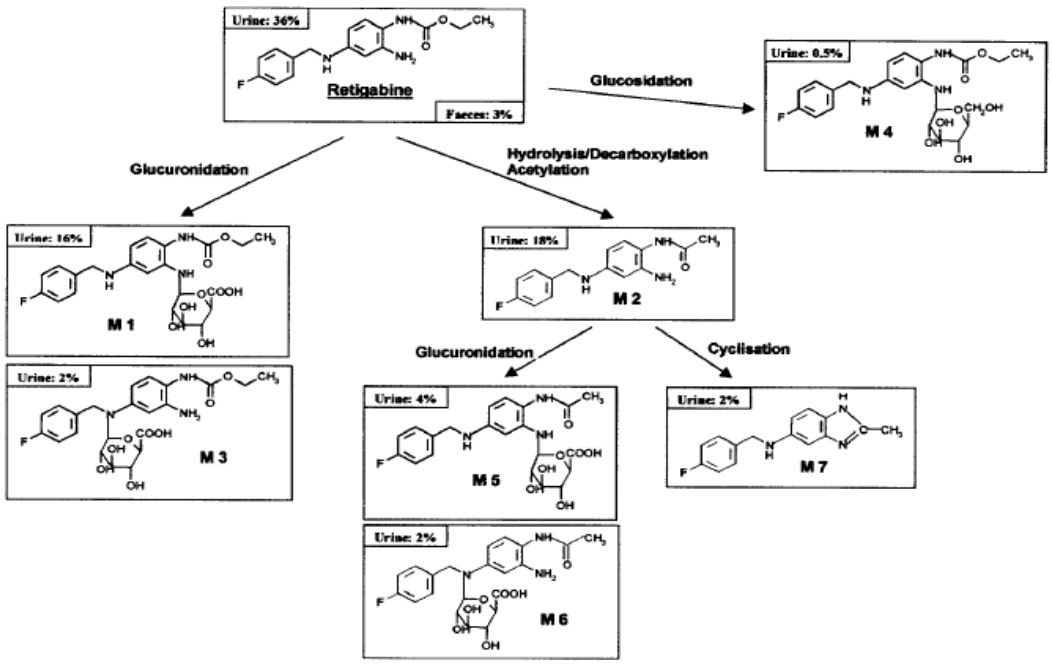
B. METABOLISM

N-acetylation is a minor pathway in mice, while rats produced significant amounts of NAMR and dogs produced negligible amounts or none (see **Tables IVB.1.1** and **IVB.2.1**). In metabolism studies using radiolabeled RTG (~10 mg/kg in CD-1 mice; 8.25 mg/kg in S-D rats), the percentages of total radioactivity in plasma for [14C]-RTG ranged between 4.4% and 6.1% in mice versus 57% and 38% in rats at the same time points (0.5, 1.5, and 4 hr) indicating more extensive metabolism in mice. The percent radioactivity made up by NAMR was not calculated, but in rats the highest levels of radioactivity in plasma appeared 4 h after dosing and were represented predominantly by RTG-N-glucuronide and NAMR which had similar relative peak areas, while NAMR was below the level of detection in mouse plasma at the dose used. Based on Cmax or AUC, parent drug represented approximately 50 and 5% of total radioactivity in plasma of rats and dogs, respectively, following oral administration of a dose of 8.25 mg/kg [14C]-RTG. RTG and NAMR represented only a small fraction of the plasma circulating [14C]-RTG-derived material in humans administered an oral dose of 200 mg. The contribution of RTG and NAMR to the Cmax of total radioactivity was in the range of 4.9 to 13.4% and 2.0 to 5.0 %, respectively. The contribution of RTG and NAMR to the AUC of total radioactivity was in the range of 3.3 to 8.4% and 3.5 to 6.5%, respectively. Therefore, other RTG metabolites account for more than 90% of total radioactivity in the plasma and RTG N-glucuronide appeared to be the dominant metabolite. Based on the mean plasma concentrations of RTG and total radioactivity, unchanged drug accounted for 3.9 to 11.0% of the total radioactivity at all time points, indicating extensive metabolism.

Overall, the metabolism of retigabine is dominated by *N*-glucuronidation. In the plasma of rats, dogs, and humans, the retigabine N2-glucuronide, in which the site of glucuronidation is the primary amino group in position 2 (**Figure IIIB.1;** M1), predominates over the N4-glucuronide (glucuronidation of the secondary

amino group in position 4; M3). Thus, the N2-glucuronide represents the major plasma metabolite in these species. This is supported by the amounts of the N-glucuronides quantified in human urine samples after a single oral 200-mg dose of [^{14}C]-retigabine (ie, 16% and 2% of the administered dose). Comparison of the in vivo and in vitro kinetics of retigabine *N*-glucuronidation identified a constant ratio between retigabine and retigabine *N*-glucuronide in vivo in humans and dogs indicating probable enterohepatic circulation of retigabine via retigabine *N*-glucuronide. Since retigabine N-glucuronides were shown to be substrates of β-glucuronidase, it was suggested that the observed in vivo equilibrium between retigabine and retigabine N-glucuronides reflects bidirectional glucuronidation and de-glucuronidation processes. Rats did not show this phenomenon. Studies to identify the isoforms of UGTs that contribute to retigabine glucuronidation involved inhibition of retigabine glucuronidation by bilirubin and lamotrigine. Lamotrigine, which is mainly glucuronidated by UGT1A4, inhibited the in vitro retigabine N-glucuronidation in human liver microsomes by about 80% indicating that is isoenzyme plays an important role in formation of retigabine N-glucuronide in humans. In humans, it is know that two isoenzymes, UGT1A1 and UGT1A4, are involved in the formation of bilirubin glucuronides, with UGT1A4 playing only a minor role. Bilirubin glucuronidation in rats is catalyzed mainly by UGT1A1 and to a lower extent by UGT1A2. Formation of retigabine N-glucuronidation was inhibited by only 25% in the presence of bilirubin (330 mM) in human microsomes, while in rat liver microsomes bilirubin inhibited retigabine N-glucuronidation by 90%. These results indicated that the UGT1A1 and UGT1A2 are more important for retigabine glucuronidation in rats compared to humans; in humans UGT1A4 seems more important. Additionally, UGT1A1, UGT1A3, and UGT1A9 are thought to contribute to retigabine N-glucuronidation in humans.

Figure IIIB.1



Scheme 9: Metabolic pathways of [^{14}C]retigabine identified in healthy male volunteers after oral administration of a 200-mg dose in a capsule. Excreted amounts in urine (0-72 h) and faeces (0-96 h) were expressed as percentage of dose administered. In addition, three metabolites (RT129, RT137, RT168) of unknown structure excreted in faeces amounted to a total of 7.5% of the dose on average.

IV. TOXICOLOGY

Toxicology studies consisted of general toxicity of up to 13 weeks in mice, 26 weeks in rats, and 1 year in dogs; *in vitro* and *in vivo* genetic toxicology studies; fertility and early embryonic development in rats, embryo-fetal development in rats and rabbits and pre- and post-natal development study in rats; juvenile rat studies; and carcinogenicity studies in rats and neonatal mice. Additional studies were conducted with the major metabolite (NAMR); these included repeat-dose studies in rats and dogs and in vitro genotoxicity studies. In humans, RTG is mainly metabolized by N-glucuronidation and by formation of NAMR, an N-acetyl metabolite. These human metabolites are found in rat, mouse, and rabbit plasma, but circulating levels of NAMR are lower in these species than in humans (much lower in rabbits and mice). Dogs do not form NAMR. Therefore, 3-month repeat dose studies of NAMR in rats and dogs were conducted.

A. Single-Dose

Single-dose oral toxicity studies of RTG and RHCl were conducted in mice and rats (**Table IVA.1.1**).

Single dose oral (gavage) administration to NMRI mice at doses from 21.5 to 1000 mg/kg (propylene glycol vehicle) produced hypokinesia (males>females), sunken sides (males), and slight to moderate clonic convulsions (females) at 215 mg/kg and hypokinesia, stilted or staggered gait, decreased muscle tone, loss of righting reflex, hypothermia, and moderate to severe clonic convulsions (all animals) at 1000 mg/kg. Two HD males died within 24 hours. Necropsy findings included a tightly filled bladder and black contents in the intestine of 1 HD male and a reddened intestine in 3 HD females.

In another study in NMRI mice with single oral gavage doses of from 10 to 464 (males) or 21.5-1470 mg/kg (females), clonic convulsions were seen at ≥46.4 mg/kg in males and ≥464 in females, and other signs included tremor, decreased muscle tone, low body surface temperature, sunken sides, loss of the righting, pinnar or corneal reflex, and abnormal respiration, present immediately after dose administration. Deaths occurred between 2 hours and 2 days after treatment at the HD in both sexes (1/5 males, 5/5 females). Necropsy revealed a reddened stomach or a reddened mucous membrane of the glandular stomach in animals that died.

When RTG was administered to Wistar rats by oral gavage at doses ranging from 4.64 to 100 mg/kg, convulsions, tremor, decreased muscle tone, staggered gait, loss of righting reflex, and hypokinesia were seen at ≥46.4 mg/kg. One HD male died 4 days after treatment. No treatment-related gross necropsy findings were reported.

After single oral gavage administration of doses from 4.64 to 464 mg/kg to Wistar rats, clonic convulsions on handling, a decrease in muscle tone, loss of righting reflex, tremor, and ataxia were seen at 46.4 mg/kg in most animals (only given to females) and moderate to severe clonic convulsions, decreased muscle tone, and loss of righting reflex at 100 mg/kg in both sexes. Two females died within 4 days of treatment, and the jejunum of 1 was filled with a red liquid at necropsy. At 215 mg/kg, clonic convulsions, tremor, staggered gait, decreased muscle tone, general loss of reflexes, low body surface temperature, and abnormal respiration were observed in most animals. Three males died within 3 days of treatment; all females died within 2 days. Necropsy found red liquid in the jejunum in 2 of the females. At 464 mg/kg (given to males only), moderate to severe clonic convulsions, decreased muscle tone, loss of righting and pinna reflexes, staggered gait, ataxia, tremor, and loss of pain reflex were seen, and all animals died within 24 hours after treatment.

Table IVA.1.1 NOAELs, Onset of Clinical Signs and Deaths in Acute Toxicity Studies using Rodents

Route	Species	Test substance	NOAEL for clinical signs (mg/kg)	Dose showing onset of clinical signs (mg/kg)	Dose levels where deaths observed (mg/kg)
Oral / gavage	Mouse [N]	RTG	215	215	1000
	Mouse [N]	RHC1	215	46.4	≥464
	Rat [W, C]	RTG	46.4	25	≥100
	Rat [W]	RHC1	46.4	46.4	≥100
Intravenous	Mouse [N]	RHC1	10	10	≥21.5
	Rat [W]	RHC1	21.5	21.5	≥46.4

N NMRI
W Wistar
C CD

B. Repeat Dose

1. Mice

In a TK study (Report No. D-23129/3000922937) in which RTG was administered by oral gavage to CD1 mice for 10 days at doses of 30 (males), 60 (males), or 120 mg/kg (males and females), no mortality was observed but clinical observations included decreased motor activity, dyspnea and tachypnea at the MD and HD, and ataxia at the HD. Low carriage, tremors, and hypersensitivity were seen in HD females and salivation, rough hair coat, yellow discoloration of the perineal pelage, distended abdomen, and rales were observed in HD males; a higher proportion of females displayed clinical signs compared to males despite higher exposures to parent in males. Distended abdomen was considered to reflect distended bladder as a result of inhibition of micturation. Macroscopic observations included distended bladder (2/21 males at MD, 3/21 males at HD, thickened bladder walls (5/21 males, 1/21 female at HD), and discolored bladder (1/21 males at HD).

In an 8-week dose range-finding study in NMRI mice (D-23129/3000914253) in which escalated doses of up to 1000 mg/kg (for only 1 week) were administered in the diet, no mortality occurred but hypokinesia, clonic convulsions, piloerection, and ptosis were seen at the HD. Only macroscopic examination was performed and revealed pale kidneys and tightly filled urinary vesicles in 5/10 HD group (1000 mg/kg) mice. TK data (**Table IVB.1.1**) showed markedly higher levels in males than in females at higher doses.

Table IVB.1.1 Plasma exposures to retigabine and NAMR in 8-week dietary study in NMRI mice

Dose		147 mg/kg	464 mg/kg	681 mg/kg	1,000 mg/kg
Male Mice					
AUC_{0-24} ng/mL·h	RTG	16,930	71,340	150,390	432,960
AUC_{0-24} ng/mL·h	NAMR	3,856.2	3,421.5	6,030.6	12,074.2
Female Mice					
AUC_{0-24} ng/mL·h	RTG	15,730	35,270	66,130	96,920
AUC_{0-24} ng/mL·h	NAMR	597.6	1,463.2	1,686.2	3,507.5

NAMR: N-acetyl metabolite of RTG (AWD21-360)
Values were calculated from sampling intervals of 6 hours and a maximum of two samples per time point

In a 13-week dietary study in CD1 mice at doses of 50, 120, and 225 mg/kg (Report No.D-23129/9321020042; conducted by (b) (4) 4/20/00, GLP), treatment-related (T-R) deaths were seen at the MD and HD and clinical observations occurred at all doses in a dose-related (D-R) incidence and frequency. The most prominent observations were consistent with effects on the urinary bladder (distended abdomen, abdominal discoloration, and yellow discoloration of the perineal pelage), or were changes that were considered secondary to the effects on the urinary bladder (probable dermatitis characterized by focal lesions, swollen areas, discoloration and nodules on the tail, penis, scrotum, abdomen, feet, and/or legs). Body weight (BW) gain (pretest to week 13) was significantly higher (14%) in HD females and was thought to reflect urinary retention. T-R hematology changes included increased platelet counts (25% in HD females), WBCs (72% in HD males), lymphocytes (44% in HD males), neutrophils *(*232% in HD males), mononuclear cells (148% in HD males), and eosinophils (125% in HD females). These were attributed to inflammatory changes of the skin. T-R clinical chemistry changes included increased BUN (39-46% in MD and HD males) and BUN:creatinine ratio *(*37-40% in MD and HD in males). These were attributed to decreased glomerular filtration and were associated with microscopic changes in the kidneys in these groups. Another clinical chemistry parameter affected by retigabine was total bilirubin (TBIL), which was increased (42-108%) in males from all dose groups and (74%) in HD females. This was thought to be due to interference of retigabine with the analytical method used to measure bilirubin, as was reported in an earlier study performed by (b) (4) or competition of retigabine with hepatic glucuronyltransferase enzymes (which metabolize bilirubin, and which are the main pathway for metabolism of retigabine in many species). At final necropsy, T-R lesions included macroscopic and microscopic dilatation of the urinary bladder, ureters, and renal pelvises in males at all doses (**Table IVB.1.2**). Additional microscopic changes included urinary bladder mucosal epithelial hyperplasia, submucosal edema, and serosal mixed cell inflammation; urethral dilatation, and bone marrow myeloid hyperplasia in MD and HD males. Urinary bladder dilatation, mononuclear cell infiltration, and serosal mixed cell inflammation were seen in MD and HD females. The urinary system changes were thought to result from direct effects of retigabine on the urinary bladder smooth muscle and/or due to obstruction of urine flow resulting in uremia. Urine accumulated in the bladder resulting in secondary dilatation of the ureters, renal pelves, and urethra. In addition, macroscopic and microscopic inflammatory skin lesions occurred in the perineal region of male mice and were considered to be secondary to urine scald. The lesions in the urinary system and bone marrow were accompanied by increased BUN (urinary lesions), total WBCs, neutrophil, and monocyte counts (bone marrow myeloid hyperplasia) that occurred in MD and HD males. Increased (10-16%) absolute and relative kidney weights were also seen in treated males and HD females. Liver weights were also slightly increased (8-10%) in HD males. TK data from this study are shown in **Table IVB.1.3**.

Table IVB.1.2 Microscopic findings in 13-week dietary study in CD1 mice

GROUP INCIDENCES (WITH AVERAGE SEVERITIES) OF RETIGABINE-RELATED MICROSCOPIC OBSERVATIONS AT FINAL NECROPSY

	MALE DOSAGE (mg/kg)				FEMALE DOSAGE (mg/kg)			
LESION	0	50	120	225	0	50	120	225
Urinary Bladder[a]	14	14	13	11	15	15	15	13
Dilatation	0	6	8	11	0	0	1	3
	(0.0)	(1.1)	(1.3)	(2.5)	(0.0)	(0.0)	(0.1)	(0.2)
Epithelial hyperplasia	0	0	3	5	0	1	0	1
	(0.0)	(0.0)	(0.4)	(0.6)	(0.0)	(0.1)	(0.0)	(0.1)
Mononuclear cell infiltration	0	0	1	1	1	1	8	5
	(0.0)	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)	(0.5)	(0.5)
Mixed cell inflammation	0	2	2	0	0	0	0	2
	(0.0)	(0.3)	(0.2)	(0.0)	(0.0)	(0.0)	(0.0)	(0.2)
Intracytoplasmic eosinophilic inclusions	0	0	3	2	0	1	1	1
	(0.0)	(0.0)	(0.2)	(0.3)	(0.0)	(0.1)	(0.1)	(0.1)
Edema	0	2	3	0	0	0	0	0
	(0.0)	(0.3)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Ureters[a]	13	14	13	11	15	15	14	12
Dilatation	0	6	5	5	0	0	0	0
	(0.0)	(0.4)	(0.5)	(0.8)	(0.0)	(0.0)	(0.0)	(0.0)
Urethra[a]	13	14	13	11	14	15	15	13
Dilatation	0	0	2	6	0	0	0	0
	(0.0)	(0.0)	(0.4)	(0.9)	(0.0)	(0.0)	(0.0)	(0.0)
Kidneys[a]	14	14	13	11	15	15	15	13
Pelvic Dilatation	1	4	5	5	0	0	0	0
	(0.1)	(0.5)	(0.7)	(0.8)	(0.0)	(0.0)	(0.0)	(0.0)
Bone Marrow[a]	14	14	13	11	15	0	0	13
Myeloid hyperplasia	0	0	3	8	0	0	0	0
	(0.0)	(0.0)	(0.2)	(1.1)	(0.0)	(0.0)	(0.0)	(0.0)
Skin[a]	14	1	4	11	15	1	0	13
Neutrophilic inflammation	0	1	2	2	0	0	0	0
	(0.0)	(4.0)	(2.0)	(0.5)	(0.0)	(0.0)	(0.0)	(0.0)
Mixed cell inflammation	0	0	2	1	0	0	0	0
	(0.0)	(0.0)	(1.0)	(0.3)	(0.0)	(0.0)	(0.0)	(0.0)

a: Number examined.
( ): Average severity (0 = no microscopic lesion, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe).

Table IVB.1.3 TK data in 13-week dietary study in CD1 mice

Daily Dose (mg/kg)	M: 50	M: 120	M: 225	F: 50	F: 120	F: 225
Dosing Day 94						
C_{max} (ng/mL)	358	1,512	1,971	BLQ	442	581
AUC $_{0-24}$ (ng·h/mL)	5,441	22,750	35,077	BLQ	2,783	4,742
AUC $_{0-24}$ / Dose	109	190	156	n.a.	23	21

N = up to 3 per time point and dose BLQ: below limit of quantification

In a 13-week dietary study in B6C3F1 mice at doses of 120, 225, and 500 mg/kg (Report No. D-23129/3000922904; conducted by (b) (4), 4/19/00, GLP), there were 14 deaths considered T-R (2 MD males, 12 HD [11 males and 1 female]) and T-R clinical observations were seen at all doses. As in CF1 mice, these were thought to be consistent with effects on the urinary bladder (distended abdomen, abdominal discoloration, abdominal masses, and yellow discoloration of the perineal pelage), or were changes that were considered secondary to the effects on the urinary bladder (probable dermatitis characterized by focal lesions, swollen areas, and discoloration of the tail, penis, scrotum, inguinal region, feet, and/or abdomen). Hematology changes included small (<10%) decreases in RBC parameters in MD and HD males, and increased PLT (14 and 42% in MD and HD males), WBC (30% in HD males), NEU (53 and 195% in MD and HD males), and MONO (97% in HD males). The RBC effect was attributed to the compromised health status, while the increased WBCs were attributed to inflammatory changes of the skin (reflecting the probable dermatitis described above) or urinary bladder. T-R clinical chemistry changes included increased ALT (50-115% in males at all doses), BUN (30-70% in males at all doses and 27% in HD females); BUN:creatinine ratio *(*16-57% in males from all groups and 27% in HD females). The increased ALT was associated with microscopic changes in the liver. Increased BUN and *B/CR* was associated with microscopic changes in the kidneys. Total bilirubin (TBIL) was increased 70-200% in males and up to 100% higher in females, but, again, this was attributed to interference of retigabine with the analytical method used to measure bilirubin or competition of retigabine with hepatic glucuronyltransferase. Hepatic centrilobular hypertrophy was seen primarily in males, consistent with induction of hepatic enzymes. At necropsy, urinary bladders were distended and/or renal cortices were discolored. Microscopically, urinary bladders were dilated and/or renal tubular degeneration was present. In the TK groups, 2 MD males and 7 HD males were found dead or electively euthanized. RTG-related deaths were considered to result from urinary tract disease likely resulting from obstruction and subsequent uremia. Macroscopically, at final necropsy, T-R urinary bladder distention, thickened wall, abnormal content, and calculus, renal cortical discoloration, and renal pelvic dilatation were noted as were multiple skin abrasions. Microscopically (**Table IVB.1.4**), urinary bladders were dilated with an attenuated muscular layer. Increased intracytoplasmic eosinophilic inclusions were noted in urinary bladder transitional epithelium. HD males had intraluminal debris in the urinary bladder. Renal pelvic dilation and renal tubular degeneration were observed primarily in HD males and females. As before, the microscopic findings were attributed to altered urinary bladder peristalsis leading to urinary bladder distention, increased hydrostatic pressure, and uremia. These findings were observed in multiple mouse studies with RTG. Skin lesions, noted microscopically in the perineal area were attributed to urine scalding. At final necropsy, increased liver weights were seen at all doses in males and in MD and HD females. Microscopically, centrilobular hepatocellular hypertrophy was found in males at all doses and in 1 HD female. Discolored thyroids noted at final necropsy correlated with thyroid follicular cell hypertrophy in males and females and intracolloidal red brown pigment in HD males and females. Vacuolated follicular epithelial cells and an increased incidence of necrotic epithelial cells (single cell necrosis) were seen in treated males and females. The thyroid lesions were considered to be secondary to the centrilobular hepatocellular hypertrophy, since this was associated with increased UDP glucuronyltransferase enzymes, which "can increase T3 and T4 metabolism and result in a compensatory follicular cell hypertrophy to restore thyroid hormone homeostasis." TK data from this study are shown in **Table IVB.1.5**.

Table IVB.1.4 Microscopic findings in 13-week dietary study in B6C3F1 mice

Protocol: 98044 GROUP INCIDENCES OF MICROSCOPIC OBSERVATIONS Printed: 07-Feb-00

Controls from group(s): 1

Tissues With Diagnoses	Animal sex:	-- Animals Affected -- Males --				-- Animals Affected -- Females --			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
	No. in group:	15	15	15	11	15	15	15	14
GALLBLADDER ..... Number examined:		15	0	0	11	15	0	0	13
HEART ..... Number examined:		15	0	0	11	15	0	0	14
MYOCARDIAL DEGENERATION		3	0	0	2	0	0	0	0
MONONUCLEAR CELL INFILTRATE		0	0	0	0	0	0	0	1
ILEUM ..... Number examined:		15	0	0	11	15	0	0	14
JEJUNUM ..... Number examined:		15	0	0	11	15	0	0	14
KIDNEYS ..... Number examined:		15	15	15	11	15	15	15	14
MONONUCLEAR CELL INFILTRATE		3	10	8	1	2	5	5	8
MIXED CELL INFLAMMATION		2	0	0	0	0	0	0	0
TUBULAR DEGENERATION		1	0	0	5	1	0	1	4
PELVIC DILATATION		0	0	0	1	0	0	0	1
PAPILLARY NECROSIS		0	0	0	1	0	0	0	0
TUBULAR CAST		0	0	0	1	0	0	0	1
TUBULAR BASOPHILIA		0	0	1	0	0	0	0	1
TUBULAR NECROSIS		0	0	0	0	0	0	0	1
CYST		0	1	0	0	0	0	0	0
LIVER ..... Number examined:		15	15	15	11	15	15	15	14
EXTRAMEDULLARY HEMATOPOIESIS		5	1	2	1	4	2	2	5
MONONUCLEAR CELL INFILTRATE		2	2	2	3	5	6	5	2
NECROSIS		0	0	0	0	0	0	0	1
MIXED CELL INFLAMMATION		0	0	0	1	1	0	0	0
CENTRILOBULAR HYPERTROPHY		0	11	15	11	0	0	0	1
FATTY CHANGE		0	0	0	0	0	0	0	0
SINGLE CELL NECROSIS		0	1	0	0	4	5	5	2
VACUOLATION		0	0	0	0	0	0	0	2
LUMBAR CORD ..... Number examined:		15	0	0	11	15	0	0	14
LUNG ..... Number examined:		15	0	0	11	15	0	0	14
ALVEOLAR MACROPHAGES		1	0	0	0	1	0	0	0
GRANULOMA		0	0	0	0	1	0	0	0
GRANULOMATOUS INFLAMMATION		0	0	0	0	0	0	0	1
URINARY BLADDER ..... Number examined:		15	15	15	11	15	15	15	14
DILATATION		0	4	14	11	0	0	0	2
INTRALUMINAL DEBRIS		0	0	1	6	0	0	0	0
MONONUCLEAR CELL INFILTRATE		0	0	6	2	5	6	6	4
MIXED CELL INFLAMMATION		0	0	0	3	0	0	0	1
EDEMA		0	0	1	0	0	1	0	1
FIBROSIS		0	0	0	0	0	0	0	0
INTRACYTOPLASMIC EOSINOPHILIC INCLUSIONS		0	15	15	11	0	15	15	14
UTERUS ..... Number examined:						15	0	0	14
ATROPHY						0	0	0	0
VAGINA ..... Number examined:						15	0	0	14
NEUTROPHILIC INFLAMMATION						0	0	0	0

All Diagnoses; Phases: All; Death types: Scheduled FS; Date of death range: 17-Sep-99 To 21-Sep-99

Table IVB.1.5 TK parameters for 13-week dietary study in B6C3F1 mice

PARAMETER[a]	SEX	GROUP V (120 mg/kg)	GROUP VI (225 mg/kg)	GROUP VII (500 mg/kg)
C_{max} (ng/mL)	Male	1281 ± 287	2379 (n = 2)	9228 (n = 2)
"	Female	535 ± 128	1381 ± 756	3768 ± 1168
AUC_{0-24} (ng·hr/mL)	Male	16929 ± 1853	40790 ± 4267	123382 ± 18725
"	Female	5654 ± 1004	13973 ± 3278	65143 ± 10653
T_{max} (time; day 95)	Male	10 pm	10 pm	10 pm
"	Female	6 pm	10 pm	10 pm

a: Data for C_{max} and AUC_{0-24} presented as mean ± standard error.

2. Rats

In repeated oral (gavage) studies in rats (4, 5, 13 weeks; previously reviewed, IND 53,950, review dated 9/11/97), clinical signs of CNS toxicity (hypoactivity, decreased muscle tone, ataxia, prostration, tremors, convulsions) were seen at >8.25 mg/kg and deaths occurred at doses as low as 60.5 mg/kg (base). There were consistent findings indicating effects on the liver (increased ALT and ALP, increased liver weights, centrilobular hypertrophy) and thyroid (increased T4, follicular cell hypertrophy/hyperplasia). The thyroid effects were thought to be secondary to induction of liver enzymes. Both effects were reversible. Decreases in absolute brain weights (5-8%) were seen in male rats in both the 5- and 13-week studies. Bladder effects were somewhat inconsistent in the rat studies and appeared to be less pronounced than in mice. Increased urinary bladder weights, reddish discoloration of the urinary bladder observed macroscopically, and histopathological findings of congestion of submucosal vessels and ectasia of the urinary bladder were increased primarily in males at the HD dose of 68.1 mg/kg in one of the two 13-week oral gavage studies in rats (Report No. D-23129/832094). TK parameters from this study are shown in **Table IVB.2.1.** In the 13-week dietary study in S-D rats (Report No.: D-23129/9321020143), 1 HD (150 mg/kg) female that died on day 71 exhibited a distended abdomen, decreased feces, decreased motor activity, and ataxia; distention of the urinary bladder macroscopically; and multifocal degeneration of the muscularis in the urinary bladder wall accompanied by edema and congestion microscopically. Death was thought likely uremia-induced due to the decreased ability to void urine. Another HD female rat had mild diffuse edema and transitional cell hyperplasia of the urinary bladder at final necropsy.

Dose selection for the rat carcinogenicity study was based on the results of a 26-week study in Wistar rats, with oral (gavage) doses of 0, 5.11, 17.8, and 61.9 mg/kg (Report No. D-23129/3000899032, previously reviewed, IND review dated 10/28/02).There was 1 death of a HD male that was considered possibly T-R (after first dose). This animal exhibited clinical signs, including clonic convulsions, but no cause of death was determined. CNS signs (clonic convulsions [severe in nearly all HD animals during first week], decreased activity, ataxia, hypersensitivity, loss of righting) were seen at ≥17.8 mg/kg, and decreased BW gain (BW 10% below C, SS in males only) and clinical chemistry changes (slight ↑ ALT, AP) were seen at the HD. Histopathology findings were limited to hepatocellular hypertrophy and thyroid follicular cell hypertrophy in HD males and females. No urinary bladder effects were reported. According to the sponsor's dose rationale, the HD in this study exceeded the MTD based on the 1 death; therefore, the HD for the carcinogenicity study was reduced to 51.1 mg/kg. Follow-up expanded histological examination including GFAP-staining was conducted on brain tissue from C (5/sex) and HD (10/sex) rats

from this study to assess possible NMDA-receptor mediated neurotoxicity. There were no reported neurohistopathological changes related to treatment.

In another 26-week rat study with dietary administration (Report No. D-23129/3000913454, previously reviewed, IND review dated 10/28/02) in which Wistar rats were administered doses of 0, 31.6, 68.1, 82.5, 100, or 147 mg/kg, clinical signs similar to those seen in the gavage study were first seen at ≥82.5 mg/kg; 1 HD male died (killed in extremis on day 177); and histopathological changes were seen in the mesenteric lymph nodes (increased deposition of a yellow brown pigment [lipofuscin] in macrophages), pancreas (focal congestion) and intestinal tract (increased diameter, pigment deposition) at ≥68.1 mg/kg, in the urinary bladder (ulcerations of the bladder mucosa associated with diffuse hyperplasia and with sandy uroliths) at ≥100 mg/kg, and in the kidney (renal pelvic dilatation) and thyroid (follicular cell hypertrophy) at 147 mg/kg. The HD male that died had marked focal necrosis of the liver. According to the report it was unclear whether this was T-R or secondary to the poor general condition of the animal. Three HD males had moderate to marked ulcerations of the urinary bladder mucosa associated with diffuse hyperplasia and sandy uroliths. Diffuse hyperplasia of the urinary bladder mucosa was also seen in 2 MHD males, 1 of which had sandy uroliths and the other focal hemorrhages, and uroliths were noted in 1 MLD male. No bladder findings were reported in females. BW effects were more pronounced with dietary administration and were considered excessive; mean BWs were decreased by 11, 17, 22, 23, and 36% in males and 14, 15, 16, 21, and 22% in females from the respective dose groups compared to C at the end of the 26-week study (food consumption reduced by similar amounts). Microscopic urinary bladder findings from this study are shown in **Table IVB.2.2**. Comparison of TK parameters from the two 6-month studies are shown in **Table IVB.2.3**.

In response to Division concerns about the major human metabolite NAMR (unusual toxicity in dogs [see below], plasma metabolite levels in rats below those expected in humans), a 13-week toxicity study of the metabolite was conducted in Wistar rats (20/sex/grp) at doses of 0 (PG vehicle), 10, 30, or 120 (males)/100 (females) mg/kg/day (Report No. PR2007-017, conducted by (b) (4) report dated 5/508, GLP). Dose selection was based on results of a 7-day rat study of NAMR with doses of 100, 300, and 1000 mg/kg in which mortality was seen at the MD (females) and HD (dosing stopped on day 3 due to excessive mortality) and clinical signs at these doses (including decreased activity, impaired righting reflex, impaired limb function, splayed limbs, hunched posture, tremors) were considered severe. RBC parameters were increased somewhat at 300 mg/kg, while total leukocytes, neutrophils, and monocytes were increased in 1 of 2 females at this dose, thought to be consistent with an inflammatory response. Total bilirubin, primarily unconjugated, was increased in MD males (6-fold) and females (5-fold). Increases in ALT (2-fold) and creatinine (2-fold) were seen in MD males and ALP (3-fold) was increased in MD males and females. Thus, there was evidence of kidney and liver toxicity at ≥300 mg/kg. Increased creatinine at the MD and increased BUN and creatinine at the HD were indicative of renal failure, possibly due to urinary bladder changes. Distention of the urinary bladder with urine was observed macroscopically at necropsy in females receiving ≥300 mg/kg. One HD TK female that died prior to study completion exhibited mild dilation of the right kidney, consistent with the urinary bladder distension observed in treated animals. In the 13-week study, mortality was observed in 1 C female, 4 MD (2 males and 2 females), and 1 HD female on Days 90, 66, 63, 28, 65, and 91, respectively. The cause of death in these animals was undetermined but they were not considered T-R. Clinical signs were limited to increased incidences of salivation, discolored hair, audible breathing, and rales. There were no apparent T-R effects on body weight, food consumption, ophthalmoscopy, hematology, urinalysis, immunologic (leukocyte phenotype determinations, natural killer cell activity), or macroscopic parameters. Small (<2X) but SS increases in phosphorus, ALP, AST, ALT, and bilirubin were observed in HD males. Increased cholesterol (SS at HD) and total bilirubin (all doses) were observed in treated females. As in other studies, the increased bilirubin was thought to be at least partially analytical artifact. Liver weights were increased in males at all dose levels and in MD and HD females, and this correlated with increases in AST, ALT, and total bilirubin and with minimal to mild centrilobular hypertrophy at the HD. This and thyroid follicular cell hypertrophy and c-cell hyperplasia, seen primarily at the MD and HD, were the only histopathology

findings reported. All effects observed at the end of dosing were partially or completely resolved at the end of recovery. TK parameters of NAMR (3/sex/group/time pt) are shown in **Table IVB.2.4.**

Table IVB.2.1 TK parameters for RTG and NAMR in 13-week toxicity study of RTG in Wistar rats

Pharmacokinetic Parameters of RTG (Day 1 and Week 13)								
	male				female			
Dose (mg/kg)	**5.11**	**12.10**	**28.70**	**68.10**	**5.11**	**12.10**	**28.70**	**68.10**
	Day 1							
t_{max} (h)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
C_{max} (ng/mL)	629.73	1,039.96	1,675.37	3,771.11	681.47	1,017.39	1,886.26	5,154.39
AUC_{0-24} (ng·h/mL)	4,224.14	8,013.93	12,871.77	34,995.38	4,960.54	7,950.07	18,003.97	36,268.32
$t_{1/2}$ (h)	nc	3.70	7.58	nc	3.60	2.87	5.71	nc
	Week 13							
t_{max} (h)	1.50	3.00	0.50	1.50	0.50	0.50	1.50	0.50
C_{max} (ng/mL)	628.70	1,180.58	1,740.37	3,175.56	630.04	1,399.19	2,353.25	4,744.00
AUC_{0-24} (ng·h/mL)	5,308.75	13,385.32	23,217.15	43,361.25	5,705.89	14,109.08	27,800.26	45,391.43
$t_{1/2}$ (h)	nc	3.84	5.37	9.72	nc	3.56	3.75	10.72
Accumulation Factor	1.26	1.67	1.80	1.24	1.15	1.77	1.54	1.25

Pharmacokinetic Parameters of NAMR (Day 1 and Week 13)								
	male				female			
Dose (mg/kg)	**5.11**	**12.10**	**28.70**	**68.10**	**5.11**	**12.10**	**28.70**	**68.10**
	Day 1							
t_{max} (h)	3.00	3.00	6.00	6.00	3.00	3.00	6.00	3.00
C_{max} (ng/mL)	265.62	456.70	588.68	1,375.69	285.77	478.89	817.77	1,166.20
AUC_{0-24} (ng·h/mL)	3,471.21	5,467.02	9,745.30	23,552.03	3,925.04	6,130.41	12,675.10	23,060.71
$t_{1/2}$ (h)	nc	4.84	13.31	nc	3.60	4.42	10.44	40.09
	Week 13							
t_{max} (h)	3.00	6.00	6.00	6.00	3.00	6.00	6.00	6.00
C_{max} (ng/mL)	242.31	575.59	986.67	1645.84	266.32	583.98	1158.98	1184.70
AUC_{0-24} (ng·h/mL)	3,164.80	8,553.04	16,140.76	25,097.54	3,064.69	8,717.70	16,515.37	20,375.70
$t_{1/2}$ (h)	nc	6.59	12.34	nc	nc	6.31	8.05	12.25
Accumulation Factor	0.91	1.56	1.66	1.07	0.78	1.42	1.30	0.88

Given are the arithmetic mean plasma concentrations of RTG and NAMR from up to 3 animals per time point (nc = not calculated). Dose linearity of AUC_{0-24} was shown in the single-dose range from 5.11 to 68.1 mg/kg RTG with r^2 from 0.961 - 0.997 for RTG and 0.823 - 0.996 for the metabolite NAMR (the range comprises males and females and Day 1 and Week 13)

Accumulation factor = AUC_{0-24}(13 weeks) / AUC_{0-24}(Day 1)

Table IVB.2.2 Microscopic bladder findings in 26-week rat study with dietary administration

SEX :						MALE
DOSE GROUP:	01	02	03	04	05	06
NO.ANIMALS:	10	10	10	10	10	10
URINARY BLADDER CONT'D.	10	10	10	10	10	10
- Ulceration focal						
GRADE 3 :	-	-	-	-	-	2
GRADE 4 :	-	-	-	-	-	1
TOTAL AFFECTED :	-	-	-	-	-	3
MEAN SEVERITY :	-	-	-	-	-	3.3
- Hyperplasia diffuse						
GRADE 1 :	-	-	-	-	1	1
GRADE 2 :	-	-	-	-	1	-
GRADE 3 :	-	-	-	-	-	2
TOTAL AFFECTED :	-	-	-	-	2	3
MEAN SEVERITY :	-	-	-	-	1.5	2.3
- Uroliths/mineraliz.						
GRADE 1 :	-	-	1	-	1	-
GRADE 2 :	-	-	-	-	-	1
GRADE 5 :	-	-	-	-	-	1
TOTAL AFFECTED :	-	-	1	-	1	2
MEAN SEVERITY :	-	-	1.0	-	1.0	3.5
- Hemorrhage focal						
GRADE 2 :	-	-	-	-	1	-
TOTAL AFFECTED :	-	-	-	-	1	-
MEAN SEVERITY :	-	-	-	-	2.0	-

Table IVB.2.3 TK parameters for RTG in 4- and 26-week toxicity studies in Wistar rats

RETIGABINE EXPOSURE IN RATS

Regimen	Time	Dosage (mg/kg/day)	C_{max} (ng/mL) M	C_{max} (ng/mL) F	AUC (ng•hr/mL) M	AUC (ng•hr/mL) F	ERs (Relative to human 400 mg TID dose; no carbamazepine or phenytoin)[a] C_{max} M	C_{max} F	AUC M	AUC F	ERs (Relative to human 400 mg TID dose; with carbamazepine and/or phenytoin)[b] C_{max} M	C_{max} F	AUC M	AUC F
Once daily gavage[c]	Week 4	8.25	925	843	6006	5310	0.4	0.4	0.1	0.1	0.5	0.4	0.2	0.2
		26.1	2249	1962	15078	14580	0.9	0.8	0.4	0.4	1.2	1.0	0.5	0.5
		82.5	3109	3868	28464	37435	1.3	1.6	0.7	0.9	1.6	2.0	0.9	1.2
Once daily gavage[d]	Week 26	5.11	398	532	2837	2154	0.2	0.2	0.07	0.05	0.2	0.3	0.09	0.07
		17.8	649	1640	7060	10044	0.3	0.7	0.2	0.3	0.3	0.9	0.2	0.3
		61.9	3640	2866	30463	24781	1.5	1.2	0.8	0.6	1.9	1.5	1.0	0.8
Diet[e]	Week 26	31.6	994	641	20418	11815	0.4	0.3	0.5	0.3	0.5	0.3	0.7	0.4
		68.1	2254	1920	47194	36139	0.9	0.8	1.2	0.9	1.2	1.0	1.5	1.2
		82.5	1969	1896	39901	36962	0.8	0.8	1.0	0.9	1.0	1.0	1.3	1.2
		100	2665	2432	55660	52701	1.1	1.0	1.4	1.3	1.4	1.3	1.8	1.7
		147	4045	3801	90251	75925	1.7	1.6	2.2	1.9	2.1	2.0	2.9	2.4

Table IVB.2.4 TK parameters in 13-week rat toxicity study of NAMR

Table E. Toxicokinetic Parameters Estimated on the Mean Concentration of N-AMR vs. Time Data Following Once Daily Oral Administration of N-AMR in Rats

Day	Sex	Nominal Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{0-24} (hr*ng/mL)
1	Male	10	3	1953	15868	19068
		30	1	4260	48381	48381
		120	6	10960	160318	160318
	Female	10	3	2343	26192	26192
		30	1	4223	58225	58225
		100	1	12740	149173	149173
91	Male	10	1	2780	22369	22369
		30	1	7797	59714	59714
		120	3	14867	155734	155734
	Female	10	1	3630	34593	34593
		30	1	7697	71665	71665
		100	1	17400	167797	167797

3. Dogs

In a 28-Day Oral (TID Capsule) Toxicity Study in Dogs (Report No. D-23129/9321020031), using only two doses (45 and 60 mg/kg/day), there were no T-R deaths; clinical observations at both doses included GI distress (emesis and diarrhea) and CNS effects (tremors, ataxia, and convulsions), and reduced heart rate (but no ECG effects), increased platelets and bilirubin, and histopathological abnormalities in the liver, thymus and urinary bladder were reported (**Table IVB.3.1**). T-R changes in the liver were described as a loss and alteration of hepatocytes in lobes subadjacent to the gallbladder with hepatocytes more basophilic than normal. Dose-dependent thymic atrophy was seen in both sexes, with the thymic cortex reduced in size compared to the medulla and the density of lymphocytes reduced in the cortex and/or medulla. Minimal to mild degeneration and necrosis of the urinary bladder smooth muscle was present in most RTG-treated animals (females more affected than males). A detrusor myopathy seen in treated dogs was thought to be related to drug-induced relaxation of the urinary bladder muscle layer. Two HD males (#303 and 304) had minimal multifocal fibrosis or mild multifocal degeneration of the myocardium (respectively). In the latter, basophilic histiocytes were present between the myocardial fibers. The fibrosis (dog #303) was considered sufficiently advanced to have occurred prior to RTG exposure, and the degeneration (dog #304) was thought to have been secondary to convulsions occurring in this animal on day 24. Thus, neither of these heart findings was considered T-R in the study report. Plasma drug levels were not analyzed in this study (plasma samples were destroyed due to thawing during shipment), but data from a 14-day study with similar doses are shown in **Table IVB.3.2**.

Table IVB.3.1 Histopathology findings in 28-day oral dog toxicity study

INCIDENCE OF MICROSCOPIC LESIONS ASSOCIATED WITH ORAL EXPOSURE TO RETIGABINE IN DOGS

		Males			Females		
Diagnosis	Dose level (mg/kg/day)	0	45	60	0	45	60
THYMUS	number examined	4	4	4	4	4	4
atrophy							
	none	4	2	1	2	1	1
	minimal	0	1	2	1	0	0
	mild	0	1	1	0	2	1
	moderate	0	0	0	1	1	2
LIVER	number examined	4	4	4	4	4	4
hepatocyte degeneration/loss							
	none	4	0	2	4	1	1
	minimal	0	3	2	0	0	3
	mild	0	1	0	0	3	0
URINARY BLADDER	number examined	4	4	4	4	4	4
degeneration/necrosis, smooth muscle							
	none	4	2	3	4	0	1
	minimal	0	0	0	0	1	2
	mild	0	1	1	0	3	1
	moderate	0	1	0	0	0	0

Table IVB.3.2 Individual TK parameters in 14-day oral dog toxicity study

		3 x 12.8 mg/kg: 38.4 mg/kg/day				3 x 21.5 mg/kg: 64.5 mg/kg/day			
		DOG #1	DOG #2	DOG #3	DOG #4	DOG #5	DOG #6	DOG #7	DOG #8
		m	m	f	f	m	m	f	f
C_{trough}	ng/ml	1011	1183	508	473	1679	1438	1559	423
C_{max}	ng/ml	1361	2645	823	908	5540	3620	2364	971
AUC_{0-24h}	h ng/ml	18367	48711	13700	10106	59678	56459	42369	13239

C_{max}: the highest value observed after one of the three administrations within the 24 h cycle

In the 13-week oral toxicity study in dogs (Report No. D-23129/3000896861; 8.25, 17.8, and 38.3 mg/kg), GI and CNS signs (hypokinesia, disturbances of coordination, loss of righting reflexes, decrease of muscle tone) were seen at doses >8.25 mg/kg, and the only histopathological finding considered T-R by the sponsor was lymphoid depletion, which was attributed to stress. However, D-R increases in gallbladder and urinary bladder changes were apparent (**Table IVB.3.3**). Additional histopathological investigations were performed after finalization of the study report (Amendment Report No. 2, dated 10/24/01) based on the results of the 28-day dog study that revealed RTG-related findings in the liver tissue underlying the gallbladder. When selected liver samples from the current study were re-examined, the histopathologic evaluation indicated that the liver tissue directly underlying the gallbladder was affected by focal fibrosis/fibroplasia, mononuclear inflammatory cell infiltrate(s), pigment deposition, and an increased incidence and grade of acute hemorrhage (**Table IVB.3.4**). According to the pathology report, "A minimal to moderate focal fibrosis/fibroplasia was almost exclusively limited to a small rim of liver tissue underlying the gallbladder, only a few liver cell plates in thickness. Bile duct like structures were entrapped by this mature fibrotic tissue which also contained minimal to slight amounts of mononuclear inflammatory cell infiltrates. This gives the impression that the fibrotic tissue had replaced previously lost hepatocytes." Pigment deposition in macrophages and Kupffer cells, thought to be a mixture of hemosiderin and bile pigment, was associated with this fibrosis/fibroplasia, and acute hemorrhage was also frequently associated with the lesion. Because the lesions were said to be confined to a small rim of liver tissue adjacent to the gallbladder, they were not thought to reflect a general hepatotoxic effect. TK parameters from the 13-week study are shown in **Table IVB.3.5**.

Table IVB.3.3 Histopathology findings in 13-week dog study

TEST ARTICLE : D-23129 PATHOL. NO.: 89686 NOL
TEST SYSTEM : DOG, 13 WEEKS,, ORAL DATE : 27-FEB-96
SPONSOR : (b) (4) PATHDATA SYSTEM V3.6B

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

	DOSE GROUP:	01		02		03		04	
	SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO.ANIMALS:	4	4	4	4	4	4	4	4
LIVER	NO.EXAM.:	4	4	4	4	4	4	4	4
- Mononucl.infiltr.foc		1		2	1				
- Polymorph.infiltr.fo						1			
- Microgranuloma(s)		4	4	4	4	4	4	4	4
- Iron-pos.pigment			2		1		1	2	2
- Vacuoliz.hepatocytes		1				2			1
- Hyaline globules						1			
- Apoptotic bodies						1			
- Prolif.oval cell foc				1		1			
- Arteritis focal		1							
GALLBLADDER	NO.EXAM.:	4	4	4	4	4	4	4	4
- Mononucl.infiltr.dif			1	2				3	1
- Mucosal edema dif.								1	
- Hyperemia focal		1							
KIDNEYS	NO.EXAM.:	4	4	4	4	4	4	4	4
- Tubular lipofuscin		4	4	4	4	4	4	4	4
- Glomerulopathia foc.				1		2			
- Mononucl.infiltr.foc				1	2			1	1
- Mineralization focal		4	4	4	4	4	4	4	4
- Vacuolation tubule/s			1		1		3		
- Hyaline cast(s)				1			1		
- Basoph.tubule(s) foc			1		1				
- Dilatat.Bowmanns cap		4	3	1	3	3	3		2
- Hyperpl.urothel.foc.		2	1	1		1		1	
- Fibrosis/-plasia foc			1	2		1			
URINARY BLADDER	NO.EXAM.:	4	4	4	4	4	4	4	4
- Mononucl.infiltr.foc			1		2	1			
- Mixed infiltr.focal									1
- Hemorrhage focal								1	1
- Hyperemia					2				1
- Hyperpl.trans.epith.						1			
- Prolif.smooth muscle								1	1

Table IVB.3.4 Histopathology findings in 13-week dog study

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TEST ARTICLE : D-23129 PATHOL. NO.: 10002 NOL
TEST SYSTEM : DOG, 13 WEEKS,, ORAL DATE : 01-JUN-01
SPONSOR : (b) (4) PathData System V5.1b

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0
liver adjancet to gallbladder only

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4
LIVER AT GALLBLADDER :	4	4	4	4	4	4	4	4
- Vacuoliz.hepatoc.dif:	1	4	1	-	1	2	-	1
- Hemorrhage acute :	1	1	2	2	2	2	3	1
- Congestion :	1	2	-	-	1	-	-	2
- Depos.pigment diff. :	1	-	3	4	3	3	3	4
- Oval cell hyperpl. :	-	1	-	-	-	-	-	2
- Mononucl.infiltr.foc:	-	-	3	4	3	2	3	4
- Fibrosis/-plasia foc:	-	-	2	4	3	3	3	4
- Microgranuloma(s) :	-	-	1	-	2	2	1	2

Table IVB.3.5 TK parameters in 13-week dog toxicology study of retigabine

Dose	M: 8.25	F: 8.25	M: 17.8	F: 17.8	M: 38.3	F: 38.3
Week 2 of treatment						
C_{max} (ng/mL)	359	856	700	739	1,235	917
t_{max} (h)	2-4	2	0.5-4	2-4	1-4	1-8
$AUC_{0.5-24}$ (ng·h/mL)	3,316	6,641	8,674	7,598	11,984	9,436
$C^{ss:min}$ (ng/mL)	27.6	76.2	162.8	71.0	196.0	147.7
Week 12 of treatment						
C_{max} (ng/mL)	359	672	639	697	2615	1,170
t_{max} (h)	2-8	1-4	2-8	1-8	1-8	0.5-8
$AUC_{0.5-24}$ (ng·h/mL)	4,519	6,044	8,850	9,771	37,901	15,538
$C^{ss:min}$ (ng/mL)	65.6	92.0	91.8	281.7	933.1	276.2
R_A ($C^{SS:min}$; week 12 vs. 2)	2.54	1.20	0.65	4.82	8.09	2.73

N = 4 (low and mid dose), n = 6 (high dose)
R_A = accumulation ratio

In the 1-year oral toxicity study (Report No.: D-23129/3000912148, conducted by (b) (4) report dated 2/12/98, GLP) in dogs (4/sex/group + 2/sex control and HD recovery) with doses of 0, 4.64, 12.1, and 31.6 mg/kg (in gel caps; batch # 96020014), GI and CNS signs (hypokinesia, hypothermia, clonic convulsions, and coordination disturbances) were seen at the MD and HD, but there were no T-R deaths (1 C female sacrificed moribund). Clinical signs occurred sporadically throughout the dosing period. Body weight effects were minimal (slight NS decrease at HD during some intervals). Ophthalmic exams (weeks 13 and 26 in C and HD, week 52 in all animals) did not indicate any T-R effects. ECG measurements carried out during weeks 3, 26, and 52 just before dose administration did not indicate any effects of treatment. On hematology parameters (weeks 4, 13, 26, and 52) only transient decreases in Hb and PT and increased neutrophils at the HD) were observed. Evaluation of clinical chemistry (weeks 4, 13, 26, and 52) found consistent D-R increases in total bilirubin and decreases in blood urea and a transient increase in ALT and GDH, seen primarily at week 4 and more pronounced in females (ALT up to 4X C at HD). There were no urinalysis changes. There were no histopathological changes considered T-R in the original study report, although lymphocytic infiltration of the gallbladder and lymphocytic pyelitis in the kidney were increased D-D in treated animals. However, this report was amended to include additional histopathological investigations performed after completion of the study and finalization of the original report in response to the findings in the liver tissue underlying the gallbladder in the 28-day dog study. According to the amended report, this localization was not a protocol tissue and therefore not included during the original histopathological examination. Three additional tissue samples of the gallbladder with attached liver were examined from all animals, and the liver tissue attached to the gallbladder, present in the original slides, was also re-examined histologically. These additional histopathological examinations replicated the findings from previous studies, showing T-R changes in the liver tissue underlying the gallbladder consisting of focal fibrosis/fibroplasia, mononuclear inflammatory cell infiltrate(s), and pigment deposition (**Table IVB.3.6**). The pigment deposition and the focal fibrosis/fibroplasia showed a D-R increase in grade. All findings were said to be confined to a small rim of liver tissue adjacent to the gallbladder and not in other liver tissue samples examined, so again were not thought to be indicative of a general hepatotoxic effect. TK parameters from the 12-month study are shown in **Table IVB.3.7**. The metabolite NAMR was found only in trace amounts reflecting the known lack of N-acetyl transferase activity in the dog.

Table IVB.3.6 Histopathology findings in 1-year dog study

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SUMMARY TABLES — RCC NO.: 912148

TEST ARTICLE : D-23129 — *PATHOL. NO.*: 10003 NOL
TEST SYSTEM : DOG, 52 Week, oral — *DATE* : 11-JUN-01
SPONSOR : (b) (4) — *PathData System V5.1b*

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS
LIVER ADJANCENT TO GALLBALDDER

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO.ANIMALS:	4	4	4	4	4	4	4	4
LIVER AT GALLBLADDER :	4	4	4	4	4	4	3	2
- Mononucl.infiltr.foc:	1	-	1	2	3	4	2	2
- Microgranuloma(s) :	1	-	-	2	-	1	1	-
- Activat.Kupffer cell:	-	1	-	-	-	1	-	-
- Vacuoliz.hepatocytes:	-	-	4	3	3	3	2	1
- Fibrosis/-plasia foc:	-	-	2	3	3	4	3	2
- Deposition pigment :	-	-	1	3	4	4	3	2
- Congestion :	2	2	2	1	1	2	3	-
- Hemorrhage acute :	3	1	1	1	1	-	1	1

Table IVB.3.7 TK parameters in 1-year dog toxicology study

Dose	M: 4.64	F: 4.64	M: 12.1	F: 12.1	M: 31.6	F: 31.6
Week 1 of treatment						
C_{max} (ng/mL)	269	202	538	539	939	908
t_{max} (h)	2.0	2.0	3.0	2.0	3.0	2.0
AUC $_{0.5\text{-}24}$ (ng·h/mL)	2,085	1,843	4,787	4,574	12,436	8,327
Week 27 of treatment						
C_{max} (ng/mL)	594	444	848	593	1,511	2,235
t_{max} (h)	0.5	3.0	3.0	2.0	4.0	2.0
AUC $_{0.5\text{-}24}$ (ng·h/mL)	5,720	4,540	8,445	4,574	22,667	22,315
Week 51 of treatment						
C_{max} (ng/mL)	302	410	563	1,045	1,440	2,274
t_{max} (h)	1.5	3.0	3.0	2.0	4.0	2.0
AUC $_{0.5\text{-}24}$ (ng·h/mL)	2,674	4,313	5,841	10,729	25,334	22,963

Median of n = 3 - 4 per dose

Because dogs do not produce appreciable amounts of the major human metabolite NAMR, the Division asked the sponsor to evaluate the metabolite directly. This was done in a 13-week study (Report No. PR2005-067, conducted by (b) (4) dated 8/29/06; **Table IVB.3.8**) in which doses of 0 (empty gel cap), 30, 100, or 600/300/200/100 mg/kg of the N-acetyl metabolite of RTG were administered to dogs (6/sex/grp). HD animals were not dosed on several days (9, 10, 11, 30, 31) due to what were considered excessive clinical signs of toxicity; and the HD was reduced from 600 to 300, 200, and 100 mg/kg on days 6, 12, and 32, respectively. Despite this, severe toxicity continued in many animals and resulted in animals being treated with medication and/or given dosing holidays through the end of study. Between days 43 and 85, 13 animals in the LD (1 male, 2 female), MD (3 male, 3 female), and HD (2 male, 2 female) groups were sacrificed in moribund condition. The clinical signs of systemic toxicity observed in these animals included anorexia, vomiting, ataxia, hypoactivity, fever, interdigital cysts/cellulitis, excessive salivation, hunched posture, thin appearance, and dehydration. Substantial BW loss was seen in these animals (10 dogs lost more than 10% of peak BW). T-R clinical signs in all treatment groups included vomiting, excessive salivation, discolored feces and urine, ataxia, hypoactivity, mucoid feces, cold to the touch, tremors, and cage sores/interdigital swelling. Overall BW gain was 76%, 35%, and 41% below C in LD, MD, and HD males and 88%, 41%, and 47% in LD, MD, and HD females, respectively. There were no ophthalmoscopic or ECG changes related to treatment. The most prominent T-R effect appeared to involve an effect on neutrophils, primarily in the form of moderately to markedly decreased absolute neutrophil count. However, the pattern was somewhat inconsistent, particularly among animals sacrificed because of poor health; some were markedly neutropenic and others exhibited a moderate to marked neutrophilia. Absolute lymphocyte count generally appeared unaffected by treatment. Individual animals from all dose groups also exhibited notably decreased RBC parameters and platelet counts and increased absolute monocyte count. Although these animals were said to lack an appropriate regenerative response to their reduced red cell mass, their absolute reticulocyte counts were not unusually low. According to the report, "the effect on red cell mass was most consistent with that commonly observed secondary to chronic inflammatory conditions and inconsistent with a direct effect on erythrocyte precursors and erythropoiesis." The pattern of neutrophil responses was considered atypical and thought to suggest "the possibility of effects on neutrophil function and or myelopoiesis." The only urinalysis finding was increased urine bilirubin which as in other studies was considered to be interference with the test method rather than an effect on bilirubin metabolism.

Microscopically, prominent lesions were observed in most treated animals from all dose groups, especially those sacrificed in a moribund condition. These were described in the report as:

> "moderate to marked myeloid hyperplasia in the bone marrow and spleen and slight to marked centrilobular myeloid cell infiltrate in the liver… the bone marrow was densely populated with immature cells of indeterminate origin, myeloid cells that had clusters of cells that could be identified as band cells, and polymorphonuclear cells. Megakaryocytes were present, but erythroid elements were reduced in amount, especially in animals sacrificed prior to Day 62. The red pulp of the spleen was densely populated with the same immature cells of indeterminate origin and myeloid elements as seen in the bone marrow. This correlated with the enlarged spleen reported grossly. Lymphoid follicles in the white pulp were similar in number and size to controls. Changes in the liver consisted of a thick, large cuff of myeloid cells around central veins. The cells were similar to those in the bone marrow, with some differentiation to band cells and polymorphonuclear cells. Sinusoids were heavily populated with mononuclear cells that were often in small clusters. These changes in the liver were similar to those of extensive extramedullary hematopoiesis, except maturation to mature myeloid elements was reduced. Kupffer cells were often slightly larger than normal and contained small amounts of pigment and cellular debris. Only an occasional degenerate or necrotic hepatocyte was observed, most likely due to the compression of myeloid cells."

Inflammatory lesions (acute inflammation, chronic/active inflammation, chronic inflammation), which were considered secondary to the immature myeloid cell hyperplasia, occurred in a variety of organs in treated animals from all dose groups. Although an increase in myeloid elements may accompany inflammatory

lesions, the myeloid hyperplasia in animals sacrificed in moribund was said to be greater than expected for the inflammatory process observed. The proliferation of immature myeloid cells was thought to have increased susceptibility to secondary infections. Lesions found in final-sacrifice animals were variable in degrees of severity. Animals sacrificed at a later date had more differentiation of myeloid elements in the liver toward mature polymorphonuclear cells. In several MD and HD animals that survived to termination, the lesions consisted of a narrow rim of small mononuclear cells around the central vein. Increased erythroid elements in the bone marrow and spleen of these animals were diagnosed as extramedullary hematopoiesis. Several animals had a narrow cuff of mononuclear cells around blood vessels in the subcapsular area of the kidney cortex. Hemorrhage, edema, and inflammation of the urinary bladder were seen in a few MD and HD males and females.

Because there was no NOAEL in the initial 13-week dog study of NAMR, a second 13-week study was conducted with doses of 0, 3, 10, and 30 mg/kg QD or 10 mg/kg TID (Report No. PR2007-003, conducted by (b) (4) dated 4/8/08) and an 8-week recovery period. At the interim sacrifice on day 30, lower neutrophil counts (<3 E3/µL) were seen on day 26 in 1 HD QD male and 2 HD TID males, one of which also had a moderately low platelet count. Two HD QD animals (one male and one female) were sacrificed moribund on days 78 and 50, respectively, and 3 HD TID animals (one male and two females) were sacrificed moribund on days 69, 76, and 57, respectively. Clinical signs included thin appearance, vomiting, hypoactivity, superficial pyodermal and oral ulceration, red oral discharge, fecal changes, and elevated body temperature. The moribund state for these animals was considered T-R and was associated with myelotoxicity (myeloid hyperplasia) observed histopathologically in bone marrow. Extramedullary hematopoiesis was also present in the liver for all moribund sacrifice and in spleen for female moribund sacrifices. The 3 HD TID animals also had multi-organ inflammation that included brain and heart involvement and which was considered to have contributed to the moribund condition. Alterations in the clinical pathology data that were observed in HD animals (QD and TID) during the remainder of the treatment period included lower RBC, platelet, and neutrophil counts. At terminal sacrifice, slight to moderate bone marrow granulocytic hyperplasia, with complete maturation (all stages represented in appropriate portions), was noted for 4 HD QD and 1 HD TID dogs. Four of these had lower peripheral blood neutrophil counts but 1 HD QD female had a high neutrophil count. After recovery, there were no cytological abnormalities observed in bone marrow smears from any dogs. Microscopic changes at terminal sacrifice consisted of myeloid hyperplasia in femoral and sternal bone marrow and increased extramedullary hematopoiesis in the spleen, and liver all primarily at the HD. Myeloid hyperplasia in femoral and sternal bone marrow was also present for 1 MD female. Representative hematology changes and bone marrow cytology results for the unscheduled and scheduled sacrificed animals (groups 3, 4, and 5 are MD, HD QD, and HD TID) are shown in **Table IVB.3.9**. All microscopic changes were reversible following the recovery period. ECGs (including QT and QTc interval) performed prior to scheduled sacrifice (1.5 –5.5 hours postdose) were unaffected by treatment. TK parameters in this study are shown in **Table IVB.3.10**.

Table IVB.3.8 Summary of 13-week dog study of NAMR

Daily Dose (mg/kg/day)	0		30		100		100+	
No. of Animals - Toxicokinetic[a]	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
Toxicokinetics: AUC $_{(0\text{-}24hr)}$ (%CV) [ng·hr/mL]								
Week 14	-	-	24900 (24)	13500 (37.4)	36400 (40.3)	55900 (52.5)	29500 (52.5)	34400 (48.8)
Toxicokinetics: C_{max} (%CV) [ng/mL]								
Week 14	-	-	7440 (14.9)	4100 (49.1)	12300 (49.0)	13100 (73.6)	5570 (104.9)	9390 (74.2)
No. of Animals - Main Study[a]	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
Noteworthy Findings								
No. of Animals Sacrificed in a Moribund Condition	M: 0	F: 0	M: 1	F: 2	M: 3	F: 3	M: 2	F: 2
Clinical Signs[b]								
Vomitus	4 (8)	5 (19)	6 (72)	6 (39)	6 (188)	6 (219)	6 (209)	6 (178)
Excessive Salivation	1	0	3	4	5	6	6	6
Discolored Feces	0	0	2	0	2	3	3	6
Discolored Urine	0	0	1	2	4	4	6	4
Ataxia	0	0	0	0	0	1	5	1
Hypoactivity	0	0	1	2	0	4	5	3
Mucoid Feces	2 (5)	5 (16)	5 (24)	6 (21)	6 (55)	6 (27)	6 (25)	5 (47)
Cold to Touch	0	0	0	2	1	4	5	6
Tremors	0	0	0	2	0	2	3	5
Body Weight Gains (%)[c]								
Dosing Days 1-96	1.7 (0.43)	1.7 (1.09)	-76*	-88	-35	-41	-41	-47
Hematology[c]								
Hemoglobin (g/dL)								
Dosing Day 49	15.0 (0.86)	14.9 (0.59)	-8.7	-12.1*	-12.7	-14.8*	-21.3*	-6.7
Dosing Day 90	15.5 (0.69)	15.3 (0.98)	-14.2	-18.3	-15.5	-20.3	-18.1*	-9.2
Absolute Segmented Neutrophils (E3/uL)								
Dosing Day 49	7.52 (2.331)	7.71 (1.954)	-6.6	-27.4	-37.0	-54.5	-16.4	-15.7
Dosing Day 90	8.05 (0.764)	7.84 (1.646)	-14.7	-18.6	-26.5	13.0	-35.2	-10.8
Absolute Monocytes (E3/uL)								
Dosing Day 49	0.57 (0.221)	0.50 (0.200)	80.7	124.0	100.0*	94.0	182.5*	98.0
Dosing Day 90	0.48 (0.120)	0.49 (0.118)	37.5	165.3	158.3*	153.1	177.1*	10.2
Clinical Chemistry[c]								
Alkaline Phosphatase (U/L)								
Dosing Day 49	59 (10.8)	64 (17.1)	23.7	70.3	33.9	112.5	50.8	12.5
Dosing Day 90	47 (10.4)	55 (20.3)	72.3	21.8	44.7	385.5	51.1	18.2
Albumin (g/dL)								
Dosing Day 49	3.1 (0.04)	3.2 (0.10)	-6.5	-12.5	-9.7	-15.6*	-16.1*	-9.4*
Dosing Day 90	3.1 (0.08)	3.1 (0.14)	-16.1*	-22.6*	-16.1*	-22.6*	-12.9*	-6.5*
Albumin/Globulin Ratio								
Dosing Day 49	1.1 (0.13)	1.3 (0.18)	-9.1	-23.1	-18.2	-30.8	-27.3	-23.1
Dosing Day 90	1.1 (0.16)	1.2 (0.11)	-27.3	-33.3*	-36.4	-33.3*	-27.3	-16.7*
Organ Weights[c]								
Spleen/Brain Weight Ratio	52.657	34.533	-0.93	81.7	158*	60.5	33.7	42.8
Macroscopic Observations[d]								
Lymph Node								
Large	0	0	1	0	1	0	1	1
Foot/Foot Pad/Skin (foot)								
Abrasion/crusted/perforated/ulcerated	0	0	0	1	0	1	0	1
Mucosa								
Discolored (stomach/duodenum/cecum/colon)	0	0	1	2	2	1	0	1
Lung								
Adhesion	0	0	0	0	1	0	1	0
Discolored	0	0	1	0	1	0	1	0
Urinary Bladder								
Thickened	0	0	0	0	1	0	0	0
Spleen								
Discolorerd	0	0	0	2	0	0	0	0
Histopathology[d]								
Liver								
Infiltrate, myeloid cell, centrilobular	0	0	2	4	5	4	3	0
Spleen								
Myeloid hyperplasia	0	0	0	2	1	1	1	0
Bone Marrow (sternum)								
Myeloid hyperplasia	0	0	6	4	5	6	5	5

M = males
F = females
- = Not applicable
* = Statistically significant from Group 1 at $p \leq 0.05$. Statistical significance is based on the actual data (not the percent differences).
a Number of animals/sex/group at study start.
b Values listed indicate the number of animals that showed the sign. Values in parentheses indicate the number of days that the sign was present for all toxicity animals regardless of the number of times it may have been recorded on a particular day.
c Group means are shown for controls. Percent differences from controls are shown for treated groups. Values in parentheses indicate the standard deviation.
d Values listed indicate the number of animals with each finding.

Table IVB.3.9 Hematology and bone marrow cytology results in 13-week dog study of NAMR (days)

Group/Sex	Animal No.	Status	RBC <5 E6/µL	PLT <150 E3/µL	NEUT <3 E3/µL	NEUT <1 E3/µL	Bone Marrow*	Fibrinogen >550 mg/dL
4/M	H46478	DP SS1					Ery-hypo / G-hyper	
4/M	H46479	DP SS1			26		NCA	
4/M	H46482	DP FPS	54,86	40,54	86	40	G-hyper	
4/M	H46485	RP FPS			86		NCA	
4/M	H46486	Moribund					NCA	
5/M	H46488	DP SS1		26		26	NCA	
5/M	H46489	DP SS1					SUFA	
5/M	H46493	Moribund	54	40,54	26	54	NCA	40,54
5/M	H46494	DP FPS	86 (RTN - reco)	86 (RTN - reco)	40	54,86	NCA	54
5/M	H46497	DP FPS			54,86		G-hyper	
3/F	H46514	DP FPS		54, incr. on 86			NCA	
4/F	H46516	DP SS1					Ery - plentiful at SS1	
4/F	H46520	DP FPS	86	86			G-hyper	86
4/F	H46523	Moribund		40			NCA	
4/F	H46524	DP FPS			40		G-hyper	
4/F	H46525	RP FPS			54	86	NCA	
4/F	H46526	DP FPS	86		54,86		G-hyper	
5/F	H46531	Moribund			54		NCA	
5/F	H46532	Moribund	54	40, (unrem. on 54)	40		NCA	40,54

*all animals RTN at recovery
RTN = Returned to Normal
NCA = no cytologic abnormalities
SUFA = sample unsuitable for analysis
G = granulocytic
Ery = erythroid
No. = number
incr. = increased
reco = recovery
unrem = unremarkable
hypo = hypoplasia
hyper = hyperplasia

DP = dosing phase
RP = recovery phase
SS1 = scheduled sacrifice 1 (interim)
FPS = final phase sacrifice (terminal)

Table IVB.3.10 TK parameters in 13-week dog study of NAMR

Day 1		
N-acetyl metabolite of Retigabine (mg/kg/dose)	**C_{max} (ng/mL)**	**AUC $_{(0-24)}$ (ng*hr/mL)**
3 (QD)	552 (M) 333 (F)	1956 (M) 975 (F)
10 (QD)	1167 (M) 1561 (F)	3963 (M) 5870 (F)
30 (QD)	5683 (M) 4037 (F)	20993 (M) 16032 (F)
10 (TID)	1596 (M) 1636 (F)	14353 (M) 16803 (F)
Week 4		
N-acetyl metabolite of Retigabine (mg/kg/dose)	**C_{max} (ng/mL)**	**AUC $_{(0-24)}$ (ng*hr/mL)**
3 (QD)	385 (M) 133 (F)	1376 (M) 667 (F)
10 (QD)	976 (M) 1675 (F)	3295 (M) 6238 (F)
30 (QD)	4286 (M) 3428 (F)	16259 (M) 15455 (F)
10 (TID)	1324 (M) 972 (F)	14444 (M) 9955 (F)
Week 13		
N-acetyl metabolite of Retigabine (mg/kg/dose)	**C_{max} (ng/mL)**	**AUC $_{(0-24)}$ (ng*hr/mL)**
3 (QD)	448 (M) 262 (F)	1900 (M) 1288 (F)
10 (QD)	1650 (M) 2318 (F)	4637 (M) 8597 (F)
30 (QD)	4524 (M) 5436 (F)	14177 (M) 23145 (F)
10 (TID)	2048 (M) 1302 (F)	20462 (M) 14440 (F)

4. Monkey

In an oral gavage dose range-finding study (Report No. D-23129/3000922893) in monkeys (2/sex/grp) in which doses of 0, 10, 20, 40, 60, 90, 120, or 180 mg/kg were given for up to 10 days, RTG-induced clinical signs consisting of ataxia, decreased motor activity, ptosis, and salivation occurred with a D-R frequency and incidence at ≥40 mg/kg. According to the study report, "one of the 4 monkeys (female #31) exhibited decreased motor activity and ataxia ~2 hours postdosing, and became immobile ~18 hours post-dosing; this monkey remained sedated (immobile, which improved to decreased motor activity and ataxia) for 2 days. During this period, this monkey also had isolated episodes of tremors, salivation, and dyspnea." This animal required special medical treatment which included heating, hydration, and dietary supplements. The plasma concentration of RTG was unusually high in this monkey: 5043, 4514, and 7635 ng/ml at the 8, 12, and 24 hour postdosing. At these same time points, plasma drug concentrations

in other monkeys from this group ranged from 818 to 1355 ng/ml. This animal did not return to a state of normal appearance and behavior until 3 days post-dosing. Due to the severity of the effects at the HD, this group was not dosed further. There was no D-D mortality in the study, although 1 HD male died due to an intubation error, confirmed by pathology. There were no T-R effects on BW, hematology, or clinical chemistry. Histopathology was only performed on the animal that died, but no urinary bladder effects were reported in this monkey. TK data from this study is shown in **Table IVB.3.11**. NAMR was not detected, indicating that this human metabolite is not formed in monkeys.

Table IVB.3.11

Dose (mg/kg)	10	20	40	60	90	120	180
Day 1 / Males							
C_{max} (ng/mL)	310	515	950	915	1,408	2,057	1,630
AUC $_{0-\infty}$ (ng·h/mL)	2,669	4,638	6,514	13,452	24,529	20,181	28,394
Day 10 / Males							
C_{max} (ng/mL)	332	499	1,730	1,404	1,342	3,819[1]	n.a.
AUC $_{0-24}$ (ng·h/mL)	4,245	4,511	13,393	17,365	17,523	33,463[1]	n.a.
Day 1 / Females							
C_{max} (ng/mL)	406	434	1,318	940	1,351	1,891	4,399
AUC $_{0-\infty}$ (ng·h/mL)	1,524	2,685	11,546	10,760	18,379	27,581	65,888
Day 10 / Females							
C_{max} (ng/mL)	355	352	1,379	1,524	773	2,830	n.a.
AUC $_{0-24}$ (ng·h/mL)	1,474	2,316	12,316	15,528	12,973	27,736	n.a.

[1] Data derived from 1 animal otherwise n=2 n.a.: not applicable

C. Genotoxicity

Genetic toxicity testing was conducted with drug substance synthesized using the early (b) (4) As discussed in the original IND review (dated 9/11/97), genotoxicity testing of the salt indicated clear mutagenic potential (positive in Ames with S9, positive for chromosomal aberrations in human lymphocytes with and without activation) while the free base (D-23129) appeared to be less genotoxic or non-genotoxic *in vitro*, which the sponsor attributed to an improved impurity profile. Since the latter is the drug substance used in clinical studies and intended for the final drug product, genotoxicity data generated for retigabine (b) (4) are the most relevant. The studies conducted on D-23129 were: numerous Ames tests, two in vitro chromosomal aberration tests in human lymphocytes, an in vitro CHO cell hprt gene mutation assay, and an in vivo mouse micronucleus test. The N-acetyl metabolite was also evaluated for mutagenic activity in an Ames test and chromosomal aberration test in CHO cells. A number of impurities identified in batches of retigabine manufactured using the most recent synthetic process were tested in the Ames test, and one impurity (b) (4) was also tested in a combined in vivo rat micronucleus and comet assay.

1. Ames Test

a. Retigabine drug substance

A number of retigabine (RTG) lots manufactured by the (b) (4) process were tested in GLP Ames tests. These include lots used in the pivotal nonclinical evaluations and which were synthesized by the intended manufacturer of drug substance for commercial use. Although the tested lots were all manufactured using the same basic synthesis process, according to the sponsor (b) (4) Three lots (96020011, 96020014, and 9805003) in particular were evaluated multiple times after initial positive results. In a study using Lot 96020011 (D-23129/3000922217, conducted by (b) (4) report dated 3/8/01, GLP), a weak positive response (revertants increased up to ~2X vehicle control) was observed with tester strain TA98 in the presence of rat S9 using the plate incorporation method (**Table IVC.1a.1**; TA1538 not tested). Two subsequent Ames assays conducted with this lot, one in the same laboratory (Report D-23129/9321020149, (b) (4) 12/2/02, GLP) and one at (b) (4) (Report D-23129/3000922678, 10/5/01, GLP), were negative. Ames test results with lot 96020014 were positive (revertants ~2-3X control) in strains TA98 and TA1538 with S9 in a non-GLP study (D-23129/3000918224, 12/4/98) conducted at the (b) (4) Lot 96020014 was subsequently retested (D-23129/3000918180, (b) (4) 5/3/99, GLP) only in strains TA98 and TA1538 in the presence of S9. A SS dose response was detected in TA1538 with S9 in the plate incorporation assay (**Table IVC.1a.2**), but the maximum increase did not reach 3-fold. In the pre-incubation assay, a SS dose response was also detected in TA98 with S9 in this same study (**Table IVC.1a.3**), but did not reach the 2-fold threshold. Lot 9805003 also produced a mutagenic signal (revertants 2-3X control) in the initial non-GLP study (Report D-23129/3000918224 above). In a follow-up study (Report D-23129/3000918191, (b) (4) dated 5/3/99, GLP), SS trends were seen but the magnitude of the increase in revertants did not meet the criteria for a positive result (ie, 2 or 3X; **Tables IVC.1a.4-5**). Lot No. 0005005, which was used in the carcinogenicity studies, tested Ames negative (Report 0-23129/3000922498, (b) (4) 3/8/01, GLP) and also contained the lowest level of the mutagenic impurity (b) (4) of any tested ((b) (4) vs range of (b) (4) in clinical batches). The most recently tested batch made with the current manufacturing process (Batch No. 02080030A-050 released on 4/11/08), which is said to include “minor but important improvements to the synthetic scheme,” also tested Ames negative (Report PR2008-027, conducted by (b) (4) 6/17/08, GLP) but showed the same tendency for small increases in revertant frequencies in TA98 with S9 (up to 1.9X C in confirmatory assay; **Tables IVC.1a.6**; TA1538 not tested). In Ames tests conducted with less pure material synthesized by the old process, positive signals were most often seen in TA98 and TA1538, where revertants were increased up to 60-fold.

Table IVC.1a.1.

Test Article Id : Retigabine (Batch 96020011)
Study Number : AA37AS.502002. (b) (4) Experiment No : B1
Study Design : Initial Assay via Plate Incorporation

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	23 ± 6	85 ± 11	10 ± 1	7 ± 1	14 ± 5
33	23 ± 2	80 ± 5	14 ± 4	4 ± 4	10 ± 2
100	16 ± 3	93 ± 9	12 ± 2	6 ± 4	12 ± 3
333	19 ± 3	79 ± 9	12 ± 1	4 ± 3	6 ± 5
667	14 ± 5	80 ± 12	9 ± 5	5 ± 2	9 ± 1
1000	16 ± 4	79 ± 13	11 ± 2	4 ± 1	8 ± 3
3333	10 ± 3	68 ± 19	8 ± 3	3 ± 2	5 ± 3
5000	11 ± 8	72 ± 8	10 ± 8	2 ± 1	5 ± 1
Positive	179 ± 24	349 ± 42	270 ± 23	683 ± 167	97 ± 8

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	14 ± 5	103 ± 18	14 ± 4	7 ± 2	13 ± 4
33	18 ± 4	104 ± 9	10 ± 4	6 ± 1	12 ± 3
100	20 ± 3	98 ± 8	7 ± 2	8 ± 1	14 ± 3
333	24 ± 1	94 ± 5	8 ± 1	7 ± 2	13 ± 1
667	32 ± 4	100 ± 6	11 ± 2	9 ± 3	14 ± 5
1000	24 ± 5	89 ± 12	6 ± 2	5 ± 1	9 ± 2
3333	22 ± 8	74 ± 4	5 ± 4	2 ± 1	2 ± 2
5000	12 ± 4	67 ± 9	4 ± 1	6 ± 1	3 ± 2
Positive	301 ± 59	520 ± 19	55 ± 3	62 ± 6	150 ± 37

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Table IVC.1a.2

TABLE 6 **D-23129 (batch 96020014): Mutagenicity Test in *Salmonella typhimurium* Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay Plate Incorporation Method - Confirmatory Assay: Summary Data**

		MEAN REVERTANTS/PLATE					
		TA98			TA1538		
DOSE (µg/plate)	S9	mean	std	MI*	mean	std	MI*
0	+	34.3	± 5.69		18.7	± 2.08	
50	+	36.3	± 3.79	1.06	20.7	± 0.58	1.11
158	+	38.3	± 10.50	1.12	27.3	± 2.08	1.46
500	+	42.0	± 2.65	1.22	25.0	± 4.00	1.34
1580	+	42.7	± 3.51	1.24	31.0	± 4.36	1.66
5000	+	41.3	± 6.66	1.20	31.0	± 5.00	1.66
P value		0.232			0.004	**	
POS-LD	+	253.7	± 26.95	7.40	200.0	± 24.88	10.70
POS-HD	+	2012.0	± 117.58	58.66	1546.0	± 55.43	82.67

Data presented as the mean of triplicate plates +/- the standard deviation;* Mutagenic Index.
** P value significant at ≤ 0.05.
POS-LD = low dose positive control (2.5 µg/plate 2-Aminoanthracene).
POS-HD = high dose positive control (12.5 µg/plate 2-Aminoanthracene).

Table IVC.1a.3

TABLE 8 **D-23129 (batch 96020014): Mutagenicity Test in *Salmonella typhimurium* Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay Pre-Incubation Method: Summary Data**

		MEAN REVERTANTS/PLATE					
		TA98			TA1538		
DOSE (µg/plate)	S9	mean	std	MI*	mean	std	MI*
0	+	29.0	± 5.29		24.0	± 4.58	
50	+	33.0	± 4.58	1.14	23.0	± 5.29	0.96
158	+	35.0	± 1.73	1.21	25.0	± 5.57	1.04
500	+	33.3	± 0.58	1.15	21.3	± 4.04	0.89
1580	+	38.7	± 7.51	1.33	27.3	± 2.52	1.14
5000	+	42.3	± 7.64	1.46	M		
P value		0.017 **			0.163		
POS-LD	+	330.3	± 121.94	11.39	266.7	± 49.34	11.11
POS-HD	+	1311.0	± 265.61	45.21	1032.3	± 83.29	43.01

Data presented as the mean of triplicate plates +/- the standard deviation;* Mutagenic Index;
M = all plates excluded due to presence of microcolonies.
** P value significant at ≤ 0.05.
POS-LD = low dose positive control (2.5 µg/plate 2-Aminoanthracene).
POS-HD = high dose positive control (12.5 µg/plate 2-Aminoanthracene).

Table IVC.1a.4

TABLE 4 **D-23129 (batch 9805003): Mutagenicity Test in *Salmonella typhimurium* Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay Plate Incorporation Method - Initial Assay: Summary Data**

		MEAN REVERTANTS/PLATE					
		TA98			TA1538		
DOSE (µg/plate)	S9	mean	std	MI*	mean	std	MI*
0	+	35.3	± 5.13		18.7	± 2.08	
50	+	40.3	± 2.08	1.14	29.0	± 1.00	1.55
158	+	50.3	± 17.01	1.42	29.7	± 1.53	1.59
500	+	39.3	± 2.31	1.11	22.0	± 1.73	1.18
1580	+	39.7	± 3.21	1.12	31.3	± 6.66	1.67
5000	+	32.3	± 7.09	0.92	35.3	± 2.08	1.89
P value		0.976			0.002 **		
POS-LD	+	M			292.0	± 19.97	15.61
POS-HD	+	1740.3	± 78.34	49.30	1482.3	± 86.07	79.27

Data presented as the mean of triplicate plates +/- the standard deviation; M = all plates excluded due to presence of microcolonies; * Mutagenic Index.
** P value significant at ≤ 0.05.
POS-LD = low dose positive control (2.5 µg/plate 2-Aminoanthracene).
POS-HD = high dose positive control (12.5 µg/plate 2-Aminoanthracene).

Table IVC.1a.5

TABLE 6 **D-23129 (batch 9805003): Mutagenicity Test in *Salmonella typhimurium* Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay Plate Incorporation Method - Confirmatory Assay: Summary Data**

		MEAN REVERTANTS/PLATE					
		TA98			TA1538		
DOSE (µg/plate)	S9	mean	std	MI*	mean	std	MI*
0	+	32.7	± 3.79		29.0	± 8.54	
50	+	33.3	± 4.93	1.02	29.3	± 5.03	1.01
158	+	37.3	± 0.58	1.14	26.7	± 6.81	0.92
500	+	39.3	± 9.29	1.20	32.0	± 2.65	1.10
1580	+	40.0	± 2.00	1.22	40.7	± 5.03	1.40
5000	+	43.7	± 4.51	1.34	41.0	± 2.65	1.41
P value		0.030 **			0.003 **		
POS-LD	+	256.3	± 11.93	7.84	245.0	± 18.52	8.45
POS-HD	+	1989.7	± 133.08	60.85	1554.7	± 27.74	53.61

Data presented as the mean of triplicate plates +/- the standard deviation;* Mutagenic Index.
** P value significant at ≤ 0.05.
POS-LD = low dose positive control (2.5 µg/plate 2-Aminoanthracene).
POS-HD = high dose positive control (12.5 µg/plate 2-Aminoanthracene).

Tables IVC.1a.6 Summary of Results - Initial and Retest of the Toxicity-Mutation Assay

Test Article Id : Retigabine
Study Number : AC11FA.503. (b) (4)
Experiment Nos. : B1/B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Vehicle	15 ± 5	94 ± 4	12 ± 2	10 ± 0	20 ± 1
1.5	11 ± 3	102 ± 11	13 ± 7	9 ± 4	22 ± 1
5.0	17 ± 6	97 ± 16	12 ± 0	9 ± 5	19 ± 5
15	21 ± 1	109 ± 6	13 ± 6	10 ± 4	15 ± 4
50	14 ± 2	95 ± 1	12 ± 3	8 ± 0	16 ± 5
150	15 ± 1	87 ± 5	14 ± 8	10 ± 2	14 ± 4
500	16 ± 5	76 ± 0	13 ± 6	14 ± 1	18 ± 5
1500	11 ± 2	95 ± 1	15 ± 4	9 ± 3	11 ± 4
5000	14 ± 6	97 ± 1	9 ± 10	10 ± 1	16 ± 1
Positive	180 ± 49	667 ± 42	405 ± 42	997 ± 69	187 ± 33

Activation Condition : Rat Liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A	TA100[a]
Vehicle	27 ± 3	111 ± 11	6 ± 2	10 ± 4	22 ± 5	97 ± 5
1.5	27 ± 1	78 ± 6	11 ± 0	9 ± 1	24 ± 4	115 ± 7
5.0	22 ± 5	79 ± 6	6 ± 4	5 ± 1	19 ± 1	119 ± 4
15	24 ± 4	86 ± 1	9 ± 2	7 ± 2	20 ± 6	121 ± 15
50	26 ± 2	0 ± 0	7 ± 5	10 ± 1	23 ± 5	105 ± 10
150	43 ± 16	75 ± 14	8 ± 0	9 ± 1	18 ± 3	107 ± 4
500	30 ± 1	78 ± 1	9 ± 4	10 ± 2	16 ± 3	101 ± 21
1500	42 ± 9	77 ± 1	9 ± 1	8 ± 4	22 ± 0	97 ± 6
5000	35 ± 5	72 ± 6	14 ± 1	13 ± 3	12 ± 1	86 ± 2
Positive	531 ± 124	371 ± 30	83 ± 4	90 ± 28	220 ± 23	1076 ± 78

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL
a = Data from Experiment B2

Test Article Id : Retigabine
Study Number : AC11FA.503.(b) (4)
Experiment No : B3

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Vehicle	13 ± 3	133 ± 3	19 ± 7	11 ± 1	24 ± 4
50	13 ± 1	140 ± 24	19 ± 4	10 ± 2	26 ± 4
150	14 ± 4	134 ± 4	18 ± 6	11 ± 2	25 ± 6
500	18 ± 3	142 ± 6	21 ± 2	8 ± 4	21 ± 3
1500	14 ± 6	141 ± 14	14 ± 3	7 ± 5	13 ± 1
5000	11 ± 4	129 ± 1	19 ± 6	9 ± 2	13 ± 2
Positive	117 ± 28	597 ± 93	313 ± 20	921 ± 217	155 ± 17

Activation Condition : Rat Liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Vehicle	27 ± 7	148 ± 10	16 ± 3	10 ± 2	27 ± 1
50	36 ± 8	155 ± 17	16 ± 7	10 ± 6	22 ± 2
150	50 ± 2	139 ± 31	13 ± 7	7 ± 4	30 ± 3
500	40 ± 9	157 ± 13	15 ± 3	11 ± 1	24 ± 6
1500	44 ± 8	125 ± 9	12 ± 4	10 ± 3	19 ± 2
5000	22 ± 2	130 ± 13	13 ± 4	9 ± 2	12 ± 3
Positive	770 ± 46	736 ± 31	132 ± 30	85 ± 14	287 ± 122

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL

b. Ames Test – N-acetyl metabolite and impurities

Ames tests were conducted on the active human plasma metabolite NAMR as well as several identified or potential impurities containing an aromatic amine group that is a structural alert for genotoxicity.

In a GLP study (Report No. D-23129/3000922915, (b) (4), 5/9/00), NAMR (batch Km3263a) was Ames negative with and without metabolic activation.

The sponsor identified nine impurities (synthesis by-products or degradation products) containing an (b) (4) that is a structural alert for possible genotoxicity: (b) (4) When these were tested in the Ames assay, (b) (4) was clearly positive in strain TA98 with activation (Report D-23129/3000921993, (b) (4) 6/26/01, GLP; **Table IVC.1b.1**). However, according to the sponsor the specific lot of (b) (4) tested was shown to have (b) (4) impurity by HPLC, so a purer lot of (b) (4) was prepared as the (b) (4) which had less than (b) (4) impurity by HPLC and was considered negative when tested in a comparable study in the same laboratory (Report D-23129/9321020151, (b) (4) 11/28/02, GLP), although there was some suggestion of a (b) (4) increase (**Table IVC.1b.2**). This led the sponsor to conclude that (b) (4) is not inherently mutagenic and that the initial positive response was due to impurities "that have no relevance to RTG drug substance." The other eight impurities were considered negative in the Ames test.

Several synthetic process starting materials and intermediates were also identified as potential genotoxins (**Figure IVC.1b.1**). One of the (b) (4), is a known mutagen, and two (b) (4)

[redacted] were Ames positive when tested by the sponsor. Three compounds [redacted] were either reported to be or found to be negative in the Ames assay [redacted]

[redacted] is a [redacted] that is reportedly Ames positive in strains TA98 and TA100, with and without metabolic activation (**Table IVC.1b.3**) as well as clastogenic in CHO cells in the absence of metabolic activation [redacted] The [redacted], a well-known mutagen contained in many dyes, has been reported to induce tumors in mice [redacted]

[redacted] is a synthesis intermediate that was strongly positive in the Ames test (Report D-23129/3000921003, conducted by [redacted] report dated 3/22/01; **Table IVC.1b.4-5**). In the plate incorporation test without metabolic activation SS increases in numbers of revertants were seen in strains TA98, TA1537 and TA1538 (up to 23X for strain TA98) and with metabolic activation SS increases in revertants were found in strains TA98, TA100, TA1537 and TA1538 (up to 15X for strain TA1537). In the preincubation test without metabolic activation SS increases in revertants occurred in strains TA98 and WP2 *uvr* A (up to 15X for strain TA98), and with metabolic activation revertants were increased in strains TA98, TA100, TA1535, TA1538 and WP *uvr* A (up to 30X for strain TA1538).

[redacted] is a synthesis intermediate (and potential human metabolic intermediate) which also produced clearly positive results in the Ames test, particularly in tester stain TA98 in the presence of metabolic activation (Report WD2008/01821/00, conducted by [redacted], report dated 3/13/09; **Table IVC.1b.6**). In the plate incorporation test at concentrations of 15, 50, 100, and 150 µg per plate (limited by toxicity), increases in revertant colonies were observed in strain TA98 in the absence (up to 3X control) and in the presence of S9 (up to 4X). In the preincubation test at concentrations of 5, 15, 50, 100, and 150 µg per plate (limited by toxicity), increases in revertant colonies were also seen in strain TA98 in the presence of S9 (up to 8X). These results with the hydrochloride salt of [redacted] confirmed initial Ames testing with the [redacted] (Report D-23129/3000922544, conducted by [redacted], dated 9/20/01), which produced a very similar positive response (**Table IVC.1b.7**). In the initial mutagenicity assay using the plate incorporation method (concentrations of up to 500 ug/plate with and without S9), positive responses were observed in the presence of S9 activation with tester strains TA 98 (3-fold maximum increase) and TA 1537 (4-fold) and in the absence of S9 activation with tester strain TA98 (3-fold). In the confirmatory mutagenicity assay using the preincubation method (concentrations of up to 150 ug/plate without and 500 ug/plate with S9), a positive response was observed in the presence of S9 activation with tester strain TA98 (8-fold). Although the greatest response was seen with metabolic activation, it should be noted that in both studies in tests without activation, and particularly with preincubation, concentrations tested were lower than those tested with S9 due to cytotoxicity.

Figure IVC.1b.1 Synthetic scheme for retigabine

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Table IVC.1b.7 Ames test of retigabine impurity (b) (4)

Test Article Id (b) (4)

Study Number : AA44YU.502002. (b) (4) Experiment No : B1
Study Phase : Initial Mutagenicity Assay via Plate Incorporation

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	11 ± 3	114 ± 20	9 ± 5	3 ± 0	11 ± 1
1.5	10 ± 5	115 ± 41	10 ± 2	3 ± 2	7 ± 3
5.0	6 ± 1	119 ± 21	13 ± 5	6 ± 3	8 ± 2
15	13 ± 6	130 ± 20	7 ± 3	3 ± 1	10 ± 3
50	25 ± 7	130 ± 14	7 ± 3	7 ± 2	9 ± 2
75	31 ± 10				
150	4 ± 1	0 ± 0	0 ± 0	1 ± 1	0 ± 0
500		0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive	87 ± 3	644 ± 17	120 ± 15	667 ± 297	53 ± 4

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	19 ± 7	117 ± 10	9 ± 3	4 ± 2	11 ± 1
1.5		131 ± 29	8 ± 0	6 ± 2	8 ± 3
5.0	19 ± 6	130 ± 29	10 ± 2	4 ± 1	8 ± 3
15	49 ± 5	156 ± 14	10 ± 2	8 ± 3	8 ± 2
50	63 ± 11	176 ± 11	7 ± 2	17 ± 7	10 ± 3
100	37 ± 4				
150	48 ± 7	178 ± 12	4 ± 4	10 ± 3	6 ± 3
500	23 ± 4	0 ± 0	1 ± 1	0 ± 1	1 ± 1
Positive	890 ± 39	1261 ± 31	119 ± 38	144 ± 53	270 ± 49

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Test Article Id : (b) (4)

Study Number : AA44YU.502002. (b) (4) Experiment No : B2
Study Phase : Confirmatory Assay via Preincubation

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	14 ± 3	118 ± 13	11 ± 4	5 ± 1	12 ± 2
0.50	10 ± 3	136 ± 11	14 ± 4	4 ± 1	11 ± 2
1.5	11 ± 3	98 ± 13	14 ± 1	4 ± 3	9 ± 2
5.0	13 ± 4	108 ± 15	13 ± 2	4 ± 1	8 ± 2
15	10 ± 2	7 ± 2	2 ± 1	2 ± 1	2 ± 1
50	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 1
150	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive	235 ± 19	391 ± 13	153 ± 19	364 ± 22	180 ± 21

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 3	133 ± 26	11 ± 4	6 ± 1	11 ± 2
1.5	20 ± 5	133 ± 8	16 ± 1	3 ± 1	8 ± 2
5.0	38 ± 5	144 ± 31	14 ± 3	5 ± 2	9 ± 3
15	104 ± 16	157 ± 11	11 ± 3	9 ± 3	12 ± 3
50	108 ± 9	149 ± 10	15 ± 1	9 ± 4	7 ± 3
150	87 ± 22	199 ± 11	11 ± 3	17 ± 8	11 ± 2
500	3 ± 1	3 ± 1	2 ± 1	2 ± 1	2 ± 1
Positive	674 ± 157	581 ± 130	72 ± 5	75 ± 21	126 ± 33

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

2. In vitro mammalian cell studies

The in vitro studies in mammalian cells included assessment of retigabine in CHO cells and human lymphocytes and assessment of the metabolite NAMR and the related substance FBI in CHO cells.

a. Retigabine

When retigabine (batch No. 96020014) was tested for the ability to induce gene mutations at the *hprt* locus in cultured Chinese Hamster Ovary (CHO) cells in the presence and absence of metabolic activation (rat S9) at concentrations up to 5000 ug/ml (no toxicity seen in preliminary test at up to 2000 ug/ml), no SS increases in mutant frequency were seen (study report no. D-23129/3000914286, conducted by (b) (4) report dated 9/29/97); apparent concentration-related increases (up to 2.3X) in MF seen in the absence of metabolic activation did not reach the pre-set criterion of 3X negative control (**Table IVC.2a.1**). Precipitate was noted at all concentrations. This increase was not replicated in the confirmatory assay, but culture contamination was a problem in the repeat assay. The sponsor's conclusion was that retigabine was negative in this assay.

Table IVC.2a.1

DOSE*			PLATE COUNTS							
(µg/mL)	FLASK	S9	1	2	3	4	5	TOTAL	MF	IMF
0	A	-	2	4	4	4	4	18	19.7	-
0	B	-	1	3	4	8	2	18	19.0	-
	MEAN								19.4	-
500	A	-	5	3	6	4	7	25	20.0	0.6
500	B	-	4	15	7	6	7	39	41.8	22.4
	MEAN								30.9	11.5
750	A	-	NC					-	-	-
750	B	-	NC					-	-	-
	MEAN								-	-
1000	A	-	1	2	2	4	2	11	19.9	0.5
1000	B	-	3	4	8	4	7	26	65.8	46.4
	MEAN								42.9	23.5
2000	A	-	9	5	6	5	8	33	33.3	13.9
2000	B	-	11	17	17	10	14	69	55.7	36.3
	MEAN								44.5	25.1
2500	A	-	CD					-	-	-
2500	B	-	NC					-	-	-
	MEAN								-	-
3000	A	-	CD					-	-	-
3000	B	-	NC					-	-	-
	MEAN								-	-
4000	A	-	CD							
4000	B	-	1	8	8	7	5	29	43.7	24.3
	MEAN								43.7	24.3
5000	A	-	3	4	3	0	2	12	30.9	11.5
5000	B	-	0	0	3	5	2	10	12.0	-7.4
	MEAN								21.5	2.1
EMS 150	A	-	CD							
EMS 150	B	-	25	24	22	30	23	124	142.5	123.2
	MEAN								142.5	123.2 **
EMS 300	A	-	38	43	58	47	58	244	301.2	281.9
EMS 300	B	-	27	30	38	28	34	157	239.1	219.7
	MEAN								301.2	281.9 **
							p value	0.5242		

MF = mutant frequency per million clonable cells; IMF = Induced Mutant Frequency = MF dose - mean MF Solvent Controls
* = Precipitate in culture media at all test article doses during treatment period
** = greater than 3 times the mean solvent control culture MF
CD = culture discontinued during expression period due to excessive toxicity
NC = culture not selected for cloning

Retigabine (batch Nos. OF06023 and 96020014) was also tested for the ability to induce structural chromosomal damage in cultured human blood lymphocytes in the presence or absence of metabolic activation (report no. D-23129/9321020150, conducted by [redacted](b) (4) report dated 12/3/02). Both retigabine batches induced chromosome aberrations following continuous treatment for 20+0 hours in the absence of S9 at concentrations that were considered at, or very close to, the cytotoxicity limit; however, increases in aberrations were seen at the lowest concentration in the absence of notable cytotoxicity (**Table IVC.2a.2**; blackouts in original).

These results agreed with those of an earlier study (report no. D-23129/3000914297, conducted by [redacted](b) (4) report dated 6/12/97) in which retigabine (batch no. 96020014; concentrations ranging from 12.5 to 200 ug/ml) induced a dose dependent clastogenic response (predominantly chromatid and chromosome breaks) when tested by exposing human lymphocytes continuously for 1.5 cell cycles (22 hr), with SS increases at 100 and 150 ug/ml (**Table IVC.2a.3**). The MI was significantly decreased among treated cultures (maximal about 50%) and the PI was not altered. The positive response was verified in a repeat assay. In the assays with metabolic activation, there were no SS increases at either time point.

Table IVC.2a.2

20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2, batch #OF0602[redacted]
Donor sex: female

Treatment (μg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	Mitotic Index (mean)
Solvent	A	100	1	1		4.9
	B	100	1	1		5.7
	C	ND	ND	ND		6.7
	D	ND	ND	ND		6.4
	Totals	200	2	2		(5.9)
45.75	A	100	2	1		6.1
	B	100	[redacted]			5.1
	Totals	200	9	7	NS	(5.6)
77.48	A	100	5	5		4.3
	B	100	[redacted]			4.3
	Totals	200	14	11	$p \leq 0.01$	(4.3)
106.3	A	100	[redacted]			3.0
	B	100				2.8
	Totals	200	22	19	$p \leq 0.001$	(2.9)
NQO, 2.50	A	100	[redacted]			
	B	100				
	Totals	200	55	51	$p \leq 0.001$	

Binomial Dispersion Test $\chi^2 = 4.32$, NS
Note: solvent replicates C and D scored for mitotic index only
§ Statistical significance (Appendix 5)
ND = not determined
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

Table IVC.2a.2 (cont.)

20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2, batch #96020014
Donor sex: female

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Significance §	Mitotic Index (mean)
Solvent	A	100	2	1		5.3
	B	100	0	0		6.0
	C	ND	ND	ND		6.1
	D	ND	ND	ND		5.0
	Totals	200	2	1		(5.6)
45.75	A	100	4	3		5.8
	B	100	4	3		4.7
	Totals	200	8	6	$p \leq 0.05$	(5.3)
77.48	A	100	[redacted]			4.1
	B	100	6	5		3.5
	Totals	200	13	11	$p \leq 0.01$	(3.8)
106.3	A	100	[redacted]			2.2
	B	100				3.5
	Totals	200	17	14	$p \leq 0.001$	(2.9)

Binomial Dispersion Test $\chi^2 = 1.41$, NS
Note: solvent replicates C and D scored for mitotic index only
§ Statistical significance (Appendix 5)
ND = not determined
NS = not significant
Numbers highlighted exceed historical negative control range (Appendix 6)

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Table IVC.2a.3

TABLE 14. CHROMOSOMAL ABERRATION DATA FOR HUMAN BLOOD LYMPHOCYTES TREATED WITH D-23129 (ILS #97-02) CONTINUOUSLY FOR 1.5 CELL CYCLE DURATIONS IN THE ABSENCE OF METABOLIC ACTIVATION - SUMMARY DATA

DOSE (µg/mL)	NO. CELLS	CHROMOSOMAL ABERRATION TYPES													CA/Cell		%DC		P-Value+
		G'	G*	B'	B*	DM	TR	QR	Dic	Rg	CR	>10	PCh	PCe	+gaps	-gaps	+gaps	-gaps	
MMC-0.05	100	5	15	43	22	0	1	1	0	0	0	0	0	0	0.870	0.670	49.00	48.00	<0.001*
0.0	200	2	1	3	1	0	0	0	0	0	0	0	0	0	0.035	0.020	3.50	2.00	-
12.5								Not Scored											
25.0								Not Scored											
50.0	200	2	0	4	1	0	0	0	0	0	0	0	0	0	0.035	0.025	3.50	2.50	0.500
100.0	200	5	4	11	3	0	0	0	0	0	0	0	0	0	0.115	0.070	10.50	6.50	0.022*
150.0	200	3	4	9	5	0	1	0	0	0	0	0	0	0	0.110	0.075	9.50	6.50	0.022*
200.0								Not Scored Due to Excessive Toxicity											
														ONE-TAILED TREND TEST P VALUE				0.003*	

Abbreviations: G',G* = chromatid and chromosome gaps, respectively; B',B* = chromatid and chromosome breaks, respectively; DM = double minute; TR = triradial; QR = quadriradial; Dic = dicentric; Rg = ring; CR = other complex rearrangement; >10 = more than 10 aberrations per cell; PCh = pulverized chromosome; PCe = pulverized cell; CA/Cell = number of aberrations per cell (excluding PCh and PCe); %DC = percentage of metaphase cells with at least one (excluding PCh and PCe); MMC = mitomycin C. P-Value+ based on pairwise comparison against concurrent control using one-tailed Fisher's exact test. *Significantly different from control data at $p < 0.05$.

b. N-acetyl metabolite and impurities

The N-acetyl metabolite of retigabine (NAMR) was tested in the in vitro mammalian chromosomal aberration test using CHO cells (study no. PR2007-002, conducted by [redacted](b) (4) report dated 10/29/07, GLP) and found to induce structural chromosome aberrations under all conditions (4 hours in the presence and absence of S9, 20 hours in the absence of S9) at the highest concentration, 1000 ug/ml, which decreased the MI by about 50% or greater (**Table IVC.2b.1**).

The impurity [redacted](b) (4) was negative in a chromosomal aberration assay in CHO cells (PR2006-074, [redacted](b) (4) 1/31/07).

Table IVC.2b.1

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SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	12.2	200	200	0.000	±0.000	0.5	0.0
N-Acetyl Metabolite of Retigabine									
250	-S9	4	12.2	200	200	0.000	±0.000	0.0	0.0
500	-S9	4	12.4	200	200	0.005	±0.071	0.5	0.5
1000	-S9	4	6.8	200	200	0.135	±0.422	0.5	11.0**
MMC, 0.2	-S9	4	7.4	200	100	0.150	±0.359	0.0	15.0**
DMSO	+S9	4	13.1	200	200	0.000	±0.000	1.5	0.0
N-Acetyl Metabolite of Retigabine									
250	+S9	4	13.4	200	200	0.005	±0.071	1.5	0.5
500	+S9	4	13.3	200	200	0.005	±0.071	0.0	0.5
1000	+S9	4	5.3	200	200	0.105	±0.353	1.0	9.5**
CP, 10	+S9	4	4.1	200	100	0.270	±0.601	0.5	20.0**
DMSO	-S9	20	14.8	200	200	0.000	±0.000	0.0	0.0
N-Acetyl Metabolite of Retigabine									
150	-S9	20	11.5	200	200	0.015	±0.158	1.5	1.0
300	-S9	20	7.1	200	200	0.010	±0.100	0.5	1.0
1000	-S9	20	5.5	200	200	0.115	±0.364	1.0	10.0**
MMC, 0.1	-S9	20	6.4	200	100	0.340	±1.265	0.5	17.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's exact test.

3. In vivo studies

a. Retigabine

Retigabine (batch no. 96020014) was evaluated in an in vivo mouse micronucleus assay (report no. D-23129/3000914308, conducted by [redacted](b) (4) report dated 6/12/97, GLP) in B6C3F1 mice at doses of 25, 50, and 100 mg/kg (males) or 50, 100, and 200 mg/kg (females). Clinical signs were seen at the HD and included lethargy, shallow breathing, tremors, and convulsions (females) and there was 1 HD death (female). The frequency of MN-PCE was not considered by the sponsor to be significantly increased at either sample time (**Table IVC.3a.1**). A SS increase was seen in LD females at the 48 hr time point, but was dismissed because of magnitude of the change and the lack of a dose response. CP induced a positive MN-PCE response.

An older batch (RDx824) was also negative when tested at a single dose (316 mg/kg) in a mouse micronucleus assay (study D-20443/3000887657, [redacted](b) (4) 12/16/92, GLP). At this dose, clinical signs included hypokinesia, clonic convulsions, decrease of muscle tone with abdominal position, loss of righting reflex with dorsal position, and stilted gait. Two males and one female died. There were no increases MN-PCE frequencies 24, 48, or 72 hrs after administration.

Retigabine (batch no. 96020014) was negative (no increase in NNG counts) in a UDS assay (study D-23129/3000914310, conducted by [redacted](b) (4); report dated 6/26/97; GLP) in male Fischer rat hepatocytes at oral (gavage) doses of 25, 50, or 100 mg/kg after exposure for 2 and 16 hours. At the HD, 2/4 animals were found dead between 13 and 15 hours of exposure. The 2 remaining animals were perfused after 15 and 16 hours of exposure, and were negative in the UDS assay. The same dose was negative after 2 hours of exposure. Dose selection was based on the results of a dose-range finding study in which doses of 150 and 200 mg/kg were lethal. The positive control, N-2-fluorenylacetamide (10 mg/kg), was positive after exposures for either 2 or 16 hours.

Table IVC.3a.1. Summary of mouse micronucleus results for retigabine

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Table 8. *In Vivo* Mouse Bone Marrow Erythrocyte Micronucleus Assay with D-23129. Micronucleus Experiment: Summary Micronucleated PCE and %PCE for Male Mice

Sample Time (Hr)	Dose (mg/kg)	N	MN-PCE/1000 PCE Mean	sem	P-Value	%PCE Mean	sem	P-Value+
24	CP	5	18.5	1.85	<0.001*	51.7	2.40	0.038*
	Sham	5	1.4	0.24	-	61.2	2.23	-
	0	5	1.0	0.27	0.730	60.0	2.36	0.722
	25	5	1.5	0.42	0.212	62.5	3.26	0.567
	50	5	1.1	0.33	0.500	58.9	1.36	0.700
	100	5	1.9	0.43	0.069	60.5	2.57	0.890
	P-Value++		0.063			0.789		
48	Sham	5	1.6	0.29	-	57.7	1.90	-
	0	5	2.2	0.78	0.209	53.0	2.31	0.159
	25	5	1.4	0.37	0.878	60.4	2.11	0.047*
	50	5	2.5	0.89	0.385	56.0	1.81	0.342
	100	5	1.7	0.86	0.739	58.0	5.48	0.430
	P-Value++		0.635			0.467		

MN-PCE data based on 2000 PCE scored per mouse; %PCE data based on 1000 erythrocytes scored per mouse. Solvent was propylene glycol. P-Value+ based on pairwise comparison of concurrent solvent control against sham control and other dose groups against the concurrent solvent control using one-tailed Chi-square test for MN-PCE data and two-tailed student's t test for %PCE data.
P-Value++ based on one-tailed trend test for MN-PCE data and ANOVA for %PCE data.
*Significantly different from control data at p <0.05.

Table 12. *In Vivo* Mouse Bone Marrow Erythrocyte Micronucleus Assay with D-23129.
Micronucleus Experiment: Summary Micronucleated PCE and %PCE for Female Mice

Sample Time (Hr)	Dose (mg/kg)	N	MN-PCE/1000 PCE Mean	scm	P-Value	%PCE Mean	scm	P-Value+
24	CP	5	10.4	1.81	<0.001*	54.2	2.91	0.023*
	Sham	5	2.1	1.00	-	62.4	3.56	-
	0	5	0.8	0.34	0.987	64.5	2.24	0.633
	50	5	1.3	0.51	0.191	61.9	1.93	0.394
	100	5	0.8	0.20	0.401	57.5	3.36	0.119
	200	5	0.6	0.29	0.605	62.9	3.03	0.684
	P-Value++		0.827			0.326		
48	Sham	5	1.2	0.34	-	61.2	1.28	
	0	5	1.3	0.34	0.500	63.1	2.20	0.471
	50	5	2.5	0.65	0.037*	60.4	1.50	0.332
	100	5	1.1	0.40	0.581	63.0	2.80	0.961
	200	5	1.8	0.54	0.236	52.0	3.35	0.028*
	P-Value++		0.442			0.024*		

MN-PCE data based on 2000 PCE scored per mouse; %PCE data based on 1000 erythrocytes scored per mouse.
Solvent was propylene glycol. P-Value+ based on pairwise comparison of concurrent solvent control against sham control and other dose groups against the concurrent solvent control using one-tailed Chi-square test for MN-PCE data and two-tailed student's t test for %PCE data. P-Value++ based on one-tailed trend test for MN-PCE data and ANOVA for %PCE data.
*Significantly different from control data at p <0.05.

b. Impurities

(b) (4)

In a combined micronucleus and Comet assay (Study no. WD2009/00868/00, conducted by (b) (4) 7/30/09, GLP) the (b) (4) referred to as (b) (4) was administered orally (gavage, 10ml/kg) at doses of 0 (vehicle: 1% (v/v) aqueous methylcellulose), 375, or 750 mg/kg/day on 3 consecutive days to male rats (S-D, 5/group). The micronucleus positive control cyclophosphamide (CPA), was given orally once only on Day 2 to 3 male rats at a dose of 20 mg/kg. The comet positive control ethyl methane sulphonate (EMS) was given orally once only on Day 3 to 5 male rats at a dose of 250 mg/kg. Rats were killed approximately 3 hours after receiving their final dose (CPA group killed 24 hours final dose) and femoral bone marrow smears were prepared for micronucleus assessment (2000 PCE/animal) and single cell liver suspensions were prepared for comet assessment. The liver was chosen as the target organ based on the Ames test results showing greater mutagenic activity in the presence of metabolic activation. For the comet end point, slides were prepared, cells lysed and the DNA left to 'unwind', then electrophoresed (pH>13), neutralized and stained with propidium iodide. Slides were analyzed by fluorescence microscopy using the COMET Assay IIITM image analysis system. Three slides per tissue were prepared from each animal and 50 cells per slide were analyzed for the presence of comets, to give a total number of 150 cells per tissue per animal. In addition, the presence of excessively damaged cells (% clouds) was scored manually from a total of 100 cells, as an indicator of cytotoxicity. According to the report, administration of three consecutive daily doses (approximately 21-24 hours apart) with a single harvest approximately 3 hours after the final dose enabled the evaluation of cells exposed to (b) (4) at time points of 24 and 48 hours prior to sampling for the micronucleus test and 3, 24 and 48 hours prior to sampling for the Comet assay. This appears to be acceptable according to published protocols, eg, (b) (4)

Use of a single sex and dose selection were based on a dose range-finding study (oral doses of 100, 250, 500, 750, and 1000 mg/kg/day for 3 days) in which clinical signs (subdued behavior, labored breathing, piloerection, unsteady and/or tiptoe gait, low and/or hunched posture, red staining around nostrils) and mortality (1 male and 1 female found dead on Days 2 and 3, respectively) were seen at 1000 mg/kg and there was no clear sex difference in response.

In the main study, there were no deaths and no notable clinical signs. Decreased body weight gain was seen at both doses (25% and 35% compared to VC). No T-R macroscopic findings were observed in the livers of treated animals. Group mean values for MPCE/2000 PCE at both doses of (b) (4) were similar to the concurrent vehicle control and fell within the laboratory historical control range (**Table IVC.3b.1)**. For the Comet assay, there was a small non-D-D increase in the comet response in the liver of (b) (4) treated rats based on means; and group tail moment and % tail DNA measurements were increased in individuals from treated groups (**Table IVC.3b.2**), with the (similar) highest values found in animals 71 (LD) and 76 (HD). Notable was the variability across slides in these animals. These increases were dismissed by the sponsor because they were considered non-D-D and fell within the historical control ranges, which also exhibited high variability (0.02-0.5 and 0.1-3.64 for tail moment and % tail DNA, respectively). There was no indication of excessive toxicity (% clouds) in the cells examined. Vehicle and positive control values indicated a valid assay. A separate PK study was conducted using the same (b) (4) doses and blood samples from the hepatic portal vein and femoral artery indicated that the target organs were exposed to (b) (4) Mean AUC_{0-t} and Cmax values from hepatic portal vein blood samples were 10.9 µg.h/ml and 10.97 µg/ml at the LD and 20.9 µg.h/ml and 14.47 µg/ml at the HD, respectively.

Table IVC.3b.1 Summary of in vivo rat micronucleus and Comet assay results for (b) (4)

Study Type: Genetic Toxicology In Vivo - Micronucleus **Study No:** R28619

Test Article	Nominal Dose[1] (mg/kg/day)	No. of Animals Analysed[2]	Group Mean %PCE	Group Mean MPCE[3]
Vehicle control	0	5 M	52	3.56
(b) (4)	375	5 M	52	2.18
	750	5 M	49	1.58
CPA[4]	20	3 M	59	40.37

1. Expressed in terms of the parent compound
2. M = Male
3. Group mean micronucleated PCE (MPCE) per 2000 PCE analysed
4. Positive control (Cyclophosphamide (CPA)) induced a clear unequivocal positive response

Study Type: Genetic Toxicology In Vivo - Comet **Study No:** R28619

Test Article	Nominal Dose[1] (mg/kg/day)	No. of Animals Analysed[2]	Liver Group Tail Moment[3] (arbitrary unit)	Liver % Tail DNA[3]	Group Mean % Clouds[3]
Vehicle control	0	5M	0.08 (0.03)	0.93 (0.32)	1.40 (0.49)
(b) (4)	375	5M	0.12 (0.04)	1.36 (0.51)	1.20 (0.61)
	750	5M	0.10 (0.05)	1.16 (0.46)	1.27 (0.64)
EMS[4]	250	5M	4.02 (0.87)	18.05 (1.95)	1.60 (0.60)

1. All doses are expressed in terms of parent compound
2. M = Male
3. Figures in parenthesis = standard deviation
4. Positive control (Ethyl methane sulphonate (EMS)) induced a clear unequivocal positive response

Table IVC.3b.2 Individual Comet assay results for (b) (4)

Comet Assay Test Data

Individual and Mean Results of Comet Analysis in the Liver of Male Rats - Vehicle Control 1% (w/v) aqueous methylcellulose

Animal No.	Slide No.	Median Tail Moment per Slide	Average Tail Moment per Animal	Group Tail Moment (SD)	Median % Tail DNA per Slide	Average % Tail DNA per Animal	Group % Tail DNA (SD)	% Clouds	Average % Clouds	Group Mean % Clouds (SD)
66	7	0.07	0.04	0.08 (0.03)	1.04	0.54	0.93 (0.32)	0	1.33	1.40 (0.49)
	26	0.01			0.23			2		
	41	0.03			0.36			2		
67	17	0.07	0.06		0.85	0.68		0	1.67	
	36	0.09			0.82			2		
	56	0.03			0.36			3		
68	10	0.08	0.07		0.71	0.97		2	2.00	
	28	0.12			1.82			2		
	58	0.02			0.37			2		
69	1	0.03	0.12		0.51	1.25		0	0.67	
	27	0.24			2.39			0		
	37	0.08			0.84			2		
70	19	0.16	0.11		1.77	1.23		1	1.33	
	22	0.16			1.61			1		
	43	0.02			0.32			2		

(SD) = Standard deviation

Individual and Mean Results of Comet Analysis in the Liver of Male Rats – 375 mg/kg/day (b) (4)

Animal No.	Slide No.	Median Tail Moment per Slide	Average Tail Moment per Animal	Group Tail Moment (SD)	Median % Tail DNA per Slide	Average % Tail DNA per Animal	Group % Tail DNA (SD)	% Clouds	Average % Clouds	Group Mean % Clouds (SD)
71	3	0.14	0.17	0.12 (0.04)	1.82	2.09	1.36 (0.51)	0	1.33	1.20 (0.61)
	12	0.08			0.73			1		
	38	0.30			3.71			3		
72	29	0.26	0.16		2.63	1.65		3	2.00	
	32	0.13			1.27			1		
	50	0.09			1.05			2		
73	6	0.15	0.09		1.74	1.04		3	1.33	
	31	0.04			0.50			1		
	46	0.08			0.89			0		
74	11	0.19	0.13		1.68	1.22		0	0.33	
	44	0.17			1.76			0		
	51	0.02			0.21			1		
75	30	0.04	0.07		0.40	0.80		1	1.00	
	52	0.03			0.32			0		
	55	0.15			1.67			2		

(SD) = Standard deviation

Individual and Mean Results of Comet Analysis in the Liver of Male Rats – 750 mg/kg/day (b) (4)

Animal No.	Slide No.	Median Tail Moment per Slide	Average Tail Moment per Animal	Group Tail Moment (SD)	Median % Tail DNA per Slide	Average % Tail DNA per Animal	Group % Tail DNA (SD)	% Clouds	Average % Clouds	Group Mean % Clouds (SD)
76	16	0.08	0.17	0.10 (0.05)	1.14	1.93	1.16 (0.46)	0	1.00	1.27 (0.64)
	25	0.33			3.50			2		
	35	0.10			1.15			1		
77	8	0.10	0.12		0.76	1.18		0	0.33	
	42	0.11			1.26			0		
	54	0.15			1.53			1		
78	24	0.00	0.07		0.06	0.79		1	1.67	
	40	0.16			1.77			3		
	49	0.05			0.55			1		
79	2	0.10	0.09		1.31	1.07		1	1.33	
	9	0.09			1.05			1		
	57	0.08			0.85			2		
80	18	0.07	0.05		1.21	0.81		1	2.00	
	23	0.02			0.42			3		
	39	0.07			0.79			2		

(SD) = Standard deviation

(b) (4)

The impurity (b) (4) was negative in a rat micronucleus assay (D-23129//3000922386, (b) (4) 11/05/01, GLP; **Table IVC.3b.3**), and the impurity (b) (4) was negative in a mouse micronucleus assay (Study No. PR2006-076, (b) (4) 4/5/07, GLP).

Table IVC.3b.3 Summary of rat micronucleus assay with (b) (4) (b) (4)

Rat micronucleus test

Table 1

SUMMARY

Dose in mg/kg b.w.p.o.	Sampling time (h)	Number of polychromatic erythrocytes scored per group*	Ratio PCE/NCE#			Micronucleated polychromatic erythrocytes mean frequency per 1000 PCE			Significance
			males	females	treatment group*	males	females	treatment group*	
0	24	20000	0.55	0.56	0.56	0.9	0.9	0.9	-
100	24	20000	0.79	0.59	0.69	0.9	0.5	0.7	n.s.
300	24	20000	0.80	0.74	0.77	1.0	1.0	1.0	n.s.
1000	24	20000	0.91	0.72	0.82	0.9	0.9	0.9	n.s.
0	48	20000	0.89	0.60	0.75	1.1	1.1	1.1	-
1000	48	20000	0.77	0.36	0.57	1.2	0.7	1.0	n.s.
Cyclophosphamide									
27 mg/kg b.w. i.p.	24	20000	0.33	0.33	0.33	10.2	7.0	8.6	s.

s. significant at $p \leq 0.05$
n.s. not significant at $p \leq 0.05$
* males and females combined
PCE polychromatic erythrocytes
NCE normochromatic erythrocytes
per 1000 counted cells

D. CARCINOGENICITY

1. TWO YEAR ORAL (GAVAGE) CARCINOGENICITY STUDY OF RETIGABINE IN CRL:WI(GLX/BRL/HAN)IGSBR RATS (Study. No.: 782A-101-050-04; conducted by (b) (4) ; report dated 12/27/07; GLP)

a. Methods

Retigabine (Lot Nos.: 0206102 for approximately 41 weeks and 0005005 for remainder) was administered orally (gavage) to Wistar rats (Crl:WI(Glx/BRL/Han)GSBR; 50-70 rats/sex/group main, 5-20/sex/grp TK) at doses of 0 (water control, WC), 0 (propylene glycol vehicle control, VC), 5, 20, or 50 mg/kg/day for at least 104 weeks. Mortality, clinical signs, ophthalmoscopic examinations, physical examinations, body weights, food consumption, hematology, coagulation, clinical chemistry, urinalysis, TSH and T4 evaluation, macroscopic and microscopic pathology (all animals) were evaluated. TK parameters for retigabine and its N-acetyl metabolite were determined in plasma samples collected at selected time points.

Dose selection was based on the results of a 26-week study in Wistar rats, with oral (gavage) doses of 0, 5.11, 17.8, and 61.9 mg/kg. There was 1 death of a HD male that was considered possibly treatment-related (after first dose). This animal exhibited clinical signs, including clonic convulsions, but no cause of death was determined. CNS signs (convulsions, decreased activity, ataxia, hypersensitivity, loss of righting) were seen at ≥17.8 mg/kg, and decreased BW gain (10%; SS in males only) and clinical chemistry changes (↑ ALT, AP) were seen at the HD. Histopathology findings were limited to hepatocellular hypertrophy and thyroid follicular cell hypertrophy in HD males and females. According to the sponsor's dose selection rationale, the HD in this study exceeded the MTD based on the 1 death; therefore, the HD for the carcinogenicity study was reduced to 51.1 mg/kg. The Exec-CAC agreed that the sponsor was probably close to the MTD based on the results of the 26-week study. However, they recommended that a planned 2nd range-finding study include a higher dose (ie, bracket the HD in the 26-week study) in order to confirm that they have correctly identified the MTD (I53,950, SN155; see protocol review and minutes dated12/10/02). In a second dose range-finding study, oral (gavage) administration to Wistar rats at doses of 5.11, 12.1, 28.7, or 68.1 mg/kg/day for 13 weeks resulted in 2 unscheduled deaths at the HD (2 females after 36 and 48 days of treatment). No treatment-related (T-R) effects were seen on body weights, food consumption, hematology, urinalysis, or ophthalmoscopic examination. T-R findings included clinical signs (waddling gait, tremor, ventral recumbency, uncoordinated movements) at the two highest doses; increased phospholipid, T4, and ALT at the HD; increased urinary bladder weights accompanied by histopathology (congestion of submucosal vessels and an increased incidence and/or severity of ectasia) in HD males; and hepatocellular hypertrophy and thyroid follicular cell hypertrophy at the 2 highest doses. These findings were generally consistent with the results of the 26-week study.

b. Results

i. Mortality, body weight, ophthalmology, clinical pathology, gross necropsy

A dose-related increase in mortality was seen in treated groups compared to controls. Survival was 42/50 and 35/50 in WC, 25/50 and 30/50 in VC (statistically significant [SS] decrease in males compared to WC), 33/50 and 20/50 in LD (both sexes SS compared to WC), 38/70 and 34/68 in MD (both sexes SS compared to WC; 2 MD females died accidentally), and 18/70 and 24/70 in HD (both sexes SS compared to WC and VC)

males and females, respectively. This gives mortality rates of 16%, 50%, 34%, 46%, and 74% in males and 30%, 40%, 60%, 50%, and 66% in females from the WC, VC, LD, MD, and HD groups, respectively.

T-R clinical signs, seen in both sexes sporadically throughout the treatment period primarily at the MD and HD, consisted of increased incidences of decreased activity, cool to touch, decreased muscle tone, tremors, and ataxia noted within one hour of dose administration. A SS decrease in BW was seen in HD males during weeks 3-7 and 9-104 compared to vehicle controls. No SS differences were seen in females. Final BWs were 559, 491, 506, 494, and 434 (12% below VC) in males and 360, 315, 313, 319, and 294 in females in the WC, VC, LD, MD, and HD groups, respectively (SS in HD males only). RBC parameters were increased (SS) in MD and HD males at termination. Serum bilirubin was increased (SS) in HD males at weeks 52, 78, and 105 and 106. There were no notable effects on ophthalmology findings, urinalysis, or hormone (TSH/T4) levels. D-R increased incidences of macroscopic findings of distended stomach and discolored lungs were observed in both sexes (**Table IVD.1.1**).

ii. Non-neoplastic (**Tables IVD.1.2** and **1.4**)

Histopathological changes considered T-R by the sponsor were limited to D-D decreases in colloid in thyroid follicles ("colloid was faintly basophilic and often had a clumped appearance") and reduced thyroid size (3/50 and 1/50 LD, 26/70 and 7/70 MD, and 39/70 and 16/70 HD males and females, respectively). There were clear D-R (expected) effects on the bladder (dilatation, hemorrhage, hyperplasia, inflammation, uroliths) and kidney (pelvic dilatation, papillary necrosis) that were inexplicably ignored in the pathology report. There were also D-R increases in bile duct congestion, lung congestion and suppurative inflammation, lymphoid depletion, prostate inflammation, decreased secretion and inflammation of the seminal vesicles, splenic pigment deposition, thyroid cysts, and uterine dilatation in treated rats.

iii. Neoplastic (**Tables IVD.1.3** and **1.5**; FDA statistical review)

The incidence of testicular interstitial cell tumors was elevated in HD males and showed a SS increasing dose-trend in the sponsor's analysis: 4, 2, 0, 1, and 6% in WC, VC, LD, MD, and HD group males, respectively. Because the incidences were within the historical range they were considered to be spurious by the sponsor. When tumor types were combined (in some cases inappropriately) in the skin/subcutaneous tissue or preputial gland in males, SS increases were seen in the LD and/or the MD groups compared to the WC but not the VC group. When each tumor type in these tissues was evaluated separately, only the increase in preputial gland carcinoma was SS in the LD and MD groups compared to WC but not VC. These statistical differences were not considered to be biologically relevant by the sponsor because of the lack of a D-R. None of the tested tumor types was considered to have a SS positive dose-response relationship in the FDA statistical analysis.

iv. Toxicokinetics (**Table IVD.1.6**)

Cmax and AUC levels of parent and metabolite increased fairly dose-proportionally over the dose range studied. Values increased between weeks 2 and 26, then decreased somewhat. There was no clear sex difference. Human exposure levels (AUC) are expected to be ~31 µg.hr/ml for retigabine and 23 ug.hr/ml for NAMR after the highest proposed therapeutic dose of 1200 mg/day.

c. Conclusion

Chronic oral administration of retigabine to rats at doses of 5, 20, or 50 mg/kg was associated with clinical signs, decreased BW and increased mortality at the HD, but there were no clearly T-R increases in tumor incidence. Dose selection appears to have been appropriate; however, the HD mortality rate was unexpectedly high, and the FDA statistical reviewer (see stat review) stated that, "Based on the survival criterion Haseman proposed, it may be concluded that not enough rats were exposed to the high dose for a sufficient amount of time in either sex, especially in females." But it appears that adequate numbers remained at the end of the study for the study be considered valid (18 males and 24 females).

Table IVD.1.1 Rat carcinogenicity study results: survival and body weight effects

Daily Dose (mg/kg)	0 (Vehicle)		5		20		50		0 (Water)	
Gender	M	F	M	F	M	F	M	F	M	F
No of Animals										
at Start	50	50	50	50	70	70	70	70	50	50
Died/Sacrificed	25	20	17	30	32	36	52	46	8	15
Terminal Sacrifice	25	30	33	20	38	34	18	24	42	35
Survival (%)	50	60	66	40	54	49	26	34	84	70
Body Weight (g, %)[a]	490.5	315.2	3.2	-0.7	0.8	1.2	-11.5**	-6.7	13.9**	13.5**
Food Consumption (g/day, %)[a]	14.0	11.7	4.3	3.4	2.9	-3.4	-15.7	-10.3	36.4**	20.5**

* $p < 0.05$
** $p < 0.01$
Statistical significance is based on group means (not on the percent differences)

Table IVD.1.2 Rat carcinogenicity study: clinical pathology, gross and microscopic pathology

Daily Dose (mg/kg)	0 (propylene glycol)		5 mg/kg/day		20 mg/kg/day		50 mg/kg/day		0 (water)	
Gender	M	F	M	F	M	F	M	F	M	F
Noteworthy Findings										
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Hematology-(%[a]) PG control	25	28	33	20	37	33	18	24	41	34
RBC	8.04	8.06	4.7	0.9	7.5*	-0.9	13.6**	2.0	2.4	-0.7
HB	15.3	15.6	3.3	-1.3	4.6	-0.6	10.5*	1.9	2.6	0
HCT	41.5	44.7	3.4	-1.1	5.5	-1.3	12.0**	1.3	3.6	-1.1
Clinical Chemistry (%[a])-PG Control	25	29	31	19	37	33	18	24	41	35
TBili	0.25	0.26	12	3.8	16*	3.8	28**	7.7	8	0
Gross pathology –No. examined	(50)	(50)	(50)	(50)	(70)	(70)	(70)	(70)	(50)	(50)
Distended stomach	3	1	3	5	2	9	14	15	0	1
Discolored lungs	11	5	7	10	15	12	20	20	0	1
Histopathology- No. examined	(50)	(50)	(50)	(50)	(70)	(70)	(70)	(70)	(50)	(50)
Decreased/altered thyroid follicles	0	0	3	1	26	7	39	16	1	1

- No noteworthy (biologically significant) findings
Dunnett's test (two-tailed); * $p < 0.05$; ** $p < 0.01$
[a] = For controls, termination group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on group means (not on percent differences
For statistical purposes, comparisons were made to the propylene glycol group (PG control).

Table IVD.1.3 Rat carcinogenicity study: neoplastic lesions

Daily Dose (mg/kg) Gender		R/C	0 (prop. glycol) Male	0 (water) Male	5 mg/kg/day Male	20 mg/kg/day Male	50 mg/kg/day Male
Summary of Statistical Analysis ([a])							
Skins Lesion (A)			(50)	(50)	(50)	(69)	(70)
Squa Cell Papilloma/Carcinoma/Kera	(a)	C	2	1	7	1	1
Preputial Gland (A)			(50)	(50)	(48)	(69)	(67)
Squamous Cell Papilloma	(a)	C	2	0	4	2	0
Squamous Cell Carcinoma	(a)	C	5	2	5	4	3
Squamous Cell Papilloma/Carcinoma	(a)	C	6	2	9	6	3
Carcinoma/Squamous Cell Carcinoma	(a)	C	6	2	9	9	6
Carcinoma	(a)	C	3	0	5	7	3
Adenoma/Carcinoma	(a)	C	3	0	6	7	3
Aden/Carc/Squa Cell Papi/Carc	(a)	C	7	2*	14	11	6
Testis (A)			(50)	(50)	(50)	(70)	(70)
Interstitial Cell Tumor	(a)	C	1	2	0	1	4
Thyroid (A)			(50)	(50)	(50)	(70)	(70)
C-Cell Adenoma/C-Cell Carcinoma	(a)	C	2	7	7	5	2

(a) Number of animals with tumor; R/C Spontaneous tumor incidence rate <1% (R=Rare tumor) or ≥ 1% (C=Common tumor);
(A) Number of tissues examined
Statistical Significance: Rare tumor - p≤0.025 (trend), p≤0.05 (pairwise); Common tumor - p≤0.005 (trend), p≤0.01 (pairwise)
*Statistically significant at the defined significance level

Table IVD.1.4 Selected histopathology incidences (CEL1-5: VC, LD, MD, HD, WC)

782A-101-050-04 Terminal Sacrifice Male Rat	GROUP CEL1			GROUP CEL2			GROUP CEL3			GROUP CEL4			GROUP CEL5		
	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total
URINARY BLADDER (CONTINUED)															
Dilatation					1	1		2	2	2	7	9			
Edema															
Hemorrhage		1	1		1	1		1	1		5	5			
Hyperplasia, Epithelium								1	1	1	6	7		1	1
Inflammation, Chronic										1	2	3			
Inflammation, Chronic Active							1	1	2		4	4		1	1
Inflammation, Suppurative					1	1					5	5			
Mineralization										1		1			
Uroliths											2	2			
VERTEBRA (NO. EXAMINED)		(1)	(1)												
ZYMBAL'S GLAND (NO. EXAMINED)														(1)	(1)
Carcinoma														1	1
SYSTEMIC NEOPLASMS (TOTAL ANIMALS															
EXAMINED)	(25)	(25)	(50)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(52)	(70)	(42)	(8)	(50)
Mesothelioma#		1	1				1		1						
Histiocytic Sarcoma#		1	1												
Malignant Schwannoma#					1	1									
Malignant Lymphoma#				1		1									

no. of animals with diagnosis

782A-101-050-04 Terminal Sacrifice Female Rat	GROUP CEL1			GROUP CEL2			GROUP CEL3			GROUP CEL4			GROUP CEL5		
	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total
TRACHEA (CONTINUED)															
Dilated Mucosal Glands	5	1	6		6	6	6	1	7	5	2	7	5	4	9
Hemorrhage		1	1		1	1									
Inflammation, Chronic										1		1	1		1
Inflammation, Chronic Active					1	1									
Inflammation, Suppurative		1	1		1	1		3	3		1	1			
Necropurulent Exudate					1	1		2	2		2	2			
Necrosis, Epithelium		1	1					3	3						
URETER (NO. EXAMINED)							(1)		(1)					(1)	(1)
Dilatation							1		1					1	1
URETHRA (NO. EXAMINED)															
URINARY BLADDER (NO. EXAMINED)	(30)	(19)	(49)	(20)	(30)	(50)	(34)	(36)	(70)	(23)	(45)	(68)	(35)	(14)	(49)
Mesothelioma					1	1									
Calculus															
Dilatation								2	2		5	5			
Edema				1		1									
Hemorrhage														1	1
Hyperplasia, Epithelium		1	1				1		1						
Inflammation, Chronic							1		1				2		2
Inflammation, Chronic Active		1	1	1		1									
Inflammation, Suppurative															
Mineralization															
Uroliths															

Table IVD.1.5 Selected tumor incidences (CEL1-5: VC, LD, MD, HD, WC)

782A-101-050-04 Terminal Sacrifice Male Rat	GROUP CEL1			GROUP CEL2			GROUP CEL3			GROUP CEL4			GROUP CEL5		
	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total
SUBCUTANEOUS TISSUE (NO. EXAMINED)	(2)	(2)	(4)	(1)	(1)	(2)	(3)	(1)	(4)		(3)	(3)	(3)	(1)	(4)
Fibroma							1		1		1	1			
Fibrosarcoma	1		1												
Granular Cell Tumor													1		1
Hemangiosarcoma							1		1				1		1
Histiocytic Sarcoma		1	1												
Lipoma															
Malignant Schwannoma					1	1									
Osteosarcoma							1		1						
Trichoepithelioma															
Undifferentiated Sarcoma	1		1											1	1
TAIL (NO. EXAMINED)	(1)		(1)	(3)		(3)	(1)		(1)	(1)	(2)	(3)	(1)		(1)
TESTIS (NO. EXAMINED)	(25)	(25)	(50)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(52)	(70)	(42)	(8)	(50)
Hemangioma	1		1												
Interstitial Cell Tumor	1		1				1		1	3	1	4	2		2
Mesothelioma		1	1												
THYMUS (NO. EXAMINED)	(25)	(23)	(48)	(32)	(17)	(49)	(33)	(31)	(64)	(17)	(51)	(68)	(33)	(7)	(40)
Histiocytic Sarcoma		1	1												
Malignant Lymphoma				1		1									
Malignant Schwannoma															
Thymoma	1	1	2	4		4					1	1	1		1
THYROID (NO. EXAMINED)	(25)	(25)	(50)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(52)	(70)	(42)	(8)	(50)
C-Cell Adenoma	1	1	2	4	2	6	3	1	4	2		2	6		6
C-Cell Carcinoma				1		1	1		1				2		2

782A-101-050-04 Terminal Sacrifice Male Rat	GROUP CEL1			GROUP CEL2			GROUP CEL3			GROUP CEL4			GROUP CEL5		
	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total	Sched. Sac.	Unschd. Sac. & Death	Total
PITUITARY (NO. EXAMINED)	(25)	(24)	(49)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(50)	(68)	(42)	(8)	(50)
Adenoma, Pars Distalis	9	5	14	5	3	8	4	3	7	2	1	3	6	4	10
Adenoma, Pars Intermedia					1	1							1		1
Carcinoma, Pars Distalis				1		1									
Meningeal Sarcoma														1	1
PREPUTIAL GLAND (NO. EXAMINED)	(25)	(25)	(50)	(33)	(15)	(48)	(38)	(31)	(69)	(17)	(50)	(67)	(42)	(8)	(50)
Adenoma					1	1									
Carcinoma	3		3	4	1	5	4	3	7	1	2	3			
Squamous Cell Carcinoma	4	1	5	3	2	5	2	2	4		3	3	2		2
Squamous Cell Papilloma	1	1	2	3	1	4	1	1	2						
PROSTATE (NO. EXAMINED)	(24)	(25)	(49)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(51)	(69)	(42)	(8)	(50)
Adenoma													1		1
SALIVARY GLAND, MANDIBULAR (NO. EXAMINED)	(25)	(25)	(50)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(52)	(70)	(42)	(8)	(50)
Malignant Lymphoma				1		1									
Malignant Schwannoma															
SCIATIC NERVE (NO. EXAMINED)	(25)	(25)	(50)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(52)	(70)	(42)	(8)	(50)
SEMINAL VESICLE (NO. EXAMINED)	(24)	(25)	(49)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(51)	(69)	(42)	(8)	(50)
Histiocytic Sarcoma		1	1												
SKELETAL MUSCLE, BICEPS FEMORIS (NO. EXAMINED)	(25)	(25)	(50)	(33)	(17)	(50)	(38)	(32)	(70)	(18)	(52)	(70)	(42)	(8)	(50)

Table IVD.1.6 Toxicokinetic parameters of retigabine and its N-acetyl metabolite

	Week 78							
	Retigabine				N-AMR			
Retigabine (mg/kg/day)	Cmax (ng/mL)		AUC(0-t) (ng·hr/mL)		Cmax (ng/mL)		AUC(0-t) (ng·hr/mL)	
	M	F	M	F	M	F	M	F
5	530	763	3456	4375	215	283	2064	2569
20	1809	1403	13558	9957	583	677	4676	6024
50	2780	2997	20782	23963	965	1104	8923	11056

N-AMR: N-acetyl metabolite of retigabine

2. RETIGABINE: NEONATAL MOUSE CARCINOGENICITY STUDY (Study No.: 05-2898; conducted by [redacted] (b) (4) report dated 11/14/07; GLP)

a. Methods

Neonatal CD-1 mice (28/sex/group) were dosed orally (gavage) once on postnatal day (PND) 8 and once on PND 15 with 0 (distilled water; WC), 0 (propylene glycol vehicle; VC), 32, 64 or 96 mg/kg retigabine (Lot No. 0005005), or 2 mg/kg diethylnitrosamine (DEN; positive control article; PC). The dose volume was 0.02 mL/animal on PND 8 and 5 mL/kg on PND 15 for all dose groups. At approximately 1 year of age, all surviving animals were euthanized and necropsied. Parameters evaluated during the study were: viability, clinical observations, body weights, hematology (termination), clinical chemistry (termination), macroscopic observations and microscopic pathology (all tissues in VC and HD; lung, liver and gross lesions only in WC, LD, MD, and PC).

A conventional 2-yr mouse carcinogenicity study was not considered feasible by the sponsor, due to the dose-limiting urinary bladder toxicity observed in this species with repeat-dose administration of relatively short duration. They proposed replacing the standard study design with the neonatal mice model (discussed in ICH S1B as an alternative test) with dosing on postnatal days 8 and 15 followed by monitoring for one year, as per the ILSI protocol and as described in a publication by McClain et al. (2001). Dose selection was based on the results of two 28-day studies in neonatal mice. In the first study (Report no. D-23129/9321020102; [redacted] (b) (4), doses of 0.5, 2, 8, 16, and 32 mg/kg were administered orally (gavage) on PNDs 8 and 15. There were 2 deaths considered T-R: 1 female given 8 mg/kg and 1 female given 32 mg/kg died after the first dose on Day 8. However, the first mouse exhibited no clinical signs prior to being found dead and the second exhibited only decreased motor activity. No cause of death was determined. Clinical signs (convulsions/myoclonus, decreased activity, hypersensitivity) were seen at ≥8 mg/kg. These were consistent with CNS signs observed in adults, but, as pointed out in the study report, young animals were considerably more sensitive than adults (eg, convulsions at ≥100 mg/kg in adults). There were no BW effects. The only histopathology findings were increased hepatic mitotic figures and urinary bladder dilatation, which appeared to be increased in treated animals, but not in a dose-related manner. The Exec-CAC concurred with the use of the neonatal mouse assay, but recommended that the sponsor also consider using the p53 or TgrasH2 assays. The committee did not agree that the sponsor had correctly identified an MTD based on the results of the 28-day dose range-finding study, particularly for a 2-dose protocol. They recommended that the sponsor include higher doses in their planned 2nd dose range-finding study in order to clearly establish that they have reached the MTD. It was also recommended that a positive control group be included in the definitive study (I53,950, SN155; see protocol review and minutes dated12/10/02).

In a second dose range-finding study ([redacted] (b) (4) Study No. 05-2897; Sponsor Study No. PR2005-040), neonatal mice were dosed on PNDs 8 and 15 with 0, 16, 32, 48, 64, or 96 mg/kg retigabine and observed until Day 28. T-R mortality was apparently higher at the HD (5/16) compared to C (1/24), although the small numbers of animals and the fact that one HD litter was cross fostered due to the illness of the original dam may have affected the outcome. Clinical signs seen in all treated groups after dosing included decreased activity, lethargy and hyper reactivity. At ≥32 mg/kg additional observations included twitching/trembling, ataxia, circling behavior, irregular/labored breathing and prostration. Severity of the findings was generally D-R and was greater after dosing on PND 8 than PND 15. At the HD, prostration, ataxia and irregular breathing were still evident in some animals on the days following dosing and appeared to affect feeding behavior. BW gain prior to weaning (PND 21) was lower in males at ≥32 mg/kg and in females at ≥48 mg/kg. After weaning, there was evidence of some compensatory growth among treated groups. Overall BW gains between PND 9 and PND 29 were generally comparable to C

at all doses except the HD, for which the overall BW gain was 20-30% lower than C. At termination, SS decreases in erythroid parameters were noted in males at ≥ 32 mg/kg. There were no T-R effects on clinical chemistry parameters. Decreased absolute and relative thymus weights were noted in HD females. There were no other T-R changes in organ weights or macroscopic findings at necropsy. Based on these findings, 96 mg/kg was selected as the HD in this study. TK results from adult CD1 mice receiving retigabine by oral gavage for 10 days in a separate study (Report No. D-23129/3000922983) are shown in **Table IVD.2.1.**

b. Results

i. Mortality, body weight, clinical pathology

Mortality was increased slightly in the MD group, but this was not considered T-R by the sponsor because of the absence of a D-R (**Table IVD.2.2**). Most of the deaths occurred immediately after dosing on PND 8 and 15. Clinical signs were noted after retigabine administration on PND 8 and/or PND 15, with D-R increases in incidence and/or duration of ataxia, decreased activity, irregular/labored breathing and twitching. In addition, lethargy was seen at all doses on PND 8, but only at the HD on PND 15, and prostration was noted at MD and HD on PNDs 8 and 15. Clinical signs that were generally present only on PND 8 included hypothermia at all dose and circling behavior and absence of milk bands at MD and HD. Clinical signs at all doses noted only on PND 15 included eyes partially closed and splayed stance. Signs were not longer evident within a few days of dosing and subsequent observations during the study were sporadic and not considered T-R.

Body weight (BW) and BW gain were statistically significantly (SS) decreased during the dosing phase at the HD compared to VC and the decreases persisted throughout the maintenance phase. BW and BW gain were also decreased at the MD during the dosing phase. At the HD, BWs in males and females were decreased 24% and 27%, respectively on PND 12 (4 days following the first dose) and 26% and 24%, respectively, on PND 18 (3 days following the second dose). BW gains in males and females were decreased 49% and 53%, respectively on PND 12 and 41% and 37%, respectively, on PND 18. This was attributed to decreased feeding during prolonged periods of lethargy and prostration, as evidenced by absence of milk bands in many animals in this group. During the maintenance phase of the study, there was some catch-up growth, but BWs remained lower than the VC such that on Day 323 (end of the study) BWs were decreased 14% and 17% in HD males and females, respectively, and overall BW gains were decreased 14% and 18%, respectively. At the MD, there were slight decreases in BW and BW gain after dosing. In males, BW was decreased 8% on both PND 12 and PND 18 and BW gain was decreased 15% and 12% on PND 12 and PND 18, respectively. In females, BW and BW gain were decreased on PND 12 (10% and 18%, respectively). During the maintenance phase, BW and BW gains in MD males and females were similar to controls.

Although there were occasional small but SS increases in reticulocytes in MD and HD males during the study, these were attributed to normal variability. There was a small but SS increase in serum bilirubin in HD males (0.15mg/dl versus 0.11 mg/dl in VC). Other SS changes, including decreased phosphorus in males at all doses, decreased sodium in MD and HD females, and decreased calcium in HD females were minimal and attributed to normal variability.

ii. Non-neoplastic

There were no clearly T-R non–neoplastic findings in retigabine-treated mice. In the PC group, 6/28 males and 5/28 females had bronchioloalveolar hyperplasia (minimal to moderate), which was considered to be associated with the high incidence of bronchioloalveolar neoplasms. The incidences of this finding in other groups were 1, 0, 1, 0, 1 and 0, 0, 0, 1, 1 in WC, VC, LD, MD and HD males and females, respectively. Foci of basophilic hepatocellular alteration (may represent an early stage in neoplastic development) were present in 4/27 males and 14/28 females. Hepatocellular necrosis/acute-subacute inflammation and hepatocellular intracytoplasmic eosinophilic inclusions were also seen in the hepatocellular neoplasms in this group.

iii. Neoplastic

Spontaneous neoplasms in the neonatal mouse model occur mainly in the lungs and liver. In this study, incidences of lung and liver neoplasms (adenomas, carcinomas, and adenomas and carcinomas combined) in water and vehicle controls were generally comparable to those reported by McClain et al. (Toxicol Pathol 299(Suppl):128-37, 2001).

There was an increased incidence of lung nodules/masses in HD retigabine-treated mice (males) as well as in the PC group (**Table IVD.2.3**). Microscopically, the nodules/masses correlated with bronchioloalveolar adenomas or carcinomas. The incidence of nodules/masses in the liver was also increased in the PC group. These correlated with hepatocellular adenomas or carcinomas.

Although there were no SS differences in the incidences of lung and liver neoplasms in retigabine-treated groups, lung neoplasms were increased in MD and HD males (**Table IVD.2.4**). Notably, bronchioloalveolar carcinoma was only seen in HD retigabine males (8%) and PC mice (4 and 7% in males and females). The overall incidence of lung carcinomas reported by McClain et al. (2001) is 1.4% (5/360).

High incidences of bronchioloalveolar adenomas/carcinomas in the lungs and hepatocellular adenomas/carcinomas in the liver were seen in PC group males and females. Males had higher incidences of pulmonary and hepatocellular neoplasms than females, which was also consistent with the findings reported by McClain et al. (**Table IVD.2.5**).

c. Conclusion

Administration of retigabine to neonatal mice on PND 8 and 15, at doses of 32, 64 and 96 mg/kg induced a small but D-R increase in the frequency of lung neoplasms compared to the water and vehicle (propylene glycol) controls: percent incidences for combined bronchioalveolar adenoma/carcinoma were 4, 4, 8, and 12% in C (combined), LD, MD, and HD group males. Notably, bronchioloalveolar carcinoma was only seen in HD retigabine males (8%) and positive control mice (4 and 7% in males and females). The expected high incidences of lung and liver tumors were seen in the (diethylnitrosamine) positive control group.

Table IVD.2.1 TK data (retigabine) from adult CD1 mice receiving RTG for 10 days by gavage

Gender	Dose (mg/kg/day)	Parameter C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng·hr/mL)	C_{max}/Dose	AUC_{0-24}/Dose
Male	30	136 ± 11	8.0	1044 ± 346	4.53 ± 0.36	34.8 ± 11.5
	60	211 ± 25	8.0	3110 ± 499	3.52 ± 0.41	51.8 ± 8.3
	120	542 ± 88	2.0	5832 ± 1353	4.52 ± 0.73	48.6 ± 11.3
Female	120	496 ± 104	1.0	3478 ± 876	4.14 ± 0.87	29.0 ± 7.3

Table IVD.2.2

TABLE 3.2.1. MORTALITY SUMMARY

Test or Control Article	Water (control)		PG[a] (vehicle control)		Retigabine						DEN[b] (positive control)	
Dose (mg/kg)	0		0		32		64		96		2	
SEX	M	F	M	F	M	F	M	F	M	F	M	F
NO. OF ANIMALS: Initial	27	29	28	28	29	27	28	28	28	29	28	28
DOSING PHASE												
Found Dead	0	0	1	2	1	2	3	3	3	3	0	0
Elective Euthanasia	3	5	0	0	0	0	0	0	0	0	0	0
Total Unscheduled Deaths	3	5	1	2	1	2	3	3	3	3	0	0
MAINTENANCE PHASE												
Found Dead	1	1	3	0	0	1	3	2	0	1	3	2
Moribund Euthanasia	3	0	0	0	0	0	1	1	1	0	1	1
Humane Euthanasia	0	0	0	1	2	0	1	0	0	0	0	0
Total Unscheduled Deaths	4	1	3	1	2	1	5	3	1	1	4	3
PERCENT SURVIVORSHIP[c]	83.3	95.8	85.7	89.3	89.7	88.5	71.4	78.6	85.7	89.3	85.7	89.3

[a]PG = propylene glycol
[b]DEN = diethylnitrosamine
[c]Survivorship at 12 months. Values used to calculate percent survivorship exclude electively euthanized animals and one missing female in the 32-mg/kg retigabine group.

Table IVD.2.3

TABLE 3.6.1.1. MACROSCOPIC FINDINGS IN LUNG AND LIVER FOR MAINTENANCE PHASE ANIMALS

Test or Control Article	Water (control)		PG[a] (vehicle control)		Retigabine						DEN[b] (positive control)	
Dose (mg/kg)	0		0		32		64		96		2	
SEX	M	F	M	F	M	F	M	F	M	F	M	F
NO. OF ANIMALS	24	24	27	26	28	24	25	25	25	26	28	28
LUNG												
Nodule(s)/Mass(es)	0	1	0	0	0	0	0	1	3	1	9	4
%	0	4	0	0	0	0	0	4	12	4	32	14
LIVER												
Nodule(s)/Mass(es)	1	0	0	0	1	0	1	0	1	0	26	2
%	4	0	0	0	4	0	4	0	4	0	93	7

[a]PG = propylene glycol
[b]DEN = diethylnitrosamine

Table IVD.2.4

TABLE 3.6.2.1. NEOPLASTIC FINDINGS IN LUNG AND LIVER FOR MAINTENANCE PHASE ANIMALS

Test or Control Article	Water (control)		PG[a] (vehicle control)		Retigabine						DEN[b] (positive control)	
Dose (mg/kg)	0		0		32		64		96		2	
SEX	M	F	M	F	M	F	M	F	M	F	M	F
NO. OF ANIMALS	24	24	27	26	28	24	25	25	25	26	28	28
LUNG: No. examined	24	24	26	26	28	24	25	25	25	26	28	28
Bronchioloalveolar adenoma/carcinoma combined	1	1	1	0	1	0	2	1	3	1	14[c,*]	12[c,*]
% Incidence	4	4	4	0	4	0	8	4	12	4	50	43
Bronchioloalveolar adenoma	1	1	1	0	1	0	2	1	1	1	14*	11*
% Incidence	4	4	4	0	4	0	8	4	4	4	50	39
Bronchioloalveolar carcinoma	0	0	0	0	0	0	0	0	2	0	1	2
% Incidence	0	0	0	0	0	0	0	0	8	0	4	7
LIVER: No. examined	24	24	25	26	28	24	24	25	25	25	27	28
Hepatocellular adenoma/carcinoma combined	3	0	1	0	1	0	2	1	1	0	26[d,*]	12[d,*]
% Incidence	13	0	4	0	4	0	8	4	4	0	96	43
Hepatocellular adenoma	3	0	1	0	1	0	2	1	1	0	24*	12*
% Incidence	13	0	4	0	4	0	8	4	4	0	89	43
Hepatocellular carcinoma	0	0	0	0	0	0	0	0	0	0	17*	1
% Incidence	0	0	0	0	0	0	0	0	0	0	63	4

[a]PG = propylene glycol

[b]DEN = diethylnitrosamine

[c]One male and 1 female had both a bronchiolosalveolar adenoma and carcinoma.

[d]Fifteen males and 1 female had both a hepatocellular adenoma and hepatocellular carcinoma.

[e]Significantly different from the water control at the 0.05 level using Fisher's exact two-tailed test.

Table IVD.2.5

TABLE 2.—ILSI ACT studies: Spontaneous tumor incidence in control neonatal CD-1 mice.

	% Incidence (# of mice)	
Tumor type	Males	Females
Liver, adenoma	6.1 (22/360)	0.0 (0/360)
Liver, carcinoma	0.3 (1/360)	0.3 (1/360)
Lung, adenoma	6.7 (24/360)	7.5 (27/360)
Lung, carcinoma	1.4 (5/360)	1.1 (4/360)
Harderian gland, adenoma	0 (0/360)	0.6 (2/360)

E. REPRODUCTIVE TOXICITY

1. Examination of the Influence on the Fertility and General Reproductive Performance of Male and Female Sprague Dawley Rats after Oral Administration (Report. No. 0-23129/3000913511; dated 3/9/99; conducted by (b) (4) GLP)

a. Methods

Rats (SD, 25/sex/grp) received oral (gavage) doses of 0 (water), 0 (propylene glycol vehicle) 4.64, 14.7, or 46.4 mg/kg for 4 (males) or 2 (females) weeks prior to and during mating (max 7 days) and early gestation (GD 7). Sperm evaluations and histopathological investigations of the reproductive organs were performed in 5 unmated satellite males per dose group after the cessation of 4 weeks treatment. Females were sacrificed and laparotomized on day 15 of pregnancy and reproductive parameters were determined.

Strain: SPF Sprague-Dawley rats (Crl: CD)
Drug: batch No.: 96020014

Dose selection: Dose selection was based on the results of dose range-finding studies (b) (4) Study No. 913487 - treatment of male animals, Study No. 913500 - treatment of female animals). In these studies, doses of 0, 4.64, 10.0, 21.5, or 46.4 mg/kg were administered to male and female rats (10/sex/grp). Males were treated for 4 weeks prior to mating with untreated females and continuing until sperm positive. Clinical signs (hypokinesia, coordination disturbances, and/or salivation) were observed at ≥10 mg/kg. Body weight gain was decreased at the HD (15 and 24% compared to C and VC). There were no effects on mating behavior, fertility, sperm (viability, count, morphology), histopathology, or fetal development. Females were treated for 2 weeks prior to cohabitation and continuing through implantation (GD 7). Clinical signs (hypokinesia, coordination disturbances, stilted gait, salivation, and/or sunken sides) were seen at ≥21.5 mg/kg (persisting for the major part of the treatment and mating periods at HD). There were no body weight effects and no observed effects on mating, fertility, or offspring development.

b. Results

i. Mortality and Clinical Observations

Although 4 males (3 LD, 1 HD) died, these were not considered T-R (attributed to gavage error).

D-R increases of the incidence, duration, and frequency of clinical signs (hyperkinesia, hypokinesia, coordination disturbances, tremor, intensified nervousness/aggressiveness, vocalization by touching, and salivation) were seen at the MD and HD.

ii. Body Weight

BW gain was reduced in HD males during the precohabitation treatment (19 and 15% compared to C and VC, SS). A minimal (NS) decrease was noted in HD females. No significant changes in absolute BW were observed.

iii. Fertility and Litter Data

There were no apparent effects on estrus cyclicity in females and no clearly T-R effects on sperm parameters (decreased epididymal sperm head count at LD due to 1 individual (87)). Mating behavior and success were unaffected by treatment (**Table IVE.1.1**).

There was a possible effect on preimplantation reproductive parameters: corpora lutea, implantations and living fetuses were decreased slightly (NS) at the HD (**Table IVE.1.2**). No abnormalities were observed upon external inspection of fetuses on GD 15.

iv. Necropsy

There were no T-R changes in absolute and relative weights of testes, epididymides, and prostate/seminal vesicles and no T-R histopathological findings in the testis, epididymidis, prostate, and seminal vesicles of the males examined (5/group).

c. Conclusions

Treatment of male and female rats with retigabine (oral gavage doses of 4.64, 14.7, or 46.4 mg/kg) prior to and during mating produced clinical signs of toxicity (HD) and reductions in BW gain (HD males) and slight decreases in corpora lutea and implantations (HD) in the mated animals.

Table IVE.1.1

Mating Results Summary

Dose Group Dose	Animals Mated Successfully	Copulation Index %	Animals Pregnant	Fertility Index %
1 Control Drinking Water	24/24*	100	23/24	96
2 Control Propylene Glycol	25/25	100	24/25	96
3 4.64 mg/kg b.w.	22/22**	100	22/22	100
4 14.7 mg/kg b.w.	25/25	100	24/25	96
5 46.4 mg/kg b.w.	24/24**	100	23/24	96

* Female 155 excluded because of partial vaginal aplasia.
** Male mating partners died prematurely, females 209, 213, 215, and 273 excluded.

Table IVE.1.2

REPRODUCTION DATA SUMMARY
F0 GENERATION – GESTATION PERIOD

	GROUP 2 CONTROL/PROP	GROUP 1 CONTROL	GROUP 3 4.64	GROUP 4 14.7	GROUP 5 46.4
NUMBER OF DAMS	24	23	22	24	23
CORPORA LUTEA	426	417	403	439	389
MEAN (+)	17.8	18.1	18.3	18.3	16.9
ST.DEV.	2.3	2.5	2.0	1.9	2.8
PRE-IMPLANTATION LOSS	32	35	33	25	39
% OF CORP. LUTEA (#)	7.5	8.4	8.2	5.7	10.0
MEAN (+)	1.3	1.5	1.5	1.0	1.7
ST.DEV.	1.5	1.5	2.1	1.4	2.4
NUMBER OF DAMS AFFECTED	15	15	13	13	15
IMPLANTATION SITES	394	382	370	414	350
% OF CORP. LUTEA (#)	92.5	91.6	91.8	94.3	90.0
MEAN (+)	16.4	16.6	16.8	17.3	15.2
ST.DEV.	2.3	1.8	1.7	1.8	2.3
POST-IMPLANTATION LOSS	34	30	23	49	36
% OF IMPL. SITES (#)	8.6	7.9	6.2	11.8	10.3
MEAN (+)	1.4	1.3	1.0	2.0	1.6
ST.DEV.	1.4	1.0	1.5	1.9	1.5
NUMBER OF DAMS AFFECTED	18	19	11	22	19
IMPLANTATION SITE SCARS	0	0	0	0	0
EMBRYONIC/FETAL DEATHS TOTAL	34	30	23	49	36
EMBRYONIC RESORPTIONS	21	15	18	25	15
% OF IMPL. SITES (#)	5.3	3.9	4.9	6.0	4.3
MEAN (+)	0.9	0.7	0.8	1.0	0.7
ST.DEV.	1.3	0.8	1.3	1.1	0.9
NUMBER OF DAMS AFFECTED	11	11	8	14	9
FETAL RESORPTIONS	13	15	5	24	21
% OF IMPL. SITES (#)	3.3	3.9	1.4	5.8	6.0
MEAN (+)	0.5	0.7	0.2	1.0	0.9
ST.DEV.	0.8	1.0	0.4	1.9	1.6
NUMBER OF DAMS AFFECTED	9	9	5	9	11
FETUSES					
TOTAL FETUSES	360	352	347	365	314
% OF IMPL. SITES (#)	91.4	92.1	93.8	88.2	89.7
MEAN (+)	15.0	15.3	15.8	15.2	13.7
ST.DEV.	2.7	2.1	1.7	3.1	2.8
LIVE FETUSES	360	352	347	365	314
DEAD FETUSES	0	0	0	0	0
ABNORMAL FETUSES	0	0	0	0	0

*/** : Dunnett-Test based on pooled variance significant at level 5% (*) or 1% (**)
#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)
+ : Steel Test significant at level 5%

2. Examination of the influence of D-23129 on the pregnant rat and the fetus by oral administration (Report No. 0-23129/3000911733; (b) (4) dated 5/22/98; GLP)

a. Methods

Retigabine was administered to pregnant rats (S-D, 20/group) from GD 6 to 17 at oral gavage doses of 0 (saline), 0 (propylene glycol vehicle), 10, 21.5, or 46.4 mg/kg. Dams were monitored for clinical signs, body weight and food consumption during gestation, and for postmortem findings. They were sacrificed on GD 20 for evaluation of pregnancy outcome, intrauterine death, fetal weight, and fetal abnormalities (1/2 of fetuses for visceral examination by Wilson section, 1/2 for skeletal examination after clearing and staining with Alizarin red S).

Strain: SPF Sprague-Dawley (Crl: CD)
Drug batch No.: 96020014

Dose selection: Doses were based on an embryo-fetal range-finding study (Report no. 9763/96) in which rats (S-D, 2/group) received oral (gavage) doses of 4.6, 10, 21.5, or 46.4 mg/kg on GDs 6 to 17. Slight and transient reduction in maternal body weight gains were observed at the HD. Slightly increased incidences of skeletal and soft tissue variations (dilation of the renal pelvis) were seen at the HD; however, only 14 fetuses were evaluated.

b. Results

i. Maternal effects

No T-R deaths occurred. T-R clinical signs included abdominal position at the MD and HD and shaking of the head and chewing motions at the HD, all seen throughout the dosing period. Corrected maternal BW gain was only slightly (NS) decreased at the HD (44.5 vs 45.9 in VC). No maternal plasma level data were collected.

ii. Developmental effects

(1) There were no T-R differences in reproductive parameters (corpora lutea, implantation sites, resorptions and live fetuses, pre- and post-implantation loss; **Table IVE.6.1**). Fetal BW was not affected by treatment.

(2) There were no apparent T-R differences in fetal external, visceral, or skeletal malformations (only 2 malformed fetuses: brachynathia in 1 LD and brachynathia and cleft palate in 1 HD fetus); however, skeletal variations (misaligned sternebrae, accessory 14th rib) and incomplete ossification (skull) were D-D increased (SS at the HD; **Table IVE.6.2**).

c. Conclusions

Treatment of pregnant rats with retigabine (oral gavage doses of 0, 10, 21.5, or 46.4 mg/kg) from GD 6 to 17 produced very slight (seemingly inadequate) maternal toxicity (HD) and increased incidences of skeletal variations (SS at HD).

Table IVE.6.1

Parameter		Group 1 Control 1	Group 2 Control 2	Group 3 10.0 mg/kg	Group 4 21.5 mg/kg	Group 5 46.4 mg/kg
Corpora lutea	total	325	326	308	354	339
	per dam	16.3	16.3	15.4	17.7	17.0
Implantation sites	total	307	317	300	347	327
	per dam	15.4	15.9	15.0	17.4	16.4
Resorptions	total	**9	24	18	**8	*12
	per dam	0.5	1.2	0.9	0.4	0.6
Early resorptions	total	9	21	11	6	9
	per dam	0.5	1.1	0.6	0.3	0.5
Late resorptions	total	0	3	7	2	3
	per dam	0.0	0.2	0.4	0.1	0.2
Live fetuses	total	**298	293	282	**340	*315
	per dam	14.9	14.7	14.1	17.0	15.8
Pre-implantation loss	mean %	5.1	2.6	5.5	2.0	4.1
Post-implantation loss	mean %	2.8	7.8	5.7	2.4	3.6

* $p \leq 0.05$
** $p \leq 0.01$

Table IVE.6.2

TABLE 10 — D-23129 - Teratology Study in Rats
SUMMARY OF ALL CLASSIFIED FETAL SKELETAL OBSERVATIONS

		TEST GROUP 2 Control 2	TEST GROUP 3 10.0 mg/kg	TEST GROUP 4 21.5 mg/kg	TEST GROUP 5 46.4 mg/kg
Litters Evaluated	N	20	20	20	20
Fetuses Evaluated	N	147	142	170	159
Live	N	147	142	170	159
Dead	N	0	0	0	0
TOTAL MALFORMATIONS					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.7	0.0	0.6
Litter Incidence	N	0	1	0	1
	%	0.0	5.0	0.0	5.0
Affected Fetuses/Litter	MEAN%	0.0	5.0	0.0	0.5
	S.D.	0.0	22.4	0.0	2.2
TOTAL VARIATIONS					
Fetal Incidence	N	9	12	13	21 *
	%	6.1	8.5	7.6	13.2
Litter Incidence	N	6	8	7	10
	%	30.0	40.0	35.0	50.0
Affected Fetuses/Litter	MEAN%	5.7	12.3	7.6	12.1
	S.D.	10.0	23.9	13.3	14.9

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P≤0.05 ** = P≤0.01 (Fisher or Chi-square test)

2. Retigabine: Embryo-fetal toxicity study in rats (Study No. PR2006-013; [redacted] (b) (4) dated 12/28/07; GLP)

a. Methods

In this repeat rat embryo-fetal development study, retigabine was administered to pregnant rats (S-D, 20/group) from GD 6-17 at oral gavage doses of 0 (propylene glycol vehicle), 20, 45, or 60 mg/kg. Due to excessive toxicity (5/20 unscheduled deaths, associated with prostration, decreased activity, hypothermia and labored breathing), dosing at the HD was terminated on GD 13. Dams were monitored for viability, clinical signs, body weight and food consumption during gestation, and for postmortem findings. They were sacrificed on GD 20 for evaluation of pregnancy outcome, intrauterine death, fetal weight, and fetal abnormalities (1/2 of fetuses for visceral examination by Wilson section, 1/2 for skeletal examination after clearing and staining

with Alizarin red S). Blood samples for TK analyses were collected from all animals on GD 6 and from the remaining groups on GD 17.

Strain: SPF Sprague-Dawley (Crl: CD)
Drug batch No.: 0005005

Dose selection: Doses were based on an embryo-fetal range-finding study carried out at (b) (4) (Study No. 06-4308) with doses of 40, 70 and 100 mg/kg. After the first dose (on GD 6) MD and HD animals showed hypoactivity and hyperreactivity to touch and sound. In subsequent dosing days hypoactivity and prostration were always observed post-dosing at ≥70 mg/kg. In addition there was 50% mortality during the first 3 days of dosing at the HD, while the remaining HD animals showed decreased body weights (-5.0% vs. Control) and decreased feed consumption (-40% vs C). Dosing at the HD was stopped and the remaining animals were euthanized on GD 9. Fifty percent of MD animals were found moribund or had to be euthanized due to poor condition during the first week of treatment. The mean BW of MD dams showed a deficit of approximately -14% vs C by GD 12. Dosing at the MD was stopped on GD 12. Despite the suspension of dosing, a slight decrease in fetal weights was observed at the MD. There were no external malformations in any of the groups. No T-R adverse effects were observed among the dams, in pre- or post implantation loss, or in the weight of the fetuses of the LD.

b. Results

i. Maternal effects

5/20 HD females were euthanized in moribund condition, while a sixth animal was found dead on GD 14. These unscheduled deaths were associated with prostration, decreased activity, hypothermia and labored breathing. The majority of HD animals exhibited decreased activity and/or were found prostrate post-dose throughout the dosing period. Some also exhibited hyperactivity upon handling at approximately 1 hr post dose. Decreased activity and prostration were also noted in a lower proportion of MD rats, mostly during the first 4 days of dosing. These clinical signs were not observed at the LD. In addition to the clinical signs described above, a few MD and HD animals also exhibited hypothermia, piloerection, hunched appearance and labored breathing toward the end of the dosing periods.

BW gain over the entire dosing period (GDs 6-18) was decreased somewhat (NS) at the MD (8%) and HD (8%). At the HD, there were SS deficits in BW gain for the intervals 6-14 (-44%), and 6-16 (-20%), and BW was 9% below C on GD 12 before dosing was stopped on GD 13. At the MD, SS deficits in BW gain were seen for the intervals 6-14 (-25%) and 6-16 (-15%), but there was no effect on BW at the end of the dosing period.

The TK results are summarized in **Table IVE.6.1**.

ii. Developmental effects

(1) There were no T-R differences in reproductive parameters (resorptions and live fetuses, post-implantation loss; **Table IVE.6.2**). There were no T-R effects on uterine weights (80.1, 80.3, 83,1, and 83.0 gm in C, LD, MD, and HD), while fetal BWs (3.9, 4.0, 3.9, and 3.8 g) were only very slightly (NS) decreased at the HD.

(2) There were no apparent T-R differences in fetal external, visceral, or skeletal malformations or variations, but incomplete ossification of vertebrae (sacral and thoracic) appeared to be D-D increased up to the MD (**Table IVE.6.3**).

c. Conclusions

Treatment of pregnant rats with retigabine (oral gavage doses 20, 45, or 60 mg/kg) from GD 6-17 produced severe (excessive) maternal toxicity at the HD and incomplete axial skeletal ossification at the LD and MD (HD dosing stopped on GD 13).

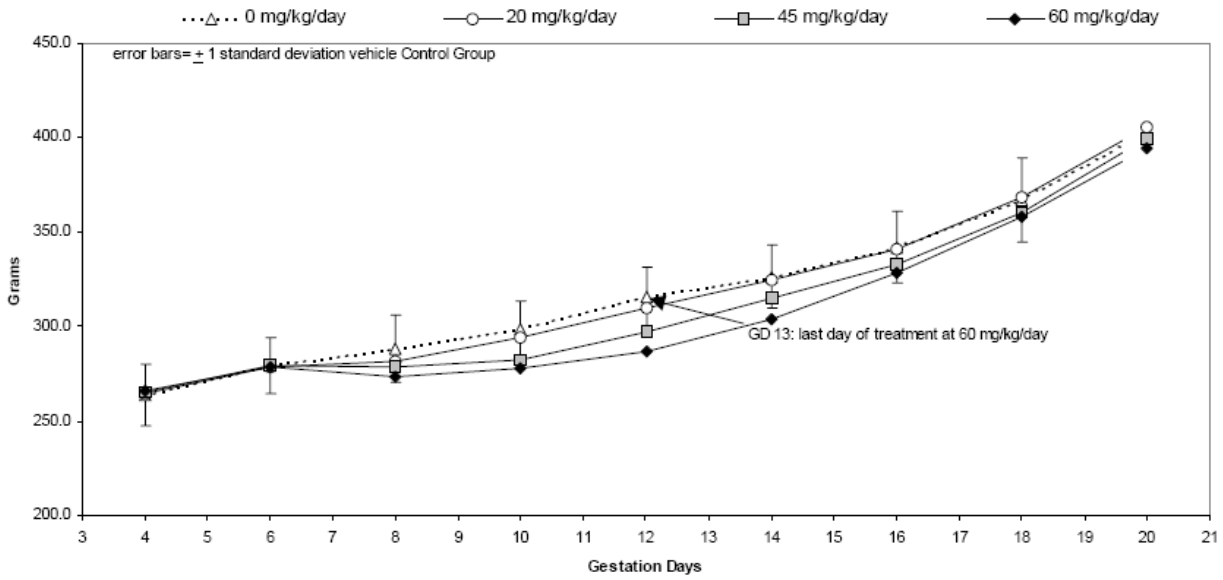

Table IVE.6.1

Dose (mg/kg/day)		AUC 0-24 (ng.h/mL) GD 6	AUC 0-24 (ng.h/mL) GD 17	C_{max} (ng/mL) GD 6	C_{max} (ng/mL) GD 17	Accumulation ratios (GD 17/GD6)
20	Retigabine	10910.55	12049.98	996	1072	1.1
	N-AMR	9655.38	10743.20	551	754	1.11
45	Retigabine	18500.75	17624.13	1818	1630	0.95
	N-AMR	16759.25	17459.38	842	921	1.04
60	Retigabine	25524.50	-	2545	-	-
	N-AMR	18752.50	-	860	-	-

N-AMR: N-acetyl metabolite of retigabine

Table IVE.6.2

Females	Summary of Female Reproductive Data									Table 9	
	Corpora Lutea	Implant-ations	Resorptions Early	Resorptions Late	Resorptions Total	Live Young Male	Live Young Female	Live Young Total	Sex Ratio (% Male)	Implantation Loss Pre (%)	Implantation Loss Post (%)
Group 1 – 0 mg/kg/day											
Mean	14.6	13.6	1.1	0.0	1.1	6.1	6.4	12.5	50.6	9.3	7.3
SD	3.20	3.45	1.92	0.00	1.92	2.50	2.29	3.64	18.47	18.09	12.93
N	17	17	17	17	17	17	17	17	17	17	17
Group 2 – 20 mg/kg/day											
Mean	14.4	13.6	0.9	0.0	0.9	6.4	6.2	12.6	50.2	5.1	6.8
SD	1.90	1.91	0.90	0.00	0.90	2.00	1.56	1.93	11.09	6.80	6.07
N	17	17	17	17	17	17	17	17	17	17	17
Group 3 – 45 mg/kg/day											
Mean	14.9	14.5	0.7	0.0	0.7	6.6	7.2	13.8	47.9	3.0	4.5
SD	2.51	2.57	1.00	0.00	1.00	2.11	1.95	2.42	11.89	5.05	6.51
N	19	19	19	19	19	19	19	19	19	19	19
Group 4 – 60 mg/kg/day											
Mean	15.5	14.5	0.8	0.0	0.8	5.9	7.8	13.7	45.2	7.4	6.1
SD	2.93	3.13	0.93	0.00	0.93	1.32	2.45	3.22	11.14	7.90	7.26
N	13	13	13	13	13	13	13	13	13	13	13

Table IVE.6.3

	Summary of Fetal Observations Major abnormalities in CAPITALS, ossification parameters in *italics*								Table 12
		Fetuses				Litters			
Group		1	2	3	4	1	2	3	4
Number examined	External	231	245	275	232	17	18	19	13
	Visceral	107	106	133	111	17	17	19	13
	Skeletal	106	109	129	111	17	17	19	13
Blood vessels									
AORTIC ARCH, RETRO-ESOPHAGEAL				1	1			1	1
Innominate artery, short					1				1
Right, subclavian, arises from aortic arch				1	2			1	1
Umbilical artery, left			1	2	1		1	2	1
Brain									
Cerebellum, subdural, hemorrhage		4				3			
Caudal vertebrae									
BRACHYURY; or slightly shortened tail		2				1			
Cervical vertebrae									
4th, vertebral arch, incompletely ossified		2	1	1		2	1	1	
4th, vertebral arch, small, misshapen		1				1			
5th, vertebral arch, incompletely ossified		3	1			3	1		
5th, vertebral arch, small, misshapen		1				1			
6th, vertebral arch, incompletely ossified		3	2	3		3	1	2	
7th, vertebral arch, incompletely ossified		1				1			

Counts								
Cervical vertebral centra, > 3 ossified	3	6	2		2	2	1	
Cervical vertebral centra, 3 ossified	3		1		2		1	
Sacrocaudal arch/centra, 1 to 3 fused	20	17	19	10	10	9	12	7
Sacrocaudal arch/centra, 4+ fused	16	13	11	3	7	6	7	3
Sacrocaudal ventral process, > 3 ossified	3	4	4		1	2	4	
Sacrocaudal ventral process, 1 to 3 ossified	78	77	80	61	17	17	18	12
Eye								
Aqueous humor, hemorrhage	1	1			1	1		
Lens, oval, bilateral	1	1			1	1		
Girdles								
Bilateral, ischium, incompletely ossified			1				1	
Bilateral, pubis, incompletely ossified	2		2		2		1	
Ischium, incompletely ossified		1	2			1	1	
Pubis, incompletely ossified			2				2	
Left, scapula cranial margin, misshapen		1	1			1	1	
Gonads								
Testes - bilateral, displaced cranially	1				1			
Testis - left, displaced cranially			1				1	
Testis - left, displaced medially			1				1	
Testis - right, displaced cranially		1	3	1		1	3	1
Testis - right, displaced medially	1	1	2	2	1	1	2	2
Head								
Cranial region, subcutaneous, hemorrhage		1	1			1	1	
Heart								
DEXTROCARDIA; VENTRICULAR SEPTUM, MEMBRANOUS VSD; AZYGOS VEIN, ADDITIONAL		1				1		
Jaw/palate/mouth								
Jaw, lower, hemorrhage, subcutaneous	1			1	1			1
Tongue, protruding	1				1			
Nasal region, hemorrhage, subcutaneous		1				1		
Kidney/ureter								
Ureter - bilateral, dilated			1				1	
Limbs								
Forelimb - left, subcutaneous, hemorrhage	1		1		1		1	
Hindlimb - left, subcutaneous, hemorrhage			1				1	
Liver/diaphragm								
Left lobe, hemorrhage				1				1
Liver median lobe, hemorrhage			1	1			1	1
Liver post. caudate lobe, fissure(s)	1			1	1			1
Liver post. caudate lobe, misshapen	1		2	1	1		1	1
Liver, additional lobe	15	11	16	8	9	10	9	5
Median lobe/diaphragm, raised area(s), thinning	5	5	1	3	4	4	1	2
Lumbar vertebrae								
1st, vertebral centrum, dumbbell ossification			1				1	
1st vertebral centrum, incompletely ossified	1				1			
1st and 2nd vertebral centra, incompletely ossified			1				1	
5th, vertebral centrum, asymmetrically ossified			1				1	
Ossification parameters, head								
Bilateral, frontal, incompletely ossified		1				1		
Bilateral, jugal, incompletely ossified			1	1			1	1
Bilateral, parietal, incompletely ossified	4	5	4	3	3	4	3	1
Bilateral, squamosal, incompletely ossified	1	1	1		1	1	1	
Hyoid, unossified	8	1	8	5	6	1	3	3

Ossification parameters, head								
Interparietal, incompletely ossified	14	10	12	3	8	6	5	2
Jugal, incompletely ossified	1	1	1		1	1	1	
Left, parietal, incompletely ossified	1	2	1		1	2	1	
Left, squamosal, incompletely ossified	1				1			
Parietal, incompletely ossified	2	3	2		1	3	2	
Presphenoid, unossified				1				1
Right, squamosal, incompletely ossified	3	1	1		3	1	1	
Supraoccipital, incompletely ossified	6	4	8	6	5	4	3	5
Paw bones								
2nd digit, metacarpal, bilateral forelimb, incompletely ossified		1				1		
2nd digit, metacarpal, left forelimb, incompletely ossified			1				1	
2nd digit, metacarpal, right forelimb, incompletely ossified			1				1	
4th digit, metacarpal, left forelimb, incompletely ossified			1				1	
5th digit, metatarsal, bilateral hindlimb, unossified	1				1			
Phalanges bilateral forelimb, 3 ossified		3				1		
Paws and limbs								
Forelimb - left, carpal flexure (minimal)				1				1
Ribs								
13th, right rib, short, absent costal cartilage				1				1
14th, bilateral rib	3	10	3	3	3	6	3	3
14th, complete bilateral rib with associated costal cartilage	1				1			
14th, left rib	11	4	3	4	6	3	3	3
14th, right rib	1	6	1	1	1	4	1	1
Rib(s) kinked, and/or irregularly ossified			4				2	
Cervical rib		1	2			1	2	
Sacral vertebrae								
Sacrocaudal vertebral arch, incompletely ossified	1	3	6	1	1	3	4	1
Skull bones								
Interparietal, fissure			1				1	
Sternebrae								
1st, center, incompletely ossified, 2nd to 6th unossified	1				1			
2nd, center, incompletely ossified	2		1	2	2		1	2
4th, center, incompletely ossified; 5th center, bipartite ossification	1				1			
5th, center, incompletely ossified	8	7	6	10	7	5	6	6
5th, center, unossified	22	11	22	14	10	6	12	7
5th, hemicenter, offset alignment			1				1	
6th, center, incompletely ossified	29	25	35	27	12	10	14	12
6th, center, unossified	9	3	1	6	6	3	1	3
Suture (skull)								
Parietal/interparietal, sutural bone	2				2			
Thoracic vertebral centra								
10th, vertebral centrum, bipartite ossification	1			1	1			1
10th, vertebral centrum, dumbbell ossification		1	3			1	3	
10th, vertebral centrum, incompletely ossified	2	8	12	5	2	6	8	4
11th, vertebral centrum, asymmetrically ossified				1				1
11th, vertebral centrum, bipartite ossification		2	3			2	1	
11th, vertebral centrum, dumbbell ossification	1			1	1			1
11th, vertebral centrum, incompletely ossified	2	5	7	8	1	3	5	7
12th, vertebral centrum, asymmetrically ossified		1		2		1		2
12th, vertebral centrum, bipartite ossification		3	1	1		3	1	1
12th, vertebral centrum, dumbbell ossification	1		1	1	1		1	1
12th, vertebral centrum, incompletely ossified		4	8	5		4	8	4
13th, vertebral centrum, asymmetrically ossified	1				1			
13th, vertebral centrum, bipartite ossification	2			1	2			1
13th, vertebral centrum, dumbbell ossification		1				1		
13th, vertebral centrum, incompletely ossified			3	4			3	3
1 incomplete/bipartite/dumbbell/asymmetric ossification	9	19	29	12	6	11	16	10
2 incomplete/bipartite/dumbbell/asymmetric ossification		1	5	7		1	5	5
>2 incomplete/bipartite/dumbbell/asymmetric ossification	1	3	3	4	1	3	1	2
20 thoracolumbar vertebrae	2				1			

7. Examination of the influence of D-23129 on the pregnant rabbit and the fetus by oral administration (Report No. D-23129/3000911722; dated 5/26/98; conducted by (b) (4) GLP)

a. Methods

Retigabine was administered to pregnant rabbits (NZW, 20/group) from GD 6-20 at oral gavage doses of 0 (saline), 0 (vehicle: propylene glycol), 5.62, 12.1, or 26.1 mg/kg. Does were monitored for clinical signs, body weight and food consumption during gestation, and for postmortem findings. They were sacrificed on GD 29 for evaluation of pregnancy outcome, intrauterine death, fetal weight, and fetal abnormalities (visceral examination by fresh dissection and skeletal examination after clearing and staining with Alizarin red S).

Strain: rabbit/Himalayan/CHR. (b) (4)
Drug batch No.: 96020014
Dose selection: Doses were based on an embryo-fetal range-finding study (Study D-23129/3000911744) in which doses of 2.15, 4.64, 10.0, or 21.5 mg/kg were given to pregnant Himalayan rabbits (2/group) from GD 6-20. There was no drug-related mortality. Maternal toxicity was limited to slightly decreased BW gain and food consumption at the HD. There were no apparent effects on pregnancy parameters as assessed by the numbers of corpora lutea, implantations, live fetuses, post-implantation loss, fetal weight, or external abnormalities.

b. Results

i. Maternal effects

One HD doe died on GD16 of unknown cause. Since only 17/22 were pregnant at the HD, with the death there were only 16 litters. No T-R clinical signs or BW effects were noted. Two HD does aborted their litters and there was 1 HD complete litter resorption.

ii. Developmental effects

(1) Post-implantation loss was increased slightly at the HD (NS), primarily due to 1 total litter loss, but there was no decrease in live fetuses when this doe was excluded (**Table IVE.7.1**). Fetal BW was slightly but D-D decreased (6% at HD; NS).
(2) Viability of fetuses (atypical endpoint in EFD studies) was impaired at the HD: 11 fetuses died within 6 hours, 2 fetuses within 24 hours after laparotomy. This was attributed to low fetal BW. External examination of the fetuses revealed only 1 malformed fetus (omphalocele) in the VC group. Visceral examination was not discussed and no table of visceral abnormalities was provided. No skeletal malformations were noted in any group. Increases in fetal and litter incidences of several individual skeletal variations and in total skeletal variations were seen in treated groups, primarily at the HD (**Table IVE.7.3**). Increases (SS) in incomplete ossification of the skull and missing ossification of the talus were seen at the HD.

c. Conclusions

Treatment of pregnant rabbits retigabine (oral gavage doses of 5.62, 12.1, or 26.1 mg/kg) from GD 6-20 produced reductions in fetal BW (HD) and increased incidences of skeletal variations and retardations (SS at HD). This study was not adequate because of the lack of maternal toxicity at the HD and the apparent failure to examine visceral abnormalities. In addition, the dearth of abnormalities in general makes the study suspect.

Table IVE.7.1

Parameter		Group 1 Control 1	Group 2 Control 2	Group 3 5.62 mg/kg	Group 4 12.1 mg/kg	Group 5 26.1 mg/kg
Corpora lutea	total	147	148	150	154	128
	per dam	7.7	7.4	7.5	7.7	7.5
Implantation sites	total	132	135	136	140	116
	per dam	6.9	6.8	6.8	7.0	6.8
Resorptions	total	11	11	14	8	12
	per dam	0.6	0.6	0.7	0.4	0.7
Early resorptions	total	7	9	10	7	11
	per dam	0.4	0.5	0.5	0.4	0.6
Late resorptions	total	4	2	4	1	1
	per dam	0.2	0.1	0.2	0.1	0.1
Live fetuses	total	121	124	122	132	104
	per dam	6.4	6.2	6.1	6.6	6.5[12]
Pre-implantation loss	mean %	10.2	8.5	10.0	8.7	9.3
Post-implantation loss	mean %	9.8	8.6	11.2	4.8	15.0

* Significantly different from the controls at $p \leq 0.05$
** Significantly different from the controls at $p \leq 0.01$

[12] obtained with 16 dams with viable fetuses

Table IVE.7.2

TABLE 8 — D-23129 - Teratology Study in Rabbits
MEAN PLACENTAL AND FETAL BODY WEIGHTS--GRAMS

		TEST GROUP 2 Control 2	TEST GROUP 3 5.62 mg/kg	TEST GROUP 4 12.1 mg/kg	TEST GROUP 5 26.1 mg/kg
PLACENTAL WEIGHTS					
of all Viable Fetuses	LITTER MEAN	4.80	4.70	4.52	4.71
	S.D.	1.05	0.68	0.98	1.48
	N	20	20	20	16
of Male Fetuses	LITTER MEAN	4.94	4.88	4.58	4.72
	S.D.	1.15	0.69	1.01	1.42
	N	20	17	20	16
of Female Fetuses	LITTER MEAN	4.69	4.60	4.46	4.43
	S.D.	1.01	0.67	1.06	1.24
	N	20	20	19	14
FETAL WEIGHTS					
of all Viable Fetuses	LITTER MEAN	36.1	36.7	35.0	33.9
	S.D.	6.0	4.8	4.5	7.7
	N	20	20	20	16
of Male Fetuses	LITTER MEAN	37.0	37.7	35.1	34.0
	S.D.	6.0	3.7	4.2	7.1
	N	20	17	20	16
of Female Fetuses	LITTER MEAN	35.5	36.3	34.9	32.3
	S.D.	6.8	5.2	5.8	7.9
	N	20	20	19	14

SIGNIFICANTLY DIFFERENT FROM CONTROL: ** = P≤0.01 (Dunnett or Student)

Table IVE.7.3

TABLE 10

D-23129 - Teratology Study in Rabbits
SUMMARY OF FETAL SKELETAL VARIATIONS

		TEST GROUP 2 Control 2	TEST GROUP 3 5.62 mg/kg	TEST GROUP 4 12.1 mg/kg	TEST GROUP 5 26.1 mg/kg
Litters Evaluated	N	20	20	20	16
Fetuses Evaluated	N	124	122	132	104
Live	N	124	122	132	104
Dead	N	0	0	0	0
ACCESSORY 13TH RIB(S)					
Fetal Incidence	N	10	12	11	18 *
	%	8.1	9.8	8.3	17.3
Litter Incidence	N	4	8	9	9 *
	%	20.0	40.0	45.0	56.3
SKULL: 1 WINDOW IN PARIETALE					
Fetal Incidence	N	0	0	1	2
	%	0.0	0.0	0.8	1.9
Litter Incidence	N	0	0	1	2
	%	0.0	0.0	5.0	12.5
STERNEBRA(E) BIPARTITE					
Fetal Incidence	N	2	1	1	2
	%	1.6	0.8	0.8	1.9
Litter Incidence	N	2	1	1	1
	%	10.0	5.0	5.0	6.3
STERNEBRA(E) FUSED (SEVERITY: SLIGHT)					
Fetal Incidence	N	0	2	4 *	1
	%	0.0	1.6	3.0	1.0
Litter Incidence	N	0	2	4	1
	%	0.0	10.0	20.0	6.3
STERNEBRA(E) MISALIGNED (SEVERITY: SLIGHT)					
Fetal Incidence	N	3	1	2	8 *
	%	2.4	0.8	1.5	7.7
Litter Incidence	N	2	1	2	8 *
	%	10.0	5.0	10.0	50.0
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence	N	13	15	18	28**
	%	10.5	12.3	13.6	26.9
Litter Incidence	N	6	10	12	14**
	%	30.0	50.0	60.0	87.5

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P≤0.05 ** = P≤0.01 (Fisher or Chi-square test)

8. Retigabine: embryo-fetal toxicity study in rabbits (Exp. No. PR2006-015; report dated 1/151/08; conducted by (b) (4) GLP)

1. Methods

Retigabine was administered to pregnant rabbits (NZW; 20/group) from GD 6-19 at oral gavage doses of 0 (vehicle: propylene glycol), 10, 40, or 60 mg/kg. Blood samples for TK analyses were collected on GD 6 and 19, at 5 time-points, with 4 animals contributing to each time-point. Plasma concentrations of retigabine and the N-acetyl metabolite were determined. Does were monitored for clinical signs, body weight and food consumption during gestation, and for postmortem findings. They were sacrificed on GD 29 for evaluation of pregnancy outcome, intrauterine death, fetal weight, and fetal abnormalities (visceral examination by fresh dissection (Staples) and skeletal examination after clearing and staining with Alizarin red S).

Strain: New Zealand White SPF
Drug batch No.: 0005005

Dose selection: Doses were based on the results of a preliminary embryo-fetal toxicity study in rabbits (PR2006-014; 0, 30, 60 and 120 mg/kg) in which clinical signs (decreased activity, prostration, and 1 case of clonic convulsions) and decreased feed consumption (-30 to –50%) were seen at the HD (dosing was stopped on GD 10), and decreases in feed consumption were seen at the MD (dosing was stopped on GD 16) and LD.

2. Results

a. Maternal effects

One HD animal (4537) and 2 MD animals (3534, and 3545) were euthanized in moribund condition, “or for other humane reasons,” between GD 14 and 18. These unscheduled deaths were associated with decreased feed consumption (-70 to -90%). One other MD animal (3543) was euthanized because of prolonged reduction in feed consumption, but the association with treatment was considered equivocal. T-R signs included unsteady gait, decreased activity, prostration, slow/labored breathing, aggressive behavior, and vocalization (1 animal) at the HD, and lethargy and decreased fecal output at the MD. There were no significant (statistically or biologically) effects on BW gain or BW over the treatment period (**Figure IVE.8.1**). A number of rabbits were found not pregnant in each group (roughly 5-15%). In addition to the 3 MD and 1 HD animals that were euthanized, 3 pregnant animals were “excluded because of poor acclimation to feed.” Therefore, the number of pregnant animals at termination was 17, 16, 14, and 18, in C, LD, MD, and HD groups, respectively. TK data are shown in **Table IVE.8.1**.

There were no T-R effects on reproductive parameters (**Table IVE.8.2**).

b. Developmental effects

i. There were no effects on post-implantation loss (**Table IVE.8.2**) or fetal BW (means: 38.3, 37.4, 40.0, 39.6 gm)
ii. There were no clear effects on incidences of fetal abnormalities; however, fetal and litter rates of some individual visceral (malpositioned origin of carotid artery) and skeletal variations (costal cartilage not connected to sternum, 13th rib, wavy ribs, fused maxilla) appeared to be increased in a D-R manner (**Table IVE.8.3**).

3. Conclusions

Treatment of pregnant rabbits with retigabine (oral gavage doses of 10, 40, or 60 mg/kg) from GD 6-19 produced maternal toxicity at the MD and HD (CNS signs and decreased food consumption)

but had no clear effects on embryo-fetal development in fetuses examined on GD 29 (slight D-R increases in some visceral and skeletal variations).

Figure IVE.8.1 Maternal body weight in rabbit embryo-fetal development study

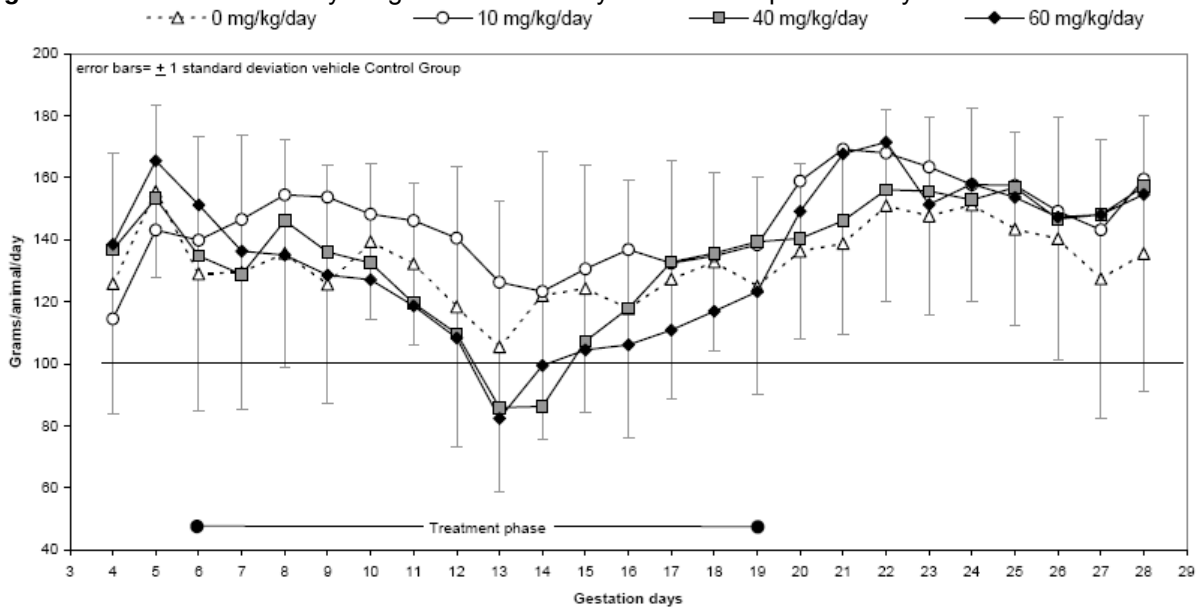

Table IVE.8.1 TK parameters in rabbit embryo-fetal development study of retigabine

Dose (mg/kg/day)		AUC $_{0-24}$ (ng.h/mL) GD 6	AUC $_{0-24}$ (ng.h/mL) GD 19	C_{max} (ng/mL) GD 6	C_{max} (ng/mL) GD 19	Accumulation ratios (GD 19/GD6)
10	Retigabine	1414.11	872.53	310	279	0.62
	N-AMR	166.93	111.47	47	48	.67
40	Retigabine	6776.92	6845.75	1485	1798	1.01
	N-AMR	791.57	892.28	193	235	1.13
60	Retigabine	10595.49	18493.37	1403	1473	1.75
	N-AMR	1142.78	2177.87	203	210	1.91

N-AMR: N-acetyl metabolite of retigabine

Table IVE.8.2

Females	Summary of Cesarean Section Data									Table 8	
	Corpora Lutea	Implant-ations	Resorptions Early	Resorptions Late	Resorptions Total	Live Young Male	Live Young Female	Live Young Total	Sex Ratio (%)	Implantation Loss Pre (%)	Implantation Loss Post (%)
Group 1 – 0 mg/kg/day											
Mean	11.1	9.6	0.6	0.1	0.6	4.5	4.5	8.9	50.5	14.5	6.8
SD	2.80	3.24	0.80	0.24	0.93	2.10	2.32	3.1	17.04	16.72	10.27
N	17	17	17	17	17	17	17	17	17	17	17
Group 2 – 10 mg/kg/day											
Mean	12.2	11.3	0.1	0.1	0.3	4.4	6.5 +	10.9	40.7	7.4	2.6
SD	4.00	3.71	0.34	0.34	0.45	1.75	2.56	3.56	11.82	6.62	4.24
N	16	16	16	16	16	16	16	16	16	16	16
Group 3 – 40 mg/kg/day											
Mean	11.3	10.2	0.2	0.4	0.6	4.9	4.9	9.8	50.6	10.5	5.5
SD	2.27	2.81	0.54	0.74	0.85	1.82	1.96	2.86	12.78	9.85	7.86
N	16	16	16	14	14	14	14	14	14	14	14
Group 4 – 60 mg/kg/day											
Mean	11.1	9.6	0.3	0.5	0.8	4.7	4.2	8.9	51.8	13.7	6.9
SD	2.30	2.93	0.56	1.02	1.48	1.78	1.63	2.73	12.67	15.35	12.04
N	19	19	19	18	18	18	18	18	18	18	18

*Significantly different from control mean; p≤0.05.
**Significantly different from control mean; p≤0.01.
If no asterisks, no statistically significant differences from control mean.

Table IVE.8.3

	Summary of Fetal Observations[a] Major Abnormalities in CAPITALS	Table 11

Group		Fetuses 1	2	3	4	Litters 1	2	3	4
Number examined	External	158	180	136	160	18	17	14	18
	Visceral	158	180	136	160	18	17	14	18
	Skeletal	159	180	137	161	18	17	14	18
Multiple, major									
ACEPHALY (INCOMPLETE); BASIOCCIPITAL SMALL, MISSHAPEN, BRIDGE OF OSSIFICATION WITH EXOCCIPITAL; HEART MALPOSITIONED, INCOMPLETE AORTIC ARCH, VENTRICULAR SEPTAL DEFECT; BENT CLAVICLES AND SCAPULAR SPINES; SCAPULAR SPINE SHORT; BILATERAL CARPAL FLEXURE, SHORT 2ND DIGITS, 4TH DIGIT PHALANGEAL ELEMENTS ABSENT; STERNEBRAE FUSED; ABSENT RIGHT THYROID		1				1			
VENTRICULAR SEPTAL DEFECT, AORTIC ARCH DISTENDED, PULMONARY TRUNK NARROW AND MALPOSITIONED		1				1			
SKULL BONES: FRONTAL, PARIETAL, FUSED, BILATERAL; FONTANELLES, ANTERIOR, POSTERIOR, SMALL; INTERPARIETAL, PARIETAL, FUSED. STERNUM: STERNEBRAE, 03RD-05TH PARTIALLY FUSED; 01ST-06TH WIDE; 01ST, ADDITIONAL CENTER ADJACENT ANTERIORLY. BLOOD VESSELS: RIGHT SUBCLAVIAN, RETRO-ESOPHAGEAL			1				1		
SKULL BONES: FRONTALS FUSED. FORELIMBS: MODERATE FLEXURE (BILATERAL)				1				1	
FORELIMB: MISSHAPEN, FLEXURE; RADIUS THIN, MALPOSITIONED; 1ST DIGIT ABSENT; (UNILATERAL)			1				1		

Blood vessels								
Carotid artery, malpositioned origin	1	4	4	7	1	2	2	5
Innominate artery, absent	1	2			1	2		
RIGHT SUBCLAVIAN, RETRO-ESOPHAGEAL (includes one fetus with other major abnormalities, above)		2				2		
Caudal vertebrae								
14th caudal vertebra, asymmetrically ossified		1	1			1	1	
14th caudal vertebra, offset		1				1		
15th caudal vertebra, asymmetrically ossified		1		1		1		1
15th caudal vertebra, misaligned		1				1		
15th caudal vertebra, offset	1				1			
15th caudal vertebra, unilaterally ossified		1				1		
16th caudal vertebra, asymmetrically ossified	1				1			
16th caudal vertebra, offset	1	3			1	3		
Caudal vertebra, incompletely ossified	3	2		2	2	2		1
Caudal vertebrae								
Tail tip, incompletely ossified		1				1		
Tail tip, kinked	5	3	1	2	3	3	1	2
Cervical vertebrae								
02nd cervical vertebral arch, incompletely ossified		2				2		
02nd left cervical hemicentrum, unilaterally ossified			1				1	
02nd left cervical hemicentrum, unossified			1				1	
03rd cervical hemicentrum, incompletely ossified			1				1	
04th cervical hemicentrum, incompletely ossified			1				1	
04th cervical hemicentrum, unilaterally ossified		1				1		
05th-06th cervical vertebral centra, incompletely ossified	1				1			
06th cervical vertebral arch, large	1				1			
Anterior tubercle of atlas, bipartite ossification		1	1			1	1	
Odontoid process, incompletely ossified	2	2			2	1		
Costal/sternal cartilage								
3rd to 6th, offset	1				1			
4th right costal cartilage, offset			1				1	
5th right costal cartilage, misaligned				1				1
7th bilateral costal cartilage, not connected to sternum	22	25	7	17	10	9	3	8
Costal/sternal cartilage								
7th unilateral costal cartilage, not connected to sternum	3	5	5	8	2	4	4	6
Right costal cartilage, 1st to 7th, misaligned			1				1	
Cranium								
General (head), domed	3				1			
Eye								
Left iris, hemorrhage	1	1	1	4	1	1	1	3
Fontanelles, Sutures								
Anterior, posterior, large	1				1			
Anterior, large sutural bone	1				1			
Frontal/frontal, small sutural bone			1				1	
Nasal/frontal, small sutural bone	1				1			
Nasal/frontal, sutural bone	1				1			
Nasal/nasal, small sutural bone	1		1		1		1	
Parietal/parietal, small sutural bone				1				1

Girdles								
Acromion process, increased ossification			1				1	
CLAVICLE, BENT, BILATERAL (includes one fetus in Group 1 with other major abnormalities, above)	2			1	2			1
Clavicle, increased ossification			1				1	
Scapula spine, short, bilateral				5				1
Metacromion process, increased ossification			1				1	
Pelvic girdle, (left sided), offset alignment	2		1		2		1	
Pelvic girdle, (right sided), offset alignment	1	3		1	1	2		1
Pubis, (left sided), incompletely ossified				1				1
Pubis, incompletely ossified	4	8		5	4	3		2
Pubis, unossified	1		1	1	1		1	1
Scapula spine, incompletely ossified	1				1			
Gonads								
Testes - bilateral, discolored	1				1			
Heart								
Large (moderate)				1				1
Kidneys/ureters								
Kidney - right, papilla, absent	1				1			
Liver/diaphragm								
Gall bladder, absent	1	1	2		1	1	2	
Gall bladder, hemorrhage, external				1				1
Gall bladder, small	3				1			
DIAPHRAGMATIC HERNIA, LEFT AND MEDIAN LIVER LOBES			1				1	
Lungs								
Azygos lung lobe, absent	3	6	3	1	3	4	2	1
Azygos lung lobe, small				2				1
Mandible								
Bilateral, dental ridge, patchy ossification				1				1
Bilateral, dental ridge, unossified area				1				1
Mandible, unossified area	1				1			
Ossification parameters, other								
Epiphyses, incompletely ossified	68	46	38	58	16	14	9	14
Epiphyses, unossified	11	27	3	9	7	7	3	4
Palate/skull base								
Hyoid body, incompletely ossified	21	15	15	15	7	9	5	5
Hyoid body, unossified	5	7	1	3	2	4	1	3
Hyoid cornu, bent	1		2	3	1		2	3
Hyoid cornu, unossified	1				1			
Paw bones								
1st digit, 1st phalanx, forelimb, unossified	1				1			
1st digit, 1st phalanx, unossified	2		2	1	1		1	1
1st digit, 2nd phalanx, hindlimb, incompletely ossified	1				1			
1st digit, metacarpal, incompletely ossified	19	13	15	15	9	7	5	6
1st digit, metacarpal, unossified	23	41	16	20	10	9	6	8
1st phalanx, 5th digit, incompletely ossified			1				1	
1st phalanx, 5th digit, unossified	1				1			
2nd digit, 2nd phalanx, incompletely ossified	1		1	1	1		1	1
2nd digit, 2nd phalanx, unossified	4	1	1		2	1	1	
2nd digit, 5th digit, unossified, forelimb	1				1			
2nd phalanx, 3rd digit, incompletely ossified	2		1		2		1	
2nd phalanx, 3rd digit, unossified		1				1		

Ribs								
04th, left rib, distal rib, kinked				1				1
06th, right rib, distal rib, associated costal cartilage, thick				1				1
06th, right rib, distal rib, thick	1				1			
06th, left rib, distal rib, thick	3		1	1	2		1	1
07th, 08th, right rib, distal rib, thick				1				1
07th, 08th, left rib, distal rib, thick	2		1	3	1		1	3
07th, 08th, bilateral rib, distal rib, thick	3		4	1	2		2	1
07th, Left rib, distal rib, kinked				1				1
07th, right rib, distal rib, thick	1		1	1	1		1	1
07th, left rib, distal rib, thick	1				1			
07th, bilateral rib, distal rib, thick	3			4	2			3
07th, left rib, increased ossification			1				1	
07th, left rib, thick, medial		1				1		
07th, right rib, focal thickening	1				1			
08th, 09th, 10th, right rib, thick, distal		1				1		
08th, 09th, 10th, right rib, thick, medial				1				1
08th, 09th, right rib, distal rib, thick				1				1
08th, 09th, left rib, distal rib, thick	1			1	1			1
08th, 09th, bilateral rib, distal rib, thick	2				2			
08th, 09th, right rib, distal rib, thick, wavy				1				1

Ribs								
08th, left rib, medial rib, focal thickening		1				1		
08th, right rib, focal thickening			1				1	
08th, right rib, thick	1		1		1		1	
09th, distal rib, focal thickening	1				1			
09th, right rib, distal rib, thick				2				2
09th, left rib, distal rib, thick				1				1
09th, bilateral rib, distal rib, thick	1		2		1		1	
10th, 11th, proximal, narrow			1				1	
11th, right rib, distal rib, ribs, increased ossification, misshapen			1				1	
12th, bilateral rib, absent		1		1		1		1
12th, left rib, non articulated, short				1				1
12th, right rib, short				1				1
13th, bilateral rib	3	1	2	3	2	1	2	2
13th, bilateral rib, complete	51	85	44	51	13	13	11	13
13th, bilateral rib, non articulated, rudimentary	3				2			
13th, bilateral rib, non articulated, short	2		2		2		2	
13th, bilateral rib, rudimentary	2			1	1			1
13th, bilateral rib, short	3	4	3	2	3	4	3	2
13th, left rib	3		2	7	2		2	3

Ribs								
13th, left rib, complete	19	17	15	15	10	9	6	9
13th, left rib, non articulated	1			1	1			1
13th, left rib, non articulated, rudimentary	7	3	6	4	6	3	4	3
13th, left rib, non articulated, short	1	6	5	3	1	5	4	3
13th, left rib, rudimentary	1	1	1	4	1	1	1	2
13th, left rib, short	7	3	3	4	5	2	3	4
13th, left rib, short, rudimentary				1				1
13th, right rib	2	1	2	5	1	1	1	4
13th, right rib, complete	11	5	15	13	6	2	8	8
13th, right rib, non articulated	1	2			1	2		
13th, right rib, non articulated, rudimentary	5	3	3	7	5	2	2	6
13th, right rib, non articulated, short	1	6	4	3	1	6	2	2
13th, right rib, rudimentary	2	3		4	2	2		3
13th, right rib, short	5	6	5	4	3	4	3	3
Cervical rib (uni- or bilateral)	6	5	9	8	4	3	4	5
Thick rib(s)	13	3	7	13	5	3	3	5
Kinked/wavy rib(s)				3				1

Skull bones								
Exoccipital, addit'l ossification site	1				1			
Frontal, incompletely ossified	1		1		1		1	
Frontal, incompletely ossified			1				1	
Frontal, fissure, misshapen	1				1			
Interparietal, incompletely ossified	1		2		1		2	
Interparietal, small		1				1		
Intra-orbital process of squamosal, narrow, short				1				1
Jugal, maxilla, bridge of ossification	3	2	2	2	3	2	2	2
Jugal, maxilla, partially fused	1	11	4	13	1	4	4	6
Jugal, maxilla, fused			1				1	
Jugal, short				1				1
Left, maxilla, mandible, fused, wide				1				1
Maxilla, incompletely ossified	2	1		1	2	1		1
Nasal(s), fissure				1				1
Nasal(s), suture			2				2	
Parietal, incompletely ossified	1				1			
Parietal, fissure	1	1	1		1	1	1	
Parietal, misshapen	1				1			
Parietal, unossified area	1	1	1		1	1	1	
Post-tympanic process of squamosal, wide				1				1
Supraoccipital, incompletely ossified	1				1			
Paw bones								
2nd phalanx, 4th digit, hindlimb, incompletely ossified		1				1		
2nd phalanx, 4th digit, hindlimb, unossified	7	3	2	1	4	2	1	1
2nd phalanx, 4th digit, incompletely ossified	2	2		2	2	2		2
2nd phalanx, 4th digit, incompletely ossified, forelimb		1				1		
2nd phalanx, 4th digit, unossified	8	1	2	1	4	1	1	1
2nd phalanx, 5th digit, incompletely ossified	11	17	11	19	6	10	5	11
2nd phalanx, 5th digit, incompletely ossified, forelimb		3	1	2		2	1	2
2nd phalanx, 5th digit, unossified	44	47	19	32	13	8	6	9
2nd phalanx, 5th digit, unossified, forelimb	1	2	2	1	1	2	2	1
5th digit, forelimb, metacarpal, incompletely ossified	1				1			
Astragalus, incompletely ossified	2	2		1	2	1		1
Astragalus, unossified	5	7	2	2	4	2	2	2
Paws and limbs								
FORELIMB - RIGHT, FLEXURE(S)			1				1	
FORELIMBS - BILATERAL, FLEXURE(S)	1		1	1	1		1	1
Sternebrae								
01st, additional center adjacent	1	1		2	1	1		2
01st, sternebral center, increased ossification	1				1			
01st, sternebral center, misshapen/wide		1	1			1	1	
01st, sternebral hemicentrum, anterior, increased ossification				1				1
01st, sternebral hemicentrum, bipartite ossification, offset				1				1
02nd, 03rd, sternebral center, offset, fused	1				1			
02nd, 03rd, sternebral center, partially fused			1				1	
02nd, 03rd, sternebral center, wide			1				1	
02nd, sternebral center, bipartite ossification, offset			1				1	
02nd, sternebral center, incompletely ossified				1				1
02nd, sternebral center, wide		1				1		
03rd, 04th, sternebral center, fused	1				1			
03rd, 04th, sternebral center, partially fused, wide		1				1		
03rd, sternebral center, offset			1				1	
04th, 05th, sternebral center, partially fused	1				1			
04th, 05th, sternebral center, partially fused, wide		1				1		
04th, sternebral center, bipartite ossification, offset	1				1			
04th, sternebral center, incompletely ossified				1				1
04th, sternebral center, offset			1				1	
04th, sternebral center, wide			1				1	

Sternebrae								
05th, sternebral center, bipartite ossification	1		1	1	1		1	1
05th, sternebral center, incompletely ossified	16	24	12	15	9	10	8	12
05th, sternebral center, unossified	78	99	63	95	16	15	12	18
05th, sternebral center, wide			1				1	
06th, sternebral center, bipartite ossification		1				1		
06th, sternebral center, dumbbell ossification, wide		1				1		
06th, sternebral center, incompletely ossified	11	7	7	4	7	4	4	2
06th, sternebral center, small			1	2			1	1
06th, sternebral center, unossified	8	9	7	9	7	5	4	5
06th, sternebral center, wide	3		1		3		1	
Thoracic vertebrae								
18 thoracolumbar vertebrae		1		6		1		3
20 thoracolumbar vertebrae	38	59	22	29	13	13	9	10
Thorax/neck								
Thymus, undescended tissue		4	3	1		3	3	1

9. Retigabine: Oral (Gavage) Perinatal and Postnatal Toxicity Study with Behavioral and Reproductive Assessments of Offspring in Rats (Report No. D-23129/9321 020048; dated 3/10/00; conducted by (b) (4) GLP)

a. Methods

Retigabine was administered to female rats (S-D, 25/group) from GD 6 to PND 20 at oral gavage doses of 0 (vehicle: propylene glycol), 4.64, 17.8, or 61.9 mg/kg. Dams were monitored for clinical signs, body weight and food consumption during gestation and lactation and were allowed to litter and rear their progeny until weaning. Progeny were examined for litter size, mortality, clinical observations, milk in stomach, body weight, reflex (acoustic startle and pupil constriction) and morphological development (including sexual maturation), motor activity, learning and memory (passive avoidance and simple water maze), estrous cycles, reproductive performance (mating and fertility indices, time to mating), hysterotomy findings, and postmortem observations.

Strain: Sprague-Dawley, Crl CD VAF
Batch No.: Lot No. 97071011

Dose selection: In the rat toxicology studies, T-R CNS signs (eg, hypokinesia, gait and coordination disturbances, abnormal body position, chewing motions, etc.) were consistent findings, particularly at 61.9 mg/kg and above. In the fertility study, doses of 14.7 mg/kg and above were associated with T-R clinical signs (eg, gait disturbances, salivation, etc.) and slight effects on body weight occurred at 46.4 mg/kg. In the first rat embryo-fetal development study, adverse clinical signs (eg, abnormal body position, chewing motions, head shaking) were observed at 21.5 mg/kg and above, while reduced food consumption occurred at 46.4 mg/kg. Skeletal variations were increased somewhat at 46.4 mg/kg (HD).

b. Results

i. Maternal effects

One HD dam (#90) was found dead on GD 21, but postmortem examination indicated dosing error as COD. Treatment-related adverse clinical signs were observed primarily in HD dams and included ataxia, increased/decreased motor activity, immobility, tremors,

abdominal posture, and coolness to the touch. One MD (#75) and 3 HD dams (#'s 85, 97, and 100) were euthanized due to total litter loss.

BW gain for the entire gestational period (GD 6 to 20) was decreased (SS) at the HD (28% compared to C; **Table IVE.9.1**). Following delivery HD animals weighed approximately 90% of C, and at the end of weaning (PD 21) maternal BWs were similar between groups. Gestation length was slightly prolonged (4% longer) in HD dams (22.3 days vs 21.4 days in C; SS). The increased gestation length was considered to be inter-related with the increased post-implantation loss and increased number of dead pups at birth at this dose (**Table IVE.9.2**). As a result of this postimplantation loss, there was a reduction in the number of live pups at birth at the HD. Maternal care (maintaining a nest area, nursing, and tending of pups) was not affected by treatment with retigabine. No TK data were collected, but exposure data can be estimated from the 26-week rat toxicity study in Wistar rats (**Table IVB.2.2**).

ii. Developmental effects

(1) There was a D-R increase in the incidence of pups found dead, missing, or selectively euthanized, with the number of pups surviving the postpartum period (viability index) being significantly reduced (SS) at the HD (**Tables IVE.9.3-4**). Most of the mortality occurred during the first 4 days postpartum. At the HD, 4 litters were responsible for the majority of the observed mortality (litters 84 [majority litter loss] and 85, 97, and 100 [total litter loss] accounted for 53 of the 65 pups found dead/missing/electively euthanized). These dams tended to be more adversely affected by drug administration during gestation (based on the incidence of adverse clinical signs and/or effects on body weight). The increased pup mortality during the early postpartum period resulted in a reduction in the average litter size at the HD throughout the remainder of the study.

2) At birth, HD pup BWs were about 10% below C (SS). Pup BWs were D-D reduced in treatment groups throughout the pre-weaning period (SS at HD; **Table IVE.9.5**). During the post-weaning period, offspring BWs continued to be D-D decreased until the end of the study (final BWs: 473, 462.2, 452.4, 418.8 in males and 272.0, 263.2, 260.7, 244.3 in females in C, LD, MD, and HD; SS at HD). For the entire postweaning period, BW gain was D-D (decreased 12% and 10% in HD males and females; both SS).

(3) There was a slight delay in the average age of attainment of a response to an auditory startle stimulus (approximately 1/2 to a full day) in the HD group, as identified by the average age at which 50% or 100% of the litter demonstrated a response(**Table IVE.9.5**). This finding was considered related to the lag in growth of pups at this dose compared to controls. There were no T-R effects on the pupil constriction response or on the age of attainment of evidence of sexual maturity in either males (balanopreputial separation) or females (vaginal patency). HD BWs were slightly lower at the age that evidence of sexual maturity was observed, consistent with the overall effects on growth at this dose.

(4) In passive avoidance testing (age at learning was 22 to 25 days), entry of HD males into the chamber occurred sooner than controls, possibly indicative of heightened impulsivity, but there were no other effects in this test (**Table IVE.9.6**). There were no clear biologically meaningful effects on motor activity when evaluated at 43 to 47 days of age. Although there were some intervals for which a SS effect was noted, there was no overall pattern or consistent effect indicative of a T-R alteration in activity levels. There was no effect noted on learning (tested at 9 to 11 weeks of age) or memory (tested 1 week after learning trial) in a simple M-maze. All groups demonstrated comparable latencies for finding the escape ramp, for number of trials required, and for the numbers of

errors made. For both time and errors, a similar value or an improvement was seen when comparing the performance on the learning trials as compared to the performance on the retention trials.

(5) There were no treatment-related effects on estrous cyclicity, time to mate, or mating and fertility indices.

3. Conclusions

Treatment of female rats with retigabine (oral gavage doses of 4.64, 17.8, or 61.9 mg/kg from GD 6 to PND 20) produced maternal toxicity as demonstrated by mortality, clinical signs, and decreased BW gain (all at HD), and developmental toxicity as evidenced by increased post-implantation loss and dead pups, decreased live pups, and decreased pup survival, growth and reflex development (primarily at HD).

Table IVE.9.1

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 10 PROTOCOL 97135 06DEC99 09:51
F0 MATERNAL BODY WEIGHT GAIN SUMMARY AND ANALYSIS (GRAMS)

TIME PERIOD	.	Vehicle-Control	4.64 mg/kg	17.8 mg/kg	61.9 mg/kg
GAIN GESTATION DAY 6 TO 17	MEAN	77.00	78.12	72.48	47.13
	STD	12.90	18.98	14.61	15.94
	N	25.00	25.00	25.00	24.00
	OVERALL P	0.01			
	PAIRWISE P		0.74	0.30	0.01
	TREND P		F	-0.19	0.01
GAIN GESTATION DAY 18 TO 20	MEAN	39.08	36.16	41.88	36.54
	STD	9.70	10.18	6.94	11.03
	N	25.00	25.00	25.00	24.00
	OVERALL P	0.15			
	PAIRWISE P		F	F	F
	TREND P		F	F	0.69
GAIN GESTATION DAY 6 TO 20	MEAN	116.08	114.28	114.36	83.67
	STD	19.63	26.38	17.36	21.98
	N	25.00	25.00	25.00	24.00
	OVERALL P	0.01			
	PAIRWISE P		0.98	0.68	0.01
	TREND P		F	-0.37	0.01
GAIN POSTPARTUM DAY 0 TO 6	MEAN	34.44	31.20	33.92	32.00
	STD	12.24	11.79	8.67	10.50
	N	25.00	25.00	24.00	21.00
	OVERALL P	0.59			
	PAIRWISE P		F	F	F
	TREND P		F	F	0.57
GAIN POSTPARTUM DAY 7 TO 13	MEAN	14.36	14.40	12.29	16.00
	STD	13.33	8.21	12.93	5.88
	N	25.00	25.00	24.00	21.00
	OVERALL P	0.56			
	PAIRWISE P		F	F	F
	TREND P		F	F	0.68

Sign(positive, negative) of Trend P-value indicates direction of trend test.
No sign indicates a two tailed test was performed.
F denotes follow up tests not appropriate.

Table IVE.9.2

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 11 PROTOCOL 97135 06DEC99 14:18
F1 BIRTH DAY SUMMARY AND ANALYSIS

PARAMETER	GROUP	N	TOTAL NUMBER	MEAN	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
NUMBER LIVE PUPS	Vehicle-Control	25	293	11.72	100	1.81		0.040	
	4.64 mg/kg	25	288	11.52	98	3.19	F		0.768
	17.8 mg/kg	25	320	12.80	109	1.55	F		0.111
	61.9 mg/kg	24	262	10.92	93	2.62	0.443		0.186
NUMBER DEAD PUPS	Vehicle-Control	25	2	0.08	100	0.28		0.405	
	4.64 mg/kg	25	4	0.16	200	0.37	F		F
	17.8 mg/kg	25	2	0.08	100	0.28	F		F
	61.9 mg/kg	24	11	0.46	573	1.14	0.134+		F
PROPORTION DEAD	Vehicle-Control	25		0.01	100	0.02		0.417	
	4.64 mg/kg	25		0.01	197	0.03	F		F
	17.8 mg/kg	25		0.01	105	0.02	F		F
	61.9 mg/kg	24		0.03	506	0.07	0.131+		F
POSTIMPLANTATION LOSS	Vehicle-Control	25	20	0.80	100	0.91		0.013	
	4.64 mg/kg	25	18	0.72	90	1.21	F		0.397
	17.8 mg/kg	25	10	0.40	50	0.58	F		0.133
	61.9 mg/kg	24	38	1.58	198	1.56	0.108+		0.097
PROPORTION POSTIMPLANTATION LOSS	Vehicle-Control	25		0.06	100	0.08		0.016	
	4.64 mg/kg	25		0.05	82	0.08	F		0.324
	17.8 mg/kg	25		0.03	47	0.04	F		0.121
	61.9 mg/kg	24		0.12	190	0.12	0.128+		0.130
LITTER SIZE	Vehicle-Control	25	295	11.80	100	1.83		0.126	
	4.64 mg/kg	25	292	11.68	99	3.21	F		F
	17.8 mg/kg	25	322	12.88	109	1.48	F		F
	61.9 mg/kg	24	273	11.38	96	2.81	0.708		F
IMPLANTATIONS	Vehicle-Control	25	311	12.44	100	1.80		0.718	
	4.64 mg/kg	25	301	12.04	97	3.47	F		F
	17.8 mg/kg	25	329	13.16	106	1.62	F		F
	61.9 mg/kg	24	299	12.46	100	2.78	0.594		F

Sign(positive, negative) of Trend P-value indicates direction of trend test.
No sign indicates a two tailed test was performed.
F denotes follow up test not appropriate.

Table IVE.9.3

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 11 PROTOCOL 97135 07DEC99 11:47
F1 MORTALITY INDICES SUMMARY AND ANALYSIS

PARAMETER	GROUP	N	TOTAL DEAD	MEAN PROPORTION	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
BIRTH INDEX	Vehicle-Control	25	2	0.01	100	0.02		0.374	
	4.64 mg/kg	25	4	0.01	204	0.03	F		F
	17.8 mg/kg	25	2	0.01	105	0.02	F		F
	61.9 mg/kg	24	11	0.04	554	0.08	0.118+		F
VIABILITY INDEX	Vehicle-Control	25	1	0.00	100	0.02		0.002	
	4.64 mg/kg	25	3	0.01	360	0.06	F		0.985
	17.8 mg/kg	25	14	0.04	1292	0.20	0.262+		0.633
	61.9 mg/kg	24	54	0.19	5596	0.36	0.001+		0.001

Birth Index is the proportion of dead pups at birth.
Viability Index is the proportion of pups dying from birth to postpartum day 21.

Sign(positive, negative) of Trend P-value indicates direction of trend test.
No sign indicates a two tailed test was performed.
F denotes follow up test not appropriate.

Table IVE.9.4

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 11 PROTOCOL 97135 07DEC99 08:12
F1 LIVE LITTER SIZE SUMMARY AND ANALYSIS

PARAMETER	GROUP	N	TOTAL LIVE	MEAN	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
DAY 0	Vehicle-Control	25	293	11.72	100	1.81		0.040	
	4.64 mg/kg	25	288	11.52	98	3.19	F		0.768
	17.8 mg/kg	25	320	12.80	109	1.55	F		0.111
	61.9 mg/kg	24	262	10.92	93	2.62	0.443		0.186
DAY 4	Vehicle-Control	25	292	11.68	100	1.82		0.002	
	4.64 mg/kg	25	285	11.40	98	3.30	F		0.772
	17.8 mg/kg	25	306	12.24	105	2.99	0.880-		0.250
	61.9 mg/kg	24	209	8.71	75	4.44	0.041		0.008
DAY 7	Vehicle-Control	25	292	11.68	100	1.82		0.002	
	4.64 mg/kg	25	285	11.40	98	3.30	F		0.772
	17.8 mg/kg	25	306	12.24	105	2.99	0.880-		0.250
	61.9 mg/kg	24	209	8.71	75	4.44	0.041		0.008
DAY 14	Vehicle-Control	25	292	11.68	100	1.82		0.001	
	4.64 mg/kg	25	285	11.40	98	3.30	F		0.778
	17.8 mg/kg	25	306	12.24	105	2.99	0.880-		0.246
	61.9 mg/kg	24	208	8.67	74	4.40	0.031		0.005
DAY 21	Vehicle-Control	25	292	11.68	100	1.82		0.001	
	4.64 mg/kg	25	285	11.40	98	3.30	F		0.778
	17.8 mg/kg	25	306	12.24	105	2.99	0.880-		0.246
	61.9 mg/kg	24	208	8.67	74	4.40	0.031		0.005

Sign(positive, negative) of Trend P-value indicates direction of trend test.
No sign indicates a two tailed test was performed.
F denotes follow up test not appropriate.

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 97135 PROTOCOL 97135 06DEC99 16:39
F1 LIVE PUP WEIGHT SUMMARY AND ANALYSIS (GRAMS)
Absolute Analysis

SEX=Male

PARAMETER	GROUP	N	MEAN	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
Birth Day	Vehicle-Control	25	6.48	100	0.53		0.003	
	4.64 mg/kg	25	6.49	100	0.60	F		0.803
	17.8 mg/kg	25	6.52	101	0.53	0.512-		0.954
	61.9 mg/kg	23	5.87	91	0.67	0.006		0.002
Day 4	Vehicle-Control	25	10.44	100	1.00		0.009	
	4.64 mg/kg	25	10.29	99	1.35	F		0.209
	17.8 mg/kg	24	10.12	97	0.75	0.109-		0.228
	61.9 mg/kg	19	9.17	88	1.24	0.002		0.001
Day 7	Vehicle-Control	25	15.04	100	1.59		0.001	
	4.64 mg/kg	25	14.47	96	1.72	0.043-		0.086
	17.8 mg/kg	24	14.08	94	1.17	0.012-		0.027
	61.9 mg/kg	19	12.77	85	1.86	0.001		0.001
Day 14	Vehicle-Control	25	28.66	100	2.75		0.001	
	4.64 mg/kg	25	27.36	95	2.78	0.034-		0.060
	17.8 mg/kg	24	26.09	91	2.26	0.001-		0.001
	61.9 mg/kg	19	23.96	84	3.91	0.001		0.001
Day 21	Vehicle-Control	25	43.94	100	6.75		0.068	
	4.64 mg/kg	25	42.69	97	5.57	0.239-		F
	17.8 mg/kg	24	40.53	92	4.73	0.030-		F
	61.9 mg/kg	19	38.53	88	7.50	0.007		F

SEX=Female

PARAMETER	GROUP	N	MEAN	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
Birth Day	Vehicle-Control	25	6.13	100	0.53		0.002	
	4.64 mg/kg	25	6.16	100	0.58	F		0.892
	17.8 mg/kg	25	6.14	100	0.44	0.550-		0.875
	61.9 mg/kg	24	5.60	91	0.63	0.005		0.002
Day 4	Vehicle-Control	25	9.92	100	0.94		0.001	
	4.64 mg/kg	25	10.00	101	1.27	F		0.925
	17.8 mg/kg	24	9.64	97	0.64	0.151-		0.336
	61.9 mg/kg	21	8.59	87	1.45	0.001		0.001
Day 7	Vehicle-Control	25	14.34	100	1.51		0.001	
	4.64 mg/kg	25	14.10	98	1.58	0.271-		0.536
	17.8 mg/kg	24	13.48	94	1.00	0.018-		0.041
	61.9 mg/kg	21	11.96	83	1.95	0.001		0.001
Day 14	Vehicle-Control	25	27.49	100	2.76		0.001	
	4.64 mg/kg	25	26.63	97	2.50	0.132-		0.291
	17.8 mg/kg	24	25.12	91	1.89	0.001-		0.001
	61.9 mg/kg	21	22.44	82	3.81	0.001		0.001
Day 21	Vehicle-Control	25	42.39	100	6.58		0.008	
	4.64 mg/kg	25	41.60	98	4.54	0.271-		0.671
	17.8 mg/kg	24	39.20	92	4.48	0.020-		0.064
	61.9 mg/kg	21	36.44	86	6.30	0.001		0.002

Sign(positive, negative) of Trend P-value indicates direction of trend test.
No sign indicates a two tailed test was performed.
F denotes follow up test not appropriate.

Table IVE.9.6

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 97135 PROTOCOL 97135 06DEC99 10:38
F1 REFLEX DEVELOPMENT SUMMARY AND ANALYSIS

PARAMETER	GROUP	N	MEAN	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
Acoustic Startle-day reaches 50%	Vehicle-Control	25	11.52	100	0.71		0.180	
	4.64 mg/kg	25	11.72	102	0.54	F		F
	17.8 mg/kg	24	11.67	101	0.48	F		F
	61.9 mg/kg	21	12.00	104	0.95	0.060		F
Acoustic Startle-day reaches 100%	Vehicle-Control	25	12.32	100	0.75		0.105	
	4.64 mg/kg	25	12.60	102	1.22	F		F
	17.8 mg/kg	24	12.75	103	1.19	0.119+		F
	61.9 mg/kg	21	13.33	108	1.65	0.017		F
Pupil Constriction-Percent responding	Vehicle-Control	25	1.00	100	0.00		0.321	
	4.64 mg/kg	25	1.00	100	0.00	F		F
	17.8 mg/kg	24	1.00	100	0.00	F		F
	61.9 mg/kg	21	1.00	100	0.02	0.163		F

Figure IVE.9.1

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 97135 PROTOCOL 97135 06DEC99 10:38
F1 REFLEX DEVELOPMENT MEANS

Measure=ACOUSTIC STARTLE

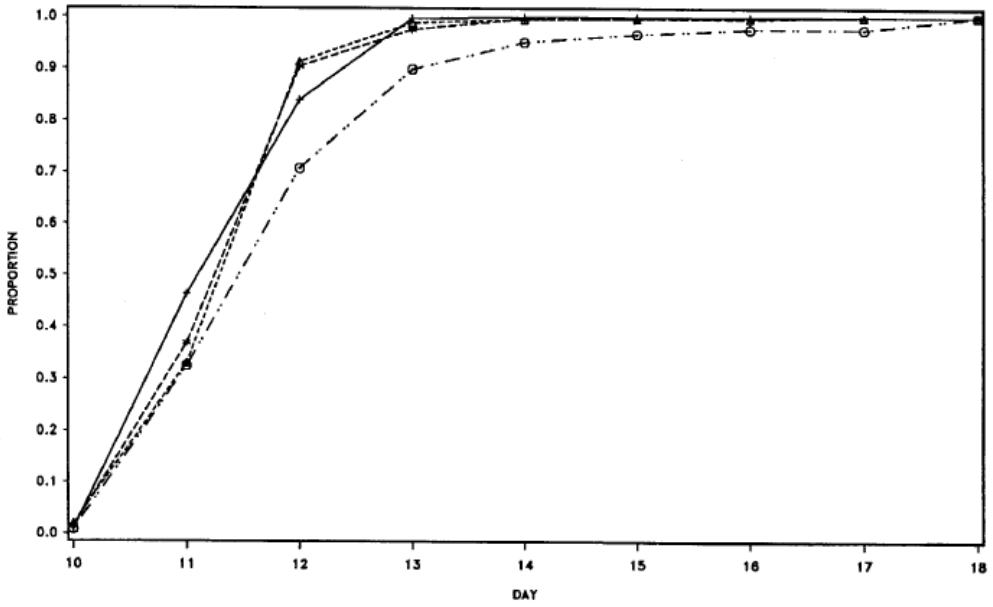

Table IVE.9.7

ORAL (GAVAGE) PERINATAL AND POSTNATAL TOXICITY STUDY WITH BEHAVIORAL AND REPRODUCTIVE ASSESSMENTS OF OFFSPRING IN RATS
TERAS 97135 PROTOCOL 97135 06DEC99 10:34
F1 PASSIVE AVOIDANCE TEST SUMMARY AND ANALYSIS

SEX=MALE

PARAMETER	GROUP	N	MEAN	PERCENT REFERENCE	STANDARD DEVIATION	TREND P-VALUE	OVERALL P-VALUE	PAIRWISE P-VALUE
LEARNING TRIAL LATENCY (sec)	Vehicle-Control	25	30.03	100	38.91		0.410	
	4.64 mg/kg	25	25.57	85	21.07	F		F
	17.8 mg/kg	24	29.03	97	29.17	F		F
	61.9 mg/kg	18	18.28	61	16.75	0.256		F
RETENTION TRIAL LATENCY (sec)	Vehicle-Control	25	118.45	100	75.44		0.749	
	4.64 mg/kg	25	139.73	118	68.64	F		F
	17.8 mg/kg	24	134.10	113	70.01	F		F
	61.9 mg/kg	18	126.60	107	70.18	0.933		F
DIFFERENCE IN LATENCIES (sec)	Vehicle-Control	25	88.42	100	83.66		0.835	
	4.64 mg/kg	25	114.16	129	70.40	F		F
	17.8 mg/kg	24	105.07	119	74.80	F		F
	61.9 mg/kg	18	108.32	123	68.33	0.430		F

F. Juvenile Animal Toxicity

1. Retigabine: Two month oral (gavage) toxicity study with recovery in juvenile rats (Study No. D-23129/9321020106, conducted by [redacted](b) (4); Report dated 12/01; GLP (GLP compliance statement included, but no raw data or data tables provided in study report; the study is thus considered non-pivotal)

a. Methods

Retigabine was administered to juvenile rats from postnatal day (PND) 14 to PND 70 at doses of 0 (PG vehicle), 0.1, 0.5, or 2 mg/kg (5 ml/kg). Approximately half of the animals in each group (non-reversal animals) were assessed for motor activity and learning and memory (passive avoidance and simple water M-maze) during the dosing period. The remaining half of the animals (reversal animals) were evaluated for the same measures and for reproductive performance during a treatment-free reversal period. All animals were evaluated for adverse effects on survival, growth, sexual maturation, and landmarks of reflex and morphological development. At approximately PND 70, the non-reversal animals were necropsied, with perfusion fixation. The remaining animals (reversal animals) were necropsied at the time of fertility assessment (males on PND 144 to 147; mated females on GD 14; females that did not mate on PND 159 to 160); this included an evaluation of embryo status and gross external anomalies. TK, hematology, clinical chemistry parameters, and macroscopic and histopathological observations were also evaluated for all animals.

Doses were based of the results of previous juvenile toxicity dose range-finding studies (Report Nos. 23129/9321020046 and D-23129/9321020047) using oral gavage doses of 0.2 to 54 mg/kg/day (PG vehicle; 2 and 10 ml/kg constant vol) administered starting on PND7. In these studies, an increased incidence of mortality occurred at doses ≥ 2 mg/kg (in the first study, mortality rates of 15, 31, 74, and 100% occurred at 0, 9, 18, and 54 mg/kg, respectively; in the second, mortality rates were 33, 36, 47, and 60% at 0, 0.6, 2, and 6 mg/kg, respectively). Postmortem examination revealed distended urinary bladders and/or dilated renal pelves, suggestive of a lower urinary tract blockage leading to uremia. In those animals found dead or electively euthanized early during the study (**Table IVF.1.1**), distended urinary bladders and occasional concurrent secondary dilation of the renal pelvis were observed. Microscopically, the urinary bladder was dilated with thin walled mucosal and muscular layers. Secondary renal pelvic dilation and dilatation of the cortical proximal convoluted tubules were observed in multiple animals. Infrequently, in these animals, dilation of the ureter was also observed. The etiology of the urinary tract dilation was unclear at the time, but physical obstruction due to intraluminal crystals or debris was not found. It was thought that the urinary bladder distention induced secondary uremia and a moribund state. The proposed mechanism involved a "neurogenic effect of retigabine on the detrusor muscle in the urinary bladder causing inhibition of bladder contractility." Adverse clinical signs consisting of moribundity, coolness to the touch, and immobility were observed. Myoclonus was also observed in a number of animals at all doses. Pup body weights were reduced by 24% or more during the first week of treatment at ≥ 6 mg/kg. Based on these data, the extremely low doses of 0.1, 0.5, and 2 mg/kg were chosen this study.

Strain: Sprague-Dawley [redacted](b) (4) CD VAF)
Drug Batch #: 97071011

b. Results

i. Mortality, Clinical signs, Body weight

There were no T-R deaths and no effects were noted on clinical observations or body weights. The deaths of 10, 11, 6, and 11 animals in the C, LD, MD, and HD groups were not considered retigabine-related

ii. Pre- and Post-weaning development

There were no T-R effects on reflex or morphological development.

iv. Behavioral testing

There were no clear effects of treatment on motor activity (between PD 34 and 36 (non-reversal) or 97 and 99 (reversal)) or on assessments of learning or memory (passive avoidance between PD 22 and 25 (non-reversal) or 84 and 89 (reversal); M-maze once between PD 56 and 59 (nonreversal) or 105 and 109 (reversal)).

v. Mating and fertility and pregnancy parameters

Reproductive performance of the treated animals was unaffected by treatment.

vi. Clinical Pathology and Necropsy

There were no T-R effects on clinical pathology parameters or on postmortem macroscopic or microscopic observations in any tissues

viii. Plasma drug levels (**Table IVF.1.2**)

The TK of retigabine and its acetyl metabolite (AWD21-360) were assessed on PNDs 21 and 70. Exposure to parent was similar in males and females and appeared to be approximately dose-proportional across the range tested. No significant differences in exposure were noted between the two study days, suggesting that after 8 daily doses (ie, on PD 21), retigabine had achieved steady-state. No sex differences were noted in the exposure to AWD21-360 and exposure increased proportionally with retigabine dose; however, exposure to AWD21-360 did increase over time, with values 1.6 to 8-fold higher on PND 70 compared to PND 21.

c. Conclusions

Oral administration of retigabine doses of 0, 0.1, 0.5, or 2 mg/kg from PND 14 to 70 produced no discernable drug-related toxicity according to the study report; however, actual study data and summary tables were not provided in this report, so this cannot be considered a valid study.

Table IVF.1.1 Microscopic findings in juvenile rat dose range-finding study

GROUP INCIDENCES (WITH AVERAGE SEVERITY) OF RETIGABINE-RELATED MICROSCOPIC OBSERVATIONS FOR UNSCHEDULED NECROPSIES

	Male				Female			
	Dosage (mg/kg/day)				Dosage (mg/kg/day)			
Lesion	0	0.6	2	6	0	0.6	2	6
Kidneys[a]	9	9	10	20	8	10	10	20
Dilated pelvis	2	1	1	5	0	0	2	9
	(0.3)	(0.3)	(0.2)	(0.3)	(0.0)	(0.0)	(0.3)	(0.9)
Tubular dilatation	3	4	7	10	2	7	6	10
	(0.7)	(0.6)	(1.0)	(0.8)	(0.5)	(1.1)	(1.0)	(0.8)
Urinary Bladder[a]	10	8	9	20	8	9	9	19
Dilation	3	3	6	18	3	6	6	17
	(0.7)	(1.1)	(2.2)	(3.0)	(1.0)	(1.4)	(2.1)	(2.7)
Ureter[a]	8	9	9	20	8	9	9	20
Dilation	0	0	1	3	0	1	0	4
	(0.0)	(0.0)	(0.2)	(0.2)	(0.0)	(0.2)	(0.0)	(0.4)

a: Number examined

( ): Average severity (0 = No microscopic lesion, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

Table IVF.1.2 PK parameters in juvenile rats

Mean (± SE) Retigabine Pharmacokinetic Parameters							
Postnatal Day	Number of Doses	Sex	Dosage (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	AUC_{0-24} /Dose
21	8	M	0.1	13.1[a]	0.9	59.8 ± 4.8	598 ± 48
			0.5	67.9[a]	1.0	302 ± 13	605 ± 26
			2	276[a]	1.5	1722 ± 378	861 ± 189
		F	0.1	17.0[a]	0.9	69.2 ± 6.6	692 ± 66
			0.5	70.4[b]	1.7	341 ± 23	683 ± 47
			2	255[a]	1.5	1412 ± 102	706 ± 51
70	57	M	0.1	18.7 ± 3.3[c]	0.5	90.5 ± 4.2	905 ± 42
			0.5	102 ± 11[c]	0.5	534 ± 53	1068 ± 106
			2	288 ± 43[c]	1.5	1674 ± 198	837 ± 99
		F	0.1	34.2 ± 1.6[c]	0.5	91.3 ± 3.9	913 ± 39
			0.5	120 ± 21[c]	0.5	395 ± 18	789 ± 36
			2	525 ± 140[c]	0.5	1501 ± 126	750 ± 63

a: n = 1

b: n = 2

c: n = 3

Mean (± SE) AWD21-360 Pharmacokinetic Parameters							
Postnatal Day	Number of Doses	Sex	Dosage (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	AUC_{0-24} /Dose
21	8	M	0.1	3.10[a]	0.9	3.83 ± 2.00	38.3 ± 20
			0.5	11.7[a]	1.8	102 ± 9.6	204 ± 19
			2	47.5 ± 15.1[b]	7.0	532 ± 86	266 ± 43
		F	0.1	3.54[a]	1.5	5.81 ± 2.82	58.1 ± 28
			0.5	15.0[c]	1.7	113 ± 11	226 ± 21
			2	59.6 ± 16.9[b]	3.0	483 ± 49	242 ± 25
70	57	M	0.1	3.39 ± 0.68[d]	0.5	26.7 ± 4.5	267 ± 45[e]
			0.5	24.6 ± 3.6[d]	3.0	165 ± 27	330 ± 54
			2	78.1 ± 13.2[d]	1.5	738 ± 109	369 ± 55
		F	0.1	7.55 ± 0.79[d]	1.5	48.8 ± 9.2	488 ± 92[e]
			0.5	36.2 ± 1.8[d]	1.5	237 ± 18	473 ± 36[e]
			2	92.1 ± 20.2[d]	7.0	954 ± 133	477 ± 66[e]

a: n = 1
b: n = 5
c: n = 2
d: n = 3
e: significantly different than postnatal day 21 values

2. Retigabine: A 12-Week Oral Gavage Toxicity Study in the Juvenile Rat Followed by a 4-Week Recovery Period (Study No. PR2008-023, conducted by (b) (4) Report dated 3/11/09; GLP)

a. Methods

Retigabine (Lot no. 0005005) was administered orally (gavage) to juvenile rats (S-D CD) at doses of 0 (vehicle: 0.5% [w/v] hydroxypropylmethylcellulose and 0.1% [v/v] Tween 80 in deionized water), 3, 10 or 30 mg/kg (2 ml/kg) for 12 weeks starting on PND 28 followed by a 4 week recovery period (**Table IVF.2.1**). Endpoints included mortality and clinical signs; body weights and food consumption; growth and physical development (including bone parameters); ophthalmology; clinical chemistry (hematology, serum chemistry, immunochemistry and urinalysis parameters); behavioral performance (FOB, motor activity, auditory startle habituation, Cincinnati water maze); mating and reproductive performance (estrous cycles, mating index, fertility index and conception rates, uterine and ovarian parameters evaluated); TK; and gross and microscopic pathology (all tissues from all main study animals (A), liver from recovery subgroup (B)).

Table IVF.2.1 Juvenile rat study design

Dose Group Identification	Dose Level (mg/kg/day)	Number of Animals							
		Main Study Subgroup (A)		Neurobehavioral / Recovery Subgroup (B)		Reproductive Subgroup (C)		Day 111 *pp* Toxicokinetic Subgroup (D)	
		Males	Females	Males	Females	Males	Females	Males	Females
1/ Control	0	10	10	10	10	15	15	4	4
2/ Retigabine	3	10	10	10	10	15	15	10	10
3/ Retigabine	10	10	10	10	10	15	15	10	10
4/ Retigabine	30	10	10	10	10	15	15	10	10

Dose Group Identification	Dose Level (mg/kg/day)	Number of Animals	
		Day 28 *pp* Toxicokinetic Subgroup (E)	
		Males	Females
1/ Control	0	4	4
2/ Retigabine	3	15	15
3/ Retigabine	10	15	15
4/ Retigabine	30	15	15

pp = post partum

Doses were based on the results of a range-finding study in juvenile rats (Ref. No. PR2007-022, conducted by [redacted](b) (4)) in which retigabine was administered by oral gavage to groups of weanling rats at two ages (PNDs 21 and 28) at dose levels of 3, 10, 30, and 60 mg/kg for up to 14 days (same vehicle and vol as above). For the 21-day old rats, clinical signs (decreased activity, tremors, uncoordination, non-sustained convulsions, hypersensitivity, lack of pinch and righting reflex and lying on side) and mortality (3/10 MHD, all HD) were noted at the two highest doses. There were minimal clinical findings at 10 mg/kg. For the 28-day old rats, clinical signs (decreased activity, tremors, uncoordination, hyperreactivity, lack of pinch and righting reflex and lying on side) and a low incidence of mortality (1/10 males) were noted at 60 mg/kg, and clinical observations and lower body weights and food consumption were noted in females at 30 mg/kg. Clinical signs were less significant at this age than in the 21-day old rats. There were no organ weights or macroscopic changes. Based on these findings the age of initiation of treatment of PND 28 and doses of 3, 10 and 30 mg/kg were chosen for the definitive study.

b. Results

i. Mortality and Clinical signs

There were no T-R deaths. One MD male and 2 MD females had decreased activity and either partly closed eyes, were lying on their side or their head was shaking following treatment on PND 28, 29 or 31. Transient effects were noted between PNDs 28 and 31 in the majority of HD males and females, and consisted mainly of decreased activity, tremors in a few (3), and occasionally erected fur, partly closed eyes, shallow breathing, head shaking, abnormal gait and/or animal lying on side. One HD female also had non-sustained convulsions and hypersensitivity following the first dose. There were no significant T-R observations during the remainder of the study.

ii. Body weight and Physical development

Although BWs were slightly lower in HD males and females, there were no SS effects. Crown-to-rump length values were unaffected by treatment. Days of attainment of landmarks of sexual maturation (vaginal opening, preputial separation) were comparable among groups.

iii. Ophthalmoscopic examination

There were no T-R effects.

iv. Behavioral evaluations

There were no clear or SS effects of treatment when subgroup B animals were tested in an FOB, for locomotor activity, or for startle habituation during treatment (PNWs 6 and 15 before daily dosing) and after recovery (PNW 19). However, locomotor activity was generally D-D increased in males at the end of treatment and after recovery (**Table IVF.2.2**) and startle response appeared to be D-D increased in males tested at PNW 19 (**Table IVF.2.3**). There were also no clear T-R effects on Cincinnati water maze assessments conducted in subgroup A during treatment (PNW 13/14 before daily dosing) and in subgroup B animals during PNW 18/19. It should be noted that group sizes (10/sex/group) are at the lower limit of what is generally accepted as adequate for neurobehavioral evaluations.

v. Reproductive assessment (subgroup C)

There was no effect of treatment on estrous cycles. When treated animals were mated on approximately PND 104, the mating index was decreased at the LD and HD and the fertility index and conception rate were decreased at all doses (**Table IVF.2.4**). In pregnant females, preimplantation loss was increased at all doses (**Table IVF.2.5**).

vi. Clinical Pathology

A tendency for D-D increased direct and decreased indirect bilirubin was noted primarily at the end of treatment in both sexes (SS at HD); total bilirubin was unaffected (**Table IVF.2.6**). Urea levels were D-D increased in males and females both at the end of treatment and after recovery (SS in HD recovery females; (**Table IVF.2.7**). There were no clear T-R effects on hematological, urinalyis, or immunochemistry (T4, T3, or TSH) parameters at the end of treatment or after recovery.

vii. Necropsy

Liver weights were increased (~10%; SS) at the HD in both sexes at the end of the treatment period. The liver weight change correlated with the centrilobular hypertrophy seen microscopically. Liver weight was not increased after the recovery period. Absolute urinary bladder weights were increased in HD males at the end of treatment. However, there was no effect on relative weights and no histopathology correlate. There were no T-R organ weight changes in rats from the recovery (B) or reproductive (C) subgroups.

There were no T-R femoral or tibial length and width changes in rats from the main and recovery groups.

There were no T-R changes in brain weight, length, or width. There were no T-R macroscopic observations.

T-R microscopic observations at the end of treatment were limited to minimal centrilobular hypertrophy in MD and HD males and HD females (**Table IVF.2.8**). These were no longer present at the end of the 4-week recovery period.

Neuropathology sections from the high dose and control animals were processed but no neuropathological evaluation was conducted.

viii. Plasma drug levels (**Table IVF.2.9**)

T1/2 values for retigabine ranged from 1.8 to 5.5 hours. The Tmax of the metabolite appeared between 4 and 24 hours post administration of the test article and the t1/2 of the metabolite ranged from 4.5 and 7.5 hours. Exposure (AUC) increased approximately dose-proportionally for all groups. The dose proportionality of the N-acetyl metabolite of retigabine (NAMR) exhibited a similar trend, with approximately dose proportional increases in exposure with increasing dose. Retigabine exposure on PND 111 was 2-4-fold greater than on PND 28.

c. Conclusions

Oral administration of retigabine to juvenile rats at doses of 0, 3, 10 and 30 mg/kg for 12 weeks beginning on PND 28 resulted in transient clinical signs (MD, HD), clinical chemistry evidence of altered renal and hepatic function (HD), possible behavioral abnormalities (increased activity and startle response, D-D tendency at all doses), impaired reproductive function (decreased fertility and increased preimplantation loss, all doses), and increased liver weight and hepatocellular centrilobular hypertrophy (MD, HD).

Table IVF.2.2 Group Mean Activity Counts

Males
Week 15 Post Partum

Interval Number	Statistic	Group 1 Control	Group 2 Retigabine 3 mg/kg/day	Group 3 Retigabine 10 mg/kg/day	Group 4 Retigabine 30 mg/kg/day
1	Mean	110.6	124.7	122.7	133.0
	SD	41.0	20.6	13.9	25.8
	N	10	10	10	10
2	Mean	80.1	77.1	84.7	92.9
	SD	33.9	23.5	17.0	16.5
	N	10	10	10	10
3	Mean	68.5	68.5	71.3	74.1
	SD	29.6	22.4	20.5	25.8
	N	10	10	10	10
4	Mean	43.7	41.6	62.3	57.7
	SD	25.9	23.0	17.2	27.7
	N	10	10	10	10
5	Mean	27.0	43.1	51.8	40.9
	SD	25.1	22.9	22.0	26.8
	N	10	10	10	10
6	Mean	30.7	37.1	35.3	41.3
	SD	21.4	16.8	23.8	18.8
	N	10	10	10	10
Total	Mean	360.6	392.1	428.1	439.9
	SD	145.5	93.9	86.8	87.4
	N	10	10	10	10
Linear Time Contrast	Mean	-69.8	-67.8	-65.1	-75.4
	SD	30.2	20.3	14.5	25.2
	N	10	10	10	10

Significantly different from control group (group 1) value: A - P <= 0.05 B - P <= 0.01 C - P <= 0.001 (Dunnett)

Males
Week 19 Post Partum

Interval Number	Statistic	Group 1 Control	Group 2 Retigabine 3 mg/kg/day	Group 3 Retigabine 10 mg/kg/day	Group 4 Retigabine 30 mg/kg/day
1	Mean	108.3	105.7	109.5	116.9
	SD	29.6	36.3	25.8	18.1
	N	10	10	10	10
2	Mean	61.0	71.3	83.5	79.1
	SD	21.4	19.4	16.1	25.1
	N	10	10	10	10
3	Mean	43.9	37.9	57.5	62.9
	SD	29.7	13.3	27.1	39.7
	N	10	10	10	10
4	Mean	18.4	36.6	40.0	53.1
	SD	12.1	15.8	19.2	24.0
	N	10	10	10	10
5	Mean	20.4	35.8	43.3	49.3
	SD	25.0	26.4	23.3	29.9
	N	10	10	10	10
6	Mean	28.4	23.2	27.3	41.8
	SD	16.7	23.1	16.8	22.4
	N	10	10	10	10
Total	Mean	280.4	310.5	361.1	403.1
	SD	90.7	82.0	98.9	129.2
	N	10	10	10	10
Linear Time Contrast	Mean	-65.4	-62.2	-65.6	-56.7
	SD	28.8	36.2	16.8	19.6
	N	10	10	10	10

Significantly different from control group (group 1) value: A - P <= 0.05 B - P <= 0.01 C - P <= 0.001 (Dunnett)

Table IVF.2.3 Group Mean Startle Habituation Data

Males
Week 19 Post Partum
Maximum Startle (voltage)

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	Trial 1-10	11-20	21-30	31-40	41-50	Mean Level	Linear Time Contrast
1	Mean	614.92	458.62	347.26	372.61	298.21	418.32	-719.43
	SD	465.90	380.37	280.81	278.12	209.13	297.72	841.15
	N	10	10	10	10	10	10	10
2	Mean	640.29	319.48	316.56	397.76	281.15	391.05	-640.00
	SD	256.62	151.65	122.26	246.94	162.91	120.01	781.78
	N	10	10	10	10	10	10	10
3	Mean	670.82	568.34	578.64	440.80	406.03	532.93	-657.12
	SD	450.16	421.00	479.90	365.41	302.84	369.52	921.04
	N	10	10	10	10	10	10	10
4	Mean	863.89	390.12	423.76	480.19	359.90	503.57	-917.91
	SD	634.96	121.03	254.65	638.76	392.94	382.22	387.02
	N	10	10	10	10	10	10	10

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Dunnett)

Table IVF.2.4 Group Mean Parental Performance

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
1	15	15	15	4.1 2.7 (N = 15)	14	100.0	93.3	93.3
2	15	15	12	3.1 2.0 (N = 12)	11	80.0	73.3	91.7
3	15	15	15	2.9 1.9 (N = 15)	12	100.0	80.0	80.0
4	15	15	12	3.0 1.4 (N = 12)	10	80.0	66.7	83.3

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon - day to mating only)
Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ *** - $P \leq 0.001$ (Fisher's)

Table IVF.2.5 Group Mean Uterine Finding

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Number of Live Embryos	Number of Dead Embryos
1	Mean	17.6	17.1	15.9	0.0
	SD	2.1	2.2	2.5	0.0
	N	14	14	14	14
2	Mean	17.4	16.0	15.0	0.2
	SD	2.1	3.8	4.0	0.4
	N	11	11	11	11
3	Mean	18.3	16.6	15.7	0.3
	SD	1.7	3.1	2.8	0.5
	N	12	12	12	12
4	Mean	16.6	15.2	14.6	0.1
	SD	5.4	5.8	5.7	0.3
	N	10	10	10	10

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	Number of Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss %	Post Implantation Loss %
1	Mean	1.2	1.2	2.46	7.23
	SD	1.1	1.1	4.34	6.77
	N	14	14	14	14
2	Mean	0.8	1.0	9.12	7.14
	SD	1.0	1.0	15.94	6.89
	N	11	11	11	11
3	Mean	0.7	0.9	9.23	5.12
	SD	0.9	1.0	14.78	5.55
	N	12	12	12	12
4	Mean	0.5	0.6	13.63	3.84
	SD	0.5	0.5	22.10	3.57
	N	10	10	10	10

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Table IVF.2.6 Group Mean Clinical Biochemical Analyses

End of Treatment
Day 112 Post Partum
Subgroup A - Males

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	AST U/L	ALT U/L	ALP U/L	TBIL mg/dL	DBIL mg/dL	IBIL mg/dL	UREA mg/dL	CREAT mg/dL
1	Mean	82.1	28.7	84.8	0.103	0.033	0.077	13.06	0.36
	SD	18.4	6.4	21.7	0.028	0.005	0.034	2.14	0.07
	N	10	10	10	10	8	10	10	10
2	Mean	94.8	41.3	82.6	0.094	0.038	0.060	12.68	0.31
	SD	38.0	43.7	17.7	0.026	0.007	0.029	1.72	0.03
	N	10	10	10	10	9	10	10	10
3	Mean	110.1	43.1	91.9	0.092	0.043	0.061	13.87	0.34
	SD	72.0	41.1	22.9	0.047	0.013	0.044	2.24	0.05
	N	10	10	10	10	8	10	10	10
4	Mean	86.2	41.3	97.9	0.091	0.047 B	0.044	14.58	0.37
	SD	30.1	21.0	21.5	0.026	0.012	0.022	1.47	0.05
	N	10	10	10	10	10	10	10	10

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

End of Treatment
Day 112 Post Partum
Subgroup A - Females

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	AST U/L	ALT U/L	ALP U/L	TBIL mg/dL	DBIL mg/dL	IBIL mg/dL	UREA mg/dL	CREAT mg/dL
1	Mean	75.2	24.2	51.3	0.097	0.037	0.075	12.22	0.35
	SD	17.1	4.8	13.4	0.019	0.008	0.021	2.29	0.05
	N	10	10	10	10	6	10	10	10
2	Mean	83.9	32.4	52.8	0.092	0.042	0.067	13.15	0.36
	SD	25.0	13.1	12.1	0.037	0.015	0.029	1.84	0.05
	N	10	10	10	10	6	10	10	10
3	Mean	72.4	23.8	50.1	0.094	0.039	0.055	12.44	0.40
	SD	7.5	2.9	13.9	0.022	0.009	0.018	2.52	0.05
	N	10	10	10	10	10	10	10	10
4	Mean	87.0	35.0	66.5	0.088	0.063 B	0.044 A	12.62	0.37
	SD	56.3	27.5	21.6	0.020	0.018	0.026	2.82	0.05
	N	10	10	10	10	7	10	10	10

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table IVF.2.7 Group Mean Clinical Biochemical Analyses

End of Recovery
Day 140 Post Partum
Subgroup B - Males

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	AST U/L	ALT U/L	ALP U/L	TBIL mg/dL	DBIL mg/dL	IBIL mg/dL	UREA mg/dL	CREAT mg/dL
1	Mean	84.5	29.6	70.3	0.120	0.033	0.107	12.20	0.34
	SD	11.3	3.7	15.2	0.030	0.005	0.026	1.99	0.05
	N	10	10	10	10	4	10	10	10
2	Mean	91.7	29.9	65.3	0.106	0.032	0.087	12.88	0.36
	SD	21.0	5.3	18.8	0.026	0.004	0.030	2.45	0.05
	N	10	10	10	10	6	10	10	10
3	Mean	93.0	35.3	82.3	0.123	0.035	0.109	12.94	0.35
	SD	31.0	22.0	22.4	0.037	0.006	0.038	1.49	0.07
	N	10	10	10	10	4	10	10	10
4	Mean	82.0	27.0	70.4	0.105	0.035	0.091	14.13	0.35
	SD	14.1	4.5	25.3	0.029	0.006	0.035	2.15	0.05
	N	10	10	10	10	4	10	10	10

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

End of Recovery
Day 140 Post Partum
Subgroup B - Females

Group 1 - Control
Group 2 - Retigabine 3 mg/kg/day
Group 3 - Retigabine 10 mg/kg/day
Group 4 - Retigabine 30 mg/kg/day

Group	Summary Information	AST U/L	ALT U/L	ALP U/L	TBIL mg/dL	DBIL mg/dL	IBIL mg/dL	UREA mg/dL	CREAT mg/dL
1	Mean	90.5	30.9	46.8	0.145	0.036	0.120	11.76	0.40
	SD	19.9	8.9	11.4	0.025	0.008	0.022	1.10	0.05
	N	10	10	10	10	7	10	10	10
2	Mean	91.7	28.9	45.9	0.132	0.037	0.106	12.94	0.40
	SD	23.1	6.1	19.6	0.035	0.008	0.021	1.70	0.07
	N	10	10	10	10	7	10	10	10
3	Mean	92.4	32.3	44.8	0.140	0.033	0.114	13.11	0.38
	SD	20.4	15.0	15.9	0.027	0.005	0.031	2.33	0.04
	N	10	10	10	10	8	10	10	10
4	Mean	90.5	28.6	61.6	0.132	0.035	0.111	15.04 C	0.40
	SD	18.8	7.6	19.9	0.025	0.008	0.020	1.89	0.05
	N	10	10	10	10	6	10	10	10

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table IVF.2.8 Incidence of Animals with Microscopic Findings - Treatment Phase

Subgroup A

MALE

DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	10	10	10
LACRIMAL GLAND	EXAMIN:	10	10	10	10
LIVER	EXAMIN:	10	10	10	10
- Hypertrophy: centrilobular		-	-	2	6
- Infiltration: mononuclear cell		2	2	-	-
- Necrosis		1	1	1	1
- Fibrosis: peribiliary		-	-	1	1
- Hyperplasia: bile duct		-	-	1	-
- Tension lipidosis		1	-	-	3
- Inflammation: granulomatous		1	-	-	-

FEMALE

DOSE GROUP		1	2	3	4
NUMBER OF ANIMALS EXAMINED		10	10	10	10
LACRIMAL GLAND	EXAMIN:	10	10	10	10
LIVER	EXAMIN:	10	10	10	10
- Hypertrophy: centrilobular		-	-	-	6
- Infiltration: mononuclear cell		3	3	1	2
- Necrosis		-	-	-	1
- Fibrosis: peribiliary		1	1	-	-
- Tension lipidosis		1	-	-	1
LUNG	EXAMIN:	10	10	10	10
- Macrophage accumulation		2	1	-	1
- Infiltration: mixed cell		-	1	1	-

Table IVF.2.9 Toxicokinetics of retigabine and NAMR in juvenile rat study

Sex	Dose Level (mg/kg/day)	Day 28 *post partum*		Day 111 *post partum*	
		C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{(0\text{-tlast})}$ (ng•h/mL)
RTG					
Males	3	274	965	282	1681
	10	676	2855	918	10059
	30	823	5364	1920	22684
Females	3	262	869	310	1817
	10	689	2984	832	8415
	30	1333	12658	1535	28719
NAMR					
Males	3	85.2	532	141	810
	10	231	1460	427	5888
	30	379	5698	668	12747
Females	3	89.3	554	183	2586
	10	268	1646	522	8132
	30	730	9647	981	21498

V. Summary and Evaluation

Pharmacology

Retigabine [D23129; N-(2-amino-4-(4-fluorobenzylamino)-phenyl)carbamic acid ethyl ester] was shown to have a broad-spectrum of activity in animal models of epilepsy based on electrically- and chemically-induced seizures (**Tables IIA.1-2**). Initial in vitro MOA studies suggested that retigabine possessed activity that distinguished it from other antiepileptic drugs, eg, its activity in several in vitro models insensitive to typical anticonvulsants, such as recurrent epileptiform discharges induced by combined application of 4-AP and bicuculline and in the low Mg2+ hippocampal slice model. Subsequent work indicated that activation of neuronal KCNQ (Kv7) voltage-activated $K(^{+})$ channels could play a role in retigabine's MOA. The KCNQ proteins make up a sub-group of the voltage-activated potassium channel family consisting of five members (KCNQ1 to 5—also named Kv7.1 to Kv7.5) encoded by single genes. The physiological importance of the KCNQ channel family was suggested by the finding that mutations in four of the five genes (KCNQ1 to 4) are linked to human pathologies; eg, mutations in KCNQ1 are associated with cardiac arrhythmias (long QT syndrome) and deafness, mutations in KCNQ4 are associated with progressive hearing loss, mutations in CNS KCNQ2 and KCNQ3 channels are associated with forms of neonatal epilepsy, and heterozygous knockout mice (KCNQ2 +/−) show increased sensitivity to chemically-induced seizures. Neuronal potassium channels, which activate close to the firing threshold of the action potential, appear to counteract the depolarizing effects of sodium and calcium channels to control neuronal excitability. Members of the KCNQ potassium channel family, primarily KCNQ2 and 3, have been proposed to underlie a current with this function called the M-current, which is considered key in the subthreshold control of firing. Retigabine appears to primarily enhance KCNQ channel activation by inducing a hyperpolarizing shift in the voltage-dependence of activation. Retigabine-treated KCNQ2/KCNQ3 channels also display an increased open probability. Blockade of the M-current results in cell-membrane depolarization as well as increased input resistance and makes the neuron more prone to action potential firing. Retigabine appears to show little selectivity between KCNQ2-5 channels; however, Tatulian et al (J Neurosci 21:5535-5545, 2001) showed that it produced a hyperpolarizing shift of the activation curves for KCNQ currents with differential potencies in the following order: KCNQ3 > KCNQ2/3 > KCNQ2 > KCNQ4, as measured either by the maximum hyperpolarizing shift in the activation curves or by the EC_{50} values (**Table IIA.3**). In this study, retigabine did not enhance cardiac KCNQ1 currents.

The finding that retigabine appears to act exclusively on KCNQ2-5 isoforms without affecting cardiac KCNQ1 channels is considered clinically important in minimizing adverse effects on cardiac function. However, "neuronal" KCNQ channels (KCNQ4 and 5) have recently been found to be expressed in vascular smooth muscle cells where they seem to play a role in controlling arterial tone (Yeung et al, Br J Pharmacol 151:758–770,2007). Flupirtine, a structural analogue of retigabine (approved as an analgesic in Europe), has been reported to lower systolic blood pressure in rats and humans, presumably via relaxation of vascular smooth muscle (Mackie and Byron, Mol Pharmacol 74:1171-79,2008). This effect was demonstrated by the sponsor in safety pharmacology studies of retigabine in dogs and pigs, where it was noted that the decrease in blood pressure was not accompanied by the expected reflex tachycardia which may indicate other nonvascular effects such as on the heart or its nervous system regulation (eg, baroreceptor neurons). A modest decrease in heart rate in response to flupirtine reported by Mackie et al (J Pharmacol Exp Ther 325:475–483,2008), was thought to possibly be a consequence of nonvascular KCNQ channel activation (eg, a reduction of sympathetic ganglionic nerve activity).

A role for KCNQ channels in controlling peripheral smooth muscle contractility appears to extend to the urinary bladder and gallbladder. In isolated rat urinary bladder tissue, retigabine reduced both the contractility and overall tonus of bladder tissue independent of the mode of stimulation, and these effects could be reversed by the KCNQ channel inhibitor XE991 (Rode et al, Eur J Pharmacol 638:121-127,2010). In another study in conscious rats undergoing continuous cystometry (Streng et al, J Urol

172:2054-2058,2004), retigabine decreased baseline and maximal bladder pressures, increased voided and infused volumes, and increased voiding intervals. These effects were blocked by the KNCQ channel antagonist linopirdine. It was suggested that KCNQ channels are potential targets for drugs for micturition control and treatment of detrusor overactivity in humans. These authors pointed out that since GABA seems to exert tonic inhibitory control of micturition centrally, retigabine, which has effects on GABAergic transmission in addition to its potassium channel effects, may have several sites of action influencing micturition. In studies submitted by the sponsor, retigabine effects on smooth muscle contractility in rat urinary bladder strips were not inhibited by glyburide (an inhibitor of ATP-sensitive potassium channels), but were markedly reduced by linopirdine, while the relaxant effect of retigabine on guinea pig gallbladder smooth muscle was only partially reversed by linopirdine (**Figure IIB.1**).

In addition to its anticonvulsant effects, retigabine has been reported to relieve pain-like behaviors (hyperalgesia and allodynia) in animal models of neuropathic pain and reduce unconditioned anxiety-like behaviors when assessed in the mouse marble burying test and zero maze. Both linopirdine and XE-991, which inhibit KCNQ channels in the low micromolar range, are said to possess cognitive enhancing properties, raising the question whether retigabine might impair cognition (Piccinin et al, J Neurophysiol 95: 3105–3112, 2006). Although retigabine has been reported to lack major effects on cognition at doses that produce anxiolytic behavior in vivo (Korsgaard et al., J Pharmacol Exp Ther 314: 282–292,2005), no studies to directly assess cognitive effects were conducted by the sponsor. The neurobehavioral testing conducted in the developmental toxicity studies was mostly sub-optimal (passive avoidance and M-maze) except in the most recent juvenile rat study with dosing starting on PND 28. Common treatment-emergent adverse events in clinical trials included memory difficulty and abnormal thinking.

ADME

PK parameters in the toxicology species are summarized in **Table IIIA.1** and TK data are included in the toxicology section. PK was similar across animal species, with rapid absorption following oral dosing (Tmax ~ 1 hr), good absorption (based on radiolabel studies, but bioavailability only ~70% in rats and ~20% in dog), moderate protein binding (~80%), and relatively short half-life (2 hr in rats, 6-10 hr in dogs). The primary routes of metabolism in animals (as well as humans) involve phase II hepatic acetylation and glucuronidation leading to the formation of NAMR and N-glucuronides of RTG and NAMR (**Table IIIA.3**; **Figures IIIA.1** and **IIIB.1**). NAMR was active but less potent than retigabine in in vitro and in vivo pharmacology studies (1/4 and 1/3 as potent in the mouse and rat MES tests; only slightly less potent effects on cardiac action potentials in vitro). The proportion of NAMR in plasma relative to parent varied (rats>rabbits>mice), but relative plasma exposure was always less than in humans where exposures to NAMR are similar to parent. Because dogs lack the necessary metabolic enzyme, N-acetyl transferase, NAMR was not detected in dog (or monkey) plasma. RTG and NAMR represented only a small fraction of the plasma circulating [14C]-RTG derived material in humans administered an oral dose of 200 mg; the contribution of RTG and NAMR to the AUC of total radioactivity was in the range of 3.3 to 8.4% and 3.5 to 6.5%, respectively. According to Ramesh Sood, a chemist in ONDQA, the metabolite NAMR is likely formed from retigabine through hydrolysis of the carbamate to (b) (4) the Ames positive synthetic impurity, followed by N-acetylation. Hence, (b) (4) would be a (b) (4) and the extent of exposure to (b) (4) would depend upon how quickly (b) (4) is acetylated. However, in rats and dogs, (b) (4) was not quantifiable in plasma following administration of RTG. (b) (4) Overall, the metabolism of retigabine is dominated by *N*-glucuronidation. All evaluated species formed N-glucuronides of RTG, although there was some evidence of species specificity for the specific structural location for glucuronidation. In the plasma of rats, dogs, and humans, the retigabine N2-glucuronide, in which the site of glucuronidation is the primary amino group in position 2 (**Figure IIIB.1;** M1), predominates over the N4-glucuronide (glucuronidation of the secondary amino group in position 4; M3). Thus, the N2-glucuronide represents the major retigabine metabolite in the plasma of rats, dogs, and humans. Urine metabolite profiles varied across species, with rats demonstrating a complex profile of over 20 metabolites/degradation products with dogs excreting only RTG and few metabolites. In contrast, the fecal metabolite profile in rats showed only one distinct

metabolite. In dog feces parent compound and two metabolites distinct from those in urine were observed. Following (oral or iv) administration to rats and dogs, RTG and its metabolites are excreted in both feces and urine, with the feces generally accounting for approximately 2/3 of the eliminated dose and urine accounting for approximately 1/3 (urinary excretion of radioactivity accounted for ~40% of the dose in male mice, ~30% in male and female rats, ~20 % in male dogs and ~50% in female dogs). Elimination patterns were similar following both oral and iv dosing. The extensive elimination of drug via the feces following iv dosing is consistent with the demonstration that large fractions of a RTG dose are excreted in bile (~60% of the dose in rats). Radioactivity was eliminated within 48 hours of dosing.

Toxicology

CNS clinical signs were notable and appeared to be dose-limiting in toxicity studies with retigabine in mice, rats and dogs; these included hypoactivity, decreased muscle tone, ataxia, prostration, tremors, and convulsions (≥46.4 mg/kg in mice and rats). Death of unknown cause but assumed to be secondary to acute neurotoxicity was seen in rodents. Rats appeared more sensitive to the acute CNS effects of RTG than mice, the maximum non-lethal oral doses were approximately 75 mg/kg for the rat and 215 mg/kg for the mouse (**Table IVA.1.1**), but this reflected the higher plasma levels for a given dose in rats. In the dose range-finding study for the neonatal mouse carcinogenicity study, T-R clinical signs included convulsion-myoclonus, decreased motor activity, ataxia, low carriage, hypersensitivity at doses ≥ 8 mg/kg (dosing on PND 8 and 15) indicating as stated in the study report "that the toxicity of retigabine is clearly higher in young rodents (neonatal and/or juvenile) in comparison with adult rodents." This was supported by the results of the juvenile rat studies, where CNS signs including ataxia, immobility, and myoclonus were seen at oral doses as low as 6 mg/kg. In an oral gavage dose range-finding study in monkeys (2/sex/grp), in which doses of up to 180 mg/kg were given for up to 10 days, CNS signs of ataxia and decreased motor activity were seen, and after a single administration of the HD, 1/4 monkeys became immobile ~18 hours post-dosing and remained sedated (immobile, which improved to decreased motor activity and ataxia) for 2 days. No further discussion of this episode was provided.

In the repeat-dose studies, urinary bladder toxicity was seen in all species in which it was examined (not examined in monkeys). The findings were attributed to altered urinary bladder peristalsis (hypotonic urinary bladder detruser muscle) leading to urinary bladder distention, increased hydrostatic pressure, and uremia. This effect has also been reported functionally in clinical trials. In mice, findings included distension, intraluminal debris/uroliths and cystitis characterized by hemorrhage with neutrophils in the wall of the bladder, edema, necrosis, epithelial hyperplasia, erosion, ulceration, and serosal fibrosis. Presumed secondary effects in the kidneys included pelvic dilatation, papillary necrosis, tubular degeneration/regeneration, and tubular dilation. Effects on the urinary bladder and kidney were seen at doses as low as 50 mg/kg (AUC ~5 ug.h/ml) in repeat-dose studies in adult mice (13-week dietary study in CD-1; **Table IVB.1.2**). In the dose range-finding study for the neonatal mouse carcinogenicity study, urinary bladder dilatation and attenuated urinary bladder epithelium were seen at ≥ 2 mg/kg.

In rat repeat-dose studies, macroscopic changes in the urinary bladder included thickening of the wall, red contents and/or white areas of the bladder wall. Macroscopic findings included luminal ectasia, congestion of submucosal vessels, ulcerations, diffuse hyperplasia of the bladder mucosa, and uroliths. Urinary bladder histopathology was first notably increased at doses of 50 (AUC~20 ug.h/ml in 2-yr carcinogenicity study) to 68.1 mg/kg (AUC~35-45 ug.h/ml in the 13-week oral gavage study), but distended bladder was D-D increased at all doses (≥5 mg/kg, AUC~3-4 ug.h/ml) in the rat carcinogenicity study and an increased incidence of urinary bladder dilatation occurred at 0.6 mg/kg in a juvenile rat study. Because similar findings (dilated urinary bladder) were found at a lower incidence in controls in the juvenile rat study, the sponsor contended that the vehicle (propylene glycol) or the dose volume (10 ml/kg) may have contributed to the mortality. But it is more likely that this reflects an age-related increased sensitivity, possibly due to urogenital system immaturity at the time dosing commenced in this study (PND 7) since the bladder effects were not seen when dosing started postweaning (PND 28) in another juvenile rat study. In rat studies of NAMR, distention of the urinary bladder was observed macroscopically at ≥300 mg/kg.

Urinary bladder pathology was also seen in dogs, and included degeneration and necrosis of the urinary bladder smooth muscle (detrusor myopathy) at ≥45 mg/kg in the 28-day study, and mononuclear infiltration of the urinary bladder, focal hemorrhage, and proliferation of bladder smooth muscle at 38.3 mg/kg/day (AUC~10 ug.h/ml) in the 13 week study. In the dog study of NAMR, urinary bladder thickening and/or inflammation was observed in 2 dogs at ≥100 mg/kg. The retigabine effects on the bladder and kidney were associated with changes in clinical chemistry parameters (creatinine and BUN) in some studies, but generally only at doses producing significant bladder and kidney lesions. Concerns about the effects of retigabine on the urinary bladder have been born out in clinical trials, where this has been a major safety focus.

A possibly related finding of retigabine on the gallbladder smooth muscle was seen in dogs, where liver damage was found in the vicinity of the gallbladder in several studies (28-day, 13-week, 1-year). In 28 day studies, the findings were described as focal necrosis, fibrosis/fibroplasia, mononuclear inflammatory cell infiltrate(s), pigment deposition, and an increased incidence and grade of acute hemorrhage in liver tissue directly underlying the gallbladder. Mechanistic studies (in vitro pathophysiology study of retigabine on gall bladder smooth muscle strip contractility; assessment of gallbladder volume by ultrasound in 28-day study) indicated that these localized lesions developed as a result of mechanical compression of those regions of the liver that were in immediate proximity to the gallbladder. Pressure necrosis of the liver was thought to result secondarily from gallbladder enlargement that occurred as a result of retigabine-mediated relaxation of the gallbladder smooth muscle. Similar hepatobiliary findings did not occur in mice or rats, where only the typical dose-dependent increases in liver weight and centrilobular hepatocellular hypertrophy were observed. Significant hepatotoxicity has not been observed in clinical trials.

An unusual toxicity was seen in dog studies of NAMR, which were conducted in response to Division concerns about coverage of the major human metabolite, which is not formed in dogs. In a 13-week study in which NAMR (HD lowered from 600 to 100 mg/kg) was directly administered to dogs (**Table IVB.3.8**), the most prominent T-R effect appeared to involve an effect on neutrophils, which manifested as variable changes in circulating neutrophil count (mainly decreased, but sometimes increased; study report: “the variable pattern of neutrophil responses was atypical and suggested the possibility of effects on neutrophil function and/or myelopoiesis, however, the mechanism was not determined”); the proliferation of immature cells of myeloid lineage in bone marrow, liver and/or spleen; and various inflammatory lesions, which were considered secondary to the immature myeloid cell hyperplasia (study report: “although the mechanism is not apparent, the proliferation of immature myeloid cells appears to have increased susceptibility to secondary infections”). There was no no-effect dose in the initial study, so a second 13-week study was performed at lower doses (HD of 30 mg/kg administered either QD or TID). Results were generally consistent with those in the original study, with decreased neutrophil counts, myelotoxicity (bone marrow myeloid hyperplasia), and multi-organ inflammation that included brain and heart involvement observed histopathologically, primarily at the HD (1 MD affected). All microscopic changes were reversible following the 8-week recovery period. Even if the NOAEL in dogs is considered to be the MD of 10 mg/kg (AUC0-24h = 4637 and 8597 ng.h/ml, in males and females, respectively), plasma levels were below those expected clinically. However, there were no comparable T-R effects on hematology or immunologic parameters (including peripheral blood leukocyte analysis and assessment of natural killer cell activity) in a 13-week rat study or NAMR at doses of up to 120 mg/kg/day in males (AUC0-24h = 155734 ng.h/ml) or 100 mg/kg/day in females (AUC0-24h = 167797 ng.h/ml). Although the sponsor offered no explanation for the effects seen in dogs, this appears to be a species-specific immunologic response to a foreign metabolite. No related hematological/immunological changes have been apparent in clinical trials despite this being highlighted as a concern early in development.

[For comparative purposes, human AUC(0-24) RTG values at 600 mg and 1200 mg RTG are ~14400 and ~30590 ng.h/ml respectively, and human AUC(0-24) NAMR values at 600 mg and 1200 mg RTG are ~12700 and~22970 ng.h/ml, respectively.]

Genetic Toxicology and Carcinogenicity

Genetic toxicity testing was first conducted with drug substance synthesized using the early (b) (4) (b) (4) As discussed in the original IND review (dated 9/11/97), genotoxicity testing of the salt indicated clear genotoxic potential (positive in Ames, positive for chromosomal aberrations in human lymphocytes with and without activation) while the free base (D-23129) appeared to be less genotoxic *in vitro*, which the sponsor attributed to an improved impurity profile. In studies conducted with the free base form, retigabine was mostly negative (intermittently weakly positive or equivocal) in the Ames test, negative in the CHO hprt gene mutation assay, positive in the human lymphocyte chromosomal aberration assay, and negative in the mouse micronucleus test. The major circulating metabolite of retigabine, NAMR, was negative in the Ames test but positive in the CHO chromosomal aberration test. Three strongly Ames positive (b) (4) containing impurities were identified (**Figure IVC.1b.1**), and all but one ((b) (4) are being controlled at the FDA limit for genotoxic impurities (b) (4) /day for all related genotoxic impurities combined; equivalent to (b) (4) or (b) (4) total at MHD; 2008 FDA draft Guidance on Genotoxic and Carcinogenic Impurities in Drug Substances and Products). The Ames positive impurity (b) (4) was considered negative when tested in a combined rat micronucleus and Comet assay in vivo. Based on this result, the sponsor concluded that (b) (4) is not an in vivo mutagen and has proposed a limit of (b) (4) (ie, < (b) (4)

Thus, while the positive responses were relatively weak, retigabine was not consistently clearly negative in the Ames test, and the Division's response to an Oct 11,1999 submission regarding the mutagenicity of retigabine is still valid:

> You have identified several highly mutagenic (b) (4) /impurities that could account for the positive mutagenic response obtained with D-20443 synthesized by the early (b) (4) process (even though it was not possible to detect any such impurities in the final product). Since these impurities should not be formed during synthesis of retigabine by the new (b) (4) process, there was some basis for expecting that the new batches would be free of mutagenic activity. However, Ames assays conducted with these new batches were not uniformly negative. Given the results with D-20443, the inability to isolate the putative mutagenic impurity(ies), and the structural similarity between some (b) (4) in the (b) (4) process and those in the new (b) (4) process (i.e., (b) (4)), the mixed results with the new batches must be viewed more cautiously than they might otherwise have been. Thus, based on the totality of the data provided, retigabine is still considered to have mutagenic (as well as clastogenic) potential and should be identified accordingly. The weight that this finding is given will depend in part on the outcome of the rodent carcinogenicity studies.

It appears that the positive Ames test results with retigabine were at least in part due to the presence of mutagenic impurites. The elimination or lowering of identified or potential retigabine impurities by modification of (b) (4) resulted in progressively "less positive" Ames test results until the negative or marginal responses seen with current clinical batches were attained. The tester strains that showed the positive results with retigabine were the same as those that were generally most responsive to the mutagenic impurities, ie, TA98 and TA1538 and/or TA1537, which all detect frameshift mutations: TA1537 has a frameshift mutation (deletion of one nucleotide), TA 1538 has a different frameshift mutation (insertion of one nucleotide) in the same gene that is mutated in TA 1537, and TA 98 is similar to 1538 but is supposed to detect more mutagens than 1538. (b) (4) are well-known frameshift mutagens (b) (4) , some of which (including (b) (4) have been shown to be carcinogenic in rodents ((b) (4) As with (b) (4) the greatest increases in revertant frequencies with retigabine and (b) (4) were seen with metabolic activation (although concentrations of (b) (4) tested without S9 were lower due to cytotoxicity). However, structural features in impurities (b) (4) and (b) (4) possibly the

presence of a nitro group, appeared to confer added mutagenic activity, since large increases in revertant frequencies were seen both with and without S9 in Ames tests of these compounds (**Tables IVC.1b.3-7**).

Because of the apparent difficulty in reducing the levels of (b) (4) to those considered acceptable for genotoxic impurities (levels in the most recent clinical batches ranged from (b) (4) additional testing was performed in an effort to mitigate concern. To this end, (b) (4) was tested in a combination in vivo micronucleus/Comet assay for its potential to induce structural chromosome damage and/or aneuploidy in polychromatic erythrocytes from bone marrow and DNA strand breaks (measured as comets) in the liver of rats (S-D). The impurity was considered negative in both assays by the sponsor. However, in the comet assay there was a small non-D-D increase in the comet response in the liver of (b) (4) treated rats based on means; and group tail moment and % tail DNA measurements were increased in individuals from treated groups (**Table IVC.3b.2**). These increases were dismissed by the sponsor because they were considered non-D-R and fell within the historical control ranges.

While the Comet assay is widely used for examining the potential for DNA damage (single strand breakage [frank strand breaks and incomplete excision repair sites], double strand breaks, and alkali-labile sites) and carcinogenesis, and appeared to be adequately conducted in this case (based on published recommendations; eg, Hartmann et al, Mutagenesis,18:45-51,2003), there is some debate about whether the assay is adequately validated for routine regulatory use. There are many biological and technical variables affecting Comet assay outcome; for example, compound-specific variables such as latency to effect and target tissue that may not be adequately accounted for in a single assay, and technical performance and assay parameters such as those related to tissue processing that have not been adequately standardized. Three sampling times (3, 24 and 48 hr) and one target tissue (liver) and species (rat) were used in the evaluation of (b) (4) Finally, though, as acknowledged by the sponsor, the Comet assay relies on a surrogate endpoint (ie, DNA damage) for detection of mutagens, since there are no in vivo tests that can measure mutation in endogenous genes. The Comet assay is considered an indicator test for detecting premutagenic lesions. So relevant questions are: what is the appropriate follow-up procedure on an Ames test positive compound? and, can an Ames test positive be "de-risked" with negative results from in vivo mammalian cell testing in the comet assay?

According to a very recent article discussing the appropriate follow-up actions from positive results of in vitro genetic toxicity testing (Dearfield et al, Environmental and Molecular Mutagenesis, 2010, online in advance of print), the Comet assay is one of several tests that may be appropriate for further evaluation of DNA reactivity in vivo in the case of a positive in vitro gene mutation assay, with the proviso, as indicated by the asterisk in Table III of the article, that there are not yet enough data available for the Comet assay, which is considered a Category #2 assay by the HESI IVGT review group (less well-characterized, less historical data than Category 1 assays like the Ames). Limitations of the Comet assay discussed in this paper include: may be unable to detect mutagens that do not produce strand breaks or alkali-labile lesions, interlaboratory variability, and lack of guidelines. The article also states that "a mutagenic response in the bacterial reverse mutation assay represents a significant hurdle to overcome in the safety assessment process since bacterial mutagens are generally regarded as DNA-reactive and the predictivity of the Ames test for rodent carcinogenicity is high." Witte et al (Tox Sci 97:21-26,2007) contend that since no combination of genetic toxicity assays provided greater predictivity of rodent carcinogenesis than the bacterial mutagenicity test alone, "positive results from a bacterial test system cannot be overruled by negative results from mammalian systems." Generally, 60% concordance and 80% positive predictivity are reported for the Ames test (Zeiger, Reg Toxicol Pharmacol 28, 85–95,1998; Snyder and Green, Mutat Res 488:151-169, 2001). But little or no Comet assay data were included in these or more recent surveys of genotoxicity and carcinogenicity test result correspondence (b) (4)

In the most extensive evaluation of the Comet assay, by Sasaki et al (Crit Rev Toxicol 30:629-799,2000*),* in vivo comet assay test results in 8 organs of the mouse for 208 chemicals selected from the IARC and NTP carcinogenicity databases indicated an impressive positive response ratio (110/117) for rodent

genotoxic (Ames positive) carcinogens and a high negative response ratio (6/30) for rodent noncarcinogens. Some of the 7 Ames positive carcinogens as well as many of the Ames positive non-carcinogens that tested negative in the Comet assay were (b) (4) (see also Sasaki et al, Mutat Res 440:1-18,1999). Based on this data, it was suggested that the comet assay provides a very good assessment of potential carcinogenicity. However, a recent publication (Kirkland and Speit, Mutat Res 654:114-132,2008) indicates that "there is still a need for standardization and validation of the Comet assay," and cautions that "there is a need to check the accuracy and reproducibility of the results so far obtained, as many of the published in vivo Comet assay results have all been generated by one group (Sasaki et al)."

Negative in vivo genotoxicity tests do not automatically negate clear positive in vitro tests (Elespuru et al., Toxicol Sci 109: 172-179,2009). Since the two assays evaluate different genotoxicity endpoints and there are inadequate data to determine the predictive value of the Comet assay relative to the Ames test; and significant questions remain about the reliability and reproducibility as well as the validity of the Comet assay (b) (4) the lack of a clear positive response in a single in vivo (Comet) assay does not remove the concern about (b) (4) arising from the clearly positive in vitro mutagenicity (Ames) test results. This is a major obstacle to approval of the NDA.

An earlier effort by the sponsor (IND53,950, SN616) to establish a higher Threshold of Toxicological Concern for (b) (4) based on an SAR assessment (linear extrapolation from data for a known carcinogen with structural similarities to (b) (4) based on the assumption that structurally similar compounds have the same genotoxic/carcinogenic mode of action and molecular target, was considered inadequate. This approach, which relies on several unsupported assumptions (eg, that we have an adequate understanding of the structural features important for mutagenic and carcinogenic activity, that the in vivo biological behavior of the structurally-related compounds would be sufficiently similar, and that the carcinogenicity of the related compound has been adequately tested) has not been accepted as sound from a regulatory standpoint. Numerous attempts to construct structure−activity relationships in order to predict (b) (4) genotoxicity/carcinogenicity have met with limited success due to the complex interrelationships between chemical and biological factors (b) (4)

The carcinogenicity studies of retigabine do not provide reassurance regarding the carcinogenic potential of the impurity of (b) (4) because the level of the impurity in the batch used in the neonatal mouse study and in most (b) (4) of the rat study ((b) (4) in batch 0005005) was lower than the proposed specification limit ((b) (4) or that measured in any clinical batch (range (b) (4) and the highest doses tested in animals ((b) (4) in mice and rats, respectively) were approximately (b) (4) the maximum human dose (MHD) of 1200 mg on a body surface area (mg/m2) basis. As stated in the EMEA Guideline on the Limits of Genotoxic Impurities (2006), "negative carcinogenicity and genotoxicity data with the drug substance containing the impurity at low ppm levels do not provide sufficient assurance for setting acceptable limits for the impurity due to the lack of sensitivity of this testing approach. Even potent mutagens and carcinogens are most likely to remain undetected when tested as part of the drug substance, i.e. at very low exposure levels." Retigabine was negative when tested at doses of up to 50 mg/kg in a 2-year study in rats; plasma exposures for retigabine and NAMR were approximately 2/3 and 1/2, respectively, those expected clinically at the MHD. A conventional carcinogenicity study was not considered feasible in mice due to urinary bladder toxicity and a neonatal mice model was substituted in which animals received only 2 doses (of up to 96 mg/kg), on post-natal days 8 and 15, followed by monitoring for one year (according to ILSI protocol (b) (4) This assay is thought to be sensitive in detecting genotoxic carcinogens. Although the response to retigabine at the doses used was weak, there appeared to be a carcinogenic signal in this assay: a small but D-D increase in the frequency of lung neoplasms was observed in treated mice compared to the water and vehicle (propylene glycol) controls. The percent incidences for combined bronchioalveolar adenoma/carcinoma were 4, 4, 8, and 12% in C (combined), LD, MD, and HD group males (**Table IVD.2.4**). Notably, bronchioloalveolar carcinoma was only seen in HD retigabine males (8%) and positive control mice (4 and 7% in males and females). This incidence is well above the control

incidence of lung carcinoma reported by McClain (1.4% in males; **Table IVD.2.5**). No plasma level data were collected in neonates, but the AUC for retigabine in adult mice at the HD (96 mg/kg) used in the neonatal study was only about 1/6 that in humans at MHD. This result in mice strengthens the concern arising from the genotoxicity data.

Reproductive and Developmental Toxicity

The reproductive and developmental toxicity of retigabine was fairly unremarkable at the doses tested, which were limited by maternal toxicity (mainly acute neurotoxicity) to doses associated with plasma levels of parent and metabolite below those expected clinically. In the initial embryo-fetal study in rats (at doses of up to 46.4 mg/kg), the only effects seen were increases in skeletal variations and incomplete ossification (**Table IVE.6.2**). Because maternal toxicity was seen at the HD, the sponsor was asked to examine higher doses. However, in the repeat study at doses of up to 60 mg/kg, maternal toxicity was excessive at the HD, resulting in deaths and cessation of dosing on GD13, and the only developmental effect was decreased ossification of some skeletal elements at the MD (45 mg/kg). The no-effect dose for embryo-fetal toxicity in rats (21.5 mg/kg/day) was associated with maternal plasma exposures (AUC) to retigabine and NAMR approximately 1/3 and 1/2 those in humans at the MHD (**Table IVE.6.1**). In the initial rabbit embryo-fetal study (doses of up to 26.1 mg/kg), reductions in fetal BW and increased incidences of skeletal variations and retardations were seen at the HD (**Table IVE.7.3**), but the study was not considered adequate because of the lack of maternal toxicity at the HD and the apparent failure to examine visceral abnormalities. In the repeat study at doses of up to 60 mg/kg, maternal toxicity was seen, fetal and litter rates of some individual visceral and skeletal variations appeared to be increased in a D-R manner (**Table IVE.8.3**), but there were no clear developmental effects. If the no-effect dose for embryo-fetal toxicity in rabbits is considered 12.1 mg/kg, this is associated with maternal plasma exposures to retigabine and NAMR that are small fractions of those in humans at the MHD. But even the highest dose tested in rabbits produces plasma levels below those expected clinically, particularly of NAMR (**Table IVE.8.1**). In the pre- and post-natal study in rats (doses of up to 61.9 mg/kg), decreased offspring survival, body weight gain, and reflex development were seen at the HD. Although no TK data were collected, plasma levels at the no-effect dose (17.8 mg/kg/day) would be expected to be similar to those at the NOAEL for the embryo-fetal study.

Juvenile rat studies of retigabine were problematic due to the increased sensitivity of young animals to neurotoxicity and urinary bladder effects which limited the doses and age range that could be examined. When dosing started on PND7 in the two dose range-finding studies, a high rate of mortality was seen due to these effects. Clinical signs of neurotoxicity included myoclonus, and distended urinary bladder and/or dilated renal pelvis were prominent postmortem findings. As in adults, the proposed mechanism involved the inhibitory effect of retigabine on urinary bladder smooth muscle contractility causing bladder dilation and associated bladder/renal lesions due to increased urinary pressure. The volume of vehicle administered was thought by the sponsor to have played a role in the toxicity seen in the initial juvenile rat studies, since a high rate of mortality and similar histopathology findings occurred in controls, but the dose-related (at constant volume) increases in mortality and microscopic findings indicated a clear drug effect (**Table IVF.1.1**). Vehicle and/or dose volume as well as urogenital system immaturity may have contributed to the background, however. Myoclonus was apparently seen only in treated animals, at doses as low as 9 mg/kg, much lower than doses producing clonic convulsions in adult rats (lowest dose about 50 mg/kg). Based on these data, extremely low doses (HD plasma exposures <1/10 clinical) were chosen for the definitive study and dosing was started on PND14. As might be expected, there was no discernable drug-related toxicity at these doses according to the study report; however, actual study data and summary tables were not provided in this report (apparently not provided to current sponsor by W-A), so this could not be considered a valid study. It was found that higher doses could be used if dosing started at weaning or later, although CNS toxicity was still greater than in adults. A dose range-finding study in which retigabine was administered on either PND 21 or 28 found clinical signs including convulsions and mortality at the two highest doses when dosing started on PND21. Clinical signs and mortality were less pronounced when dosing started on PND28. In the definitive study (dosing initiated on PND28; HD plasma exposures 1/3-1/2 clinical at start of treatment), there were no T-R deaths and clinical

signs (decreased activity, tremors, head shaking, abnormal gait, non-sustained convulsions at HD) were transient. Other toxicity observed consisted of clinical chemistry evidence of altered renal and hepatic function (↑bilrubin and urea at HD), possible behavioral abnormalities (increased activity and startle, both indicative of decreased habituation; NS but D-D tendency at all doses), impaired reproductive function (decreased fertility and increased preimplantation loss, all doses), and hepatocellular hypertrophy. Notably, no bladder histopathology was reported. The implications of these studies for pediatric use are that neonates and infants may be at increased risk of neurotoxicity and urinary bladder/renal effects associated with retigabine and that there may be long-term developmental effects even in older children. A possible mitigating factor is that urogenital system development is more advanced at birth in humans than in rodents. This is not an immediate concern for the current NDA which does not include a pediatric indication.

VI. Recommendations

The NDA is not approvable from a pharmacology/toxicology standpoint due to the high specification limit proposed for the mutagenic impurity (b) (4) (NMT (b) (4) The sponsor should either lower the specification to that considered acceptable for genotoxic impurities (b) (4) day for all related genotoxic impurities combined; equivalent to (b) (4) or (b) (4) total) or qualify the impurity by examining its carcinogenic potential directly in an appropriate bioassay, ie, 2-year rodent study or alternative assay. Recommendations concerning the proposed labeling follow.

cc:
NDA (22-345)
Div File
HFD-120/FreedL/FisherE/KeefeS

_____
J.E. Fisher, Ph.D.

Labeling Recommendations

8.1 Pregnancy

Pregnancy Category C. There are no adequate and well-controlled studies in pregnant women. POTIGA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

(b) (4)

[redacted]

8.4 Pediatric Use

The safety and effectiveness of POTIGA™ in patients under 18 years of age have not been established.

12.1 Mechanism of Action

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Impairment of Fertility

Ezogabine had no effect on fertility, general reproductive performance, or early embryonic development when administered to male and female rats at doses up to 46.4 mg/kg/day (associated with a plasma (b) (4) exposure [AUC] less than that in humans at the MRHD) prior to and during mating, and continuing in females through gestation Day 7.

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/s/

J EDWARD FISHER
11/30/2010

LOIS M FREED
11/30/2010

Executive CAC
Date of Meeting: July 20, 2010

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Wendy Schmidt, Ph.D., DAIOP, Alternate Member
Lois Freed, Ph.D., DNP, Supervisor
Ed Fisher, Ph.D., DNP, Presenting Reviewer

Author of Draft: Ed Fisher

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 22-345
Drug Name: retigabine
Sponsor: Valeant

Background: Genotoxicity testing was conducted with drug substance synthesized using the early (b) (4) Genotoxicity testing of the salt indicated clear genotoxic potential (positive in Ames, positive for chromosomal aberrations in human lymphocytes with and without activation). The free base (D-23129) appeared to be less genotoxic *in vitro*, which the sponsor attributed to an improved impurity profile. In studies conducted with the free base, retigabine was inconsistently negative (intermittently weakly positive or equivocal) in the Ames test, negative in the CHO hprt gene mutation assay, positive in the human lymphocyte chromosomal aberration assay, and negative in the mouse micronucleus test. The major circulating metabolite of retigabine, NAMR, was negative in the Ames test but positive in the CHO chromosomal aberration test. Several Ames (strongly) positive (b) (4) containing impurities were identified, and it is possible that the positive genotoxicity findings with the salt may have been due to the presence of one or more of these impurities.

A conventional 2-yr mouse carcinogenicity study was not considered feasible by the sponsor, due to the dose-limiting urinary bladder toxicity observed in this species with daily administration of relatively short duration. The sponsor proposed replacing the 2-year study with the neonatal mice model (discussed in ICH S1B) with dosing on postnatal days 8 and 15 followed by monitoring for one year, as per the ILSI protocol and as described in a publication by (b) (4) The Exec-CAC concurred with the use of the neonatal mouse assay, but recommended that the sponsor also consider using the p53 or TgrasH2 assays. The Committee did not agree that the sponsor had correctly identified an MTD based on the results of the 28-day dose range-finding study, particularly for a 2-dose protocol (I53,950, SN155; see protocol review and minutes

dated 12/10/02). Final dose selection for the neonatal mouse study was based on the results of a second dose range-finding study that tested higher doses (up to 96 mg/kg).

Mouse Carcinogenicity Study

Oral administration of retigabine to neonatal mice on PNDs 8 and 15 at doses of 0 (distilled water; WC), 0 (propylene glycol vehicle; VC), 32, 64, or 96 mg/kg induced a small but dose-related increase in the frequency of lung neoplasms in treated males compared to the water and vehicle controls; numbers affected (%) for combined bronchioloalveolar adenoma/carcinoma were: 1(4), 1(4), 1(4), 2(8), and 3(12) in WC, VC, LD, MD, and HD males, respectively. Notably, bronchioloalveolar carcinoma was only seen in 2(8%) HD retigabine males and in positive control mice (1(4%) males and 2(7%) females). (The historical control values provided by the sponsor for lung carcinomas were 1.4% (5/360) and 1.1% (4/360) in males and females.) The expected high incidences of lung and liver tumors were seen in the (diethylnitrosamine) positive control group.

Rat Carcinogenicity Study

Oral administration of retigabine to rats for 2 years at doses of 0 (water control, WC), 0 (propylene glycol vehicle control, VC), 5, 20, or 50 mg/kg was associated with clinical signs, decreased BW and increased mortality at the HD, but there were no clearly treatment-related increases in tumor incidence. According to the sponsor's analysis, there was an increase in the incidence of testicular interstitial cell tumor in HD males and, when tumor types were (inappropriately) combined in the skin/subcutaneous tissue or preputial gland in males. However, none of the tested tumor types were determined to have a SS positive dose-response relationship in the FDA statistical analysis. Dose selection appears to have been appropriate. Although the HD mortality rate was unexpectedly high, adequate numbers remained at ≈18 months and at the end of the study for the study to be considered valid (18 males and 24 females).

Executive CAC Recommendations and Conclusions:

Neonatal Mouse:

- The Committee agreed that the study was adequate.

- Regarding the lung carcinoma observed in HD males, the Committee concluded that a drug-related effect could not be ruled out since the incidence in HD males (2/25 or 8%) exceeded the background incidence (mean and range) in both concurrent and historical controls.

Rat:

- The Committee agreed that the study was adequate.

- The Committee found that the study was negative for drug-related neoplasms.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DNP
/LFreed, DNP
/EFisher, DNP
/SKeefe, DNP
/ASeifried, OND IO

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22345	ORIG-1	VALEANT PHARMACEUTICALS NORTH AMERICA	RETIGABINE

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/s/

ADELE S SEIFRIED
07/23/2010

DAVID JACOBSON KRAM
07/27/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 22-345 **Applicant: Valeant Pharm** **Stamp Date: 10/30/09**

Drug Name: retigabine **NDA/BLA Type: NDA**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?	x		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	NA		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___yes_____

Ed Fisher 12/11/09

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22345	ORIG-1	VALEANT PHARMACEUTICALS NORTH AMERICA	RETIGABINE

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/s/

J EDWARD FISHER
12/15/2009

LOIS M FREED
12/15/2009