CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-250s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-250
Submission date: April 22, 2009
Drug: dalfampridine (also referred to as fampridine)
Sponsor: Acorda Therapeutics, Inc.
Indication: to improve walking speed in individuals with multiple sclerosis (MS)
Reviewing Division: Division of Neurology Products

Introductory Comments:
The primary pharmacology/toxicology reviewer raised several safety concerns with dalfampridine. The primary reviewer recommended that the NDA not be approved until the concerns were resolved. The supervisor agreed that some of the concerns should be addressed, but concluded that the NDA could be approved since the clinical team has determined that the drug fulfills an unmet medical need. The supervisor also concluded that the remaining nonclinical concerns could be addressed after approval.

Drug impurities:
Most of the outstanding nonclinical concerns are associated with inadequately qualified impurities. The secondary review discusses how most of the concerns for impurities were resolved. However, the potential genotoxicity of one impurity (b) (4) remains unresolved. In addition, another impurity (b) (4) occurs at a level that exceeds the ICH Q3 qualification threshold. The genotoxicity and general toxicity of this impurity appear to have been adequately assessed; however, the teratogenic potential of this impurity has not been assessed.

The supervisor recommended that (b) (4) be qualified in one of two ways: either by testing in two in vitro genetic toxicity assays or by demonstrating that the impurity had been tested at ≥25 fold higher than the human plasma exposure (AUC) in one of the already completed mouse or rat carcinogenicity studies.

The supervisor also recommended that the teratogenic potential of (b) (4) be assessed in a study of embryofetal development. The primary reviewer and supervisor both discussed some concerns with the adequacy of the previously conducted rat embryofetal development. In particular, a relatively low number of litters were available for evaluation from the high dose animals. The reviewer and supervisor recommend that a high dose dalfampridine group be included in a new embryofetal study to enable comparison with the impurity and to provide additional assessment of dalfampridine at a high dose.

Conclusions:
I have discussed this NDA with the division pharm/tox supervisor and agree that the outstanding issues could be addressed after approval. The proposal to address the genotoxic potential of [redacted] (b) (4) either through genetic toxicity studies or by demonstrating that adequate levels were assessed in a carcinogenicity study is acceptable. Assessment of the embryofetal toxicity of [redacted] (b) (4) after approval is acceptable considering the indication.

I concur with the labeling suggested by the supervisor in her secondary review.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22250	ORIG-1	ACORDA THERAPEUTICS INC	FAMPRIDINE TABLETS

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/s/

PAUL C BROWN
01/21/2010

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: January 14, 2010

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

Subject: NDA 22-250 (4-aminopyridine, fampridine), re-submission date 22 April 2009
Applicant: Acorda Therapeutics

NDA 22-250 for an oral sustained-release formulation of fampridine (or 4-aminopyridine, 4-AP) was originally submitted to the Agency on January 30, 2009. This application was refused (Refusal to File letter, March 30, 2009) due to eCTD formatting deficiencies and missing clinical data. NDA 22-250 was resubmitted on April 22, 2009, and filed (Agency letter, May 5, 2009). 4-AP was developed "as a treatment to improve walking ability in patients with multiple sclerosis".

4-AP is a member of the family of mono- and di-amino pyridine derivatives and has been used for many years as a pharmacological tool for investigating and characterizing subtypes of potassium channels. 4-AP also has a history of use as a bird control agent, and is registered by the EPA under the tradename, Avitrol, for this purpose.

To support clinical development and marketing approval of 4-AP for the proposed indication, a full battery of nonclinical studies has been conducted. These studies have been reviewed in detail by Dr. Houghtling (Pharmacology/Toxicology Review and Evaluation NDA 22-250, Richard A. Houghtling, Ph.D., 12/22/2009). Based on his review, Dr. Houghtling recommends that the NDA not be approved, noting several nonclinical deficiencies:

- "…an inadequate evaluation of a potentially genotoxic impurity, (b) (4) (b) (4)
- Inadequate qualification of drug product impurity, (b) (4) , specifically "…the application lacked data from a 3-month repeat dose toxicity study and an embryofetal study in one species".
- An inadequate embryo-fetal development study in rat, due to the lack of a sufficient number of litters at the highest dose tested.

Dr. Houghtling recommends that, to address these deficiencies, the sponsor needs to conduct the following nonclinical studies, presumably prior to approval:

- An embryo-fetal development study of the (b) (4) impurity, to include a high dose of fampridine alone.
- An *in vitro* Ames assay for (b) (4).
- Either an *in vitro* chromosomal aberration assay in mammalian cells *or* an *in vitro* mouse lymphoma tk assay for (b) (4).

This memo will focus primarily, but not exclusively, on these issues.

Nonclinical findings

- **Seizures**

A primary toxicity identified in the nonclinical studies was seizures. Seizures following administration of 4-AP were observed in animals (rat, dog, rabbit, and, possibly, mouse). Fampridine-induced seizures were not clearly evident in the sponsor's toxicity studies in mouse; however, fampridine is used as a convulsant agent in animal (including mouse) models to assess anticonvulsant activity (e.g., Shih C-L *et al*. *Toxicol* 162(1):35-42, 2001; Witkin JM *et al*. *Life Sci* 83:377-380, 2008; Zappala A *et al*. *Neurosci* 141(1):167-178, 2006). Fampridine-induced seizures were evident in dog; however, following chronic administration, the data suggest that seizures and/or death (observed in all but the low dose group) may have been secondary to cardiac toxicity. Seizures have been reported to occur in humans, particularly with IR formulations, in published literature (cf. Korenke AR et al. *Ann Pharmacotherap* 42:1458-1465, 2008; Bever CT, Judge S. *Expert Opin Invest Drugs* 18(7):1013-1024, 2009) and in clinical trials conducted by the sponsor (at doses >10 mg b.i.d.).

In animals, the data suggest that drug-related seizures are related to maximum plasma levels (C_{max}) of the parent. For example, in rat, the no-effect doses for seizures were higher when fampridine was administered by gavage in divided doses (b.i.d., q.i.d.) compared to q.d. or in the diet compared to gavage dosing, at equivalent daily doses and plasma exposures (although comparative data were limited). According to the sponsor, EEG activity data in rat suggest a threshold plasma level ($C_{50\%}$) of 109-135 ng/mL for CNS toxicity, fairly consistent with "A risk of seizure…associated with high (>100 ng/mL) plasma concentrations of 4-AP in humans (following dose levels greater than the proposed dose of 10 mg b.i.d.)."

- **Reproductive and developmental toxicology studies**

The sponsor conducted a full battery of reproductive and developmental toxicology studies of fampridine. Dr. Houghtling has concluded that "For the most part, these studies were adequate…", except for the embryofetal development study in rat. Dr. Houghtling notes that there were only 14 evaluable litters at the HD, less than the ICH recommended ≥16 litters per group (cf. *Guideline for Industry Detection of Toxicity to Reproduction for Medicinal Products ICH-S5A September 1994*), and that further assessment is needed.

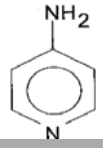
Dr. Houghtling also notes that "The fertility and early development study conducted in rats was inadequate…" due to the "…low n/grp for the uterine parameters (GD 13)…and the postnatal evaluations were conducted in 11 HD litters", but does not specify how this deficiency should be addressed.

The fertility study was designed to assess drug effects on all phases of development; doses were administered to males and females prior to and throughout the mating period and, in females, to either gestation day (GD) 13 or lactation day (LD) 21. Mating and fertility indexes and estrus cycle effects were evaluated in an adequate number of animals (29 or 35/sex/group). Uterine parameters were assessed in 11, 11, 9, and 10 CF, LDF, MDF, and HDF, respectively, sacrificed on GD 13; postnatal evaluation was conducted in only 11-12 HD litters. As Dr. Houghtling points out, these are generally considered inadequate numbers of litters per group. However, uterine parameters (implantation sites) were also examined in dams sacrificed on LD 21 (20, 17, 19, and 11 CF, LDF, MDF, and HDF, respectively), as well as in the embryofetal development study in rat, which provide some additional assessment of uterine effects. Also, postnatal evaluation was adequately assessed in a separate pre- and post-natal development study in rat, so the small number of HD litters examined postnatally in the fertility study is acceptable. Therefore, in my opinion, the study does not need to be repeated.

In the embryofetal development study in rat, there were 9 HD deaths within the first 2 weeks of dosing; most deaths (9) occurred within two days of initiation of dosing. Of the 17 surviving HDF, 3 were confirmed (using 10% ammonium sulfide solution for detection of implantations) at necropsy to be not pregnant, resulting in only 14 HDF evaluable at GD 20. Clearly, the HD of 10 mg/kg exceeded the MTD in this study; at the MD of 3 mg/kg, the plasma AUC in rats is only ≈3 times the plasma AUC in humans at the MRHD. Considering the small safety margin and the minimal number of HDF, it is not unreasonable to conclude that fampridine should be further assessed for potential adverse effects on embryofetal development in this species (*see discussion under Impurities)*. It is my opinion that any additional evaluation may be conducted post-approval, particularly since fampridine has been adequately assessed in an embryofetal development study in rabbit.

- **Impurities**

The review chemist identified seven potentially genotoxic impurities, based on the presence of structural alerts (see table below). Only one of these impurities (b) (4) has a specification limit that exceeds the qualification threshold; the sponsor conducted nonclinical studies to qualify this impurity (28-day oral toxicity study, *in vitro* Ames assay, *in vitro* chromosomal aberration assay in CHO cells). The sponsor was informed in the Agency's 74-day letter (7/2/09) that the genotoxic potential of the (b) (4) impurity had been adequately assessed based on submission of the *in vitro* genotoxicity studies, but that the genotoxic potential of the other six impurities would need to be addressed.

NAME	STRUCTURE	SPECIFICATION LIMIT	CATEGORY
fampridine		n/a	drug substance

(b) (4)

For these six impurities, the sponsor could either lower the level of each to an amount that would result in a daily intake of NMT 1.5 µg/day or demonstrate that each was negative in *in vitro* genotoxicity assays (cf. ICH Q3A (Revision 1) and Q3B(R) guidances). In addition, the Agency's letter noted that the qualification of the (b) (4)

(b) (4) impurity was incomplete; a 3-month oral toxicity study and an embryo-fetal development study, each in a single species, had not been conducted.

The sponsor provided responses to the impurity issues described in the Agency's 74-day letter in Amendments 0016 (July 21, 2009) and 0029 (September 18, 2009).

In Amendment 0016, the sponsor stated that "There are no identified potentially genotoxic impurities in the drug substance or in Fampridine-SR tablets", based on:

- Negative results in a QSAR evaluation using DEREK computational toxicology assessment of 5 impurities (b) (4)
- The fact that (b) (4) and the (b) (4) impurity have not been detected in the drug substance or product,
- The fact that in previous meetings with the sponsor, the Division "agreed that the studies [to qualify the (b) (4)] appeared to be adequate…"

The sponsor was notified in an email communication (August 7, 2009) that the DEREK QSAR assessment is not considered adequate for regulatory purposes and, therefore, the genotoxic potential of each of the identified impurities (except for the (b) (4)) would need to be addressed. Regarding the (b) (4), the Division acknowledged the previous discussions regarding qualification, but noted that "The adequacy of the data remains a matter of review."

In Amendment 0029, the sponsor stated the following:

- Based on data collected using improved assay methods, the levels of two impurities (b) (4) and (b) (4)) were documented to be at levels (i.e., (b) (4) of drug substance) consistent with a daily intake of <1.5 µg/day.
- (b) (4) "was recently identified as a metabolite in mouse and rat plasma retains [sic] from GLP toxicokinetic studies (7338-103 and 7338-104, respectively)."

The sponsor is being asked to lower the specification limits for (b) (4) and (b) (4) consistent with the new data.

Regarding (b) (4), the sponsor submitted a report providing "preliminary qualification of the (b) (4) metabolite in mouse and rat". The data on the (b) (4) impurity as a metabolite are from "preliminary quantitative results" of analysis of plasma samples retained from bridging TK studies in rat and mouse. The sponsor stated that "Following this study, a quantitative GLP validated method will be developed to quantify the (b) (4) plasma concentration as a function of dose and dosing time."

In mouse, the data were from an analysis of plasma samples collected on Day 28 of dosing (time of collection in relation to dose was not stated) from individual animals in each dose group (i.e., 1/sex/group); "Concentrations of fampridine (b) (4) were 19.2-

16.4, 58.2-66.7, and 652-478 ng/mL at 2, 12.5, and 80 mg/kg, respectively, which are the doses used in the mouse carcinogenicity bioassay.

In rat, the data were from an analysis of pooled plasma samples (one pooled sample per dose group) collected on Day 28 of dosing (time of collection in relation to dose was not stated); plasma levels of the (b) (4) were reported to be 3.84, 14.9, and 37.7 ng/mL at 2, 6, and 18 mg/kg, respectively, which are the doses used in the rat carcinogenicity bioassay.

The sponsor was asked to provide the status of the ongoing GLP study (FDA Information Request Letter, October 23, 2009). The sponsor response (October 28, 2009) was as follows:

> Acorda identified the (b) (4) in mouse and rat plasma retains that were obtained from GLP toxicokinetic (TK) studies (7338-103 and 7338-104, respectively). These data were summarized in a metabolism report (8210924) and submitted to the NDA on September 18, 2009 (Sequence 0029). This metabolism study followed good scientific practices commonly used for metabolism studies (non-GLP) and was performed at (b) (4) It is our understanding that GLP studies are not generally required for characterization of metabolites.

The Division repeated the request, noting the sponsor's statement regarding the ongoing GLP study in the preliminary report, in an Information Request Letter dated November 16, 2009. In response (submitted November 25, 2009), the sponsor indicated that "…we intend to update the recently completed toxicokinetic studies by quantifying this metabolite as a function of dose and dosing time…The bioanalysis of the (b) (4) will be performed in the available plasma samples from the following TK studies:

- Study No. 7338-103: Pharmacokinetics of Fampridine and its 3 Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites on Days 1 and 28 Following Dietary Administration of Fampridine in Mice
- Study No. 7338-104: Pharmacokinetics of Fampridine and its 3 Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites on Days 1 and 28 Following Dietary Administration of Fampridine in Rats
- Study No. 7338-105: Pharmacokinetics of Fampridine and its 3 Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Doses to Non-Pregnant and Pregnant Rats
- Study No. 7338-106: Pharmacokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Doses to Dogs
- Study No. 7338-107: Pharmacokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Dose to Pregnant Rabbits.

The anticipated completion date is 1st quarter of 2010. The sponsor has no plans to further analyze human plasma for the (b) (4) from the available information, the (b) (4) is not a major metabolite in humans.

Since these communications, in discussions with the CMC reviewer and PAL, it was determined that the structural alert for genotoxic potential upon which the CMC team based their concern is a moiety present in the parent compound and shared by all but the (b) (4) impurity. Since fampridine itself was negative in a standard battery of genetic toxicology assays, it is reasonable to assume that the impurities containing only that structural alert would not be of concern. Of the 7 potentially genotoxic impurities originally identified by the CMC team, only the (b) (4) impurity contains an additional structural alert (b) (4)); therefore, only the genotoxic potential of the (b) (4) still needs to be addressed.

The preliminary data provided in rat and mouse are not sufficient to document tha (b) (4) has been adequately assessed for genotoxic/carcinogenic potential. Generally, the genotoxic potential of an impurity is tested, preferably directly, in *in vitro* and, possibly, *in vivo* genotoxicity assays. Alternatively, if it can be demonstrated that the potentially genotoxic impurity is a human metabolite that circulates at a level substantially greater than that anticipated from its presence in the drug product, then no further assessment would be required. The genotoxic potential of the (b) (4) has not been tested, either added to the drug substance or directly. In order to consider this impurity adequately tested for carcinogenic potential, the sponsor would need to demonstrate that it circulates in either rat or mouse plasma at a sufficiently high level at the doses used in the carcinogenicity study(ies). Currently, the carcinogenic potential of a drug substance (whether identified as genotoxic or non-genotoxic in a standard battery of genetic toxicology studies) is considered adequately assessed if the plasma exposure (AUC) achieved in adequately carcinogenicity studies is ≥25 times the human plasma AUC at the MRHD; it would seem reasonable to apply this criterion (but limited to a single species) to a potentially genotoxic impurity. Therefore, to support the proposed specification limit for (b) (4), the sponsor may:

- Test the (b) (4) impurity directly in *in vitro* genetic toxicology studies (i.e., Ames assay and either an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma tk assay [with colony sizing]) or
- Demonstrate that the (b) (4) is a metabolite in either rat or mouse at sufficiently high levels (e.g., at least 25-fold the plasma AUC in humans at the MRHD) at the doses used in the carcinogenicity study(ies).

The sponsor need not conduct additional TK studies of the (b) (4) in dog or pregnant animals.

Finally, it is acknowledged that in previous communications, the sponsor was told that the studies conducted to qualify the (b) (4) impurity were adequate; however, based on further consideration and considering the intended patient population, it is my opinion that this impurity needs to be tested for the potential to adversely affect embryofetal development. As noted by Dr. Houghtling, this study should test both the

impurity and a high dose of fampridine; inclusion of a high-dose fampridine group will address the deficiency identified by Dr. Houghtling in the original embryofetal development study. Since the specification limit for this impurity has been the same throughout most of clinical development, it is my opinion that at this point a 3-month general toxicity study is not needed; the 28-day study of fampridine spiked with (b) (4) indicated no new or additional toxicity.

Conclusions and Recommendations

Dr. Houghtling has recommended that the NDA not be approved until nonclinical studies are conducted to address the genotoxic potential of drug substance/product impurity, (b (b) (4), to qualify an excipient interaction impurity, (b) (4) and to further characterize the potential for fampridine to adverse affect embryofetal development. Although this is a reasonable recommendation, particularly regarding the need to further assess the toxicity of impurities, it is my understanding that the clinical team has determined that fampridine fulfills an unmet medical need. Therefore, from a nonclinical standpoint, I have no objection to approval of the NDA based on the available nonclinical data, with the nonclinical deficiencies discussed to be addressed post-approval. The PMRs have been specified, along with timelines for their completion, in separate documents.

Recommended labeling: the following labeling is proposed, and is based on recommendations from Drs. Houghtling and Fisher, and taking into account comments from the Maternal Health Team (cf. Maternal Health Team Review, Jeanine Best, MSN, RN, PNP; 11/20/2009) and the sponsor (emails received 12/14/09, 12/16/09). Also, "fampridine" has been replaced with "dalfampridine", the new established name (changed due to concerns that the name "fampridine" may result in medication errors).

The sponsor claims that fampridine is a "selective" potassium channel blocker; however, in published literature, fampridine is characterized as a "broad-spectrum" or non-selective potassium channel blocker (e.g., Bever CT, Judge S. *Expert Opin Invest Drugs* 18(7):1013-1024, 2009; Wulff H *et al*. *Nature Rev Drug Disc* 8:982-1001, 2009)

(b) (4)

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[Tradename] is a non-selective potassium channel blocker indicated to improve walking speed in adult patients with multiple sclerosis (MS).

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of [Tradename] in pregnant women. Administration of dalfampridine to animals during pregnancy and lactation resulted in decreased offspring viability and growth at doses similar to the maximum recommended human dose (MRHD) of 20 mg/day. [Tradename] should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In developmental toxicity studies in rat and rabbit, dalfampridine was administered orally at doses up to 10 and 5 mg/kg/day, respectively, during the period of organogenesis. These doses are approximately 5 times the MRHD on a body surface area (mg/m^2) basis. No evidence of developmental toxicity was found in either species at the highest doses tested, which were maternally toxic. Oral administration of dalfampridine (1, 3, and 9/6 mg/kg/day; high dose reduced during the second week of dosing) to rats throughout the pregnancy and lactation periods resulted in decreased offspring survival and growth. The no-effect dose for pre- and post-natal developmental toxicity in rats (1 mg/kg) is approximately 0.5 times the MRHD on a mg/m^2 basis.

8.2 Labor and delivery

The effect of [Tradename] on labor and delivery in humans is unknown.

8.3 Nursing mothers

It is not known if dalfampridine is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from dalfampridine, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The mechanism by which dalfampridine exerts its therapeutic effect has not been fully elucidated. Dalfampridine is a non-selective potassium channel blocker. In animal studies, dalfampridine has been shown to increase conduction of action potentials in demyelinated and non-myelinated axons through inhibition of potassium channels.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, mutagenesis, impairment of fertility

Carcinogenesis: Two year dietary carcinogenicity studies of dalfampridine were conducted in mice and rats. In mice, the doses tested (approximately 2, 12.5, and 80 mg/kg/day) were associated with plasma exposures (AUC) up to 18 times the plasma AUC in humans at the maximum recommended human dose (MRHD) of 20 mg/day. There was no evidence of drug-related carcinogenicity.

In rats, the doses tested (approximately 2, 6, and 18 mg/kg/day) were approximately 1, 3, and 9 times the MRHD on a body surface area (mg/m^2) basis. There was a significant increase in uterine polyps at the highest dose tested.

Mutagenesis: Dalfampridine was negative in *in vitro* (bacterial reverse mutation, mouse lymphoma *tk*, chromosomal aberration) and *in vivo (*mouse bone marrow, rat erythrocyte micronucleus) genetic toxicology assays.

Impairment of Fertility: Oral administration of dalfampridine (doses of 1, 3, and 9 mg/kg/day) to male and female rats prior to and throughout mating, and continuing in females up to day 13 of gestation or day 21 of lactation resulted in no adverse effects on fertility. Reduced offspring viability and body weight were observed at 9 mg/kg/day. The mid dose (a no-effect dose) was similar to the MRHD on a mg/m^2 basis.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22250	ORIG-1	ACORDA THERAPEUTICS INC	FAMPRIDINE TABLETS

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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:	**22-250**
SUBMISSION NUMBER/CODE:	**000**
CDER STAMP DATE:	**04/22/09**
PRODUCT:	**Fampridine**
INDICATION:	**Multiple Sclerosis**
APPLICANT:	**Acorda Therapeutics**
REVIEW DIVISION:	**Division of Neurological Drug Products (HFD-120)**
REVIEWER:	**Richard A. Houghtling, Ph.D.**
SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell G. Katz, M.D.**
PROJECT MANAGER:	**James H. Reese, Ph.D.**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical perspective, it is recommended that fampridine not be approved until the following deficiencies associated with two drug impurities are addressed: first, the (b) (4) impurity should be adequately evaluated to determine if it possesses genotoxic potential and second, the (b) (4) impurity should be evaluated in an embryofetal study conducted in a single species to determine whether this impurity has the potential to cause embryotoxicity.

B. Recommendation for nonclinical studies

There are three notable deficiencies in this application, which may be addressed by additional data. The first deficiency is an inadequate evaluation of a potentially genotoxic impurity, (b) (4), which is present in both the drug substance and drug product. At present, the specification has not limited this impurity to ≤1.5 µg/day and without data to suggest this impurity lacks genotoxic potential, additional nonclinical studies are recommended. Deficiencies are noted in the qualification of the (b) (4) impurity to support a chronic use indication; the application lacked data from a 3-month repeat dose toxicity study and an embryofetal study in one species. Finally, a deficiency was found in the embryofetal toxicity study conducted in rats. In this study, the number of litters examined at the high dose of fampridine was too few. Thus, the sensitivity of this study to detect rare developmental anomalies may have been comprised by a low number of litters examined. Therefore, to address these concerns, an embryofetal study should be conducted in a single species to evaluate both the (b) (4) impurity and also include an additional group to evaluate the high dose of fampridine.

Three nonclinical studies are recommended to address the safety concerns for two drug impurities (b) (4), a drug substance and a drug product impurity, and (b) (4) a drug product, impurity. The first study should be an embryo-fetal development study to qualify the drug product impurity, (b) (4) that exceeds the qualification threshold. The second and third studies should determine the genotoxic potential of the drug substance and drug product impurity (b) (4) The second study will evaluate (b) (4) in an Ames test, and the third study will be examine this impurity in an *in vitro* chromosomal aberration assay in mammalian cells or an *in vitro* mouse lymphoma tk assay.

C. Recommendations on labeling

The following changes to the label for TRADNAME are recommended:

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8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Patients should be advised to notify their physician if they become pregnant or intend to become pregnant during therapy.

Teratogenic effects – Pregnancy Category C

In reproductive studies in rats and rabbits, fampridine was administered orally at doses up to 10 mg/kg/day and 5 mg/kg/day, respectively, during the period of organogenesis. No evidence of embryotoxicity or developmental anomalies was found in either the rat or rabbit studies, even at levels that were maternally toxic. In a study in which rats were dosed from gestation Day 7 to lactation Day 21, pup survival from Days 1 to 4 of gestation was reduced and decreased pup weight gains were observed from postnatal Days 4 to 35, but these effects were only seen at the high dose of 9 mg/kg/day (reduced to 6 mg/kg/day during the second week of the study). These dose levels represent 6.9 and 4.6 fold the human exposure at the MRHD. No treatment-related effects were observed in a battery of behavioral and developmental assessments of the F1 pups.

Adequate and well-controlled studies in pregnant women have not been conducted. AMPRIVA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Although no teratogenic effects were seen in preclinical studies it is not known if teratogenic effects would be seen in humans.

8.2 Labor and delivery

The effect of fampridine on labor and delivery in humans is unknown.

8.3 Nursing mothers

It is not known whether fampridine is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from fampridine, a decision should be made whether to discontinue nursing or to discontinue fampridine, taking into account the importance of the drug to the mother.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of action

Fampridine blocks multiple potassium channels including those found under the myelin sheath in nerve fibers of adult mammals, where they are located primarily in the paranodal and internodal membrane of the axon. In animal studies on demyelinated nerve fibers, fampridine has shown enhanced action potential conduction at concentrations of ~1 μM (94 ng/mL) with IC50 values in the range of 2–3 μM (188–282 ng/mL) recorded.

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

B. Pharmacologic activity

Fampridine is 4-aminopyridine, which is a member of the class of aminopyridine compounds. This class of structurally and functionally related compounds has been extensively studied since the late 1920's (as reviewed in Bowman and Savage, 1981). In particular, 4-aminopyridine has been utilized as an anticurare agent to reverse the neuromuscular blockade effects of curare. At doses slightly greater than those used to reverse the effects of neuromuscular blockade, pro-convulsant activity of 4-aminopyridine has been described (Fastier and McDowall, 1958). More recently, 4-aminopyridine and related compounds were shown to bind with a low affinity binding (μM to mM) within the intracellular mouth of voltage-gated potassium channels (IK_v). Binding of fampridine within the channel occludes it, thus preventing efflux of intracellular K^+ and repolarization of the membrane potential leading to prolonged action potentials and enhanced neurotransmitter release (particularly acetylcholine release at the neuromuscular junction).

C. Nonclinical safety issues relevant to clinical use

1. The primary toxicity associated with fampridine is related to CNS activation that culminates in seizure activity. In the scientific literature, Ball et al. (1979) described epileptiform seizures in human following administration of 4-aminopyridine. Similar seizure activity has been observed in most animal species treated with 4-aminopyridine. Likewise, evidence of increased CNS activity and seizures were observed throughout toxicological testing of 4-aminopyridine by Acorda.

2. During the review process, the CMC reviewer identified seven potentially genotoxic impurities, of which two were identified in the drug substance and five of which were found in the drug product. One of these impurities was common to both the drug substance and the drug product (b) (4) Thus, six different chemical structures were identified as potentially genotoxic. The sponsor has reduced the specification limit on two of these six structures. Unfortunately, the sponsor has not submitted adequate studies to evaluate the genotoxic potential of one of these identified impurities, (b) (4) According to the CMC reviewer, it is not likely that Acorda will be able to reduce each of these potentially genotoxic impurities to a level of <1.5 μg/day based upon the present specifications of the clinical batches.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-250
Review number: 1
Sequence number/date/type of submission: 000
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Acorda Therapeutics, Inc.
145 West 58th St STE 8J
New York, NY 10019

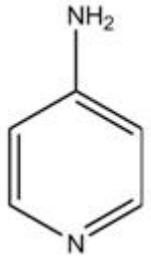
Manufacturer for drug substance: (b) (4)

Reviewer name: **Richard A. Houghtling, Ph.D.**
Division name: **Neurology Drug Products**
HFD #: **120**
Review completion date: November 12, 2009

Drug:

Trade name: AMAYA
Generic name: Fampridine
Code name: 4-AP; Avitrol, Phillips 1861; VMI 10-3; EL-970
Chemical name: 4-aminopyridine; 4-pyridinamine; γ-aminopyridine; 4-pridylamine
CAS registry number: 504-24-5
Molecular formula/molecular weight: $C_5H_6N_2$; MW: 94.12

Structure:



Relevant INDs/NDAs/DMFs:

Application	Indication
(b) (4)	
DMF 14978	Manufacture of fampridine extended release tablets by Elan.
IND 17627	Multiple Sclerosis, DNP, Commercial, ***Active***, 3/1983
(b) (4)	
IND 57123	Multiple Sclerosis, DNP, Research, ***Active,*** 10/1998
(b) (4)	

Drug class: Analeptic, Potassium Channel Blocker

Intended clinical population: Improve walking ability in patients with (b) (4) multiple sclerosis.

Clinical formulation: a single strength of 10 mg as oval-shaped, white to off-white, sustained-release matrix tablets. Each tablet contains the following excipients: hydroxypropyl methylcellulose, USP; microcrystalline cellulose, USP; colloidal silicon dioxide, NF; magnesium stearate, USP; and (b) (4). The proposed recommended dosage regimen is 10 mg twice daily.

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified, all data and information discussed below and necessary for approval of NDA 22-250 are owned by Acorda Therapeutics, Inc. or are data for which Acorda Therapeutics, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-250 that Acorda Therapeutics, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Acorda Therapeutics, Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-250.

Studies reviewed within this submission:

Study Type	Study #	Route	Study Title
Pharmacology	080121.BVK	*In vitro*	Effects of 3 test articles on cloned Kv1.1, Kv1.2 and Kv1.4 potassium channels expressed in human embryonic kidney cells (HEK293).
Safety Pharmacology	pk-pd-1994	i.v.	Development and validation of a pharmacokinetic/pharmacodynamic model of the changes seen in the EEG of Sprague-Dawley rats given 4-aminopyridine.
	Herg4AP102003	*In vitro*	Effects of 4-aminopyridine on cloned hERG channels expressed in mammalian cells.
	Purk4AP102003	*In vitro*	Effects of 4-aminopyridine on action potential parameters in isolated dog Purkinje fibers.
	TPS 486A-501-510-93	i.v.	Cardiovascular effects of 4-aminopyridine in Beagle dogs.
	(b) (4) 684-017	n/a	Determination of 4-aminopyridine in canine plasma samples
PK/ADME	(b) (4) 684-012	Oral (gavage)	Bioavailability of 4-aminopyridine in rats. SD rat
	(b) (4) 684-018	Oral (capsule)	Bioavailability study of 4-aminopyridine in Beagle dogs following oral administration to fed or fasted animals 8-Days
	M-2001-02	*In situ*	*In situ* absorption of 4-aminopyridine from the rat stomach and small intestine
	M-2001-03	i.v.	Gender differences in the pharmacokinetics of 4-aminopyridine in Sprague-Dawley rats
	HWI 6379-101	Oral (gavage)	Pharmacokinetics, tissue distribution, and mass balance of [^{14}C]4-aminopyridine in rats following a single oral dose SD rat
	HWI 6379-103	*In vitro*	*In vitro* protein binding of [^{14}C]4-aminopyridine to human, dog, and rat plasma samples
	ELN-11-010092	*In vitro*	[^{14}C]4-aminopyridine comparative *in vitro* metabolism studies with rat, dog, and human liver microsomes and precision-cut liver slices
	M-2001-029	*In vitro*	Determination of the inhibitory potential of test article on CYP450 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in cyropreserved human hepatocytes.

Study Type	Study #	Route	Study Title
PK/ADME	XT064039	*In vitro*	Reaction phenotyping: Identification of CYP enzymes involved in the metabolism of fampridine
	XT075077	*In vitro*	*In vitro* evaluation of 4-aminopyridine as an inhibitor of human cytochrome P450 enzymes
	XT073070	*In vitro*	*In vitro* evaluation of 4-aminopyridine as an inducer of cytochrome P450 expression in cultured human hepatocytes
	ELN-015	Oral (gavage)	^{14}C-4-Aminopyridine: Preliminary metabolite profiling of portal and systemic plasma after oral administration to rats SD rat
	HWI 6379-104	Oral (capsule)	Identification of [^{14}C]-4-Aminopyridine metabolites in dog urine. Beagle dog
	ELN-9-960528	Oral (gavage)	Identification of [^{14}C]-4-Aminopyridine metabolites in dog urine. Beagle dog
	M-2001-01	i.p.	4-Aminopyridine and hepatic enzyme induction SD rat
	HWI 6379-102	Oral (gavage)	Pharmacokinetics and excretion profile of [^{14}C]-4-Aminopyridine in dogs following a single oral dose Beagle dog
	M-2001-04	i.v.	Drug interactions of 4-Aminopyridine in Sprague-Dawley rats
Other Pharmacokinetic Studies	7338-105	Oral (gavage)	Toxicokinetics of fampridine and its 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate metabolites after multiple oral doses to non-pregnant and pregnant rats SD rat
	7338-103	Dietary	Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites on Days 1 and 28 Following Dietary Administration of Fampridine in Mice 28-Days, CD-1 mouse
	7338-104	Dietary	Toxicokinetics of fampridine and its 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate metabolites on days 1 and 28 following dietary administration of fampridine in rats 28-Days, SD rat

Study Type	Study #	Route	Study Title
Other Pharmacokinetic Studies	7338-106	Oral (capsule)	Toxicokinetics of fampridine and its 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate metabolites after multiple oral doses to dogs 14-Days, Beagle dog
	7338-107	Oral (gavage)	Toxicokinetics of fampridine and its 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate metabolites after multiple oral doses to pregnant rabbits 10-Days (Gestational days), NZW rabbit
Toxicology	(b) (4) 2620-102	Oral (gavage)	Acute oral toxicity study with 4-aminopyridine in rats 14-Days, SD rat
	O402RA44.001	Oral (gavage)	Acute oral toxicity study with 4-aminopyridine in rats 14-Days, SD rat
	(b) (4) 2620-103	Oral (gavage)	Acute oral toxicity study with 4-aminopyridine in rabbits 14-Days, NZ White rabbit
	0433DA44.001	Oral (capsule)	Acute oral toxicity study with 4-aminopyridine in dog. 14-Days, Beagle dog
	(b) (4) 684-019	Dietary	2-Week Range-finding dietary toxicity study in mice. CRL: CD-1 mouse
	(b) (4) 684-021	Dietary	13-Week Dietary toxicity study in mice. CD-1 BR VAF/Plus
	(b) (4) 1139-003	Oral (gavage)	A dose range-finding oral toxicity study of 4-AP in rats 7-Days, SD rat
	(b) (4) 2620-100	Oral (gavage)	2-Week oral preliminary range finding toxicity study with 4-aminopyridine in rats
	(b) (4) 684-007	Dietary	Two week palatability study in rats SD rat
	0437RA44.001	Oral (gavage)	Two week range-finding study with orally administered 4-aminopyridine in rats. SD rat
	0437RA44.003	Oral (gavage)	A 14-Day oral toxicity/pharmacokinetic study of 4-aminopyridine in rats SD rat
	(b) (4) 684-009	Oral (gavage)	4-Week oral range-finding toxicity study in rats SD rat

Study Type	Study #	Route	Study Title
Toxicology	(b) (4)-1139-002	Oral (gavage)	A 13-Week oral toxicity study of 4-AP in rats SD rat
	(b) (4) 2620-105	Oral (gavage)	13-Week oral toxicity study with 4-aminopyridine in rats with a 4-week recovery period SD rat
	(b) (4) 684-010	Dietary	26-Week dietary toxicity study in rats SD rat
	(b) (4) 1139-007	Oral (capsule)	A dose range-finding oral toxicity study of 4-AP in Dogs Phase A: Single dose; Phase B: 7 Days Beagle dog
	(b) (4) 2620-101	Oral (capsule)	2-Week oral preliminary range finding toxicity study with 4-aminopyridine in dogs Beagle dog
	0437DA44.002	Oral (capsule)	Two-week range-finding study with orally administered 4-aminopyridine in dogs Beagle dog
	0437DA44.004	Oral (capsule)	A 14-Day oral toxicity/pharmacokinetic study of 4-aminopyridine in dogs Beagle dog
	(b) (4) 1139-008	Oral (capsule)	A 13-Week oral toxicity study of 4-AP in Beagle dogs
	(b) (4) 2620-104	Oral (capsule)	13-Week oral toxicity study with 4-aminopyridine in dogs with a 4-week recovery period Beagle dog
	(b) (4) 684-008	Oral (capsule)	One year oral toxicity study in dogs Beagle dog
Genotoxicity	(b) (4) 1139-004	*In vitro*	Bacterial reverse mutation assay: AP
	(b) (4) 14477-0-401	*In vitro*	Mutagenicity test on 4-aminopyridine in the Salmonella/Mammalian-microsome reverse mutation assay (Ames test)
	(b) (4) 15558-0-431R	*In vitro*	Mutagenicity test on 4-aminopyridine in the L5178Y/TK+/- mouse lymphoma forward mutation assay with an independent repeat
	(b) (4) 1139-005	*In vitro*	*In vitro* Mammalian cell gene mutation test (L5178Y.TK +/- mouse lymphoma assay)

Study Type	Study #	Route	Study Title
Genotoxicity	(b) (4) 15558-0-437Z	*In vitro*	Mutagenicity test on 4-aminopyridine (4-AP) measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with and without metabolic activation with a confirmatory assay with multiple harvests
	(b) (4) 15558-0-455CO	Oral (gavage)	Mutagenicity test on 4-aminopyridine (4-AP) in an *In vivo* mouse micronucleus assay ICR mouse
	(b) (4) 1139-006	Oral (gavage)	Mammalian erythrocyte micronucleus test SD rat
Carcinogenicity	(b) (4) 684-022	Dietary	Two-year dietary carcinogenicity study in mice CRL: CD (ICR) BR VAF/Plus mouse
	(b) (4) 684-023	Dietary	Two-year dietary carcinogenicity study in rats SD rat
	(b) (4) 684-023 AMENDMENT	Dietary	Two-year dietary carcinogenicity study in rats SD rat
Reproductive and Developmental Toxicology	(b) (4) 684-002	Oral (gavage)	Range-finding developmental toxicity study in rats CRL: CD VAF/Plus rat
	(b) (4) 684-001	Oral (gavage)	Study of fertility and general reproductive performance in rats CRL: CD VAF/Plus rat
	(b) (4) 684-003	Oral (gavage)	Developmental toxicity study in rats CRL: CD VAF/Plus rat
	(b) (4) 684-006	Oral (gavage)	Study for toxic effects on pre- and postnatal development including maternal function in rats CRL: CD VAF/Plus rat
	(b) (4) 684-004	Oral (gavage)	Range-finding developmental toxicity study in rabbits New Zealand White rabbit
	(b) (4) 684-005	Oral (gavage)	Developmental toxicity study in New Zealand White Rabbits
Impurity Qualification	23807-0-409OECD	*In vitro*	Salmonella-Escherichia coli/Mammalian-microsome reverse mutation assay with a confirmatory assay with fampridine with 2% (b) (4)
	23807-0-437OECD	*In vitro*	Chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with 4-aminopyridine spiked with 2% (b) (4)

Study Type	Study #	Route	Study Title
Impurity Qualification	7338-100	Oral (gavage)	28-Day oral gavage toxicity and toxicokinetic study with fampridine (spiked with 2% (b) (4)) in rats SD rat
Comparative Toxicity	(b) (4) 684-026	Oral (capsule)	Comparative toxicity and bioavailability of an instant release and sustained release formulation in the beagle dog

Studies not reviewed within this submission:

Study Type	Study #	Route	Study Title
Other Toxicity Studies	(b) (4) PN-55074	intrathecal	A 7-Day intrathecal infusion toxicity study of 4-AP in the Beagle dog

Abbreviations used:

SD= single dose, LD= low dose, MD= medium dose, HD= high dose, M= male, F= female, GD= gestational day, Wk= week, Mo= month; [ss]= statistically significant, [nss]=not statistically significant, gp=group, conc=concentration; trtmt=treatment

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Fampridine is 4-aminopyridine (4-AP), which is structurally and functionally related to 3,4-diaminopyridine. The aminopyridines act intracellularly by binding with low affinity (µM to mM) within the intracellular mouth of voltage-gated potassium channels (IK_v). Binding of fampridine within the channel occludes it, thus preventing efflux of intracellular K^+ and repolarization of the membrane potential (see Sponsor's Figure 1). Fampridine was originally developed in the 1960's and marketed as an avicide and bird repellant named "Avitrol". The toxicological effect of Avitrol is mediated by enhanced neurotransmission localized at the neuromuscular junction and at neuronal synapses. In the United States, fampridine is an orphan drug that has been investigated for treatment of selective neuromuscular disorders including multiple sclerosis, Eaton-Lambert syndrome, myasthenia gravis, and it is used in cardiovascular research to block the transient outward potassium channel that affects phase I of the cardiac action potential.

Figure 1: Schematic diagrams of a representative K_v channel *(figure 2, A, B, E from Judge and Bever, 2006)*

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Mechanism of Action:
In neurons, the IK_v channels are largely expressed along axons, and these channels are localized to axonal membrane regions beneath the myelin sheath. In myelinated neurons, an action potential is rapidly conducted down an axon without degradation of the amplitude or duration of the signal. This occurs largely due to the opening of voltage-gated Na^+ channels localized at the nodes of Ranvier; and by the limited efflux of K^+ ions through the voltage-gated K^+ channels. The limited K+ efflux occurs because of the presence of the myelin sheath, which impedes diffusion of the K+ ions. As a result, the electrostatic potential driving the efflux of the K+ ions is reduced, the axon membrane potential remains depolarized and the

action potential is conducted down to the next node of Ranvier until the action potential reaches the nerve terminal. In contrast, demyelinated axons do not possess a diffusional barrier. As an action potential travels down a demyelinated axon, the depolarization of the axonal membrane opens voltage-gated K+ channels, which in turn permits diffusion of K+ ions out of the axon, and the axonal membrane repolarizes. Depending upon how much of the axon is demyelinated, the action potential may be shortened, delayed, degraded or terminated—thus, resulting in poor or failed neurotransmission.

IK_v channels are also localized on the membranes of presynaptic nerve terminals. These channels re-polarize the membrane potential to counter the large increases in intracellular Ca^{2+}, which occur in the nerve terminal and are necessary for the release of neurotransmitter. Blockade of these presynaptic potassium channels (IKv) prolongs the depolarization of the end terminal membrane resulting in a greater influx of extracellular Ca^{2+} through voltage-gated calcium channels, increased neurotransmitter release, and enhanced CNS or neuromuscular transmission.

Interestingly, $IK_{v1.3}$ channels are localized on T- and B-lymphocytes, platelets and megakaryocytes, and microglia (Beeton et al., 2001). These potassium channels function in naïve T cells to re-establish the resting membrane potential following recognition of antigen by the T cell receptor, which results in prolonged exposure to high concentrations of intracellular Ca^{2+}. Without such signaling, the T cell fails to elicit an appropriate effector function. The potential for drugs such as 4-aminopyridine that block $IK_{v1.3}$ channels to act as an immunosuppressant has been shown both *in vitro* and *in vivo* using models of delayed-type hypersensitivity and a multiple sclerosis-like model of disease in rodents, EAE (Koo et al., 1997; Beeton et al. 2001).

According to the sponsor, the precise mechanism of action which is beneficial for improved walking ability in multiple sclerosis patients is still unknown; however, it likely involves a combination of these actions. Many of the unwanted side effects of fampridine are due to the enhanced CNS neurotransmission. Most notably, fampridine-elicited CNS toxicity is associated with paresthesias, dizziness, anxiety, fatigue, tremor, gait instability, and tonic-clonic seizure. It is well established in the scientific literature and supported by sponsor's data that fampridine is a pro-convulsant.

2.6.2.2 Primary pharmacodynamics

Acorda sponsored a single study to assess the binding of 4-aminopyridine, 3-hydroxy-4-aminopyridine, and 3-hydroxy-4-aminopyridine sulfate to cloned voltage-gated potassium channels.

Effects of 3 test articles on cloned Kv1.1, Kv1.2 and Kv1.4 potassium channels expressed in human embryonic kidney cells (HEK293). (Study report 080121.BVK, (b) (4) .

The *in vitro* binding of 4-aminopyridine to cloned voltage-activated potassium channels is summarized in sponsor's Table 1, below. These data show that 4-aminopyridine binds to

human potassium channels Kv1.1, Kv1.2 and Kv1.3 with similar binding affinities, whereas neither metabolite 4-amino-3hydroxypyridine nor 4-amino-3-hydroxypridine sulfate competes well for binding sites in these channels (IC_{50} range 7,886 µM to >50,000 µM).

These data were combined by the sponsor with those found in Gutman et al. (2005) that also tested the ability of 4-aminopyridine to compete for binding sites localized on other potassium channels as represented in sponsor's Table, below.

Table 1: Summary of Effect of 4 AP and Two Primary Metabolites on Selected Voltage-Activated Potassium Channels *In Vitro*

	Gutman *et al.*, 2005	Acorda's 080121.BVK Study			Gutman *et al.*, 2005		
Channel[a]	IUPHAR[b] 4-AP IC_{50} (µM)	4-AP IC_{50} (µM)	3-hydroxy 4-AP IC_{50} (µM)	3-hydroxy 4-AP sulfate IC_{50} (µM)	Current Type	Physiological Function	Tissue Location
Kv1.1	290	242	7,866	30,341	Voltage-gated channel in neurons, skeletal muscle	Maintaining membrane potential, modulating electrical excitability in neurons and muscle	Brain, heart, retina, skeletal muscle, islets
Kv1.2	590	399	23,652	21,388	Delayed rectifier	Maintaining membrane potential, modulating electrical excitability in neurons and muscle	Brain (pons, medulla, cerebellum, inferior colliculus hippocampus, thalamus, cerebral cortex, superior colliculus, midbrain, corpus striatum, olfactory bulb; neurons associated w/ mechanoreception & proprioception), spinal cord, Schwann cells, atrium, ventricle, islet, retina smooth muscle
Kv1.3	195	--	--	--	Type N voltage-gated channel in lymphocytes	Regulation of membrane potential and calcium signaling in lymphocytes and oligodendrocytes	Brain (inferior colliculus olfactory bulb, pons/medulla midbrain, superior colliculus, corpus striatum, hippocampus, cerebral cortex), lung, islets, thymus, spleen, lymph node, fibroblasts, B lymphocytes, T lymphocytes, pre-B cells, tonsils, macrophages, microglia, oligodendrocytes, osteoclasts, platelets, testis
Kv1.4	13,000[b]	399	23,191	>50,000	A-type, fast-inactivating; KV1.4/KV1.2 heteromultimers may form presynaptic A-type	Neuronal after hypolarization	Brain (olfactory bulb, corpus striatum hippocampus, superior and inferior colliculus cerebral cortex, midbrain basal ganglia pons/medulla), lung-carcinoid, skeletal muscle, heart, pancreatic islet

	Gutman *et al.*, 2005	Acorda's 080121.BVK Study			Gutman *et al.*, 2005		
Channel[a]	IUPHAR[b] 4-AP IC_{50} (µM)	4-AP IC_{50} (µM)	3-hydroxy 4-AP IC_{50} (µM)	3-hydroxy 4-AP sulfate IC_{50} (µM)	Current Type	Physiological Function	Tissue Location
Kv1.5	270	--	--	--	Ultrarapid-activating K^+ current in heart (IKur)	Similar to the ultrarapidly activating IKur current in the heart; maintains membrane potential that modulates electrical excitability in neurons	Aorta, colon, kidney, pooled colon, kidney, stomach, smooth muscle, whole embryo, hippocampus & cortex (oligodendrocytes, microglia, Schwann cells), pituitary, pulmonary artery
Kv1.7	150	--	--	--	Possibly a component of IKur in the heart	Similar to the ultra rapidly activating IKur current in the heart	Placenta, amnion, islets (mouse), skeletal muscle, heart, pulmonary arteries
Kv3.1	29	--	--	--	Delayed rectifier	Important for the high-firing frequency of auditory & fast-spiking GABAergic interneurons; regulation of action potential duration in presynaptic terminals	Brain (cerebellum, globus pallidus, subthalamic nucleus, substantia nigra,reticular thalamic nuclei, cortical and hippocampal interneurons, inferior colliculi, cochlear and vestibular nuclei), skeletal muscle, human Louckes B cells, germ cell, lung, testis
Kv3.2	100	--	--	--	Delayed rectifier	Probably in heteromeric complexes with KV3.1; important for the high-frequency firing of fast spiking GABAergic interneurons & GABA release *via* regulation of action potential duration in presynaptic terminals	Brain (fast-spiking GABAergic interneurons of the neocortex, hippocampus, and caudate; terminal fields of thalamocortical projections), islets, mesenteric artery, Schwann cells

'--' indicates Not Determined; [a]only channels with IC_{50} < 1000 µM are listed; [b]Note: value of 1 µM listed in Gutman *et al.*, 2005 appears to be incorrect. In the cited original reference (Stuhmer *et al.*, 1989) a value of 13 mM is reported.

Additional Comments: Kv1.1 mutations and pathophysiology associated with Episodic ataxia/myokymia syndrome type 1.

Kv1.3 is therapeutic target for immunosuppressants; KV1.3 inhibitors suppress T-cell activation *in vitro* and delayed type hypersensitivity *in vivo* and have proven effective for multiple sclerosis in animals; KV1.3 expression is dramatically and exclusively increased in effector memory T cells.

Kv1.4 expression increases in rat ventricular myocytes after myocardial infarction and induction of diabetes.

Kv1.5 inhibitors have potential use in management of atrial fibrillation via blockade of IKur.

Kv3.2: Fast deactivation; knockout mice show specific alterations in their cortical electroencephalographic patterns and an increased susceptibility to epileptic seizures consistent with an impairment of a cortical inhibitory mechanism15; mammalian Shaw-related family.

The sponsor did not conduct any additional receptor binding screens to evaluate possible off-target binding sites.

2.6.2.3 Secondary pharmacodynamics

No secondary pharmacology studies were sponsored by Acorda. Review of the literature suggests that 4-aminopyridine increases synaptic transmission and neuromuscular tension (Bowman and Savage, 1981). An alternate possibility for neurologic improvement following 4-aminopyridine treatment may result from immunomodulatory effects due to blockade of K_v channels in microglia, macrophages, dendritic cells, and/or T-lymphocytes (Judge and Bever, 2006).

2.6.2.4 Safety pharmacology

Acorda sponsored a few safety pharmacology studies to evaluate the cardiovascular and neurological effects of 4-aminopyridine. Three cardiovascular studies were conducted, including an *in vivo* study in beagle dogs (Study TPS 486A-501-510-93), an *in vitro* assessment of 4-aminopyridine on cloned hERG channels expressed in mammalian cells (Study Herg4AP 102003), and a determination of the effect of 4-aminopyridine on action potential parameters *in vivo* in isolated dog Purkinje fibers (Study Purk4AP102003). A neurological study was conducted to model the PK/PD changes found in the EEG of SD rats.

Neurological effects: Acorda Therapeutics sponsored a study to determine a suitable EEG parameter that is related to the CNS toxicity found in male rats administered 4-aminopyridine, and to develop a PK/PD model by which to evaluate CNS changes as a function of the plasma concentration of 4-aminopyridine. In this study, male rats were used due to the higher plasma concentrations obtained after oral administration compared to females. Administration of 4-AP to rats was associated with significant increases in higher frequency bins (20-30 Hz, 30-40 Hz, and 40-50 Hz) indicating increased CNS activity (see sponsor's Figure 4-1, reproduced below).

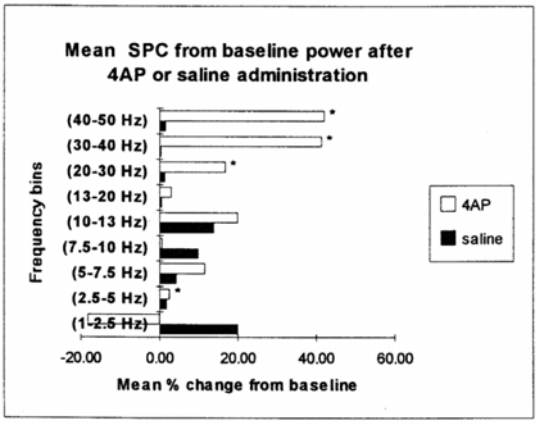


Figure 4-1: Symmetrized percent change (SPC) in power in frequency bins after 4AP or normal saline (NS) administration. n = 3. * indicates a significant change from normal saline administration, $p < 0.05$.

Pharmacodynamic data were evaluated from animals dosed with low and high doses of 4-AP. A greater predictability and confidence in the association of plasma 4-AP concentrations and CNS toxicity was identified in animals administered high doses of 4-AP. These data are provided in sponsor's Figures 4-15 and 4-16, below.

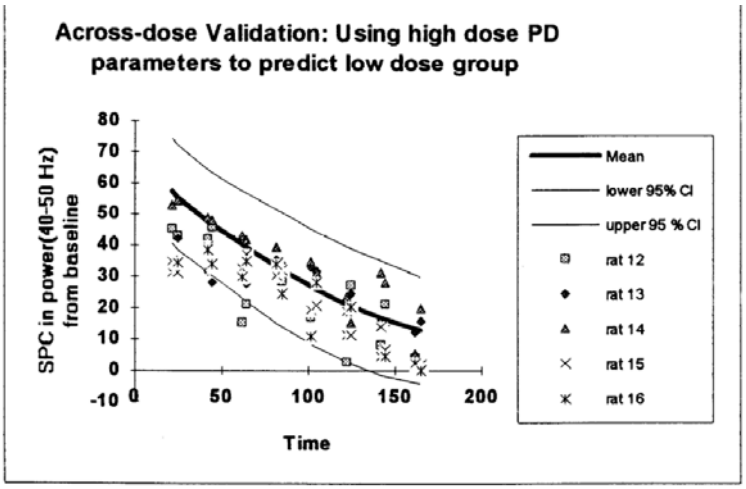


Figure 4-15: Results of across-dose validation using the pharmacodynamic parameter estimates derived from the high dose group to predict the pharmacodynamics of the low dose group of rats. Most observed data are contained within the 95% confidence limits, although the mean prediction line is biased. Six out of 80 (7.5%) fall outside the confidence limits.

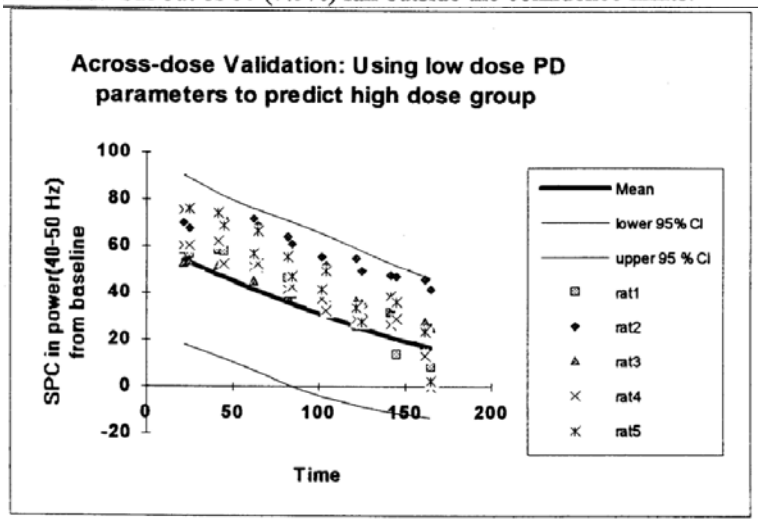


Figure 4-16: Results of across-dose validation using the pharmacodynamic parameter estimates derived from the low dose group to predict pharmacodynamics of the high dose group of rats. All observed data are contained within the 95% confidence limits, although the mean prediction line is biased.

Data from this safety pharmacology study implicate a direct relationship between plasma 4-aminopyridine concentration and the intensity of CNS toxicity as measured by changes in EEG as depicted in sponsor's Figures 4-1, 4-15, and 4-16, above. In rats, this model predicts that plasma concentrations of less than 109 ng/ml are likely to be associated with little CNS toxicity; however, plasma concentrations between 109 and 135 ng/ml represent a threshold for CNS toxicity as evidenced by effects on EEG activity power spectra. Early clinical studies (study report from 1994) found CNS toxicity at concentrations ≥100

ng/ml thus suggesting that this model may in fact be a reasonably good predictor of the human response. Likewise, several reports concluded that maintaining plasma 4-aminopyridine concentrations of <100 ng/ml reduced the seizure incidence associated with higher plasma concentrations (Stefkoski et al, 1991; Davis et al, 1990; and Jones et al, 1983). During the clinical development of fampridine-SR, seizures were observed in patients who received doses greater than the clinical dose of 10 mg b.i.d. A functional observational battery (FOB) was conducted as part of a 13-week repeat dose toxicity study in rats (b) (4)-2620-105). In this study, rats were administered fampridine at doses of 0, 0.4, 2 and 10 mg/kg/day by oral gavage. For the most part, behavior in Control, LD, and MD groups was unremarkable. In contrast, HDM and HDF had some behavioral differences.

- Increased arousal (graded somewhat high) occurred in HDM commencing Wk 4
- Aerial righting was slightly uncoordinated and occurred in HD animals at a greater incidence than for Control, LD, and MD groups beginning Wk 8.
- Tremors were limited to the HD group and were found beginning Wk 8 with an incidence of 50%, which increased to 74% by Wk 13. Withdrawal of 4-aminopyridine from Wks 13-17 resulted in the reversal of the tremor behavior.

Cardiovascular effects: Two *in vitro* studies were conducted to evaluate 4-AP effects on conductance of the delayed rectifier current (I_{Kr}, hERG) and on prolongation of the QT interval. 4-AP was shown to inhibit the hERG current (Study Herg4AP 102003) in a concentration-dependent manner. Application of 4-AP (0.3, 1, 10 and 30 mM) inhibited the hERG current by 8.2%, 31.8%, 67.6%, and 76.7%. An IC_{50} value was estimated to be 3.83 mM. This high IC_{50} value would predict a reduced liability for QT prolongation. In the isolated dog Purkinje fiber study (Purk4AP102003), concentrations of 4-AP (0.5, 5, 50 and 500 mM) were incubated with isolated dog Purkinje fibers to determine if action potential durations (APD_{30}, APD_{50}, and APD_{90}) were affected at three repolarization levels. 4-AP at the lowest concentration had no effect, whereas both 5 and 50 mM concentration-dependently increased the APD_{30}, an effect that was presumed to be due to effects on the transient outward potassium current. The highest concentration of 4-AP was found to significantly prolonged APD at all three repolarization levels tested.

Acorda Therapeutics also sponsored a GLP cardiovascular study (TPS 486A-501-510-93) in which 4-AP was administered intravenously at doses of 0.5, 1.0 or 1.5 mg/kg in anesthetized dogs. The dogs were instrumented for determination of direct blood pressure measurements and ECG recordings. A single intravenous infusion of 4-AP was administered followed by a second dose after 2 hr. No effects were found on the ECG parameters, which were recorded every 15 min for 2 hr following each dose. Peak plasma concentrations were found within 30-135 min after 4-AP administration and ranged from 1.25-2.0 µg/ml.

Abuse liability: Acorda Therapeutics did not sponsor any studies to evaluate abuse liability.

2.6.2.5 Pharmacodynamic drug interactions

Acorda Therapeutics did not sponsor any animal pharmacodynamic drug interaction studies; and did not identify any in the literature.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The sponsor submitted 17 PK/ADME and five TK bridging study reports. Only the TK studies are reviewed here; the 17 PK/ADME studies are summarized briefly.

2.6.4.2 Methods of Analysis

The sponsor submitted eight analytical method validation reports; seven were included under Section 4.2.2.1 and one under Section 4.2.2.2 Absorption. The methods for quantitating fampridine were acceptable, and LLOQs were 10 ng/ml (rat plasma) and 5 ppm (dietary formulations). The methods for quantitating fampridine and two circulating metabolites, 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate, were acceptable, and the LLOQs as measured in rat, mouse, and dog plasma were each 1.00 ng/ml.

2.6.4.3 Absorption

Acorda conducted 17 PK/ADME studies in several nonclinical species to evaluate the pharmacokinetics of 4-aminopyridine. Sponsor's Table 5 (provided below) summarizes the results of these studies as well as provides PK data from additional nonclinical species (Hartley guinea pig and mongrel dog) taken from published literature. Oral administration of fampridine is well-absorbed in Sprague-Dawley rats (M 66% and F 55%) and to a greater extent in humans (95%). In Study m-2001-03, the volume of distribution was determined to be greater than total body water for each toxicological species tested, as well as humans, indicating tissue distribution of fampridine.

Table 5: Summary of Mean Pharmacokinetic Parameters Determined for 4-AP in Rats, Guinea Pigs, Dogs, and Humans Following Single Intravenous or Oral Dose of 4-AP

Species	Gender (No.)	4-AP Dose (mg/kg); Route	HED[a] (mg/kg)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{∞} (ng·hr/mL)	Cl (mL/min/kg)	V_{dss} (L/kg)	t½ (hr)	F (%)	Reference
Sprague-Dawley rat	M (8)	2; i.v.	0.32	--	--	732	45.5	3.4	1.0	--	M-2001-03
	M (8)	2; p.o.	0.32	303.9	0.4	487	--	--	1.0	66	
	F (8)	2; i.v.	0.32	--	--	813	42.0	3.3	1.0	--	
	F (10)	2; p.o.	0.32	187.9	0.5	447	--	--	1.5	55	
Sprague-Dawley rat	M (4); F (4)	2, p.o.	0.32	230	--	547[b]	--	--	1.41	--	(b) (4) 684-012
Hartley guinea pig	M (4)	2; i.m[e]	0.43	380	0.02	764[f]	--	4.5	1.2	--	Capacio *et al.*, 1997
Beagle dog	M (4)	0.75, p.o.[i]	0.42	214.5	1.5	532[c]	--	--	--	--	(b) (4) 684-018
Mongrel dog	NS (7)	1, i.v[g].	0.55	--	--	--	21[h]	2.5	2.1	--	Rupp *et al.*, 1983
Human	M (6)	10 mg IR, p.o.	0.167[d]	46.4	1.2	285	--	--	3.7	--	ELA/G-9101
Human	M (7); F(2)	20 mg, i.v.	0.33[e]	--	--	--	9.3[1]	2.6	3.6	95	Uges *et al.*, 1982

-- indicates not applicable; animals were fasted prior to drug administration; vehicle = saline or water.
a. Human Equivalent Dose (HED); adjusted for body surface area as described in Table 1 of FDA Guidance "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" July 2005.
b. $AUC_{24\ hrs}$;
c. $AUC_{12\ hrs}$;
d. 60 kg person;
e. intramuscular;
f. $AUC_{4\ hrs}$;
g. anesthetized;
h. triexponential (n=5);
i. capsule

2.6.4.4 Distribution

Individual and mean concentrations and percent of [^{14}C]4-aminopyridine equivalents recovered from tissues were determined in a mass balance study (HWI 6379-101) conducted in male Sprague-Dawley rats administered a dose of 1 mg/kg by oral gavage. In addition, tissue elimination rates and half-life values, as well as tissue-to-blood ratios, were calculated. The following table summarizes the relevant results adapted from sponsor's Tables 7-10 that provide T_{max}, C_{max}, tissue elimination half-life ($t_{1/2}$), and tissue-to blood–ratios in male rats. Of the tissue half-lives calculated in the table below (data presented are the Mean ± SD), none of the organs had a prolonged [^{14}C]4-aminopyridine $t_{1/2}$. It is presently unknown whether fampridine distributes to

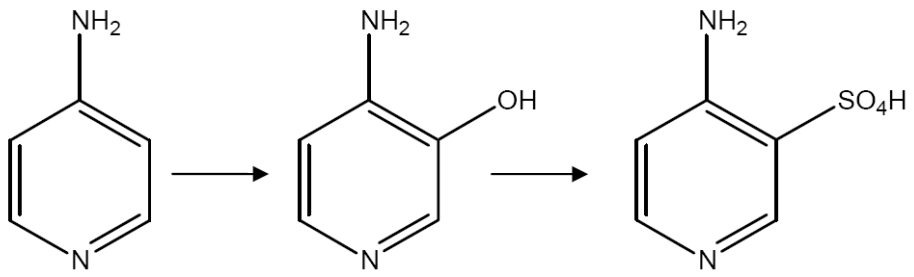
pigmented tissues, as a tissue distribution study was not conducted in pigmented animals. Fampridine was shown to be largely unbound to plasma proteins (>77% free) in rat, dog, and human.

Tissue	T_{max} (hr)	C_{max} (ng eq/g)	$t_{1/2}$ (hr)	Tissue:Blood Ratios
Blood	1	167 ± 15.8	1.21 ± 0.02	1.00
Plasma	1	181 ± 12.9	1.21 ± 0.03	1.09 ± 0.10
Carcass	1	590 ± 139	1.64 ± 0.11	5.43 ± 3.21
Cerebellum (medulla, pons, brainstem)	1	196 ± 24.6	1.24 ± 0.06	1.48 ± 0.31
Cerebrum	1	247 ± 36.5	1.63 ± 0.10	3.07 ± 1.38
Fat (reproductive area)	1	71 ± 10	1.22 ± 0.06	0.41 ± 0.10
Heart	1	350 ± 48.4	1.15 ± 0.03	2.07 ± 0.27
Kidney	1	1789 ± 236.3	1.08 ± 0.04	9.94 ± 2.63
Liver	1	725 ± 104	1.22 ± 0.03	4.17 ± 0.38
Lungs	1	463 ± 46.4	1.14 ± 0.02	2.57 ± 0.29
Muscle (thigh)	1	344 ± 39	1.18 ± 0.04	2.18 ± 0.29
Spleen	1	618 ± 58.6	1.14 ± 0.03	3.43 ± 0.46
Testes	1	248 ± 39	1.95 ± 0.18	4.33 ± 2.88
Urinary bladder	1	9552 ± 9626	1.32 ± 0.46	32.25 ± 37.44

2.6.4.5 Metabolism

Acorda conducted both *in vitro* and *in vivo* studies to determine the metabolic profile of fampridine in the nonclinical species utilized. In these studies it was determined that fampridine is metabolized to two metabolites—3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate in rats, dogs, mice, and rabbits. These metabolites are illustrated in Figure 1 (reproduced from sponsor's Pharmacokinetic Written Summary). Most of the dose in rats and dogs is excreted in the urine within the first 12 hrs, and a large proportion of it is unchanged (approximately 40%). In a radiolabeled mass-balance and metabolism study conducted in humans (Study 0496-002), the metabolites were 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate. Unlike rats and dogs, however, most of the excreted radioactivity in humans is excreted unchanged in the urine as parent drug (90.3%); and each metabolite 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate represent 4.3% and 2.6% of the radioactive dose, respectively. Thus in humans, the amount of each of the two metabolites is considered minor and, therefore, these metabolites are not of toxicological concern.

Figure 1: Diagram of Metabolism Pathway for 4-AP



4-AP **3-hydroxy-4-AP** **3-hydroxy-4-AP sulfate**

2.6.4.6 Excretion

Acorda conducted one PK and excretion profile study of [^{14}C]-4-Aminopyridine in dogs following a single oral dose of 1.03 mg/kg (12.2 µCi/kg). Results from this study indicated that the majority of the dose was excreted in the urine as shown in the following Table 1; the relevant PK parameters for single dose administration in dog are summarized in Table 2.

Table 1: Mean excretion of [^{14}C]4-Aminopyridine (0-72 hr) as percent of dose

Sex	Urine	Feces	Total
M	89.90 ± 6.13%	1.7 ± 0.72%	91.60 ± 6.84%
F	97.3 ± 0.46%	0.5 ± 0.15	97.8 ± 0.36%

Table 2: PK parameters measured in dog administered oral [^{14}C]4-Aminopyridine

Parameter	MALES (n=3)		FEMALES (n=3)	
	Mean	SD	Mean	SD
C_{max} (ng eq/g)	574	123	635	103
T_{max} (hr)	1	0.0	0.80	0.29
AUC (ng eq•hr/ml)	2030	410	1920	150
K_{el} (hr^{-1})	0.338	0.023	0.394	0.01
$t_{1/2}$ (hr)	2.1	0.14	1.8	0.04

2.6.4.7 Pharmacokinetic drug interactions

Acorda conducted a few *in vitro* studies to investigate whether 4-aminopyridine had any effect on CYP450 enzymes. Results of these studies suggested that pretreatment of CYP450 enzymes with 4-aminopyridine did not induce or inhibit these enzymes. An *in vivo* study conducted in SD rats investigated whether amitryptyline, caffeine, or baclofen interacted with 4-aminopyridine resulting in changes in its PK profile. Similar to the *in vitro* findings, pretreatment of rats by intraperitoneal injection of either amitryptiline (10 mg/kg) or caffeine (20 mg/kg) failed to change the PK parameters of an intravenous injection of fampridine (AUC, Vdss, Vdss/kg, Cl, Cl/kg, MRT, and $t_{1/2}$). In contrast, intraperitoneal injection of baclofen (3 mg/kg) resulted in a significant decrease in Cl and Cl/kg and a significant increase in fampridine exposure ($AUC_{0\text{-}inf}$), MRT, and $t_{1/2}$ after an intravenous injection of fampridine (see sponsor's Table 2.6, below). Thus, these data suggested that co-administration of baclofen, a commonly subscribed drug used concomitantly in multiple sclerosis patients, with 4-aminopyridine may result in a possible drug interaction in humans.

Parameter	4AP + Baclofen	4AP alone
Wt (kg)	0.289 (0.013)	0.291 (0.013)
$AUC_{(0-inf)}$ (µg·min/mL)	49.6* (5.2)	40.9 (7.5)
Vdss (mL)	1067.6 (274.2)	996.2 (151.3)
Vdss/kg (mL/kg)	3706.0 (999.2)	3420.5 (429.4)
Cl (mL/min)	11.7* (1.3)	15.0 (3.5)
Cl/kg (mL/min/kg)	40.5* (4.6)	51.4 (9.6)
MRT (min)	90.4* (14.5)	67.5 (9.5)
$t_{1/2}$ (min)	67.0* (11.4)	54.7 (5.9)

Table 2.6: Comparison of mean 4AP parameters obtained from baclofen-treated animals compared with placebo. * significantly different from placebo $p < 0.05$.

2.6.4.8 Other Pharmacokinetic Studies

In the Pre-NDA meeting held on 31 October 2007, Acorda Therapeutics was informed that there was a lack of critical data needed for an NDA submission, as summarized in the following nonclinical response to a pre-meeting comment (as found in FDA meeting minutes issued on 21 December 2007).

> Plasma exposure (Cmax, AUC) data in the animal species (and strains) tested in the pivotal toxicology (including general and reproductive toxicology and carcinogenicity) studies for parent compound and all major metabolites circulating in human. If these data were not collected in the pivotal (or other relevant) studies, then bridging studies will need to be conducted using similar doses and route(s) of administration. These data are needed in order to determine the relevance of the animal species to human and to document that the toxicity of parent compound and major human metabolites has been adequately tested in the nonclinical studies.

Two additional comments were provided as clarification of the proposed TK studies in the FDA Meeting Minutes sent to sponsor on 21 December 2007, as reproduced below:

- The TK bridging studies to support the embryo-fetal development and the pre- and post-natal development studies should be conducted in pregnant animals, unless you have data to document similar kinetics in pregnant and non-pregnant animals.
- The TK bridging studies need to be conducted at the same doses and with the same dosing regimen used in the definitive studies.

The sponsor submitted five bridging studies to evaluate the toxicokinetics of parent, 4-Aminopyridine, and its two major human metabolites, 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate.

1.) **Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Doses to Non-Pregnant and Pregnant Rats.**

Key findings:

- Doses used for the embryo-fetal development study were 1, 3, and 10 mg/kg/day, whereas the TK study used 1, 3, 9 mg/kg/d; doses of 1, 2, and 9 mg/kg/d were used for the pivotal fertility and early embryonic development study.
- Study is adequate for assessing the toxicokinetic profile of parent and major human metabolites to support the reproductive toxicology studies.

Study No.: 7338-105
Volume #, and page#: EDR, 4.2.3.2.1 Study 7338-105, pp. 1-352.
Conducting laboratory and location: (b) (4)
Date of study initiation: 03 April 2008
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Control No. 0000049835, 100%

Methods

Doses: Fertility and early embryonic development and embryo-fetal development: 1, 3, 9 mg/kg/day

Pre- and post-natal development: 1, 3, 9/6 mg/kg/day (dose reduction on GD11)

Species/strain: Rat/Sprague Dawley (Harlan)

Number/sex/group or time point:

Fertility and early embryonic development: M, 5/gp

Embryo-fetal development: F, 5/gp

Pre- and post-natal development: F, 5/gp

Route, formulation, volume, and infusion rate:

Oral (gavage), 0.2, 0.6 or 1.8 mg/ml solution 4-AP dissolved in distilled water (vehicle), 5 ml

Age:

Fertility and early embryonic development: M, approx. 10.5 wk of age

Embryo-fetal development: F, 9.5 or 12 wk of age

Pre- and post-natal development: F, 13.5 wk of age

Weight:

Sampling times: Sponsor provided a list of times utilized for blood collection to determine plasma concentrations of 4-aminopyridine, 3-hydroxy-4-aminopyridine, and 3-hydroxy-4-aminopyridine sulfate shown in the table below.

Scheduled Collection Time	Acceptable Deviation from Scheduled Time
0 – 15 minutes	±1 minute
16 – 30 minutes	±2 minutes
31 – 45 minutes	±3 minutes
46 – 60 minutes	±4 minutes
61 minutes – 2 hours	±5 minutes
2 hours 1 minute – 8 hours	±10 minutes
> 8 hours – 24 hours	±20 minutes
> 24 hours	±60 minutes

Housing: Fertility and early embryonic development: rats were individually housed. Diet and water were available *ad libitum*.

Dose concentration and stability:

Concentrations of 4-AP were confirmed and were within an acceptable range (97.6% to 110%) of the targeted concentration for each dose administered.

STUDY DESIGN

Segment	Group	Number of Animals	Dose Route	Target Dose Level (mg/kg/day)	Target Concentration (mg/mL)	Dose Volume (mL/kg)
I	1	5 Males	Oral	1	0.2	5
	2	5 Males	Oral	3	0.6	5
	3	5 Males	Oral	9	1.8	5
II	4	12 Females	Oral	1	0.2	5
	5	12 Females	Oral	3	0.6	5
	6	12 Females	Oral	9	1.8	5
III	7	12 Females	Oral	1	0.1	10
	8	12 Females	Oral	3	0.3	10
	9	12 Females	Oral	9/6	0.9/0.6	10

Note: For Group 9, on gestation day (GD)11, the dose level was decreased from 9 mg/kg/day to 6 mg/kg/day to mimic the design of a previous reproductive toxicology study.

Dosing Regimens

Fertility and early embryonic development (Group 1-3)

Males were administered single daily doses of 4-aminopyridine by oral gavage for 14 consecutive days. In HD group, 3/5 died on Day 2, thus this group had 2/5 with 14 days consecutive dosing and 3/5 with 12 days consecutive dosing. (The study director stated that this did not compromise the conclusions.)

Sample collection for TK analyses: Blood (0.25 ml) was collected via a jugular vein and transferred into tubes containing K_2EDTA.

- Days 1 and 14—Each animal
  - Predose and postdose (0.5, 0.75, 1, 2, 3, 6, and 24 hr) samples were collected.

Embryo-fetal development (Group 4-6)

Non-pregnant F were administered single daily doses of 4-aminopyridine by oral gavage for 14 consecutive days. In HD group, 1/12 died on Day 2, thus a replacement animal (B09265) was dosed for 12 consecutive days. On Day 15, a non-dosed male was cohabitated for mating. Females were administered single daily doses during the mating period. Mating was determined by visual inspection for copulatory plug and confirmed by visual examination of uterus during sacrifice. The mating period was extended for up to 6 days. Non-gravid females were dosed until the end of in-life period. All surviving F were euthanized on GD 13.

Sample collection for TK analyses: Blood (0.25 ml) was collected via a jugular vein and transferred into tubes containing K_2EDTA.

- Days 1 and 14, and on GD13—6 animals/4 time points
  - Predose, postdose at 0.5, 0.75, 1, 2, 3, 6, and 24 hr

Pre- and post-natal development (Group 7-9)

Pregnant F (timed-parous) were received on GD3. Dosing of 4-aminopyridine commenced on GD7; dams were administered single daily doses by oral gavage for approximately 28 consecutive days. Dosing was suspended for any dam giving birth

during the scheduled dosing time for that day. On GD11, HD was reduced from 9 mg/kg/day to 6 mg/kg/day to simulate the pivotal reproductive toxicology study design.

Sample collection for TK analyses: Blood (0.25 ml) was collected via a jugular vein and transferred into tubes containing K_2EDTA.

- Days GD7, GD16, and on lactation day (LD 14)—6 animals/4 time points
  - Predose, postdose at 0.5, 0.75, 1, 2, 3, 6, and 24 hr

Observations, Times and Results:

Antemortem observations: Frequency not well described in methods.

Fertility and early embryonic development

- HD (3/5) deaths (B09236, B09237, and B09239) occurred on Day 2, approximately 1.5 to 3.25 hrs following 24 hr sampling; animals found dead with no visible adverse observations. Possible COD, combined stress of serial blood collections and test article administration. Animals were replaced; however, these animals only had 12 days of test article administration. Clinical signs included body tremors, piloerection and nasal discharge.
- HD signs: whole body tremors, piloerection, nasal discharge, red liquid discharge preputial area, low food consumption and few feces.

Embryo-fetal development

- HD death (1/5, B09265) on Day 2. Clinical signs 1.5 hr postdose: hyperactivity and hunched posture 1.5 hr postdose; and at 2.5 hr tremors, hyperactivity, 3 seizures, rapid respirations, lateral recumbency, foamy discharge mouth.
- HD gp (B09265-B09270) hyperactive, hunched posture 1.5 hr postdose (D2); other signs included ataxia (D3, B09237), 3.75 hr postdose all HD tremors, hunched posture, cold to touch (D14), 3.25 hr postdose all HD tremors.

Pre- and post-natal development

- LDF (B09893) seizure 3 hr postdose (D1); HDF—all were hyperactive and exhibited tremors.
- HDF B09922 whole body convulsions (study day 16)
- HDF(all) postdose tremors (D15 at 2.75 hr, D29 at 2hr)

Body weights: Individual body wt recorded at randomization (Day -1) and twice weekly during test period and following parturition (Dams only).

Fertility and early embryonic development

- LDM and MDM, unremarkable.
- HDM, decreased mean body wt on D4 (-11.3%) and D7 (-9.8%) compared to pretreatment mean body wt value.

Embryo-fetal development

- LDF, D1-18 unremarkable. Increased (>10%, compared to pretreatment mean body wt).
  - Days 22 (+6.9%), 25 (+12.1%), and 32 (25.1%)

- MDF, D1-18 unremarkable. Increased (>10%, compared to pretreatment mean body wt).

- Days 22 (+7.7%), 25 (+12.1%), and 32 (+19.8%)
- HDF, D1-22 unremarkable. Increased (>10%, compared to pretreatment mean body wt).
  - Days 25 (+11.4%) and 32 (+18.3%)

Pre- and post-natal development

- LDF, increased (>10%, compared to pretreatment mean body wt).
  - Days 8 (+15.9%), 12 (+30.2%), 15 (+48.9%), 16 (+16.3%), 17 (+15.5%), 18 (+12.7%), 25 (+19.8%), and 29 (+21%)
- MDF, increased (>10%, compared to pretreatment mean body wt).
  - Days 8 (+11%), 12 (+27.8%), 15 (+46.4%), 16 (+12.6%), 25 (+14.6%), and 29 (+15.4%)
- HDF, increased (>10%, compared to pretreatment mean body wt).
  - Days 12 (+22.4%), 15 (+37.8%), 16 (+10.2%), 25 (+13%), and 29 (+15.7%)

Food consumption: Recorded twice weekly through end of in-life phase. The following tables show mean percent change from the mean pretreatment values for food consumption for each treatment group. Treatment Day -1 corresponds to pre-treatment which was set to 100% and the actual (mean food consumption value) provided.

Fertility and early embryonic development

Males	Treatment Day				
	-1	2	6	9	13
LD	100% (27g)	-18.5%	-7.4%	+3.7%	-7.4%
MD	100% (28g)	-42.8%	-14.3%	NC	-7.1%
HD	100% (26g)	-73%	-23.1%	NC	-7.7%

NC=No change from pretreatment value.

Embryo-fetal development

Females	Treatment Day								
	-1	3	7	10	13	21	24	28	31
LD	100% (20g)	-20%	-31%	-7.6%	-5%	-20%	-5%	-5%	-10%
MD	100% (19g)	-26.3%	-15.8%	+5.3%	NC	-15.8%	-5.3%	-15.8%	-42.1%
HD	100% (19g)	-47.4%	-10.5%	+5.3%	NC	-5.3%	-5.3%	-5.3%	NC

NC=No change from pretreatment value.

Pre- and post-natal development

Females	Treatment Day								
	-1	3	7	11	15	17	21	24	28
LD	100% (18g)	NC	+11.1%	+16.7%	-22.2%	+38.9%	+111%	+111%	+167%
MD	100% (18g)	-27.8%	NC	+22.2%	NC	+61.1%	+117%	+117%	+211%
HD	100% (16g)	-37.5%	+18.8%	+37.5%	+12.5%	+69.5%	+131%	+156%	+225%

NC=No Change from pretreatment value

Toxicokinetics: TK analyses were performed on rat plasma to determine concentrations of 4-aminopyridine and its two major human metabolites, 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate, to determine C_{max}, T_{max}, AUC, and $t_{1/2}$.

Fertility and early embryonic development (M)

Sponsor's summaries of the mean TK parameters for 4-Aminopyridine (parent), 3-Hydroxy-4-Aminopyridine, and 3-Hydroxy-4-Aminopyridine Sulfate are provided below.

Table 1. Summary of the Mean Toxicokinetic Parameters for Fampridine in Rat Plasma: Segment I

Dose Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}
						Day 1						
1	1	M	Mean	139	139	0.550	311	483	483	386	2.21	NA
			SD	22	22	0.112	41	48	49	2	0.38	NA
			N	5	5	5	5	5	5	3	3	NA
2	3	M	Mean	224	74.7	0.650	1017	1350	450	1297	3.13	NA
			SD	48	15.9	0.224	366	161	54	203	0.56	NA
			N	5	5	5	5	5	5	3	3	NA
3	9	M	Mean	277	30.7	1.00	2696	2696	300	2679	5.60	NA
			SD	59	6.6	0.61	521	521	58	NA	NA	NA
			N	5	5	5	5	5	5	1	1	NA
						Day 14						
1	1	M	Mean	126	126	0.550	327	517	517	NA	2.00	1.07
			SD	24	24	0.112	74	56	56	NA	0.51	0.10
			N	5	5	5	5	5	5	NA	4	5
2	3	M	Mean	252	84.0	0.650	1069	1479	493	NA	4.38	1.10
			SD	37	12.2	0.224	511	297	99	NA	2.49	0.21
			N	5	5	5	5	5	5	NA	3	5
3	9	M	Mean	545	60.5	0.500	3486	3486	387	NA	4.48	1.31
			SD	128	14.3	0	739	739	82	NA	1.11	0.26
			N	5	5	5	5	5	5	NA	4	5

Table 5. Summary of the Mean Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine in Rat Plasma: Segment I

Dose Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	M:P Ratio AUC_{0-24}
							Day 1						
1	1	M	Mean	8.53	8.53	0.500	14.3	22.9	22.9	16.8	1.55	NA	0.0468
			SD	1.18	1.18	0	3.5	9.6	9.6	3.4	0.52	NA	0.0167
			N	5	5	5	5	5	5	3	3	NA	5
2	3	M	Mean	10.7	3.57	0.550	27.7	51.9	17.3	38.8	2.73	NA	0.0382
			SD	1.3	0.43	0.112	5.0	11.2	3.8	4.7	0.18	NA	0.0048
			N	5	5	5	5	5	5	3	3	NA	5
3	9	M	Mean	12.1	1.34	0.800	140	140	15.5	133	11.8	NA	0.0530
			SD	2.4	0.27	0.274	28	28	3.1	NA	NA	NA	0.0124
			N	5	5	5	5	5	5	1	1	NA	5
							Day 14						
1	1	M	Mean	8.13	8.13	0.500	17.5	27.7	27.7	NA	1.82	1.41	0.0529
			SD	1.41	1.41	0	4.9	8.5	8.5	NA	0.48	0.75	0.0128
			N	5	5	5	5	5	5	NA	3	5	5
2	3	M	Mean	21.3	7.09	0.500	56.4	112	37.4	NA	2.56	2.18	0.0735
			SD	6.8	2.25	0	22.4	50	16.5	NA	NA	0.92	0.0169
			N	5	5	5	5	5	5	NA	1	5	5
3	9	M	Mean	35.8	3.98	0.500	199	249	27.6	NA	5.52	1.87	0.0715
			SD	8.1	0.90	0	86	53	6.0	NA	NA	0.74	0.0070
			N	5	5	5	5	5	5	NA	2	5	5

Table 9. Summary of the Mean Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate in Rat Plasma: Segment I

Dose Group	Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	M:P Ratio AUC_{0-24}
							Day 1						
1	1	M	Mean	93.9	93.9	0.550	176	287	287	260	3.84	NA	0.593
			SD	11.0	11.0	0.112	31	65	65	97	1.93	NA	0.105
			N	5	5	5	5	5	5	4	4	NA	5
2	3	M	Mean	128	42.7	0.550	482	607	202	567	3.08	NA	0.449
			SD	21	6.9	0.112	289	214	71	286	1.18	NA	0.135
			N	5	5	5	5	5	5	4	4	NA	5
3	9	M	Mean	142	15.8	0.750	1514	1514	169	1443	8.46	NA	0.576
			SD	29	3.2	0.250	252	252	28	NA	NA	NA	0.122
			N	5	5	5	5	5	5	1	1	NA	5
							Day 14						
1	1	M	Mean	95.3	95.3	0.700	203	302	302	NA	2.01	1.08	0.583
			SD	19.5	19.5	0.209	36	55	55	NA	0.56	0.26	0.075
			N	5	5	5	5	5	5	NA	28	5	5
2	3	M	Mean	159	53.1	0.500	758	943	314	NA	4.73	1.67	0.623
			SD	46	15.4	0	415	334	112	NA	2.47	0.82	0.101
			N	5	5	5	5	5	5	NA	52	5	5
3	9	M	Mean	341	37.9	0.800	2106	2106	234	NA	4.57	1.46	0.585
			SD	135	15.0	0.671	831	831	92	NA	0.70	0.72	0.129
			N	5	5	5	5	5	5	NA	3	5	5

Embryo-fetal development (F)

Sponsor's summaries of the mean TK parameters for 4-Aminopyridine (parent), 3-Hydroxy-4-Aminopyridine, and 3-Hydroxy-4-Aminopyridine Sulfate are provided below.

Table 2. Toxicokinetic Parameters for Fampridine in Rat Plasma: Segment II

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	Male:Female Ratio[a] C_{max}	Male:Female Ratio[a] AUC_{0-24}
							Day 1						
4	1	F	155	155	0.500	340	525	525	415	2.54	NA	0.897	0.920
5	3	F	232	77.3	0.500	1334	1334	445	1340	3.21	NA	0.966	1.01
6	9	F	293	32.6	0.500	3429	3429	381	NC	NC	NA	0.945	0.786
							Day 14						
4	1	F	117	117	0.750	601	601	601	NA	2.84	1.14	1.08	0.860
5	3	F	237	79.0	0.500	1451	1451	484	NA	3.90	1.09	1.06	1.02
6	9	F	607	67.4	0.750	4078	4078	453	NA	7.25	1.19	0.898	0.855
							GD 13						
4	1	F	117	117	0.500	666	666	666	NA	3.39	1.27	1.08	0.776
5	3	F	225	75.0	0.750	1519	1519	506	NA	4.23	1.14	1.12	0.974
6	9	F	502	55.8	0.500	4168	4168	463	NA	5.84	1.22	1.09	0.836

a Male:Female ratios are a comparison of Segment I Day 1 to Segment II Day 1; or Segment I Day 14 to Segment II Day 14 and GD13.

Table 6. Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine in Rat Plasma: Segment II

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	M:P Ratio AUC_{0-24}	Male:Female Ratio[a] C_{max}	Male:Female Ratio[a] AUC_{0-24}
							Day 1							
4	1	F	16.1	16.1	0.500	34.2	56.5	56.5	43.6	2.63	NA	0.108	0.530	0.405
5	3	F	25.3	8.43	0.500	64.5	140	46.7	NC	NC	NA	0.105	0.423	0.371
6	9	F	29.3	3.26	0.500	320	320	35.6	NC	NC	NA	0.0933	0.413	0.438
							Day 14							
4	1	F	13.6	13.6	0.500	35.0	59.5	59.5	NA	3.38	1.05	0.0990	0.598	0.466
5	3	F	29.5	9.83	0.500	85.0	166	55.3	NA	6.13	1.19	0.114	0.722	0.675
6	9	F	56.4	6.27	0.750	432	432	48.0	NA	7.37	1.35	0.106	0.635	0.576
							GD 13							
4	1	F	11.0	11.0	0.750	31.8	63.1	63.1	NA	4.00	1.12	0.0947	0.739	0.439
5	3	F	21.9	7.30	0.500	77.7	154	51.3	NA	5.65	1.10	0.101	0.973	0.727
6	9	F	47.2	5.24	0.500	423	423	47.0	NA	5.74	1.32	0.101	0.758	0.589

a Male:Female ratios are a comparison of Segment I Day 1 to Segment II Day 1; or Segment I Day 14 to Segment II Day 14 and GD13.

Pre- and post-natal development (F)

Table 10. Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate in Rat Plasma: Segment II

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	M:P Ratio AUC_{0-24}	Male:Female Ratio[a] C_{max}	Male:Female Ratio[a] AUC_{0-24}
							Day 1							
4	1	F	104	104	1.00	236	363	363	NC	NC	NA	0.691	0.903	0.791
5	3	F	154	51.3	0.500	904	904	301	909	3.55	NA	0.678	0.831	0.671
6	9	F	166	18.4	0.500	2115	2115	235	NC	NC	NA	0.617	0.855	0.716
							Day 14							
4	1	F	65.5	65.5	0.500	362	362	362	NA	2.99	1.00	0.602	1.45	0.834
5	3	F	133	44.3	0.500	856	856	285	NA	4.22	0.947	0.590	1.20	1.10
6	9	F	436	48.4	0.750	2132	2132	237	NA	7.22	1.01	0.523	0.784	0.988
							GD 13							
4	1	F	45.1	45.1	0.750	149	313	313	NA	6.38	0.862	0.470	2.11	0.964
5	3	F	113	37.7	1.00	818	818	273	NA	4.41	0.905	0.539	1.41	1.15
6	9	F	301	33.4	0.500	2212	2212	246	NA	5.78	1.05	0.531	1.13	0.952

a Male:Female ratios are a comparison of Segment I Day 1 to Segment II Day 1; or Segment I Day 14 to Segment II Day 14 and GD13.

Sponsor's summaries of the mean TK parameters for 4-Aminopyridine (parent), 3-hydroxy-4-aminopyridine, and 3-hydroxy-4-aminopyridine sulfate are provided below.

Table 3. Toxicokinetic Parameters for Fampridine in Rat Plasma: Segment III

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	Male:Female Ratio[b] C_{max}	Male:Female Ratio[b] AUC_{0-24}	Segment III:II Ratio[c] C_{max}	Segment III:II Ratio[c] AUC_{0-24}
							GD 7								
7	1	F	150	150	0.500	654	654	654	660	3.78	NA	0.927	0.739	0.968	1.25
8	3	F	273	91.0	0.500	1711	1711	570	1830	6.75	NA	0.821	0.789	1.18	1.28
9	9	F	438	48.7	0.500	3797	3797	422	NC	NC	NA	0.632	0.710	1.49	1.11
							GD 16								
7	1	F	136	136	0.750	750	750	750	NA	2.56	1.15	0.926	0.689	1.16	1.25
8	3	F	262	87.3	0.750	2036	2036	679	NA	5.23	1.19	0.962	0.726	1.11	1.40
9	6	F	452	75.3	0.750	3253	3253	542	NA	6.63	NA	NA	NA	NA	NA
							LD 14								
7	1	F	152	152	0.500	699	699	699	NA	3.46	1.07	0.829	0.740	1.30	1.05
8	3	F	332	111	0.750	1813	1813	604	NA	2.96	1.06	0.759	0.816	1.48	1.19
9	6	F	612	102	0.500	3462	3462	577	NA	3.96	NA	NA	NA	NA	NA

b Male:Female ratios are a comparison of Segment I Day 1 to Segment III GD 7; or Segment I Day 14 to Segment III GD 16 and LD14.
c Segment III:II ratios are a comparison of Segment III GD 7 to Segment II Day 1; or Segment III GD 16 to Segment II Day 14; or Segment III LD 14 to Segment II GD 13.

Table 7. Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine in Rat Plasma: Segment III

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	M:P Ratio AUC_{0-24}	Male:Female Ratio[b] C_{max}	Male:Female Ratio[b] AUC_{0-24}	Segment III:II Ratio[c] C_{max}	Segment III:II Ratio[c] AUC_{0-24}
								GD 7								
7	1	F	15.4	15.4	0.500	37.7	65.9	65.9	50.7	2.87	NA	0.101	0.554	0.347	0.957	1.17
8	3	F	27.5	9.17	0.500	171	171	57.0	179	5.98	NA	0.100	0.389	0.304	1.09	1.22
9	9	F	35.6	3.96	0.500	371	371	41.2	NC	NC	NA	0.0977	0.340	0.377	1.22	1.16
								GD 16								
7	1	F	9.43	9.43	0.500	26.8	46.6	46.6	NA	3.96	0.707	0.0621	0.862	0.594	0.693	0.783
8	3	F	18.9	6.30	0.750	64.0	143	47.7	NA	10.6	0.836	0.0702	1.13	0.783	0.641	0.861
9	6	F	28.3	4.72	0.500	214	214	35.7	NA	5.87	NA	0.0658	NA	NA	NA	NA
								LD 14								
7	1	F	10.3	10.3	1.00	32.0	51.1	51.1	NA	2.45	0.775	0.0731	0.789	0.542	0.936	0.810
8	3	F	22.4	7.47	0.750	69.9	132	44.0	NA	3.61	0.772	0.0728	0.951	0.848	1.02	0.857
9	6	F	41.6	6.93	1.00	199	199	33.2	NA	3.60	NA	0.0575	NA	NA	NA	NA

b Male:Female ratios are a comparison of Segment I Day 1 to Segment III GD 7; or Segment I Day 14 to Segment III GD 16 and LD14.
c Segment III:II ratios are a comparison of Segment III GD 7 to Segment II Day 1; or Segment III GD 16 to Segment II Day 14; or Segment III LD 14 to Segment II GD 13.

Table 11. Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate in Rat Plasma: Segment III

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AR AUC_{0-24}	M:P Ratio AUC_{0-24}	Male:Female Ratio[b] C_{max}	Male:Female Ratio[b] AUC_{0-24}	Segment III:II Ratio[c] C_{max}	Segment III:II Ratio[c] AUC_{0-24}
								GD 7								
7	1	F	69.9	69.9	0.750	300	300	300	302	3.39	NA	0.459	1.34	0.957	0.672	0.826
8	3	F	116	38.7	0.500	937	937	312	998	6.80	NA	0.548	1.10	0.648	0.753	1.04
9	9	F	192	21.3	0.750	1668	1668	185	NC	NC	NA	0.439	0.740	0.908	1.16	0.789
								GD 16								
7	1	F	44.0	44.0	1.00	128	218	218	NA	3.17	0.727	0.291	2.16	1.38	0.672	0.602
8	3	F	92.5	30.8	0.750	637	637	212	NA	4.90	0.680	0.313	1.72	1.48	0.696	0.744
9	6	F	130	21.7	0.750	1003	1003	167	NA	5.94	NA	0.308	NA	NA	NA	NA
								LD 14								
7	1	F	52.8	52.8	0.750	268	268	268	NA	3.71	0.893	0.383	1.80	1.13	1.17	0.856
8	3	F	108	36.0	0.750	604	604	201	NA	3.59	0.645	0.333	1.48	1.56	0.952	0.738
9	6	F	224	37.3	1.00	1148	1148	191	NA	4.19	NA	0.332	NA	NA	NA	NA

b Male:Female ratios are a comparison of Segment I Day 1 to Segment III GD 7; or Segment I Day 14 to Segment III GD 16 and LD14.

c Segment III:II ratios are a comparison of Segment III GD 7 to Segment II Day 1; or Segment III GD 16 to Segment II Day 14; or Segment III LD 14 to Segment II GD 13.

2.) **Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites on Days 1 and 28 Following Dietary Administration of Fampridine in Mice**

Key findings:

- Dietary administration of increasing doses of 4-aminopyridine resulted in increased plasma C_{max} and $AUC_{0\text{-}24h}$ values that were not consistently dose proportional.
  - Similarly, C_{max} and $AUC_{0\text{-}24h}$ for metabolites M1 and M2 increased with dose although not consistently dose proportionally.
- 3-Hydroxy-4-Aminopyridine (M1) is a major metabolite in both sexes based on $AUC_{0\text{-}24h}$.
  - M ≈ 84.1-106% of parent
  - F ≈ 52.2-67.8% of parent
- 3-Hydroxy-4-Aminopyridine Sulfate (M2) is a major metabolite in both sexes based on $AUC_{0\text{-}24h}$.
  - M ≈ 164-300% of parent
  - F ≈ 199-273% of parent

Study No.: 7338-103
Volume #, and page#: EDR, 4.2.3.2.1 Study 7338-103, pp. 1-195.
Conducting laboratory and location: (b) (4)
Date of study initiation: 13 March 2008
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Control No. 0000049835, 100%

Methods

Doses: 2 (LD), 12.5 (MD), and 80 (HD) mg/kg/day
Species/strain: Mouse/ICR Hsd:ICR(CD-1)
Number/sex/group or time point: 42/sex/group
Route, formulation, volume, and infusion rate: Oral, Dietary admixture (prepared weekly),
Age: ≈5 wk of age
Weight: data not provided
Sampling times: 1, 4, 8, 12, 16, 20 and 24 hr postdose feeding

Housing: During test period, mice were individually housed in stainless steel wire-mesh cages. Diet (Certified Rodent Diet #5002) and water were available *ad libitum*.

Dose concentration and stability:
Diet samples collected for Wk 1 and 4 were analyzed for homogeneity. Diet sample also collected for Wk 2 LD due to out-of-specification results for Wk 1 (LDF). Homogeneity samples were also analyzed to confirm concentrations. Except for Week 1 LDF, all Week 1 and 4

4-AP concentrations were within 15% of theoretical mean and ≤ 5% variability from the mean. Week 2 reanalysis performed for LDM and LDF, both formulations were within the specifications for homogeneity and concentration.

Unique study design or methodology (if any): see sponsor's table below.

Group	Number of Animals	Dose Route	Target Dose Level (mg/kg/day)
1	42 males/42 females	Diet	2
2	42 males/42 females	Diet	12.5
3	42 males/42 females	Diet	80

Note: Each mouse was given test article in feed daily for 1 day or for 28 consecutive days. After appropriate anesthetization, blood samples were taken from 3 mice/sex/time point by cardiac puncture on Day 1 and Day 28.

Sample collection for TK analyses: Post feeding on Days 1 and 28, mice (3 mice/sex/group) were anesthetized using isoflurane and blood (≈ 1 ml) was collected into tubes containing K_2EDTA 1, 4, 8, 12, 16, 20, and 24 hr.
TK Analysis: Whenever possible, C_{max}, T_{max} and AUC were calculated.

Observations, Times and Results:
Antemortem observations: Frequency not well described in methods.

- HDM (A06249) died on D8, COD was unknown.
- MDF (2/20; A06318 and A06332) non-formed feces.

Body weights: Individual body wt recorded at randomization (Day 15 of acclimation) and twice weekly during test period.

- M, F mean body wt increased during the treatment period in all dose groups.

Food consumption: Recorded twice weekly through end of in-life phase.

- Day 1, M, F showed dose-related decrease in mean food consumption.
- Day 27, M (unremarkable); F, the decreased food consumption was less evident.

Animal disposition: Following blood sample collection, mice were euthanized by overdose of isoflurane. Carcasses were not retained.

Toxicokinetic analyses: Evaluated concentrations of 4-aminopyridine, 3-hydroxy-4-Aminopyridine (M1), and 3-hydroxy-4-aminopyridine sulfate (M2) were quantitated and toxicokinetic parameters were determined on Days 1 and 28.

4-Aminopyridine (parent)

- Day 1, exposures increased with increase in dose.
  - M, dose range 1.66 to 41.2 mg/kg/day
  - F, dose range 1.13 to 36.5 mg/kg/day
- Day 28, exposures increased with increase in dose from 2 to 80 mg/kg/day.
- Increases in mean plasma C_{max} and AUC_{1-24} were not consistently dose proportional.

3-Hydroxy-4-Aminopyridine (M1)

- Day 1, exposures increased with increase in dose.
  - M, dose range 1.66 to 41.2 mg/kg/day
  - F, dose range 1.13 to 36.5 mg/kg/day
- Day 28, exposures increased with increase in dose from 2 to 80 mg/kg/day.
- Increases in mean plasma C_{max} and AUC_{1-24} were not consistently dose proportional.
- Mean plasma AUC1-24 metabolite to parent ratios ranged in M from 0.849 to 1.06 and in F from 0.518 to 0.679.
- Sponsor concluded, "3-hydroxy-4-aminopyridine is a major circulating metabolite of fampridine following dietary administration in the mouse".

3-Hydroxy-4-Aminopyridine Sulfate (M2)

- Day 1, exposures increased with increase in dose.
  - M, dose range 1.66 to 41.2 mg/kg/day
  - F, dose range 1.13 to 36.5 mg/kg/day
- Day 28, exposures increased with increase in dose from 2 to 80 mg/kg/day.
- Increases in mean plasma C_{max} and AUC_{1-24} were not consistently dose proportional.
- Mean plasma AUC1-24 metabolite to parent ratios ranged in M from 1.65-3.04 and in F from 1.98 to 2.72.
- Sponsor concluded, "3-hydroxy-4-aminopyridine is a major circulating metabolite of fampridine following dietary administration in the mouse".

The toxicokinetic parameters evaluated and calculated for 4-Aminopyridine (Table 1), 3-Hydroxy-4-Aminopyridine (Table 3), and 3-Hydroxy-4-Aminopyridine Sulfate (Table 5) are reproduced from the sponsor's original study report.

Table 1. Toxicokinetic Parameters for Fampridine in Mouse Plasma

Dose Group	Fampridine Dose[a] Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	AUC_{0-24} (ng·hr/mL)
				Day 1				
1	1.66	M	14.6	8.79	1.00	146	88.1	154
	1.13	F	7.37	6.52	4.00	108	95.2	109
2	10.1	M	39.3	3.89	12.0	647	64.0	666
	6.54	F	37.7	5.76	4.00	568	86.9	578
3	41.2	M	291	7.06	12.0	3727	90.5	3782
	36.5	F	166	4.54	12.0	2572	70.5	2593
				Day 28				
1	2	M	18.4	9.19	4.00	272	136	284
		F	13.3	6.67	4.00	161	80.6	168
2	12.5	M	74.1	5.93	4.00	1080	86.4	1131
		F	51.3	4.11	1.00	624	49.9	667
3	80	M	320.3	4.00	1.00	5346	66.8	5664
		F	317.3	3.97	8.00	5257	65.7	5419

a Actual (mean) fampridine dose levels are presented on Day 1 and nominal on Day 28.

Table 3. Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine in Mouse Plasma

Dose Group	Fampridine Dose[a] Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} M/P Ratios
				Day 1					
1	1.66	M	9.48	5.71	1.00	127	76.3	131	0.866
	1.13	F	6.16	5.45	4.00	70.1	62.0	71.0	0.652
2	10.1	M	37.2	3.68	12.0	588	58.2	604	0.909
	6.54	F	21.2	3.25	24.0	328	50.2	334	0.578
3	41.2	M	214	5.19	12.0	3339	81.0	3375	0.896
	36.5	F	100	2.75	24.0	1416	38.8	1428	0.551
				Day 28					
1	2	M	16.4	8.22	4.00	231	115	239	0.849
		F	8.44	4.22	8.00	109	54.7	114	0.679
2	12.5	M	54.2	4.34	4.00	979	78.3	1029	0.906
		F	30.8	2.46	1.00	374	29.9	401	0.600
3	80	M	333	4.16	4.00	5692	71.2	5974	1.06
		F	183	2.29	8.00	2724	34.0	2828	0.518

a Actual (mean) fampridine dose levels are presented on Day 1 and nominal on Day 28.

Table 5. Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate in Mouse Plasma

Dose Group	Fampridine Dose[a] Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} M/P Ratios
				Day 1					
1	1.66	M	17.1	10.3	1.00	272	164	280	1.86
	1.13	F	15.7	13.9	4.00	234	207	237	2.17
2	10.1	M	74.0	7.33	12.0	1090	108	1116	1.69
	6.54	F	82.4	12.6	24.0	1150	176	1176	2.02
3	41.2	M	507	12.3	24.0	6164	150	6203	1.65
	36.5	F	375	10.3	12.0	5646	155	5705	2.20
				Day 28					
1	2	M	47.9	23.9	16.0	559	280	581	2.06
		F	30.7	15.4	8.00	438	219	459	2.72
2	12.5	M	114	9.12	4.00	2101	168	2187	1.94
		F	118	9.47	24.0	1490	119	1605	2.39
3	80	M	964	12.1	8.00	16268	203	16984	3.04
		F	658	8.23	12.0	10408	130	10797	1.98

a Actual (mean) fampridine dose levels are presented on Day 1 and nominal on Day 28.

The C_{max} data (units: ng/ml) are summarized in the table below.

DOSE	MALES			FEMALES		
	2 mg/kg/d	**12.5 mg/kg/d**	**80 mg/kg/d**	**2 mg/kg/d**	**12.5 mg/kg/d**	**80 mg/kg/d**
4-AMINOPYRIDINE (PARENT)						
1st	14.6	39.3	291	7.37	37.7	166
28th	18.4	74.1	320.3	13.3	51.3	317.3
3-HYDROXY-4-AMINOPYRIDINE (M1)						
1st	9.28	37.2	214	6.16	21.2	100
28th	16.4	54.2	333	8.44	30.8	183
3-HYDROXY-4-AMINOPYRIDINE SULFATE (M2)						
1st	17.1	74	507	15.7	82.4	375
28th	47.9	114	964	30.7	118	658

The $AUC_{0\text{-}24h}$ data (units: ng•hr/ml) are summarized below.

DOSE	MALES			FEMALES		
	2 mg/kg/d	12.5 mg/kg/d	80 mg/kg/d	2 mg/kg/d	12.5 mg/kg/d	80 mg/kg/d
4-AMINOPYRIDINE (PARENT)						
1st	154	647	3727	109	578	2593
28th	284	1131	5664	168	667	5419
3-HYDROXY-4-AMINOPYRIDINE (M1)						
1st	131	604	3375	71	334	1428
28th	239	1029	5974	114	401	2828
3-HYDROXY-4-AMINOPYRIDINE SULFATE (M2)						
1st	280	1116	6203	237	1176	5705
28th	581	2187	16984	459	1607	10797

3.) **Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites on Days 1 and 28 Following Dietary Administration of Fampridine in Rats**

Key findings:

- No females were examined in this study—there are no TK data to support the females in the carcinogenicity study.
- Dietary administration of increasing doses of 4-aminopyridine resulted in increased plasma C_{max} and $AUC_{0\text{-}24h}$ values that were not consistently dose proportional.
    - Similarly, C_{max} and $AUC_{0\text{-}24h}$ for metabolites M1 and M2 increased with dose although not consistently dose proportionally.
- 3-hydroxy-4-aminopyridine (M1) is a major metabolite in males.
    - M ≈ 84.1-106% of parent
- 3-hydroxy-4-aminopyridine sulfate (M2) is a major metabolite in males.
    - M ≈ 164-300% of parent

Study No.: 7338-104
Volume #, and page#: EDR, 4.2.3.2.1 Study 7338-104, pp. 1-140.
Conducting laboratory and location: (b) (4)
Date of study initiation: 13 March 2008
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Control No. 0000049835, 100%

Methods

Doses: 2 (LD), 6 (MD), and 18 (HD) mg/kg/day
Species/strain: Rat/Hsd:Sprague Dawley SD
Number/sex/group or time point: 5M/gp

Route, formulation, volume, and infusion rate: Oral, Dietary admixture (prepared weekly)
Age: ≈10.5 wk of age
Weight: Not provided.
Sampling times: 1, 4, 8, 12, 16, 20 and 24 hr postdose feeding

Housing: During test period, rats were individually housed. Diet (Certified Rodent Diet #5002) and water were available *ad libitum*.

Dose concentration and stability:
Diet samples collected for Wk 1 and 4 were analyzed for homogeneity and for Wk 2 to measure concentration of test article. Homogeneity (Wk 1) and concentrations measured during Wk 2 were within 15% of theoretical mean and ≤ 5% variability from the mean. Week 4, the homogeneity varied >5% for the bottom sample of the MD and the top sample of the HD.

Unique study design or methodology (if any): see sponsor's table below.

Group	Number of Male Animals	Dose Route	Target Dose Level (mg/kg/day)
1	5	Diet	2
2	5	Diet	6
3	5	Diet	18

Note: Each rat was given test article in feed daily for 28 consecutive days.

Sample collection for TK analyses: Post feeding on Days 1 and 28, blood was collected from the jugular vein at 1, 4, 8, 12, 16, 20, and 24 hr. On Day 28, time points later than 1 hr postdose were extended ≈30 min due to aggressive animal behavior.
TK Analysis: Whenever possible, C_{max}, T_{max} and AUC were calculated.

Observations, Times and Results:
Antemortem observations: Frequency not well described in methods.

- Some aggressive animal behavior observed during handling on D27-29.

Body weights: Individual body weights recorded at randomization (Day 15 of acclimation) and twice weekly during test period.

- LD, mean body wt increased from D1-27 with a mean body weight gain of +62 g.
- MD, mean body weight decreased from D1-6 (-6 g) on D2 which lasted to D6; D7-D27 mean body weight increased (+49 g), with a mean body weight gain from D1-D27 of +43 g.
- HD, mean body weight decreased from D1-2 (-16 g), decreased from D2-6 (-7 g); D7-D27 mean body weight increased (+45 g), with a mean body weight gain from D1-D27 of +22 g.

Food consumption: Recorded twice weekly through end of in-life phase.

- Day 1, all animals had low food consumption on day of first TK analysis.
  - LD, mean value 16 ± 2 g
  - MD, mean value 11 ± 2 g
  - HD, mean value 9 ± 2 g
- Ranges of mean food consumptions observed on subsequent days were higher.
  - LD, 21-23 g/day
  - MD, 20-22 g/day
  - HD, 17-20 g/day

Animal disposition: Following blood sample collection, rats were euthanized by overdose of isoflurane. Carcasses were not retained.

Toxicokinetic analyses: Evaluated concentrations of 4-aminopyridine, 3-hydroxy-4-aminopyridine (M1), and 3-hydroxy-4-aminopyridine sulfate (M2) were quantitated and toxicokinetic parameters were determined on Days 1 and 28.

4-Aminopyridine (parent)

- Day 1, exposures increased with increase in dose.
  - M, dose range 0.8 to 4.1 mg/kg/day
- Day 28, exposures increased with increase in dose.
- Increases in mean plasma C_{max} and AUC_{1-24} were not consistently dose proportional.

3-Hydroxy-4-Aminopyridine (M1)

- Day 1, exposures increased with increase in dose.
  - M, dose range 0.8 to 4.1 mg/kg/day
- Day 28, fampridine exposures increased with increase in dose.
- Increases in mean plasma C_{max} and AUC_{1-24} were not consistently dose proportional.
- Mean plasma AUC_{1-24} metabolite to parent ratios ranged from 0.0463 to 0.0738.
- Sponsor concluded, "3-hydroxy-4-aminopyridine is a minor circulating metabolite of fampridine following dietary administration in the rat".

3-Hydroxy-4-Aminopyridine Sulfate (M2)

- Day 1, exposures increased with increase in actual mean dose level.
  - M, dose range 0.8 to 4.1 mg/kg/day
- Day 28, exposures increased with increase in dose.
- Increases in mean plasma C_{max} and AUC_{1-24} were not consistently dose proportional.
- Mean plasma AUC1-24 metabolite to parent ratios ranged from 0.477 to 0.630.
- Sponsor concluded, "3-hydroxy-4-aminopyridine is a major circulating metabolite of fampridine following dietary administration in the rat".

The toxicokinetic parameters evaluated and calculated for 4-Aminopyridine (Tables 1 and 2), 3-Hydroxy-4-Aminopyridine (Tables 4 and 5), and 3-Hydroxy-4-Aminopyridine

Sulfate (Tables 7 and 8) are reproduced from the sponsor's original study report. The sponsor did not provide the actual fampridine dose level observed on Day 28.

Table 1. Summary of the Mean Toxicokinetic Parameters for Fampridine in Male Rat Plasma: Day 1

Dose Group	Actual (mean) Fampridine Dose Level (mg/kg/day)		C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day
1	0.8	Mean	22.7	28.4	8.80	292	289	361
		SD	4.1	NA	8.67	70	69	NA
		N	5	NA	5	5	5	NA
2	1.8	Mean	53.8	29.9	4.80	692	678	377
		SD	10.3	NA	1.79	84	88	NA
		N	5	NA	5	5	5	NA
3	4.1	Mean	86.4	21.1	5.80	1377	1348	329
		SD	18.9	NA	3.19	163	152	NA
		N	5	NA	5	5	5	NA

Notes: Target fampridine dose levels are 2, 6, and 18 mg/kg/day, respectively for Groups 1 through 3.
DN: Dose normalized

Table 2. Summary of the Mean Toxicokinetic Parameters for Fampridine in Male Rat Plasma: Day 28

Dose Group	Target Fampridine Dose Level (mg/kg/day)		C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)
1	2	Mean	47.1	23.6	2.80	582	548	274
		SD	3.8	NA	1.64	56	56	NA
		N	5	NA	5	5	5	NA
2	6	Mean	107	17.8	2.80	1398	1318	220
		SD	17	NA	1.64	254	235	NA
		N	5	NA	5	5	5	NA
3	18	Mean	316	17.5	2.20	4922	4679	260
		SD	26	NA	1.64	895	893	NA
		N	5	NA	5	5	5	NA

DN: Dose normalized

Table 4. Summary of the Mean Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine in Male Rat Plasma: Day 1

Dose Group	Actual (mean) Fampridine Dose Level (mg/kg/day)		C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	M/P Ratio
1	0.8	Mean	1.59	1.99	12.0	14.5	14.5	18.2	0.0509
		SD	0.31	NA	10.6	1.8	1.8	NA	0.0062
		N	3	NA	3	3	3	NA	3
2	1.8	Mean	3.78	2.10	4.80	44.7	43.8	24.3	0.0661
		SD	0.79	NA	1.79	9.0	8.8	NA	0.0183
		N	5	NA	5	5	5	NA	5
3	4.1	Mean	6.30	1.54	12.8	89.5	88.0	21.5	0.0652
		SD	1.49	NA	7.7	12.9	12.2	NA	0.0050
		N	5	NA	5	5	5	NA	5

Notes: Target fampridine dose levels are 2, 6, and 18 mg/kg/day, respectively for Groups 1 through 3.
DN: Dose normalized

Table 5. Summary of the Mean Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine in Male Rat Plasma: Day 28

Dose Group	Target Fampridine Dose Level (mg/kg/day)		C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	M/P Ratio
1	2	Mean	2.58	1.29	5.80	26.7	24.9	12.4	0.0463
		SD	0.58	NA	3.19	8.4	7.7	NA	0.0166
		N	5	NA	5	5	5	NA	5
2	6	Mean	6.23	1.04	4.20	80.2	75.6	12.6	0.0571
		SD	1.33	NA	2.49	18.6	17.3	NA	0.0065
		N	5	NA	5	5	5	NA	5
3	18	Mean	23.6	1.31	3.00	361	342	19.0	0.0738
		SD	2.8	NA	3.08	56	55	NA	0.0073
		N	5	NA	5	5	5	NA	5

DN: Dose normalized

Table 7. Summary of the Mean Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate in Male Rat Plasma: Day 1

Dose Group	Actual (mean) Fampridine Dose Level (mg/kg/day)		C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	M/P Ratio
1	0.8	Mean	14.4	18.1	8.80	168	166	208	0.576
		SD	2.4	NA	8.67	54	54	NA	0.134
		N	5	NA	5	5	5	NA	5
2	1.8	Mean	36.7	20.4	4.80	432	423	235	0.630
		SD	6.6	NA	1.79	53	52	NA	0.097
		N	5	NA	5	5	5	NA	5
3	4.1	Mean	54.8	13.4	10.6	715	698	170	0.519
		SD	17.5	NA	8.5	99	91	NA	0.055
		N	5	NA	5	5	5	NA	5

Notes: Target fampridine dose levels are 2, 6, and 18 mg/kg/day, respectively for Groups 1 through 3.
DN: Dose normalized

Table 8. Summary of the Mean Toxicokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate in Male Rat Plasma: Day 28

Dose Group	Target Fampridine Dose Level (mg/kg/day)		C_{max} (ng/mL)	DN C_{max} (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	AUC_{1-24} (ng·hr/mL)	DN AUC_{1-24} (ng·hr/mL)/(mg/kg/day)	M/P Ratio
1	2	Mean	24.3	12.1	2.80	294	277	138	0.511
		SD	5.4	NA	1.64	49	44	NA	0.108
		N	5	NA	5	5	5	NA	5
2	6	Mean	57.3	9.56	3.60	734	695	116	0.523
		SD	13.7	NA	2.88	197	186	NA	0.084
		N	5	NA	5	5	5	NA	5
3	18	Mean	140	7.78	3.60	2305	2204	122	0.477
		SD	30	NA	2.88	338	325	NA	0.063
		N	5	NA	5	5	5	NA	5

DN: Dose normalized

The mean C_{max} ± SD (units: ng/ml) are summarized below.

DOSE	MALES[a,b]		
	2 mg/kg/d	**6 mg/kg/d**	**18 mg/kg/d**
	4-AMINOPYRIDINE (PARENT)		
1st	22.7 ± 4.1	53.8 ± 10.3	86.4 ± 18.9
28th	47.1 ± 3.8	107 ± 17	316 ± 26
	3-HYDROXY-4-AMINOPYRIDINE (M1)		
1st	1.59 ± 0.31 (3)	3.78 ± 0.79	6.30 ± 1.49
28th	2.58 ± 0.58	6.23 ± 1.33	23.6 ± 2.8
	3-HYDROXY-4-AMINOPYRIDINE SULFATE (M2)		
1st	14.4 ± 2.4	36.7 ± 6.6	54.8 ± 17.5
28th	24.3 ± 5.4	57.3 ± 13.7	140 ± 30

[a]Actual mean dose levels for 1st dose were 0.8, 1.8, and 4.1 mg/kg/d
[b]Data derived from n < 5 rats/dose, (n).

The mean $AUC_{0\text{-}24h}$ (units: ng•hr/ml) ± SD are summarized below.

DOSE	MALES[a,b]		
	2 mg/kg/d	**6 mg/kg/d**	**18 mg/kg/d**
	4-AMINOPYRIDINE (PARENT)		
1st	292 ± 70	692 ± 84	1377 ± 163
28th	582 ± 56	1398 ± 254	4922 ± 895
	3-HYDROXY-4-AMINOPYRIDINE (M1)		
1st	14.5 ± 1.8 (3)	44.7 ± 9.0	89.5 ± 12.9
28th	26.7 ± 8.4	80.2 ± 18.6	361 ± 56
	3-HYDROXY-4-AMINOPYRIDINE SULFATE (M2)		
1st	168 ± 54	432 ± 53	715 ± 99
28th	294 ± 49	734 ± 197	2305 ± 338

[a]Actual mean dose levels for 1st dose were 0.8, 1.8, and 4.1 mg/kg/d
[b]Data derived from n < 5 rats/dose, (n).

4.) **Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Doses to Dogs**

Key findings:

- Exposure to fampridine generally increased with an increase in dose from 0.75 to 3.0 mg/kg/day, and the increases in mean plasma C_{max} and AUC_{0-24h} were generally dose proportional.
    - Similarly, metabolites 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate also increased with increase in dose and these increases in mean plasma C_{max} and AUC_{0-24h} were generally dose proportional.
- Slight gender differences exist in mean plasma C_{max} and AUC_{0-24h} values for fampridine and its two metabolites, the difference of which was <2-fold apart.
- After 14 days of twice-daily administration of fampridine, there was no accumulation of parent drug or its two metabolites, 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate.

Study No.: 7338-106
Volume #, and page#: EDR, 4.2.3.2.1 Study 7338-106, pp. 1-199.
Conducting laboratory and location: (b) (4)
Date of study initiation: 02 April 2008
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Control No. 0000049835, 100%

Methods

Doses: 0.75 (LD), 1.5 (MD), and 3.0 (HD) mg/kg/day
Species/strain: Dog/Beagle
Number/sex/group or time point: 3/sex/dose
Route, formulation, volume, and infusion rate: Oral, Capsule, dose divided equally for twice-daily dosing ≈6 h apart.
Age: ≈6 months of age
Weight: not provided
Sampling times: predose and after 1st dose at 0.5, 1, 1.5, 3, 6 (prior to 2nd dose), and after 2nd dose at 6.5, 7, 7.5, 9, 12, 24 hr on D1 and D14

Housing: Animals were housed in individual, stainless steel cages during acclimation and test period. Diet (Certified Canine Diet #5007) and water were available *ad libitum*.
Dose preparation: Individual capsules were prepared weekly.

Sample collection for TK analyses: On Days 1 and 14, predose and 0.5, 1, 1.5, 3, 6 hr (prior to 2nd daily dose), 6.5, 7, 7.5, 9, 12, and 24 hr postdose (prior to

administration of first dose on day 2), blood was collected (≈ 3 ml) via a jugular vein. Samples were collected into tubes containing K_2EDTA and processed for plasma.

- HDM (H09639) and HDF (H09643) were euthanized on Day 1 due to severe clinical effects. A blood sample was collected from HDM at sacrifice, but not from HDF due to aggressive behavior and agitation.

TK Analysis: Whenever possible, C_{max}, T_{max}, $t_{1/2}$, and AUC were calculated.

Observations, Times and Results:

Antemortem observations: Frequency not well described in methods.

- HDM (1/3; H09639) died on D1.
- HDF (1/3; H09643) died on D1.

Body weights: Individual body weights were recorded at randomization (Day 16 of acclimation) and twice weekly during test period.

- Unremarkable.

Food consumption: Recorded twice weekly through end of in-life phase.

- Unremarkable.

Animal disposition: Surviving animals were transferred to the (b) (4) stock colony.

Toxicokinetic analyses: Evaluated concentrations of 4-aminopyridine, 3-hydroxy-4-aminopyridine (M1), and 3-hydroxy-4-aminopyridine sulfate (M2) were quantitated and toxicokinetic parameters were determined on Days 1 and 14 using a validated LC/MS/MS method.

Fampridine (Parent)

- Exposure generally increased with increase in dose from 0.75 to 3.0 mg/kg/day.
  - Increases were generally dose proportional.
- Readily absorbed after oral capsule administration; after reaching C_{max}, concentrations decreased on Days 1 and 14 with a mean half-life of 1.02 to 1.26 hr and 1.27 to 1.42 hr, respectively.
- Slight differences between the sexes; however, any difference was <2-fold for either C_{max} or $AUC_{0\text{-}24h}$.
- No apparent accumulation of the drug, as C_{max} and $AUC_{0\text{-}24h}$ values were similar on Days 1 and 14.
- Mean T_{max} values were similar on Days 1 and 14 in both sexes.
  - In males, Day 1 means ranged 0.5 to 1.67 hr and Day 14 means ranged 1.0 to 1.2 hr.
  - In females, Day 1 means ranged 0.67 to 0.75 hr and Day 14 means ranged 0.83 to 1.0 hr.

Mean C_{max} ± SD (units: ng/ml), $AUC_{0\text{-}24h}$ (units: ng•hr/ml), and $t_{1/2}$ (hr) are summarized below.

Study Day	Sex	FAMPRIDINE											
		C_{max}						$AUC_{0\text{-}24h}$			$t_{1/2}$		
		LD		MD		HD[a,b]							
		D1	D2	D1	D2	D1	D2	LD	MD	HD[a,b]	LD	MD	HD [a,b]
1	M	91.7 ± 15.4	76.1 ± 30	168 ± 48	203 ± 23	387	355	420± 7	887± 129	1747	1.03	1.20	1.2
	F	108 ± 8	111 ± 3	204 ± 25	174 ± 29	367	332	512± 112	869± 81	1795	1.26	1.02	N.C.
14	M	94.2 ± 17.8	112 ± 12	196 ± 12	211 ± 64	214	521	492± 30	1097± 167	1946	1.35	1.42	1.27
	F	120 ± 46	130 ± 41	192 ± 33	233 ± 28	436	398	580± 125	1028± 165	2195	1.32	1.35	1.34

[a]Study Day 1, due to death of 1M and 1F, n=2/sex
[b] Study Day 14, due to vomiting and early termination, TK data from 1 animal/sex

3-Hydroxy-4-Aminopyridine (M1)

- Exposure generally increased with increase in dose from 0.75 to 3.0 mg/kg/day.
  - Increases were generally dose proportional.
- Readily absorbed after oral capsule administration, after reaching C_{max}, concentrations decreased on Days 1 and 14 with a mean half-life of 1.17 to 1.44 hr and 1.50 to 1.58 hr, respectively.
- Slight differences between the sexes; however, any difference was <2-fold for either C_{max} or $AUC_{0\text{-}24h}$.
- No apparent accumulation of the drug, as C_{max} and $AUC_{0\text{-}24h}$ values were similar on Days 1 and 14.
- Mean T_{max} values were similar on Days 1 and 14 in both sexes.
  - In males, Day 1 means ranged 0.5 to 1.33 hr and Day 14 means ranged 1.0 to 3 hr.

- In females, Day 1 means ranged 0.67 to 1.33 hr and Day 14 means ranged 0.83 to 1.0 hr.

Mean C_{max} ± SD (units: ng/ml), AUC_{0-24h} (units: ng•.hr/ml), and $t_{1/2}$ (hr) are summarized below. Doses were administered twice daily, and thus in the following table, C_{max} values are provided for each dose administered (i.e., AM dose = D1 and PM dose = D2).

Day	Sex	3-Hydroxy-4-Aminopyridine (M1)											
		C_{max}						AUC_{0-24h}			$t_{1/2}$		
		LD		MD		HD[a,b]							
		D1	D2	D1	D2	D1	D2	LD	MD	HD[a, b]	LD	MD	HD[a, b]
1	M	62.9 ± 22.9	105 ± 57	105 ± 57	116 ± 27	255	292	300 ± 70	524 ± 109	1343	1.19	1.44 ± 0.13	1.26
	F	63.9 ± 13.4	150 ± 42	150 ± 42	140 ± 15	179	235	329 ± 36	678 ± 68	1119	1.39 ± 0.19	1.17	1.32
14	M	65.4 ± 17.3	58.5 ± 20	89.8 ± 47	102.3 ± 33	152	253	274 ± 65	527 ± 147	1116	1.55	1.58	1.52
	F	52.7 ± 7.9	72.5 ± 13.1	129 ± 26	154 ± 68	179	248	318 ± 28	665 ± 72	1268	1.56	1.52	1.50

[a]Study Day 1, due to death of 1M and 1F, n=2/sex
[b] Study Day 14, due to vomiting and early termination, TK data from 1 animal/sex

3-Hydroxy-4-Aminopyridine Sulfate

- Exposure generally increased with increase in dose from 0.75 to 3.0 mg/kg/day.
  - Increases were generally dose proportional.
- Readily absorbed after oral capsule administration, after reaching C_{max}, concentrations decreased on Days 1 and 14 with a mean half-life of 1.82 to 2.97 hr and 2.2 to 2.75 hr, respectively.
- Slight differences between the sexes; however, any difference was <2-fold for either C_{max} or AUC_{0-24h}.
- No apparent accumulation of the drug, as C_{max} and AUC_{0-24h} values were similar on Days 1 and 14.
- Mean T_{max} values were similar on Days 1 and 14 in both sexes.
  - In males, Day 1 means ranged 1.0 to 2.0 hr and Day 14 means ranged 1.33 to 2.0 hr.
  - In females, Day 1 means ranged 1 to 1.83 hr and Day 14 means ranged 1.0 to 1.83 hr.

Mean C_{max} ± SD (units: ng/ml), AUC_{0-24h} (units: ng•.hr/ml), and $t_{1/2}$ (hr) are summarized below. Doses were administered twice daily, and thus in the following table, AUC values are provided for each dose administered (i.e., AM dose = D1 and PM dose = D2).

Day	Sex	3-Hydroxy-4-Aminopyridine Sulfate (M2)											
		C_{max}						AUC_{0-24h}			$t_{1/2}$		
		LD		MD		HD[a,b]							
		D1	D2	D1	D2	D1	D2	LD	MD	HD[a, b]	LD	MD	HD[a, b]
1	M	244 ± 87	246 ± 68	381 ± 166	493± 99	988	1091	300 ± 70	524 ± 109	1343	1.19	1.44 ± 0.13	1.26
	F	195 ± 69	251 ± 90	404 ± 202	473± 127	609	1021	329 ± 36	678 ± 68	1119	1.39 ± 0.19	1.17	1.32
14	M	246 ± 72	213 ± 71	341 ± 142	410 ± 161	970	851	274 ± 65	527 ± 147	1116	1.55	1.58	1.52
	F	166 ± 41	308 ± 83	429 ± 137	557 ± 52	657	1060	318 ± 28	665 ± 72	1268	1.56	1.52	1.50

[a]Study Day 1, due to death of 1M and 1F, n=2/sex
[b] Study Day 14, due to vomiting and early termination, TK data from 1 animal/sex

The mean ± SD of C_{max} (ng/ml) and AUC_{0-24h} (units: ng•hr/ml) values are summarized below.

Fampridine and its two metabolites—3-Hydroxy-4-Aminopyridine (M1) and 3-Hydroxy-4-Aminopyridine Sulfate (M3) in Beagle Dogs

Day	Sex	Dose	4-AP Parent		M1*			M3*		
			C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}	M/P Ratio	C_{max}	AUC_{0-24}	M/P Ratio
1	M	LD	76.1±30	420± 7	63± 13.4	300± 70	0.713± 0.157	246± 68	2004± 492	4.76± 1.11
		MD	203±23	887±129	116± 27	524± 109	0.607± 0.19	493± 99	3186± 844	3.70± 1.35
		HD	355	1747	292	1343	0.782	1091	9052	5.24
	F	LD	111±3	512± 112	80.5± 19.6	329± 36	0.674± 0.2	251± 90	2149± 213	4.40± 1.25
		MD	174±29	869±81	140± 15	678± 68	0.784± 0.084	473± 127	3838± 551	4.49± 1.10
		HD	332	1795	235	1119	0.643	1021	7270	4.15
14	M	LD	112±12	492± 30	58.5±20	274± 65	0.563±0. 166	213± 71	2008± 761	4.15± 1.85
		MD	211±64	1097± 167	102.3 ±33	527± 147	0.498±0. 196	410 ±161	3747± 1283	3.56± 1.61
		HD	521	1946	253	1116	0.574	851	8422	4.33
	F	LD	130±41	580± 125	72.5± 13.1	318± 28	0.569±0. 146	308± 83	2265± 382	4.03± 1.03
		MD	233±28	1028± 165	154± 68	665± 72	0.658±0. 133	557± 52	4454± 676	4.35± 0.45
		HD	398	2195	248	1268	0.578	1060	8167	3.72

M1 = 3-hydroxy-4-aminopyridine
M3 = 3-hydroxy-4-aminopyridine sulfate

5.) **Toxicokinetics of fampridine and its 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate metabolites after multiple oral doses to pregnant rabbits**

Key findings:

- Early study termination on Day 10 due to persistent loss of body wt and decreased food consumption of all dose groups.
  - Dosed from GD6 until GD15

Study No.: 7338-107
Volume #, and page#: EDR, 4.2.3.2.1 Study 7338-107, pp. 1-166.
Conducting laboratory and location: (b) (4)
Date of study initiation: 14 May 2008
GLP compliance: Yes
QA report: yes (X) no ( )

Drug, lot #, and % purity: 4-aminopyridine, Control No. 0000049835, 100%

Methods

Doses: 1 (LD), 3 (MD), and 5 (HD) mg/kg/day
Species/strain: Rabbit/New Zealand White [Hra:(NZW)SPF]
Number/sex/group or time point: 7/group
Route, formulation, volume, and infusion rate: Oral, gavage
Age: ≈5.2 mos of age
Weight: F, 2.94 to 3.86 kg
Sampling times: 1, 4, 8, 12, 16, 20 and 24 hr postdose feeding

Housing: During 2-day acclimation, animals were individually housed in suspended stainless steel wire-mesh cages. Diet (Certified Rabbit High Fiber Diet #5325) and water were available *ad libitum*.

Dose concentration and stability: Dose concentrations ranged within 94.4% to 97.9% of the targeted concentration.

Sample collection for TK analyses: Blood (≈ 0.5 ml) was collected from the auricular artery or by jugular venipuncture and transferred to tubes containing K_2EDTA at predose, 0.25, 0.5, 1, 2, 4, 8, and 24 hr postdose on study days 1 and 10. The 24 hr collection occurred prior to the dose administered on day 2.

TK Analysis: Whenever possible, C_{max}, T_{max}, $t_{1/2}$, and AUC were calculated.

Observations, Times and Results:

Antemortem observations: Frequency not well described in methods.

- 2 animals euthanized before study terminated early on study Day 10.
  - HD (F18554)—Study Day 6 due to continued body tremors and convulsions, which occurred 1.25 hr postdose.
  - LD (F18543)—Study Day7 due to aspiration of dose material during dosing procedure.
- Study terminated on Day 10 due to poor animal condition in all groups. In the following sponsor provided table is a list of medications used for treatment.

- ***Study Day 1***: Sponsor's table listing postdose observations in the rabbits following the first dose of fampridine as reproduced below.

Study Day 1:

Animal Number	Group	Postdose Observations: RR and BT within 1 hour	RR within 1 hour	IR and BT at 2 hours	Convulsion at 4.5 hours
F18540	1	√			
F18541	1	√			
F18546	2	√			
F18548	2		√	√	
F18549	2			√	
F18553	3		√	√	
F18554	3		√	√	√
F18555	3		√		
F18556	3			√	
F18557	3		√	√	
F18558	3		√	√	
F18559	3		√	√	

BT Body tremors
IR Irregular respirations
RR Rapid respirations

All animals received Lactated Ringer's solution as part of a palliation plan. In addition, the following medicants were administered.

Animal Number	Group	Medicant: Lacri-Lube	Diazepam	Baytril	Enrofloxacin
F18539	1			√	
F18542	1			√	
F18544	1			√	
F18545	1			√	
F18546	2			√	
F18552	2			√	
F18553	3		√		
F18554	3	√	√	√	
F18555	3			√	
F18556	3			√	
F18557	3			√	
F18558	3				√
F18559	3	√	√	√	

- ***Study Days 2-10***: LD 1/7 had irregular respirations on Day 2 within 0.5 hr postdose. MD 1/7 noted in a seizure ≈8.5 hr postdose (F18548); on Day 3, same animal had rapid respirations within 15 min postdose, which occurred again on Day 4 (2/7 from MD). From Day 3 onward, many HD does experienced one or more of the following: body tremors, rapid respirations, and non-formed feces. From Days 5-10, HD experienced body tremors from 2-3 hr postdose. On Days 8-10, MD and HD groups experienced body tremors ≈3 hr postdose.

Body weights: Individual body weights recorded at randomization (Day 1 of acclimation) and on study days 1, 3, and 8.

- M, F, mean body wt increased during the treatment period in all dose groups.

Food consumption: Measured on study days 1, 5, and 8.

- Due to low food consumption, study day 4, nutritional supplements were offered (broccoli, lettuce, apple, or oats) on a daily basis.
- Majority of animals in all dose groups had decreased food consumption on Day 5 compared to Day 1.

Animal disposition: Prematurely euthanized after 10 days of treatment using Beuthanasia-D; pregnancy was confirmed by visual examination of the uterus. Carcasses were not retained.

Toxicokinetic analyses: Evaluated concentrations of 4-aminopyridine, 3-hydroxy-4-aminopyridine (M1), and 3-hydroxy-4-aminopyridine sulfate (M3) were quantitated and toxicokinetic parameters were determined on Days 1 and 10. Pregnant does (GD6) were utilized for this study; and were euthanized on GD15 after 10 days of treatment.

Fampridine (Parent)

- Exposure generally increased with increase in dose from 1 to 5 mg/kg/day.
- Readily absorbed after oral capsule administration; after reaching C_{max}, concentrations decreased on Days 1 and 10 with a mean half-life of 2.03 to 2.97 hr and 2.67 to 3.04 hr, respectively.
- No apparent accumulation of the drug, as C_{max} and $AUC_{0\text{-}24h}$ values were similar on Days 1 and 10.
- Mean T_{max} values were similar on Days 1 and 10.
    - On Day 1 means ranged 0.29 to 0.61 hr and Day 10 means ranged 1.08 to 2.71 hr.
- Dose proportionality was provided in the following table from sponsor.

Table 2. Dose Proportionality Ratios of Fampridine in Pregnant Rabbit Plasma

Interval	Actual Dose Level Increase	Pregnant Females C_{max}	Pregnant Females $AUC_{0\text{-}24}$
Day 1	1.0:3.0:5.0 - fold	1.0:1.9:2.5 - fold	1.0:2.9:4.9 - fold
Day 10	1.0:3.0:5.0 - fold	1.0:2.1:3.7 - fold	1.0:2.5:4.3 - fold

Mean ± SD C_{max} (units: ng/ml), AUC_{0-24h} (units: ng•.hr/ml), and $t_{1/2}$ (hr) are summarized below.

Study Day	Sex	Fampridine								
		C_{max}			AUC_{0-24h}			$t_{1/2}$		
		LD[a]	MD	HD[b]	LD[a]	MD	HD[b]	LD[a]	MD	HD[b]
1	F	155± 27	301± 93	389± 93	694± 95	2027± 330	3430± 621	2.03± 0.27	2.77± 0.08	2.97
10	F	147± 63	310± 73	537± 191	1016± 800	2538± 728	4397± 1334	2.82± 1.03	3.04± 0.55	2.67± 0.23

[a]LD n=6, one euthanized on Study Day 7 due to gavage error
[b]HD n=6, one euthanized on Study Day 6 due to tonic/clonic grand mal seizure, tremors

3-Hydroxy-4-Aminopyridine (M1)

- Exposure generally increased with increase in dose of fampridine from 1 to 5 mg/kg/day.
- Readily absorbed after oral capsule administration; after reaching C_{max}, concentrations decreased on Days 1 and 10 with a mean half-life of 2.57 to 3.24 hr and 3.09 to 3.46 hr, respectively.
- No apparent accumulation of the drug, C_{max} and AUC_{0-24h} values were <2.0-fold different on Days 1 and 10.
- Mean T_{max} values were similar on Days 1 and 10.
    - On Day 1 means ranged 0.42 to 1.93 hr and Day 10 means ranged 1.17 to 2.58 hr.
- Dose proportionality was provided in the following table from sponsor.

Table 4. Dose Proportionality Ratios of 3-Hydroxy-4-Aminopyridine in Pregnant Rabbit Plasma

Interval	Actual Dose Level Increase	Pregnant Females	
		C_{max}	AUC_{0-24}
Day 1	1.0:3.0:5.0 - fold	1.0:2.0:2.9 - fold	1.0:3.7:6.0 - fold
Day 10	1.0:3.0:5.0 - fold	1.0:3.1:3.9 - fold	1.0:4.0:6.7 - fold

Mean ± SD C_{max} (units: ng/ml), AUC_{0-24h} (units: ng•hr/ml), and $t_{1/2}$ (hr) are summarized below.

Study Day	Sex	3-Hydroxy-4-Aminopyridine (M1)								
		C_{max} (ng/ml)			AUC_{0-24h} (ng•hr/ml)			$t_{1/2}$ (hr)		
		LD[a]	MD	HD[b]	LD[a]	MD	HD[b]	LD[a]	MD	HD[b]
1	F	61.5± 8.1	124± 21	178± 37	315± 52	1175± 129	1889± 281	2.57± 0.54	3.24± 0.09	3.02± 0.56
10	F	71± 31.6	220± 42	275± 77	422± 114	1679± 287	2825± 283	3.09± 1.21	3.46± 0.41	3.46± 0.21

[a]LD n=6, one euthanized on Study Day 7 due to gavage error
[b]HD n=6, one euthanized on Study Day 6 due to tonic/clonic grand mal seizure, tremors

3-Hydroxy-4-Aminopyridine Sulfate (M3)

- Exposure generally increased with increase in dose of fampridine from 1 to 5 mg/kg/day.
- Readily absorbed after oral capsule administration; after reaching C_{max}, concentrations decreased on Days 1 and 10 with a mean half-life of 2.57 to 3.24 hr and 3.09 to 3.46 hr, respectively.
- No apparent accumulation of the drug, C_{max} and $AUC_{0\text{-}24h}$ values were <2.0-fold different on Days 1 and 10.
- Mean T_{max} values were similar on Days 1 and 10.
    - On Day 1 means ranged 0.42 to 1.93 hr and Day 10 means ranged 1.17 to 2.58 hr.
- Dose proportionality was provided in the following table from sponsor.

Table 6. Dose Proportionality Ratios of 3-Hydroxy-4-Aminopyridine Sulfate in Pregnant Rabbit Plasma

Interval	Actual Dose Level Increase	Pregnant Females	
		C_{max}	$AUC_{0\text{-}24}$
Day 1	1.0:3.0:5.0 - fold	1.0:2.0:3.6 - fold	1.0:3.2:5.3 - fold
Day 10	1.0:3.0:5.0 - fold	1.0:2.3:2.6 - fold	1.0:2.9:4.2 - fold

Mean ± SD C_{max} (units: ng/ml), $AUC_{0\text{-}24h}$ (units: ng•hr/ml), and $t_{1/2}$ (hr) are summarized below.

Study Day	Sex	3-Hydroxy-4-Aminopyridine Sulfate (M3)								
		C_{max} (ng/ml)			$AUC_{0\text{-}24h}$ (ng•hr/ml)			$t_{1/2}$ (hr)		
		LD[a]	MD	HD[b]	LD[a]	MD	HD[b]	LD[a]	MD	HD[b]
1	F	251± 41	494± 120	909± 240	1593± 325	5146± 838	8425± 1401	3.27± 0.23	3.78± 0.27	3.47± 0.46
10	F	241± 95	557± 159	621± 202	1664± 641	4782± 1262	7001± 1428	3.77± 0.49	3.99± 0.48	4.71± 1.47

[a]LD n=6, one euthanized on Study Day 7 due to gavage error
[b]HD n=6, one euthanized on Study Day 6 due to tonic/clonic grand mal seizure, tremors

Mean ± SD of C_{max} (ng/ml) and AUC_{0-24h} (units: ng•hr/ml) values are summarized below. Included in this table is the metabolite to parent ratios of exposures calculated.

Fampridine and its two metabolites—3-Hydroxy-4-Aminopyridine (M1) and 3-Hydroxy-4-Aminopyridine Sulfate (M3) in pregnant Rabbits

Day	Sex	Dose	4-AP Parent		M1			M3		
			C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}	M/P Ratio	C_{max}	AUC_{0-24}	M/P Ratio
1 GD6	F	LD	155± 27	694± 95	61.5± 8.1	315± 52	0.45 ± 0.04	251± 41	1593± 325	2.30± 0.36
		MD	124± 21	2027± 330	124± 21	1175± 129	0.59 ± 0.11	494± 120	5146± 838	2.57± 0.46
		HD	389± 93	3430± 621	178± 37	1889± 281	0.56 ± 0.11	909± 240	8425± 1401	2.50± 0.45
10 GD15	F	LD	147± 63	1016± 800	71±3 1.6	422± 114	0.57 ± 0.26	241± 95	1664± 641	2.25± 1.05
		MD	220± 42	2538± 728	220± 42	1679± 287	0.69 ± 0.16	557± 159	4782± 1262	1.93± 0.36
		HD	537± 191	4397± 1334	275± 77	2825± 283	0.68 ± 0.16	621± 202	7001± 1428	1.66± 0.39

- Metabolite M3, 3-hydroxy-4-aminopyridine sulfate is found at approximately a 4-5:1 to Metabolite M1 on Day 1 and at approximately a 2.4-3.9:1 to M1 on Day 10.
- In pregnant rabbits, both metabolites, M1 and M3, were considered major metabolites.

2.6.4.8 Other Pharmacokinetic Studies

Acorda Therapeutics sponsored two additional pharmacokinetic studies, i.e., an evaluation of the potential of 4-aminopyridine to be a substrate/inhibitor of P-glycoprotein and a comparison between immediate- and sustained-release fampridine that are reviewed below.

- **Assessment of 4-Aminopyridine as substrate and inhibitor of P-glycoproteins in different cell systems.** (b) (4) Study No. 8ACORP1; EDR, 4.2.2.7.1, pp. 1-43; 4-Aminopyridine, Lot No. 49835, assigned potency 100%; GLP/QA).

Key findings:

- 4-Aminopyridine is not a P-glycoprotein substrate, and it does not inhibit P-glycoprotein at concentrations up to 100 µM.

MDR1-MDCK cells, MDCK cells, and Caco-2 cells were utilized to evaluate whether 4-aminopyridine was a substrate or inhibitor of P-glycoprotein transport. MDR1-MDCK

and MDCK cells were utilized to measure apical to basolateral and basolateral to apical P-glycoprotein transport of 4-aminopyridine (0.7, 7, and 70 µM). In MDR1-MDCK cells, the efflux ratios of 4-aminopyridine ranged from 0.8 to 1.0, whereas, the efflux ratio of digoxin (positive control) was 123. Similarly, in MDCK cells 4-aminopyridine produced reflux ratios of 1.0, whereas digoxin was transported at an efflux ratio of 19.5. Clearly, 4-aminopyridine was not a substrate for P-glycoprotein transport (see sponsor's Tables 6 and 7, as reproduced below). Caco-2 cells were used to determine if 4-aminopyridine inhibited P-glycoprotein by measurement of digoxin transport. Fampridine pretreatment failed to reduce the efflux ratio of digoxin, which was 18.3. In contrast, two known inhibitors of P-glycoprotein transport, cyclosporine A (CsA) and ketoconazole, each reduced digoxin efflux ratios to 0.9 and 2.5, respectively.

Table 6. Permeability and Recovery of 4-Aminopyridine and Digoxin in MDR1-MDCK Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
4-aminopyridine (0.7 µM)	P_{app} ($\times 10^{-6}$cm/s)	3.1	3.7	2.4	3.1 ± 0.6	3.0	3.0	3.0	3.0 ± 0.0	1.0
	% Recovery	78.8	76.7	73.8	76.4 ± 2.5	79.6	79.5	79.6	79.6 ± 0.1	
4-aminopyridine (7 µM)	P_{app} ($\times 10^{-6}$cm/s)	3.7	3.7	3.6	3.7 ± 0.0	3.1	3.2	2.9	3.0 ± 0.1	0.8
	% Recovery	99.6	97.6	97.9	98.4 ± 1.1	103	91.2	101	98.3 ± 6.2	
4-aminopyridine (70 µM)	P_{app} ($\times 10^{-6}$cm/s)	4.0	3.4	3.9	3.8 ± 0.3	3.6	3.8	3.9	3.7 ± 0.1	1.0
	% Recovery	111	105	109	108 ± 2.8	110	109	108	109 ± 1.0	
Digoxin (10 µM)	P_{app} ($\times 10^{-6}$cm/s)	1.9*	0.1	0.1	0.1 ± 0.0	14.1	12.7	16.8	14.5 ± 2.1	123
	% Recovery	89.4*	87.2	89.7	88.4 ± 1.8	92.5	89.4	92.2	91.4 ± 1.7	

*The measured concentration was unusually high and its value was excluded from calculations.

Table 7. Permeability and Recovery of 4-Aminopyridine and Digoxin in MDCK Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
4-aminopyridine (0.7 µM)	P_{app} ($\times 10^{-6}$cm/s)	0.5*	0.5*	0.5*	0.5	0.5*	0.5*	0.5*	0.5	N.D.
	% Recovery	85.1*	84.0*	84.7*	84.6	84.9*	86.7*	85.0*	85.5	
4-aminopyridine (7 µM)	P_{app} ($\times 10^{-6}$cm/s)	0.5	0.3	0.6	0.5 ± 0.1	0.5	0.5	0.5	0.5 ± 0.0	1.0
	% Recovery	97.8	96.9	99.1	97.9 ± 1.1	105	98.7	104	102 ± 3.2	
4-aminopyridine (70 µM)	P_{app} ($\times 10^{-6}$cm/s)	0.5	0.5	0.5	0.5 ± 0.0	0.6	0.5	0.6	0.5 ± 0.0	1.0
	% Recovery	96.9	96.2	96.8	96.7 ± 0.4	102	96.0	101	100 ± 3.2	
Digoxin (10 µM)	P_{app} ($\times 10^{-6}$cm/s)	0.1	0.1	0.1	0.1 ± 0.0	1.5	1.7	1.3	1.5 ± 0.2	19.5
	% Recovery	67.2	87.7	88.6	81.2 ± 12.1	81.6	77.5	91.1	83.4 ± 7.0	

*The concentrations of the test compound in the receiver side were below the lowest quantification limit (5 nM); the value of 5 nM was used to estimate the permeability and recovery values.
N.D.: Not determined. Because reliable P_{app} values were not obtained, the efflux ratio could not be determined.

5.6 P-gp Inhibitor Assessment

Table 9. Permeability and Recovery of Digoxin in Caco-2 Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
Digoxin (10 µM)	P_{app} ($\times 10^{-6}$cm/s)	0.9	0.9	0.9	0.9 ± 0.0	14.4	13.4	14.9	14.2 ± 0.8	16.0
	% Recovery	101	102	102	102 ± 0.7	103	99.3	114	106 ± 7.7	
Digoxin + 4-aminopyridine (100 µM)	P_{app} ($\times 10^{-6}$cm/s)	1.1	0.9	1.2	1.0 ± 0.2	16.6	19.1	21.9	19.2 ± 2.7	18.3
	% Recovery	88.4	94.5	100	94.3 ± 5.7	105	102	104	104 ± 1.3	
Digoxin + CsA (10 µM)	P_{app} ($\times 10^{-6}$cm/s)	2.8*	3.2*	3.3	3.3	2.7	3.0	3.0	2.9 ± 0.2	0.9
	% Recovery	104*	120*	121	121	110	105	103	106 ± 4.1	
Digoxin + Ketoconazole (10 µM)	P_{app} ($\times 10^{-6}$cm/s)	1.3	2.3	2.3	2.0 ± 0.6	4.6	4.5	5.3	4.8 ± 0.4	2.5
	% Recovery	100	101	109	103 ± 4.7	107	102	100	103 ± 3.1	

*The cell monolayer failed post-experiment LY criteria; thus their values were excluded from average calculation.

Table 10. Concentration of 4-Aminopyridine as a P-gp Inhibitor

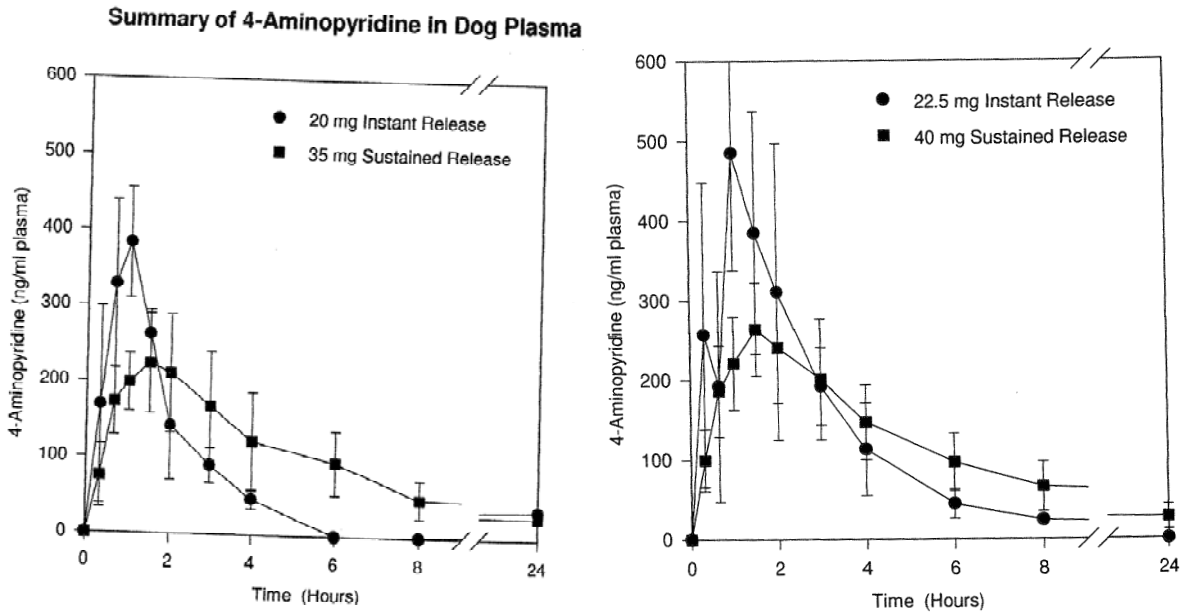
Nominal Concentration (µM)	Replicate 1	Replicate 2	Replicate 3	Average ± SD
100	105	104	105	105 ± 0.6

- **Comparative toxicity and bioavailability of an instant release and sustained release formulation in the beagle dog.** (b) (4)
  Study report No. (b) (4)-684-026; GLP/QA; 3/95, EDR 4.2.2.7.1, pp. 1-171).

The bioavailability of 4-Aminopyridine (Lot C08078) as an immediate release capsule was compared to Fampridine-SR tablets of the following strengths 5 mg (Lot PD13154), 7.5 mg (PD13159), 12.5 mg (PD13161) in ten male beagle dogs (8-9 mos. of age). This dose range-finding study examined 4-aminopyridine as an oral immediate-release (n=5) or sustained-release formulation (n=5) to determine a dose that produced toxicity and to perform blood level profiling at certain doses. The 22.5 mg dose of the immediate-release formulation of 4-aminopyridine resulted in toxicity including: convulsion (1/5), prostration (2/5), and marked ataxia (2/5). The dog that convulsed was euthanized for humane reasons due to presence of the following clinical signs: marked body tremors, impaired hindlimb function (both), marked ataxia, prostration, a blue discolored tongue (prominent 2 hr postdose), slight to marked salivation, and marked mydriasis both eyes. The sustained-release formulation dosing was increased from 22.5 mg/animal to 25 mg, 27.5 mg, 30 mg, 35 mg, and 40 mg/animal on Day 80. At 40 mg/animal, clinical signs had included tremors (5/5), soft stool (1/5), increased salivation (1/5), and mydriasis (4/5). Comparison of plasma 4-aminopyridine levels following doses of immediate- and sustained-release formulations (20 mg/animal IR vs. 35 mg/animal SR and 22.5 mg/animal IR vs. 40 mg/animal SR) are shown in sponsor's Figure 1 below. These comparisons are also presented in the following reviewer-generated table that summarizes data found in sponsor's Table 3.

4-AP Formulation	Dose (mg/animal)	C_{max} (ng/ml)	AUC_{1-12h} (ng.hr/ml)	Tmax (h)
IR	20	385±74.3	753±117	0.87±0.18
	22.5	482±86.5	1113±263	1.3±0.27
SR	35	236±51.1	1082±444	1.3±0.58
	40	269±53.4	1352±293	1.5±0.35

Figure 1



- Test article-related findings included discolored feces, salivation, mydriasis, and whole body tremors.
- Increased incidence of body tremors was observed for IR at doses of 5-15 mg/animal (1/5), 17.5 mg/animal (5/5), 20 mg/animal (5/5), and 22.5 mg/animal (5/5); however, body tremors were not observed in dogs treated with the SR formulation until doses reached 35 mg/animal (3/5) and 40 mg/animal (5/5).
- Both IR and SR increased salivation and mydriasis in dogs at a dose of 17.5 mg/animal.
- Incidence of discolored feces was higher in the SR group than IR group, which was partially considered undigested test article, but also included green or dark brown colorations. Sponsor suggests that these may be associated with local effects on the gastrointestinal tract, which had not been observed in previous studies using the immediate release formulation.
- The PK of the IR and SR formulations are clearly different. Plasma C_{max} and AUC increase greater than dose proportionately with the IR formulation, but dose proportionately with the SR formulation.

- This study suggests that the SR formulation of 4-aminopyridine appeared to reduce the toxicity profile observed for the IR formulation by lowering the C_{max}.

2.6.4.9 Discussion and Conclusions

See section entitled "Overall Summary and Evaluation".

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The toxicology summary is included in the overall summary and evaluation section.

2.6.6.2 Single-dose toxicity

Single dose toxicity studies were conducted in rat, rabbit, and dog. In rat (Study # (b) (4) 2620-102), oral administration of fampridine resulted in LD_{50} values of 14.3 mg/kg (male) and 22.1 mg/kg (female). Administration of fampridine q.i.d. (every 6 hr) (Study #0402RA44.001) increased the LD_{50} value to 40.4 mg/kg (males and females); and a NOAEL for death or visible lesions was established at 10 mg/kg/day. In rabbits, the LD_{50} value was 22.7 mg/kg for both sexes combined (study (b) (4) 2620-103); and a NOAEL for death/seizures was 20 mg/kg/day. It is noted that there is a very steep dose-response relationship for seizures and death. An acute lethal dose was not established in dogs (Study #0433DA44.001). The following Sponsor's Table 1 summarizes the single dose toxicology studies completed.

Table 1: Completed Single-Dose Toxicology Studies

Study Title	Test System [No. Animals]	Dose (mg/kg) (Route)	Study Duration	Study Number
Acute Oral Toxicity Study with 4-Aminopyridine in Rats	Rats (Sprague-Dawley) [5/Sex/Group]	Males: 11.25, 12.5, 15, 20 Females: 20, 21.5, 22.5, 25 (Oral Gavage)	14-Days	(b) (4) 2620-102
Acute Oral Toxicity of 4-Aminopyridine in Rats	Rats (Sprague-Dawley) [4/Sex/Group]	0, 10, 20, 40, 80 (Oral Gavage)	14-Days	0402RA44.001
Acute Oral Toxicity Study with 4-Aminopyridine in Rabbits	Rabbits (NZ White) [5/Sex/Group]	20, 22.5, 25, 30 (Oral Gavage)	14-Days	(b) (4) 2620-103
Acute Oral Toxicity of 4-Aminopyridine in the Dog	Dogs (Beagle) [1/Sex/Group]	1, 5 (Oral Capsule)	14-Days	0433DA44.001

2.6.6.3 Repeat-dose toxicity

Mouse

1.) **2-Week Range-Finding Dietary Toxicity Study in Mice**

Study no.: 684-019
Volume #, and page #: EDR, 4.2.3.2 (b) (4) 684-019 2-Week Range-Finding Dietary Toxicity Study in Mice, pp. 1-67
Conducting laboratory and location: (b) (4)
Date of study initiation: 23 March 1994
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot C4886, Assume 100%

Methods

Doses: 0, 5, 10, 20 (LD), 40 (MD-1), 60 (MD-2), 80 (HD) mg/kg/day
Species/strain: Mouse/CD-1
Number/sex/group or time point (main study): 5/sex/dose
Route, formulation, volume, and infusion rate: Dietary admixture
Satellite groups used for toxicokinetics or recovery: none
Age: 6 weeks of age
Weight: Males, 28-36 g; Females, 23-27 g
Unique study design or methodology:

Beginning on April 6, 1994, the test article was offered in the diet to yield dosage levels of 5, 10, 20 and 40 mg/kg/day. On April 15, 1994, the dosage level for the 5 mg/kg/day group was increased to 60 mg/kg/day and the 10 mg/kg/day group was increased to 80 mg/kg/day. Each weekly food admixture was divided into seven portions and kept frozen until fed to the animals. Test article and untreated diet were offered daily for a period of 14 days. Following two weeks of treatment, all animals were euthanized on (b) (4)

Observations and Results:

Mortality/Morbidity: Three times daily, and twice daily on weekends

- All mice survived until scheduled sacrifice.

Clinical signs: At least once weekly, detailed observations were performed and appearance and condition, behavior and activity, excretory functions, respiration, orifices, eyes and palpable masses were noted.

- No test article-related clinical findings were observed at any dose level.

Body weights: Individual body weights were recorded prior to study initiation and weekly during the study.

- In males, control mice increased body weight +12.9% compared to the pre-study mean value. All 4-aminopyridine dose groups had reduced body weight compared to the control group at both week 1 and week 2. The greatest reduction

in body weight was found in the HD group (-14.3%), which was not statistically significant.

- In females, control mice increased body weight +8.0% compared to the pre-study mean value. Week 1, mice either had no change in body weight or a slight reduction (-4.0%). Week 2, body weight was reduced in all 4-aminopyridine dose groups (range, -3.7% to -11.1%). A significant ($p \leq 0.01$) reduction in mean body weight was observed for MD-2 and HD groups compared to the mean body weight of control.

Food and Compound consumption: Individual food and compound consumption were measured daily, but data were presented as weekly intervals.

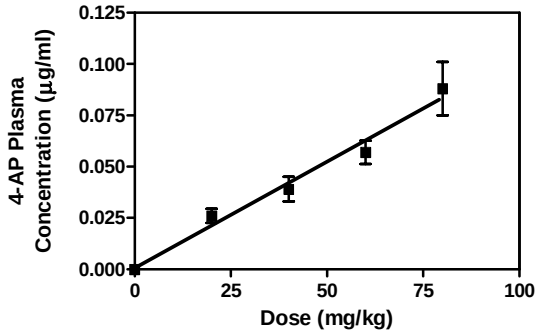
- Food consumption was unaffected by test article at any dose tested.

Plasma analysis: Plasma collected at necropsy was evaluated for 4-aminopyridine concentrations as measured by HPLC.

- Plasma 4-AP concentrations were approximately linear with respect to oral dose administered. Sponsor's data from Appendix B are summarized in the reviewer generated table and figure, below.

Dose (mg/kg)	Plasma 4-AP (µg/ml)
0	LLOQ
20	0.026 ± 0.011
40	0.039 ± 0.019
60/5	0.057 ± 0.018
80/10	0.088 ± 0.041

LLOQ = Lower than the Limit of Quantitation



Terminal procedures: All animals surviving to terminal sacrifice were examined postmortem for external abnormalities including palpable masses which if found were correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities *in situ* and the organs were removed and examined. All abnormalities were recorded and the tissues discarded.

- No test article-related macroscopic observations were found.

2.) **13-Week Dietary Toxicity Study in Mice**

Study no.: 684-021
Volume #, and page #: EDR, 4.2.3.2.1. Mouse—Oral-Medium, pp. 1-269
Conducting laboratory and location: (b) (4)
Date of study initiation: 03 June 1994
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot# P104-33-1, assumed 100%

Methods

Doses: C: 0, LD: 2.4, MD: 12, HD: 60/80[a]/120[b] mg/kg
[a]Increased to 80 mg/kg at week 6; [b]Increased to 120 mg/kg at week 9.
Species/strain: Mouse, Crl: CD-1 (ICR) BF VAF/Plus
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Dietary admixture, control mice received basal laboratory diet, fresh diet offered daily for 13 weeks

- Dietary admixture of 4-Aminopyridine was sampled for determination of 4-AP homogeneity, stability and concentration and was within acceptable limits.

Satellite groups used for toxicokinetics or recovery: TK, 5/sex/group
Age: 4 wks
Weight: Males, 19-23 g; Females, 17-22 g
Sampling times: TK measured at study week 4; Clinical laboratory studies collected at study termination.

Housing: Mice were individually housed in stainless steel wire-mesh cages. Diet and water were available *ad libitum*, except for an overnight period prior to clinical pathology testing when food, but not water, was withheld.

Observations and Results:

Mortality/Morbidity: Three times daily on weekdays and twice daily on weekends and holidays.

- CF (#63236) died on study Day 64, and cause of death was unknown.
- 6 HDF died during weeks 9-11 (within 2-16 days of increasing the dose from 80 to 120 mg/kg/day).
    - Cause of death, unknown (Day 75, #63319; Day 68, #63327; Day 78, #63326; Day 72, #63331; Day 77, #63332, Day 64, #63333).
- MDM and HDF from the TK group died on study Day 18.

Clinical signs: Signs of overt toxicity were monitored at the times of mortality/morbidity checks. At least once/week, detailed observations of appearance and condition, behavior and activity, excretory functions, respiration, orifices, eyes and palpable masses were conducted.

- Unremarkable.

Body weights: Individual body weights were recorded pretest and weekly throughout the study.

- HDM, mean body weight was decreased significantly during weeks 10-12 (-10.8%, -13.2%, and -8.1%, respectively) compared to controls, following the dose increase from 80 to 120 mg/kg/day.
- In LD, MD and HD females, mean body weight was significantly decreased compared to control. A time-dependent reduction in body weight was observed at

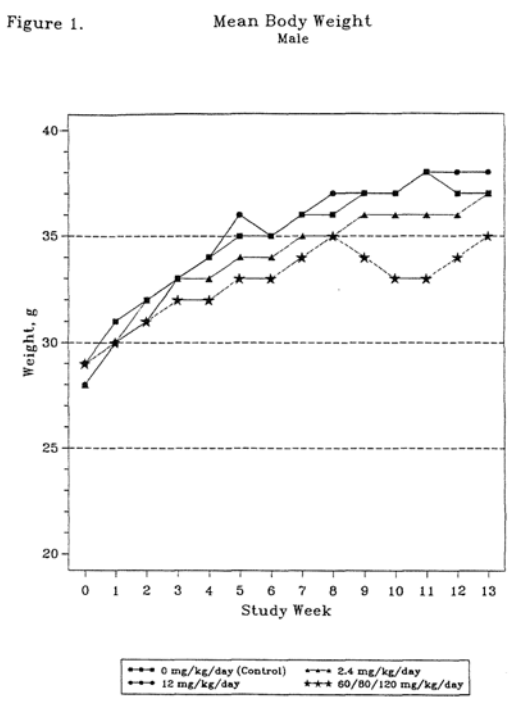
each dose. Significant body weight reduction was observed during weeks 9 to 12 in the HD, weeks 11 and 12 in the MD, and weeks 10-12 in the LD groups.

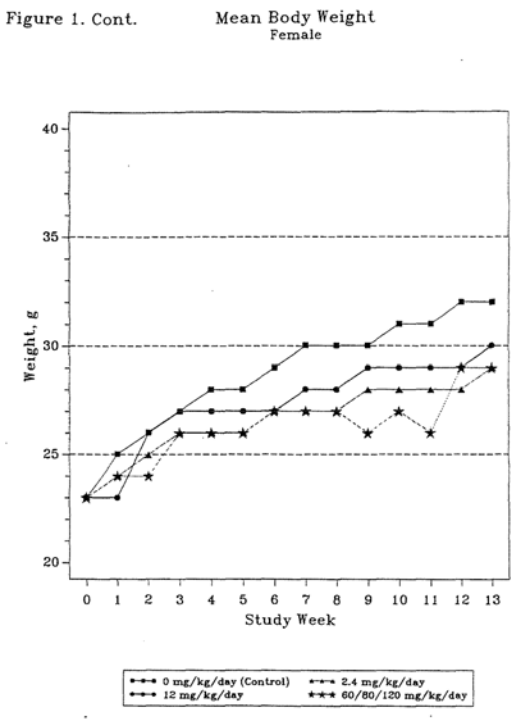
These body weight changes are summarized in the following reviewer generated Table and in sponsor's Figure 1 as reproduced below.

4-Aminopyridine (dietary admixture) treatment reduced female mouse body weight compared to control (% Control).

Treatment (Weeks)	4-Aminopyridine		
	LD	MD	HD
9	-6.7%	-3.3%	-13.3%**
10	-9.7%*	-6.4%	-12.9%**
11	-9.7%**	-6.4%*	-16.1%**
12	-12.5%**	-9.4%*	-9.4%*
13	-9.4%	-6.2%	-9.4%

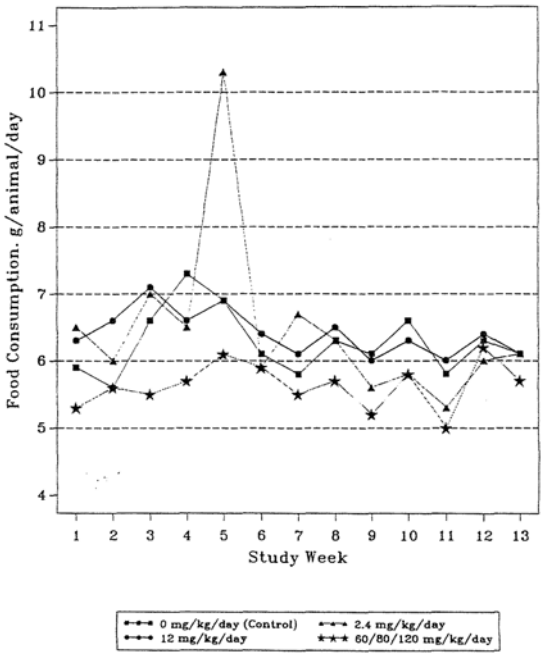
*p≤0.05; **p≤0.01 compared to the control group





Food consumption: Individual food consumption was measured daily and weekly totals were calculated during the study. Compound consumption was calculated weekly except at weeks 9 and 10 during which time the dose level was increased.

- Males, food consumption was unremarkable.
- HDF, food consumption decreased throughout the study; however, C, LD, and MD groups were similar. Food consumption in females is shown below in sponsor's Figure 2.



Clinical pathology: Animals were fasted overnight prior to clinical pathology sample collection but had *ad libitum* access to water. Blood samples were collected (orbital sinus) and hematological testing was conducted on the first 5/sex/group and biochemical testing was conducted on the next 5/sex/group. In some groups, both hematological and biochemical testing were conducted from the same animals.

Hematology: The following parameters were evaluated: RBC, Hb, Hct, MCV, MCHC, MCH, Plt, Diff. Leuk.

- Unremarkable.

Clinical chemistry: The following parameters were evaluated: ALT, AST, LDH, Urea Nitrogen, and glucose.

- LD and MD groups were unremarkable.
- HDM (1/5) and HDF (2/5) had elevated AST, ALT and LDH, which was considered possibly treatment-related by the sponsor. The following table summarizes the mean percent of HD liver enzymes compared to Control.

Mean percent from control

HD	AST	ALT	LDH
M	+84.4%	+157%	+14.7%
F	+82.6%	+126%	+86.2%

Toxicokinetics: Plasma samples for TK analysis were collected from the last surviving 5/sex/group.

- Two TK animals were found dead on study and were necropsied (limited to macroscopic assessment; histopathology was not required); MDM (Day 18, #63290) and HDF (Day 18, #63339). In the MDM, a 0.5 cm liver-colored nodule

was present near the hilus of the liver (moderate), multifocal red fluid was found in the subcutis skin at the thorax level (moderate) and the duodenum was focally perforated (trace) and red discolored (mild) were noted.

- Plasma 4-aminopyridine concentrations increased with increasing dose in both sexes.
- Week 4, plasma 4-aminopyridine concentrations are summarized in sponsor's Table 5.3, below.

Table 5.3.
Determination of the Concentration of Test Article in Mouse Plasma.

Group	Sex	Dosage Level (mg/kg/day)	Mean Concentration Found (µg/ml)	Standard Deviation
1	M	-	-	
	F	-	-	
2	M	2.4	0.001	0.0007
	F	2.4	0.00175	0.00126
3	M	12	0.0083	0.0046
	F	12	0.0058	0.0034
4	M	60	0.0808	0.0176
	F	60	0.0395	0.0156

Gross pathology: A complete postmortem examination was performed on the first 10/sex/group that survived to terminal sacrifice and on all animals that died on study. Animals were examined for external abnormalities and palpable masses, which if found were correlated with antemortem signs. Abdominal, thoracic, and cranial cavities were examined for abnormalities *in situ* and the organs removed and examined.

- No test article-related macroscopic observations were found in any treatment group of either sex that died prior to terminal sacrifice or following 13 weeks of oral dietary administration of 4-AP. Any macroscopic observations made were considered spontaneous, incidental, and/or agonal and unrelated to 4-AP administration.

Organ weights: The following organs were weighed: adrenal (2), brain, heart, kidney (2), liver, ovary (2), spleen, and testis with epididymis (2).

- HDM, mean body weight was significantly reduced (-9.4%) compared to Control.
  - Organ weights were unremarkable.
- MDM, a significant increase in the ratio of kidney weight to body weight was found (+13.5%).
- Female, mean body weight was unaffected by 4-AP.
  - HDF, a statistically significant ($p \leq 0.01$) reduction in the mean absolute liver weight, liver to body weight ratio, and liver to brain weight ratio were found when compared to Control. Decreased liver weight was considered 4-AP treatment-related.

  LDF, mean absolute liver weight was also decreased significantly ($p \leq 0.05$); however, this effect was no longer apparent when normalized to body weight. Additionally, the reduced absolute liver weight was observed only in females, and no histological changes were observed in liver, suggesting that this effect was unlikely related to 4-aminopyridine treatment.

Histopathology: Protocol-designated organs and tissues were collected and placed in a phosphate-buffered neutral formalin solution. The pituitary was fixed *in situ*. The following list of organs and tissues were collected from all animals:

adrenal (2), aorta, bone (femur and sternum), bone marrow (femur and sternum), bone marrow smear, brain (fore, mid, and hind), eye including optic nerve (2), gallbladder, gastrointestinal tract (esophagus, stomach—glandular and nonglandular, duodenum, jejunum, ileum, cecum, colon, rectum), gonads—ovary (2) or testis with epididymis (2), gross lesions, Harderian gland (2), heart, kidney (2), larynx, liver—all lobes, lung with mainstem bronchi (2), lymph nodes—mandibular (2) and mesenteric, mammary gland—females only, pancreas, pituitary, prostate and seminal vesicle (2), salivary gland—mandibular and sublingual, sciatic nerve, skeletal muscle—thigh, skin, spinal cord—thoracic, spleen thymus, thyroid/parathyroid (2), trachea, urinary bladder, uterus—both horns, uterus—cervix, and vagina.

From the first ten animals in the C and HD groups and all animals found dead on study (irrespective of dose group), all tissues and organs were collected and microscopically evaluated. Gross lesions found in the LD and MD groups that survived to terminal sacrifice (identified in the first 10/sex/group were also evaluated). All tissues examined were stained using hematoxylin and eosin.

Adequate Battery: yes (X)
Peer review: yes (X)

- Several animals died on study including 1 CF, 1 MDM, and 7 HDF, the cause of death was listed by the study pathologist as unknown and none was attributed to the test article.

No test article-related microscopic observations were found in any treatment group in either sex following 13 weeks of oral dietary administration of 4-AP. Any microscopic observations made were considered spontaneous, incidental, and/or agonal and unrelated to 4-AP administration.

Rat

Acorda conducted eight repeat dose toxicity studies in rats that are reviewed below.

1.) **A Dose Range-Finding Oral Toxicity Study of 4-AP in Rats**

Study no.: 1139-003
Volume #, and page #: EDR, 4.2.3.2.1 (b) (4) 1139-003
Conducting laboratory and location: (b) (4)
Date of study initiation: 12 June 2006
GLP compliance: Yes
QA report: yes (X) no ( )

Drug, lot #, and % purity: 4-Aminopyridine, Lot No. 155292, purity 100.5%.

Methods

Doses: Phase A: 3 (Grp 1, LD), 25 (Grp 2, HD), 8 (Grp 3, LMD), and 15 (Grp 4, HMD) mg/kg/day administered once at each dose level.
Phase B: 0 (Grp 5), 1 (Grp 6, 9), 4 (Grp 7, 10), and 8 (Grp 8, 11) mg/kg/day administered once daily for 7 consecutive days.
Species/strain: Rat/Crl: CD(R) (SD)
Number/sex/group or time point (main study): Phase A: 3/sex/group; Phase B: 5/sex/group
Route, formulation, volume, and infusion rate: Oral, gavage, 10 ml/kg/dose
Satellite groups used for toxicokinetics or recovery: Phase B: TK 9/sex/group
Age: ≈ 6 wks of age
Weight: Phase A: Male, 171-191 g; Female, 154-169 g
Phase B: Male, 197-221 g; Female, 156-193 g

Observations and Results:

Cageside observations: Twice daily observed for morbidity, mortality, injury, and availability of food and water.

Phase A:

- For each sex, HD (2/3) rats were found dead on Day 1, and COD was undetermined.
  - Males #6004, #6006 and Females #6063, #6064.

Phase B:

- No deaths were found in main-study animals. However, several HD deaths occurred in the TK study, which was attributed to the bleeding/anesthetic procedure used. When changed to a procedure without anesthetic, no additional deaths occurred. One LDF found dead on Day 7 was considered related to the bleeding procedure.

Clinical signs: Detailed observations were made for each animal on dosing days at 1, 2, and 4 hrs postdose and once daily for three consecutive non-dosing days (Phase A); and for each animal 1 hr postdose in the main study (Phase B). Each animal was examined for clinical signs of disease, toxicity, injury, and evidence of masses.

Phase A:

- LD (both sexes)—Unremarkable.
- LMD, HMD, and HD groups (both sexes)—tremors and rapid respirations for most survivors as summarized in the reviewer-generated table, below.

		DAY 1									Day 2	Day 3
		1 hr			2 hr			4 hr				
Sign	**Sex**	**LMD**	**HMD**	**HD**	**LMD**	**HMD**	**HD**	**LMD**	**HMD**	**HD**	**HD**	**HD**
Tremor	**M**	3/3	3/3	3/3	3/3	3/3	1/1	2/3	3/3	1/1		
	F	3/3	3/3	3/3	3/3	3/3	1/1	2/3	1/3	1/1		
Rapid Resp.	**M**	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	1/1	1/1	
	F	3/3	3/3	3/3	3/3	3/3	1/1	3/3	3/3	1/1	1/1	1/1

- Additional clinical signs were observed for HDM and HDF and the notable findings are summarized in the following table.

Clinical Sign	HD Males		HD Females				
	Day 1 Post Dose		Day 1 Post Dose			Day 2	Day 3
	1 hr	2 hr	1 hr	2 hr	4 hr		
Convulsions Tonic		1/3	1/3				
Clonic	1/3	1/3	3/3	1/1			
Ataxia	2/3	2/3	2/3	1/1	1/1		
Vocalization	1/3	1/3				1/1	
Decreased Activity	2/3	2/3					
Limb Fn. Impaired	1/3	1/3					
Splayed		1/3					
Righting reflex		1/3					
Material near: Nose						1/1	
Mouth						1/1	1/1

Body weights: Individual body weights were recorded within one day of arrival, prior to randomization, and daily prior to test article administration (Phases A and B).
Phase A: Unremarkable
Phase B:

- Days 4-7, HDM mean body weight (range, -7.3% to -8.1%) was decreased and statistically significant compared to CM.
- LDM, MDM, and females were unremarkable.

Food consumption: Recorded daily throughout treatment for both study Phases A and B.

- Food consumption was unaffected by test article during Phase A.
- MD and HD (both sexes) consumed less food than Control, and the decrease was statistically significant [ss, $^{*}p<0.05$, $^{**}p<0.01$].
    - MDM, decreased food consumption as a percent of Control for Days 2-3 was -15.6%* and -17.2%*, respectively.
    - HDM, decreased food consumption as a percent of Control for Days 1-5 ranged from -33%** to -19.4%** and for Day 6 was -12.6%.
- LD, unremarkable (both sexes)

Clinical pathology: Evaluations were performed on all Phase B study animals at study termination. Prior to terminal sacrifice, animals were fasted overnight but had *ad libitum*

access to water. Animals were euthanized by carbon dioxide inhalation, and blood samples were collected via cardiac puncture.

Hematology: The following parameters were evaluated: RBC, Hb, Hct, MCV, MCHC, MCH, Retic, RBC morphology, Plt, WBC, Neut, Lymph, Mono, Eos, Baso, Others (as needed).

Phase B:

- Dose-dependent decrease of reticulocytes (absolute and percent) for MD and HD (M+F) that was significantly different from Control. The following reviewer generated table summarizes the percent difference between 4-AP treated and Control. Statistics were calculated based upon the absolute number and percent of reticulocytes and are noted as $^{*}p<0.05$; $^{**}p<0.01$ in the table.

Reticulocytes	Sex	4-Aminopyridine LD	MD	HD
Absolute	M	-2.1%	-24.9%*	-35.2%**
	F	-9.4%	-22.2%*	-30.1%**
Percent	M	-3.1%	-26.1%**	-39%**
	F	-8.6%	-23.9%*	-28.8%**

- The dose related decrease in absolute number and percent of reticulocytes present in both males and females suggested this observation was test article related.

Coagulation: The following parameters were evaluated: APTT and PT.

- Unremarkable.

Clinical chemistry: Serum samples were collected to evaluate the following parameters: Na, K, Cl, Ca, p, ALP, ALT, AST, GGT, TBili, BUN, SDH, Creat, TP, Alb, Glob, A:G, Glucose, and Chol. [ss, as $^{*}p<0.05$; $^{**}p<0.01$]

- Unremarkable.

Urinalysis: Animals were housed in stainless steel metabolism cages and urine was collected for at least 16 hrs. The following parameters were assessed: Color, Clarity, SG, microscopic evaluation of Urine sediment, TotVol, pH, Pro, Glu, Bil, Ket, Nit, Leukocytes, Blood, and Urobilinogen.

- Unremarkable.

Plasma analysis: Blood samples were collected from TK animals to determine the plasma 4-aminopyridine concentrations. Samples were collected from cohorts of 3/sex/group prior to dosing and at 1, 2, 4, 8, and 24 hr postdose on Days 1 and 7. None of the TK animals was fasted prior to blood collection. Sponsor did not provide a data summary table; however, sponsor's Tables 4 and 5 provide the raw data concentrations collected on Days 1 and 7, respectively (see below).

- Plasma 4-AP concentration increased relative to the dose administered.
- Maximum concentrations generally occurred between 1-2 hr postdose.

- LD 4-AP concentrations were BLOQ by 8 hr postdose Days 1 and 7.
- MD and HD 4-AP concentrations were BLOQ by 24 hr postdose.

Table 4. 4-Aminopyridine Concentrations in Rat Plasma (Day 1)

Subject			Calculated Concentration, ng/mL					
			Day1					
Animal No.	Group	Sex	0Hr	1Hr	2Hr	4Hr	8Hr	24Hr
6033	9	M						(b) (4)
6034	9	M						
6035	9	M						
6092	9	F						
6093	9	F						
6094	9	F						
6042	10	M						
6043	10	M						
6044	10	M						
6101	10	F						
6102	10	F						
6103	10	F						
6051	11	M						
6052	11	M						
6053	11	M						
6110	11	F						
6111	11	F						
6112	11	F						
R6051	11	M						
6036	9	M						
6037	9	M						
6038	9	M						
R6038	9	M						
6095	9	F						
6096	9	F						
6097	9	F						
6045	10	M						
6046	10	M						
6047	10	M						
6104	10	F						
6105	10	F						
6106	10	F						
6054	11	M						
R6054	11	M						
6055	11	M						
R6055	11	M						
6056	11	M						
6113	11	F						
6114	11	F						
6115	11	F						
6039	9	M						
6040	9	M						
6041	9	M						
6098	9	F						
6099	9	F						

Table 4 (continued)

Subject			Calculated Concentration, ng/mL					
			Day1					
Animal No.	Group	Sex	0Hr	1Hr	2Hr	4Hr	8Hr	24Hr
6100	9	F						(b) (4)
6048	10	M						
6049	10	M						
6050	10	M						
6107	10	F						
6108	10	F						
6109	10	F						
6057	11	M						
6058	11	M						
6059	11	M						
6116	11	F						
6117	11	F						
6118	11	F						

Note: Any value below the LLOQ (b) (4) is reported as 0.000.
ADP: Animal Died Prior to Collection.

Table 5. 4-Aminopyridine Concentrations in Rat Plasma (Day 7)

Subject			Calculated Concentration, ng/mL					
			Day 7					
Animal No.	Group	Sex	0Hr	1Hr	2Hr	4Hr	8Hr	24Hr
6033	9	M						
6034	9	M						
6035	9	M						
6092	9	F						
6093	9	F						
6094	9	F						
6042	10	M						
6043	10	M						
6044	10	M						
6101	10	F						
6102	10	F						
6103	10	F						
R6051	11	M						
6052	11	M						
6053	11	M						
6110	11	F						
6111	11	F						
6112	11	F						
6036	9	M						
6037	9	M						
R6038	9	M						
6095	9	F						
6096	9	F						
6097	9	F						
6045	10	M						
6046	10	M						
6047	10	M						
6104	10	F						
6105	10	F						
6106	10	F						
R6055	11	M						
6057	11	M						
6113	11	F						
6114	11	F						
6115	11	F						
6039	9	M						
6040	9	M						
6041	9	M						
6098	9	F						
6099	9	F						
6100	9	F						
6048	10	M						
6049	10	M						

(b) (4)

Table 5 (continued)

Subject			Calculated Concentration, ng/mL					
			Day 7					
Animal No.	Group	Sex	0Hr	1Hr	2Hr	4Hr	8Hr	24Hr
6050	10	M						
6107	10	F						
6108	10	F						
6109	10	F						
6058	11	M						
6059	11	M						
6116	11	F						
6117	11	F						
6118	11	F						

(b) (4)

Note: Any value below the LLOQ (b) (4) is reported as 0.000.
ADP: Animal Died Prior to Collection.

Gross pathology: A complete gross necropsy examination was performed on all animals found dead on study in Phase A and on all main study animals at scheduled necropsy (Day 8) in Phase B. Necropsy included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with associated organs and tissues.

Phase A: Premature decedents

- HDM (2)—(1/2) Lung, consolidated (moderate) and Trachea, fluid, white (severe); (1/2)—unremarkable.
- HDF (2)—(2/2) Lung, red discoloration (moderate)

Phase B: Main study

- Unremarkable.

Organ weights: In all surviving Phase B animals, organ weights were recorded at scheduled necropsy. Absolute organ weights and organ weights relative to body and brain weights were calculated. Paired organs were weighed together. Thyroid/parathyroid and pituitary were weighed after fixation. The following organs were weighed: adrenal (2), brain—cerebrum, midbrain, cerebellum, medulla/pons, epididymis (2), eye including optic tract, ovary (2), testis (2), heart, kidney (2), lacrimal gland, exorbital (2), liver (3 sections), lung (whole), prostate and seminal vesicle (2), salivary gland—mandibular and sublingual, salivary gland—parotid (2), spleen, thymus, tureter (2) and uterus—both horns/cervix.

- All groups, unremarkable.

Histopathology: Tissues were preserved in 10% neutral buffered formalin except for the eyes and testes, which were preserved in Davidson's fixative. Tissues were collected and preserved, but microscopic evaluation was not performed. The following tissues were collected: adrenal (2), aorta, bone with marrow (femur and sternum), brain (cerebrum, midbrain, medulla/pons, and cerebellum), epididymis (2), eye w/optic nerve (2), gastrointestinal tract—esophagus, stomach—glandular and nonglandular, duodenum, jejunum, ileum, cecum, colon, and rectum, gonads—ovary (2) and testis (2), gross lesions, heart, joint—tibiofemoral, kidney (2), lacrimal gland, exorbital (2), larynx, liver—3 sections collected, lung collected whole, lymph nodes—mandibular and

mesenteric, mammary gland, pancreas, Peyer's patch, pituitary gland, prostrate and seminal vesicle, salivary gland—parotid (2), sciatic nerve, skeletal muscle—*biceps femoris*, skin, spinal cord (cervical, thoracic, and lumbar), spleen, thymus, thyroid/parathyroid, tongue, trachea, ureter (2), urinary bladder, uterus—both horns/cervix, and vagina.

- Microscopic evaluation was not performed.

2.) **2 Week Oral Range-Finding Toxicity Study in Rats**

Study no.: (b) (4) Study No. 684-007
Volume #, and page #: EDR 4.2.3.2. rat-oral-short, pp. 1-59.
Conducting laboratory and location: (b) (4)
Date of study initiation: 09 October 1992
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C4886, purity assumed 100%

Methods

Doses: 0 (C), 2 (LD), 10 (MD1), 20 (MD2), and 40 (HD) mg/kg/day
Species/strain: Rat, Charles River CD®
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: Oral, 4-AP offered in the diet
Age: 4 wks
Weight: Males, 114-138 g; Females, 105-119 g

Results

Mortality/morbidity: twice, daily.

- All animals survived to terminal sacrifice.

Clinical signs: twice, daily for overt toxicity at the time of the mortality/morbidity checks and detailed observations were conducted once during each study week.

- No treatment-related effects were observed.

Body weights: Pre-test and weekly during the study.

- Dose-dependent reduction of mean MD2 and HD group body weight in both sexes compared to respective controls.
  - Statistical significance achieved during week 1 in MD2 and HD groups for both sexes; and during week 2 in HD group (both sexes).
- In males, during week 1 of treatment mean body weight gain was reduced in a dose-dependent manner in 4-AP treated groups (LD, +63g; MD1, +59g; MD2, +40g; and HD, +13g) compared to the Control (+69g). During week 2, a trend was observed in the LD, MD1, and HD groups resulting in reduced mean body weight gain compared to the control. A slight increase was observed in MD2.
- In females, mean body weight gain in the Control was +44g (Week 1) and +21g (Week 2). Oral 4-AP treatment reduced mean body weight gain in a dose-dependent manner (LD, +40g; MD1, +34g; MD2, +21g; and HD, +11g) during

Week 1, but increased mean group body weight gain in a dose-related manner during Week 2 (LD, +22g; MD1, +17g; MD2, +32g; and HD, +33g).

Food consumption: weekly measurements for individual consumption.

- Week 1, males and most females were unremarkable; HDF had a significant increase in food consumption.
- Week 2, males consumed less food in a dose-dependent manner that reached significance in HDM (-9.4%), whereas food consumption in females was unremarkable except for HDF which had increased food consumption (+9.8%).

3.) **2-Week Oral Preliminary Range Finding Toxicity Study with 4-Aminopyridine in Rats**

Study no.: (b) (4)-2620-100
Volume #, and page #: EDR, 4.2.3.2.1 (b) (4)-2620-100, pp. 1-232
Conducting laboratory and location: (b) (4)
Date of study initiation: 20 December 1990
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot No. P85-12-4, Assumed 100%

Methods

Doses: 0 (C), 0.3 (LD), 1 (MD-1), 3 (MD-2), 10 (HD) mg/kg/day
Species/strain: Rat/Sprague Dawley Crl:CD® BR
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: Oral (gavage), 4-AP dissolved in distilled water, 1 ml/kg
Satellite groups used for toxicokinetics or recovery: none.
Age: 6-weeks of age
Weight: Male, 176-228 g; Female, 146-184 g

Observations and Results:

Mortality/Morbidity: Twice daily, AM/PM; approximately 1 hr postdose toxic effects of the drug administration were carefully assessed.

- All animals survived to scheduled sacrifice.

Clinical signs: Detailed observations were performed once prior to treatment and weekly during weeks 1 and 2.

- Treatment-related clinical signs were observed in MD2 and HD groups.
  - Tremors and/or sensitivity to external stimuli observed.
- Additional HD group observations included red crust on nose, rhinorrhea, and salivation in males; and vocalization, epistaxis, and/or seizures (partial or clonic static).

Body weights: Individual body weights were recorded on day of randomization, prior to treatment, and at weeks 1 and 2.

- No significant differences were reported in the mean body weight of 4-AP treated compared to Controls for either sex on Days 0, 7, or 14.
- A statistically significant reduction in mean body weight gain was found in HDM at each interval Days 0-7, 7-14, and 0-14. HDF had consistently less mean body weight gain than the Control at each interval, but statistical significance was not achieved.

Food consumption: Measured and recorded at weeks 1 and 2.

- During the interval Day 0-7, a statistically significant reduction in food consumption was noted in HDM. HDF had mean food consumption values that were consistently lower than the Controls, but statistical significance was not achieved.

Clinical pathology: Animals were fasted overnight; samples were collected 1-2 hr postdose for plasma drug concentrations, routine hematology and serum chemistry

Hematology: The following parameters were evaluated: RBC, Hgb, Hct, MCV, MCHC, MCH, RBC morphology, Plt, WBC, leukocyte differential, corrected leukocyte count.

- In females, mean RBC count was significantly elevated in MD2 (+4.5%). A significant increase in red cell mass was found in HD group (RBC, +8.8%; Hgb, +9.6%; and Hct, +8.5%) compared to the Control.
    - According to the study pathologist, the increased RBC count (MD2) and RBC mass (HD) was attributed to dehydration (i.e., secondary to a mild decrease in plasma water) and not directly due to fampridine treatment.

Serum chemistry: The following parameters were evaluated: glucose, creatinine, albumin, TBili, ALT, AST, ALP, BUN, Ca, P_i, TP, Alb, Glob, T Chol, Na, K, Cl.

- HD females had significantly increased inorganic phosphorus; and HD males had a minimal but significant increase in ALT.

TK analysis: Blood samples were collected from the retro orbital sinus within 1-2 hr of the 16th dose in animals under ketamine hydrochloride paralysis. Plasma samples were stored frozen until 4-AP drug concentrations were determined by HPLC analysis.

- No plasma TK results were provided in this study report.

Terminal procedures: All animals were weighed and necropsied. Necropsies were performed on all animals.

Gross pathology: The external surface, all orifices, cranial cavity, carcass, nasal cavity and paranasal sinuses, external surfaces of the brain and spinal cord and cut surfaces of the brain and spinal cord, thoracic, abdominal, and pelvic cavities and their viscera and cervical tissues and organs were examined for each animal.

- No macroscopic findings were considered test article-related.

Organ weights: The following organs were weighed: adrenals, kidneys, ovaries, heart, brain, liver, and testes. Organ-to-terminal body weight ratios and organ-to-brain weight ratios were calculated.

- No remarkable differences were noted in body weights upon comparison between the treatment groups and the controls.
- Mean absolute ovary weight in HD females and mean absolute liver weight in MD-1, MD-2 and HD females was significantly decreased when compared to the mean absolute ovary and liver weight of Control.
    - All organ to body weight ratios for females were without statistical significance.
    - MD-1, MD-2 and HD females also had significantly reduced liver-to-brain ratios compared to the control females.
- All 4-AP treated males had similar mean organ weight data both absolute and relative to Control.

Histopathology: From each animal, the following tissues were preserved in 10% neutral-buffered formalin: adrenals, ileum, kidneys, ovaries, heart, brain, liver, lesions, and testes.

- Histopathology was not performed.

4.) **Two-Week Range-Finding Study with Orally Administered 4-Aminopyridine in Rats**

Study no.: 0437RA44.001
Volume #, and page #: EDR 4.2.3.2. 0437RA44.001 pp. 1-190
Conducting laboratory and location: (b) (4)
Date of study initiation: 08 March 1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot No. P96-230-3, purity not supplied by (b) (4)

Methods

Doses: 0 (C), 1 (LD), 3 (LMD), 6 (HMD), and 12 (HD) mg/kg/day
Species/strain: Rat/Sprague Dawley CRL:CD (SD) BR
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: Oral, gavage, 10 ml/kg total daily dose, administered as 2.5 ml/kg four times a day.
Age: 5 weeks of age
Weight: Male, 237-272 g; Female 158-195 g
Unique study design or methodology (if any): Divided into 4 equal doses, administered approximately 6 hr apart.

Observations and Results:
Mortality/Morbidity: Daily prior to the first dose of the day.

- No deaths were found on study.

Clinical signs: Recorded daily prior to each dose administration.
- No treatment-related clinical signs were noted.

Body weights: Individual body weights were recorded on day of randomization and Days 1, 8 and 14. Day 15 body weights (fasted overnight) were recorded at necropsy prior to terminal sacrifice.
- On Day 8, HD males had a significant reduction in absolute mean body weight (-10.4%) and mean body weight gain (-67.6%) compared to the Control.
- Mean body weight (absolute and change) in females were similar to those observed in the Control.

Food consumption: Recorded daily through Day 14; [ss] $^{*}p<0.05$ and $^{**}p<0.01$ compared to control.
- In males, mean food consumption was reduced for HMD on Days 2-13 ([ss] Days 2^{**} and 3^{*}) and for HD on Days 2-12, 14 ([ss] Days $2\text{-}3^{**}$, Days $4\text{-}7^{*}$).
- In females, mean food consumption was unremarkable.

Clinical pathology: Animals were fasted overnight. Blood samples were collected on Day 15 one hour postdose.

Hematology: The following parameters were evaluated: RBC, Hgb, Hct, MCV, MCHC, MCH, Plt, and WBC (Total and Differential).
- Unremarkable.

Serum chemistry: The following parameters were evaluated: GLU, GLOB, TBILI, ALB, TP, AST, ALT, BUN, ALP, CREA, A/G ratio, Na, K, Cl, Ca, and Phos.
- Unremarkable.

TK analyses: Plasma samples were collected for estimation of plasma 4-Aminopyridine concentration from each animal approximately 1 hr postdose.
- *It is noted in the study report that the plasma samples were stored at* (b) (4) *No data were provided regarding plasma 4-Aminopyridine concentrations.*

Gross pathology: A complete gross necropsy examination was performed on all animals. Evaluation included examination of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with associated organs and tissues.
- No macroscopic findings were attributed to 4-aminopyridine treatment.

Organ weights: The following organs were weighed (paired organs weighed together): liver, adrenal glands, brain, kidneys, ovaries and testes.
- No differences in the group mean absolute organ weights or organ weight ratios were attributed to 4-aminopyridine treatment in either sex.

Histopathology: Control, HDM, and HDF were subjected to histopathological evaluation of organs and tissues collected at terminal necropsy. The following organs and tissues were preserved in 10% neutral buffered formalin: adrenal glands, aorta, brain (cerebral cortex, medulla, pons, cerebellar), cecum, cervix, colon, duodenum, epididymides, esophagus, extraorbital lacrimal glands, eyes w/optic nerve, femur-including articular surface, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs inflated w/mainstream bronchus, mammary gland, mesenteric lymph node, ovaries, pancreas, peripheral nerve, pituitary gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, midthoracic, and lumbar), spleen, sternum with bone marrow, stomach, testes, thigh musculature, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

- No treatment-related changes found in HDM and HDF tissues.

5.) **A 14-Day Oral Toxicity/Pharmacokinetic Study of 4-Aminopyridine in Rats**

Study no.: 0437RA44.003
Volume #, and page #: EDR 4.2.3.2. 0437RA44.003 pp. 1-292
Conducting laboratory and location: (b) (4)

Date of study initiation: 03 December 1996
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot No. P96-230-3, purity not supplied by (b) (4)

Methods

Doses: 0 (C), 6/4 (LD), 12 (MD), and 24/16 (HD) mg/kg/day
Species/strain: Rat/Sprague Dawley CRL:CD (SD) BR
Number/sex/group or time point (main study): 8/sex/group
Route, formulation, volume, and infusion rate: Oral, gavage, 10 ml/kg total daily dose, administered as 3.33 ml/kg three times a day.
Satellite groups used for toxicokinetics or recovery: 12/sex/group for TK
Age: 6 weeks of age
Weight: Male, 207-267 g; Female, 174-232 g
Unique study design or methodology (if any): Divided into 3 equal doses, administered approximately 4 hr apart. Vehicle = deionized water

Group	Daily Dosage t.i.d. (mg/kg)	Dose Volume t.i.d. (mL/kg)	Concentration (mg/mL)	Total Daily Dosage (mg/kg)	No. of Rats	
					Male	Female
1. Control	0	3.33	0	0	8	8
2. 4-Aminopyridine	2/1.33[a]	3.33	0.6/0.4[a]	6/4[a]	8	8
3. 4-Aminopyridine	4	3.33	1.2	12	8	8
4. 4-Aminopyridine	8/5.33[b]	3.33	2.4/1.6	24/16[b]	8	8

[a] 2 mg/kg t.i.d. was administered on Day 1 and 1.33 mg/kg t.i.d. was administered thereafter.
[b] 8 mg/kg t.i.d. was administered on Days 1 and 2. On Day 3 rats were dosed once at 8 mg/kg and then twice at 4 mg/kg (total of 16 mg/kg/day). On Day 4 and thereafter rats were administered 5.33 mg/kg t.i.d.

Observations and Results:

Mortality/Morbidity: Twice daily

- HD (2M and 3F) died within the first 4 days of treatment; none of these rats had any drug-related gross or microscopic pathological findings.
  - Day 1: Male #7055, prior to death tremors observed post-dose 1 and 2 (slight), pre-dose 3 (minimal) and immediately post-dose 3 (severe).
  - Day 2: Female # 7061 COD, unknown
  - Day 3: Female #7060 COD, unknown ; and Female #7064 COD, unknown
  - Day 4: Male #7051, COD attributed to prostatitis and cystitis. Gross and microscopic findings included hemorrhage in both the urinary bladder and prostate.
- Control, 1M #7001 sacrificed moribund on Day 11.
  - Several macroscopic findings with microscopic correlates were reported and COD was attributed to severe suppurative prostatitis and bone marrow depletion.

Clinical signs: Recorded several times daily throughout study that commenced with treatment initiation.

- Significant clinical signs were noted and related to CNS activation in all treatment groups with increasing incidence and severity with dose, especially during the first 4 days of treatment (prior to dose reduction) and in HD (both sexes) throughout the study.
  - Most prevalent clinical sign was tremors.

Body weights: Body weights were recorded twice prior to treatment and weekly at the start of treatment. [ss, $^{*}p<0.05$, $^{**}p<0.01$]

- Males
  - Day 8, mean body weight was reduced in MD (-6.3%) and HD (-8.5%) compared to control. Day 14, the decreased body weight MD (-10.4%*) and HD (-12.6%**) reached statistical significance compared to controls.
  - A significant dose-dependent reduction in total weight gain compared to the Controls, LD (-20.8 %*), MD (-43.1%**), and HD (-50%**) was found.
- Females
  - Day 8, a dose-related decrease in mean body weight was observed LD (-5.5%), MD (-7.8%) and HD (-4.1%) compared to Control. Day 14, body

weight was slightly less than the previous week LD (-6.0%), MD (-9.0%), and HD (-4.3%).
  - A significant dose-dependent reduction in total weight gain compared to the Controls, LD (-36.1 %*), MD (-69.4%**), and HD (-33.3%**) was found.

Food consumption: Recorded weekly throughout study, commencing with treatment.

- In males, a dose-dependent and statistically significant (all doses**) reduction in food consumption was found on Day 8. Day 14 was not statistically significant, although all treatment groups remained reduced compared to Control.
  - Day 8, LD (- 17%), MD (-23.4%), HD (-30.7%)
  - Day 14, LD (-9.4%), MD (-6.1%), HD (-6.7%)
- In females, a dose-related and statistically significant reduction in food consumption was found on Day 8. Day 14 was not statistically significant, and the dose groups largely recovered the weight lost in the first week.
  - Day 8, LD (- 12.4%), MD (-19.5%**), HD (-23.7%**)
  - Day 14, LD (-9.1%), MD (-7.7%), HD (+1.4%)

Ophthalmoscopic examination: Conducted pre-dose and at pre-study termination by a board certified veterinary ophthalmologist (b) (6)

- No definitive drug-related findings.
  - One HD male (#7052) had chorioretinitis and pinpoint retinal hemorrhages in the right eye. The veterinary ophthalmologist listed the potential causes of this type of lesion as infection, nonseptic inflammation, and toxic effect on the retina or choroid of the test compound; the cause was not established antemortem.

Clinical pathology: Animals were fasted overnight. Blood samples were collected on Day 15 one hour postdose.

Hematology: The following parameters were evaluated: RBC, Hgb, Hct, MCV, MCHC, MCH, Plt, and WBC (Total and Differential).

- Males
  - HD, mean HGB (-5.4%*) and HCT (-5.8%*) were significantly reduced compared to Control; WBC (-12.3%) and RBC (-6.3%) were reduced compared to Control.
  - A dose-dependent decrease in absolute neutrophil count was found at all doses of 4-aminopyridine (range -14.2% to -26.5%). None of the other cell types showed a dose-related effect.
- Females
  - Unremarkable.

Coagulation: PT and APTT were evaluated.

- Coagulation parameters were unaffected by 4-aminopyridine treatment.

Serum chemistry: The following parameters were evaluated: GLU, GLOB, TBILI, ALB, TP, AST, ALT, BUN, ALP, CHOL, CREA, A/G ratio, Na, K, Cl, Ca, and Phos.

- No biologically relevant or statistically significant findings were attributed to the test article.

Urinalysis: The following parameters were evaluated: Specific gravity, Appearance/Color, and a semi-quantitative Estimation was made of pH, PROT, GLU, KET, UROB, BILI, Blood, LEUK, NIT and microscopic examination of spun deposit.

- No 4-aminopyridine-related findings, any significant findings were considered incidental.

Pharmacokinetic analyses: Plasma was collected using EDTA as the anticoagulant. Animals were bled at the following times postdose 20 min, 1 hr 20 min, 4 hr 20 min and approximately 18 hr post-dose. The following sponsor provided table details the sample collections for PK analyses.

Group/Treatment	Dosage t.i.d. (mg/kg)	Dose Volume t.i.d. (mL/kg)	Number of Animals		Dose Day/Subgroup			
			Males	Females	1/A	1/B	14/A	14/B
5. 4-Aminopyridine	2/1.33	3.33	12	12	3♂ 3♀	3♂ 3♀	3♂ 3♀	3♂ 3♀
6. 4-Aminopyridine	4	3.33	12	12	3♂ 3♀	3♂ 3♀	3♂ 3♀	3♂ 3♀
7. 4-Aminopyridine	8/5.33	3.33	12	12	3♂ 3♀	3♂ 3♀	3♂ 3♀	3♂ 3♀

- Subgroup A animals were bled at 5, 20 and 80 minutes and 4 hours 20 minutes post the last daily dose.
- Subgroup B animals were bled 15 minutes, 2, 6 and approximately 18 hours post the last daily dose.
- Animals bled on Day 1 were sacrificed following the last sample time. All rats bled on Day 14 were sacrificed on Day 15 for gross and microscopic evaluation.

The reviewer notes that toxicokinetic data were not provided.

Gross pathology: Animals were sacrificed on Day 15 and complete necropsies were performed on all surviving animals in Groups 1-7 (Groups 1-4, main study; Groups 5-7, PK study). Necropsy evaluation included evaluation of the external body surface, all orifices, cranial, thoracic, and abdominal cavities and their contents.

- No macroscopic findings were attributed to 4-aminopyridine treatment.

Organ weights: Organ weights were measured from main study animals, which included the following: Adrenals, Brain (whole), Heart, Kidneys, Liver, Ovaries, Spleen, and Testes. [ss, $^{*}p<0.05$]

- Males
  - Mean terminal body weight was reduced by 4-aminopyridine in a dose-dependent manner (LD, -4.8%; MD, -9.9%*; and HD -11.3%*) compared to Control.
  - Absolute mean heart weight was reduced in a dose-dependent manner (LD, -8.1%; MD, -17%*; HD -19.2%*) compared to Control.

- Mean heart to body weight ratio largely accounted for the difference in mean absolute heart weight. Although there still was, a dose-related decrease observed (LD, -2.2%; MD, -6.5%; HD, -8.7%).
- Mean heart to brain weight ratio was reduced in a dose-dependent manner (LD, -5.3%; MD, -17.1%*; HD, -19.5%*) that reached statistical significance in both MD and HD groups compared to Control.

- Females
  - Mean terminal body weight was similar between Control and 4-aminopyridine treatment.
  - No trends or significant changes were observed in absolute organ weights that were attributed to 4-aminopyridine.

The sponsor concluded that there was no direct effect of 4-aminopyridine on organ weights. The reduction in the mean absolute heart weight and heart weight relative to brain weight were explained as the result of decreased body weight; and because the mean heart weight relative to body weight in the MD (-6.5% of Control) and HD (-8.7% of Control) were similar to Control.

The reviewer disagrees with the sponsor's interpretation of these data primarily because a dose-related decrease is still present after normalizing for the body weight. Although not statistically significant, the dose-dependent reduction in mean heart to body weight ratio implies a remaining 4-aminopyridine-dependent effect.

Histopathology: The organs and tissues collected from the animals at terminal necropsy were preserved in 10% neutral buffered formalin for histopathological evaluation. Control and HD groups (main and PK studies) were microscopically examined. Microscopic changes were graded on a scale of one to four, representing severities of minimal, slight, moderate, and marked.

The tissues collected included the following: adrenal glands, aorta, brain (whole), cecum, cervix, colon, duodenum, epididymides, esophagus, extraorbital lacrimal glands, eyes with optic nerve, femur—including articular surface, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs inflated with mainstem bronchus, mammary gland, mesenteric lymph node, ovaries, pancreas, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, midthoracic, and lumbar), spleen, sternum with bone marrow, stomach, testes, thigh musculature, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

- No treatment-related changes were found in tissues evaluated in the HD group compared to Controls of either sex.

- Any changes observed were considered by the study pathologist to be comparable in incidence and the usual type commonly seen in rats of this strain.

6.) **4-Week Oral Range-Finding Toxicity Study in Rats**

Study no.: (b) (4) Study No. 684-009
Volume #, and page #: EDR 4.2.3.2. rat-oral-short, pp. 1-136.
Conducting laboratory and location: (b) (4)
Date of study initiation: 02 December 1992
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C4886, purity assumed 100%

Methods

Doses: 0 (C), 5 (LD), 10 (MD1), 20 (MD2), and 30 (HD) mg/kg/day
Species/strain: Rat, Charles River CD® BR (VAF/Plus)
Number/sex/group or time point (main study): 10/sex/group
Age: 4 wks
Weight: Males, 142-177 g; Females, 150-181 g
Route, formulation, volume, and infusion rate: Oral, 4-AP offered in the diet at dose levels of 5, 10, 20 and 30 mg/kg/day. Control rats received the basal diet on the same regimen. Treated diet was available *ad libitum* seven days a week for 4 weeks. To reach optimal dosing accuracy, fampridine concentration in the diet was adjusted, based on body weight and food consumption data.

Observations and Results:
Mortality/morbidity: twice, daily.

- All animals survived to the terminal sacrifice.

Clinical signs: twice, daily for overt toxicity at the time of the mortality/morbidity checks and detailed observations were conducted at least weekly. Examinations included evaluations of appearance and condition, behavior and activity, excretory function, respiration, orifices, eyes and palpable masses.

- HD group, males (6/10) and females (6/10) had greatest incidence of clinical signs associated with mouth, nose, lungs, and CNS activation (convulsion). These data are summarized in the following table made by the reviewer.

Summary of clinical signs for Control and HD (M+F)

Clinical Sign	C	HD
No visible abnormalities	**19/20**	**12/20**
Abrasion	1/20 (1@1)	1/20 (1@2)
Convulsions		1/20 (1@1)
Labored breathing		3/20 (3@1)
Rales		1/20 (1@2)
Focal swelling		1/20 (1@1)
Red/brown material around nose		2/20 (2@1)
Hair loss face		2/20 (2@1)
Stained fur, anogenital region		2/20 (1@1 1@2)

Recurring observations: recurring, frequently observed signs were documented by weekly estimates of the proportion (%) of animals affected.

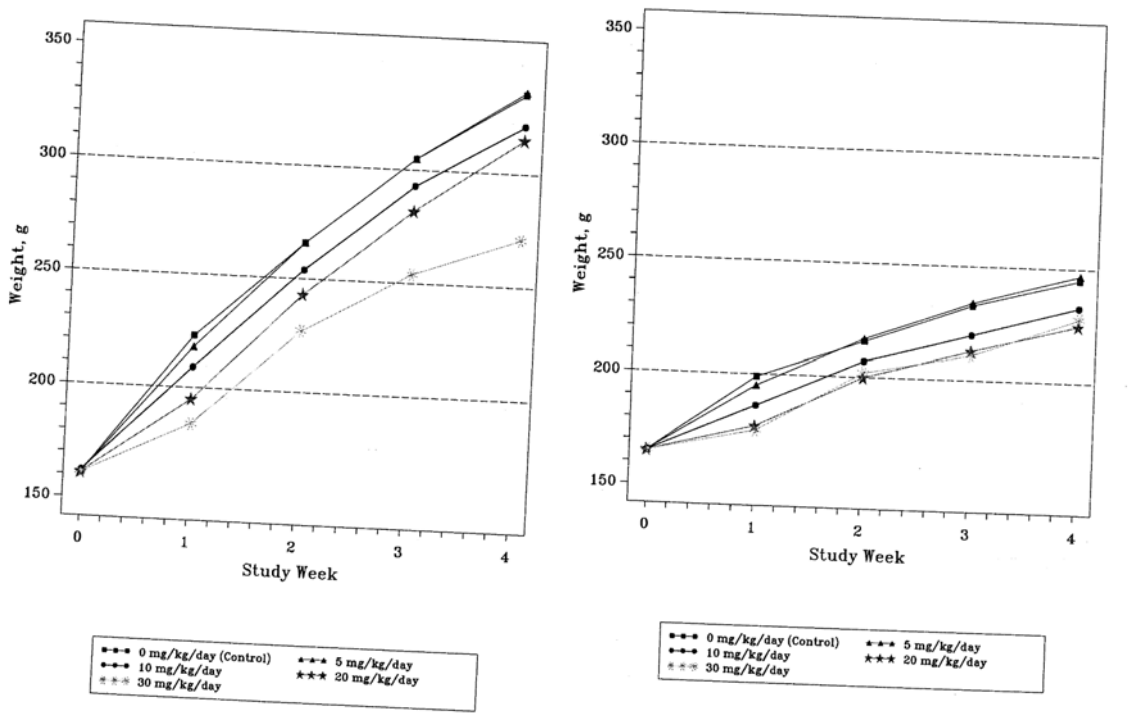
- Increase in activity and aggressive behavior was observed in all HD animals of both sexes during handling (Weeks 3 and 4).

Body weights: Pre-test and weekly during the study, [**ss, p<0.01]

- Males
  - LD body weight gain was similar to Control.
  - LMD and HMD weight gains were reduced compared to mean Control weight gain (see sponsor's Figure 1 below).
  - HD body weight gain plateaued between weeks 3 and 4 of treatment, and a significant reduction of mean HD body weight was apparent compared to Control (-19.0%**).
- Females
  - LD body weight gain was similar to Control.
  - Mean LMD weight gain (-4.9%) was reduced compared to Control.
  - Mean HMD (-8.2%) and HD (-7%) weight gain was similar, but reduced and delayed as compared to Control. See sponsor's Figure below.

Figure 1. Mean Body Weight

MALE **FEMALE**



Food consumption: weekly measurements for individual consumption, [**ss, p<0.01]

- Week 1
  - LMD (M, -19.1%; F, -16.7%), HMD (M, -28.2%; F, -28.7%), and HD (M, -38.1%; F, -22.3%) mean food consumption was significantly reduced compared to Control.

- Week 4
  - LMD (M, -3.2%; F, -5.2%) and HMD (M, -3.2%; F, -7.7%) mean food consumption was similar to Control, in both sexes.
  - HDM (-10.8%**) mean food consumption was significantly reduced compared to Control, whereas HDF food consumption was increased (+5.1%).

4-Aminopyridine consumption:

- The amount of 4-aminopyridine consumed was calculated for each dose group and the data (adapted from sponsor's Table 5.1.1.5—B) were presented in the reviewer generated table below. Overall, the mean 4-aminopyridine consumed at each dose group approximated the targeted dose for both sexes.

Mean 4-Aminopyridine consumed for each dosage group

Dose group	MALE	FEMALE
Control	0.0	0.0
5 ppm	5.2	4.9
10 ppm	10.5	13.3
20 ppm	23.8	19.9
30 ppm	32.6	31.1

Plasma drug levels: From the first five survivors for each group, plasma was collected prior to study termination (between 5:54 and 6:29 AM) for 4-AP concentration determination measured by HPLC.

- Male, plasma 4-AP concentrations ranged from 77.4 to 271 ng/ml and were dose proportional.
    - Female, plasma 4-AP concentrations ranged from 70 to 333 ng/ml and were dose proportional.

Rat plasma 4-aminopyridine concentrations following 4-week dietary administration.

Dose group	MALE	FEMALE
Control	0.0	0.0
5 ppm	77.4 ± 13.4	70.2 ± 22.6
10 ppm	121 ± 29.7	108 ± 22.8
20 ppm	204 ± 41.6	200 ± 50.3
30 ppm	271 ± 73.6	333 ± 73.9

Anatomic pathology: All animals received a complete postmortem examination in which the skin was reflected from a ventral midline incision and any abnormalities were correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined. The organs were removed, examined, and where required by protocol, placed in a fixative. The pituitary was fixed *in situ*. Representative samples of protocol-designated organs and tissues were collected at necropsy, placed in phosphate-buffered neutral formalin where appropriate, and saved for possible future examination. The tissues collected included the following: adrenal glands (2)^{w}, aorta, bone with bone marrow (femur), bone marrow smear (2), brain^{w} (fore, mid, and hind), eyes with optic nerve (2), gastrointestinal tract—esophagus, stomach—glandular and non glandular, duodenum, jejunum, ileum, cecum, colon, gonads—ovary^{w} (2) and testes^{w} with epididymis (2), gross lesions, heart^{w}, kidney (2)^{w}, liver^{w} (2 lobes examined, 3 sections collected), lung with bronchi (2), lymph nodes—mandibular and mesenteric, mammary gland, pancreas, prostate and seminal vesicle (2), salivary gland—mandibular and sublingual, sciatic nerve, skeletal muscle—thigh, skin, spinal cord—thoracic, spleen^{w}, thymus, thyroid/parathyroid (2), trachea, ureter (2), urinary bladder, uterus—both horns, uterus—cervix, and vagina.

Macroscopic findings:

- Unremarkable, no treatment-related findings.

Microscopic findings:

- Histopathology was not performed.

Organ weights: In the above list of organs/tissues demarcated with [w] were weighed, and the appropriate weight ratios were calculated. Organ weights were represented as absolute and relative to body and brain weights. [ss, [*]p<0.05, [**]p<0.01]

- HD males
  - Significant decreases in the mean absolute heart (-23.8%[*]), kidney (-14.6%[**]), and liver (-25%[**]) weights were found compared to Control.
  - Decreases in mean relative organ:body weight ratios were observed in the heart (-5.5%) and in the liver (-7.7%[*]).
  - Significant decreases in the mean relative organ to brain ratios were observed for the heart (-23.8%[*]), kidney (-15.4%[**]), and liver (-25.5%[**]).
- MD and HD females
  - Mean relative brain to body weight ratios were increased in the MD (+11.6%[*]) and increased in the HD (+14.5%) compared to Control.
  - Mean relative liver to brain weight ratios were decreased in the MD (-15.1%[**]) and in HD (-14.4%[**]).

The sponsor notes that the toxicological significance of the changes observed in mean absolute and mean relative changes in the heart, liver and kidney weights were unknown, since microscopic analyses were not performed.

- *The reviewer noted in males:*
  - *A dose-dependent decrease in mean absolute heart weight—LD (-4.3%), LMD (-7.9%), HMD (-11.5%), HD (-23.8%); and a dose-dependent decrease in mean relative heart to body weight ratio was also observed—LD (-7%), LMD (-7.4%), HMD (-12.7%), and HD (-24.3%).*
  - *The significant decreases observed in the liver and kidney were limited to the HD group and not accompanied by dose-dependent changes at the lower doses.*

7.) **13-Week Oral Toxicity Study with 4-Aminopyridine in Rats with a 4-Week Recovery Period**

Study no.: (b) (4) 2620-105
Volume #, and page #: EDR 4.2.3.2. rat-oral-medium, pp. 1-685.
Conducting laboratory and location: (b) (4)

Date of study initiation: 05 February 1991
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, P85-12-4, purity assumed 100%

Methods

Doses: 0 (C), 0.4 (LD), 2 (MD), 10 (HD) mg/kg/day
Species/strain: Rat, Charles River CD® BR
Number/sex/group or time point (main study): 10/sex/group
Number/sex/group (TK study): 5/sex/group
Number/sex/group (4-wk Recovery): 5/sex/Control and HD
Age: 4-5 wks; dosing initiation, approximately 7 wks
Weight: Males, 220-279 g; Females, 156-217 g
Route, formulation, volume, and infusion rate: Oral, gavage

Observations and Results:

Mortality/morbidity: twice, daily.

- HDF, 3 found dead during course of the study. Animal B16847 was found dead during week 7. Animals B16831 and B16844 were found dead during week 9.
    - Cause of death was undetermined.
- All other animals survived to termination.

Clinical signs: One to two hours post-dose, careful cageside examination was performed once daily. Particular attention was paid to any abnormal CNS behavior. A thorough physical examination was conducted at each weighing interval.

- Clinical observations made at the weekly physical examinations were considered few and not treatment-related.
- Within 1 hr of dosing, HDM and HDF showed the following treatment-related observations: tremors, excessive salivation, partial seizures (2/10 M; 3/10 F), clonic-tonic seizures (2/10 M; 1/10 F), rhinorrhea, lacrimation (females only), chromodacryorrhea, and red crust on the nose.
    - These observations persisted throughout the dosing period.
    - Clonic-tonic seizures were observed periodically in 1-2 males throughout the dosing period (B16777, wks 2, 9, 12, and 13; B16778, wk 13) and in one female (B16847) during week 7.
    - Partial seizures were observed in the same 2/10 HDM (B16777, wk 9; B16778, wks 2-13) and in 3/10 HDF (B16833, wks 8, 9, and 12; B16834, wk 3; and B16836, wks 6, 10, and 11).
- No post-dose clinical signs were observed for Control, LD, or MD groups.
- During the recovery period, none of the animals exhibited these treatment-related observations.

Neurobehavioral Assessments: A Functional Observational Battery (FOB), was conducted prior to treatment and during weeks 4, 8, 13 and 17. During week 4, the initial FOB was conducted approximately 18-25 hours after the previous day's dose. Thereafter, the FOB was conducted immediately after dosing due to the number of clinical signs being observed in treated animals after dosing, and lack of a clear effect observed during week 4 (when the battery was conducted prior to dosing). The following parameters were evaluated for each animal as it was removed from its cage: ease of cage removal, ease of handling, palpebral closure, lacrimation, color of tears, salivation, respiration, appearance of fur, piloerection, writhing, and vocalizations. Each animal was

placed into a plexiglas arena on a flat surface for 1-minute period and the following behaviors were evaluated: posture, gait, arousal, bizarre behavior, stereotyped behavior, circling, tremors, and convulsions. During the 1-minute observation period, the latency to first step, number of rears, and number of convulsions that occurred were counted and recorded. At the conclusion of the 1-minute period, the number of urine pools (polyuria) and fecal boli (or diarrhea) was counted and recorded. Each animal was also evaluated in the testing arena for the following: approach response, touch response, catalepsy withdrawal, and righting reflex.

The following reflexes and physiological parameters were also evaluated at the same intervals as the FOB testing: tail flick latency, grip strength (fore and hindlimbs), landing foot splay, startle, pupil reflex, and automated motor activity.

- HD group showed treatment-related effects during FOB assessment at Weeks 8 and 13.

The following reviewer-generated table summarizes the incidence and severities of treatment-related effects observed in the FOB assessment.

Combined incidence of selected FOB parameters for male and female rats

Dose	Tremors			Righting Reflex, Slightly Uncoordinated				Arousal, Somewhat High			
	8	13	17	4	8	13	17	4	8	13	17
C	0/20	0/20	0/10	3/20	1/20	1/20	0/10	0/20	0/20	1/20	0/10
HD	**10/20**	**14/19**	0/10	3/20	**7/20**	**3/19**	0/10	**6/20**	0/20	0/19	0/10

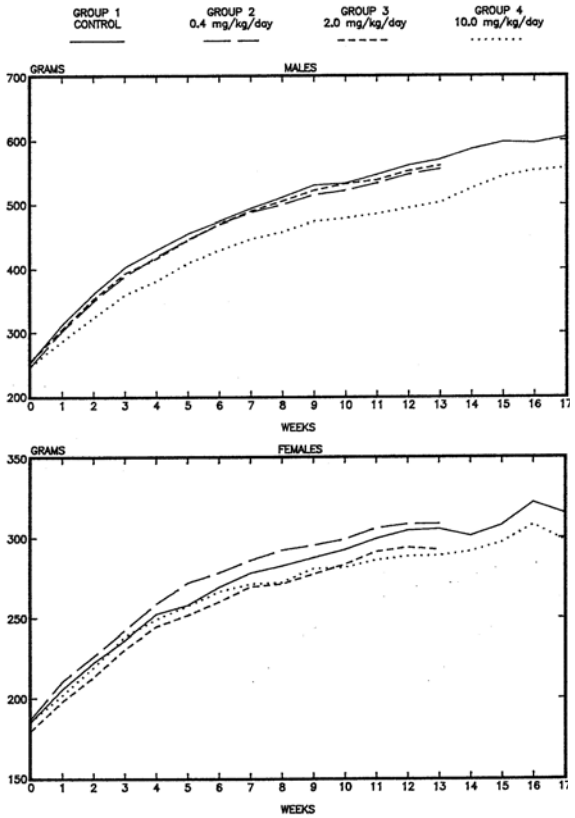
Dose	Salivation			Soiled Fur	
	8	13	17	13	17
C	0/20	0/20	0/10	0/20	0/10
HD	**2/20 (1@1; 1@2)**	**3/19 (2@1; 1@2)**	0/10	**3/19 (2@1; 1@2)**	0/10

Severity (#@score), where 1=slight, 2=moderate

- No consistent treatment-related findings were found for the following FOB parameters:
  - Ease of cage removal, ease of handling, palpebral closure, lacrimation, tear color, piloerection, respiration, writhing, vocalizations, posture, gait, bizarre behavior, stereotyped behavior, circling, approach response, touch response, catalepsy withdrawal, forelimb grip strength, hindlimb grip strength, total activity counts, auditory startle, landing foot splay, tail flick latency, step latency, number of fecal boli, number of convulsions, number of urine pools, number of rears or papillary reflex.

Body weights: Measured and recorded at randomization, pretreatment FOB, at initiation, and weekly thereafter. Reproduced below, sponsor's Figure 5 shows the mean body weights of control and 4-aminopyridine treated groups.

- HDM, mean absolute body weight was significantly decreased compared to Control during Weeks 1-14; and HDM mean body weight gain was significantly decreased for Weeks 0-1, 1-2, 2-3, 7-8 compared to the Control. A significant increase was observed during Week 14-15 and 15-16 during the recovery period.
- Mean body weight gain was significantly reduced for LDM and MDM during Weeks 7-8 and Weeks 0-1, respectively.
- Female, mean absolute body weight was decreased [nss] over the 13-week treatment period.
- LDF, unremarkable.



Food consumption: Food consumption data were collected weekly for weeks 0-13 (terminal sacrifice) and 13-17 (recovery). These data are summarized in sponsor's Figure 6, below.

- Food consumption was significantly decreased for MDM, HDM, and HDF during the first interval (Weeks 0-1).
- HDM had a significant reduction in food consumption for interval Weeks 1-2, 2-3, and 3-4; however, during the recovery period, a significant increase in food consumption was observed at Weeks 14-15 compared to Control.

Ophthalmoscopy: Indirect ophthalmoscope and 1% Mydriacyl® were used to examine all rats prior to treatment initiation, main study termination (Week 13), and recovery

termination (Week 17). Animals with eye lesions identified prior to treatment were excluded.

- The ophthalmology report noted no 4-AP treatment related effects at study termination (Week 13) and following a 4-week recovery period (Week 17) and was signed and dated by (b) (6)

Clinical pathology: Blood samples were collected from all animals scheduled for terminal sacrifice at study weeks 13 and 17 for hematology and clinical chemistry. The following hematological parameters were evaluated: cell morphology, RBC, Hct, Hgb, WBC, Diff, MCH, MCHC, MCV, and Platelet.

- HDM, mean number of monocytes was significantly reduced compared to Control
  - Recovery HDM monocyte number was similar to Control.
- Recovery HDM had a significant increase in HCT that was not evident in HDM at terminal sacrifice.

The following clinical chemistry parameters were examined: ALT, Albumin, ALK P, AST, BUN, Calcium, T Chol, Chloride, Creat, globulin, In Phos, Sodium, T Bili, and T Prot. [ss, $^{*}p<0.05$]

- HD group had a few sporadic alterations in clinical chemistry parameters.
  - HDF, ALK P* and IN PHOS* were significantly increased at terminal sacrifice compared to Control.
    - Recovery HDF, ALK P and IN PHOS were similar to Control.
  - HDM, Cl^{-*} was significantly increased at terminal sacrifice compared to Control.
    - Recovery HDM Cl^{-*} was similar to Control.

Plasma drug levels: Plasma samples were collected approximately 1-2 hours post dose after the first day and during the last week of dosing. In the reviewer-generated table below, the results of the plasma drug concentrations were summarized from sponsor's Text Table 1.

Plasma 4-Aminopyridine Concentrations (ng/ml)

Sex	Week	4-Aminopyridine (Mean ± SD)		
		LD	MD	HD
M	1	4.75 ± 4.027	158.4 ± 33.8	427.74 ± 258.69
	13	30.41 ± 18.44	126.99 ± 51.49	703.56 ± 348.68
F	1	0.0 ± 0.0	109.62 ± 33.83	412.94 ± 264.72
	13	17.31 ± 13.27	124.27 ± 39.21	402.10 ± 168.55

- Plasma 4-AP concentrations increased in a dose-dependent manner.
- Plasma concentrations were approximately dose-proportionate between MD and HD groups for both sexes and at Weeks 1 and 13. Week 13 plasma concentrations were approximately dose-proportionate between LD and MD groups for both sexes. In contrast, Week 1 LD plasma concentrations were low to undetectable.

Terminal studies:

Gross pathology-All animals found dead and/or sacrificed in extremis during the study were subjected to gross postmortem examination. All surviving animals (Wk 13 and Wk 17) were fasted overnight, weighed on the day of scheduled necropsy, and necropsies were performed.

- Unremarkable, no notable gross pathology or treatment-related findings were observed.

Organ weights-The following organs were weighed: adrenals, brain (including brainstem), heart, kidneys, liver, ovaries, and testes with epididymides. Paired organs were weighed and reported separately in appendices and reported as combined weight in tables. Organ-to-terminal-body-weight and organ-to-brain-weight ratios were calculated. The statistically significant organ weight changes are summarized below in sponsor's Text Table 2.

- HD group, a treatment-related decrease in terminal body weight was observed.
- HDF, a significant increase in liver-to-terminal body weight ratio compared to the corresponding control.

Text Table 2
Statistically Significant Organ Weight Data

	Absolute Organ Weight			Organ-to-Terminal-Body-Weight Ratio			Organ-to-Brain-Weight Ratio		
Organ	2	3	4	2	3	4	2	3	4
Nonrecovery Males									
Terminal Body Weight			↓						
Adrenal	↑		↑	↑		↑	↑		↑
Testes						↑			
Kidney									↓
Nonrecovery Females									
Terminal Body Weight			↓						
Brain with Stem				↓		↑			

Key: ↓ = Significantly decreased, $p \leq 0.05$.
↑ = Significantly increased, $p \leq 0.05$.

Histopathology-The following list includes the tissues from each animal that were preserved in 10% neutral-buffered formalin: adrenals, aorta, brain (with brainstem), colon, cecum, rectum, duodenum, esophagus, exorbital lacrimal glands, eyes (in Bouin's fixative), femur with articular surface, femur (bone marrow), gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mesenteric lymph node, ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicles, skin, spinal cord (cervical, lumbar, and mid-thoracic), spleen, sternum with marrow, stomach, testes with epididymides, thigh muscle, thymus, thyroid with parathyroids, trachea, urinary bladder, and uterus. All preserved tissues from Control and HD groups, and the liver, lungs, heart, and kidneys from the animals in the LD and MD groups were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined

microscopically. For the brain, at least 10 step sections were examined microscopically. The following reviewer-generated table summarizes the notable histopathology results from Table 15B and 15C of sponsor's study report that resulted in review of all dose groups. The reviewer also noted three additional tissues (prostate, glandular stomach, and thymus) which showed findings in the HD group; however, the sponsor did not evaluate all dose groups for these tissues. Most of the findings resolved following a 4-week recovery period, except in the kidney.

	Terminal				Post Recovery	
Tissue/Organ	**C**	**0.4**	**2**	**10**	**C**	**10**
Heart[a]	**0/30**	**2/30**	**1/30**	**1/27**	**3/10**	**1/10**
Inflammation, chronic		2/30	0/30	1/27	0/10	1/10
subacute		0/30	1/30	0/27	3/10	0/10
Liver	**1/30**	**3/30**	**1/30**	**3/27**	**0/10**	**0/10**
Necrosis	0/30	2/30	1/30	1/27		
Inflammation, chronic	1/30	1/30	0/30	2/27		
Kidney	**2/30**	**1/30**	**1/30**	**5/27**	**1/10**	**5/10**
Inflammation, chronic	2/30	0/30	1/30	1/27	1/10	1/10
Tubule, regeneration	0/30	0/30	0/30	1/27	0/10	1/10
Dilatation, tubular	0/30	0/30	0/30	1/27	0/10	1/10
pelvic	0/30	0/30	0/30	1/27	0/10	1/10
Proteinaceous casts	0/30	1/30	0/30	1/27	0/10	1/10

	Terminal		Recovery	
Tissue/Organ	**C**	**10**	**C**	**10**
Prostate	**0/15**	**3/15**	**0/5**	**0/5**
Microconcretions		2/15		
Inflammation, chronic		1/15		
Stomach, glandular	**0/30**	**6/27**	**1/10**	**0/10**
Inflammation, perivascular	0/30	1/27	0/10	0/10
Erosion	0/30	4/27	1/10	0/10
Inflammation, acute	0/30	1/27	0/10	0/10
Thymus, malignant	**0/30**	**0/27**	**0/10**	**1/10**
Squamous cell carcinoma				1/10

[a]Findings in the heart limited to males.
NE= Not examined; **Bold** corresponds to total incidence of microscopic findings in each tissue/organ.

- One HDM (B16760) had a malignant squamous cell carcinoma of the thymus, which is described below from the pathologist's report.
  - "Small cystic mass occupying a lobule was found, which contained keratin and cellular debris. The cyst was lined by poorly differentiated neoplastic stratified squamous epithelium; and the cells had pleomorphic nuclei with multiple nucleoli and indistinct cytoplasmic intercellular bridges. A moderate number of mitotic figures were observed. Smaller nests of

neoplastic cells were observed on the periphery of the mass which contained foamy or vacuolated cells, suggesting differentiation toward adnexal structures."

8.) **A 13-Week Oral Toxicity Study of 4-AP in Rats**

Key Study Findings

- Deaths occurred in HDM (3/15) and HDF (5/15) during main study and in TK group; in males, urinary tract obstruction (2/3) and spinal cord hemorrhage and necrosis (1/3) caused death and for females, COD was undetermined.
- NOAEL = 3 mg/kg/day
- NOEL = 1 mg/kg/day

Study no. (b) (4) 1139-002
Volume #, and page #: EDR 4.2.3.2. rat-oral-long, pp. 1-1726.
Conducting laboratory and location: (b) (4)
Date of study initiation: 01 August, 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, P175-4-1, purity 100.5%; Vehicle = distilled water

Methods

Doses: 0 (C), 1 (LD), 3(MD), and 9 (HD) mg/kg/day
Species/strain: Rat Crl:CD® (SD)
Number/sex/group or time point (main study): 15/sex/group
Route, formulation, volume, and infusion rate: Oral, gavage, 10 ml/kg/dose
Satellite groups used for toxicokinetics or recovery: TK: C, 6/sex; LD, MD, HD 12/sex; Recovery: C and HD, 10/sex
Age: ≈ 6wks
Weight: Males, 135-171 g; Females, 131-152 g

Observations and Results

Mortality/morbidity: twice, daily.

Main study

- HDM (3/15) and HDF (5/15) died on study.
  - 2/3 HDM euthanized *in extremis* on Days 45 and 91 for #2129 and #2123, respectively, due to clinical signs associated with 4-AP administration. 1/3 HDM found dead on Day 73 (#2126).
    - COD 2M (#2123 and #2126) had urinary tract obstruction and 1M (#2129) had hemorrhage/necrosis of the spinal cord.
  - 5/5 HDF found dead on Days 49 (#2159), 64 (#2152), 73 (#2157), 85 (#2136), and 87 (#2150).
    - No COD was determined.

TK (necropsy was not performed as per protocol)

- HDM (2/12)—#2209 found dead on Day 21; #2203 euthanized *in extremis* Day 73.

- HDF (1/12)—#2244 found dead on Day 4.

Clinical signs: Detailed clinical examination was performed weekly; and occasionally, observations were made at unscheduled times.

- LD and MD, unremarkable.
- HD, several clinical signs were observed for both sexes. The following reviewer-generated table summarizes the individual clinical findings found in sponsor's Appendix D.

Incidence[a] of clinical signs observed for HD but absent for C, LD, and MD Grps

Week Present:	3		4		7		11		13	
Clinical Sign	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Increased activity	3/25	14/25								
Hypersensitivity to touch	16/25	24/25		1/25[b,c]			1/23[c]			3/22[c]
Tremors	24/25	25/25				3/24	1/23[c]	1/22		3/22[Y]
Rapid breathing	25/25	25/25		1/25[b]			1/23			
Splayed limbs		1/25				1/25				
Tonic convulsion				1/25[b]				1/22	1/23[*]	3/22
Clonic convulsion				1/25	3/24	4/24[Y]				1/22[X]
Self mutilation					1/24				1/23[*]	
Twitching					1/24	1/24	1/23			
Impaired limb function					1/24					
Vocalization					2/24	1/24	1/23	1/22		1/22[Y]
Jumping							1/23[Z]	1/22	1/23[*]	2/22[Y]
Prostration					1/24					
Stereotypy									1/23[*]	1/22
Piloerection									1/23[*]	
Moribundity									1/23	

[a]Incidence corresponds to # animals affected/total # animals present at time of observation; deaths only occurred in the HD group.
[b]Signs occurred for same F #2156.
[c]Clinical sign also occurred Wk 3.
[X]F #2155 also 1/4 of clonic convulsions observed Wk 7.
[Y]F #2150 experienced multiple clinical signs.
[*]M #2118 experienced multiple clinical signs.

- During the recovery period, none of the animals (0/10 per sex) exhibited these treatment-related observations.

Neurobehavioral Assessments: A Functional Observational Battery (FOB), was conducted prior to treatment and during weeks 4, 8, 13 and 17 (sponsor did not provide information as to when the FOB was conducted in relation to dosing). The following

parameters were evaluated for each animal included: activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration. Amount (qualitative or quantitative) of defecation and urination were also recorded. Forelimb and hindlimb grip strength was measured and hindlimb splay was quantitatively measured. Pain perception was evaluated as latency of response to thermal stimulus to a hot plate apparatus (52ºC). [ss, $^{*}p<0.05$; $^{**}p<0.01$]

Activity/Arousal

- All dose groups, unremarkable.

Neuromuscular Assessment

- LD, unremarkable.
- MDM and HDM—increased hindlimb grip strength and decreased hindlimb splay (Weeks 4, 8, and 13)
- MDF and HDF—decreased hindlimb splay (Weeks 4 and 8)
- Recovery HD group—unremarkable

Sensorimotor Measurement

- HD, males (Week 8, +81%* of CM) and females (Weeks 4, +74%* of CF and 8, +110%** of CF) had an increased thermal response to hotplate.

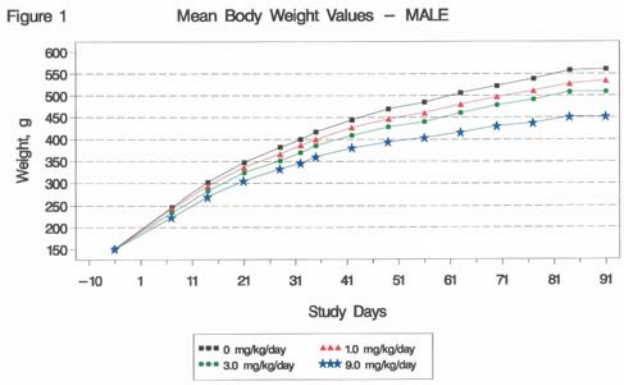
Autonomic Measurement

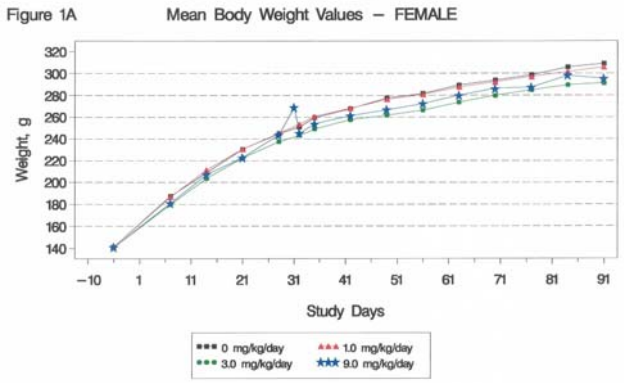
- All doses, unremarkable

Physiological Measurement

- Unremarkable.

Body weights: Measured and recorded at arrival, prior to randomization, and weekly. The following sponsor's Figures 1 and 1A illustrate the effect of 4-AP treatment on mean male and female bodyweight, respectively. [ss, $^{*}p<0.05$; $^{**}p<0.01$]





Main study

- LDM, LDF, MDF, and HDF mean absolute body weight unaffected by 4-AP.
- MDM (-9.3%**) and HDM (-19.3%**) showed a dose-dependent decrease in mean absolute body weight compared to CM after 13 Weeks of 4-AP treatment.
- Recovery HDM (-10.8%*) mean absolute body weight had increased from Week 13; however, it remained significantly decreased compared to CM.

TK study

- LDM, unremarkable.
- Mean absolute body weight for LDF, MDF, HDF was decreased -11.8%, -10.9%, and -14.7% compared to CF, respectively.
- Mean absolute body weight for MDM and HDM was decreased -7.3% and -15.8% compared to CM, respectively.

The following reviewer-generated table summarizes the overall mean body weight gain (0-91 days) for main study and TK groups and mean body weight gain 0-119 days for recovery groups. In the table, the absolute mean body weight gain of the Control group is provided. For the 4-AP treated groups, the percent of difference from Control is provided.

Sex	Main				Recovery		TK			
	C	LD	MD	HD	C	HD	C	LD	MD	HD
M	410.8g	-6.5%	-12.7%	-26.6%	411.4g	-14.8%	378.2g	-0.8%	-9.3%	-21.6%
F	168.1g	-2%	-10.5%	-8.1%	175.6g	-2.9%	187.8g	-21.1%	-18.9%	-26.8%

Food consumption: Food consumption was collected weekly.

- Food consumption was unremarkable for LDM and LDF.
- MDM had significantly less food consumption than CM during Weeks 1-8, 11, and 12.
- HDM had significantly less food consumption than CM from Week 1-13. During the recovery period, food consumption increased almost to that of controls.
- Females were less affected and subtle differences were noted for MDF during Week 1 and for HDF during Weeks 1 and 3; overall, the effect in females was unremarkable.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted on all animals by a veterinary ophthalmologist, pretest, and prior to terminal and recovery necropsies.

- All dose groups were unremarkable for both sexes.

Clinical pathology: All main study and recovery animals were evaluated for clinical pathology. Animals were fasted overnight prior to sample collection, but had access to drinking water. Blood samples were collected for clinical pathology and for coagulation parameters. Clinical chemistry was evaluated from serum samples.

Hematology

The following hematological parameters were examined: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, Platelets, Abs. Retic, Percent Retic, Neutro., Lymph, Monos, Eos, Baso, and Lg. unstained cells.

Summary of notable hematological findings for males represented as the percent difference from CM.

Hematological Parameter	LD	MD	HD	HD Recovery
Leukocytes	-2.8%	-4.6%	-18.1%*	+4.2%
Platelets	-11.2%	-8.3%	-13.6%	+2.3%
Neutrophils			-15%	+2.5%
Lymphocytes	-2.8%	-4.6%	-17.8%*	+5.6%
Monocytes		-14.3%	-26.8%	**-21%**
Eosinophils	-14.9%	-22.4%	-32.9%	-4.4%
Basophils	-18.1%	-19.3%	-36.1%**	+2.9%
Lg. Unstained Cells	-19.4%	-23.4%	-37.9%*	**-12%**

- HDM had significantly lower leukocytes, lymphocytes, basophils, and large unstained cells than CM. Greater than 10% difference in cell counts were found for platelets, neutrophils, monocytes, and eosinophils. After 4-Week recovery, most of these differences resolved; however, the dose-dependent decrease in monocyte number remained for HD recovery group compared to CM (-21%).
- Sponsor suggested that none of these effects was 4-AP related.

Coagulation

Coagulation parameters, APTT and PT were examined and shown to be unremarkable for all dose groups of both sexes.

Clinical chemistry

The following clinical chemistry parameters were examined: ALT, Albumin, ALK P, AST, BUN, Calcium, T Chol, Chloride, Creat, globulin, In Phos, Sodium, T Bili, and T Prot.

- HD group had a few sporadic alterations in clinical chemistry parameters; however, none of these was test article-related.

Plasma analysis: Plasma samples were collected on Days 1 and 90 at 1, 2, 4, 8, and 24 hrs postdose. Samples from Control animals were also collected on Days 1 and 90; these animals were not fasted overnight.

PK/TK analysis: PK/TK analysis was performed by (b) (6) Test article PK/TK parameters were calculated for parent and, if appropriate, the metabolite(s).

In the reviewer-generated table below, the results of the plasma drug concentrations were summarized from sponsor's Text Table 1.

4-Aminopyridine TK parameters following oral administration for 1 or 90 days

Sex	Wk	4-Aminopyridine (Mean ± SD)								
		1 mg/kg			3 mg/kg			9 mg/kg		
		C_{max}	AUC	T_{max}	C_{max}	AUC	T_{max}	C_{max}	AUC	T_{max}
M	1	98.8	242	1.00	168	733	1.00	256	1390	1.00
	13	131	373	1.00	291	1100	1.00	500	2620	1.00
F	1	92.9	208	1.00	152	636	1.00	242	1250	1.00
	13	131	271	1.00	225	995	1.00	469	2570	1.00

- Plasma 4-AP concentrations increased in a dose-dependent manner.
- Plasma concentrations were approximately dose-proportionate between MD and HD groups for both sexes and at Weeks 1 and 13. Week 13 plasma concentrations were approximately dose-proportionate between LD and MD groups for both sexes.

Terminal studies:

Gross pathology-Main study animals euthanized *in extremis,* found dead, and surviving animals at scheduled terminal and recovery sacrifices were necropsied. Animals were examined externally for abnormalities and any findings were correlated with antemortem findings. Abdominal, thoracic, and cranial cavities were examined for abnormalities. Designated organs and tissues were placed in fixative, stained and examined microscopically.

- Unremarkable, no notable gross pathology or treatment-related patterns were observed.

Organ weights-Body weights and designated organs were weighed for all surviving animals at scheduled necropsy. Organ weight ratios relative to body and brain weights were calculated. Paired organs were weighed together. The thyroid/parathyroid gland and pituitary gland were weighed following fixation.

The following organs were weighed: adrenals, brain (including brainstem), heart, kidneys, liver, ovaries, and testes with epididymides. Paired organs were weighed and reported separately in appendices and reported as combined weight in tables. Organ-to-terminal-body-weight and organ-to-brain-weight ratios were calculated.

- HD group, a treatment-related decrease in terminal body weight was observed.
- HDF, a significant increase in liver-to-terminal body weight ratio compared to the corresponding control.

Histopathology-Microscopic examination of fixed hematoxylin and eosin-stained organ/tissue sections were performed by a board certified veterinary pathologist. A four step grading system (minimal, mild, moderate, severe) was used to describe the severity of the lesions. The following list includes the tissues from each animal that were preserved in 10% neutral-buffered formalin:

adrenals, aorta, brain (with brainstem), colon, cecum, rectum, duodenum, esophagus, exorbital lacrimal glands, eyes (in Bouin's fixative), femur with articular surface, femur (bone marrow), gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mesenteric lymph node, ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicles, skin, spinal cord (cervical, lumbar, and mid-thoracic), spleen, sternum with marrow, stomach, testes with epididymides, thigh muscle, thymus, thyroid with parathyroids, trachea, urinary bladder, and uterus.

All preserved tissues from Control and HD groups were examined microscopically. No notable treatment-related patterns were observed.

Chronic Toxicology Study

9.) **26 Week Dietary Toxicity Study in Rats**

Study no.: (b) (4) Study No. 684-010
Volume #, and page #: EDR 4.2.3.2. rat-oral-long, pp. 1-449.
Conducting laboratory and location: (b) (4)
Date of study initiation: 28 January 1993
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C4886, purity assumed 100%

Methods

Doses: 0, 2, 6.5, and 20 mg/kg/day

Species/strain: Rat CD® BR VAF/Plus
Number/sex/group or time point (main study): 20/sex/group
Route, formulation, volume, and infusion rate: Oral, 4-AP offered in the diet
Satellite groups used for toxicokinetics or recovery: TK 5/sex/grp, 1 & 6 months
Age: 4 wks
Weight: Males, 132-161 g; Females, 121-146 g

Observations and Results

Mortality: Rats were observed for mortality/morbidity twice daily throughout the study.

- 2 HDM died (#44554 found dead, Day 141; prior to death, aggressive behavior, decreased defecation, body surface staining and labored breathing were observed). TK animal 44560 was euthanized *in extremis* soon after the 3-month blood collection on Day 88 (prior to sacrifice, decreased activity, tremors and convulsions were observed).

Clinical signs: Rats were observed twice daily for signs of overt toxicity at the times of the mortality/morbidity checks, detailed clinical examinations were made on a weekly basis.

- HDM and HDF were reported to experience several clinical signs, which were attributed to the test article whereas, LD and MD groups experienced clinical signs similar to those of Controls. Notable clinical signs are summarized in the following reviewer-generated table.

Affected System	Clinical Signs	Control		HD		
		M	F	M	F	M+F
CNS	Decreased activity	0/25	0/25	1/25	2/25	6%
	Ptyalism	0/25	0/25	1/25	0/25	2%
	Aggressive behavior	0/25	0/25	14/25	5/25	38%
	Tremors	0/25	0/25	1/25	0/25	2%
	Convulsions	0/25	0/25	2/25	0/25	4%
Respiratory	Breathing, labored	0/25	0/25	9/25	4/25	26%
Secretions/ Immune	Nose, red/brown material	1/25	0/25	11/25	2/25	26%
	Body surface stained	0/25	0/25	6/25	5/25	22%
	Eyes/eyelids, Red area	0/25	1/25	7/25	2/25	18%
	Focal swelling	0/25	0/25	5/25	3/25	16%
	Decubital ulcer	0/25	0/25	2/25	0/25	4%

Body weights: Individual body weights were measured prior to study initiation and weekly during the study. [ss, $^{**}p<0.01$]

- HDM (-19.8%**) and HDF (-11.9%**) had reduced mean body weight compared to Controls. For both sexes, all doses of 4-aminopyridine decreased body weight.
  - Males, fampridine decreased body weight for LD (-3.9%), MD (-7.4%) and HD (-19.8%**) groups compared to Control.
  - Females, fampridine decreased body weight for LD (-2.0%), MD (-7.1%) and HD (-11.9%**) groups compared to Control.

These results are graphically represented in the following sponsor's Figure 1.

Figure 1. Mean Body Weight Male

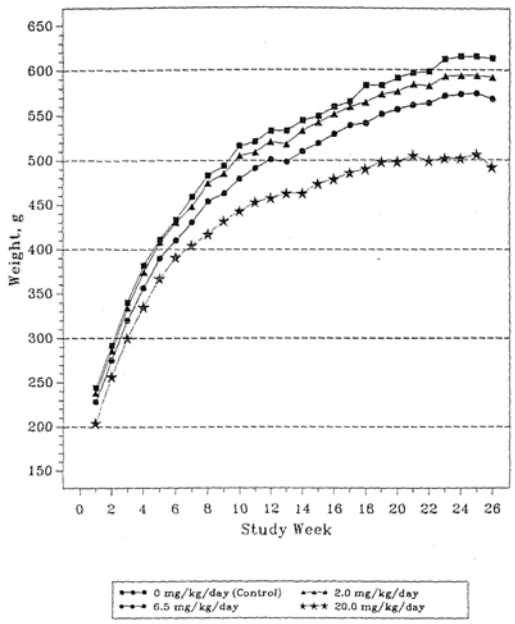
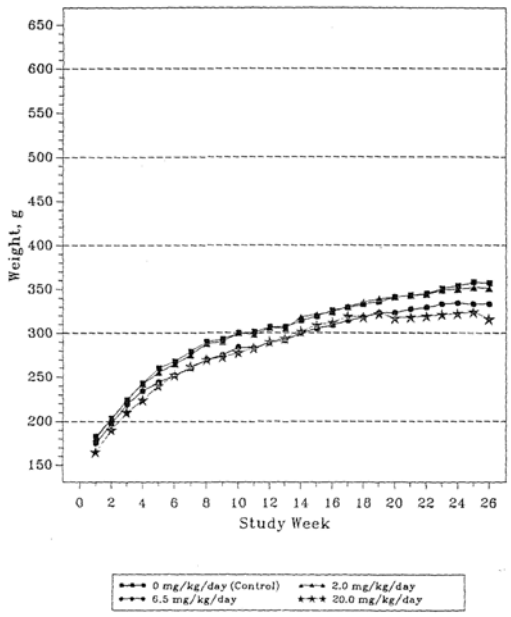


Figure 1. Cont. Mean Body Weight Female



Food consumption: Individual food consumption was determined weekly during the study.

- HDM had decreased mean food consumption compared to Control (-9.3%, nss).
- In contrast, food consumption in females was unremarkable at any dose.

Ophthalmoscopy: An indirect ophthalmoscopic examination of the cornea, conjunctiva, sclera, iris, and fundus was performed by a veterinary ophthalmologist during the acclimation period, at week 13 and prior to terminal sacrifice at week 26.

- All animals examined during the acclimation period had normal ophthalmoscopic findings pre-test.
- After treatment with fampridine for 13- and 26-wk, an increase in the incidence and severity (one eye vs. both eyes) of conjunctivitis was found. Males tended to be more affected than females. None of the control animals had conjunctivitis at either the 13-wk or the 26-wk examinations. The incidence/severity was

treatment-related, but not dose-dependent. The following reviewer-generated table summarizes the results that were provided in Appendix H of sponsor's study report.

	Fampridine								
	LD			MD			HD		
Exam:	**Pre**	**13**	**26**	**Pre**	**13**	**26**	**Pre**	**13**	**26**
M+F	0/40	6/40	4/40	0/40	2/40	2/40[c]	0/40	4/40	2/38[e]
M	0/20	5/20[a]	4/20[b]	0/20	2/20	2/20	0/20	2/20[d]	1/18
F	0/20	1/20	0/20	0/20	0/20	0/20	0/20	2/20	1/20

Shaded area corresponds to progression from one to both eyes in individual animals.
[a]All animals had only one eye affected.
[b]Comparison of wk 13 to wk 26, conjunctivitis had resolved in one male and one female, remained the same in two males, and progressed from one to both eyes for two other males.
[c]Same animals as week 13.
[d]One HDM had conjunctivitis in both eyes (note: this animal died prior to wk 26 exam).
[e]All of the animals in this group had both eyes affected; one female progressed from wk 13, whereas the male was a new case involving both eyes.

- Sponsor did not attribute these findings to the test article.

Clinical Pathology: Clinical laboratory studies were conducted at 13 wks and at study termination. Blood samples were collected following an overnight fasting period (water available *ad libitum*); and urine was collected during the fasting period. TK animals were not included in this assessment.

- Hematology: Occasional significant differences in hematological parameters were noted between 4-AP and Control groups. Sponsor attributed each of these to normal variation.
    - Sponsor did note a non-significant trend for HDM and HDF to have decreased RBC count, Hgb, and Hct that was >10% from Controls. These data are summarized in the following reviewer-generated table.

Hematology Parameter	Sex	Fampridine		
		LD	MD	HD
Erythrocytes	**M**	-3.6%	-2.1%	-14.0%
	F	-9.3%	-3.4%	-14.6%
Hemoglobin	**M**	-1.3%	0.0%	-12.2%
	F	-8.9%	-3.2%	-11.5%
Hematocrit	**M**	-2.8%	-1.6%	-12.7%
	F	-9.7%	-3.9%	-12.6%

Clinical chemistry: Blood biochemistries were performed during week 13 and at terminal sacrifice. [ss, $^{**}p<0.01$]

Terminal sacrifice:

- HDM and HDF, T. Bili (M, -33%**; F, -67%**) and Albumin (M, -17.6%**; F, -31.8%**) were decreased compared to Controls.
- HDF, Ca^{2+} concentration (-9.2%**) and T. Prot. (-17.3%**) were decreased compared to Control. HDF also had a trend toward higher concentrations of phosphorus (6.4%), alkaline phosphatase (186%), and urea nitrogen (162%).

Urinalysis: At week 13 and at terminal sacrifice, urine was collected during the fasting period. No test article-related changes were noted by the sponsor at either week 13 or at the terminal sacrifice. Although not statistically significant, in both males and females, treatment with fampridine resulted in a dose-dependent increase in urine volume, as indicated in the reviewer-generated table.

Percent Change in Urine Volume and Specific Gravity from Control

Parameter	Sex	Fampridine		
		LD	MD	HD
Urine Volume	M	117%	153%	130%
	F	104%	128%	170%
Specific Gravity	M	-1.9%	-7.8%	1.9%
	F	-1.9%	-7.8%	-8.7%

- Similar to the dose-dependent increase in urine volume, a dose-related decrease in the specific gravity of the urine was also observed.

Gross pathology: All animals were examined. The skin was reflected from a ventral midline incision, and any abnormalities were identified and were correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined and were placed in fixative where required by protocol. The pituitary was fixed *in situ*. Representative samples of protocol designated organs and tissues were collected and placed into phosphate-buffered neutral formalin; a full complement of organs and tissues was collected from all animals. Selected macroscopic observations are in the following reviewer-generated table. Four gradations of severity were indicated (1=trace, 2=mild, 3=moderate, 4=severe).

Tissues with macroscopic observations combined males and females.

Organ/Tissue	Treatment			
	C	LD	MD	HD
Lung Focus/Foci	**1/40** 1@1	**4/40** 3@1 1@2	**2/40** 2@1	**5/40** 4@1 1@2
Soft Tissue, Foot Swollen Ulcer	**0/40**	**1/40**	**0/40**	**6/40** **3/40** 3@2 **3/40** 2@3 1@4
Stomach, Glandular Focus, foci	**0/40**	**0/40**	**0/40**	**2/40** 1@2 1@3

#Affected/Total Animal number Incidence @ severity score
Shaded area = includes animal(s) which died on study.

Toxicokinetics: Plasma 4-AP levels were measured after 4- and 26-weeks using a separate set of rats/sex/grp. The data are shown in the following reviewer-generated table.

Wks	4-AMINOPYRIDINE (ng/ml)					
	MALES			FEMALES		
	LD	MD	HD	LD	MD	HD
4	33.8±7.58	101±9.10	210±38.6	27.3±3.45	88.5±36.9	192±66
26	39.2±9.42	52.5±37.6	230±93.2	21.4±5.74	48.5±30.1	185±36.5

Organ weights: The following organs and tissues were weighed: adrenal (2), brain, heart, kidney (2), liver, ovary (2), spleen, and testis (2). Organ weights were presented as absolute values and as ratios to either terminal body or terminal brain weight.

- All of the tissues weighed were affected by fampridine treatment; however, not all were dose-dependent.

The following three reviewer-generated tables summarize the percent difference of fampridine-treated to Control for mean organ weights. These tables summarize absolute, relative to body weight, and relative to brain weight, respectively.

Absolute organ weight: Percent change from control following 4-aminopyridine treatment.

Organ/Tissue	MALES			FEMALES		
	LD	MD	HD	LD	MD	HD
Body weight	-3.9%	-7.4%	-19.8%**	-2.0%	-7.1%	-11.9%**
Brain	-2.4%	-0.5%	4.8%	-2.5%	1.5%	0.5%
Adrenal	7.1%	13.3%	20.0%	3.2%	-1.1%	10.6%
Heart	-3.2%	-8.0%	-10.6%*	7.9%	-7.9%	-3.2%
Kidney	-5.3%	-6.3%	-8.6%	6.7%	-4.6%	-6.7%
Liver	-3.4%	-4.7%	-19.6%*	3.9%	-12.2%**	-11.1%*
Spleen	2.0%	-7.0%	-13.1	12.3%	0.0%	4.6%
Testis	0.0%	0.1%	-14.4%	n/a	n/a	n/a
Ovary	n/a	n/a	n/a	-1.3%	15.7%	9.8%

***, **Significantly different from the control group; *p<0.05; **p<0.01 n/a = not applicable**

Organ:body weight ratio: Percent change from control following 4-aminopyridine treatment.

	MALES			FEMALES		
Organ/Tissue	**LD**	**MD**	**HD**	**LD**	**MD**	**HD**
Brain	1.7%	6.7%	33.6%**	-0.5%	9.4%*	14.4%**
Adrenal	11.2%	21.6%	53.4%**	5.2%	8.6%	26.8%**
Heart	8.7%	-8.7%	12.0%*	10.3%	-1.4%	11.7%
Kidney	-1.6%	1%	14.8%**	9.0%*	2.6%	5.8%
Liver	0.1%	0.1%	0.1%	6.2%	-5.5%	-0.2%
Spleen	7.4%	0.0%	9.2%	15.2%	3.3%	19%
Testis	0.0%	0.1%	-14.4%	n/a	n/a	n/a
Ovary	n/a	n/a	n/a	1.1%	24.8%**	24.2%**

***, **Significantly different from the control group; *p<0.05; **p<0.01 n/a = not applicable**

Organ:brain weight ratio: Percent change from control following 4-aminopyridine treatment.

	MALES			FEMALES		
Organ/Tissue	**LD**	**MD**	**HD**	**LD**	**MD**	**HD**
Brain	9.8%	15.8%	15.4%	6.2%	-1.6%	14.4%
Adrenal	-1.2%	-7.6%	-14.7%**	10.4%	-9.7%	-3.8%
Heart	-3.0%	-5.5%	-12.3%**	10.3%*	-5.5%	-6.8%
Kidney	-1.1%	-6.7%	-22.4%**	7.1%	-13.2%**	-12.2%*
Liver	4.6%	-6.7%	-16.4%*	15.2%	-2.0%	3.3%
Spleen	2.3%	1.1%	-8.0%	n/a	n/a	n/a
Testis/Ovary	n/a	n/a	n/a	1.8%	14.6%	9.1%

***, **Significantly different from the control group; *p<0.05; **p<0.01 n/a = not applicable**

- In females, adrenal, liver, and ovary were affected by fampridine treatment.
  - A dose-dependent increase in the absolute weight of the ovary, and weight ratios of ovary to body and brain were found.
  - Brain and adrenal gland ratios to body weight were increased in a dose-dependent manner, which was statistically significant in the HD group. This significant effect is most likely the result of reduced body weight gain found in the HD group.
  - A dose-dependent decrease was found in the absolute weight of the liver; however, normalization of liver weight to both body and brain weights eliminated this effect.
- In males, brain, adrenal, heart, kidney, liver, spleen, and testis were affected by fampridine treatment.
  - A dose-dependent increase was found in the adrenal gland for absolute weight and relative to body weight, but decreased relative to brain weight.
  - A dose-dependent decrease in the organ to brain weight ratio occurred in the heart, liver, kidney, and spleen.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Microscopic analyses were performed on a full complement of organs and tissues for all animals in the control and HD group. All gross lesions were examined in all animals. A four-step grading system of trace, mild, moderate, and severe was used.

The following list of tissues and organs were examined microscopically and tissues demarcated [W] were weighed:

adrenal glands (2) [W], aorta, bone with bone marrow (femur), bone marrow smear (2), brain[W] (fore, mid, and hind), eyes with optic nerve (2), gastrointestinal tract—esophagus, stomach—glandular and non glandular, duodenum, jejunum, ileum, cecum, colon, gonads—ovary[W] (2) and testes[W] with epididymis (2), gross lesions, heart[W], kidney (2) [W], liver[W] (2 lobes examined, 3 sections collected), lung with bronchi (2), lymph nodes—mandibular and mesenteric, mammary gland, pancreas, prostate and seminal vesicle (2), salivary gland—mandibular and sublingual, sciatic nerve, skeletal muscle—thigh, skin, spinal cord—thoracic, spleen[W], thymus, thyroid/parathyroid (2), trachea, ureter (2), urinary bladder, uterus—both horns, uterus—cervix, and vagina.

- HDM had the highest incidence of glandular dilatation of the glandular stomach, which was graded at trace to mild severity. Because of this finding, the sponsor also evaluated the remaining dose groups as is shown in sponsor's summary table.

MICROSCOPIC INCIDENCE OF GASTRIC GLANDULAR DILATATION
MALES AND FEMALES

Dosage	0 mg/kg/day (Control)		2.0 mg/kg/day		6.5 mg/kg/day		20.0 mg/kg/day	
Sex	M	F	M	F	M	F	M	F
Number Examined	20	20	20	20	20	20	20	20
STOMACH, GLANDULAR								
- Dilatation, glandular								
- trace	2	4	5	6	5	2	9	5
- mild	1	1	6	2	3	1	3	1

According to the study pathologist, the stomach was considered a target organ. In males, the glandular dilatation was test article- and dose-related. However, in females, there was no apparent drug-related effect. The study pathologist notes that these lesions were graded as trace or mild and were not considered life threatening. The description of the lesion has been reproduced from the original pathologist report.

Microscopically, the lesion consisted of trace to mild dilatation of the gastric fundic glands. In affected animals, fundic glands were dilated exhibiting a distinct lumen. Dilatation resulted in reduction in size and flattening of the chief cells in the basilar portion of the gland. Parietal cells appeared to be unaffected. Dilated glands sometimes contained amorphous eosinophilic debris within the glandular lumens. Occasional pyknotic nuclei were seen within the dilated glands. Affected glands had an increased number or density of cell nuclei. This appeared to be a relative increase secondary to the reduction of cell cytoplasm.

All other microscopic findings in other organs were considered by the study pathologist to be of incidental or spontaneous findings.

Dog

Sponsor submitted two repeat dose toxicology studies conducted in Beagle dogs. These studies are reviewed below.

1.) **13-Week Oral Toxicity Study with 4-Aminopyridine in Dogs with a 4-Week Recovery Period**

Key study findings:

- Death of one HDM (G28791 occurred during week 12 (Day 79), COD undetermined; 30-60 min post second dose tremors were observed; no adverse clinical signs were observed prior to death.
- 4-Aminopyridine-induced tremors observed in all HD dogs throughout the dosing phase of the study.
    - Greatest incidence occurred 30-60 min following the second dose; the majority were graded slight (4/8); however, moderate (3/8 dogs) and severe (1/8 dog) tremors were also noted.
    - During week 1, ataxia was observed in MDF (1/4), HDM (1/4), and HDF (2/4); and mydriasis was observed in HD (3/8).
        - HDM developed ataxia within 30 min of second dose on Day 2 followed by convulsions and prostration.

Study no.: (b) (4) 2620-104
Volume #, and page #: EDR 4.2.3.2.1, pp. 1-360
Conducting laboratory and location: (b) (4)
Date of study initiation: 05 February 1991
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot # P85-12-4, assumed purity of 100%, designated by the sponsor as 99+%.

Methods

Doses: 0 (C), 0.3 (LD), 1.0 (MD), and 3.0 (HD) mg/kg/day
Species/strain: Beagle dog
Number/sex/group or time point (main study): 4/sex/group

Route, formulation: Oral, capsule; control group received empty capsule; divided dose administered twice daily 6 hr apart
Satellite groups used for toxicokinetics or recovery:
1-month recovery: 2/sex/Control and HD
Age: 5.75 to 6.75 mos
Weight: M, 6.4-9.8 kg; F, 6.0-7.9 kg

Observations, Times and Results:
Mortality: Dogs were observed for mortality/morbidity at least twice daily throughout the study.

- Day 79, one HDM (#28791) found dead, COD was undetermined.
  - During interval prior to death, no clinical signs were noted; tremors were often observed within 30-60 min of the second dose.

Clinical signs: Dogs were observed three times within 0.5 hrs following each daily dose and once daily following the second dose (≈ 0.5 hr of dosing). A thorough physical exam was conducted by a laboratory technician at each weighing.

- Convulsion (severe) and prostration—HDM #28794 (Day 2).
- Tremor increased with dose, MD (2/8) and HD (12/12) groups (see table below).
- Ataxia increased with dose, MD (1/8) and HD (3/8) groups.
  - Week 1, four animals—MDF (#28789, slight Day 5), HDM (#28794, severe Day 2), and HDF (#28798, gradation not specified Day 4; #28799, slight Day 5).

Days (Mean ± SD) tremor observed for fampridine-treated and recovery groups

Clinical Sign	MALES			FEMALES		
	MD	HD	HD Recovery	MD	HD	HD Recovery
Tremor						
# Affected	0/4	6/6	1/2	2/4	6/6	0/2
slight		31.3±13.8	1[c]	1[d]	48.7±9.4	
moderate		1[a]			2.5±2.1[e]	
severe		4[a]				

[a]HDM (#28794) most severely affected (seizure, ataxia, prostration, and tremors).
[c]Day 93
[d]Day 5, same day as ataxia;
[e]N= 2, HDF (#28800 and #28801) experienced both slight and moderate tremors.

Body weights: Individual body weights were recorded pretest and weekly during the study. Terminal body weights were taken for all animals that died on study and all remaining animals at the time of the scheduled sacrifice.

- Mean terminal body weight was unremarkable for LD and MD groups (both sexes). HDM mean body weight was decreased compared to CM (-8.2%), and HDF mean body weight was decreased compared to CF (-11.8%). Recovery HDM were unremarkable compared to CM, whereas the mean body weight of recovery HDF remained decreased compared to CF (-10.7%).

- MD and HD mean body weight gain decreased for both sexes—MDM (-15%), MDF (-15.4%), HDM (-25%), and HDF (-34.6%) compared to Controls.
- Body weight gain was not affected at the LD.

Food consumption: Individual food consumption was measured weekly during the study.
- Mean food consumption was unremarkable for all dose groups.

Ophthalmoscopic examination: Prior to dosing initiation and during week 13, dogs were examined by a veterinarian using an indirect ophthalmoscopic examination and administered Mydriacyl® (1%) as a mydriatic.
- The signed ophthalmology report ((b) (6) dated 6/4/92) stated that at the study's termination, no 4-AP or dose-related ophthalmoscopic abnormalities related to treatment were observed and that any findings were considered incidental.

Neurologic examination: Prior to study initiation, during Week 13 (surviving animals), and during Week 17 (recovery animals), a neurological examination was performed by the staff veterinarian ((b) (6) Evaluations included assessment of gait, auditory startle response, pain perception, and papillary, extensor thrust, placement, righting and patellar reflexes.
- According to the signed Neurological Report (dated 6/3/92), tests of general neurological function were performed and minor changes occurred in the extensor thrust, placement, and righting reflexes of animals in all groups and thus represented normal variation. No differences were noted among groups, individuals within a group, or between sexes. No 4-AP effects were observed on neurological function.

ECG: Prior to study initiation, during Week 13 (surviving animals), and during Week 17 (recovery animals), an ECG was performed on each dog. Leads I, II, III, A_{VR}, A_{VL}, and A_{VF} were recorded and the tracings were evaluated by a consultant veterinary cardiologist, (b) (6).
- The signed electrocardiography report (dated 16 August 1991) by (b) (6) stated that there were no drug-related effects on ECG.

Blood pressure: Prior to study initiation, during Week 13 (surviving animals), and during Week 17 (recovery animals), mean arterial, systolic, and diastolic blood pressures were recorded for each dog.
- No changes in blood pressure of either sex were attributed to 4-AP treatment as is shown in sponsor's Table 4, below.

Table 4
Summary of Heart Rate and Blood Pressure Values
13-Week Oral Toxicity Study with 4-Aminopyridine in Dogs
with a 4-Week Recovery Period

Group		Heart Rate (B/M)			Mean Arterial Pressure (mm Hg)			Systolic Pressure (mm Hg)			Diastolic Pressure (mm Hg)		
		PreRx	Wk 13	Wk 17	PreRx	Wk 13	Wk 17	PreRx	Wk 13	Wk 17	PreRx	Wk 13	Wk 17
						Males							
1 - 0 mg/kg/day	Mean	137	142	160	104	117	112	135	135	119	91	102	104
	S.D.	15.1	13.3	28.3	12.4	18.9	2.8	12.1	22.1	3.5	15.1	18.3	2.1
	N	6	6	2	6	6	2	6	6	2	6	6	2
2 - 0.3 mg/kg/day	Mean	140	139		110	105		127	123		95	99	
	S.D.	28.3	24.9		5.7	5.6		13.2	9.9		5.4	6.6	
	N	4	4		4	4		4	4		4	4	
3 - 1.0 mg/kg/day	Mean	130	105		100	101		117	128		89	89	
	S.D.	11.5	10.0		14.2	19.2		16.4	20.5		18	20.7	
	N	4	4		4	4		4	4		4	4	
4 - 3.0 mg/kg/day	Mean	123	120	130	114	106	107	127	130	144	105	93	97
	S.D.	15.1	24.5	14.1	18.5	14.2	10.6	11.7	8.8	31.8	18.1	15.5	17
	N	6	5	2	6	5	2	6	5	2	6	5	2
						Females							
1 - 0 mg/kg/day	Mean	147	143	150	98	114	126	120	140	156	82	99	111
	S.D.	24.2	42.7	42.4	26.4	16.1	14.1	28.5	23.7	19.8	25.1	14.2	9.2
	N	6	6	2	6	6	2	6	6	2	6	6	2
2 - 0.3 mg/kg/day	Mean	135	140		92	116		113	135		81	105	
	S.D.	25.2	28.3		18.6	18.4		14.6	12.2		17.8	24.9	
	N	4	4		4	4		4	4		4	4	
3 - 1.0 mg/kg/day	Mean	125	110		84	115		91	138		74	105	
	S.D.	19.1	11.5		36.9	9.7		41.8	10.1		30.9	4.2	
	N	4	4		4	4		4	4		4	4	
4 - 3.0 mg/kg/day	Mean	130	130	140	94	108	105	116	133	127	81	94	91
	S.D.	16.7	24.5	28.3	27.4	32.5	2.1	33.6	28.7	14.1	21.9	30	0.7
	N	6	6	2	6	6	2	6	6	2	6	6	2

NOTES: B/M = beats/minute
PreRx = Pretreatment

Clinical pathology: Prior to study initiation, during Weeks 4 and 13 (surviving animals), and during Week 17 (recovery animals), blood samples were collected from the jugular vein of fasted dogs prior to collection at each interval. Results from the clinical pathology studies were reported by (b) (6)

- Hematological parameters examined include cell morphology, corrected Wbc, Rbc, Hgb, Hct, Wbc, Leuk differential, MCV, MCHC, MCH, M/E ratio, and Platelet.
  - Unremarkable.
- Clinical chemistry parameters examined include ALT, ALK P, AST, Bun, Albumin, Calcium, Chloride, ck, Creat, Globulin, Glucose, In Phos, Potas, Sodium, T Bili, T Chol, T Prot.
  - Unremarkable.
- The signed clinical pathology report (dated June 8, 1992) by (b) (6) . stated that none of the changes in hematologic or biochemical analyses was considered 4-AP treatment-related.

Urinalysis: Not performed.

Toxicokinetics: Plasma 4-AP concentrations were determined on Day 1 and during weeks 4 and 13. Samples were collected approximately 1-2 hr after the second dose.

- Week 1, 4 HD dogs (2M and 2F) had high plasma 4-aminopyridine concentrations (range: 293.9 to 343.2 ng/ml).
    - The HDM #28791 that died on study Day 79 had 4-AP concentrations measured at week 1 of 310.2 ng/ml and week 4 of 144.2 ng/ml.

Individual plasma fampridine concentrations are summarized in the following table provided by sponsor in Appendix 7.

Amount of 4-Aminopyridine (ng/ml)

Males				Females			
Number	Week 1	Week 4	Week 13	Number	Week 1	Week 4	Week 13
Group 1 - 0 mg/kg/day							
28762	-	-	-	28768	-	-	-
28763	-	-	-	28769	-	-	-
28764	-	-	-	28770	-	-	-
28765	-	-	-	28771	-	-	-
28766	-	-	37.10[a]	28772	-	-	-
28767	-	-	-	28773	-	-	-
Group 2 - 0.3 mg/kg/day							
28774	-	-	11.16[a]	28778	42.33[a]	-	-
28775	-	-	-	28779	-	38.36[a]	-
28776	-	32.60[a]	-	28780	-	-	-
28777	-	-	-	28781	-	-	-
Group 3 - 1.0 mg/kg/day							
28782	44.41[a]	129.0	28.60[a]	28786	75.73	38.29[a]	35.37[a]
28783	30.27[a]	-	60.86	28787	63.72	73.94	26.87[a]
28784	83.07	88.46	12.70[a]	28788	-	72.33	5.006[a]
28785	54.17	74.67	33.86[a]	28789	48.96	43.18[a]	22.09[a]
Group 4 - 3.0 mg/kg/day							
28790	65.09	129.8	89.45	28796	343.2	164.0	176.2
28791	310.2	144.2	b	28797	179.3	205.4	-
28792	163.6	188.5	139.3	28798	118.2	179.6	252.4
28793	226.4	168.3	95.91	28799	114.3	137.9	111.9
28794	320.3	229.8	298.1	28800	293.9	258.6	79.84
28795	193.8	155.4	154.0	28801	114.2	33.28[a]	85.75

[a] Value was below the standard curve and was considered semiquantitative (below the lowest standard of the standard curve).
[b] Animal died during Week 12.

Key: - = None detected.

Gross pathology: All animals, those that died on study and those that survived to scheduled sacrifice at Weeks 13 (terminal) and 17 (recovery), were subjected to gross postmortem examination. Examination of the following parameters at necropsy included: all orifices, carcass, cervical tissues and organs, cranial cavity, external surface of the

body, brain, and spinal cord. Cut surfaces of brain were examined at time of trimming, nasal cavity and paranasal sinuses, thoracic, abdominal, and pelvic cavities and their viscera.

Premature decedent: HDM, #28791 found dead on Day 79, COD was undetermined.

- Macroscopic findings included dark-red kidneys, lung, thymus, gastrointestinal organs, and severely enlarged thymus.
- Microscopic findings included multiple organ hemorrhages (brain with stem, heart, and liver) at minimal to moderate severities and congestion (lymph node, lung, and thymus) at moderate severity.

Terminal sacrifice (Week 13): Unremarkable, for all doses tested.

Recovery sacrifice (Week 17): Unremarkable, for all doses tested.

Organ weights: Organ weights of all animals scheduled for terminal and recovery sacrifices were recorded and the appropriate weight ratios were calculated. The organs weighed included adrenals, brain and brainstem, heart, kidneys, liver (with drained gallbladder), ovaries, testes/epididymides, and thyroid/parathyroids. [ss, $^{*}p<0.05$].

- HDF mean absolute thyroid weight was decreased (-25.6%*) compared to control; and ratios to body weight (-20%) and brain weight (-27%) were reduced compared to CF.
- Recovery HDF, thyroid weights were unremarkable.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (), no (X)

All preserved tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically from all animals. In the brain, at least 10 sections were examined microscopically. The following list includes the tissues (when present) collected from each animal and preserved in 10% neutral-buffered formalin.

adrenals, aorta (thoracic), sternum with bone marrow, brain (medulla/pons, cerebellar cortex, and cerebral cortex), colon, cecum, rectum, duodenum, jejunum, ileum, esophagus, eyes with optic nerves, femur with bone marrow, gallbladder, heart, kidneys, liver, lesions, lung with bronchi, mammary gland (F only), mandibular and mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, salivary glands (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, lumbar, and mid-thoracic spinal cord), spleen, stomach, testes and epididymides, thymus, thyroid/parathyroids, trachea, urinary bladder and uterus.

In the signed Pathology Report (dated 6/5/92), [redacted] (b) (6) concluded that the cause of death of HD male (#28791) could not be determined morphologically, and none of the histomorphologic lesions could be attributed to 4-aminopyridine treatment. The following reviewer generated table summarizes the incidence of focal brain hemorrhage of MD and HD survivors.

13-Week Main Study			
Brain (medulla/pons, cerebral and cerebellar cortices)	**C**	**4-AP**	
		MD	**HD**
	M+F	**M+F**	**M+F**
Focal hemorrhage Grade[b]	2/8 Min.	5/8 Min.	2/7[b] Min-Mod.
4-Week Recovery			
Brain (medulla/pons, cerebellar cortex, and cerebral cortex)	**C**	**4-AP**	
		MD	**HD**
	M+F	**M+F**	**M+F**
Focal hemorrhage Grade	1/4 Min.	NE	2/4[c] Slight

NE=Not Examined
[a]Graded scale: slight, minimal, moderate and severe
[b]HDF had multi-focal hemorrhage in cerebellum and perivascular areas of cerebrum
[c]HDM had multi-focal hemorrhage in cerebellum.

Chronic Toxicology Study

2.) **One Year Oral Toxicity Study in Dogs**

Key study findings:

- NOAEL=0.75 mg/kg administered b.i.d (1.5 mg/kg/day)
- Target organ: CNS
- Mortalities: MDF (2/6), HDM (4/6) and HDF (4/6) related to seizures in these animals. Sponsor's pathology report listed cause of death as the following:
  - Myocardium congestion of mild to moderate severity and epicardium or endocardium hemorrhage of moderate severity (i.e., sudden cardiovascular failure).
  - Several HD CODs listed as unknown; however, common to most was multi-organ, multifocal hemorrhage of varying severities involving the adrenal gland (cortex and medulla), brain, spinal cord (cervical and/or thoracic), heart (endocardium or epicardium), and lung.

Study no.: (b) (4) 684-008
Volume #, and page #: EDR 4.2.3.2.1, pp. 1-609.
Conducting laboratory and location: (b) (4)
Date of study initiation: September 28, 1992
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot# C4886, purity assumed 100%

Methods

Doses: 0 (C), 0.375 (LD), 0.75 (MD), and 1.5 (HD) mg/kg, b.i.d. (i.e., 0, 1.5, 3.0 mg/kg/day)
Species/strain: Beagle dog

Number/sex/group or time point (main study): 4/sex/group (52-week study)
Route, formulation, volume, and infusion rate: Oral, capsule, two equally divided doses of (0.375, 0.75, and 1.5 mg/kg) were administered twice daily, morning and afternoon with at least 6 hr between doses; control animals received an empty gelatin capsule on same schedule.
Satellite groups used for toxicokinetics or recovery: none
Age: ≈ 5 mos of age
Weight: Male, 8.0-11.5 kg; Female, 7.6-10.5 kg
Sampling times: A 26-wk interim sacrifice was conducted (2/sex/group); except for HD groups, which had significant deaths.
Study Design:

- One HDM was replaced during study on December 2, 1992 (28 doses administered; 14 days), because the animal had become excessively aggressive and was difficult to dose; data from this animal was excluded from summary and individual tables.

Observations, Times and Results:
Mortality: Dogs were observed for mortality/morbidity at least twice daily throughout the study.

- HD group, four males and four females did not survive to scheduled sacrifice.
  - Males, #3667, #3688 and #3674 were found dead on days 29, 193, and 341 respectively; #3664 was euthanized *in extremis* on day 56.
  - Females, #3692 and 3717 were found dead on days 90 and 191, respectively; females #3695 and 3709 were euthanized *in extremis* on days 173 and 193, respectively.
- MD group, two females did not survive to scheduled sacrifice.
  - Females, #3714 and #3707 were found dead on days 171 and 305, respectively.

The following table summarizes the number of survivors at Weeks 26 and 52.

Treatment Grp mg/kg/day	Survivors/Initial Total			
	Week 26		Week 52	
	M	F	M	F
0	6/6	6/6	4/4	4/4
0.75	6/6	6/6	4/4	4/4
1.5	6/6	5/6	4/4	3/4
3.0	4/6	4/6	2/4	2/4

Clinical signs: The sponsor separated clinical signs into two designations, "pharmacotoxic observations" that corresponded to signs observed approximately 1 hr following the AM dose and "clinical signs" that corresponded to twice daily observations made for signs of overt toxicity at the time of the morbidity/mortality checks. Detailed clinical examinations were performed weekly, which included the occurrence, size, location, and description of palpable masses. The sponsor also separated the incidences of findings based upon these two distinctions. The two types of observations have been combined in the following reviewer-generated table.

Combined Clinical Signs and Pharmacotoxic Observations

Clinical Signs		MALES				FEMALES			
		MD		HD		MD		HD	
Sign	Grade	N	Freq	N	Freq	N	Freq	N	Freq
Convulsion	**Total (N)**			**6/6**	**13**	**4/6**	**5**	**3/6**	**4**
	Slight					1/6	2		
	Moderate								
	Marked			3/6	3	1/6	1	1/6	1
	No Grade			6/6	10	2/6	2	2/6	3
Tremors[a]	**Total (N)**	**5/6**	**9**	**6/6**	**164**	**5/6**	**44**	**5/6**	**43**
	Slight	5/6	8	5/6	135	5/6	38	5/6	33
	Moderate	1/6	1	3/6	11	3/6	3	4/6	5
	Marked			6/6	18	3/6	3	2/6	4
	No Grade							1/6	1
Ptyalism	**Total (N)**	**1/6**	**1**	**5/6**	**24**	**5/6**	**8**	**4/6**	**18**
	Slight	1/6	1	2/6	10	2/6	3	2/6	5
	Moderate			1/6[b]	1	1/6	1	4/6	12
	Marked			1/6[b]	13	3/6	4	1/6	1
Ataxia	**Total (N)**			**5/6**	**15**	**2/6**	**5**	**1/6**	**2**
	Slight			2/6	3	2/6	1		
	Moderate			1/6	4	1/6	1	1/6	2
	Marked			4/6	8	1/6	3		
Found dead				3		2		3	
In extremis				1				1	

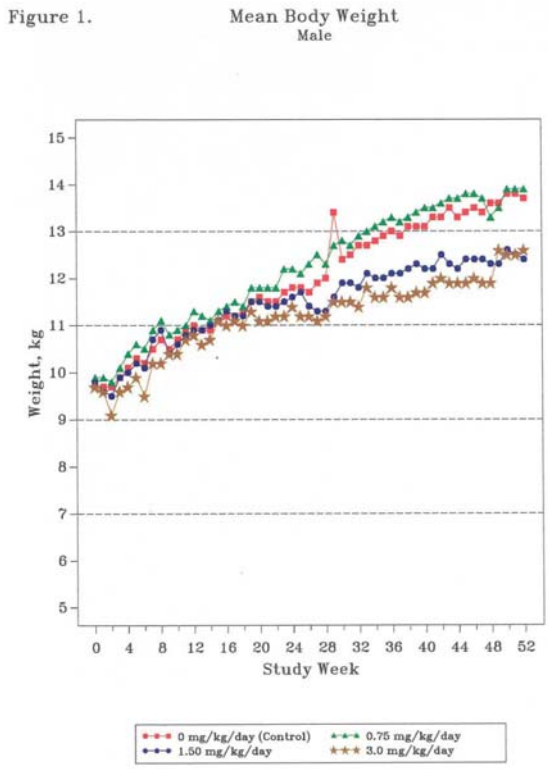
[a]Tremors includes tremors and body tremors

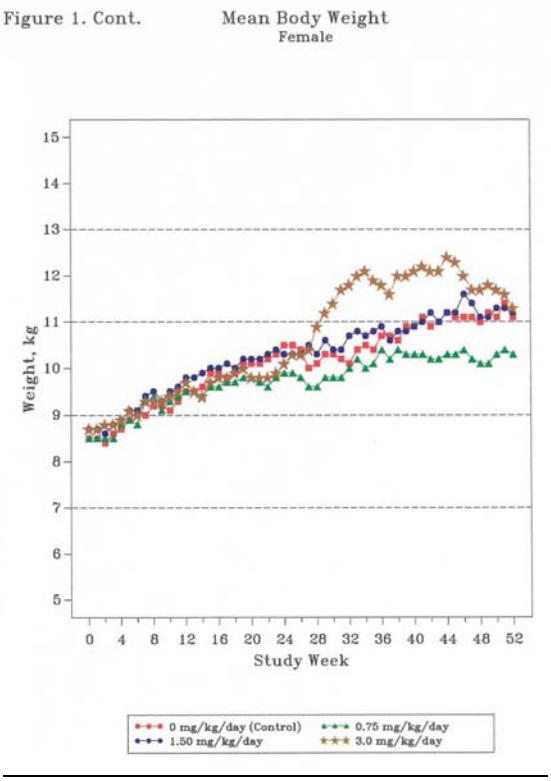
- Most of these clinical signs were absent in Control and LD groups.

Body weights: Individual body weights were recorded pretest and weekly during the study. Terminal body weights were recorded for all animals that died on study and all survivors at the time of the scheduled sacrifice. The following table summarizes the mean group body weight and body weight gain for fampridine-treated groups compared to Control for both 26-Wk and 52 Wk measurements. There was an increase in body weight, but not body weight gain, in HDF after wk 26.

	Interim (26-Week)				Terminal (52-Week)			
	Body weight		Gain		Body weight		Gain	
Fampridine	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
LD	+5.1%	-5.8%	+20%	-31.6%	+1.4%	-7.2%	-5.3%	-25.9%
MD	-2.6%	-1.0%	-20%	-15.8%	-9.5%	+0.9%	-28.9%	-22.2%
HD	-4.3%	-1.0%	-35%	-26.3%	-10.2%	+1.8%	-47.4%	-33.3%

- Mean body weight for males and females during the 52-week study were recorded and plotted in sponsor's Figure 1 (see below).





Food consumption: Individual food consumption was measured weekly during the study.

- Unremarkable.

Ophthalmoscopy: All animals received an indirect ophthalmoscopic examination of the cornea, conjunctiva, sclera, iris, and fundus during the acclimation period, and at 3, 6 and 12 months by a veterinary ophthalmologist.

- M and F were unremarkable.

Physical examination: A staff veterinarian conducted a routine physical examination during the acclimation period and at months, 3, 6, 9 and 12. Additional examinations were arranged when necessary. The examination consisted of a determination of general condition and included an examination of the head, neck, thorax, abdomen, external reproductive organs, skin, and extremities. Heart and lung sounds were assessed.

- 12th-month examination, surviving HDM (1/2) had mild whole body tremors and dilated pupils (slow response to light).
- Prior to *in extremis* euthanasia, HDM (day 56) and 2 HDF (days 173 and 193, respectively), each had prolonged seizure with severe tremors at the time of euthanasia. The HDM was reported in continuous seizure for greater than 30 min prior to euthanasia.

Neurologic examination: During the last month of study, all survivors were subjected to a neurologic examination performed by a board certified veterinary neurologist, (b) (6) In the "Final Report of Beagle Neurologic Examinations for the (b) (6) Study" signed and dated (October 28, 1993), Dr. (b) (6) reported results of a blinded neurological examination of the behavior of 28 beagle dogs.

- Abnormal behavior identified for 6 dogs and normal behavior of 22 dogs.
    - 3 dogs—Generalized tremors, appeared anxious, and had dilated pupils.
    - 1 dog—Excessive salivation.
    - 1 dog—No neurological abnormalities, but had hyperactive behavior.
    - 1 dog—Questionable occasional tremors.

After breaking the blind the 6 abnormal behaving dogs were identified as MD (1/6) and HD (5/6). These dog behaviors were further stratified based upon time from last dose and previous clinical signs.

- Dogs exhibiting generalized tremors, anxious, and dilated pupils were each dosed 2 – 4 hr prior to the neurological exam. Each of these dogs also had previously been observed to show episodes of tremor, dilated pupils, and convulsions.
- The dog with excessive salivation had been dosed 3 hr 25 min prior to the neurological examination and previously showed neurologic signs.
- The dog with hyperactive behavior had been dosed 5 hr 48 min prior to the assessment and previously showed tremors.
    - The dog with the questionable occasional tremors was from the MD group and was examined 1 hr 43 min after dosing; and previously, tremors were observed in this animal.
    - Control and LD dogs showed normal neurological behavior.

ECG: Pretest and at months 3, 6 and 12; an ECG was performed on each dog. Leads I, II, III, A_{VR}, A_{VL}, and A_{VF} were recorded at paper speed 25 mm/second. The tracings were evaluated by a consultant veterinary cardiologist, (b) (6)

- No test article-related abnormalities were noted during the ECG examinations.

Clinical pathology: Clinical laboratory studies were conducted prior to initiation of dosing and on all surviving animals at 1, 3, 6, and 12-months of study. Blood samples were obtained from the jugular vein following an overnight fasting period in which food and water were removed.

- Hematology: The following parameters were evaluated: RBC, Hb, Hct, MCV, MCHC, MCH, Retic, RBC morphology, Plt, WBC, Neut, Lymph, Mono, Eos, Baso, Others (as needed).
    - No test article-related findings were noted in the parameters evaluated.
- Clinical chemistry: Blood samples were collected from the orbital plexus under isoflurane anesthesia and collected in serum separator tubes to evaluate the following parameters: ALT, AST, ALP, GGT, TBili, BUN, Creat, Ca, Phos, TP, Alb, Glob, A:G, Glucose, Chol, Trig, Na, K, Cl.
    - No test article-related biochemical changes in the parameters evaluated were noted.

- Urinalysis: Urine was collected from animals during the fasting period and the following parameters were assessed: Color, Clarity, SG, microscopic evaluation of Urine sediment, TotVol, pH, Pro, Glu, Bil, Ket, Nit, Leukocytes, Blood, and Urobilinogen.
    - No test article-related changes were noted in the parameters evaluated.

Organ weights: Organs were trimmed free of fat and connective tissue and weighed. Organ weights of all animals scheduled for sacrifice including the 6-month interim and the terminal sacrifices were recorded along with terminal body weights and the appropriate weight ratios were calculated. The following organs were weighed:

Adrenal (2)	Liver (2 lobes examined, 3 sections collected)
Brain (7 sections)	Pituitary
Thyroid/parathyroid (2)	Heart
Kidney (2)	Spleen

Gonads—Ovary (2) or Testis with epididymus (2)

6-Month interim sacrifice

- Unremarkable.

Terminal sacrifice

- Two HD males (#3671 and #3678) at study termination had significantly higher group mean absolute brain weight compared to the control group; and both showed a higher individual brain weight compared to the control group. HD females were not similarly affected. No 6-month interim sacrifice occurred in the HD group.
    - Dr. Leander Tryphonas reviewed all of the brain sections from all dogs; he did not find any evidence of treatment-related histological changes in the brains of the HD males (#3671 and #3678). A similar increase in brain weight was not found in treated females; thus, the sponsor concluded that the higher brain weight in the HD males was "unlikely treatment related".

Gross pathology: All animals surviving to scheduled necropsy and premature decedents were subjected to a complete necropsy examination. Animals were euthanized by sodium pentobarbital deep anesthesia followed by exsanguination.

All survivors at 52 weeks had an upper body perfusion with 10% neutral buffered formalin at the recommendation of a consultant pathologist. Perfusion of the body was not done for dogs examined at the 6-month interim sacrifice or for those sacrificed *in extremis* prior to the 6-month interval. Prior to sacrifice, dogs were injected with approximately 800 IU of heparin followed by induction of deep anesthesia with sodium pentobarbital solution and then euthanized by exsanguination. A full set of required tissues was collected from all dogs and preserved in 10% neutral buffered formalin solution. The following organs and tissues were collected for microscopic evaluation:

Adrenal (2), aorta (thoracic), bone marrow—femur and sternum, brain (7 sections), gastrointestinal tract—colon, cecum, rectum, duodenum, jejunum, ileum, and esophagus, eye with optic nerve (2), gallbladder, heart, kidneys, liver, lesions, lung with bronchi, mammary gland (F only), mandibular and mesenteric lymph nodes, ovaries, pancreas,

pituitary, prostate, salivary glands (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, lumbar, and mid-thoracic spinal cord), spleen, stomach, testes and epididymides, thymus, thyroid/parathyroids, trachea, urinary bladder, ureter (2) and uterus—both horns and cervix.

The study pathologist, (b) (6), examined all hematoxylin and eosin stained paraffin sections of all protocol-required tissues. All sections of brain from all dogs, including the sections previously examined by (b) (6) and all special stained sections were examined by the consultant neuropathologist, Dr. (b) (6) (signed neuropathology report provided).

In brain, three standard H&E-stained paraffin sections of fore-, mid- and hind-brain were prepared for all dogs necropsied at the six-month interim sacrifice and for all dogs that died on study or that were sacrificed *in extremis* prior to 5/29/93 (day 192). Additional H&E-stained paraffin sections of brain were made, at the recommendation of the consulting neuropathologist, which included the following structures: olfactory bulb, piriform cortex, amygdale, hippocampus, cingulate cortex, neocortex, medial geniculate body, substantia nigra, and area postrema.

- Duplicate special stained sections were made using Bodian stain and cresyl violet in combination with luxol fast blue. After day 192 (5/29/93), brain sections from animals that died *in extremis* or at scheduled necropsy were prepared as special stained sections.
- Results from the Neuropathologist report (b) (6) signed and dated 13 July 1994) are provided below.

6 Month Interim Sacrifice

Group 1 (0 mg/kg/day)

No abnormal findings were detected in the CNS of control dogs.

Group 2 (0.75 mg/kg/day)

No abnormal findings were detected in the CNS of dogs treated with 0.75 mg/kg/day orally.

Group 3 (1.50 mg/kg/day)

No abnormal findings were detected in the CNS of one female [3693] and two male dogs [3668 and 3670] treated with 1.50 mg/kg/day orally for 6 months.

Slight vacuolation of the piriform cortex was present in one dog [3714], a female dog found dead on (b) (6) This change affected the second layer of the piriform cortex and was largely due to an acute swelling of the neuronal satellite oligodendrocytes. Affected oligodendrocytes had a small dark nucleus and a swollen watery cytoplasm which impinged upon nearby neurons causing scalloping of the neuronal outline. The cerebral cortex of the neopallion was less affected. The hippocampus was unaffected, excepting the dentate gyrus the inner aspect of which was mildly vacuolated.

Recent multifocal hemorrhages without secondary tissue damage were present in the neocortex and medulla oblongata of this dog [3714]. Both ring and ball hemorrhages were detected.

Mild autolysis was present.

Group 4 (3.00 mg/kg/day)

No abnormal findings were detected in the CNS of one female dog euthanized in extremis [3695].

Minimal vacuolation of the neuronal satellite oligodendrocyte cells was present in the piriform cortex of one male dog euthanized in extremis [3664]. Mild to moderate vacuolation of the piriform and cerebral cortices were present in two dogs found dead (one male [3667] and one female [3692]). Vacuolation of the piriform cortex was characterized by acute swelling of perineuronal satellite oligodendrocytes, microvacuolation and disruption of the interneuronal neuropil, and reduced stainability of the neuropil. Vacuolation of the cerebral cortex was less pronounced and affected the deeper cortical layers.

The two male dogs [3664 and 3667] and one female dog [3692] had in addition mild recent multifocal ring and or ball type hemorrhages without secondary tissue damage. The hemorrhages were randomly distributed in both dogs, but were more common in one male dog [3667].

Mild to moderate autolysis was present in all dogs found dead.

1 Year Study

Group 1 (0 mg/kg/day)

No abnormal findings were detected in the CNS of control dogs.

Group 2 (0.75 mg/kg/day)

No abnormal findings were detected in the CNS of dogs treated with 0.75 mg/kg/day orally.

Group 3 (1.50 mg/kg/day)

No abnormal findings were detected in the CNS of four male and three female dogs treated with 1.50 mg/kg/day orally. Mild focal hemorrhage was present in one female dog [3707] which died on study.

Group 4 (3.00 mg/kg/day)

No abnormal findings were detected in the CNS of two male [3671 and 3678] and three female [3702, 3705, 3709] dogs treated with 3.0 mg/kg/day orally.

Moderate vacuolation, seen in two male dogs [3674 and 3688], was present in the olfactory bulb of one dog and in the piriform cortex, cingulate cortex, and neocortex of the other. Vacuolation of the piriform cortex was characterized by acute swelling of perineuronal satellite oligodendrocytes, microvacuolation and disruption of the interneuronal neuropil, and reduced stainability of the neuropil. Vacuolation of the cerebral cortex was less pronounced, affected the deeper cortical layers, and, occasionally, was centered around small blood vessels. The cortex surrounding the middle and inner thirds of sulci was most commonly affected. Occasional neurons within affected areas contained an intracytoplasmic lake of amorphous material.

Recent mild to moderate multifocal ring and/or ball type hemorrhages were present in two male [3674 and 3688] and one female [3717] dogs. Most commonly affected areas included the medulla oblongata and the neocortex. A somewhat larger recent perivenular hemorrhage was present in the female dog.

The male [3688] dog also had edema in the neocortex. Mild to moderate autolysis was present in all dogs found dead.

Premature decedents

Deaths occurred on study for 2 MDF, 4 HDF, and 4HDM; each death was associated with antemortem observations including convulsions as well as findings consistent with cardiac and/or respiratory arrest.

- Two MD females #3714 and #3707 were found dead on Days 171 and 305, respectively.
  - COD #3714 attributed to heart, myocardium congestion of mild severity; the study pathologist noted that the multiple organ congestion was indicative of acute general passive venous congestion suggesting a sudden cardiovascular failure.
  - COD #3707 was unknown. Multifocal hemorrhage was found in adrenal gland bilaterally in the cortex and medulla at moderate severity, in heart endocardium at moderate severity, and in spinal cord at the cervical and thoracic levels with severities of mild and moderate, respectively.
- Four HD males died prematurely during the study, three were found dead #3667 (Day 29), #3674 (Day 341), and #3688 (Day 193); one #3664 was sacrificed *in extremis* (Day 56).
  - COD #3667 attributed to heart, multifocal epicardium, and endocardium hemorrhage of moderate severity; the study pathologist noted that the multiple organ congestion was indicative of acute general passive venous congestion suggesting a sudden cardiovascular failure. These microscopic findings correlated with the macroscopic findings of multifocal red foci associated with the heart epicardium (trace severity).

  - COD #3674 was unknown. Hemorrhage was present in several tissues including: bilaterally in the adrenal glands (cortex and medulla, each moderate severity), heart (endocardium, moderate severity), spinal cord (cervical and thoracic, each mild severity), and thymus (multifocal, moderate severity).
  - COD#3688 was unknown. Macroscopic findings were found in the heart, lung, thymus, and urinary bladder. In the heart, multiple red foci were identified in the epicardium (mild severity). Both the lung (multiple lobes, moderate) and thymus (severe) had diffuse red discoloration. Microscopically, multifocal hemorrhage was found in the heart epicardium (mild) and in the thymus (severe). In the lung, diffuse congestion (moderate) and multifocal hemorrhage (moderate) were observed. No microscopic correlates were found in the urinary bladder. Additional evidence of hemorrhage was found bilaterally in the adrenal cortex (mild).
  - COD#3664 attributed to heart, congestion of the myocardium (mild). The study pathologist noted that the multiple organ congestion was indicative of acute general passive venous congestion suggesting a sudden cardiovascular failure.
- Four HD females died prematurely during the study, two were found dead—#3692 (Day 90) and #3717 (Day 191), and two were sacrificed *in extremis*—#3695 (Day 56) and #3709 (Day 173).
  - COD #3692 attributed to heart, hemorrhage of the endocardium (moderate). Macroscopically, congestion was found in brain (diffuse, moderate), liver (multilobar, mild), and lung (multilobar, severe); and the meninges had focal red discoloration (mild). Microscopically, hemorrhage was found in several tissues including: bilateral adrenal glands (cortex and medulla, each moderate), rib bone (subperiosteal, moderate), brain had both multifocal hemorrhage (mild) and congestion (mild), heart endocardium (moderate) and heart congestion present (mild), meninges multifocal (moderate), spinal cord (cervical, thoracic, and lumbar, each mild), thymus multifocal (mild), and cervix multifocal mild.
  - #3695 COD attributed to heart, myocardium congestion of mild severity. Macroscopic findings occurred in all lobes of the lung (multifocal dark red discoloration foci, 0.5-1.5 cm, and moderate), ileocolic orifice (black discoloration, moderate), fundic stomach (generalized red discoloration, moderate) and trachea (froth fluid in the lumen, moderate). Microscopically, congestion and hemorrhage were found in many tissues.
  - #3709 COD was unknown. Macroscopic findings were diffuse red discoloration of the duodenum (mild), single red focus in the spleen (mild), and diffuse fluid distension of the stomach. Microscopically, hemorrhage was found bilaterally in the adrenal glands (cortex and medulla, each moderate) and multifocal in cervical (mild) and thoracic (trace) spinal cord. Congestion was found in the duodenum mucosa (mild) and multifocal in the spleen (mild).

- #3717 COD was unknown. Macroscopically, multifocal red foci were found in the mucosa of the duodenum. Microscopically, hemorrhage was found in the following tissues: bilateral adrenal glands (cortex, mild and medulla, moderate), spinal cord (lumbar, mild), and thymus (moderate). In addition, congestion was reported in the duodenum mucosa (mild).

Terminal sacrifice

No treatment-related microscopic changes in dogs sacrificed at study termination were observed by the study pathologist.

The following list of organs and tissues were collected for microscopic analysis:

Adrenal (2), aorta (thoracic), bone marrow—femur and sternum, bone marrow smear[a], brain (7 sections)[f], gastrointestinal tract—colon, cecum, rectum, duodenum, jejunum, ileum, and esophagus, eye with optic nerve (2)[b], gallbladder, heart, kidneys, liver, lesions, lung with bronchi[c], mammary gland (F only)[d], mandibular and mesenteric lymph nodes, ovaries, pancreas, pituitary, prostate, salivary glands (mandibular), sciatic nerve, skeletal muscle, skin, spinal cord (cervical, lumbar, and mid-thoracic spinal cord), spleen, stomach, testes and epididymides, thymus[d], thyroid/parathyroids[e], trachea, urinary bladder, ureter (2) and uterus—both horns and cervix.

[a]Bone marrow smears collected at scheduled sacrifices only.
[b]Eyes fixed in Davidson's fixative.
[c]Lungs inflated with formalin via the trachea.
[d]At times these tissues were not visible by the unaided eye because of physiologic variation in size. However, tissue from the region was fixed for microscopic evaluation.
[e]Parathyroids could not always be identified microscopically. If in the plane of a section, these tissues were examined. If noted grossly enlarged, all cases were microscopically examined.
[f]Brain was sectioned to obtain when possible sections of the olfactory bulb, piriform cortex, amygdale, hippocampus, cingulate cortex, cerebral cortex, medial geniculate body, substantia nigra, and area postrema.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )

Plasma Drug Concentrations: Plasma was collected at 0, 0.5, 1, 2, and 4 hours of morning dosing during weeks 18, 26, 40 and 52 and measured using HPLC. Individual samples were collected from dogs showing seizure activity in order to determine plasma 4-aminopyridine concentrations.

- 4-Aminopyridine was rapidly absorbed from the stomach following oral administration. C_{max} was achieved within 1 hr of test article administration.
  - Peak plasma concentrations of 4-AP were approximately proportional to dose level.
- Average plasma concentration decreased at the 2 hr collection in all dose groups. Maximum concentration reached at each dose group and sex appeared relatively unaffected by repeated dosing.
- The greatest variability in plasma concentrations was noted at 0.5 hr post dose with differences of 3 to 4-fold between individuals of the same group and sex.

More uniform plasma concentrations were observed at the later collection times. The sponsor suggested that this might explain some of the variability observed in the physiological effects of the test article.

- Sponsor's Table 3 (reproduced from Appendix B) summarizes plasma drug concentrations obtained from dogs during seizure activity.
    - High plasma 4-AP concentrations suggest a possible relationship between C_{max} and the generation of seizure activity.

Aminal #	Group	CONC. (ng/ml)	Comments
3707	3F	682	last dosed 9/18/93 at 7:29 blood collected 8:36
3708	3F	112	last dosed 7/31/93 at 7:21 blood collected between 10:27 and 11:30
3708	3F	164	last dosed 9/18/93 at 7:29 blood collected 10:24
3714	3F	381	lasted dosed 5/7/93 at 7:34 blood collected 11:05
3664	4M	386	last dosed 1/12/93 at 13:45 blood collected 16:20
3667	4M	68.7	last dosed 12/9/92 at 18:14 blood collected in the evening after last dose Animal was given an I.V. bolus of diazepam to facilitate blood collection. *Sample was seriously hemolyzed
3671	4M	238	last dosed 10/16/93 at 7:21 blood collected 9:17
3688	4M	513	last dosed 5/28/93 at 8:22 blood collected 10:50
3702	4F	461	last dosed 11/4/93 at 7:36 blood collected between 9:36-9:49

2.6.6.4 Genetic Toxicology

Sponsor submitted seven genetic toxicology study reports—five were *in vitro* studies and two were *in vivo* studies conducted in mouse and rat. Each of these studies is reviewed below.

In vitro studies

1.) Mutagenicity test on 4-aminopyridine in the salmonella/mammalian-microsome reverse mutation assay (Ames test)

Key findings:

- 4-Aminopyridine was non-mutagenic in the absence or presence of metabolic activation in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538.
- However, this study failed to test bacterial strains *S. typhimurium* TA102 or *E.coli* WP2 *uvr*A and thus may be insensitive to hydrazines, some oxidizing mutagens and cross-linking agents.

Study no.: (b) (4) 14477-0-401

Volume #, and page #: EDR, 4.2.3.3.1 (b) (4) 144770-0-401
Conducting laboratory and location: (b) (4)

Date of study initiation: 27 March 1991
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, 99+% pure

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were used.

Doses used in definitive study: Six concentrations (100, 333, 667, 1000, 3330, and 5000 µg/plate) were selected.

Basis of dose selection: A preliminary study to determine the cytotoxicity of 4-aminopyridine in the TA100 strain was conducted in both the absence and presence of the S9 mix at concentrations of 6.67 to 5000 µg/plate. Metabolic activation was achieved using Aroclor-induced male SD rat liver S9 mix. No cytotoxicity was observed in the presence or in the absence of S9 as evidenced by normal background lawn and no observed decrease in the number of revertants/plate.

Negative controls: Deionized water for all tester strains in both the presence and absence of S9 mix.

Positive controls: 2-aminoanthracene (+S9 mix, TA98, TA100, TA1535, TA1537 and TA1538), 2-nitrofluorene (-S9 mix, TA98 and TA1538), sodium azide (-S9 mix, TA100 and TA1535), and ICR-191 (-S9 mix, TA1537).

Incubation and sampling times: Tester strains were exposed to the test article using the plate incorporation assay as described by Ames et al. (1975) and Maron and Ames (1983). Incubations occurred at 37 ± 2 ºC for 48 ± 8 hr. All concentrations of the test article, and negative and positive controls were plated in triplicate. Plates were scored immediately following the incubation or stored at 5 ± 3 ºC, until colony counting and bacterial lawn evaluation could be made.

Results

Study validity: For tester strains TA98, TA100, TA1535, TA1537 and TA1538, assay validity was demonstrated. However, according to OECD 471, it is well established that these strains of bacteria have G-C base pairs at the primary reversion site that may not detect certain oxidizing mutagens, cross-linking agents and hydrazines. This assay, therefore, lacks a strain of bacteria, either *E. coli* or *S. typhimurium* TA102, which have an A-T base pair at the primary reversion site that is sensitive to those mutagenic agents listed above. Therefore, the results of this Ames test must be interpreted with caution.

Assay validity was demonstrated. The bacterial lawn was examined for evidence of cytotoxicity or test article precipitate. The criteria established for a positive result was replicated from sponsor's study report (p.19), as shown below.

1. Tester Strains TA98 and TA100

For a test article to be considered positive, it must produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate must be accompanied by a dose response to increasing concentrations of the test article.

2. Tester Strains TA1535, TA1537 and TA1538

For a test article to be considered positive, it must produce at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate must be accompanied by a dose response to increasing concentrations of the test article.

The condition of the bacterial lawn was examined macroscopically and microscopically for determination of cytotoxicity and test article precipitate.

Study outcome: In the first study, 4-aminopyridine, with or without metabolic activation, was not cytotoxic or mutagenic in tester strains TA98, TA100, TA1535, and TA 1538 (see sponsor's Table 3, below). The mean vehicle control values for tester strain TA1537 were not within the historical control range and thus the study was repeated. In the repeat study, similar to the findings in the other strains, 4-aminopyridine (98.8 to 4940 µg/plate) did not produce cytotoxicity and was not mutagenic in tester strain TA1537 (see

sponsor's Table 4, below). In each experiment, the positive controls were markedly elevated compared to the vehicle controls in the absence or in the presence of S9 mix.

The following data tables (Tables 3-4) were reproduced from the sponsor's study report (pp. 27-28).

TABLE 3

SUMMARY OF TEST RESULTS

TEST ARTICLE ID: 4-Aminopyridine

EXPERIMENT ID: 14477-B1

DATE PLATED: 05-Apr-91 VEHICLE: Deionized water

DATE COUNTED: 08-Apr-91 PLATING ALIQUOT: 100 µl

	DOSE/PLATE	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION										BACKGROUND LAWN*
		TA98		TA100		TA1535		TA1537		TA1538		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver												
VEHICLE CONTROL		38	7	128	13	12	5	105⊗	17	23	5	1
TEST ARTICLE	100 µg	36	6	116	8	12	4	NC	-	20	4	1
	333 µg	37	6	137	4	9	4	NC	-	20	1	1
	667 µg	40	8	120	16	15	3	NC	-	20	1	1
	1000 µg	27	5	124	9	11	6	NC	-	22	5	1
	3330 µg	36	3	120	6	11	6	NC	-	19	5	1
	5000 µg	36	8	122	13	13	5	NC	-	19	9	1
POSITIVE CONTROL **		1135	30	1164	79	151	9	NC	-	1226	41	1
MICROSOMES: None												
VEHICLE CONTROL		16	1	109	7	11	4	86⊗	11	14	6	1
TEST ARTICLE	100 µg	16	3	95	7	9	4	NC	-	12	3	1
	333 µg	22	3	100	17	9	2	NC	-	11	3	1
	667 µg	20	6	105	8	10	5	NC	-	10	2	1
	1000 µg	17	2	108	15	11	3	NC	-	10	3	1
	3330 µg	17	5	96	9	12	1	NC	-	12	2	1
	5000# µg	13	3	104	19	7	3	NC	-	10	2	1
POSITIVE CONTROL ***		118	10	501	107	371	65	NC	-	136	18	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate
TA1538 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate
TA1538 2-nitrofluorene 1.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

⊗ The mean vehicle control values for tester strain TA1537 were not within the acceptable range. See retest data, Table 4.

NC = Not counted due to unacceptable mean vehicle control value. See retest data, Table 4.

TABLE 4

INDIVIDUAL PLATE COUNTS
AND
MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION

TEST ARTICLE ID: 4-Aminopyridine

EXPERIMENT ID: 14477-B2

DATE PLATED: 12-Apr-91 VEHICLE: Deionized water

DATE COUNTED: 15-Apr-91 PLATING ALIQUOT: 100 µl

DOSE/PLATE		REVERTANTS PER PLATE TA1537 1	2	3	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION TA1537 MEAN	S.D.	BACKGROUND LAWN*
MICROSOMES: Rat Liver							
VEHICLE CONTROL		17	9	3	10	7	1
TEST ARTICLE	98.8 µg	13	9	9	10	2	1
	329 µg	15	14	5	11	6	1
	659 µg	9	8	6	8	2	1
	988 µg	9	8	5	7	2	1
	3290 µg	10	8	4	7	3	1
	4940 µg	9	8	7	8	1	1
POSITIVE CONTROL **		170	119	118	136	30	1
MICROSOMES: None							
VEHICLE CONTROL		9	6	2	6	4	1
TEST ARTICLE	98.8 µg	8	8	7	8	1	1
	329 µg	11	9	7	9	2	1
	659 µg	12	7	5	8	4	1
	988 µg	14	8	7	10	4	1
	3290 µg	12	8	6	9	3	1
	4940 µg	11	10	7	9	2	1
POSITIVE CONTROL ***		473	460	447	460	13	1

** TA1537 2-aminoanthracene 2.5 µg/plate *** TA1537 ICR-191 2.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal 2 = slightly reduced 3 = moderately reduced
4 = extremely reduced 5 = absent 6 = obscured by precipitate
sp = slight precipitate mp = moderate precipitate (requires hand count) hp = heavy precipitate (requires hand count)

2.) **Bacterial Reverse Mutation Assay**

Key findings:

- 4-Aminopyridine was not mutagenic in the absence or presence of metabolic activation in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and in *E.coli* WP2 *uvr*A.

Study no.: (b) (4) 1139-004

Volume #, and page #: EDR, 4.2.3.3.1 (b) (4) 1139-004
Conducting laboratory and location: (b) (4)

Date of study initiation: 29 August 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, P175-4-1, purity 100.5%

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 were used as well as *Escherichia coli* tester strain WP2 *uvr*A.

Doses used in definitive study: Concentrations tested were 1.5, 5, 15, 50, 150, 500 and 5000 µg/plate; the concentrations selected for the confirmatory assay were 50, 150, 500, 1500 and 5000 µg/plate.

Basis of dose selection: A preliminary study to determine the cytotoxicity of 4-aminopyridine was performed using overnight cultures of TA 98, TA100, TA1535, TA1537 and WP2 *uvr*A tester strains on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9.

- No cytotoxicity was observed in the presence or in the absence of S9.

Negative controls: Deionized water for all tester strains in both the presence and absence of S9 mix.

Positive controls: The following positive controls were utilized: 2-aminoanthracene (all *Salmonella* strains 1 µg/plate and WP2*uvr*A 10 µg/plate), 2 nitrofluorene (TA 98, 1 µg/plate), sodium azide (TA100 and TA1535, 1 µg/plate), 9-amionoacridine (TA1537, 75 µg/plate), and methyl methanesulfonate (WP2*uvr*A, 1000 µg/plate).

Incubation and sampling times: Tester strains were exposed to the test article using the plate incorporation assay as described by Ames et al. (1975) and Maron and Ames (1983). Incubations occurred at 37 ± 2 ºC for 48 to 72 hr. All dose levels of the test article, and negative and positive controls were plated in triplicate. Plates were scored immediately following the incubation or stored at 2 to 8 ºC, until colony counting and bacterial lawn evaluation could be made.

Study validity: The condition of the bacterial lawn background was evaluated for evidence of toxicity and precipitation.

Assay validity was demonstrated. The bacterial lawn was examined for evidence of cytotoxicity or test article precipitate.

Result interpretation: According to the sponsor, tester strains TA1535 and TA1537 were considered positive if the increase in mean revertants at the peak of the dose response was ≥ 3-times the mean vehicle control value. Tester strains TA98, TA100 and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was 2 times the mean vehicle control value.

A positive test article result requires a dose-related increase in the number of mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of 4-aminopyridine. An equivocal result was determined when a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as a positive. For example, a dose-response increase without reaching the threshold cited or a non-dose responsive increase that is equal to or greater than the respective threshold.

Results

Analysis of the test article solutions revealed that the concentrations of 4-aminopyridine were within 96.8% and 94.0% of their respective targeted concentrations.

No precipitation or cytotoxicity was noted in any of the tester strains incubated with 4-aminopyridine for either the first study at concentrations ranging from 1.5 to 5000 µg/ml or the second study using concentrations from 50 to 5000 µg/ml. The absence or presence of metabolic activation of 4-aminopyridine did not affect the cytotoxicity or mutagenicity to tester strains TA98, TA100, TA1535, TA 1537, and WP2 *uvr*A (see sponsor's Table 21, below). In each tester strain, the positive controls were markedly elevated compared to the vehicle controls in the absence or in the presence of S9 mix.

The following data tables (Tables 21-22) were reproduced from the sponsor's study report (pp. 36-37).

Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay

Table 21

Test Article Id : 4-AP
Study Number : AB34YG.503.BTL Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Vehicle	12 ± 2	140 ± 10	18 ± 6	5 ± 2	17 ± 3
1.5	7 ± 1	106 ± 15	19 ± 3	7 ± 1	16 ± 2
5.0	9 ± 4	101 ± 16	15 ± 4	5 ± 1	12 ± 1
15	10 ± 0	97 ± 35	17 ± 6	5 ± 1	17 ± 3
50	9 ± 0	110 ± 5	11 ± 1	8 ± 4	18 ± 8
150	11 ± 1	127 ± 18	18 ± 3	8 ± 2	15 ± 1
500	14 ± 0	74 ± 23	16 ± 0	8 ± 3	20 ± 1
1500	12 ± 1	109 ± 0	13 ± 4	5 ± 4	18 ± 4
5000	10 ± 4	127 ± 6	21 ± 1	6 ± 3	23 ± 1
Positive	116 ± 6	538 ± 40	212 ± 33	599 ± 57	143 ± 15

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Vehicle	14 ± 4	119 ± 4	15 ± 0	8 ± 1	22 ± 7
1.5	14 ± 1	134 ± 15	16 ± 6	9 ± 4	18 ± 2
5.0	14 ± 1	112 ± 13	10 ± 6	7 ± 6	22 ± 4
15	12 ± 3	153 ± 2	13 ± 1	6 ± 0	27 ± 6
50	16 ± 4	122 ± 11	13 ± 3	7 ± 1	21 ± 5
150	14 ± 4	99 ± 1	10 ± 2	5 ± 1	19 ± 1
500	13 ± 1	126 ± 2	13 ± 4	5 ± 0	18 ± 1
1500	16 ± 2	137 ± 10	13 ± 2	11 ± 2	18 ± 1
5000	12 ± 3	106 ± 14	16 ± 2	6 ± 3	18 ± 3
Positive	326 ± 64	497 ± 27	59 ± 1	44 ± 13	69 ± 0

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay

Table 22

Test Article Id : 4-AP
Study Number : AB34YG.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 2	99 ± 11	15 ± 1	7 ± 1	24 ± 2
50	15 ± 3	103 ± 6	12 ± 4	5 ± 1	22 ± 4
150	15 ± 3	102 ± 12	12 ± 2	5 ± 3	18 ± 4
500	14 ± 6	88 ± 7	16 ± 3	5 ± 2	21 ± 1
1500	15 ± 3	93 ± 7	15 ± 2	8 ± 3	22 ± 5
5000	15 ± 1	95 ± 11	17 ± 3	7 ± 2	16 ± 5
Positive	134 ± 30	495 ± 32	425 ± 44	1044 ± 56	281 ± 20

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	24 ± 4	96 ± 11	17 ± 2	10 ± 1	29 ± 2
50	18 ± 3	98 ± 7	19 ± 2	6 ± 2	24 ± 4
150	18 ± 2	103 ± 19	16 ± 4	9 ± 3	21 ± 4
500	22 ± 5	87 ± 10	16 ± 3	9 ± 2	23 ± 5
1500	25 ± 3	96 ± 5	13 ± 3	7 ± 3	17 ± 5
5000	17 ± 1	111 ± 4	10 ± 2	8 ± 3	19 ± 4
Positive	214 ± 43	465 ± 97	70 ± 8	81 ± 1	88 ± 5

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

- In two separate assays, 4-Aminopyridine was non-mutagenic in the absence or presence of metabolic activation (S9 mix) in *S. typhimurium* strains TA98, TA100, TA1535, TA 1537, and *Escherichia coli* strain WP2 *uvr*A.

3.) Mutagenicity test on 4-aminopyridine in the L5178Y TK+/- mouse lymphoma forward mutation assay with an independent repeat

Key findings:

- 4-Aminopyridine was negative for inducing forward mutation at the TK locus in mouse lymphoma cells in the absence and presence of metabolic activation.

Study no.: (b) (4) 15558-0-431R
Study type: *In vitro* Mutagenesis and Clastogenesis
Volume #, and page #: EDR 4.2.3.3.1 (b) (4) 15558-0-431R, pp. 1-48
Conducting laboratory and location: (b) (4)
Date of study initiation: 04 March 1993
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot # C4886, purity unknown

Methods

Strains/species/cell line: L5178Y/TK+/- Mouse Lymphoma

Doses used in definitive study:

Trial 1:	-S9	100, 200, 400, 600, 800, 1000 µg/ml
	+S9	100, 200, 400, 600, 800, 1000 µg/ml
Trial 2:	-S9	100, 200, 400, 600, 800 µg/ml
	+S9	200, 400, 600, 800, 1000, 1200 µg/ml

Basis of dose selection: A cytotoxicity assay was performed to select the concentrations of 4-aminopyridine for the definitive study. At concentrations of 4-AP ranging from 313 to 5000 µg/ml, the pH was basic and thus stock material was adjusted to pH 7.0-7.2. A second cytotoxicity assay was performed utilizing the treatment medium (Fischer's medium) as the diluent. Due to pH-dependent cytotoxicity in the first test, results from the second cytotoxicity assay were utilized to determine concentrations of 4-AP to use in the mutagenicity study.

A range-finding cytotoxicity assay was conducted in the absence and presence of S9 metabolic activation. Concentrations of 4-aminopyridine tested were 9, 19, 38, 78, 156, 313, 625, 1250, 2500, and 5000 µg/ml. Results are presented in sponsor's Table 1. In the absence of S9 metabolic activation, 4-aminopyridine was found to be slightly more cytotoxic; 4-AP concentrations 9-38 µg/ml were nontoxic, 78-625 µg/ml were moderately toxic, and ≥1250 µg/ml were completely cytotoxic. In the presence of S9 mix, 4-AP concentrations 9-78 µg/ml were nontoxic, 156-625 µg/ml were moderately toxic, 1250

µg/ml was highly toxic, and ≥2500 µg/ml was completely cytotoxic to the mouse lymphoma cells. Based on these results the concentrations for the definitive study were selected.

TABLE 1

CYTOTOXICITY ASSAY WITH 4-AMINOPYRIDINE

SAMPLE IDENTITY: 4-Aminopyridine
ASSAY NUMBER: 15558
TEST DATE: MARCH 11, 1993
SOLVENT: TREATMENT MEDIUM
COMMENTS ON TREATMENT: FOUR-HOUR EXPOSURE PERIOD.

APPLIED CONCENTRATION µg/ml	WITHOUT S9 ACTIVATION		WITH S9 ACTIVATION	
	CELL DENSITY/ML ($X10^5$)[a]	% NEGATIVE CONTROL[b]	CELL DENSITY/ML ($X10^5$)[a]	% NEGATIVE CONTROL[b]
NC[c]	21.8	100.0	11.5	100.0
9.77	NCT	NCT	NCT	NCT
19.5	NCT	NCT	NCT	NCT
39.1	NCT	NCT	NCT	NCT
78.1	16.3	74.8	NCT	NCT
156	14.7	67.4	10.5	91.3
313	12.2	56.0	7.5	65.2
625	7.0	32.1	8.0	69.6
1250	0.0	0.0	1.2	10.4
2500	0.0	0.0	0.0	0.0
5000	0.0	0.0	0.0	0.0

[a]Cell density determined by hemocytometer approximately 24 hours after treatment initiation.
[b]Relative to negative control cell density for all treatments.
NC[c] = Negative (media) control.
NCT = Not counted; nontoxic dose level.

Negative controls: treatment medium (Fischer's medium). Three negative control cultures were utilized in the pivotal mutation assays.

Positive controls: Methyl methanesulfonate without S9 metabolic activation; 3-Methylcholanthrene (MCA) with metabolic activation.

S9 Metabolic Activation System: Aroclor 1254-induced S9 fraction of Sprague Dawley rat liver.

Incubation and sampling times: 4 hr at 37ºC in the absence and presence of S9.

Mutagenesis Criteria: According to the sponsor, the following criteria must be met in order to demonstrate mutagenesis:

1. A concentration-related or toxicity-related increase in mutant frequency,
2. Confirmation of a mutagenic response in a second mutation assay,
3. If mutant frequency obtained for a single dose at or near the highest testable toxicity is ≥ 4-times the concurrent background mutant frequency, the trial is considered mutagenic.
4. To term a test article positive (mutagenic), the increase must be repeatable in the second mutation assay.
5. Either applied concentration or toxicity (percent relative growth) may be used to establish whether the increase in mutant frequency is related to an increase in effective treatment.
6. Treatments that result in an induction of <10% relative growth are included in the assay but are not used as primary evidence for mutagenicity for risk assessment.

Results

Mutation Assay: Two mutation assay trials were conducted in the absence and presence of metabolic activation. In both trials, concentration-related cytotoxicity was obtained.

Nonactivation conditions (-S9 mix)

The data for Trials 1 and 2 are summarized in the following sponsor's tables. 4-aminopyridine was considered negative in the absence of S9.

TABLE 2

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL 1

A. TEST ARTICLE: 4-Aminopyridine
B. GENETICS ASSAY NO: 15558
C. VEHICLE: FISCHER'S MEDIUM
D. SELECTIVE AGENT: 3 µg/ml TFT
E. TEST DATE: 03/16/93

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
NONACTIVATION CONTROLS[e]			AVG NEGATIVE CONTROL				AVG NEGATIVE CONTROL			
NEGATIVE CONTROL	12.8	21.9	31.1		74	539	89.8		100.0	27.5
NEGATIVE CONTROL	13.2	20.5	30.1		48	546	91.0		100.0	17.6
NEGATIVE CONTROL	16.2	19.4	34.9	32.0	104	435	72.5	84.4	100.0	47.8
MMS 10 NL/ML	6.7	18.4	13.7		557	143	23.8		12.1	779.0[f]
MMS 15 NL/ML	8.3	7.5	6.9		618	135++	22.5		5.7	915.6[f]
TEST COMPOUND			RELATIVE TO NEGATIVE CONTROL (%)				RELATIVE TO NEGATIVE CONTROL (%)			
100 µg/ml	15.7	14.4	78.5		133	687	135.7		106.5	38.7
200 µg/ml	16.9	17.3	101.5		85	574	113.3		115.0	29.6
400 µg/ml	10.8	18.8	70.5		123	536	105.8		74.6	45.9
600 µg/ml	8.5	12.6	37.2		115	737	145.5		54.1	31.2
800 µg/ml	2.7*	21.8	22.7		131	549	108.4		24.6	47.7
1000 µg/ml	1.2*	5.7	5.9		73	454	89.7		5.3	32.2

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]NEGATIVE CONTROL = MEDIUM CONTROL; MMS = METHYL METHANESULFONATE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 61.9 X 10E-6

* NOT SPLIT BACK

++ONE PLATE CONTAMINATED; VALUE DETERMINED BY WEIGHT PROPORTION

TABLE 3

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL 2

A. TEST ARTICLE: 4-Aminopyridine
B. GENETICS ASSAY NO: 15558
C. VEHICLE: FISHER'S MEDIUM
D. SELECTIVE AGENT: 3 µg/ml TFT
E. TEST DATE: 03/30/93

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
NONACTIVATION CONTROLS[e]			AVG NEGATIVE CONTROL				AVG NEGATIVE CONTROL			
NEGATIVE CONTROL	19.2	17.6	37.5		156	533	88.8		100.0	58.5
NEGATIVE CONTROL	14.2	16.5	26.0		130	736	122.7		100.0	35.3
NEGATIVE CONTROL	14.7	16.9	27.6	30.4	150	713	118.8	110.1	100.0	42.1
MMS 10 NL/ML	8.6	12.3	11.8		1006	359	59.8		21.1	560.4[f]
MMS 15 NL/ML	6.8	12.9	9.7		854	229	38.2		11.1	745.9[f]
TEST COMPOUND			RELATIVE TO NEGATIVE CONTROL (%)				RELATIVE TO NEGATIVE CONTROL (%)			
'00 µg/ml	18.4	18.2	122.4		119	576	87.2		106.7	41.3
00 µg/ml	12.4	22.0	99.7		146	595	90.1		89.8	49.1
·00 µg/ml	11.5	17.0	71.5		160	567	85.8		61.3	56.4
600 µg/ml	7.4	17.8	48.1		164	494	74.8		36.0	66.4
800 µg/ml	1.9*	8.3	9.1		181	594	89.9		8.2	60.9

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]NEGATIVE CONTROL = FISCHER'S MEDIUM; MMS = METHYL METHANESULFONATE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 90.6 X 10E-6

* NOT SPLIT BACK

Activation conditions (+S9 mix)

The data for Trials 1 and 2 are summarized in the following sponsor's tables, 4-aminopyridine was considered negative in the presence of S9.

Study validity: This study is valid as provided by the sponsor, as each criterion described above was met.

Study outcome: 4-Aminopyridine is negative for inducing forward mutation at the TK locus in mouse lymphoma cells under the conditions of testing.

TABLE 4

MUTATION ASSAY WITH ACTIVATION - TRIAL 1

A. TEST ARTICLE: 4-Aminopyridine
B. GENETICS ASSAY NO: 15558
C. VEHICLE: FISCHER'S MEDIUM
D. SELECTIVE AGENT: 3 µg/ml TFT
E. TEST DATE: 03/16/93

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
S9 ACTIVATION INDUCED[e]	S9 BATCH NO: 0391									
			AVG NEGATIVE CONTROL					AVG NEGATIVE CONTROL		
NEGATIVE CONTROL	10.7	12.5	14.9		402	755	125.8		100.0	106.5
NEGATIVE CONTROL	8.8	17.2	16.8		330	727	121.2		100.0	90.8
NEGATIVE CONTROL	7.6	18.5	15.6	15.8	347	608	101.3	116.1	100.0	114.1
MCA 2 µg/ml	7.5	10.7	8.9		1090	530	88.3		42.8	411.3[f]
MCA 4 µg/ml	1.9*	4.8	1.6		787	457	76.2		6.6	344.4[f]
TEST COMPOUND			RELATIVE TO NEGATIVE CONTROL (%)				RELATIVE TO NEGATIVE CONTROL (%)			
100 µg/ml	7.4	22.4	116.6		300	517	74.2		86.5	116.1
00 µg/ml	8.8	19.3	119.4		224	472	67.8		81.0	94.9
00 µg/ml	8.2	14.7	84.8		321	802	115.1		97.6	80.0
600 µg/ml	6.6	16.2	75.2		339	723	103.8		78.1	93.8
800 µg/ml	5.2	21.4	78.3		255	607	87.1		68.2	84.0
1000 µg/ml	4.5	17.1	54.1		320	679	97.5		52.7	94.3

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]NEGATIVE CONTROL = FISCHER'S MEDIUM; MCA = METHYLCHOLANTHRENE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 207.6 X 10E-6

* NOT SPLIT BACK

TABLE 5

MUTATION ASSAY WITH ACTIVATION - TRIAL 2

A. TEST ARTICLE: 4-Aminopyridine
B. GENETICS ASSAY NO: 15558
C. VEHICLE: FISHER'S MEDIUM
D. SELECTIVE AGENT: 3 µg/ml TFT
E. TEST DATE: 03/30/93

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
S9 ACTIVATION INDUCED[e]	S9 BATCH NO: 0391		AVG NEGATIVE CONTROL				AVG NEGATIVE CONTROL			
NEGATIVE CONTROL	5.0	27.0	15.0		274	454++	75.7		100.0	120.7
NEGATIVE CONTROL	5.2	26.1	15.1		303	491	81.8		100.0	123.4
NEGATIVE CONTROL	8.0	19.2	17.1	15.7	310	513	85.5	81.0	100.0	120.9
MCA 2 µg/ml	4.7	15.1	7.9		1058	431	71.8		44.6	491.0[f]
MCA 4 µg/ml	2.8*	7.2	2.4		801	304	50.7		9.6	527.0[f]
TEST COMPOUND			RELATIVE TO NEGATIVE CONTROL (%)				RELATIVE TO NEGATIVE CONTROL (%)			
200 µg/ml	8.2	17.4	101.0		347	529	108.8		109.9	131.2
00 µg/ml	6.4	18.1	82.0		337	532	109.5		89.8	126.7
500 µg/ml	5.3	24.3	91.1		289	497	102.3		93.2	116.3
800 µg/ml	3.2*	24.3	51.6		362	596	122.6		63.3	121.5
1000 µg/ml	2.1*	17.0	36.1		314	441	90.7		32.7	142.4
1200 µg/ml	1.2*	4.1	8.7		321	514	105.8		9.2	124.9

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]NEGATIVE CONTROL = FISCHER'S MEDIUM; MCA = METHYLCHOLANTHRENE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 243.3 X 10E-6

* NOT SPLIT BACK

++ONE PLATE CONTAMINATED; VALUE DETERMINED BY WEIGHT PROPORTION

4.) *In vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay) Study Report

Key findings:

- 4-Aminopyridine was considered negative for inducing forward mutation at the TK locus in mouse lymphoma cells under the conditions of testing.

Study no.: (b) (4) 1139-005

Volume #, and page #: EDR 4.3.3.1. *In vitro* (b) (4) 1139-005, pp. 1-55
Conducting laboratory and location: (b) (4)
Date of study initiation: 16 August 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot # P175-4-1, 100.5%

Basis of dose selection: A cytotoxicity assay was performed to select the concentrations of 4-aminopyridine for the definitive study. A range-finding cytotoxicity assay was conducted in the absence and presence of S9 metabolic activation after 4 hr exposure to test article and in the absence of S9 after 24 hr exposure to test article. Concentrations of 4-aminopyridine tested were 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, and 941 µg/ml. Results are presented in sponsor's Table 1.

In the absence of S9 metabolic activation, a 4-hr exposure to 4-aminopyridine was found to be slightly more cytotoxic; 4-AP concentrations 0.15-150 µg/ml were nontoxic and 500-941 µg/ml were moderately toxic. Twenty-four hr exposure to 4-AP concentrations resulted in similar values for nontoxic concentrations 0.15-150 µg/ml; however, 500 µg/ml was highly toxic, and at 941 µg/ml 4-AP was completely toxic to the mouse lymphoma cells. In the presence of S9 mix, a 4-hr exposure to 4-AP concentrations 0.15-500 µg/ml were nontoxic and 941 µg/ml was moderately toxic to the mouse lymphoma cells. Based on these results the concentrations for the definitive study were selected.

TABLE 1

PRELIMINARY TOXICITY ASSAY USING 4-AP
4-Hour Exposure

Test Article Concentration (µg/mL)	Cell Concentration ($X10^6$)[a] Day 1	Day 2	Suspension Growth Total[b]	% Control[c]
WITHOUT ACTIVATION				
Solvent 1	1.131	1.617	20.3	
Solvent 2	1.117	1.554	19.3	
.15	1.125	1.547	19.3	98
.5	1.142	1.555	19.7	100
1.5	1.198	1.423	18.9	96
5	1.156	1.485	19.1	96
15	1.185	1.268	16.7	84
50	1.217	1.470	19.9	100
150	1.200	1.441	19.2	97
500	0.909	1.344	13.6	69
941	0.714	1.361	10.8	55
WITH S9 ACTIVATION				
Solvent 1	1.105	1.495	18.4	
Solvent 2	0.992	1.460	16.1	
.15	1.142	1.476	18.7	109
.5	1.097	1.453	17.7	103
1.5	1.101	1.488	18.2	106
5	1.117	1.469	18.2	106
15	1.109	1.469	18.1	105
50	1.116	1.518	18.8	109
150	1.145	1.469	18.7	108
500	1.027	1.489	17.0	99
941	0.781	1.060	9.2	53

Solvent = water

1 and 2 are duplicate cultures

[a] - Cultures containing $<0.3x10^6$ cells/mL on day 1 and 2 are considered to have 0% total suspension growth.

[b] - Total suspension growth $= \frac{\text{Day 1 cell conc.}}{0.3x10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$

[c] - % of control suspension growth $= \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$

TABLE 1 (Cont.)

PRELIMINARY TOXICITY ASSAY USING 4-AP
24-Hour Exposure

Test Article Concentration	Cell Concentration ($x 10^6$) Day 0	Day 1	Day 2	Suspension Growth Total	% of Control
Solvent:	0.915	0.945	1.278	40.91	100%
Solvent:	0.894	0.928	1.337	41.08	100%
0.15	0.863	0.961	1.173	36.04	88%
0.5	0.893	0.933	1.377	42.51	104%
1.5	0.920	0.964	1.310	43.05	105%
5	0.931	0.965	1.347	44.83	109%
15	0.877	0.941	1.308	39.97	98%
50	0.923	0.951	1.417	46.05	112%
150	0.937	1.013	1.228	43.17	105%
500	0.417	0.393	0.913	5.55	14%
941	0.151	0.063	0.086	NG	0%

Units = µg/mL

Solvent = water

1 and 2 are duplicate cultures

Cultures containing $<0.3x10^6$ cells/mL on day 0, 1, and 2 are considered to have 0% total suspension growth.

$$\text{Total suspension growth} = \frac{\text{Day 0 cell conc.}}{0.3x10^6 \text{ cells/mL}} \times \frac{\text{Day 1 cell conc.}}{\text{Day 0 adjusted cell conc.}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$$

$$\text{\% of control suspension growth} = \frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$$

Negative controls: treatment medium (Fischer's medium). Duplicate negative control cultures were utilized in the pivotal mutation assays.

Positive controls: Methyl methanesulfonate without S9 metabolic activation; 7,12 Dimethylbenz(a)nthrene with metabolic activation.

S9 Metabolic Activation System: Aroclor 1254-induced S9 fraction of Sprague Dawley rat liver.

Incubation and sampling times: 4 hr at 37ºC in the absence and presence of S9 and 24 hr at 37ºC in the absence of S9.

Mutagenesis Criteria: According to the sponsor, the following criteria must be met in order to demonstrate mutagenesis:

a. A concentration-related or toxicity-related increase in mutant frequency and one or more concentrations with ≥10% total growth showed mutant frequencies ≥100 mutants per 10^6 clonable cells over the background level,
b. If spontaneous mutant frequencies were elevated and >100 mutants/10^6 clonable cells, then a doubling of mutant frequency was also required.
c. An equivocal result occurred if the mutant frequency in treated cultures was between 55 and 99 mutants/10^6 clonable cells over the background level.
d. A result was negative if the mutant frequency was <55/10^6 clonable cells over the background level.

Results

Mutation Assay: Two mutation assays (4hr and 24 hr) were conducted in the absence of metabolic activation. An additional mutation assay was conducted following a 4 hr incubation with 4-AP in the presence of S9. In all assays, concentration-related cytotoxicity was obtained.

Nonactivation conditions (-S9 mix)

The data for the 4 and 24 hr incubation of mouse lymphoma cells with 4-AP in the absence of S9 mix are summarized in the following sponsor's tables.

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH 4-AP IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	41	42	59	47 ±8	218	214	222	218	±3	43		
Solvent	2	48	34	51	44 ±7	165	236	188	196	±30	45		
Mean Solvent Mutant Frequency= 44													
600	A	57	45	50	51 ±5	215	228	173	205	±23	49	5	76
600	B	46	45	25	39 ±10	198	217	179	198	±16	39	-5	71
700	A	34	30	53	39 ±10	205	199	244	216	±20	36	-8	76
700	B	38	50	35	41 ±6	228	234	202	221	±14	37	-7	74
800	A	40	31	50	40 ±8	230	314	321	288	±41	28	-16	104
800	B	66	55	65	62 ±5	245	231	198	225	±20	55	11	82
850	A	47	72	60	60 ±10	216	247	227	230	±13	52	8	68
850	B	45	40	45	43 ±2	204	212	205	207	±4	42	-2	51
941	A	33	64	47	48 ±13	214	214	205	211	±4	45	1	77
941	B	41	52	39	44 ±6	245	240	249	245	±4	36	-8	79
Positive Control - Methyl Methanesulfonate (µg/mL)													
15		190	189	189	189 ±0	107	120	101	109	±8	346	302	32
20		153	130	123	135 ±13	45	68	51	55	±10	495	451	14

Solvent = water

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency per 10^6 surviving cells $= \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$

[b] - Induced mutant frequency per 10^6 surviving cells = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth $= \frac{(\text{\% suspension growth} \times \text{\% cloning growth})}{100}$

TABLE 6

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH 4-AP IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Extended Treatment Assay (24-hour exposure)

Test Article Concentration		TFT Colonies Counts			Mean	SD	VC Colonies Counts			Mean	SD	Mutant Frequency per (10^6)	Induced Mutant Frequency	% Total Growth
Solvent	1	59	50	25	45	± 18	186	153	166	168	± 17	53		100%
Solvent	2	60	18	40	39	± 21	165	177	182	175	± 9	45		100%
Average Solvent Mutant Frequency = 49														
100	A	52	31	50	44	± 12	148	152	142	147	± 5	60	11	84%
100	B	84	32	78	65	± 28	170	178	199	182	± 15	71	22	115%
250	A	92	21	53	55	± 36	199	195	197	197	± 2	56	7	96%
250	B	38	27	33	33	± 6	160	189	230	193	± 35	34	-15	85%
300	A	*	*	*			*	*	*					
300	B	54	38	41	44	± 9	191	195	*	193	± 3	46	-3	60%
400	A	32	60	17	36	± 22	190	166	218	191	± 26	38	-11	35%
400	B	40	54	47	47	± 7	210	190	166	189	± 22	50	1	38%
500	A	114	28	38	60	± 47	171	156	181	169	± 13	71	22	13%
500	B	55	48	60	54	± 6	158	245	190	198	± 44	55	6	20%
550	A	40	33	27	33	± 7	169	195	184	183	± 13	36	-13	21%
550	B	55	41	39	45	± 9	222	215	175	204	± 25	44	-5	18%
Positive Control: MethylMethaneSulfonate (MMS)														
7.5		163	149	160	157	± 7	89	47	85	74	± 23	427	378	22%
5		186	145	164	165	± 21	52	53	47	51	± 3	651	602	14%

Units = μg/mL

Solvent = water

A and B or 1 and 2 are duplicate cultures

* - Culture lost prior to cloning

[a] - Mutant frequency per 10^6 surviving cells = $\frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$

[b] - Induced mutant frequency per 10^6 surviving cells = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = $\frac{(\text{\% suspension growth} \times \text{\% cloning growth})}{100}$

Activation conditions (+S9 mix)

The data for the 4 hr incubation of mouse lymphoma cells with 4-AP in the presence of S9 mix are summarized in the following sponsor's table.

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH 4-AP IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

Test Article Concentration (µg/mL)		TFT Colonies Counts				Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	32	45	45	41	±6	197	198	207	201	±4	41		
Solvent	2	40	38	38	39	±1	201	179	194	191	±9	40		
Mean Solvent Mutant Frequency= 40														
600	A	48	34	45	42	±6	250	235	251	245	±7	35	-6	107
600	B	40	42	27	36	±7	214	218	215	216	±2	34	-7	94
700	A	32	35	26	31	±4	209	216	248	224	±17	28	-13	91
700	B	30	30	48	36	±8	217	195	212	208	±9	35	-6	86
800	A	45	31	38	38	±6	244	190	219	218	±22	35	-6	85
800	B	48	46	44	46	±2	198	210	184	197	±11	47	6	75
850	A	60	50	58	56	±4	199	195	188	194	±5	58	17	70
850	B	48	40	52	47	±5	245	230	241	239	±6	39	-1	90
941	A	57	35	35	42	±10	198	234	242	225	±19	38	-3	78
941	B	33	28	58	40	±13	244	189	210	214	±23	37	-3	84
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)														
.75		210	234	248	231	±16	173	157	159	163	±7	283	243	56
1		211	234	210	218	±11	169	169	130	156	±18	280	239	55

Solvent = water

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency per 10^6 surviving cells $= \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$

[b] - Induced mutant frequency per 10^6 surviving cells = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth $= \frac{(\text{\% suspension growth} \times \text{\% cloning growth})}{100}$

Study validity: This was a valid study as each criterion described by the sponsor was met.

Study outcome: Due to a lack of sufficient cytotoxicity achieved for the 4-hr treatment ±S9, a conclusion that this assay is negative for inducing forward mutation at the TK locus in mouse lymphoma cells under these testing conditions is premature.

5.) **Mutagenicity test on 4-aminopyridine (4-AP): Measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay with multiple harvests.**

Key findings:

- 4-Aminopyridine cultured with CHO cells under non-activating or activating (+S9 mix) did not induce chromosomal aberrations.

Study no.: (b) (4) 15558-0-437Z

Volume #, and page #: EDR, 4.2.3.3.1, (b) (4) 15558-0-437Z, pp.
Conducting laboratory and location: (b) (4)
Date of study initiation: 04 March 1993
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4 Aminopyridine (4-AP), Lot No. C4886 Raw material, no purity is provided.

Methods

Strains/species/cell line: Chinese Hamster Ovary cells (CHO-WBL)

Range-finding Assay: Concentrations of 4-aminopyridine tested for both non-activating and activating assays were the following: 0.164, 0.491, 1.64, 4.91, 16.4, 49.1, 164, 491, 1640, and 4910 µg/ml.

Doses used in definitive study:

Non-activating Assay, 24 hr harvest: 150, 200, 250, 300, 350, 400, and 450 µg/ml
Activating Assay, 4 hr harvest: 150, 200, 250, 300, 350, 400, and 450 µg/ml
Metabolic activation was achieved using Aroclor-induced male SD rat liver S9 mix.

Basis of dose selection: range-finding study.

Negative controls: culture medium in the absence and presence of metabolic activation.

Positive controls: Mitomycin C (0.04 and 0.08 µg/ml) was used in the absence of metabolic activation, and cyclophosphamide (0.75 and 1.0 µg/ml) was used in the presence of metabolic activation. Only one of the two concentrations was analyzed for the aberration assays.

15558-0-4372

TABLE 2

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 24.1 Hours After Initiation ofTreatment

Assay No.: 15558 Trial #: 1 Lab #:3163 Metabolic Activation: Without

Compound: 4 Aminopyridine Date: March 17, 1993

		CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP g	CHROMATID TYPE ctb	cte	CHROMOSOME TYPE csb	cse	OTHERS GT	TOTALS -g	+g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:		A 100	2.0	0								0	0	
		B 100	2.0	0					1	1		2	2	
	TOTAL	200							1	1		2	2	
		%	2.0	0	-				0.5	0.5		1.0	1.0	-
SOLVENT CONTROL:		A 100	4.0	0								0	0	
Sterile Deionized Water		B 100	3.0	0								0	0	
10.0 µl/ml	TOTAL	200										0	0	
		%	3.5	0								0.0	0.0	
POSITIVE CONTROL:														
Mitomycin C	0.080 µg/ml	25	12.0	0			3	10				12	12	
		%	12.0	0	-		12	40				48	48	+
TEST ARTICLE:	300 µg/ml	A 100	3.0	0								0	0	
		B 100	1.0	0						1		1	1	
	TOTAL	200								1		1	1	
		%	2.0	0	-					0.5		0.5	0.5	-
	350 µg/ml	A 100	5.0	0								0	0	
		B 100	5.0	0		1						0	1	
	TOTAL	200				1						0	1	
		%	5.0	0	-	0.5						0.0	0.5	-
	400 µg/ml	A 100	2.0	0						2		2	2	
		B 100	2.0	0								0	0	
	TOTAL	200								2		2	2	
		%	2.0	0	-					2		1.0	1.0	-
	450 µg/ml	A 100	4.0	0								0	0	
		B 100	6.0	0					2	1		3	3	
	TOTAL	200							2	1		3	3	
		%	5.0	0	-				1.0	0.5		1.5	1.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, p <0.01.

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TABLE 3

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 24.0 Hours After Intiation of Treatment

Assay No.: 15558 Trial #: 2 Lab #:3243 Metabolic Activation: Without

Compound: 4 Aminopyridine Date: March 24, 1993

	CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS: GAP g	CHROMATID TYPE ctb	CHROMATID TYPE cte	CHROMOSOME TYPE csb	CHROMOSOME TYPE cse	OTHERS GT	TOTALS -g	TOTALS +g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:	A 100	2.0	0								0	0	
	B 100	2.0	0								0	0	
TOTAL	200										0	0	
	%	2.0	0	-							0.0	0.0	-
SOLVENT CONTROL:	A 100	2.0	0								0	0	
Sterile Deionized Water	B 100	2.0	0						1		1	1	
10.0 µl/ml TOTAL	200								1		1	1	
	%	2.0	0						0.5		0.5	0.5	
POSITIVE CONTROL:													
Mitomycin C 0.040 µg/ml	25	4.0	0		1	7	6	1			12	13	
	%	4.0	0	-	4	28	24	4			48	52	+
TEST ARTICLE: 350 µg/ml	A 100	3.0	0			1					1	1	
	B 100	3.0	0								0	0	
TOTAL	200					1					1	1	
	%	3.0	0	-		0.5					0.5	0.5	-
400 µg/ml	A 100	2.0	0								0	0	
	B 100	4.0	0				1				1	1	
TOTAL	200						1				1	1	
	%	3.0	0	-			0.5				0.5	0.5	-
450 µg/ml	A 100	2.0	0		1	2			1		3	4	
	B 100	3.0	0		1				1		1	2	
TOTAL	200				2	2			2		4	6	
	%	2.5	0	-	1.0	1.0			1.0		2.0	3.0	-
500 µg/ml	A 100	4.0	0			1					1	1	
	B 100	2.0	0								0	0	
TOTAL	200					1					1	1	
	%	3.0	0	-		0.5					0.5	0.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, $p < 0.01$.

TABLE 4

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 48.0 Hours After Initiation of Treatment

Assay No.: 15558 Trial #: 2 Lab #:3243 Metabolic Activation: Without

Compound: 4 Aminopyridine Date: March 24, 1993

		CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP g	CHROMATID TYPE ctb	cte	CHROMOSOME TYPE csb	cse	OTHERS GT	TOTALS -g	+g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:		A 100	2.0	0			1		1	1		3	3	
		B 100	1.0	0			1	1		1		3	3	
	TOTAL	200					2	1	1	2		6	6	
		%	1.5	0	-		1.0	0.5	0.5	1.0		3.0	3.0	-
SOLVENT CONTROL:		A 100	4.0	0						1		1	1	
Sterile Deionized Water		B 100	2.0	0				1		1		2	2	
10.0 µl/ml	TOTAL	200						1		2		3	3	
		%	3.0	0				0.5		1.0		1.5	1.5	
TEST ARTICLE:	350 µg/ml	A 100	3.0	0		1		1				1	2	
		B 100	2.0	0								0	0	
	TOTAL	200				1		1				1	2	
		%	2.5	0	-	0.5		0.5				0.5	1.0	-
	400 µg/ml	A 100	2.0	0				2				2	2	
		B 100	1.0	0		1	2			1		3	4	
	TOTAL	200				1	2	2		1		5	6	
		%	1.5	0	-	0.5	1.0	1.0		0.5		2.5	3.0	-
	450 µg/ml	A 100	3.0	1		1		2		1		3	4	
		B 100	1.0	0			3		2			5	5	
	TOTAL	200				1	3	2	2	1		8	9	
		%	2.0	0.5	-	0.5	1.5	1.0	1.0	0.5		4.0	4.5	-
	500 µg/ml	A 100	3.0	2		4		1				1	5	
		B 100	3.0	2		3		3		1		4	6	
	TOTAL	200				7		4		1		5	11	
		%	3.0	2	-	3.5		2.0		0.5		2.5	5.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, $p < 0.01$.

TABLE 5

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 24.1 Hours After Initiation of Treatment (6-Hour Treatment)

Assay No.: 15558 Trial #: 1 Lab #:3163 Metabolic Activation: With

Compound: 4 Aminopyridine Date: March 17, 1993

		CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP g	CHROMATID TYPE ctb	cte	CHROMOSOME TYPE csb	cse	OTHERS GT	TOTALS -g	+g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:		A 100	2.0	3								0	0	
		B 100	3.0	3								0	0	
	TOTAL	200										0	0	
		%	2.5	3	-							0.0	0.0	-
SOLVENT CONTROL:		A 100	3.0	2								0	0	
Sterile Deionized Water		B 100	2.0	0						1		1	1	
30.0 µl/ml	TOTAL	200								1		1	1	
		%	2.5	1						0.5		0.5	0.5	
POSITIVE CONTROL:														
Cyclophosphamide 1.00 µg/ml		25	4.0	2	-		4	5	1			6	6	
		%					16	20	4			24	24	+
TEST ARTICLE:	750 µg/ml	A 100	3.0	3								0	0	
		B 100	1.0	2						1		1	1	
	TOTAL	200								1		1	1	
		%	2.0	2.5	-					0.5		0.5	0.5	-
	1000 µg/ml	A 100	4.0	3		1						0	1	
		B 100	1.0	4					1			1	1	
	TOTAL	200				1			1			1	2	
		%	2.5	3.5	-	0.5			0.5			0.5	1.0	-
	1250 µg/ml	A 100	0.0	0								0	0	
		B 100	3.0	3		2	1	1				2	3	
	TOTAL	200				2	1	1				2	3	
		%	1.5	1.5	-	1.0	0.5	0.5				1.0	1.5	-
	1500 µg/ml	A 100	3.0	0				2	1			1	1	
		B 100	2.0	4								0	0	
	TOTAL	200						2	1			1	1	
		%	2.5	2	-			1.0	0.5			0.5	0.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, p <0.01.

TABLE 6

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 24.0 Hours After Initiation of Treatment (6-Hour Treatment)

Assay No.: 15558 Trial #: 2 Lab #:3223 Metabolic Activation: With

Compound: 4 Aminopyridine Date: March 23, 1993

		CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP g	CHROMATID TYPE ctb	CHROMATID TYPE cte	CHROMOSOME TYPE csb	CHROMOSOME TYPE cse	OTHERS GT	TOTALS -g	TOTALS +g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:	A	100	3.0	3						1		1	1	
	B	100	1.0	0				1		1		2	2	
	TOTAL	200						1		2		3	3	
		%	2.0	1.5	-			0.5		1.0		1.5	1.5	-
SOLVENT CONTROL: Sterile Deionized Water 15.0 µl/ml	A	100	2.0	2				1	1	1		3	3	
	B	100	4.0	3			1	1		1		2	2	
	TOTAL	200					1	2	1	2		5	5	
		%	3.0	2.5			0.5	1.0	0.5	1.0		2.5	2.5	
POSITIVE CONTROL: Cyclophosphamide 1.00 µg/ml		50	1.0	3		2	1	9	2	1		11	13	
		%	1.0	3	-	4	2	18	4	2		22	26	+
TEST ARTICLE: 750 µg/ml	A	100	2.0	2			1			1		2	2	
	B	100	5.0	5			1		1			2	2	
	TOTAL	200					2		1	1		4	4	
		%	3.5	3.5	-		1.0		0.5	0.5		2.0	2.0	-
1000 µg/ml	A	100	5.0	5		1				2		2	3	
	B	100	3.0	5				1		1		2	2	
	TOTAL	200				1		1		3		4	5	
		%	4.0	5	-	0.5		0.5		1.5		2.0	2.5	-
1250 µg/ml	A	100	3.0	8			1					1	1	
	B	100	2.0	6				1	1			2	2	
	TOTAL	200					1	1	1			3	3	
		%	2.5	7	-		0.5	0.5	0.5			1.5	1.5	-
1500 µg/ml	A	100	4.0	9		2	1	1	2			4	6	
	B	100	3.0	5				1				1	1	
	TOTAL	200				2	1	2	2			5	7	
		%	3.5	7	-	1.0	0.5	1.0	1.0			2.5	3.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, $p < 0.01$.

TABLE 7

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 48.25 Hours After Initiation of Treatment (6-Hour Treatment)

Assay No.: 15558 Trial #: 2 Lab #:3223 Metabolic Activation: With

Compound: 4 Aminopyridine Date: March 23, 1993

	CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS							
					GAP g	CHROMATID TYPE ctb	CHROMATID TYPE cte	CHROMOSOME TYPE csb	CHROMOSOME TYPE cse	OTHERS GT	TOTALS -g	TOTALS +g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:	A 100	2.0	0						1		1	1	
	B 100	2.0	0						1		1	1	
TOTAL	200								2		2	2	
	%	2.0	0	-					1.0		1.0	1.0	-
SOLVENT CONTROL:	A 100	3.0	0				1		2		3	3	
Sterile Deionized Water	B 100	2.0	0								0	0	
15.0 µl/ml TOTAL	200						1		2		3	3	
	%	2.5	0				0.5		1.0		1.5	1.5	
TEST ARTICLE: 750 µg/ml	A 100	2.0	0			1					1	1	
	B 100	2.0	0								0	0	
TOTAL	200					1					1	1	
	%	2.0	0	-		0.5					0.5	0.5	-
1000 µg/ml	A 100	2.0	0						1		1	1	
	B 100	2.0	0					1			1	1	
TOTAL	200							1	1		2	2	
	%	2.0	0	-				0.5	0.5		1.0	1.0	-
1250 µg/ml	A 100	1.0	0					1			1	1	
	B 100	2.0	1								0	0	
TOTAL	200							1			1	1	
	%	1.5	0.5	-				0.5			0.5	0.5	-
1500 µg/ml	A 100	1.0	0								0	0	
	B 100	4.0	0						1		1	1	
TOTAL	200								1		1	1	
	%	2.5	0	-					0.5		0.5	0.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, p <0.01.

TABLE 8

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Harvested 24.0 Hours After Initiation of Treatment (6-Hour Treatment)

Assay No.: 15558 Trial #: 2 Lab #:3223 Metabolic Activation: Without

Compound: 4 Aminopyridine Date: March 23, 1993

		CELLS SCORED	% POLY-PLOID	% ENDO REDUPLI-CATION	JUDGE-MENT (+/-)*	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS							
						GAP g	CHROMATID TYPE ctb	cte	CHROMOSOME TYPE csb	cse	OTHERS GT	TOTALS -g	+g	JUDGEMENT (+/-)*
NEGATIVE CONTROL:	A	100	1.0	0		1						0	1	
	B	100	3.0	2						1		1	1	
	TOTAL	200			-	1				1		1	2	
		%	2.0	1		0.5				0.5		0.5	1.0	-
SOLVENT CONTROL: Sterile Deionized Water 15.0 µl/ml	A	100	2.0	1						1		1	1	
	B	100	3.0	3							1	1	1	
	TOTAL	200								1	1	2	2	
		%	2.5	2						0.5	0.5	1.0	1.0	
TEST ARTICLE: 750 µg/ml	A	100	2.0	6		1	1		1			1	2	
	B	100	2.0	2						2		2	2	
	TOTAL	200				1	1		1	2		3	4	
		%	2.0	4	-	0.5	0.5		0.5	1.0		1.5	2.0	-
1000 µg/ml	A	100	3.0	6					1	1		2	2	
	B	100	2.0	8		1				2		2	3	
	TOTAL	200				1			1	3		4	5	
		%	2.5	7	-	0.5			0.5	1.5		2.0	2.5	-
1250 µg/ml	A	100	3.0	2		2				1		1	3	
	B	100	4.0	8				1	1	1		3	3	
	TOTAL	200				2		1	1	2		4	6	
		%	3.5	5	-	1.0		0.5	0.5	1.0		2.0	3.0	-
1500 µg/ml	A	100	2.0	3			1					1	1	
	B	100	2.0	1					1	1		2	2	
	TOTAL	200					1		1	1		3	3	
		%	2.0	2	-		0.5		0.5	0.5		1.5	1.5	-

ctb:chromatid break cte:chromatid exchange csb:chromosome break cse:chromosome exchange
others:fragmentation etc.
*Significantly greater than the solvent control, $p < 0.01$.

Study validity: This was a valid study; each criterion described by the sponsor was met.

Study outcome: 4-Aminopyridine was considered negative for inducing chromosomal aberrations in CHO cells under the conditions employed in this assay.

In vivo studies

6.) **Mutagenicity Test on 4-Aminopyridine in an *in vivo* mouse micronucleus assay**

Key findings:

- MTD established at 9.0 mg/kg (oral gavage).
- Single oral administration of 4-Aminopyridine at 2.25, 4.5, and 9.0 mg/kg did not induce a statistically significant dose-related increase in micronucleated PCEs. None of the individual doses resulted in a statistically significant increase in micronucleated PCEs at any of the three time points assessed (24, 48 or 72 hr). Single oral administration of cyclophosphamide resulted in a statistically significant increase in micronucleated PCEs in both sexes (2.22% ± 0.2%, males; 2.36% ± 0.16%, females). This increase was approximately 111-fold greater than the vehicle control.

Study no.: (b) (4)-15558-0-455co

Volume #, and page #: EDR, 4.2.3.3.2.1. Study (b) (4)-15558-0-455CO, pp. 1-40
Conducting laboratory and location: (b) (4)
Date of study initiation: 04 March 1993
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine (4-AP), C4886 Raw

Methods

Strains/species/cell line: ICR Mouse

Doses used in definitive study: 2.25, 4.5, and 9.0 mg/kg; oral gavage

Basis of dose selection: Dose range finding assay utilized oral gavage for administration of doses of 5, 16.25, 27.5, 38.75, and 50 mg/kg. 4-Aminopyridine was dissolved in sterile deionized water; the dosing volume was 10 ml/kg. At study initiation, mice weighed 27.9 to 31.8 g (males) and 24.6 to 30 g (females) and were approximately 9-weeks of age. Each dose group consisted of 3/sex. Each animal was administered a single dose of 4-aminopyridine. Animals were monitored after dosing and daily for toxic effects and mortalities. Animals were sacrificed after 24, 48, and 72 hrs.

During the dosing (all dose levels), animals experienced tremors. Animals in dose groups ≥16.25 mg/kg had convulsions. Immediately after dosing, all animals (males and

females) from the 27.5 mg/kg, 38.75 mg/kg, and 50 mg/kg groups were found dead; in the 16.25 mg/kg group, all females and 2 males were found dead after dosing, and the remaining male died 45 minutes after dosing. Mice dosed with 5 mg/kg 4-AP experienced tremors, but they appeared normal and remained healthy until the end of the observation period.

Due to the lack of a dose response, a second dose selection study was conducted using 3/sex/group to test 4-aminopyridine at 9 and 12 mg/kg. Mice were approximately 9 weeks of age and weighed 30.9 to 35.7 g (males) and 26.9 to 29 g (females). Immediately following dosing, all mice in both dose groups experienced tremors. After 30 minutes, two males and two females were found dead in the 12 mg/kg dose group. At 9 mg/kg, approximately two hours post-dose, animals appeared normal and were healthy until the end of the observation period. From the mortality data, it was concluded that the Maximum Tolerated Dose in mice was 9 mg/kg. This MTD was selected as the maximum dose for use in the micronucleus assay.

Negative controls: Vehicle control was sterile deionized water.

Positive controls: Cyclophosphamide (80 mg/kg; oral gavage)

Incubation and sampling times: Test article and vehicle control dosed mice were sacrificed 24, 48, and 72 hours after dosing. The positive control dosed group was sacrificed after 24 hr.

Results

Study validity: Yes.

Data are summarized by sex and dose groups for the different time points. The criteria for a positive response of the test article or positive control were a statistically significant dose-related increase in micronucleated PCEs or a reproducible and statistically significant positive response for at least one dose level. A negative response was considered when a test article induced neither a statistically significant dose response nor a statistically significant and reproducible increase at one dose level.

Study outcome: Approximately 45 min following dosing, all animals from the 2.25, 4.5 and 9.0 mg/kg dose groups experienced tremors. After 1.5 hr, the 9.0 mg/kg dose group were still experiencing tremors, whereas, animals in the lower dose groups (2.25 and 4.5 mg/kg) were behaving normally. After 5 hrs, all animals in the study were behaving normally and remained so until the end of the study.

Administration of 4-Aminopyridine at 2.25, 4.5, and 9.0 mg/kg did not induce a statistically significant dose-related increase in micronucleated PCEs. None of the individual doses resulted in a statistically significant increase in micronucleated PCEs at any of the three time points assessed (24, 48 or 72 hr).

Administration of cyclophosphamide resulted in a statistically significant increase in micronucleated PCEs in both sexes (2.22% ± 0.2%, males; 2.36% ± 0.16%, females). This increase was approximately 111-fold greater than the vehicle control.

The results of this assay are summarized in sponsor's Table 1 (reproduced below from p. 19 Study Report).

15558-0-455CO

TABLE 1

MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: Elan Corporation plc

TEST ARTICLE: 4 Aminopyridine (4-AP)

ASSAY: 15558

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E.			RATIO PCE:NCE MEAN ± S.E.	
			MALES	FEMALES	TOTAL	MALES	FEMALES
VEHICLE CONTROL STERILE DEIONIZED WATER	10 ml/kg	24	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.44 ± 0.12	0.66 ± 0.11
		48	0.06 ± 0.02	0.02 ± 0.02	0.04 ± 0.02	0.45 ± 0.08	0.70 ± 0.06
		72	0.08 ± 0.04	0.10 ± 0.05	0.09 ± 0.03	0.44 ± 0.12	0.67 ± 0.11
POSITIVE CONTROL CYCLOPHOSPHAMIDE	80 mg/kg	24	2.22 ± 0.20*	2.36 ± 0.16*	2.29 ± 0.13*	0.45 ± 0.08	0.49 ± 0.10
TEST ARTICLE	2.25 mg/kg	24	0.06 ± 0.02	0.04 ± 0.04	0.05 ± 0.02	0.41 ± 0.11	0.54 ± 0.05
		48	0.10 ± 0.00	0.14 ± 0.07	0.12 ± 0.03	0.58 ± 0.20	0.61 ± 0.11
		72	0.08 ± 0.04	0.02 ± 0.02	0.05 ± 0.02	0.34 ± 0.04	0.52 ± 0.15
	4.50 mg/kg	24	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.53 ± 0.17	0.53 ± 0.11
		48	0.02 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.47 ± 0.13	0.74 ± 0.12
		72	0.08 ± 0.04	0.02 ± 0.02	0.05 ± 0.02	0.31 ± 0.08	0.56 ± 0.09
	9.0 mg/kg	24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.51 ± 0.09	0.55 ± 0.14
		48	0.06 ± 0.04	0.14 ± 0.04	0.10 ± 0.03	0.38 ± 0.10	0.82 ± 0.09
		72	0.02 ± 0.02	0.12 ± 0.06	0.07 ± 0.03	0.57 ± 0.14	0.51 ± 0.11

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* Significantly greater than the corresponding vehicle control, $p<0.05$.

7.) **Mammalian Erythrocyte Micronucleus Test**

Key findings:

- MTD established at approximately 15 mg/kg (oral, gavage) in both males and females based on mortality at 20 mg/kg in both males (3/5) and females (1/5).
- Single oral administration of 4-Aminopyridine in rats did not induce a statistically significant dose-related or reproducible increase in the percent of multinucleated PCEs at any dose tested following 24 and 48 hr of exposure.
- 24 hr cyclophosphamide treatment in rats induced a statistically significant increase in the percent of multinucleated PCEs that was ≈ 60-fold greater than the negative vehicle control.

Study no.: (b) (4)-1139-006

Volume # and page #: EDR, 4.2.3.3.2.1., (b) (4)-1139-006 Study Report, pp. 1-57
Conducting laboratory and location: (b) (4)
Date of study initiation: 11 August 2006
GLP compliance: Yes
QA reports: yes (X)
Drug, lot #, and % purity: 4-Aminopyridine, Lot No. P175-4-1, Purity 100.5%

Methods

Strains/species/cell line: Sprague Dawley Rat

Doses used in definitive study: 4-Aminopyridine doses of 3.75, 7.5 and 15.0 mg/kg; doses were diluted in a distilled water vehicle and a dose volume of 10 ml/kg was utilized for single oral gavage administration.

Basis of dose selection: Dose selection was based on an oral dose range-finding toxicity study conducted in rats at 10, 15, or 20 mg/kg 4-aminopyridine. All rats showed ataxia and lethargy following dosing. Rats receiving either 15 or 20 mg/kg had tremors. Deaths occurred only in the HD group of both sexes. Thus, an approximate MTD was set at 15 mg/kg that was utilized as the high dose in the definitive study.

Negative controls: Distilled water was used as the vehicle control in this study.

Positive controls: A single dose of cyclophosphamide (40 mg/kg) was administered by oral gavage.

Incubation and sampling times: The following table provided by the sponsor describes the study design for the definitive micronucleus assay.

Treatment (10 mL/kg)	Number of Rats/Sex Dosed	Number of Rats/Sex Used for Bone Marrow Collection After Dose Administration	
		24 hr	48 hr
Vehicle Control: Distilled water	10	5	5
Test Article: 4-AP			
Low dose (3.75 mg/kg)	5	5	0
Mid dose (7.5 mg/kg)	5	5	0
High dose (15 mg/kg)	15*	5	5
Positive Control: CP (40 mg/kg)	5	5	0

*Including 5 replacement rats/sex to ensure the availability of five rats for micronucleus analysis.

Evaluation

The incidence of micronucleated polychromatic erythrocytes was determined per 2000 PCEs for each rat and per 10,000 PCEs for each treatment group. Bone marrow toxicity was evaluated by the proportion of PCEs to total ECs for each rat and treatment group.

The sponsor's criteria for determining if the test article elicited a positive or negative response in the micronucleus assay were the following:

- A positive response requires that a dose-responsive increase in micronucleated PCEs be observed and one or more doses be statistically elevated relative to the vehicle control ($p \leq 0.05$ accepted as statistically significant).
    - However, it is noted that statistically significant values that did not exceed the range of historical negative or vehicle controls (Appendix I in the original study report) would have been judged as not biologically significant.
- A negative response requires that no statistically significant increase in micronucleated PCEs above the concurrent vehicle control and no evidence of dose response be observed at any sampling time.

A valid test was established when the number of micronucleated PCEs in the vehicle control groups did not exceed the historical control range. The incidence of micronucleated PCEs in the positive control group must also be significantly increased relative to the vehicle control group ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Results

In the definitive micronucleus assay, there were no premature deaths. All rats treated with 4-aminopyridine HD (15 mg/kg) showed clinical signs of lethargy and tremors. Rats treated with 3.75 and 7.5 mg/kg appeared normal following dosing and throughout the observation period. The incidence of micronucleated PCEs per 10,000 scored (2000 PCEs/rat) and the proportion of PCEs per total erythrocytes for each treatment group by sacrifice time are presented in sponsor's Table 8.0-4 (see below, from p. 23 of the study report).

Table 8.0-4: Summary of Bone Marrow Micronucleus Analysis Following a Single Oral Dose of 4-AP in Sprague Dawley Rats

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Difference from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Distilled water	M	24	5	0.541 ± 0.05	---	0.2 ± 0.27	2 / 10000
	F	24	5	0.547 ± 0.07	—	0.2 ± 0.27	2 / 10000
4-AP							
3.75 mg/kg	M	24	5	0.514 ± 0.04	-5	0.1 ± 0.22	1 / 10000
	F	24	5	0.520 ± 0.04	-5	0.0 ± 0.00	0 / 10000
7.5 mg/kg	M	24	5	0.564 ± 0.05	4	0.2 ± 0.27	2 / 10000
	F	24	5	0.501 ± 0.04	-8	0.1 ± 0.22	1 / 10000
15 mg/kg	M	24	5	0.508 ± 0.06	-6	0.1 ± 0.22	1 / 10000
	F	24	5	0.545 ± 0.05	0	0.4 ± 0.22	4 / 10000
Cyclophosphamide							
40 mg/kg	M	24	5	0.416 ± 0.01	-23	12.9 ± 2.30	*129 / 10000
	F	24	5	0.386 ± 0.04	-29	11.7 ± 3.09	*117 / 10000
Distilled water	M	48	5	0.542 ± 0.04	---	0.0 ± 0.00	0 / 10000
	F	48	5	0.536 ± 0.05	—	0.0 ± 0.00	0 / 10000
4-AP							
15 mg/kg	M	48	5	0.504 ± 0.01	-7	0.2 ± 0.27	2 / 10000
	F	48	5	0.491 ± 0.03	-8	0.2 ± 0.45	2 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables).

Study validity: The number of micronucleated PCEs evaluated was acceptable and the criteria for a negative and positive result were clear.

Study outcome: None of the doses of 4-Aminopyridine tested resulted in a dose-responsive increase in micronuclei. In contrast, single oral administration of cyclophosphamide induced a statistically significant increase in the number of micronucleated polychromatic erythrocytes in rat bone marrow. Therefore, under the conditions of the present assay 4-aminopyridine was found to be negative in the micronucleus test using male and female Sprague Dawley rats.

2.6.6.5 Carcinogenicity

Sponsor conducted two lifetime carcinogenicity bioassays in two rodent species—mouse and rat, each of which is reviewed below. The meeting minutes from the Executive CAC meeting held August 11, 2009 have been appended.

1.) **Study title**: **Two Year Dietary Carcinogenicity Study in Mice**

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The mouse carcinogenicity bioassay was an adequate study. Doses of 4-AP selected produced clear toxicities including decreased body weight, reduced food consumption, and reduced survival of HDM and HDF compared to Controls. HDF were euthanized prematurely during Week 100 due to this reduced survival rate; however, this sacrifice did not compromise the outcome of this bioassay.

Evaluation of tumor findings: No test article-related tumors were identified.

Study no.: 684-022
Volume #, and page #: eCTD 4.2.3.4.1.1, pp. 1-1994
Conducting laboratory and location: (b) (4)

Date of study initiation: 22 December 1994
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine (Fampridine Raw Material), Lot # C08078, 100% assumed

CAC concurrence: Not requested

Methods

Doses: 0, 0, 2.0, 12.5, and 80 mg/kg/day
Controls 1 and 2 are same, untreated Control diet.
Basis of dose selection: MTD, dose-related reduction in survival as determined in study 684-021 that evaluated dietary administration of 4-aminopyridine at doses of 2.4, 12, 60/80/120 mg/kg for 13 weeks. At week 6, the high dose (60 mg/kg) was increased to 80 mg/kg without any serious adverse events. At week 9, the high dose was increased to 120 mg/kg; however, several HDF died within 2-16 days of the increase.
Species/strain: Crl:CD-1® (ICR) BR VAF/PLUS™ mice
Number/sex/group (main study): 60/sex/Control 1; 70/sex/Control 2, LD, MD, HD
TK was conducted on the first 10 surviving mice/sex from Control 2, LD, MD, and HD groups were used for plasma analysis at 12 months and at termination.

Route, formulation, volume: Dietary admixture
Frequency of dosing: Daily
Age: Approximately 4 wks.
Animal housing: Mice were housed individually.
Restriction paradigm for dietary restriction studies: Access to food and water was *ad libitum*.
Drug stability/homogeneity: Dosage formulations were shown to be homogenous and accurately prepared ranging within 83-98% of nominal
Dual controls employed: Yes.
Interim sacrifices: none
Deviations from original study protocol: All HDF were sacrificed prematurely during study week 100 due to poor survival.

Observations and Results

Mortality: Mice were checked twice daily for morbidity and mortality.

- Reduced survival was found in HDM and HDF (see table below).
  - Due to reduced survival, the HDF group was euthanized at week 100.
- Survival rates between Controls 1 and 2 and treated groups—LDM, LDF, MDM, and MDF were similar throughout most of the study (see Kaplan-Meier plots as reproduced from the sponsor in Figures 1A (males) and 1B (females), below).

The following table summarizes the survival rates of mice administered 4-aminopyridine in a 2-year carcinogenicity bioassay as analyzed by the sponsor.

Treatment Group	Survival Analyses			
	Males		Females	
	Rate	%	Rate	%
Control 1	26/60	43	27/60	45
Control 2	29/60	48	24/60	40
LD	34/60	57	18/60	30
MD	24/60	40	24/60	40
HD	16/60	27	14*/60	23*

*HD females sacrificed prematurely during week 100 due to reduced survival.

The following survival curves were provided by the sponsor for male (Figure 1A) and female (Figure 1B) mice.

Figure 1A: Males

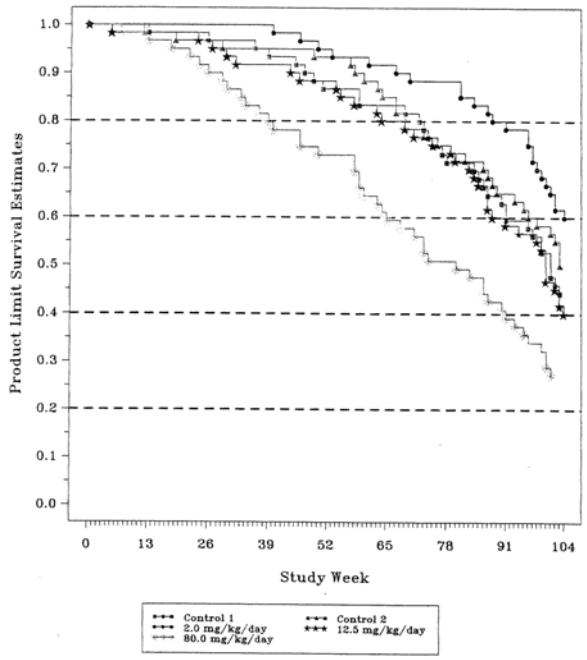
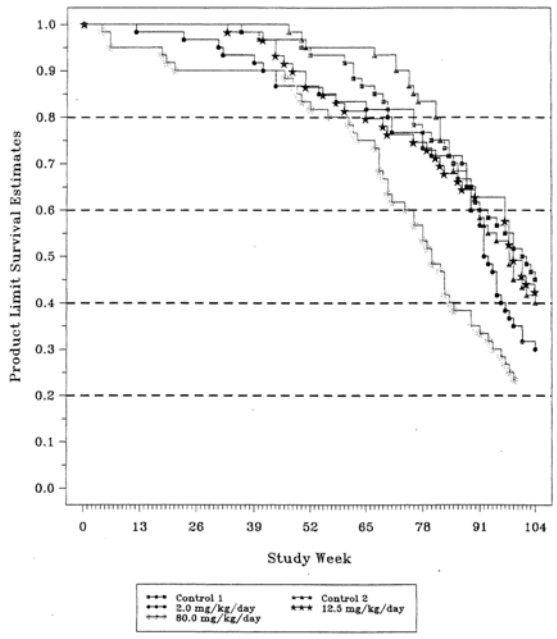


Figure 1B: Females



Clinical signs: Mice were observed for overt toxicity twice daily at the time of the morbidity/mortality checks. Weekly, detailed observations of appearance, condition, behavior and activity, excretory functions, respirations, orifices and eyes as well as the occurrence, size, location, and description of palpable masses were performed.

- The clinical signs noted in this study were considered by the Sponsor to be commonly seen in laboratory mice and thus incidental. The sponsor did not consider the convulsions noted in the following table drug related.
    - A notable exception was an increased incidence of convulsions that occurred in HDM and HDF, commencing in males during the interval of 27-39 weeks and in females during weeks 40-52. Convulsions continued to be observed throughout the study until study termination at week 100 (HDF) or week 104 (HDM).

The following table summarizes the incidence of convulsion and the mean number of wks convulsion was noted per mouse. Data were adapted from sponsor's Table 2.

	MALES				**FEMALES**			
	C	**LD**	**MD**	**HD**	**C**	**LD**	**MD**	**HD**
#Affected/Total	21/130	3/70	9/70	19/70	5/130	0/70	1/70	8/70
% Affected	16.2	4.3	12.8	27.1	3.8	0	1.4	11.4
Mean Weeks of Convulsion/Mouse	9.1	4	7.2	8.8	3.4	0	7	8

- The incidences of palpable masses were similar among all dose groups in both sexes compared to Controls.

Body weights: Individual body weights were recorded pretest, then weekly thereafter until study termination.

- HDM and HDF had decreased mean body weight compared to their respective Controls, which occurred in the beginning and throughout the study period.
    - Statistically significant body weight decreases occurred throughout the study and were approximately 10% less than Control body weights. These data are summarized in sponsor's Table 5.2.3 reproduced below, which show the mean body weight results observed at study termination.

Table 5.2.3.
Summary of Mean Body Weights in Grams

Dosage Level (mg/kg/day)	Male	A	B	Female	A	B
Control 1	39	NA	(+2.6)	36	NA	(-2.7)
Control 2	38	(-2.6)	NA	37	(+2.8)	NA
2	38	(-2.6)	(0.0)	38	(+5.6)	(+2.7)
12.5	39	(0.0)	(+2.6)	36	(0.0)	(-2.7)
80	34[2]	(-12.8)	(-10.5)	32[1,4,*]	(-11.1)	(-13.5)

A - % Difference from Control 1 B - % Difference from Control 2
[1]Significantly different from Control 1; p≤0.05 [2]Significantly different from Control 1; p≤0.01
[4]Significantly different from Control 2; p≤0.01 *Study Week 98 NA - Not Applicable

- Weights of animals in the LD and MD groups (both sexes) were similar to their respective Controls throughout the study.

Food consumption: Individual food consumption was measured daily and reported on a weekly basis for the first 14 weeks, then every fourth week thereafter. Compound consumption was calculated for the same intervals.

- Mean food consumption in HDF was slightly lower than Control.
    - At study termination, mean food consumption (g/animal/day) was significantly reduced compared to Control.
    - Compound consumption (mg/kg/day) measured at Week 98 was slightly less than the targeted 80 mg/kg/day dosage (-2.0%).
- Mean food consumption was similar in LDF, MDF, and all male dose groups throughout the study.

At study termination, the mean food and compound consumption data were summarized by the sponsor in Table 5.2.4, which is reproduced below.

Table 5.2.4.
Average Food and Compound Consumption

Dosage Level (mg/kg/day)	Food Consumption (g/animal/day)						Compound Consumption (mg/kg/day)	
	Male	A	B	Female	A	B	Male	Female
Control 1	5.2	NA	(-1.9)	5.5	NA	(-1.9)	NA	NA
Control 2	5.2	(+1.9)	NA	5.6	(+1.9)	NA	NA	NA
2	5.2	(0.0)	(-1.9)	5.4	(+1.9)	(0.0)	2.1	2.1
12.5	5.3	(+1.9)	(0.0)	5.6	(+3.7)	(+1.9)	12.8	12.3
80	5.3	(0.0)	(-1.9)	5.0* [2,4]	(-3.7)	(-5.5)	80.2	78.4*

A - % Difference from Control 1
B - % Difference from Control 2
[2]Significantly different from Control 1; p≤0.01
[4]Significantly different from Control 2; p≤0.01
*Study Week 98 NA - Not Applicable

Organ weight: Organs and tissues listed in the sponsor table below (see histopathology section) demarcated (*) were trimmed free of fat and connective tissue and weighed. Absolute organ weights and terminal body weights were measured at study termination. Relative organ weights were calculated as ratios to total body weight and to brain weight.

- No treatment-related body or organ weight differences were noted in the LD or MD groups of either sex.
- HDM (-10.5% and -8.1%) and HDF (-13.9%) each showed a treatment-related decrease in group mean body weight compared to Control.
    - Statistically significant increases in relative brain weight were reported in HDM and HDF, liver to body weight ratios in HDM, and kidney absolute and relative to brain weight in HDF were found.
    - A non-significant increase in the ratio of heart to body weight in HDM (+11.9% vs. C1) was found.
        - The sponsor attributed these significant differences as secondary to the lower body weight of the group, as no treatment-related morphologic changes were found in these tissues.

Gross pathology: Selected findings of animals that died on study are summarized in the following table that presents incidence (#affected/died) and severity of the finding (#findings @ severity code) in which 1= trace, 2= mild, 3= moderate, and 4= severe. Controls are pooled.

- In the animals that survived to terminal sacrifice, there were no notable macroscopic observations that were dose-related and/or elevated in the HD group of either sex.

TISSUE	FINDING	MALES C	MALES LD	MALES MD	MALES HD	FEMALES C	FEMALES LD	FEMALES MD	FEMALES HD
Kidney	Mottled, mild	0/65	0/27	0/36	0/44	0/70	0/42	0/36	1/46 1@2
Liver	Focus, Foci, tan	2/65 1@2 1@4	0/27	02/36 2@1	2/44 1@1 1@2	3/70 2@1 1@3	1/42 1@2	1/36 1@2	0/46
Lung	Discoloration, red	8/65 6@2 2@3	4/27 3@2 1@3	6/36 6@2	11/4 4 1@1 6@2 4@3	13/70 1@1 11@2 1@3	5/42 1@1 2@2 1@3 1@4	4/36 3@2 1@3	10/46 1@1 7@2 2@3
LN, mandibular	Enlarged	1/65 1@3	0/27	3/36 2@2 1@4	5/44 3@2 2@3	6/70 1@1 4@2 1@3	2/42 1@3 1@4	7/36 1@1 1@2 4@3 1@4	3/46 1@1 2@2
Ovary	Discoloration, black					0/70	0/42	1/36 1@2	1/46 1@2
Skin	Abrasion	4/65 2@2 2@3	2/27 1@2 1@3	5/36 1@2 2@3 2@4	8/44 1@2 3@3 4@4	7/70 3@2 3@3 1@4	1/42 1@3	1/36 1@3	4/46 4@4
	Alopecia	7/65 3@1 4@2	3/27 1@1 1@2 1@3	5/36 2@1 2@2 1@3	5/44 2@1 3@2	7/70 3@1 3@2 1@3	5/42 2@1 2@2 1@3	6/36 1@1 5@2	11/46 4@1 7@2
	Scab	2/65 1@1 1@2	0/27	4/36 2@1 2@3	2/44 2@4	2/70 1@1 1@3	4/42 3@2 1@3	0/36	4/46 3@2 1@3
Stomach, glandular	Focus, red	0/34	0/27	1/36 1@1	6/44 3@1 3@2	5/70 2@1 3@2	3/42 1@2 2@2	1/36 1@2	1/46 1@1

Histopathology: Organs and tissues listed in the sponsor's Table provided below were processed and prepared for microscopic examination using hematoxylin-eosin stained paraffin sections. A full battery of organs and tissues was prepared and examined for Control 1 (it is noted that the sponsor did not provide an explanation why Control 2 was not also examined) and HD, and all mice in all groups dying or euthanized *in extremis* during the course of the study (excluding mice from the 52-Week TK study). Liver was also microscopically examined for males in Control 2, LD and MD groups. All gross lesions and tissue masses were prepared and microscopically examined.

- The slides were reviewed by a veterinary pathologist. Gradable lesions were defined using a four-step grading system of trace, mild, moderate, and severe for comparison between groups.

  Peer review: yes ( ), no (X).

  The list of organs/tissues examined was the following:

  adrenal (2), aorta, bone with bone marrow (femur and sternum), bone marrow smear, brain (fore, mid, and hind), eye including optic nerve with contiguous Harderian gland (2), gallbladder, gastrointestinal tract (esophagus, stomach

(glandular and nonglandular), duodenum, jejunum, ileum, cecum, colon, and rectum), gonads (ovary (2) or testis with epididymis (2).

Non-neoplastic: The following table summarizes selected microscopic findings from animals that died on study and reports the incidence (#affected/died) and severity of each finding (#findings @ severity code) in which, 1= trace, 2= mild, 3= moderate, and 4= severe. Controls are pooled.

		MALES				FEMALES			
Tissue	**Finding**	**C**	**LD**	**MD**	**HD**	**C**	**LD**	**MD**	**HD**
Ear	Dermatitis	10/65 2@1 4@2 4@3	2/27 2@2	4/31 1@1 3@2	6/44 1@1 3@2 2@3	6/70 2@1 3@2 1@3	5/42 1@1 2@2 2@3	4/36 4@2	10/46 2@1 6@2 2@3
	Dermatitis, ulcerative	1/65 1@2	0/27	1/31 1@3	2/44 2@3	0/70	0/42	0/36	1/46 1@2
Lung	Abscess	2/65 1@2 1@3	1/27 1@2	2/31 1@2 1@4	1/44 1@4	2/70 1@1 1@3	1/42 1@3	1/36 1@3	1/46 1@3
Skin	Bacterial colonies	0/65	0/27	1/31 1@4	2/44 1@2 1@4	2/70 2@2	0/42	0/36	0/46
	Dermatitis	11/65 2@1 4@2 3@3 2@4	3/27 2@3 1@4	7/31 1@2 3@3 3@4	11/44 2@2 2@3 7@4	8/70 1@1 2@2 2@3 3@4	5/42 1@2 2@3 2@4	0/36	8/46 2@1 2@3 4@4
	Exudate, epidermal surface	0/65	0/27	1/31 1@4	2/44 2@3	2/70 2@2	0/42	0/36	0/46
Stomach, glandular	Necrosis	3/65 1@1 2@2	3/27 3@2	1/31 1@2	3/44 3@2	1/70 1@1	5/42 1@1 4@2	1/36 1@1	1/46 1@2
	Necrosis, coagulative	0/65	0/27	0/31	2/44 1@1 1@2	0/70	1/42 1@2	0/36	0/46

From the above table, an increase in the incidence and severity of skin inflammation is noted. In the following table, data from both sexes were combined that illustrate a possible relationship between 4-aminopyridine treatment and skin inflammation.

Skin inflammation and possible related findings

		Males + Females			
		C	**LD**	**MD**	**HD**
Ear	Dermatitis	16/135 4@1 7@2 5@3	7/69 1@1 4@2 2@3	8/67 1@1 7@2	16/90 3@1 9@2 4@3
	Dermatitis, ulcerative	1/135 1@2	0/69	1/67 1@3	3/90 1@2 2@3
Skin	Dermatitis	19/135 3@1 6@2 5@3 5@4	8/69 1@2 4@3 3@4	7/67 1@2 3@3 3@4	19/90 2@1 2@2 4@3 11@4
	Exudate, epidermal surface	2/135	0/69	1/67 1@4	2/90 1@1 1@2
	Bacterial colonies	2/135 2@2	0/69	1/67 1@4	2/90 1@2 1@4

Neoplastic: The following sponsor summary of neoplastic lesions (see Table 12, below). No statistically significant treatment-related findings were observed in either males or females. Sponsor's summary table of neoplastic lesions (Table 12) follows below.

Table 12 **Summary of Neoplastic Lesions - Males**
0 to Termination

Organ Lesion	Type	Peto Trend Test Probability	0 mg/kg/day	2 mg/kg/day	12.5 mg/kg/day	80 mg/kg/day
Harderian Gland						
Adenoma	I	0.9808	5			0
Lung						
Adenoma, alveolar bronchiolar	I	0.5865	10			6
Carcinoma, alveolar bronchiolar	M	0.9516	4			0
Testis						
Interstitial cell tumor, benign	I	0.8111	1			0
Thyroid Gland						
Adenoma, follicular	I	0.7836	1			0
Liver (Control 1)						
Adenoma, hepatocellular	I	0.8676	8	9	7	2
Carcinoma, hepatocellular	M	0.0470	0	2	3	2
Liver (Control 2)						
Adenoma, hepatocellular	I	0.9564	11	9	7	2
Carcinoma, hepatocellular	M	0.0482	0	2	3	2

684-022

I - Incidental
F - Fatal
M - Mixed

Table 12 Cont.

Summary of Neoplastic Lesions - Females
0 to Termination

Organ Lesion	Type	Peto Trend Test Probability	0 mg/kg/day	80 mg/kg/day
Adrenal Gland, Medulla				
Pheochromocytoma, benign	I	1.0000	1	0
Harderian Gland				
Adenoma	I	0.9757	5	0
Liver (Control 1)				
Adenoma, hepatocellular	I	1.0000	1	0
Lung				
Adenoma, alveolar bronchiolar	I	0.9187	16	7
Carcinoma, alveolar bronchiolar	M	0.3523	5	3
Mesothelioma, benign	I	1.0000	1	0
Mammary Gland				
Adenoma	I	0.2216	0	1
Ovary				
Adenoma	I	0.2635	3	1
Pituitary Gland				
Adenoma	M	0.7708	4	0
Thyroid Gland				
Adenoma, follicular	I	1.0000	1	0
Uterus				
Leiomyoma	I	0.3955	3	2
Polyp	I	0.9654	5	0
Uterus, Cervix				
Leiomyoma	I	0.1088	0	3
Polyp	I	1.0000	1	0

I - Incidental
F - Fatal
M - Mixed

Toxicokinetics: During study week 52, blood samples were obtained by cardiac puncture immediately following the end of the dark cycle (between 7:00 and 8:00 AM) from the first 10 surviving mice/sex/group (except Control 1) which were anesthetized with CO_2.

- Plasma concentrations of 4-aminopyridine were approximately dose proportional for LD, MD and HD groups.

Plasma 4-aminopyridine concentrations collected at study weeks 52, 100 and 104 are summarized below from sponsor's Table 5.2.5.3.

	Plasma 4-Aminopyridine (ng/ml)[a]							
	Control		**LD**		**MD**		**HD**	
Week	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
52	0 [b]	0[b]	3.89 ± 6.46	12.0 ± 19.0	40.4 ± 30.3	40.7 ± 30.1	239 ±121	404 ± 338
100	NA	NA	NA	NA	NA	NA	NA	243±92.9
104	0 [b]	2.00 ± 6.32	8.59 ± 4.51	13.2 ± 7.11	67.0 ± 28.0	84.3 ± 61.6	365 ± 209	NA

[a]Group mean ± SD
[b]Test article not detected (≤3.60 ng/ml)

2.) **Study title**: **Two Year Dietary Carcinogenicity Study in Rats**

Adequacy of the carcinogenicity study and appropriateness of the test model: Throughout this study, mean body weight of MD and HD groups (both sexes) was decreased (>10%) compared to respective Controls. At terminal sacrifice, mean body weight of HDM and of HDF was decreased significantly compared to Controls. Thus, prior to the NDA submission, the Agency requested that all dose groups (rather than only Control and HD) be evaluated for tumors. As requested, Acorda Therapeutics amended their original study report to include the additional LD and MD analyses. Toxicokinetic analyses conducted at weeks 26, 52, and 104 confirmed that plasma 4-AP concentrations were approximately dose proportional among the dose groups in both sexes. For these reasons, the carcinogenicity bioassay was considered an adequate study.

Evaluation of tumor findings: Both the sponsor and the reviewing statistician identified a statistically significant increase in the incidence of benign uterine polyp of the HD group. A peer review of the initial findings was confirmed; however, additional analyses performed failed to find any supporting microscopic evidence of preneoplastic correlates in the endometrium.

Study no.: 684-023
Volume #, and page #: EDR 4.2.3.4.1. Rat Amendment
Conducting laboratory and location: (b) (4)

Date of study initiation: 14 May 1994

GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, Lot # CAS#504-24-5 (09 May 1994) and C06284 (31 May 1995), purity assumed 100%
CAC concurrence: not requested

Methods

Doses: 0, 2.0, 6.0, and 18.0 mg/kg
Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: Rat, Charles River Crl:CD® BR (VAF/Plus)
Number/sex/group (main study): 70/sex/Grp Control 1, LD, MD, HD;
60/sex/Grp Control 2
Route, formulation, volume: Dietary admixture
Frequency of dosing: Daily
Satellite groups used for toxicokinetics or special groups: First 10 rats/sex/group.
Age: Approximately 4 wks; males 162-201g; females 141-171g
Animal housing: At study initiation, all animals were individually housed in stainless steel cages with wire-mesh floors. In early April 1995, some of the HD males had an early occurrence of decubital ulcers and thus the animals were changed to shoe box cages with hard wood bedding. On April 14, 1995 all males were housed individually in shoe box cages with hard wood bedding (week 43). Females were kept in the wire-mesh floor cages because foot ulcerations developed later than those observed in males.
Restriction paradigm for dietary restriction studies: N/A
Drug stability/homogeneity: Dosage formulations were shown to be homogenous and stable. Three separate analyses found the dose groups to be within acceptable ranges of the targeted concentrations—LD (91-114%), MD (94-99%), and HD (101-105%).
Dual controls employed: Yes
Interim sacrifices: None
Deviations from original study protocol: None.

Observation and Results

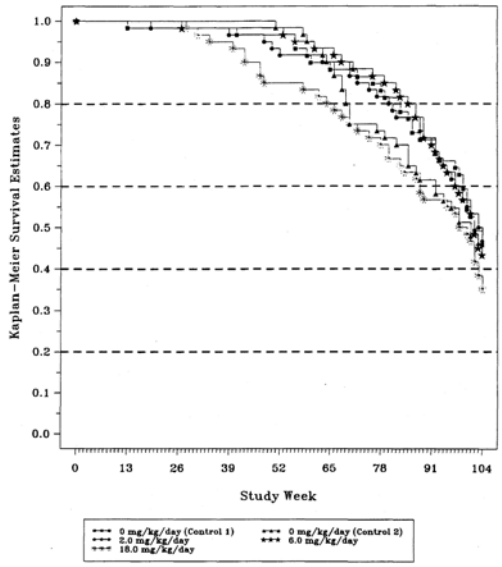
Mortality: Rats were checked twice daily for morbidity and mortality.
Survival data for each of the Control and 4-aminopyridine groups are shown in the following table and plotted by sponsor as shown in Sponsor's Figure 1. Decreased survival in HDM occurred during the end of the first year of the study and persisted to the end of the study. This decrease was not statistically significant.

Treatment Group	Survival Analyses			
	Males		Females	
	Rate	%	Rate	%
Control 1	27	45	25	42
Control 2	27	45	20	33
LD	28	47	25	42
MD	26	43	27	45
HD	21	35	28	47

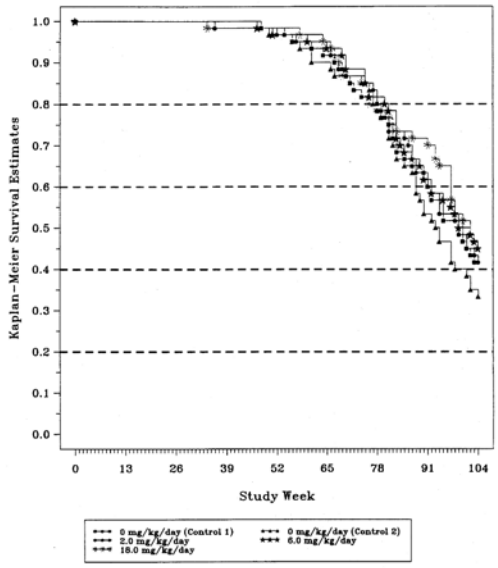
The Kaplan-Meier survival estimates were plotted in sponsor's Figure 1 for males and females (as reproduced below).

Figure 1. Survival Curves

Males



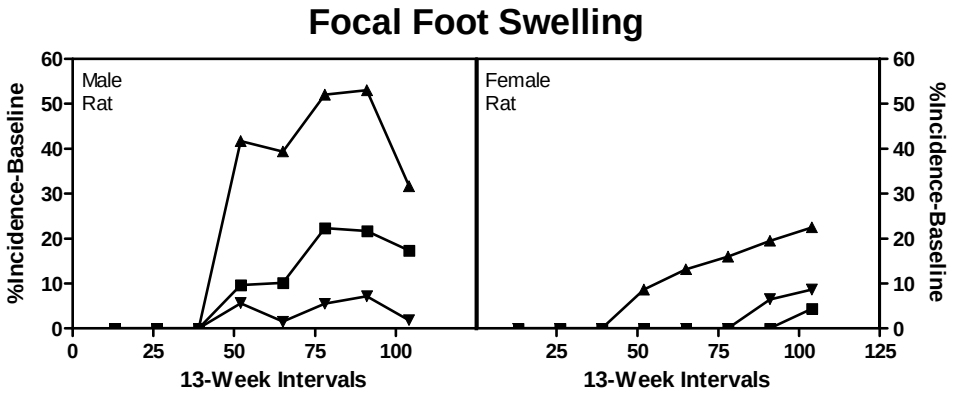
Females

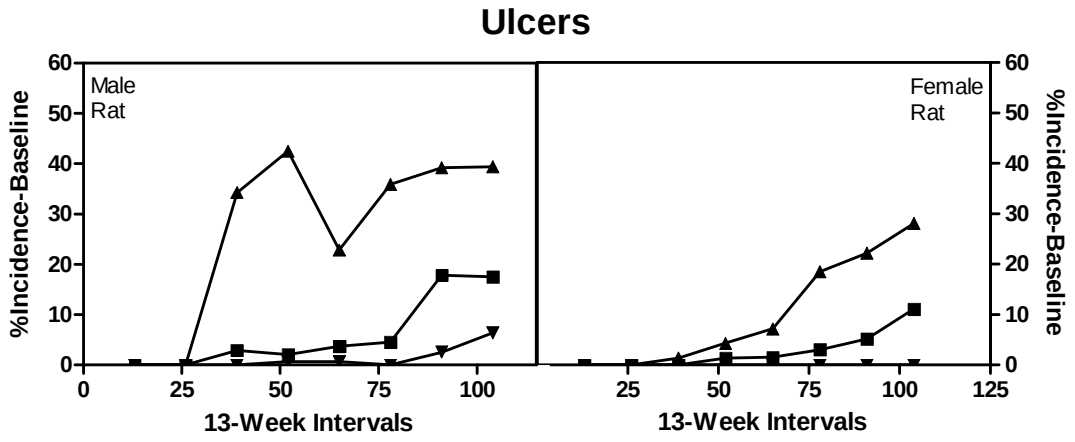


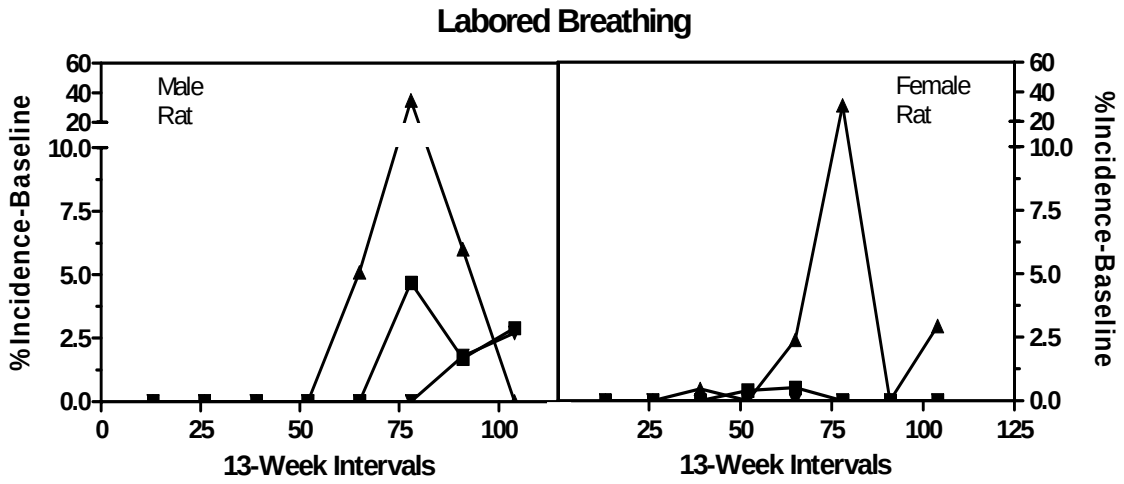
Clinical signs: Rats were observed for overt toxicity twice daily at the time of the morbidity/mortality checks. Weekly, detailed observations of appearance, condition, behavior and activity, excretory functions, respirations, orifices and eyes as well as the occurrence, size, location, and description of palpable masses were performed.

Incidence of clinical signs observed during 12-week intervals throughout the study.

	Wk Interval	Pooled Control 1+2		LD		MD		HD	
		M	F	M	F	M	F	M	F
Foot, Ulceration	14-26	0/129	0/130	0/70	0/70	0/70	0/70	3/70	0/70
	27- 39	0/129	0/130	0/69	0/70	2/69	0/70	24/70	1/70
	40-52	1/128	0/130	1/68	0/69	2/69	1/70	29/67	3/69
	53-65	1/116	0/128	1/65	0/68	3/67	1/67	14/59	5/69
	66-78	2/116	0/119	1/63	0/64	4/64	2/64	21/56	12/65
	79-91	3/103	2/104	3/56	0/54	12/58	4/57	21/50	13/54
	92-104	4/87	4/75	5/46	2/39	10/45	7/43	20/41	16/48
	Total Grp Incidence	**11**	**6**	**11**	**2**	**33**	**15**	**132**	**50**
	# Animals affected	**4**	**5**	**7**	**1**	**17**	**4**	**36**	**20**
Foot, Focal Swelling	14-26	0/129	0/130	0/70	0/70	0/70	0/70	0/70	0/70
	27- 39	0/129	0/130	0/69	0/70	0/69	0/70	0/70	1/70
	40-52	4/128	4/130	6/68	0/69	9/69	1/70	30/67	5/69
	53-65	6/126	13/128	4/65	2/68	10/67	2/67	26/59	16/69
	66-78	12/116	23/119	10/63	8/64	21/64	12/64	35/56	23/65
	79-91	26/103	33/104	18/56	21/54	27/58	18/57	39/50	28/54
	92-104	46/87	33/75	27/46	19/39	32/45	20/43	35/41	30/48
	Total Grp Incidence	**94**	**96**	**65**	**50**	**99**	**53**	**165**	**103**
	# Animals affected	**8**	**14**	**9**	**5**	**25**	**13**	**41**	**28**







4-AP
LD 2 mg/kg
MD 6 mg/kg
HD 18 mg/kg
Baseline %Incidence
(Controls I&II)

Observation	Wk Interval	Pooled Control 1+2 M	Pooled Control 1+2 F	LD M	LD F	MD M	MD F	HD M	HD F
Behavior	53-65	0/126	0/127	0/65	1/68	1/67	0/67	0/59	1/69
Tremors	66-78	0/116	0/119	0/63	1/64	0/64	0/64	0/56	1/65
	79-91	0/103	0/104	0/56	0/54	0/58	0/57	2/50	0/54
	92-104	0/87	0/75	0/46	1/39	0/45	0/43	0/41	0/48
	Total Grp Incidence				**3**	**1**		***2***	***2***
	Animals affected				3	1		2	1
Convulsions	27-39	0/129	0/130					1/70	
	40-52	0/128	0/130					1/67	
	Total Grp incidence							**2**	
	Animals affected							***1***	
Labored Breathing	14-26	0/129	0/130	3/70	0/70	0/70	0/70	1/70	0/70
	27-39	0/129	0/130	0/69	0/70	0/69	0/70	3/70	1/70
	40-52	0/128	1/130	1/68	1/69	1/69	1/70	7/67	2/69
	53-65	2/126	3/128	0/65	2/68	1/67	1/67	4/59	4/69
	66-78	6/116	2/119	0/63	3/64	4/64	1/64	21/56	2/65
	79-91	0/103	0/104	1/56	2/54	1/58	2/57	3/50	0/54
	92-104	3/87	0/75	3/46	0/39	3/45	0/43	0/41	2/48
	Total Grp incidence	**11**	**6**	**8**	**8**	**10**	**4**	**39**	**11**
	Animals affected	9	6	***6***	***7***	***8***	***4***	***10***	***7***
Limb function, impaired	14-26	0/129	0/130	0/70	0/70	1/70	0/70	0/70	0/70
	27-39	0/129	1/130	0/69	0/70	1/69	0/70	0/70	0/70
	40-52	0/128	0/130	0/68	0/69	2/69	0/70	0/67	0/69
	53-65	1/126	0/128	0/65	1/68	1/67	0/67	1/59	1/69
	66-78	0/116	0/119	2/63	1/64	2/64	1/64	2/56	1/65
	79-91	0/103	0/104	2/56	0/54	2/58	0/57	5/50	0/54
	92-104	4/87	0/75	2/46	0/39	3/45	0/43	7/41	0/48
	Total Grp incidence	**5**	**1**	**6**	**2**	**12**	**1**	**15**	**2**
	Animals affected	5	1	***3***	***1***	***8***	***1***	***8***	***1***

Body weights: Body weight was measured prior to the study initiation, weekly and at study termination.

- In the beginning, throughout and at study termination, HDM and HDF females had reduced mean body weight compared to their respective Controls (sponsor's Table 5.2.3 below and Figure 2 Male and Female).

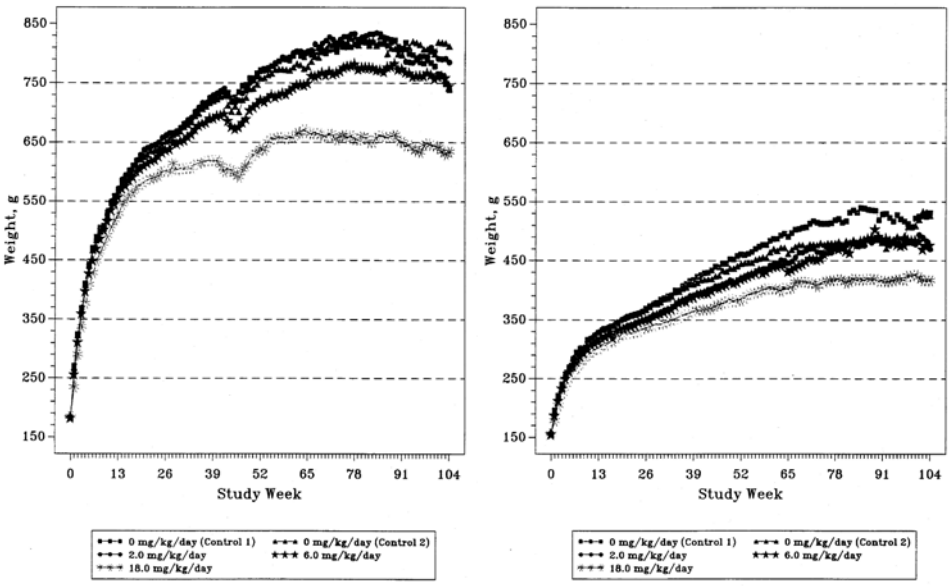
Table 5.2.3.
Summary of Mean Body Weights (grams)

Dosage Level (mg/kg/day)	Male			Female		
		A	B		A	B
0 (Control 1)	739	NA	(-9.1)	531	NA	(+0.8)
0 (Control 2)	813	(+10.0)	NA	527	(-0.8)	NA
2.0	786	(+6.4)	(-3.3)	470	(-11.5)	(-10.8)
6.0	746	(+0.9)	(-8.2)	477	(-10.2)	(-9.5)
18.0	634[1,3]	(-14.2)	(-22.0)	416[2,3]	(-21.7)	(-21.1)

NA - Not Applicable
A - Percent Difference from Control 1 B - Percent Difference from Control 2
[1]Significantly different from Control 1; p≤0.05
[2]Significantly different from Control 1; p≤0.01
[3]Significantly different from Control 2; p≤0.01

Figure 2. Mean body weight

MALE FEMALE



Food consumption: Individual food consumption was recorded daily and reported weekly during the first 13 weeks of study and monthly thereafter. Compound consumption was calculated weekly for weeks 1-13 and monthly thereafter.

- Overall, the average food consumption for the treated groups was similar to that of the control groups.
- The average food consumption values for two years are summarized in sponsor's Table 5.2.4 (below).

Table 5.2.4.
Average Food Consumption (g/animal/day)

Dosage Level (mg/kg/day)	Male			Female		
		A	B		A	B
0 (Control 1)	27.2	NA	(0.0)	20.8	NA	(+1.0)
0 (Control 2)	27.2	(0.0)	NA	20.6	(-1.0)	NA
2.0	27.4	(+0.7)	(+0.7)	20.6	(-1.0)	(0.0)
6.0	27.0	(-0.7)	(-0.7)	20.6	(-1.0)	(0.0)
18.0	25.9[1,2]	(-4.8)	(-4.8)	20.2	(-2.9)	(-1.9)

NA - Not Applicable
A - Percent Difference from Control 1
B - Percent Difference from Control 2
[1] Significantly different from Control 1; $p \leq 0.01$
[2] Significantly different from Control 2; $p \leq 0.01$

Compound consumption: Table 5.2.5 provided by the sponsor summarizes the mean compound consumed by each dose group for both sexes. The intended amount of compound consumed was achieved for most scheduled dosage levels except for a slight reduction observed in HDF (-1.7%).

Table 5.2.5.
Summary of Compound Consumption

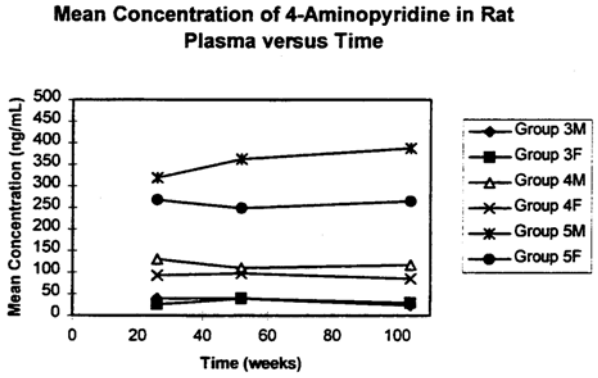
Dosage Level (mg/kg/day)	Mean compound consumption (mg/kg/day) Males	Females
0 (Control 1)	NA	NA
0 (Control 2)	NA	NA
2.0	2.07	2.02
6.0	6.13	6.00
18.0	18.06	17.70

NA - Not applicable

Toxicokinetics: Plasma samples were analyzed from animals at weeks 26, 52 and 104. Concentrations of the test article were performed using HPLC.

- The following Figure and Table provided by the sponsor show the 4-aminopyridine concentrations found in the plasma collected at the three time intervals.

SD = Standard deviation



Mean Concentration of 4-Aminopyridine (ng/mL) in Plasma Versus Time

Week	Group					
	3 Males	3 Females	4 Males	4 Females	5 Males	5 Females
26	40.2	26.4	131	92.7	319	268
52	39.8	39.7	110	97.7	362	249
104	24.4	29.3	117	85.3	387	265

The concentration of 4-aminopyridine in plasma collected at the 3 time intervals was approximately proportional to dose level. The figure presented above indicates that for a given dose level similar concentrations of the test article were found in the plasma of males and females at the various time intervals. Differences in concentration of test article in plasma between males and females is most evident at the 18.0 mg/kg/day dose rather than at the 2.0 mg/kg/day and 6.0 mg/kg/day doses.

Histopathology:
Adequate battery of tissues: Yes
Peer review: yes (X), no ( )

The incidence of endometrial stromal polyps was peer reviewed by (b) (6) , Veterinary Pathologist. Additionally, examination of the uterus and uterus, cervix of all females from all dose groups was performed by (b) (6)) who also reviewed an audited draft of histopathology data.

Non-neoplasm:

Macroscopic observations

The incidence and severity of ulceration of the feet was increased in a dose-related manner for both sexes, thus suggesting a 4-aminopyridine-dependent effect. Associated with this finding was the enlargement of several regional lymph nodes, which was considered by the sponsor to be

secondary to the foot inflammation. A summary of the pertinent macroscopic changes from time 0 to termination is provided in sponsor's Table 5.3.1, as reproduced below.

Table 5.3.1.
Pertinent Macroscopic Changes
0 to Termination

Group Number	Control 1		Control 2		3		4		5	
Dosage mg/kg/day	0		0		2.0		6.0		18.0	
Sex	M	F	M	F	M	F	M	F	M	F
Foot										
Ulcer, plantar	1	3	2	2	7	1	16	4	36	20
-trace				1						1
-mild		1			1		2		3	2
-moderate	1				2		3		10	3
-severe		2	2	1	4	1	11	4	23	14
Ulcer, palmar										
-mild to severe									4	2
Lymph Node, Axillary										
Enlarged,										
-trace to severe		5		6	1	7	3	5	7	8
Lymph Node, Iliac										
Enlarged,										
-trace to severe	1	1		2	1	2	1	2	5	7
Lymph Node, Inguinal										
Enlarged,										
-mild to severe		1		1	1	3	1	3	9	7
Lymph Node, Popliteal										
Enlarged,										
-moderate									2	1

- Although the sponsor suggested that 4-aminopyridine treatment exacerbated the ulceration of the foot which is a common finding in longer term studies in rats; this reviewer notes that the incidence of this finding was extremely low in Controls.
- The incidence of the lymphoid tissue enlargement is markedly less than the incidence of foot ulcerations suggesting these two occurrences may not be completely dependent on one another as the sponsor has suggested.
- Any remaining macroscopic changes were considered incidental, spontaneous or agonal in origin and thus unrelated in occurrence to the dietary intake of 4-aminopyridine.

Microscopic findings

Non-neoplastic: The following table summarizes the relevant microscopic findings as shown in Sponsor's Table 5.3.2.

Table 5.3.2.
Pertinent Microscopic Changes
0 to Termination

Group Number	Control 1		Control 2		3		4		5	
Dosage mg/kg/day	0		0		2.0		6.0		18.0	
Sex	M	F	M	F	M	F	M	F	M	F
Bone Marrow, Femur										
Hypercellular, -mild to moderate	4	9	2	3	3	3	2	3	15	2
Bone Marrow, Sternum										
Hypercellular, -mild to moderate	3	5	1	3	3	3	1	1	12	1
Foot										
Inflammation,	4	10	4	4	9	5	25	13	41	28
-mild	1						3	2	3	2
-moderate	2	8	2	4	9	4	12	9	24	14
-severe	1	2	2			1	10	2	14	12
Lymph Node, Axillary										
Hyperplasia, reactive, -mild to moderate		4		6		6	3	4	6	7
Lymph Node, Iliac										
Hyperplasia, reactive, -mild to moderate				2	1	2	1		5	7
Lymph Node, Inguinal										
Hyperplasia, reactive, -mild to moderate					1	2	1	2	8	6
Lymph Node, Popliteal										
Hyperplasia, reactive, -mild to moderate									2	1

- Microscopically, regional lymph nodes were characterized by reactive hyperplasia, which the sponsor attributed to the inflammation and ulceration of the feet.
- Pathogenesis of the foot ulceration was unclear; however, the pathologist noted no test article-induced changes in the metatarsal bones or blood vessels, which might have predisposed these rats to foot ulceration and inflammation.
- Morphologically identical ulcerations were observed in both Controls. The increased incidence of ulceration in the 4-aminopyridine-treated groups was considered a

treatment-related exacerbation. In both Control and 4-AP treated rats, foot ulcers were not associated with preneoplastic or neoplastic changes.

- Femoral or sternal bone marrow hypercellularity was also attributed to a secondary effect of foot inflammation.
- Higher incidence of marrow hypercellularity was determined in H&E sections and correlated with increased severity of ulceration in individual animals.

Neoplasm: The sponsor reported an increased incidence of benign uterine endometrial polyps (see sponsor's Table 1, below), which was peer reviewed by [redacted] (b) (6) (veterinary pathologist). In Dr. [redacted] (b) (6) report, the incidence of uterine polyps was evaluated for all dose groups, and he concluded that the increased incidence in HDF was a "fortuitous incidence variation and not a compound-related effect." During the peer review, Dr. [redacted] (b) (6) was unable to identify any neoplastic or preneoplastic changes in the endometrium. Comparison of polyps from in Controls and HDF were morphologically similar; and no similar finding was observed in the mouse two-year bioassay.

TABLE 1

Incidence of Endometrial Stromal Polyps and other Primary Uterine Neoplasms

Dose Group Dose Level (mg/kg/day)	Control 1 0	Control 2 0	Low 2.0	Mid 6.0	High 18.0
Number of Female Rats/Group	60	60	60	60	60
Endometrial Polyps					
Uterus, horn	1	2	4	1	9
Uterus, cervix	0	0	0	1	0
Total Incidence/% Incidence	1/1.67%	2/3.33%	4/6.67%	1/1.67%	9/15%
Other Primary Tumors					
Fibroma	0	0	0	1	0
Sarcoma, stromal-cell	0	0	1	0	0
Adenoma	0	0	1	0	0
Adenocarcinoma	0	0	0	0	1

Statistical analyses performed by the sponsor suggested a significant trend and pairwise comparisons showed a significant difference between Control 1 and HDF.

polyp, benign										
Overall Rates (a)	1/60	(1.67%)	2/60	(3.33%)	4/60	(6.67%)	1/60	(1.67%)	9/60	(15.00%)
Fisher Exact Test; P-value			1.000		0.364		1.000		0.017	
Cochran-Armitage Trend Test; P-value	0.008									
Peto Test; P-value	0.011									
Onset Rate Test; P-value	0.740									

Independent analysis of the tumor datasets by Dr. Mohammad Atiar Rahman, (statistician in the Division of Biometrics-6) also found a statistically significant increase in the incidence of uterine polyp was identified (as shown below). Thus, the reviewer and the Executive CAC concluded

that the increase in uterine polyps was a positive finding for the presence of a neoplasm in rats treated with dietary 4-aminopyridine.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons

Sex	Organ Name	Tumor Name	Cont. N=60	Low N=60	Med N=60	High N=60	Dos Resp	Cont 1 vs. L	C 1 vs. M	C 1 vs. H
Male	skin, subcutis	fibroma, benign	2	5	1	7	0.0333	0.2172	0.5082	0.0546
Female	uterus#	polyp, benign	3	4	1	9	0.0027*	0.1876	0.4305	0.0050*

#All tests for uterus tumors were performed using the combined control (Pooling Control 1 and Control 2, N=120).

Using α=0.05, the incidences of benign fibroma of skin subcutis in male rats and of benign polyps of uterus in female rats were considered to have statistically significant positive dose response relationships. Also, the increased incidence of benign polyps in uterus in high dose group was considered to be statistically significant compared to combined control.

2.6.6.6 Reproductive and developmental toxicology

Sponsor submitted three rat reproductive and developmental toxicology studies (fertility and early embryonic development, embryo-fetal development, pre- and postnatal development) and one embryo-fetal development study in rabbits. These studies are reviewed below.

Fertility and early embryonic development

1.) **Study of Fertility and General Reproductive Performance in Rats**

Key study findings:

- The fertility and early embryonic development study conducted in rats was inadequate because there was low n/grp for the uterine parameters (GD13) and the postnatal evaluations were conducted in 11 HD litters.
- NOAEL = 1 mg/kg/day for orally administered 4-aminopyridine to Crl:CD® VAF/Plus® rats of either sex.
    - Based upon mortalities, antemortem (pre- and post-dose seizures and tremors) and necropsy findings, reduced body weight and food consumption and inhibited maternal body weight gain at 3 and/or 9 mg/kg/day.
- NOAEL = 9 mg/kg/day for reproductive performance.
- NOAEL = 3 mg/kg/day for developmental toxicity.
    - Based upon reduced pup viability at 9 mg/kg/day.

Study no.: 684-001
Volume #, and page #: EDR 4.2.3.5.1., pp. 1-257
Conducting laboratory and location: (b) (4)
Date of study initiation: 14 October 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C4886, assumed 100%

Methods

Doses: 0 (Control), 1 (LD), 3 (MD), 9 (HD) mg/kg/day
Species/strain: Rat/Crl:CD® VAF/Plus®
Number/sex/group: 35/sex/grp
Age: Males, 45 days of age; Females, 87 days of age
Weight: Males, 205-240 g; Females, 238-271 g
Route, formulation, volume, and infusion rate: Oral, gavage, 5 ml/kg
Vehicle: distilled water
Satellite groups used for toxicokinetics: TK not measured

Study design: Dosing occurred approximately 1.75 to 4.25 hours after initiation of the light phase. Dosage volumes were based on individual body weights.

- Male rats were administered vehicle or 4-aminopyridine by oral gavage 60 days prior to and during the mating period until euthanasia.
- Females were administered vehicle or 4-aminopyridine 14 days prior to mating and until euthanasia on gestation day 13 or lactation day 21.
- Females that failed to deliver on day 21 were dosed until presumed gestation day 24.
- Offspring were not dosed directly.

Housing: Rats were individually housed. Diet and water were available *ad libitum*.

Parameters and endpoints evaluated include mortality, clinical signs, body weight, food consumption, gross pathology of adults. F_0 tissues were preserved in 10% neutral buffered formalin, but histopathology was not conducted.

Female-specific data included estrous cyclicity, pre- and post-implantation loss, and mean gestation lengths. Copulatory interval was also determined.

Fetuses from dams found dead were externally examined and discarded. Pups from dams found dead during lactation were necropsied.

Justification of Route of Administration: Intended route of administration to humans is oral; therefore, this study employed the same route.

Justification of Dosage Selection: Sponsor selected doses based upon the results of a dose range-finding study conducted by (b) (4) (684-002). In this study, 4-aminopyridine (3, 5, 7, 10 and 13 mg/kg/day) was administered by oral gavage to groups of five mated females on gestation days (GD) 0 to 15. Maternal toxicity occurred at 13 mg/kg/day, as evidenced by mortality and clinical sign (e.g., convulsions, abnormal vocalizations, and tremors).

Observations, Times and Results:

Dosage formulation: Dosing formulations were prepared during study weeks 1-4, 8, and 12; 4-aminopyridine concentrations were quantified by HPLC.

- LD, MD, and HD 4-aminopyridine dosing formulations were acceptable with mean values of 102, 103, and 103% of target concentrations, respectively.

Mortality: Animals were checked daily for mortality and for overt signs of toxicity.

- 3 HDM and 10 HDF died prior to scheduled euthanasia. HDM died on Days 42, 70, and 79 and HDF died on Days 2, 3, 4, 12, 24, 36, 37, and 40. Antemortem observations included pre- and post-dose tremors and postdose convulsions. A summary of the clinical signs for all animals is provided in the clinical signs section below. Most deaths occurred post-dose (3/3 HDM and 6/10 HDF); however, the sponsor did not specify how long following dosing the deaths occurred. Necropsy revealed lung discoloration in 2/3 HDM (moderate-severe) and 7/10 HDF (1 moderate and 6 severe).
- 1 MDF died prior to scheduled euthanasia on Day 41, which was attributed to gavage error and not test-article, as a ruptured esophagus was found at necropsy.
- 100% of Control and LD animals survived.

Clinical signs: Twice daily, at dosing and approximately 1 hr post-dose.

- MD and HD groups, males and females were noted to experience both pre- and post-dose tremors and post-dose convulsions, increased salivation and material around the nose, mouth, and/or eyes, all of which were considered test article-related.
- The sponsor suggests that other clinical signs in the treatment groups were noted at single incidences or were comparable to the rates observed in the Control and thus were not considered test article-related.
- *The reviewer disagrees with sponsor's interpretation of the remaining clinical signs; the following Table illustrates the incidence of clinical signs that were either dose-related or observed only in the HD group.*
    - *Treatment-related CNS activation was observed, as evidenced by increased incidence of post-dose convulsions in HDM and HDF and dose-dependent increased incidence of pre- and post-dose tremors in MDF and HDM. Additional signs include pre- and/or post-dose incidences of aggressive behavior, impaired limb function, increased activity, and increased salivation.*
    - *Presence of labored breathing, rales, and gasping may reflect impaired lung function although of low incidence, these findings are consistent with findings of red discolored lungs in premature decedents in the HD group.*

Observed clinical signs pre- (BD) and post-dose (PD) in MD or HD, which were absent in Control and LD groups.

	MD				HD			
	Male		Female		Male		Female	
Clinical sign	**BD**	**PD**	**BD**	**PD**	**BD**	**PD**	**BD**	**PD**
Died				1 (3)[a]		3 (9)	5 (14)	5 (14)
Tremors		26 (77)	1 (3)	1 (3)	3 (9)	35 (100)	7 (20)	33 (94)
Convulsions						2 (6)		6 (17)
Increased salivation	2 (6)	7 (20)	2 (6)	14 (40)	1 (3)	27 (77)	1 (3)	17 (49)
Impaired limb function					1 (3)	1 (3)	1 (3)	1 (3)
Increased activity	1 (3)	1 (3)			2 (6)	4 (11)		
Gasping					1 (3)	1 (3)		
Labored breathing					1 (3)	2 (6)		
Rales							1 (3)	1 (3)
Aggressive behavior					1 (3)	1 (3)		

Incidence/35 rats/sex; (% affected)
[a]COD =gavage error

Body weight: In males, individual body weights were measured weekly from randomization until euthanasia; in females, individual body weights were measured weekly until evidence of copulation occurred or animal euthanized. In dams selected for sacrifice on gestation day 13 (GD13), body weights were recorded on GD0, GD7, and GD13. Dams allowed to deliver were weighed on GD0, GD7, GD13, and GD20 as well as on lactation days 0, 7, 14, and 21. In

nongravid females, body weights were recorded on the above presumed gestation days; however, these data were not included in the summary data.

- HDM, a statistically significant reduction in body weight (-4.5 to -6.7% compared to control) was found during weeks 1-12.
- MDF and HDF body weights were also significantly reduced on gestation days 7 (-5.2% and -4.6%), 13 (-6.2% and -4.8%) and 20 (-8.3% and -9%) when compared to the Control.
    - Maternal body weight gain was significantly decreased over the entire gestation interval (GD 0-20) and during GD 0-7 and GD 13-20.

Food consumption: Individual food consumption was recorded weekly for all animals prior to mating; during cohabitation, no measurement was made. During the post-mating period, food consumption in males was recorded weekly until euthanasia; food consumption in females was recorded on the same days as body weight during the gestation and lactation periods. Food consumption in nongravid females was measured at the same times as for other females; however, these data were excluded from summary data.

- HDM, statistically significant reductions in food consumption occurred during weeks 1-4 (range from -19.3% at week 1 to -5.3% at week 4) and week 8 (-7%).
- MDF and HDF, during study week 1 significant reductions in food consumption were noted (-15.6% and -22.6%, respectively).
    - In HDF, these reductions in food consumption paralleled the effect on body weight.
- MDF and HDF, a statistically significant reduction in food consumption was noted during GD 0-20 (-11.1% and -9.6%, respectively) and during GD 0-7 (-10.8% and -9.2%, respectively) and GD 7-13 (-13.4% and -9.3%, respectively).
    - Although not statistically significant, a similar magnitude of reduced food consumption was also found during GD 13-20 (-11.4% and -11%).
- HDF had a statistically significant decrease in food consumption during lactation days 0-7 (-17.3%) compared to Control.

Toxicokinetics: No TK data were collected during this study; instead refer to bridging study 7338-105, entitled "Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Doses to Non-Pregnant and Pregnant Rats". This study is reviewed in the PK/ADME section 2.6.4.8. Other Pharmacokinetic Studies as study number 1.

Estrous cycle determination: Ten days prior to dosing, vaginal smears of all females were made to establish cyclicity; and vaginal smears were also collected during the 15-day premating treatment period and during the mating period until evidence of copulation was observed during the mating period. Estrous cycling in rats was evaluated as published by Fox and Laird (1970)[1].

- No test article-related differences in estrous cyclicity were found (see sponsor's Table 11, appended below).

[1]Fox, R.R and Laird, C.W. (1970). Sexual Cycles. In: *Reproduction and Breeding Techniques for Laboratory Animals,* E.S.E. Hafez, ed., Lea and Febiger, Philadelphia, PA 107-122.

TABLE 11. Summary of Estrous Cycle Abnormalities

Study Period Estrous Abnormality	0 mg/kg/day (Control) No.	(%)	1 mg/kg/day No.	(%)	3 mg/kg/day No.	(%)	9 mg/kg/day No.	(%)
	Number and Percentage[a] of Animals with Abnormalities							
Untreated Premating								
Long Diestrus	11	(31)	13	(37)	15	(43)	17	(49)
Short Diestrus	5	(14)	9	(26)	4	(11)	10	(29)
Long Estrus	22	(63)	23	(66)	19	(54)	14	(40)
Treated Premating								
Long Diestrus	16	(46)	17	(49)	22	(63)	26	(74)
Short Diestrus	6	(17)	10	(29)	11	(31)	10	(29)
Long Estrus	26	(74)	26	(74)	25	(71)	22	(63)
Treated Mating								
Long Diestrus	8	(23)	10	(29)	9	(26)	7	(24)[b]
Short Diestrus	1	(3)	1	(3)			1	(3)[b]
Long Estrus	4	(11)	3	(9)			1	(3)[b]

[a]Based on 35 animals per group
[b]Based on 29 animals per group
Long diestrus - Diestrus occurring for more than 3 consecutive days
Short diestrus - Diestrus occurring for less than 2 consecutive days
684-001 Long estrus - Estrus occurring for 2 or more consecutive days

Fertility parameters:

1. Maternal observations at uterine examination on gestation day 13 are presented in sponsor's Table 12.
   - No test article-related differences in the number of viable embryos were noted between the control and treatment groups; and no embryo was found dead.
   - Post-implantation loss was comparable among groups.
   - Although the percent preimplantation loss increased with 4-aminopyridine treatment in the LD and MD groups, the lack of a similar increase in the HD group suggests this difference is not test article-related.
   - Total resorption occurrence in Control dams and 4-aminopyridine-treated dams was similar and was due to early resorptions. Late resorptions were not found in any of the dams.

TABLE 12. Summary of Maternal Observations at Uterine Examination*

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	9 mg/kg/day
Animals on Study	N	12	12	12	10
Pregnant	N	11	11	9	10
	%	92.0	92.0	75.0	100
Animals with Viable Embryos	N	11	11	9	10
Animals with all Resorptions	N	0	0	0	0
Female Mortality	N	0	0	0	0
	%	-	-	-	-
Number Evaluated	N	11	11	9	10
Corpora Lutea	Mean	16.7	18.6	15.7	16.5
	S.D.	2.53	1.96	1.41	1.18
	N	11	11	9	10
	Total	184	205	141	165
Implantation Sites	Mean	15.3	16.1	13.7	15.4
	S.D.	4.08	2.34	2.55	1.43
	N	11	11	9	10
	Total	168	177	123	154
Postimplantation Loss	Mean	1.3	1.1	0.2	1.1
	S.D.	1.27	1.04	0.44	1.20
	N	11	11	9	10
	Total	14	12	2	11
Preimplantation Loss[a]	%	8.7	13.7	12.8	6.7
Postimplantation Loss[b]	%	8.3	6.8	1.6	7.1

684-001

[a] $\frac{\text{Corpora Lutea - Implantations}}{\text{Corpora Lutea}} \times 100$

[b] $\frac{\text{Implantations - Viable Fetuses}}{\text{Implantations}} \times 100$

*No statistical significance observed
- - Not applicable
S.D. - Standard deviation
N - Number of animals

TABLE 12. Cont. Summary of Maternal Observations at Uterine Examination*

			0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	9 mg/kg/day
Resorptions	Total	Mean	1.3	1.1	0.2	1.1
		S.D.	1.27	1.04	0.44	1.20
		N	11	11	9	10
		Total	14	12	2	11
	Early	Mean	1.3	1.1	0.2	1.1
		S.D.	1.27	1.04	0.44	1.20
		N	11	11	9	10
		Total	14	12	2	11
	Late	Mean	0.0	0.0	0.0	0.0
		S.D.	0.00	0.00	0.00	0.00
		N	11	11	9	10
		Total	0	0	0	0
Dead Embryos		N	0	0	0	0
Live Embryos		Mean	14.0	15.0	13.4	14.3
		S.D.	4.12	2.45	2.92	2.41
		N	11	11	9	10
		Total	154	165	121	143

684-001

S.D. - Standard deviation
N - Number of animals
*No statistical significance observed

2. A summary of the mating study parameters is presented below in sponsor's Table 13. Each male was cohabitated with one female of the same strain, source, age and treatment group until evidence of copulation (presence of sperm plug or vaginal inspection for sperm); at which time, the female was removed and returned to an individual cage. Any female failing to show evidence of copulation after the initial 10-day mating period was housed for an additional 5 days with another male which was sperm positive during the initial 10 days. Copulatory intervals were calculated for females with evidence of copulation during the mating period.
   - Male and female fertility and copulatory (i.e., mating) indices were not different among groups.
   - Mean copulatory (i.e., mating) interval and gestation lengths were similar among groups.
   - 4-aminopyridine did not affect reproductive mating parameters in males or females.

TABLE 13. Summary of Mating Parameters*

Dosage Level	Male Copulatory Index: No. of Males Mated[a] / No. of Males Paired	Male Copulatory Index: %	Male Fertility Index: Fertile Males / No. of Males Paired	Male Fertility Index: %	Female Copulatory Index[b]: No. of Females Mated[a] / No. of Females Paired	Female Copulatory Index[b]: %	Female Fertility Index[b]: No. Gravid Females / No. of Females Paired	Female Fertility Index[b]: %	Copulatory Interval (days): Mean	Copulatory Interval (days): S.D.	Copulatory Interval (days): N	Gestation Length (days): Mean	Gestation Length (days): S.D.	Gestation Length (days): N
0 mg/kg/day (Control)	33/35	94.3	30/35	85.7	31/35	88.6	31/35	88.6	3.2	2.83	33	22.2	0.42	19
1 mg/kg/day	32/35	91.4	28/35	80.0	32/35	91.4	28/35	80.0	3.0	2.19	29	22.2	0.43	15
3 mg/kg/day	34/35	97.1	28/35	80.0	35/35	100	28/35	80.0	2.8	2.45	33	22.4	0.71	17
9 mg/kg/day	26/29	89.7	25/29	86.2	28/29[c]	96.6	27/29[c]	93.1	4.1	3.25	27	21.8	1.08	11

684-001

[a]Mated is defined as having had evidence of copulation either by presence of sperm, copulatory plug, gravid status at uterine examination or delivery of a litter
[b]Includes animals scheduled for gestation day 13 uterine examination as well as those allowed to deliver
[c]Does not include six females which died prior to pairing
*No statistical significance observed
S.D. - Standard deviation
N - Number of animals

3. Summary of uterine observations on Lactation Day 21 is found in sponsor's Table 17 (appended below).

- MD, reduced mean number of implantation sites (-15.3%) and mean number of delivered offspring (-16.8%) were found compared to the Control.
- The sponsor concluded the lack of an effect in the HD pups suggested that the MD effect was representative of biological variation.

TABLE 17. Summary of Uterine Observations on Lactation Day 21

Dosage Level (mg/kg/day)	Number of Implantation Sites: Mean	Implantation Sites: S.D.	Implantation Sites: N	Number of Offspring Delivered: Mean	Offspring Delivered: S.D.	Offspring Delivered: N	Number of Remaining Sites[a]: Mean	Remaining Sites: S.D.	Remaining Sites: N
0 mg/kg/day (Control)	15.0	1.78	20	13.7	1.87	20	1.3	1.03	20
1 mg/kg/day	14.7	1.21	17	13.9	1.54	17	0.8	0.95	17
3 mg/kg/day	12.7	4.00	19	11.4	4.15	19	1.3	1.49	19
9 mg/kg/day	14.1	2.98	11	13.0	3.13	11	1.1	1.22	11

684-001

[a]Implantation sites not corresponding to delivered offspring; values represent postimplantation loss and/or complete cannibalization
S.D. - Standard deviation
N - Number of animals

F_1 generation

1. Offspring viability for Control, LD, MD, and HD dams is summarized in sponsor's Table 14.
   - *No significant differences or dose-related findings in offspring survival were apparent at birth, and after lactation Days 4, 7, 14, and 21 days after birth.*
   - On Lactation Day 4 (prior to culling), the following reviewer-generated table summarizes the number of litters and number of pups that died during Day 0 to Lactation Day 4 interval.

Group	# Affected Litters	# Pups died Day 0-Lactation Day 4
Control	3/20	5
LD	5/17	5
MD	5/19	9
HD	4/12	19

   - HD offspring survival was lower on Lactation Day 4 (prior to culling) when compared with the number of live offspring at Day 0 (84.6% versus 98.1%).
     - 19 pups died from 4 separate HD litters (2 litters each lost one pup, 1 litter lost 5 pups, and 1 litter was entirely lost, 12 pups).
   - After culling, HD offspring survival was almost 100% at Lactation Days 7, 14, and 21.

The percent of live LD and MD offspring were comparable to that of the Control at Lactation Days 4, 7, 14, and 21.

TABLE 14. Cont. Summary of Offspring Viability*

	Offspring Survival Index (Day of Age)									
	0		4		7		14		21	
	No. Live Offspring at Day 0		No. Live Offspring at Day 4 BR		No. Live Offspring at Day 7		No. Live Offspring at Day 14		No. Live Offspring at Day 21	
Dosage Level	Total Born	%	No. Live Offspring at Day 0	%	No. Live Offspring at Day 4 AR	%	No. Live Offspring at Day 7	%	No. Live Offspring at Day 14	%
0 mg/kg/day (Control)	271/274	98.9	265/271	97.8	198/199	99.5	198/198	100	198/198	100
1 mg/kg/day	236/236	100	230/236	97.5	164/170	96.5	164/164	100	164/164	100
3 mg/kg/day	197/203	97.0	188/197	95.4	149/151	98.7	144/149	96.6	144/144	100
9 mg/kg/day	156/159	98.1	132/156	84.6	107/108	99.1	107/107	100	106/107	99.1

*No statistical significance observed
BR - Before reduction
AR - After reduction

684-001

TABLE 14. Summary of Offspring Viability

Dosage Level	Total Litter Size			No. Dead Offspring at Lactation Day 0			No. Live Offspring at Lactation Day 0		
	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
0 mg/kg/day (Control)	13.7	1.87	20	0.2	0.37	20	13.6	1.96	20
1 mg/kg/day	13.9	1.54	17	0.0	0.00	17	13.9	1.54	17
3 mg/kg/day	11.4	4.15	19	0.3	0.82	19	11.1[1]	4.18	19
9 mg/kg/day	13.3	3.11	12	0.3	0.45	10	13.0	3.13	12

[1]Significantly different from the control group; p<0.05
S.D. - Standard deviation
N - Number of animals

684-001

2. Offspring growth is summarized in sponsor's Table 15.
   - On Lactation Days 7-21, mean body weight in HD pups was significantly decreased compared to Control pups. [ss, *p<0.05 and **p<0.01 compared to control]
     - At birth (Lactation Day 0), HD pups weighed slightly less (-3.1%) than Controls. HD pups also weight slightly less than Control pups on Day 4BR by -6.3%, Day 4AR (after culling) by -5.2%, on Day 7 by -15.1%, on Day 14 by -18.3%*, and on Day 21 by -16.2%** (males) and by -16.3%** (females).
   - Control, LD, and MD pups were unremarkable.

TABLE 15. Summary of F_1 Offspring Growth

Dosage Level	Group Mean Body Weight (grams) of Live Offspring During Lactation (Day of Age)																				
	0			4BR			4AR			7			14			21					
																Male			Female		
	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
0 mg/kg/day (Control)	6.3	0.43	20	9.6	0.67	20	9.6	0.66	20	15.2	1.20	20	32.2	1.74	20	53.1	3.04	20	51.1	2.62	20
1 mg/kg/day	6.6	0.80	17	10.3	1.76	17	10.4	1.76	17	16.1	1.80	17	33.0	2.10	17	54.8	5.16	17	52.9	4.68	17
3 mg/kg/day	6.6	0.83	19	9.8	2.08	18	9.8	2.09	18	15.0	3.25	18	30.3	5.05	18	52.9	8.38	17	49.3	8.99	18
9 mg/kg/day	6.1	0.91	12	9.0	1.68	11	9.1	1.64	11	12.9[1]	2.48	11	26.3[2]	3.44	10	44.5[2]	7.36	11	42.8[2]	6.75	11

BR - Before reduction
AR - After reduction
S.D. - Standard deviation
N - Number of animals

[1]Significantly different from the control group; p≤0.05
[2]Significantly different from the control group; p≤0.01

684-001

3. Offspring assessment at necropsy resulted in a limited number of findings.

Deaths

- LD, accidental death in one pup on lactation day 7.

- MD, 13 pups were euthanized on lactation day 2 due to death of their dam and these data were excluded from the summary table.

The sponsor suggested that the increased deaths and the increased missing pups relative to the Control correlated with the reduced offspring viability; and concluded that these effects were test article-related. The incidence of cannibalized pups by MD dams was suggested by the sponsor to be test article-related.

F_1 Antemortem observations

- Observations were noted either single or low incidence and thus considered normal biological variability by the sponsor.
  - No test article-related findings.

Gross pathology (F_1)

- The developmental variation of a 14th rudimentary rib was found in one Control, one MD, and three HD pups
  - The number of HD litters affected was not specified in the sponsor's study report.
  - One Control pup had a misaligned sternebra (#4) and unossified sternebra (#5).
- HD, two pups had less than 13 full pairs of ribs; one pup had a 7th cervical rib.

Histopathology (F_1)

- Hydronephrosis and/or distended ureter(s) was found in one Control pup from a single litter, four LD pups from four litters, and six MD pups from a single litter.
  - These findings were considered by the sponsor to represent normal biological variability and unrelated to test article treatment.
- Hemorrhagic testes were observed in one pup from each treatment group.
  - These findings were considered by the sponsor to represent normal biological variability and unrelated to test article treatment.
- No malformations were observed in any dead pup.

Embryofetal Development

2.) **Developmental Toxicity Study in Rats**

Key study findings:

Adequacy of the developmental toxicity study and appropriateness of the test model: the study conducted in rats was adequate.

Evaluation of early embryonic development: The NOAEL for developmental toxicity was 3 mg/kg/day (MD) based on findings of reduced offspring viability and reduced pup body weight during lactation days 4-21.

- NOAEL < 1 mg/kg/day for orally administered 4-aminopyridine to Crl:CD® VAF/Plus® rats.
    - Based upon mortalities, antemortem (pre- and post-dose seizures and tremors) and necropsy findings, reduced body weight, reduced food consumption and maternal body weight gain were all decreased at all doses tested.
- NOAEL = 3 mg/kg/day for developmental toxicity.

Study no.: 684-003
Volume #, and page #: EDR 4.2.3.5.1., pp. 1-117
Conducting laboratory and location: (b) (4)

Date of study initiation: 14 October 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C4886, 99.9%

Methods

Doses: 0 (Control), 1 (LD), 3 (MD), 10 (HD) mg/kg/day
Species/strain: Rat/Crl:CD® VAF/Plus®
Number/sex/group: Main study: 30 F/grp
Age: 12-13 weeks of age
Weight: 232-299 g
Route, formulation, volume, and infusion rate: Oral, gavage, 5 ml/kg
Vehicle: distilled water
Satellite groups used for toxicokinetics: none

Housing: Rats were individually housed. Diet and water were available *ad libitum*.

Parameters and endpoints evaluated include mortality, clinical signs, body weight, food consumption, maternal gross pathology at cesarean section (parental tissues saved in 10% neutral buffered formalin for

possible histopathology), and fetal morphological observations—malformations and developmental variations.

Justification of Route of Administration: Oral route intended for human administration.

Justification of Dosage Selection: Sponsor selected dosages based upon the results of a dose range-finding study conducted by (b) (4) (684-002). This study administered 4-aminopyridine (3, 5, 7, 10 and 13 mg/kg/day) by oral gavage to groups of five mated females on gestation days (GD) 6 to 15. Maternal toxicity occurred at 13 mg/kg/day, as evidenced by mortality and clinical signs (e.g., convulsions, abnormal vocalizations, and tremors). No evidence of developmental toxicity was noted at any dose level.

Observations, Times and Results:

Dosing formulation: LD, MD, and HD dosing formulations were acceptable, with mean values of 102, 101, and 102% of the targeted dosing concentrations, respectively.

Mortality: Daily for mortality and overt signs of toxicity.

- Within first 14 days of gestation, 13/30 HD died. Most (9/13) died within two days of initiating 4-aminopyridine treatment (5 on GD 7, 4 on GD 8, and single deaths each on GD 10, GD 12-14).
    - In 4/5 dams that died on GD 7, mild to severe multilobar, red discoloration of the lung was detected at necropsy. Mild to moderate cloudy corneas were detected in one or both eyes in 3 of 4.
    - In 1/5 dams that died postdose on GD7, the following clinical signs were observed: tremors, convulsions, increased salivation, and/or abnormal vocalization. At necropsy, mild multilobar, red discoloration of lung and mild LN enlargement were detected in this dam.
    - In dams that died on GD 8, clinical signs were present. Convulsions (4/4), tremors (3/4), abnormal vocalizations (2/4), increased salivation (2/4), body surface and urogenital yellow staining (1/4) were observed. At necropsy, severe multilobar, red discoloration of lung and/or mild LN enlargement were detected in 2 of 4.
- All Control, LD, and MD survived to terminal euthanasia.

Clinical signs: Twice daily, at dosing and approximately 1 hr post-dose on GD6-15, and until termination on GD20.

- The following table summarizes the relevant observed clinical signs and necropsy findings for MD and HD treatment groups; none of the tabulated clinical signs was found in either Control or LD groups.

Observed clinical signs (predose, PreD and postdose, PD) and necropsy findings.

Clinical sign[1]	MD PreD	MD PD	HD PreD	HD PD
No visible abnormalities	27 (90%)		25 (83%)	
Died	0	0	5 (17)	8 (27)
Tremors	0	6 (20)	0	24 (80)
Convulsions	0	0	0	5 (17)
Increased salivation	0	0	0	5 (17)
Abnormal vocalization	0	0	0	4 (13)
High carriage	0	0	0	2 (7)
Increased activity[2]	0	0	0	1 (3)

[1]Incidence/30 rats/sex; (% affected)
[2]Clinical sign included because this sign has been observed in several other toxicology studies even though it appears rare in the present study.

Body weight: Individual body weights were recorded on GD 0, 6, 9, 12, 16, and 20. Body weights were recorded in non-pregnant females on the above presumed GD, but the data were not included in the summaries. [ss, $^{*}p<0.05$; $^{**}p<0.01$ from Control].

Body weight gain

- Decreased maternal body weight gain [ss] relative to Control in all dose gps during GD6-9 and GD6-16.
  - GD6-9 interval, mean body weight decreased -43.8% (LD)*, -87.5% (MD)**, and -156.3% (HD)** relative to Control mean body weight.
  - GD6-16 interval, mean body weight decreased -11.9% (LD)*, -19.4% (MD)**, and -29.8% (HD)** relative to Control mean body weight.
- Decreased maternal body weight gain relative to Control [ss] during adjusted overall gestation period GD0-20.
  - Gravid uterine weight was reduced in MDF -12.2%* and HDF -17.8% **.

Mean body weight

- Mean body weight was reduced [ss] in MDF and HDF on GD6 and GD9.
  - GD6, -4.7% (MD)* and -7.6% (HD)** compared to Control.
  - GD9, -4.5% (MD)* and -5.1% (HD)* compared to Control.
  - GD20, all dose groups were similar to Control.
  - GD20 adjusted for gravid uterine weight, all dose groups were similar to Control.
- LD mean body weight was similar to Control throughout the study period.

Food consumption: Individual food consumption was recorded as for body weight. The following GD intervals were evaluated: GD 0-6, GD 6-9, GD 9-12, GD 12-16, GD16-20, GD 6-16, and GD 0-20. The intervals GD 6-16 and GD 12-16 were calculated to account for possible effects on food consumption following final dose administration on GD 15. Food consumption in nongravid females was measured at the same times; however, these data were excluded from summary. [*ss, $p<0.05$; **ss, $p<0.01$ from Control]. Food consumption was decreased at all doses relative to Control.

Food consumption as a percent of Control

GD Interval	4-Aminopyridine		
	LD	MD	HD
0-6	+2.3%	-1.2%	+0.7%
6-9	-6.4%	-16.8%**	-38.7%**
9-12	-6.3%*	-22.2%**	-20.6%**
12-16	-2.6%	-5.9%	-3.9%
16-20	+5.7%*	-0.6%	+4.2%
6-16	-4.9%	-13.6%**	-21.6%**
0-20	-0.3%	-7.3%**	-10.8%**

Toxicokinetics: No TK data were collected during this study; instead refer to bridging study 7338-105, entitled "Toxicokinetics of Fampridine and its 3-Hydroxy-4-Aminopyridine and 3-Hydroxy-4-Aminopyridine Sulfate Metabolites After Multiple Oral Doses to Non-Pregnant and Pregnant Rats". This study is reviewed in the PK/ADME section 2.6.4.8. Other Pharmacokinetic Studies as study number 1.

Cesarean section observations: All surviving dams were euthanized by carbon dioxide inhalation on GD 20, immediately followed by cesarean section. The uterus was excised, pregnancy status determined, and gravid uterine weight recorded. Fetuses were removed, and the location of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea were noted. Uteri that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. The following table summarizes the relevant cesarean observations adapted from sponsor's Table 5.

Cesarean examination of maternal reproductive parameters and fetal observations from dams treated during GD 6-16.

Parameter	Control	LD	MD	HD
Corpora lutea/rat	17.9	18.5	18.8	18.4
Implantation sites/rat	16.0	16.8	17.5	16.9
Preimplantation	53	49	33	22
loss/group	10.6%	9.4%	7%	8.5%
Postimplantation	27	36	37	11
loss/group	6.0%	7.7%	8.4%	4.7%
Resorptions/group	27	36	37	11
Uterine wt. (g)	89.1	90.3	92.7	93.4
Fetal wt. (g)	3.9	3.8	3.7	3.8
Fetal sex				
M/gp	7.8	7.0*	8.4	8.5
F/gp	7.2	8.5*	7.6	7.6

*Statistically significant compared to Control ($p<0.05$).

- No test article-related differences were found between treatment groups and Control with respect to postimplantation losses, resorptions, viable fetuses/dam and fetal body weights.

Fetal morphological observations: Individual fetuses were weighed, sexed, tagged, and examined for external malformations and variations. Approximately one-half of the fetuses were placed in Bouin's solution for soft-tissue examination using a razor-blade sectioning technique. The remaining fetuses were fixed in alcohol, macerated with potassium hydroxide, stained with Alizarin Red S and cleared with glycerin by the Dawson method for subsequent skeletal examination. All gross, visceral, and skeletal alterations were classified as malformations or variations.

In the present study, there were only 14 HD litters (n= 225 viable fetuses) available for evaluation, which is marginal at best.

Malformations

Summary of total number of fetuses examined for each type of malformation[1]

	Malformations			
Fetal Examinations	**Control**	**LD**	**MD**	**HD**
Litters (#)	28	28	25	14
Fetuses—externally	421	433	401	225
Fetuses—viscerally	210	216	203	112
Fetuses—skeletally	211	217	198	113
Fetal wt. (g)	3.9	3.8	3.7	3.8
% Litters with malformations	0 0%	0 0%	2 8%	2 14.3%

[1]Data in Table adapted from Sponsor's Table 6.

Summary of fetuses with malformations and variations.

Dose mg/kg	Dam #	Fetus #	Malformation	Variation
0				
1				
3	43789	1	Microphthalmia	
	43790	15	Folded retina	
10	43833	4	Fused sternebrae: #1 and #2	14th rudimentary rib(s)
	43839	15	Cranioorachischisis Cleft lips Cleft palate Malformed skull bones: premaxillae, maxillae, jugals, squamosals and frontals malformed in two pieces; orbits smaller than normal	Vertebral arches reduced in ossification: C3-C6 Bent ribs: T5-T7

Developmental Variations

- The total number of fetuses (litters) observed with developmental variations were Control—84 (26), LD—89 (25), MD—90 (23) HD—45 (13). In total, the number of variations were similar among fampridine-treated and Control groups.
- A slight dose-related increase in the number of fetuses (litters) with Sternebra #5 or #6 unossified was observed in fampridine-treated groups (LD, 41 (18); MD, 46 (18); HD 29 (10)) compared to Control (33 (16)).
- All other variations were similar among fetuses from Control and fampridine-treated dams.

TABLE 7. Summary of the Incidence of Fetal Developmental Variations*

	0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	10 mg/k
Number of litters examined	28	28	25	1
Number of fetuses examined externally	421	433	401	22
Number of fetuses examined viscerally	210	216	203	11
Number of fetuses examined skeletally	211	217	198	11
Developmental Variations Observed	**Number of fetuses (Number of Litters)**			
Renal papillae not developed	1 (1)	1 (1)	2 (2)	
Distended ureter(s)	1 (1)	3 (3)	2 (2)	2
Hyoid unossified	1 (1)	1 (1)	2 (1)	1
Skull bones reduced in ossification: jugal, parietal and/or squamosal(s)	3 (2)	1 (1)	1 (1)	1
27 presacral vertebrae		4 (1)		
Vertebral arches reduced in ossification: C3-C6				1
Greater than 13 pairs of full ribs		5 (2)		
14th rudimentary rib(s)	52 (19)	37 (17)	48 (16)	13
7th cervical rib	3 (2)	3 (2)		1
Bent rib(s): T4-T12	2 (1)	1 (1)	1 (1)	2
Sternebra #5 or #6 unossified	33 (16)	41 (18)	46 (18)	29
Other sternebra unossified: #1 or #2		1 (1)	1 (1)	
Misaligned sternebra: #4 or #5	1 (1)	1 (1)		1
Ischium reduced ion ossification			1 (1)	
Total fetuses (litters) with developmental variations	84 (26)	89 (25)	90 (23)	45

684-003 *No statistical significance observed

Necropsy: A full necropsy was performed on all dams. Maternal tissues were preserved in 10% neutral-buffered formalin for histopathological analysis, as needed. The following table summarizes the notable findings observed in HDF.

HDF Necropsy Findings

No visible abnormalities	21/30
LN, mandibular—enlarged	3 (10) mild
Lung—multilobar, discolored red	9 (30) mild-severe
Eye—cornea, cloudy	3 (10) mild-moderate

[1]Incidence/30F

3.) ***Developmental Toxicity Study in New Zealand White Rabbits***

Key study findings:

- Maternal toxicity in MDF and HDF, NOAEL = 1 mg/kg/day.
  - 2 Deaths during treatment period, MDF (necropsy—lung, red discoloration) and HDF (necropsy—lung, red discoloration).
  - Increased clinical signs including tremor, convulsion (2 HD), labored breathing, and increased activity.
  - Inhibition of both body weight gain and food consumption was apparent.
- **HD, increased incidence of whole litter resorption**.
- **Developmental toxicity,** NOAEL = 5 mg/kg/day.

Study no.: 684-005
Volume #, and page #: EDR 4.2.3.5.2.1., pp. 1-168
Conducting laboratory and location: (b) (4)

Date of study initiation: 08 October 1992
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C 4886, Purity = 100%

Methods

Doses: 0, 1, 3, and 5 mg/kg/day
Species/strain: Rabbit/New Zealand White
Number/sex/group: 20/female/group
Weight: 22.6 kg to 3.7 kg
Age: approx. 5.5 months at insemination
Route, formulation, volume, and infusion rate: Oral, gavage, 3 ml/kg
Satellite groups used for toxicokinetics: 4F/group for TK

Study design:

Housing: Rabbits were individually housed. Diet and water were available *ad libitum*.

Parameters and endpoints evaluated include mortality, clinical signs, body weight, food consumption, gross pathology of adults (parental tissues saved in 10% neutral buffered formalin for possible histopathology).

Female specific data include survival, appearance, clinical signs, body weight, food consumption, and cesarean section observations.

Fetuses from dams found dead were externally examined and discarded. Pups from dams found dead during lactation were euthanized and necropsied.

Justification of Route of Administration: Oral route intended for human administration.

Justification of Dosage Selection: Sponsor chose dose levels based on results of (b) (4) study number 684-004; doses of 3, 5, 7, 9, and 11 mg/kg/day were evaluated. Maternal toxicity (deaths and clinical signs, including convulsions) was evident at doses of ≥5mg/kg/day. All treatment groups inhibited body weight gain. Reduced fetal weight was observed at all treatment levels.

Observations, Times, and Results

Maternal observations

Mortality:

- One MDF died on GD7 and one HDF died on GD13.
  - Clinical signs included tremors (MD and HD), labored breathing and staining in the anogenital region found post-dose Day 7 (MD)
  - Necropsy findings included red discoloration of the lungs (MD and HD); and red discoloration of mammary glands and stomach noted in the MDF.

Clinical signs: Tremors were observed in MD (10/24) and HD (20/24) females, which were not found in the Control.

- On GD 8 (1 hr postdose), two HDF (#82379 and 82382) experienced convulsions, loss of righting reflex, red material around the nose; one female was also prostrate (#82382). At 4.5 hr postdose, tremors, labored breathing, and red material around the nose and mouth were also observed 4.5 hr post-dose for these two females.

Body weight: Maternal body weights were measured on GD 0, 6, 12, 19, 24 and 29. Body weights of nongravid animals were recorded on the same days but were not included in the calculation of mean body weights. [ss, $^{*}p<0.05$; $^{**}p<0.01$]

- MD and HD, dose-related, statistically significant reduction of mean maternal body weight gain relative to control were found during the treatment period (GD6-19).
  - Following cessation of treatment, mean weight gain was greater in these groups than control. These effects on body weight are illustrated in sponsor's Figure 1 (below).
- Overall body weight gain (GD 0-29), HD was reduced and statistically significant from the Control group.
- Mean body weight was significantly decreased for HDF on GD19 (-8.5%**) and GD24 (-7.3%*), but not at the end of study GD 29 (-5.7%). Mean body weight of LDF and MDF was slightly decreased (LD, -3.9% to -4.4%; MD, -5.2% to -3.5%) compared to Control on GD24 and GD29.
- Body weight in LDF was not statistically different from the Control group during the treatment period, post-treatment period or the entire gestation period.

Overall body weight gain (based on body weight minus the weight of the gravid uteri) was reduced at all doses compared to Control.

Figure 1. Mean Maternal Body Weight

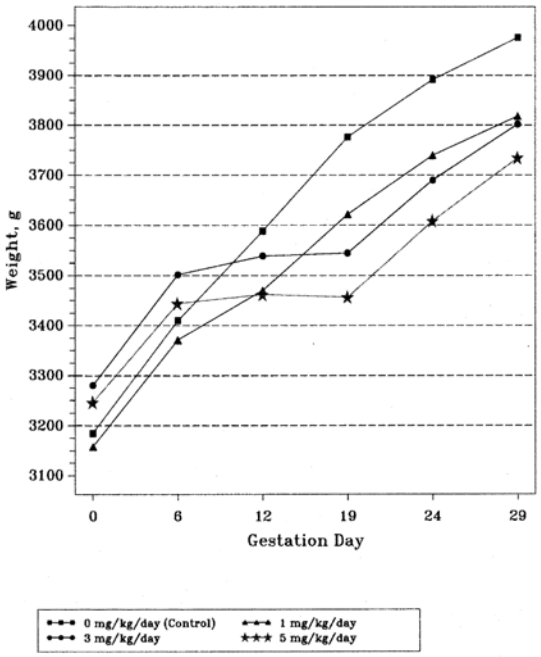
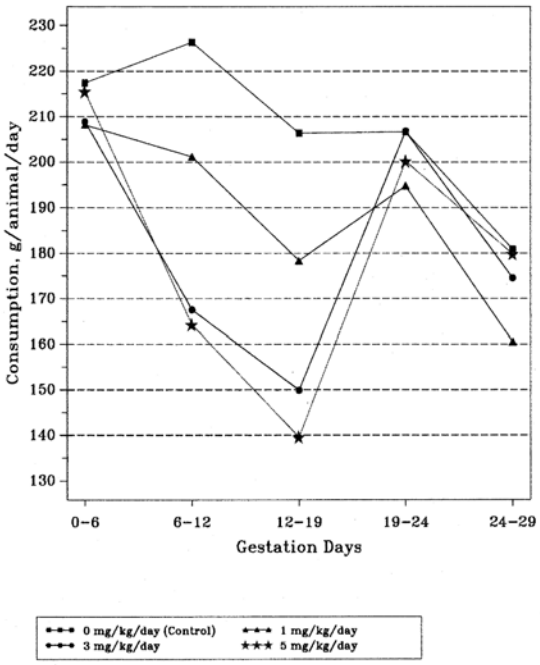


Figure 2. Mean Maternal Food Consumption



Food consumption: Food consumption was measured daily and calculated for the following intervals: GD 0-6, 6-12, 12-19, 19-24, and 24-29. Values for nongravid animals were not included in the means.

- Mean food consumption of LD, MD, and HD groups was reduced significantly, compared to the Control group during treatment intervals GD6-12, GD12-19, and GD 6-19, but were similar to controls during the intervals following treatment cessation (GD19-24 and GD24-29).
- Evaluation of the percent difference between LD, MD, and HD mean food consumption to Control is presented in the following reviewer-generated Table.

Mean food consumption as a percent of Control

GD Interval	Mean Control Consumption g/animal/day	4-Aminopyridine		
		LD	MD	HD
0-6	217.5	-4.3%	-4.8%	-0.9%
6-12	226.4	-11.1%	-26.0%	-27.4%
12-19	206.4	-13.6%	-27.3%	-32.4%
19-24	206.7	-5.8%	100%	-3.1%
24-29	180.9	-11.2%	-3.5%	-0.6%
6-19	216.3	-12.4%	-26.2%	-29.5%
0-29	209.3	-8.9%	-13%	-14.6%

Toxicokinetics: Blood samples were collected from 4 rabbits/group at 1, 2 and 4 hrs following dosing on GD14. 4-aminopyridine concentrations in plasma were evaluated by HPLC analysis and are provided in sponsor's table below.

SUMMARY OF 4-AMINOPYRIDINE LEVELS IN RABBIT PLASMA (ng/ml)

Dose Level	Animal Group	1 Hour	2 Hour	4 Hour
0 mg/kg/day (Control)	1F	0	0	0
1 mg/kg/day	2F	252 +/- 70.0	259 +/- 91.7	43.3[1]
3 mg/kg/day	3F	1410 +/- 487	592 +/- 170	136 +/- 34.1
5 mg/kg/day	4F	626 +/- 149	834 +/- 125	355 +/- 69.3

[1]Value from analysis of sample from only one animal

Terminal and necropsy evaluations: Maternal observations at cesarean section are summarized in sponsor's Table 4.

The percentage of pregnancies in the control animals (70%) was reduced when compared either to the historical control (811/910 = 89.1%) or any of the 4-aminopyridine treated groups (range = 85-90%). Due to the lack of abortions in the HD group, the abortions observed in 1 LDF and 1 MDF were considered spurious.

- Whole litter resorptions of one Control, two LDF, and three HDF occurred. Sponsor considered the 3 HDF resorptions related to 4-AP treatment.
- No adverse effects were observed in the mean number of viable fetuses, implantations, and corpora lutea, mean fetal body weight, or the fetal sex ratio.

TABLE 4. Summary of Maternal and Fetal Observations at Cesarean Section*

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	5 mg/kg/day
Animals on Study	N	20	20	20	20
Pregnant	N	14	18	17	18
	%	70.0	90.0	85.0	90.0
Aborted	N	0	1	1	0
Animals with Viable Fetuses	N	13	15	16	14
Animals with all Resorptions	N	1	2	0	3
Female Mortality	N	0	0	0	1
	%	-	-	-	5.0
Number Evaluated	N	14	17	16	17
Corpora Lutea	Mean	12.2	11.4	11.8	12.4
	S.D.	3.29	3.37	3.40	3.61
	N	13	14	16	14
	Total	158	159	188	174
Implantation Sites	Mean	6.1	7.1	5.3	5.8
	S.D.	2.56	2.23	1.95	3.09
	N	14	17	16	17
	Total	85	121	84	98
Postimplantation Loss	Mean	0.8	0.9	0.2	0.5
	S.D.	1.72	2.01	0.54	0.63
	N	14	17	16	17
	Total	11	16	3	8
Preimplantation Loss[a]	%	50.0	36.5	55.3	46.0
Postimplantation Loss[b]	%	12.9	13.2	3.6	9.2

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[a] $\frac{\text{Corpora Lutea - Implantations}}{\text{Corpora Lutea}} \times 100$ [b] $\frac{\text{Implantations - Viable Fetuses}}{\text{Implantations}} \times 100$

*No statistical significance observed
- - Not applicable
S.D. - Standard deviation
N - Number of animals

TABLE 4. Cont. Summary of Maternal and Fetal Observations at Cesarean Section*

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	5 mg/kg/day
Resorptions Total	Mean	0.8	0.9	0.2	0.5
	S.D.	1.72	2.01	0.54	0.62
	N	14	17	16	17
	Total	4	16	3	9
Early	Mean	0.8	0.8	0.2	0.5
	S.D.	1.72	2.01	0.54	0.62
	N	14	17	16	17
	Total	11	16	3	9
Late	Mean	0.0	0.1	0.0	0.0
	S.D.	0.00	0.49	0.00	0.00
	N	14	17	16	17
	Total	0	2	0	0
Dead Fetuses	N	0	0	0	0
Animals with Viable Fetuses	N	13	15	16	14
Live Fetuses	Mean	5.3	6.2	5.1	5.2
	S.D.	2.79	2.92	2.11	3.47
	N[a]	14	17	16	17
	Total	74	105	81	89
Fetal Sex Distribution					
Males	Mean	2.8	2.9	2.3	3.7
	S.D.	1.69	1.58	1.54	2.20
	N[a]	13	15	16	14
	Total	36	44	37	52
Females	Mean	2.9	4.1	2.8	2.6
	S.D.	1.80	1.83	1.34	1.60
	N[a]	13	15	16	14
	Total	38	61	44	37

684-005

*No statistical significance observed
S.D. - Standard deviation
N - Number of animals
N[a] - Number of litters

TABLE 4. Cont. Summary of Maternal and Fetal Observations at Cesarean Section*

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	5 mg/kg/day
Fetal Weights Units: grams of all Viable Fetuses	Mean	46.8	43.9	48.0	47.0
	S.D.	6.56	4.90	4.28	5.61
	N[a]	13	15	16	14
of Male Fetuses	Mean	48.4	44.6	47.1	47.7
	S.D.	4.97	4.06	4.44	5.98
	N[a]	12	13	13	13
of Female Fetuses	Mean	45.5	43.3	47.6	43.7
	S.D.	8.03	5.54	4.46	5.07
	N[a]	13	15	15	11
Uterine Weight Units: grams	Mean	361.6	429.8	344.6	406.4
	S.D.	133.56	89.39	119.30	141.44
	N	13	15	16	14

684-005

*No statistical significance observed
S.D. - Standard deviation
N - Number of animals
N[a] - Number of litters

Offspring:

Malformations

- Malformations observed in treated groups were present only in single incidences, and total incidences of malformations in the treated groups were slightly less than the Control. These findings are presented in sponsor's Table 5 below.

TABLE 5. Summary of the Incidence of Fetal Malformations

	0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	5 mg/kg/day
Number of litters examined:	13	15	16	14
Number of fetuses examined externally:	74	105	81	89
Number of fetuses examined viscerally:	74	105	81	89
Number of fetuses examined skeletally:	74	105	81	89
Malformations Observed	**Number of fetuses (Number of Litters)**			
Omphalocele		1 (1)		
Interventricular septal defect			1 (1)	
Bulbous aortic arch				1 (1)
Gallbladder agenesis				1 (1)
Vertebral malformation with or without an associated rib malformation: T7 centrum, T7 arch (one side), T9 arch, L2 arch, T7 rib (one side) or T9 rib absent; T9, T11 or L2 centrum malformed, misshapen, present on one side only; T7 arch, T8, T9, T10, T11 and/or T12, T13 and L2 centra malformed, misshapen, [one side] smaller than normal; T10 centrum or T13 and L1 arches malformed, misshapen, larger than normal; T7 and T8 ribs, T12 and T13 centra or L1 and L2 centra fused; T6, T7, T8, T9, T10, T11, T12 and/or T13 vertebrae misaligned; T6 rib malformed, misshapen, forked	3 (3)		1 (1)	1 (1)
Rib malformation: T9 and T10 ribs malformed, misshapen, fused		1 (1)		
Fused sternebrae: #3, #4 and/or #5	4 (3)			1 (1)
Total fetuses (litters) with malformations:*	7 (5)	2 (2)	2 (2)	4 (4)

684-005 *No statistical significance observed

Developmental Variations

- Unremarkable. Sponsor considered the total number of litters with any developmental variation to be comparable between control and treated groups; and thus, any variation observed was a function of normal biological variability and unrelated to treatment (see sponsor's Table 6, below).

TABLE 6. Summary of the Incidence of Fetal Developmental Variations

	0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	5 mg/kg/da
Number of litters examined:	13	15	16	14
Number of fetuses examined externally:	74	105	81	89
Number of fetuses examined viscerally:	74	105	81	89
Number of fetuses examined skeletally:	74	105	81	89
Developmental Variations Observed	**Number of fetuses (Number of Litters)**			
Left carotid arises from the innominate	22 (7)	36 (10)	6 (4)	18 (8)
Retroesophageal right subclavian	3 (1)			
Azygous lobe of lung absent	5 (5)	9 (5)	5 (3)	5 (4)
Gallbladder hypoplasia		6 (3)	2 (1)	1 (1)
Malpositioned kidney	2 (2)			
Accessory adrenal gland		1 (1)		
Hyoid arch(es) bent	6 (5)	8 (5)	12 (7)	4 (2)
Hyoid arch unossified	1 (1)			
25 presacral vertebrae		1 (1)		
27 presacral vertebrae	19 (6)	27 (11)	11 (7)	28 (10)
28 presacral vertebrae	1 (1)			
Extra vertebral ossification site				1 (1)
Greater than 12 pairs of full ribs	29 (10)	46 (14)	25 (10)	54 (13)
13th rudimentary rib(s)	15 (8)	14 (8)	25 (12)	12 (9)
7th cervical rib(s)	10 (5)	8 (5)	5 (4)	5 (5)
8th cervical ribs	1 (1)			
Sternebra #5 or #6 unossified	8 (7)	5 (4)	4 (3)	6 (3)
Misaligned sternebra: #5	2 (2)			
Extra sternebral ossification site(s)	3 (3)			1 (1)
Pubic bones unossified		1 (1)		
Total fetuses (litters) with developmental variations:*	64 (12)	92 (15)	64 (15)	76 (14)

684-005 *No statistical significance observed

- Although sponsor has concluded the incidence of developmental variations to be within historical control ranges, this reviewer noted two findings:
    - HD fetuses (54 from 13 litters) had greater than 12 pairs of full ribs compared to Control (29 from 10 litters) and an extra vertebral ossification site found in one fetus in a HD litter appears to be a relatively rare variation as it was observed in the historical control data in two fetuses from 2 litters from over 50 studies.

Prenatal and postnatal development

4.) **Study for Toxic Effects on Pre- and Postnatal development including maternal function in rats**

Key study findings:

Adequacy of the prenatal and postnatal development study and appropriateness of the test model: the prenatal and the postnatal development study were adequate. Doses selected for this study were 1, 3, and 9/6 mg/kg. Clear dose-related maternal toxicities included deaths (8 HDF within first week of dosing resulted in dose reduction from 9 mg/kg/day to 6 mg/kg/day; 1 death occurred in HDF at 6 mg/kg/day) and convulsions (HDF). Both MDF and HDF showed signs of tremors, decreased mean body weight [ss], body weight gain ([ss], LDF, MDF, HDF), and food consumption [ss] during gestation and lactation. Thus, clear dose-limiting toxicities were established. Reduced offspring viability and decreased mean pup body weight [ss] occurred in pups born from HDF compared to CF.

- NOAELs established in this study
  - Maternal toxicity = 1 mg/kg/day
  - Reproductive capability of F0 and F1 animals = 3 mg/kg/day
  - Behavior and development = 6 mg/kg/day

Study no.: 684-006
Volume #, and page #: EDR, 4.2.3.5.1.1., pp. 1-533.
Conducting laboratory and location: (b) (4)
Date of study initiation: 07 May 1994
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: 4-Aminopyridine, C4886, Purity assumed 100%

Methods

Doses: 0, 1, 3, and 9/6 mg/kg/day, the HD group was lowered from 9 to 6 mg/kg/day on GD 11 due to significant deaths.
Species/strain: Rat/Sprague-Dawley derived Charles River Crl:CD® VAF/Plus®
Number/sex/group: 30 mated females/group
Route, formulation, volume, and infusion rate: Oral, gavage, 10 ml/kg
Satellite groups used for toxicokinetics: None.

Study design: F_0 dosed beginning on GD7 through lactation Day 21. F_0 were evaluated for signs, weight, food, pregnancy duration/parturition, and necropsy. F_1 generation were reared to reproductive maturity and were evaluated for number, viability, weight, physical development, sensory/motor functions, learning/behavior/memory and fertility. F_1: Day 1 postpartum selected for behavioral testing, reproductive trial; cesarean examination of F_1 females on GD20 and F_2 fetuses for external morphological observations. The F_2 generation was evaluated to assess fertility of the F_1 offspring and for any external morphological observations.

Toxicokinetics: not conducted in this experiment, please refer to TK bridging study data.

Housing: Rats were individually housed. Diet and water were available *ad libitum*.

Justification of Route of Administration: Oral route intended for human administration.

Justification of Dosage Selection: Sponsor chose dose levels based on available data from previous studies.

Observations, Times and Results:

Dosage formulation: Dosing formulations prepared for use during study weeks 1 and 6.

- 4-Aminopyridine dosing formulations were acceptable with mean values of 97% to 99% and 99% to 100% of the target concentrations.

F_0 Survival, Appearance, and Behavior

Mortality: Animals checked daily, for mortality and for overt signs of toxicity.

Gestation:

- 8 HDF died during 1st week of dosing; therefore, the dose level was reduced from 9 mg/kg/day to 6 mg/kg/day (beginning GD 11). At 6 mg/kg/day, one dam died on GD 23.
- Test article-related clinical signs were the following: dose-related tremors in MDF and HDF, 3 animals (4 occurrences) and 15 animals (22 occurrences), respectively.
- HDF, 2 animals with abnormal gait and abnormal vocalization in 1 animal; ptyalism in 5 animals and dilated pupils in one animal.

Lactation:

- No deaths occurred.
- HDF—Tremors observed in 12; animals experienced convulsions, exposed skin area pale, and excessive lacrimation. Material around nose and ptyalism found for MDF (1/30) and HDF (4/30).

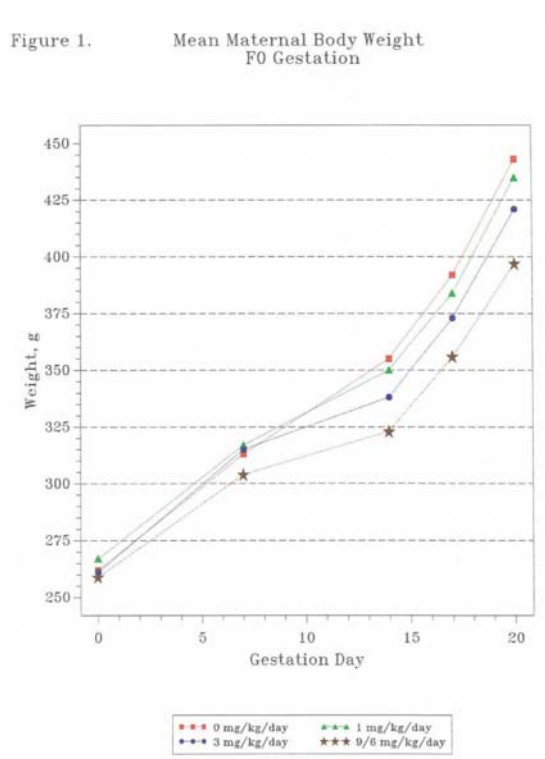
Necropsy:

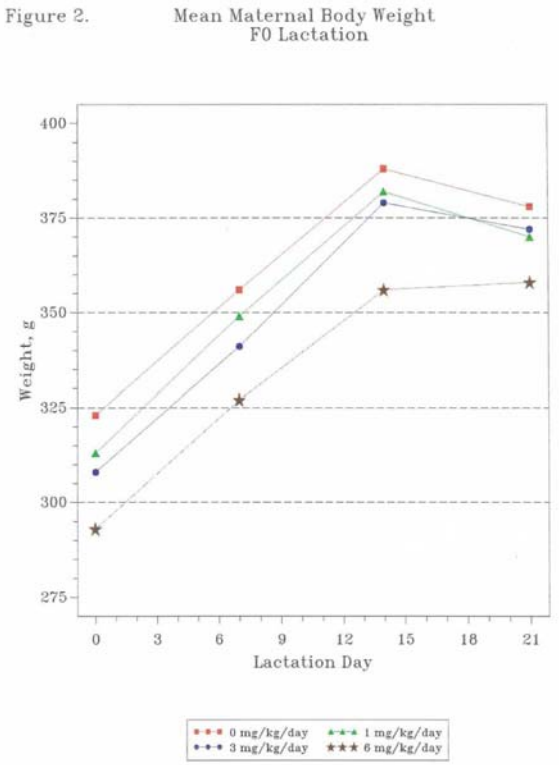
- HDF, lungs congested, multilobar (2/30) and/or multilobar, diffuse, red, discolored lungs (5/30) were observed and considered test article-related.
    - Severity—congested lungs graded moderate (#76414) or severe (#76411); and lung discoloration graded mild (3/5, #76403, 76405, 76415), moderate (1/5, #76407) and severe (1/5, #76411).
- No other findings were considered test article-related due to lack of a dose response relationship and/or occurred in only one animal.

F_0 Body weights

The following figures provided by the sponsor illustrate the decreased body weight observed in MDF and HDF during Gestation (Figure 1) and Lactation (Figure 2) and in the reviewer generated tables below.

- Significantly decreased body weight was noted during both gestation and lactation periods for the MDF and HDF, [$^{*}p<0.05$, $^{**}p<0.01$].





Maternal body weight (Mean ± SD, n) of Controls: % Control (n) for treated dams during gestation and lactation.

GD or LacD	Control	LD	MD	HD
GD0	262.3 ± 14.7 (27)	+1.6% (28)	-0.4% (23)	-1.4% (26)
GD7	313.3 ± 16.3 (27)	+1.3% (28)	+0.4% (23)	-2.9% (26)
GD14	354.5 ± 19.9 (27)	-1.2% (28)	-4.7%** (23)	-8.8%** (20)
GD17	391.7 ± 22.5 (27)	-1.9% (28)	-4.7%* (23)	-9.1%* (20)
GD20	443.4 ± 25.1 (27)	-1.9% (28)	-5.0%** (23)	-10.4%** (20)
LacD0	322.7 ± 16.8 (27)	-3.0% (28)	-4.7%** (22)	-9.1%** (19)
LacD7	356.4 ± 16.9 (27)	-2.0% (28)	-4.4%** (22)	-8.2%** (19)
LacD14	387.6 ± 19.6 (27)	-1.4% (28)	-2.2% (22)	-8.2%** (19)

LacD21	377.6 ± 19.6 (27)	-2.0% (28)	-1.4% (22)	-5.1%** (19)

Maternal body weight change (Mean ± SD, n) of Controls: % Control (n) for treated dams during gestation and lactation.

GD or LacD	Control	LD	MD	HD
GD_{0-7}	51 ± 7.6 (27)	-0.2% (28)	+5.1% (23)	-8.4% (26)
GD_{7-14}	41.1 ± 9.7 (27)	-20%** (28)	-44%** (23)	-49.9%** (20)
GD_{14-17}	37.2 ± 6.6 (27)	-9.1% (28)	-4.6% (23)	-11.6% (20)
GD_{17-20}	51.7 ± 7.0 (27)	-1.3% (28)	-7.4% (23)	-20.3%** (20)
$LacD_{0-7}$	28.3 ± 2.3 (27)	-5.6%* (28)	-20.1%** (23)	-32.5%** (20)
$LacD_{7-14}$	32.1 ± 2.3 (27)	-4.4% (28)	-9.3%** (23)	-17.1%** (20)
$LacD_{14-21}$	33.1 ± 2.2 (27)	-5.7%* (28)	-10.3%** (23)	-17.2%** (20)

[ss] from control by Anova and pairwise comparison (Dunnett-test), *p<0.05, **p<0.01

Figure 3. Mean Maternal Food Consumption F0 Gestation

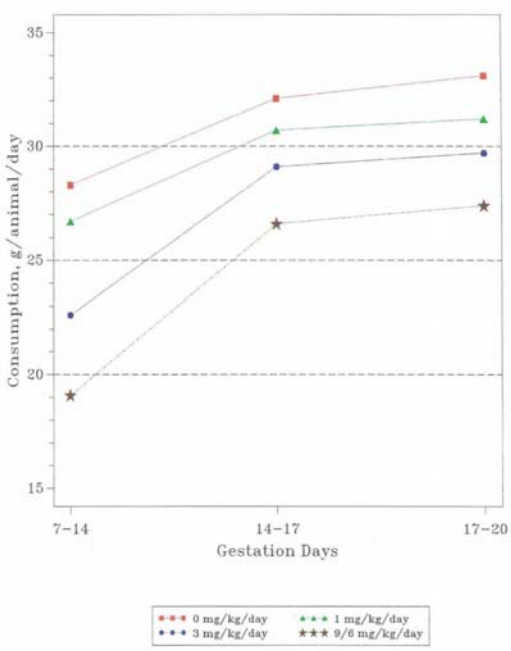
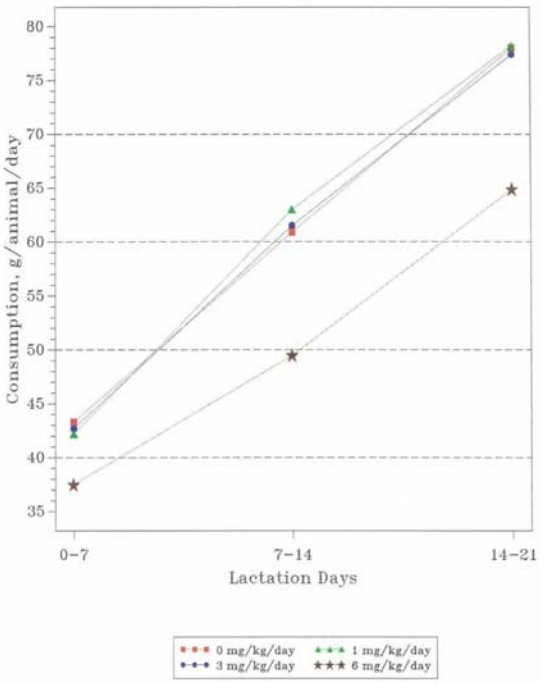


Figure 4. Mean Maternal Food Consumption F0 Lactation



- Maternal food consumption was decreased between 4-aminopyridine-treated groups compared to Control. [ss, *p<0.05; **p<0.01]
  - GD_{7-14} and $GD1_{7-20}$, all dose groups LD*, MD**, and HD**.

- GD_{14-17}, MD** and HD**
- $LacD_{7-14}$ and $LacD_{14-20}$, HD**

F_0 Reproductive parameters and F_1 generation, viability, and body weight [ss, *p<0.05 and **p<0.01].

- MDF_0
  - Decreased pup weight/litter* on Days 4 and 35.
- HDF_0
  - Decreased number of live born F1 pups*
  - Increased stillborn pups** (+300%)
  - Pups dying, missing or cannibalized** during Days 1-4 (-45.7% compared to Control)
  - Decreased number (-8.2%)** and weight** (-14.7%) of live pups/litter on Days 4-35.
- Sponsor considered the MDF and HDF effects on pup weight/litter as test article-related.

These results were summarized in sponsor's Table 13, below.

Table 13 Natural F0 Delivery Data and F1 Litter Data Summary

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	9/6 mg/kg/day
Females on Study	N	30	30	30	30
Females Mated	N	30 f	30	30	30
Mating Index	%	100.0	100.0	100.0	100.0
Females Pregnant	N	27 f	28	23	26
Female Fertility Index	%	90.0	93.3	76.7	86.7
Females with Liveborn	N	27 f	28	22	19*
Gestation Index	%	100.0	100.0	95.7	73.1
Pups Surviving Days 4 to 21	N	202 f	212	167	113
Weaning Index	%	51.0	50.8	50.3	52.6
Females Surviving Delivery	N	27 f	28	22	19
	%	90.0	93.3	73.3	63.3
Duration of Gestation	Mean	22.11 d	22.11	21.95	22.26
	S.D.	0.42	0.31	0.21	0.45
with Stillborn Pups	N	3 f	7	4	6
	%	11.1	25.0	18.2	31.6
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
with Entire Liveborn Litter Dying and/or Missing, Cannibalized					
days 0-4	N	0 f	0	0	2
	%	0.0	0.0	0.0	10.5
days 5-21	N	0 f	0	0	1
	%	0.0	0.0	0.0	5.3
days 0-21	N	0 f	0	0	3
	%	0.0	0.0	0.0	15.8

684-006 Statistical key: d=ANOVA + Dunnett-test f=Chi-square + Fishers exact test * = p<0.05

Table 13 Cont. Natural F0 Delivery Data and F1 Litter Data Summary

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	9/6 mg/kg/day
Litters with Liveborn Pups	N	27	28	22	19
Pups Delivered (total)	N	412	446	354	273
	Mean	15.26 d	15.93	16.09	14.37
	S.D.	2.75	1.92	2.18	3.24
Liveborn	N	409 f	437	350	261**
Live Birth Index	%	99.3	98.0	98.9	95.6
Stillborn	N	3 f	9	4	12**
	%	0.7	2.0	1.1	4.4
Uncertain	N	0	0	0	0
Cannibalized	N	0	0	0	0
Missing	N	6	15	13	30
Died	N	12	8	6	23
Liveborn, not culled prior to day 21	N	409	437	350	261
Pups Dying, Missing, and/or Cannibalized					
day 0	N	5 f	0	2	1
	%	1.2	0.0	0.6	0.4
days 1-4	N	8 f	20	16	45**
	%	2.0	4.6	4.6	17.2
days 5-7	N	3 f	1	1	7
	%	0.7	0.2	0.3	2.7
days 8-14	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 15-21	N	1 f	0	0	0
	%	0.2	0.0	0.0	0.0

684-006 Statistical key: d=ANOVA + Dunnett-test f=Chi-square + Fishers exact test ** = p<0.01

Table 13 Cont. Natural F0 Delivery Data and F1 Litter Data Summary

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	6 mg/kg/day
Pup Weight/Litter (grams)					
day 0	Mean	6.53 d	6.29	6.26	6.31
	S.D.	0.68	0.45	0.41	0.56
	N	26	28	22	19
day 4	Mean	10.69 d	10.11	9.79*	9.34**
	S.D.	1.42	1.19	1.09	1.08
	N	27	28	22	17
day 7	Mean	17.65 d	16.82	16.40	15.06**
	S.D.	2.20	2.25	1.98	1.32
	N	27	28	22	16
day 14	Mean	37.08 d	35.52	34.61	31.88**
	S.D.	3.79	4.68	2.82	1.46
	N	27	28	22	16
day 21	Mean	60.70 d	58.28	57.41	54.27**
	S.D.	6.36	7.24	4.51	3.43
	N	27	28	22	16
day 28	Mean	101.23 d	97.35	95.20*	91.22**
	S.D.	8.42	10.74	6.40	4.91
	N	27	28	22	16
day 35	Mean	153.74 d	150.86	147.19	141.06**
	S.D.	11.41	14.05	8.14	6.52
	N	27	28	22	16
Sex Ratio - Male Pups:Total Pups					
day 0	N	206 f	223	181	128
	%	50.4	51.0	51.7	49.0
day 21	N	105 f	105	83	54
	%	52.0	49.5	49.7	47.8

684-006 Statistical key: d=ANOVA + Dunnett-test f=Chi-square + Fishers exact test * = $p \leq 0.05$ ** = $p \leq 0.01$

Table 13 Cont. Natural F0 Delivery Data and F1 Litter Data Summary

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	9/6 mg/kg/day
Pups Surviving 4 days	N	396 f	417	332	215**
Viability Index	%	96.8	95.4	94.9	82.4
Pups Surviving 21 days	N	202 f	212	167	113
Lactation Index	%	51.0	50.8	50.3	52.6
Implantation Sites	N	453	487	393	316
per Litter	Mean	16.78 d	17.39	17.86	16.63
	S.D.	3.13	1.55	1.93	2.43
Live Pups/Litter					
day 0	Mean	15.15 d	15.61	15.91	13.74
	S.D.	2.77	2.04	2.18	3.41
day 4	Mean	14.67 d	14.89	15.09	11.32**
	S.D.	2.51	2.60	1.80	5.30
day 7	Mean	7.52 d	7.57	7.59	5.95**
	S.D.	0.85	1.03	0.59	3.12
day 14	Mean	7.52 d	7.57	7.59	5.95**
	S.D.	0.85	1.03	0.59	3.12
day 21	Mean	7.48 d	7.57	7.59	5.95**
	S.D.	0.89	1.03	0.59	3.12
day 28	Mean	7.44 d	7.50	7.59	5.95**
	S.D.	0.89	1.23	0.59	3.12
day 35	Mean	7.44 d	7.50	7.59	5.95**
	S.D.	0.89	1.23	0.59	3.12

684-006 Statistical key: d=ANOVA + Dunnett-test f=Chi-square + Fishers exact test ** = $p \leq 0.01$

F_0 Uterine examination observations on Lactation Day 21

- Unremarkable, no significant differences in the mean number of implantation sites/litter.

F_1 Behavioral and Developmental Indices

Behavioral and developmental indices were measured in the F_1 generation from the first 4 male and 4 female pups/litter. Each evaluation was performed at specific time points throughout development and these are summarized in the reviewer-generated table, below.

Behavior/Developmental Indices	**Initial day of observation**	**Last day of observation**	**Evaluation**
Static righting reflex	LD 2	Until present	15 sec
Pinna detachment	LD 2	Until present	
Cliff aversion (before eyes open)	LD 11	Until present	Animal moves away from edge
Eye opening	LD 13	Until present	Both eyes open
Air drop, righting reflex	LD16	Until present	
Neuropharmacologic evaluation[a]	LD 21	LD 21 (after weaning)	Observations-Irwin (minimum 1 min.)
Auditory response[a]	PND 33	PND 33	3 trials
Rotarod performance[a]	PND D34	PND 34	3 trials (1 min w/out falling off rod)
Vaginal opening	PND 34	Until present	
Balanopreputial cleavage	PND 40	Until present	Balanopreputial separation
Activity and emotionality[a]	PND 35		1 pup/sex/litter for 20 minutes emotionality (1st 5 min period) activity (all 20 min)
Step-through passive avoidance	PND 70-77	PND 79	

LD = Lactation Day; PND = Post-natal Day
[a]Any animal not achieving the activity and emotionality response by weaning, neuropharmacologic response, auditory or rotarod performance was euthanized.

The results of the studies described above were provided in sponsor's Tables 15-27 that showed no significant 4-aminopyridine-related effects. Shown below is sponsor's Table 15 that summarizes the group results of the reflex and physical development evaluations.

Table 15 F1 Pup Reflex and Physical Development Summary*

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	6 mg/kg/day
Static righting reflex	Mean	2.63 d	2.63	2.57	2.43
	S.D.	0.36	0.43	0.40	0.44
	Number of Litters	27	28	22	17
	Number of Pups	206	220	172	122
	pups reaching criteria, %	100	99	100	100
Pinna detachment	Mean	2.49 d	2.58	2.70	2.43
	S.D.	0.37	0.44	0.51	0.54
	Number of Litters	27	28	22	17
	Number of Pups	206	220	172	122
	pups reaching criteria, %	100	98	100	99
Cliff aversion	Mean	11.26 d	11.24	11.23	11.21
	S.D.	0.25	0.27	0.21	0.26
	Number of Litters	27	28	22	15
	Number of Pups	203	212	167	105
	pups reaching criteria, %	100	100	100	100
Eye opening	Mean	13.90 d	14.03	14.14	14.19
	S.D.	0.51	0.58	0.43	0.61
	Number of Litters	27	28	22	16
	Number of Pups	203	212	167	113
	pups reaching criteria, %	100	100	100	100
Air drop righting reflex	Mean	16.23 d	16.28	16.32	16.40
	S.D.	0.23	0.21	0.29	0.32
	Number of Litters	27	28	22	16
	Number of Pups	202	212	167	113
	pups reaching criteria, %	100	100	100	100

684-006 Statistical key: d=ANOVA + Dunnett-test
*No statistical significance observed

Table 15 Cont. F1 Pup Reflex and Physical Development Summary*

		0 mg/kg/day (Control)	1 mg/kg/day	3 mg/kg/day	6 mg/kg/day
Neuropharmacological Evaluation					
Number of Pups Tested		202	212	167	113
Number of Pups Within					
Normal Limits	N	202	209	164	111
	%	100	98.6	98.2	98.2
Urination	N		2	3	2
	%		0.9	1.8	1.8
Ataxia	N		1		
	%		0.5		
Righting reflex	N		1		
	%		0.5		
Auditory response	Mean	33.00	33.00	33.00	33.00
	S.D.	0.00	0.00	0.00	0.00
Number of Litters		27	28	22	16
Number of Pups		195	210	167	113
pups reaching criteria, %		100	100	100	100
Rotarod Performance	Mean	38.3	36.0	39.6	38.6
	S.D.	11.2	8.3	10.4	9.6
Number of litters		27	28	22	16
Number of pups		201	210	168	113
Pups reaching criteria, %		72.6	67.1	64.9	69.0
Vaginal opening	Mean	34.27 d	34.22	34.16	34.29
	S.D.	0.28	0.32	0.21	0.39
Number of Litters		27	28	22	16
Number of Pups		96	106	83	59
pups reaching criteria, %		97	96	95	92
Balanopreputial Cleavage					
Number of litters		25	25	22	16
Number of pups		25	25	24	25
Pups reaching criteria, %		100	100	100	100

684-006 *No statistical significance observed

F_1 General observations

Antemortem and necropsy evaluations: Unremarkable.

- Necropsy of F1 pups failed to show any differences in organs or tissues among the various dose groups compared to Controls.

F_1 Body weights

- HDM and HDF, F_1 pups had [ss] reduced weight gains (M, from weeks 7-19; F, from weeks 7-13).
- HD F_1 that were pregnant had [ss] reduced body weight gain during GD0-14.

F_1 Cesarean examinations

- Unremarkable, 16-23 litters/group were evaluated. No significant differences were noted between Control and treated groups on the following parameters: corpora lutea, implantation sites, preimplantation losses, live fetuses, postimplantation losses, dead fetuses, resorptions, placental weights, and fetal weights.

F_2 Fetal external morphological observations

- Unremarkable. No significant differences were found among groups.

2.6.6.7 Local tolerance

No studies conducted.

2.6.6.8 Special toxicology studies

Acorda Therapeutics submitted three additional study reports to qualify a known impurity in the drug product— (b) (4) also known as (b) (4) : two *in vitro* genetic toxicology studies (Ames test and chromosomal aberrations) and a 28-Day repeat dose toxicity study in rats.

Impurity Qualification

Three drug substance impurities were identified (b) (4) . Five drug product impurities were identified (b) (4) The chemical structures are provided in the table below.

Chemical Structure	Name	Source	Impurity/Degradant
(b) (4)			

Chemical Structure	Name	Source	Impurity/Degradant
[redacted]			

(b) (4)

The sponsor noted that some of these impurities were formed from interactions between fampridine and the excipients, (b) (4) and (b) (4) To determine whether additional testing of these impurities was necessary, the sponsor conducted literature searches and performed computational analysis using DEREK for Windows (v. 11.0). Literature searches conducted in the following databases: NLM MEDLINE/TOXLINE databases (back to 1965), National Toxicology Program, EPA Website, European Commission Joint Research Centre, ESIS: European Chemical Substances Information System failed to produce any useful scientific articles. Results from the DEREK for Windows analyses performed for mutagenicity, chromosome damage, genotoxicity and carcinogenicity using the five structures above also failed to produce any alerts for genotoxicity, mutagenicity, chromosome damage, and carcinogenicity. Thus, the sponsor considered 4 of the identified impurities (b) (4) to have no structural alerts and to not require qualification due to their presence at ≤0.2%, compliant with ICH Q3B. The amount of (b) (4) exceeded the qualification threshold of 0.5%, and therefore, the sponsor conducted three studies to qualify 4-AP spiked with 2% (b) (4)—an *in vitro* Ames test, an *in vitro* chromosomal aberration study using CHO cells, and a 28-day oral gavage repeat dose toxicity study in rat.

The specification limit of the (b) (4) was set at (b) (4), which is greater than the 0.5% permitted. For qualification, fampridine was spiked with the (b) (4) (2%) impurity.

1.) ***Salmonella-Escherichia coli*/Mammalian-microsome reverse mutation assay with a confirmatory assay with Fampridine with 2%** (b) (4)

Study no.: 23807-0-409oecd

Volume #, and page #: 4.2.3.7.6.1 Legacy Study Report, Study 23807-0-409oecd, pp. 1-26.
Conducting laboratory and location: (b) (4)
Date of study initiation: 24 April 2002
GLP compliance: Yes
QA reports: yes (X) no ( )

Drug, lot #, and % purity: Fampridine, Batch No. 0 (b) (4) retest date (b) (4) purity not listed; (b) (4), Lot No. 4-AP (b) (4) 2/13022002, purity not listed.

Methods

Strains/species/cell line: *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 *uvr*A.

Basis of dose selection: Range finding study determined the cytotoxicity of 4-aminopyridine +2% (b) (4) (± S9) for tester strains TA100 and WP2uvrA. Concentrations of the test article ranged from 6.67 to 5000 µg/plate and 2% (b) (4) was added to each concentration. No cytotoxicity was observed with either tester strain in the presence or in the absence of S9 as evidenced by normal background lawns and no concentration-related decreases in the number of revertants/plate.

Negative control: deionized water (vehicle control).

Positive controls: benzo[a]pyrene (+S9 mix, TA98), 2-aminoanthracene (+S9 mix, TA100, TA1535, TA1537, and WP2*uvr*A), 2-nitrofluorene (-S9 mix, TA98), sodium azide (-S9 mix, TA100 and TA1535), ICR-191 (-S9 mix, TA1537), and 4-nitroqunioline-N-oxide (-S9 mix, WP2*uvr*A).

S9 Mix: The S9 mix was prepared from male SD rats that had been injected with Aroclor™ 1254.

Incubation and sampling times: Tester strains were exposed to the test article for 52 ± 4 hr and incubations occurred at 37 ± 2 ºC.

Criteria: According to the sponsor, the following criteria were utilized:

1. A result was considered positive for tester strains TA98, TA100, and WP2*uvr*A when there was at least a 2-fold increase in the mean revertants/plate relative to its respective negative control and the increase in the mean number of revertants per plate was concentration-related.
2. A result was considered positive for tester strains TA1535 and TA1537 when there was at least a 3-fold increase in the mean revertants per plate relative to the respective negative control and the increase in the mean number of revertants per plate was concentration-related.

Results

Study validity: The assay as conducted met the sponsor's criteria for a valid study. All tester strains had normal background lawns in the presence of increasing concentrations of 4-AP+2% (b) (4); and no precipitate was observed.

Study outcome: Three mutagenicity assays were conducted Experiment 23807-B1—initial mutagenicity test, Experiment 23807-C1—confirmatory mutagenicity assay, and Experiment 23807-D1—repeat mutagenicity assay. Tester strains were cultured with 4-AP at 100, 333, 1000, 3330 and 5000 µg/plate and 2% (b) (4) (2% of each 4-

AP concentration). Summaries of these data were reproduced from sponsor's Tables 4, 6 and 7 (see below).

- 4-Aminopyridine spiked with 2% (b) (4) was negative in the absence and presence of metabolic activation.

Table 4 : Mutagenicity Assay Results – Summary

Test Article ID: Fampridine with 2% (b) (4)

Experiment ID: 23807-B1

Date Plated: 30-May-02 Vehicle: Water

Date Counted: 03-Jun-02 Plating Aliquot: 200 µL

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		37	3	130	17	17	2	11	5	21	4	N
Test Article	100 µg	34	1	117	20	15	4	14	5	19	7	N
	333 µg	37	6	117	10	15	2	11	4	16	1	N
	1000 µg	39	6	114	13	12	4	10	4	23	3	N
	3330 µg	38	4	114	10	17	2	12	1	17	2	N
	5000 µg	34	4	132	5	17	3	12	2	20	3	N
Positive Control[b]		523	17	707	39	198	30	147	10	366	26	N
Microsomes: None												
Vehicle Control		25	7	112	23	25	2	6	4	18	2	N
Test Article	100 µg	29	5	111	2	13	6	9	0	14	2	N
	333 µg	20	3	111	3	23	6	7	3	21	4	N
	1000 µg	18	7	108	5	20	1	9	2	15	1	N
	3330 µg	21	4	113	10	23	5	10	4	17	4	N
	5000 µg	24	6	111	9	15	9	9	2	19	1	N
Positive Control[c]		359	37	1439	66	969	34	1053	74	283	32	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate	WP2*uvr*A	4-nitroquinolone-N-oxide	1.0 µg/plate

Table 6 : Mutagenicity Assay Results – Summary

Test Article ID: Fampridine with 2% (b) (4)

Experiment ID: 23807-C1

Date Plated: 11-Jun-02 Vehicle: Water

Date Counted: 14-Jun-02 Plating Aliquot: 200 µL

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
		TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		26	7	93	7	21	6	14	4	25	2	N
Test Article	100 µg	21	4	98	14	23	5	8	1	17	9	N
	333 µg	26	2	88	9	14	5	7	1	10	2	N
	1000 µg	26	2	94	14	15	3	11	5	19	3	N
	3330 µg	22	5	87	6	17	6	9	2	16	5	N
	5000 µg	19	4	88	3	12	4	6	1	15	4	N
Positive Control[b]		292	39	502	134	112	2	126	6	612	204	N
Microsomes: None												
Vehicle Control		18	8	143	12	63	12	7	1	15	3	N
Test Article	100 µg	16	2	254	117	NC	-	7	1	21	7	N
	333 µg	22	5	149	15	NC	-	6	4	19	5	N
	1000 µg	20	3	152	19	NC	-	8	1	15	7	N
	3330 µg	17	2	130	18	NC	-	6	2	18	6	N
	5000 µg	19	4	159	14	NC	-	4	2	14	4	N
Positive Control[c]		319	20	1206	133	868	67	695	75	147	50	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate	WP2*uvr*A	4-nitroquinolone-N-oxide	1.0 µg/plate

NC = No count due to unacceptable mean vehicle control value. See retest data, Table 7.

Table 7 : Mutagenicity Assay Results – Individual Plate Counts and Summary

Test Article ID: Fampridine with 2% (b) (4)

Experiment ID: 23807-D1

Date Plated: 10-Jul-02 Vehicle: Water

Date Counted: 12-Jul-02 Plating Aliquot: 200 µL

	Dose/Plate	Revertants Per Plate TA100 1	TA100 2	TA100 3	TA1535 1	TA1535 2	TA1535 3	Background Lawn[a]
Microsomes: None								
Vehicle Control		72	68	68	3	8	7	N
Test Article	100 µg	58	73	67	10	6	9	N
	333 µg	84	78	90	7	9	9	N
	1000 µg	77	67	72	11	8	6	N
	3330 µg	64	64	72	9	17	15	N
	5000 µg	66	80	50	7	8	14	N
Positive Control[b]		1014	885	962	774	736	661	N

	Dose/Plate	Mean Revertants Per Plate with Standard Deviation TA100 Mean	TA100 S.D.	TA1535 Mean	TA1535 S.D.	Background Lawn[a]
Microsomes: None						
Vehicle Control		69	2	6	3	N
Test Article	100 µg	66	8	8	2	N
	333 µg	84	6	8	1	N
	1000 µg	72	5	8	3	N
	3330 µg	67	5	14	4	N
	5000 µg	65	15	10	4	N
Positive Control[b]		954	65	724	58	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate

2.) **Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with Fampridine Spiked with 2%** (b) (4)

Study no.: 23807-0-437OECD
Volume #, and page #: 4.2.3.7.6.1 Legacy Study Report, Study 23807-0-437oecd, pp. 1-29.
Conducting laboratory and location: (b) (4)
Date of study initiation: 24 April 2002
GLP compliance: Yes

QA reports: yes (X)
Drug, lot #, and % purity: Fampridine, Batch No. 000000 (b) (4), purity not listed; (b) (4), Lot No. 4-AP (b) (4) 2/13022002, purity not listed

Strains/species/cell line: Chinese Hamster Ovary cells (CHO-WBL).

Basis of concentration selection: Range-finding study.
Range-finding Assay: Potential cytotoxicity of 4-AP was tested in a range-finding assay (± S9). 4-AP concentrations used for this study were 9.73, 13.9, 19.9, 28.4, 40.5, 57.8, 82.6, 118, 168, 240, 343, 490, 700, and 1000 µg/mL; and the (b) (4) was present in the same cultures at concentrations of 0.137, 0.195, 0.279, 0.398, 0.568, 0.812, 1.16, 1.65, 2.35, 3.36, 4.80, 6.86, 9.80, 14.0, and 20.0 µg/mL.

Doses used in definitive study:

Non-activating Assay, 19.8 hr harvest: Fampridine 31.3, 62.5,125,188,250,375,500,750, and 1000 µg/ml and (b) (4) (2%) was present in the same cultures at concentrations of 0.625, 1.25, 2.5, 3.75, 5.0, 7.5, 10, 15, and 20 µg/ml respectively.

Activating Assay, 3 hr harvest: In the presence of rat S9 mix, fampridine 125, 250, 500, 750, and 1000 µg/ml and (b) (4) (2%) was present in the same cultures at concentrations of 2.5, 5.0, 10, 15, and 20 µg/ml, respectively.
Negative controls: vehicle control = cell culture grade water, in the absence and presence of metabolic activation.

Positive controls: -S9 mix, mitomycin C (3 hr: 0.75 and 1.0 µg/ml and 19.8 hr: 0.2 to 0.4 µg/ml); +S9 mix, cyclophosphamide (5 and 10 µg/ml).

Incubation and sampling times: Initial chromosome aberrations assay was conducted at 3 hours (± S9) and harvested after 20 hours. In the repeat assay, treatment was 19.8 hr for –S9 and 3 hr +S9. Cultures were harvested 19.8 hr from treatment initiation.

Results

Study validity: Yes, this was a valid study as each criterion described by the sponsor was met.

Study outcome: 4-Aminopyridine was considered negative for inducing chromosomal aberrations in CHO cells under the conditions employed in this assay.

Table 6: Chromosome Aberrations in Chinese Hamster Ovary Cells - Without Metabolic Activation – 19.8 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23807 Trial No.: C1 Date: 06/20/02 Lab No.: CY7222 Test Article: Fampridine Spiked with 2% [redacted] (b) (4)

				CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDO-REDUPLI-CATED CELLS	# POLY-PLOID CELLS	JUDGE-MENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGE-MENT (+/-) [d]
CONTROLS																
NEGATIVE:	McCoy's 5a		A	100		0	9		1					0	1	
			B	100		0	15		1					0	1	
			TOTAL	200					2					0	2	
			AVERAGE	**%**	-	**0.0**	**12.0**		**1.0**					**0.0**	**1.0**	
VEHICLE:	Water	100µL/mL	A	100		0	5		3	3	1			4	7	
			B	100		1	5		3	1	1			2	5	
			TOTAL	200					3	4	2			6	12	
			AVERAGE	**%**	**0**	**0.5**	**5.0**		**1.5**	**2.0**	**1.0**			**3.0**	**6.0**	
POSITIVE:	MMC	0.200µg/mL	A	50		0	4		5	8	15		20	37	40	
			B	50		0	4		7	9	18		19	37	40	
			TOTAL	100					12	17	33		39	74	80	
			AVERAGE	**%**	-	**0.0**	**4.0**	-	**12.0**	**17.0**	**33.0**		**39.0**	**74.0**	**80.0**	+
TEST ARTICLE		188µg/mL	A	100		0	9		4	2	1			2	6	
			B	100		0	9		4			3		3	7	
			TOTAL	200					8	2	1	3		5	13	
			AVERAGE	**%**	16	**0.0**	**9.0**	-	**4.0**	**1.0**	**0.5**	**1.5**		**2.5**	**6.5**	-
		250µg/mL	A	100		0	7		2					0	2	
			B	100		0	7		2	1	1			2	3	
			TOTAL	200					4	1	1			2	5	
			AVERAGE	**%**	**27**	**0.0**	**7.0**	-	**2.0**	**0.5**	**0.5**			**1.0**	**2.5**	-
		375µg/mL	A	100		2	9		6	3	2			5	11	
			B	100		1	7		3	1	1			1	4	
			TOTAL	200					9	4	3			6	15	
			AVERAGE	**%**	**37**	**1.5**	**8.0**	-	**4.5**	**2.0**	**1.5**			**3.0**	**7.5**	-
		500µg/mL	A	100		1	7		9	2				2	11	
			B	100		1	7		8	1	3	1		5	12	
			TOTAL	200					17	3	3	1		7	23	
			AVERAGE	**%**	**59**	**1.0**	**7.0**	-	**8.5**	**1.5**	**1.5**	**0.5**		**3.5**	**11.5**	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium Water = cell culture grade water MMC = Mitomycin C

[redacted] (b) (4) at 3.75, 5.00, 7.50, and 10.0 µg/mL in the cultures dosed at 188, 250, 375, and 500 µg/mL of Fampridine, respectively.

Table 8: Chromosome Aberrations in Chinese Hamster Ovary Cells - With Metabolic Activation – 3.0 Hour Treatment, 19.8 Hour Harvest

Assay No.: 23807 Trial No.: C1 Date: 06/20/02 Lab No.: CY7222 Test Article: Fampridine Spiked with 2% (b) (4)

				CELLS SCORED	% MITOTIC INDEX REDUCTION [a]	# ENDO-REDUPLICATED CELLS	# POLY-PLOID CELLS	JUDGEMENT (+/-) [b]	NUMBERS AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOME ABERRATIONS: Gaps	Simple Breaks	chte	chre	mab	TOTALS [c] -g	TOTALS [c] +g	JUDGEMENT (+/-) [d]
CONTROLS NEGATIVE:	McCoy's 5a		A	100		1	9			2				2	2	
			B	100		0	9		4		2	1		3	6	
			TOTAL	200					4	2	2	1		5	8	
			AVERAGE	%	-	0.5	9.0		2.0	1.0	1.0	0.5		2.5	4.0	
VEHICLE:	Water	100µL/mL	A	100		0	9		6	3		2		5	10	
			B	100		0	12		3		1			1	4	
			TOTAL	200					9	3	1	2		6	14	
			AVERAGE	%	0	0.0	10.5		4.5	1.5	0.5	1.0		3.0	7.0	
POSITIVE:	CP	5.00µg/mL	A	50		0	12		8	9	14	1	2	18	22	
			B	50		4	7		6	7	11		3	17	19	
			TOTAL	100					14	16	25	1	5	35	41	
			AVERAGE	%	-	2.0	9.5	-	14.0	16.0	25.0	1.0	5.0	35.0	41.0	+
TEST ARTICLE		250µg/mL	A	100		0	8		1					0	1	
			B	100		0	7		3	1	2			3	6	
			TOTAL	200					4	1	2			3	7	
			AVERAGE	%	17	0.0	7.5	-	2.0	0.5	1.0			1.5	3.5	-
		500µg/mL	A	100		0	6		2					0	2	
			B	100		0	10		1			1		1	2	
			TOTAL	200					3			1		1	4	
			AVERAGE	%	0	0.0	8.0	-	1.5			0.5		0.5	2.0	-
		750µg/mL	A	100		2	5		4	3		1		4	7	
			B	100		0	5		1			1		1	2	
			TOTAL	200					5	3		2		5	9	
			AVERAGE	%	13	1.0	5.0	-	2.5	1.5		1.0		2.5	4.5	-
		1000µg/mL	A	100		2	2		4	2	1			2	6	
			B	100		1	4		3					0	3	
			TOTAL	200					7	2	1			2	9	
			AVERAGE	%	73	1.5	3.0	-	3.5	1.0	0.5			1.0	4.5	-

chte: chromatid exchange chre: chromosome exchange mab: multiple aberrations, greater than 4 aberrations

[a] % Mitotic index reduction as compared to the vehicle control.

[b] Significantly greater in % polyploidy and % endoreduplication than the vehicle control, $p \leq 0.01$.

[c] -g = # or % of cells with chromosome aberrations; +g = # or % of cells with chromosome aberrations + # or % of cells with gaps.

[d] Significantly greater in -g than the vehicle control, $p \leq 0.01$. McCoy's 5a = culture medium Water = cell culture grade water CP = Cyclophosphamide

(b) (4) at 5.00, 10.0, 15.0, and 20.0 µg/mL in the cultures dosed at 250, 500, 750, and 1000 µg/mL of Fampridine, respectively.

3.) **28-Day Oral Gavage Toxicity and Toxicokinetic Study with Fampridine (Spiked with 2% (b) (4)) in Rats**

Key study findings:

- NOAEL for 4-aminopyridine spiked with 2% (b) (4) was 4.5 mg/kg/day for males and 10.5 mg/kg/day for females.
- CNS toxicity observed for HD group—convulsions observed in 5/20 (3M and 2F)

Study no.: 7338-100
Volume #, and page #: EDR, 4.2.3.7.6.1 7338-100, pp. 1-346
Conducting laboratory and location: (b) (4)
Date of study initiation: 29 April 2002
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Fampridine
(b) (4) Lot #4-AP- (b) (4) -1/130202200, (b) (4) purity
Formulation/vehicle: reverse osmosis water

Methods

Day 1, doses: 0, 6+2% mb, 12+2% mb, and 18+2% mb mg/kg/day
Days 2- 28, doses: 0, 4.5+2% mb, 10.5+2% mb, and 15+2% mb mg/kg/day
Species/strain: Rat/Sprague Dawley CRL:CD (SD) IGS BR
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Oral, gavage, 3 times daily within an 8 hr interval.
Satellite groups used for toxicokinetics or recovery: 12/sex/group for TK (fampridine only)
Age: 6 weeks of age
Weight: Male, 172-214 g; Female, 128-156 g
Unique study design or methodology (if any): Divided into 3 equal doses administered over 8-hr period. Vehicle = deionized water; Dose reduction occurred on Day 2 due to deaths in TK groups—HDM and HDF—on Day 1.

Observations and Results:
Mortality/Morbidity: Twice daily

TK group (Fampridine only)

- Day 1 (second dose): 1MD and 6HD animals died. Convulsions were observed in one or more of these animals; the study report did not specify the sex of these animals.
  - Replacements for each of the seven animals were made and the doses were reduced from 6, 12, and 18 mg/kg/day to 4.5, 10.5, and 15 mg/kg for

both Main study and TK groups. Both main study and TK animals received the original HD on Day 1.

- Day 3, one HD animal was found dead (sex not reported); this animal was not replaced.

Main study (Fampridine + 2% (b) (4)

- Males—three unscheduled deaths occurred; one death in each dose group on study Days 17, 19, and 23 for LD, MD, and HD, respectively. No histopathology findings were observed in the LD and MD deaths. The pathologist described the histopathology in the HD death as severe bladder distention with slight bilateral dilatation of the renal pelvis suggesting a low urinary tract blockage. Clinical chemistry was not performed on animals that died on study, and thus a correlate for the COD could not be determined.
    - None of these deaths was attributed to fampridine+2% (b) (4)

Clinical signs: Recorded several times daily throughout study.

- Notable clinical signs from main study animals are summarized in the following reviewer-generated table (based upon data from sponsor's Table 1).

Clinical signs of Fampridine + 2% (b) (4) in rats that were absent for Control and LD groups

Clinical Sign	MALES		FEMALES
	MD	HD	HD
Convulsions		2/10	3/10
Discharge—Oral/Nasal	2/10	3/10	2/10
Discharge—Genital/cloudy		1/10	
Hair coat—Rough	1/10	5/10	1/10

Body weights: Body weights were recorded twice prior to treatment and weekly after the start of treatment. [ss, $^{*}p<0.05$]

Absolute bodyweight—Males

Day	Control (g)	% of Control		
		LD	MD	HD
1	195	-1.5	-2.0	-1.0
8	253	-7.5*	-11.1*	-13.8*
15	305	-5.6*	-9.5*	-12.8*
22	348	-4.0	-9.2*	-12.6*
29	387	-3.4	-8.8	-11.9*

- Body weight gain was significantly decreased in all male dose groups during week 1 (Days 1-8) and for MDM and HDM for the entire treatment period (Days 1-29).
    - Days 1-8, LD -29.3%*, MD -43.1%*, and HD -56.9%* compared to Control.

- Days 1-29, MD -16.7%* and HD -23.4%* compared to Control.
- In females, absolute body weight and body weight gain were unaffected by 4-AP treatment.

Food consumption: Recorded weekly throughout study, commencing with treatment.

- Dose-dependent decreased food consumption occurred during week 1 (days 1-8) for both males and females.

Mean food consumption for males and females treated with vehicle control or 4-aminopyridine.

DAYS	MALES				FEMALES			
	Control (g)	% of Control			Control (g)	% of Control		
		LD	MD	HD		LD	MD	HD
1-8	193	-19.7*	-24.9*	-30.5*	150	-10.7*	-19.3*	-25.3*
8-15	209	-4.3*	-6.2	-11.2*	150	-1.3	-2.0	-4.7
15-22	214	-2.3	-2.8	-4.2	160	+1.9	-2.5	-3.7
22-29	217	-3.6	-4.6	-5.5	159	-5.0	-0.7	-6.6
1-29	817	-6.6*	-7.7*	-9.9*	619	-2.9	-5.2	-9.8*

[ss, *p<0.05]

Clinical pathology: Blood samples were collected at terminal sacrifice.

Hematology: The following parameters were evaluated: RBC, Hgb, Hct, MCV, MCHC, MCH, Plt, and WBC (Total and Differential).

- Males—Unremarkable, hematological parameters were unaffected by 4-AP treatment.
- Females—Notable changes in absolute cell counts are summarized in the following reviewer-generated table.

Increased mean cell counts observed in fampridine-treated females compared to CF

Hematological parameter	LD	MD	HD
WBC	+21.6%	+31.1%	+54.0%*
Lymphocytes	+25%	+31.2%	+54.7%*
Neutrophils	-12.5%	+37.5%	+37.5%
Monocytes	N.C.	+100%*	+100%*
Eosinophils	N.C.	N.C.	+100%*

N.C. = No change
[ss, *p<0.05]

Coagulation: PT and APTT were evaluated.

- Unremarkable, coagulation parameters were unaffected by 4-aminopyridine treatment for either sex.

Serum clinical chemistry: The following parameters were evaluated: GLU, GLOB, TBILI, ALB, TP, AST, ALT, BUN, ALP, CHOL, CREA, A/G ratio, Na, K, Cl, Ca, and Phos.

- Unremarkable, serum clinical chemistry parameters were unaffected by 4-aminopyridine treatment for either sex.

Toxicokinetic analyses: TK parameters were determined for plasma fampridine and were summarized in the following Table provided by the sponsor.

Toxicokinetic Parameters for Fampridine in Rat Plasma

Dose Group	Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	Dose Normalized C_{max}	T_{max} (Hours)	AUC_{0-t} (ng·hr/mL)	Dose Normalized AUC_{0-t}	AUC_{0-24} (ng·hr/mL)	Dose Normalized AUC_{0-24}
					Day 1				
5	6	M	211	35.2	0.500	1123	187	1752	292
		F	236	39.3	0.500	1230	205	1230	205
6	12	M	277	23.1	0.500	2399	200	2399	200
		F	227	18.9	0.500	2633	219	2633	219
7	18	M	341	18.9	0.500	4064	226	4064	226
		F	469	26.1	12.0	5847	325	5847	325
					Day 28				
5	4.5	M	122	27.2	0.500	773	172	1065	237
		F	174	38.6	1.00	564	125	716	159
6	10.5	M	235	22.3	0.500	1734	165	2570	245
		F	246	23.4	1.00	1762	168	2604	248
7	15	M	304	20.2	8.00	2633	176	3647	243
		F	370	24.7	1.00	2362	157	3533	236

Note: Dose level was lowered on Day 2 of the study.

Terminal sacrifice: All animals were sacrificed after 28 days of treatment. Necropsies were performed for unscheduled deaths and those sacrificed at study termination.

Organ weight: At terminal sacrifice, the following organs were collected and weighed: adrenal (2), brain, heart, kidney (2), liver, lung, ovary (2), pituitary gland, prostate, spleen, and testis (2). Organ-to body weight percentages and organ-to-brain weight ratios were calculated.

- Males and Females: absolute organ weights, organ to body weight ratio, and organ to brain weight ratios were unremarkable.

Macroscopic findings: Each animal was examined macroscopically at necropsy and any lesions observed were examined for histopathology and, if found, each animal from the LD and MD groups was examined.

- Unremarkable, no macroscopic findings were attributed to 4-aminopyridine treatment for either sex.

Microscopic findings: Preserved tissues from each animal of the C and HD groups, and all gross lesions, were examined in animals that died on study or at terminal sacrifice. Preserved tissues were stained with hematoxylin and eosin and examined microscopically. The following tissues (when present) were preserved in 10% neutral-buffered formalin. The tissues preserved for histopathology are listed below.

Adrenal (2), brain, cecum, colon, duodenum, epididymis (2), esophagus, eye (2), femur with bone marrow (articular surface of the distal end), heart, ileum, jejunum, kidney (2), lesions, liver, lung with mainstem bronchi, lymph node (mesenteric), mammary gland (females), ovary (2), pancreas, pituitary gland, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid with parathyroid (2), tongue, trachea, urinary bladder, uterus, and vagina.

Adequate Battery: yes (X)
Peer review: no (X)

- Unremarkable, no microscopic findings in the HD group consistent with a treatment effect of 4-aminopyridine for either sex.

OVERALL SUMMARY AND EVALUATION:

4-Aminopyridine, a non-selective voltage-gated potassium channel blocker was developed to improve 25-ft walking ability in multiple sclerosis patients. The precise mechanism of action of 4-aminopyridine is unknown. The sponsor claims that 4-aminopyridine is known to bind and occlude outward potassium conductance from neurons, which prolongs neurotransmission through regions of demyelinated neurons resulting in successful signal transmission and enhanced neurotransmitter release. However, there is insufficient information in sponsor's application to support this claim. The sponsor has minimally characterized fampridine and shown that fampridine binds with relatively low affinity within voltage-gated potassium channels (i.e., IKv1.1 IC_{50}=242 μM, IKv1.2 IC_{50}=399 μM, IKv1.3 IC_{50}=195 μM, IKv1.4 IC_{50}=399 μM, IKv1.5 IC_{50}=270 μM, IKv1.7 IC_{50}=150 μM, IKv3.1 IC_{50}=29 μM, and IKv3.2 IC_{50}=100 μM). The claim made that these channels are localized on demyelinated axons has not been well substantiated by the sponsor. Thus, the mechanism of action related to improved walking ability in multiple sclerosis patients remains largely unknown.

In the animal species used for toxicity testing, fampridine is well absorbed, has a $V_d \approx 3.4$ L/kg, which is greater than total body water, and is metabolized by CYP2E1 into two inactive metabolites, 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate. In mouse, pregnant rabbit, and dog, both inactive metabolites are produced as major metabolites. In rat, a gender difference is noted. Male rats metabolize 4-aminopyridine to both 3-hydroxy-4-aminopyridine as a minor metabolite and 3-hydroxy-4-aminopyridine sulfate as a major metabolite. In contrast, non-pregnant female rats and pregnant female rats (GD 7 to GD 13) metabolize 4-aminopyridine to the same two inactive metabolites as in males except both are major metabolites. Pregnant female rats from GD 16 to post-partum LD 14 metabolize 4-aminopyridine as do their male counterparts with 3-hydroxy-4-aminopyridine as a minor metabolite and 3-hydroxy-4-aminopyridine sulfate as a major metabolite. In humans, fampridine is also metabolized by CYP2E1 into the same two inactive metabolites, however both are present as minor circulating metabolites and 4-aminopyridine is excreted in urine largely as unchanged parent. Although differences in *in vivo* metabolic profile among the animal species, for the most part, both rat and dog are good models to evaluate the toxicological effects of fampridine.

As noted above, the toxicological studies were conducted using fampridine-IR, whereas, the clinical product was fampridine-SR. However, a single study was conducted in beagle dogs to compare these two formulations of fampridine. In this study (b) (4)-684-026), differences in PK parameters, CNS-related toxicities, and local effects on the gastrointestinal tract were noted between the fampridine-SR and -IR formulations. As an evaluation of CNS-related toxicity, the incidence of body tremors was noted. Doses of fampridine-IR ≥17.5 mg/animal resulted in body tremors in all of the dogs tested (5/5); however, fampridine-SR dosed at 35 mg/animal resulted in an incidence of 3/5 dogs and a dose of 40 mg/animal increased the incidence to all of the dogs tested (5/5). In contrast to body tremors, increased salivation and mydriasis were observed at 17.5 mg/animal for both fampridine-IR and -SR formulations. Fampridine-SR was absorbed more slowly and peak plasma concentrations did not increase with an increase in dose, whereas fampridine-IR was rapidly absorbed and peak plasma concentrations increased dose-proportionally.

Toxicology studies of fampridine in several species, including mouse, rat, rabbit, and dog, were conducted by the sponsor (Acorda Therapeutics). Additional toxicology studies conducted by either Elan Corporation Plc. or Neurorecovery, Inc. were acquired by the sponsor.

The primary toxicity following fampridine dosing was CNS activation. Common to all of the studies conducted was the occurrence of a similar pattern of CNS-related clinical signs.

Toxicological testing was conducted in both rodents and non-rodent species. In rat, several acute and subchronic toxicity studies were completed to evaluate the toxicological profile of fampridine. Initially, several 2-Wk studies determined that once

daily dosing of fampridine (0.3, 1, 3, and 10 mg/kg) by oral gavage resulted in unfavorable CNS signs (clonic static or partial seizures, tremors, and vocalizations). These clinical signs were reduced or eliminated by tid or qid dosing; however, significant mean body weight loss still occurred. Thus, multiple daily dosing strategies (e.g., bid, tid) were shown to improve the safety profile of fampridine. The NOAEL for single dose administration was 1 mg/kg/day, which is equivalent to 0.5 times the MRHD of 20 mg/day on a mg/m^2 basis. Dosing regimens using tid and qid administration of fampridine increased the NOAELs to 6 mg/kg (males) and 12 mg/kg (females) that are equivalent to approximately 3-times and 6-times the MRHD on an mg/m^2 basis, respectively. Attempts to shorten the interval between doses resulted in an increase in the unfavorable CNS signs thereby reducing the benefit gained from multiple dosing regimens. As a result, longer-term studies were conducted using a dietary admixture of fampridine. In these studies, NOAELs were established at 5 mg/kg/day in a 4-Wk study (equivalent to 2.4-times the MRHD) and at 3 mg/kg/day in a 13-Wk study (equivalent to 1.4-times the MRHD on a mg/m^2 basis). A NOAEL for CNS activation and reduced body weight was established at 2 mg/kg (equivalent to 0.97-times the MRHD on an mg/m^2 basis). A NOEL was not established in the pivotal 6-month rat study, as dilatation of the glandular stomach, of trace to mild severity was observed in both sexes. The lesion was described as a mild dilatation of the gastric fundic glands. The dilated fundic glands had a distinct lumen and a reduction in size and flattening of the chief cells in the basilar part of the gland. Parietal cells were unaffected. Within the dilated glands, amorphous eosinophilic debris was found in the glandular lumens. Pyknotic nuclei were occasionally observed within the dilated glands. The affected glands had increased number or density of cell nuclei that appeared to be a relative increase secondary to the reduction of the cytoplasm. The study pathologist did not consider these lesions to be life threatening, however, the stomach was considered a potential target organ.

In the dog, 13-Wk and 52-Wk repeat dose toxicity studies were conducted. Similar to the findings in rodents, CNS effects of 4-aminopyridine were observed in Beagle dogs following bid oral (capsule) administration. In both studies, clinical signs included tremors, ataxia, prostration, and convulsions that occurred within 30 to 60 minutes following the first and/or second dose of 4-aminopyridine. In the 52-Wk study (684-008), the most prominent sign of CNS activation was the presence of mild-severe tremors and seizures in both sexes. Prior to the interim 6-month sacrifice, many of the HD group (3 mg/kg/day, which is equivalent to 4.9-times MRHD based on mg/m^2 and 5.8-times MRHD based on AUC) died. Causes of death were attributed to either sudden cardiac failure or listed as unknown. Many of these deaths, however, occurred following antemortem observations of seizure activity. It is unclear from these data as to whether the cardiac failure found in some animals contributed to the seizure activity or whether the animals that died from an unknown cause did so following the seizure activity. It should be noted that survivors in the one-year study did not show any brain histopathology that is commonly associated with seizure activity. The sponsor has suggested that this is an indication that there is no evidence of permanent damage following fampridine-induced seizures. Overall, the CNS-activating properties of fampridine are well established in the scientific literature, and it has been well documented that this drug induces seizure activity in humans following oral

administration of fampridine (Ball et al, 1979). Thus, it is not surprising that convulsions and tremors are the primary clinical signs related to fampridine administration in rats, rabbits, and dogs.

The pivotal toxicology studies were chronic studies conducted in SD rat (6-month) and Beagle dog (one-year). As described above, chronic administration of fampridine was also associated with central nervous system toxicity. Based upon its mechanism of action to block outward potassium current from voltage-gated potassium channels, fampridine increases and prolongs action potential neurotransmission and enhances neurotransmitter release. As a result, most of the species (e.g., rat, rabbit and dog) administered fampridine showed clinical signs representing CNS-related toxicities. One exception was mouse; no evidence of seizures or seizure-like activity was observed in either repeat dose 28-Day TK study or 13-Wk repeat dose toxicity study, in which mice were, administered fampridine in their diet. However, following prolonged repeat dose administration of fampridine in the carcinogenicity study, an increased incidence of seizures was observed for HDM (19/70 = 27.1%) and for HDF (8/70 = 11.4%) compared to Control groups (M, 21/130 = 16.2%; F, 5/130 = 3.8%). In males, seizures commenced during Weeks 27-39 and in females, seizures were observed during Weeks 40-52. The mean HDM and HDF C_{max} values were 239 ng/ml and 404 ng/ml, respectively. Seizures continued to be observed throughout the remainder of the study for both HDM and HDF. At the end of the study, C_{max} values were 365 ng/ml and 243 ng/ml for HDM and HDF, respectively.

Unlike mouse, single doses of fampridine (25 mg/kg) administered by oral gavage to rats resulted in seizures observed in both sexes. Repeated oral (gavage) dosing of 4-aminopyridine (10 mg/kg) to either sex for 2-Wk or 13-Wk increased the incidence of post-dose (within 1 hr) partial seizures, tonic-static, and tonic clonic seizures. Additional clinical signs suggesting CNS activation included increased activity, hypersensitivity to touch, tremors, rapid breathing, and vocalization in the HD group. For the 13-Wk study, mean plasma 4-aminopyridine concentrations were 704 ng/ml (HDM) and 402 ng/ml (HDF). In this study, a functional observational battery was also conducted, however, no seizures were observed. The high mean plasma concentration for the HDM is greater than that measured in females largely because of the inclusion of two HDM with high plasma concentrations (899.6 ng/ml and 1196 ng/ml measured at Week 13). It is worth noting that the high plasma 4-aminopyridine concentration in these individual animals correlated well with the frequency of partial and/or clonic-tonic seizures that occurred in each individual animal. The HDM rat with the highest plasma 4-aminopyridine concentration had partial seizures from weeks 2-13 and clonic-tonic seizures during week 13. During the chronic rat study (26-Wk), 2 HDM (20 mg/kg/day, via diet) had convulsions that were associated with a mean C_{max} value range of 210 to 230 ng/ml. 4-Aminopyridine treatment in rats resulted in a greater occurrence of seizure activity and at lower plasma concentrations than those reported in mice.

The toxicity of 4-aminopyridine was evaluated in Beagle dog in a 13-Wk and a 52-Wk repeat dose study. In the 13-Wk repeat dose study, severe convulsions, ataxia, and severe tremors were observed on Day 2 in one HDM within 30 min of dosing (i.e., first 1.5 mg/kg dose). Although the convulsions and ataxia were limited to Day 2, severe tremors

persisted on Days 2-3, Days 6-7, and moderate tremors on Day 8. Plasma 4-aminopyridine concentration in this HDM was 320.3 ng/ml (Week 1), 229.8 (Week 4), and 298.1 (Week 13). Thus, it is likely that at the time of the seizures, plasma 4-aminopyridine concentrations were between 229.8 and 320.3 ng/ml. The most notable clinical sign associated with fampridine administration in this study was the presence of tremors in MDM (0/4), MDF (2/4), HDM (6/6), and HDF (6/6) compared to Control (0/6, either sex). Tremor was observed most frequently in HDF (mean = 48.7 days) than in HDM (mean = 31.3 days), but HDM had a greater mean number of days of severe tremor. The mean C_{max} values associated with tremor were 233 ng/ml (MDF), 398 ng/ml (HDF), and 521 ng/ml (HDM). In the chronic one-year study, seizure activity was noted in several animals euthanized *in extremis* and in dogs that died prematurely. Thorough examination of the brains of these animals was performed by (b) (6) Evidence of vacuolation of the cerebral cortex, recent multifocal hemorrhages, and/or perivascular or focal edema was found in dogs treated at 1.5 or 3.0 mg/kg/day. In all dogs found dead or euthanized *in extremis*, vacuolation was found. Vacuolation resulted from both the acute swelling of perineuronal satellite oligodendrocytes and to a lesser extent microvacuolation of the neuropil, especially the interneuronal and the perivascular neuropil. However, it was noted that the vacuolation found, in part, was due to agonal stage and postmortem autolysis superimposed on minimal to mild vacuolation secondary to recent seizure activity. The majority of the vacuolation occurred in the piriform cortex; however, it was largely absent from other brain regions (i.e. cerebral cortex of the neopallium, hippocampus, amygdaloid complex, and the hypothalamus) commonly associated with excitotoxin-induced seizure activity. Because of the lack of vacuolation in these other limbic structures, the pathologist concluded that the seizures in the dogs may not have been of the duration and intensity necessary to exceed the necessary thresholds to elicit such lesions and alternately, the seizures themselves may not have been entirely central in origin. This latter opinion stems from the presence of recent multifocal brain hemorrhages only in the dogs that died on study, that were suspected to be a short-lived terminal hypoxia/anoxia resulting from circulatory failure and increased venous pressure that was further supported by findings of diffuse congestion in the cerebral and meningeal blood vessels. In contrast, MD and HD dogs surviving to terminal sacrifice failed to demonstrate cumulative lesions (progressive neuronal loss and astrocytosis in the piriform cortex and other structures of the limbic system) even though these animals had histories of seizure activity. Thus, the conclusions from this study suggested that the vacuolation observed in the piriform cortex and the neopallium was secondary to seizure activity, but that the mechanism by which the seizure was induced remained "elusive".

In the reproductive toxicology studies, pregnant rabbits were administered 4-aminopyridine by oral (gavage). Following dosing, MD and HD does showed clinical signs consistent with CNS activation, such as, increased activity, labored breathing, tremor (MD and HD), and convulsion (2 HD). The mean C_{max} values for MD and HD groups were 220 ng/ml and 537 ng/ml, respectively.

Collectively, CNS-related toxicities were observed following 4-aminopyridine administration to each of the toxicological species. Comparison of group mean C_{max}

values across the toxicological species suggests that the more sensitive species are dog and rat> rabbit and the least sensitive is mouse. On a mg/m^2 basis, seizures and tremors were observed at doses equivalent to 2.4-times the MRHD of 10 mg bid (20 mg/day). At the NOAEL, the dose equivalent is reduced to 1.2-times the MRHD on a mg/m^2 basis. Based upon fampridine exposure, the AUC_{0-24} at the NOAEL for the dog is 420 ng•hr/ml, which is 1.4-times the exposure expected at the MRHD. Based upon maximum fampridine concentration, the C_{max} at the NOAEL for the dog is 121 ng/ml, which is 4.8-times the concentration expected at the MRHD.

Fampridine was evaluated in a complete battery of *in vitro* and *in vivo* genetic toxicology studies. The mutagenic and clastogenic potential of 4-aminopyridine was evaluated in the absence and presence of metabolic activation (S9 mix) in an *in vitro* Ames test, an *in vitro* L5178Y TK$^{+/-}$/mouse lymphoma assay, and an *in vitro* chromosomal aberration assay in CHO cells. The *in vitro* Ames test and chromosomal aberration assay as well as the *in vivo* erythrocyte micronucleus assay in mouse and rat were adequate. A deficiency was identified in the *in vitro* L5178Y TK study, at the 4 hr time point, because of a lack of cytotoxicity reached ± S9 mix. Although this study did not reach the acceptable cytotoxicity criterion, the weight of evidence suggested by negative findings for both the *in vitro* assays for mutagenicity and clastogenicity and *in vivo* assays for mutagenicity supports the overall conclusion that 4-aminopyridine is negative for genotoxicity

The carcinogenic potential of fampridine was evaluated in two rodent bioassays—mouse and rat. In the two-year mouse carcinogenicity study (684-022), CD-1/Crl mice were administered fampridine at doses of 0, 0, 2.0, 12.5, and 80 mg/kg/day via dietary admixture for up to 104 weeks. In both sexes, the survival rates of the HD group were reduced compared to Controls; the effect in HDF was statistically significant. As a result, all surviving HDF were euthanized at week 100; however, this unscheduled termination did not compromise the outcome of this study. Decreased body weight was observed in HD groups (M, -11.6% and F, -12.3%) compared to Controls. No other significant adverse effects were observed. No drug-related increases of any tumor type were found in this study. On a mg/m^2 basis, fampridine was tested at doses that were approximately 0.5 times, 3.1 times, and 19.6 times the MRHD of 20 mg/day. Based upon AUC_{0-24}, fampridine was tested at exposures approximately 1 times, 3.8 times, and 18.9 times the MRHD of 20 mg/day.

In the two-year rat carcinogenicity study (684-023), CD®BR (VAF/Plus)/Crl rats were administered fampridine at doses of 0, 2, 6, and 18 mg/kg/day via dietary admixture for 104 weeks. Mean body weights for HDM and HDF were decreased throughout the entire dosing period, and final mean body weights were significantly decreased (-18.1 and -21.4%, respectively) compared to Controls. In contrast, mean body weights for LD and MD groups were not significantly affected. Initially, histopathological evaluation was conducted only for the Control and HD groups. Histopathological evaluation for the LD and MD groups were subsequently conducted, at the Agency's request, because of the magnitude of the body weight effects at the HD. No drug-related increase of any tumor type was observed in males; however, a statistically significant increase in the incidence of uterine polyps was observed in females at the HD. On a mg/m^2 basis, fampridine was

tested at doses that were approximately 1 times, 2.9 times, and 8.8 times the MRHD of 20 mg/day.

Fampridine was evaluated in both rats and rabbits for potential reproductive toxicity. This assessment included studies to evaluate fertility and embryonic development (rats), developmental toxicity (rats and rabbits), and pre- and postnatal development (rats). Oral administration of fampridine (1, 3, and 9 mg/kg/day) to male and female rats prior to and throughout mating resulted in no adverse effects on fertility. Females were also dosed up to day 13 of gestation or day 21 of lactation without any adverse effects on the dams. In offspring of rats administered 9 mg/kg/day, viability and mean body weight were reduced. Body weights of HD pups were reduced compared to Control pups at birth (-3.1%) and this trend continued to lactation days 4 (-6.3%), 7 (-15.1%), 14 (-18.3%) and 21 (M, -16.2% and F, -16.3%). The no effect dose for developmental toxicity in this study was 3 mg/kg/day and was associated with a plasma exposure (AUC) approximately 5 times the expected human plasma AUC at the MRHD of 10 mg bid (20 mg/day). For the most part, these studies were adequate to assess the reproductive toxicity induced by fampridine; however, one deficiency was noted in the embryofetal study in rats (684-001). The number of litters examined for the HD group was 14. According to the ICH guidelines, it is recommended that at least 16 litters be examined to determine whether drug-induced effects are observed in embryofetal/developmental studies. The sponsor suggests that this study is adequate because the number of fetuses examined were comparable among the groups. However, this argument does not address the limited sensitivity of an examination of a reduced number of HD litters to detect rare developmental anomalies. The lack of sufficient numbers of HD litters examined for developmental anomalies raises a safety concern that rare developmental anomalies may have been missed.

In the developmental toxicity studies in rats and rabbits, doses up to 10 mg/kg/day and 5 mg/kg/day were evaluated, respectively. Although maternal toxicity was achieved in rat and rabbit studies, no evidence of embryotoxicity was observed or presence of developmental anomalies detected during organogenesis following high dose administration of fampridine ≥ 9/6 mg/kg/day. This treatment did, however, consistently reduce pup viability and mean body weight. Rat pup survival from Days 1 to 4 of gestation was reduced and decreased pup weight gains were observed from postnatal Days 4 to 35, but these effects were only seen at the high dose of 9 mg/kg/day (reduced to 6 mg/kg/day during the second week of the study). These dose levels represent 6.9 and 4.6 fold the human AUC at the MRHD. No treatment-related effects were observed in a battery of behavioral and developmental assessments of the F1 pups.

The CMC primary reviewer identified several impurities in the drug substance and/or product that possess structural alerts for potential genotoxicity. Seven potentially genotoxic impurities in the drug substance and/or drug product have been identified:

(b) (4)

In an Amendment N0012 (June 22, 2009) to the NDA, Acorda Therapeutics submitted "Derek for Windows" reports for five of the impurities listed, including: (b) (4) Each of these study reports was prepared by (b) (4)) and the release date was either 06 May 2009 (b) (4)) or 29 May 2009 (b) (4) .

For each impurity, the following endpoints were evaluated: Carcinogenicity, Chromosome damage, Genotoxicity, and Mutagenicity. In the analysis, tautomers and hydrogens (implicit and explicit) were included in the database search, LHASA predictions. The same result was found in all of the Derek for Windows reports, "No alerts for genotoxicity, mutagenicity, chromosome damage, or carcinogenicity were present. Based on these findings this impurity could be classified as a (b) (4) impurity with no alerting features".

One of the drug product impurities (b) (4)) exceeded the specification limit of < 0.5%. Therefore, Acorda Therapeutics sponsored two *in vitro* genetic toxicology studies—an Ames test to determine mutagenic potential and a chromosomal aberrations test in CHO cells to determine clastogenic potential of this impurity. In addition, a 28-day repeat-dose toxicity study was conducted in rats to qualify this impurity. Both *in vitro* and *in vivo* studies demonstrated 4-AP spiked with 2% (b) (4) were negative for mutagenicity and clastogenicity. The sponsor did not include a three month toxicity study or an embryofetal study conducted in one species to support chronic administration of this drug impurity.

For the overall conclusions and recommendation, please see the Executive Summary.

APPENDIX 1

Executive CAC
Date of Meeting: August 11, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Todd Bourcier, Ph.D., DMEP, Alternate Member
Lois M. Freed, Ph.D., DNP, Supervisor
Richard A. Houghtling, Ph.D. DNP, Presenting Reviewer

Author of Draft: Richard A. Houghtling

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

NDA #22-250

Drug Name: AMAYA (fampridine)

Sponsor: Acorda Therapeutics, Inc.

Background: AMAYA (fampridine) is a potassium channel (I_{Kv}) blocker indicated as a treatment to improve walking ability in patients with multiple sclerosis (MS).

The test article was negative in a full battery of *in vitro* (Ames test, chromosomal aberration assay in CHO cells, and tk+/- mouse lymphoma assay) and *in vivo* (rat and mouse micronucleus assay) genotoxicity assays.

ECAC concurrence was not requested for either of the 2-year carcinogenicity studies.

Mouse Carcinogenicity Study
CD-1/Crl mice were administered fampridine at doses of 0, 0, 2.0, 12.5, and 80 mg/kg/day via dietary admixture for up to 104 weeks. The HDM and HDF had a reduced survival rate compared to Controls; the effect was statistically significant only in HDF. HDF were euthanized at week 100 due to low survival; however, this sacrifice did not compromise the outcome of this study. Decreased body weight for HD groups was observed (M, -11.6% and F, -12.3%) compared to controls. HD groups exhibited an increased incidence of convulsions, which is considered part of the pharmacological effect of fampridine. No other significant adverse effects were observed. No drug related increases of any tumor type were found in this study.

Rat Carcinogenicity Study
CD®BR (VAF/Plus)/Crl rats were administered fampridine at doses of 0, 0, 2, 6, and 18 mg/kg/day via dietary admixture for 104 weeks. Mean body weights for HDM and HDF were decreased throughout the entire dosing period; final mean body weights were significantly decreased (-18.1 and -21.4%, respectively) compared to Controls. Mean body weights for LD and MD groups were not significantly affected. Initially, histopathological evaluation was conducted only for the Control and HD groups. Histopathological evaluation for the LD and MD groups were subsequently conducted, at the Agency's request, because of the magnitude of the body weight effects at the HD. No drug-related increase of any tumor type was observed in males; however, a statistically significant increase in the incidence of uterine polyps was observed in females at the HD.

Executive CAC Recommendations and Conclusions:
Mouse:

- Although prior concurrence of the exec-CAC was not obtained, the Committee agreed that the dose selection for the study was adequate, based on reduced body weight at the high dose.

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

Rat:

- Although prior concurrence of the exec-CAC was not obtained, the Committee agreed that the dose selection for the study was adequate, based on reduced body weight at the high dose.

The Committee concurred that the study was negative for drug related neoplasms in males, but positive in females with a statistically significant increase in the incidence of uterine endometrial polyps at the HD, which was above the historical incidence range.

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

cc:\
/Division File, DNP
Freed/Supervisor, DNP
Houghtling/Reviewer, DNP
Reese/CSO/PM, DNP
/ASeifried, OND IO

APPENDIX 2

REFERENCES

Ball, A.P., Hopkinson, R.B., Farrell, I.D., Hutchison, J.G.P., Paul, R., Watson, R.D.S., Page, A.J.F., Parker R.G.F., Edwards, C.W., Snow, M., Scott, D.K., Leone-Ganado, A., Hastings, A., Ghosh, A.C., and Gilbert, R.J. (1979). Human botulism caused by *Clostridium butulinum* type E: The Birmingham outbreak. *Quart. J. Med.* **48:** 473-491.

Beeton, C., Wulff, H., Barbaria, J., Clot-Faybesse, O., Pennington, M., Bernard, D., Cahalan, M.D., Chandy, K.G. and Béraud, E. (2001). Selective blockade of T lymphocyte K+ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. *Proc. Natl. Acad. Sci.* **98(24):** 13942-13947.

Bowman, W.C. and Savage, A.O. (1981). Pharmacological actions of aminopyridines and related compounds. *Rev Pure Appl. Pharmacol. Sci.* **2:** 317-371.

Koo,G.C., Blake,J.T., Talento,A., Nguyen,M., Lin,S., Sirotina,A., Shah,K., Mulvany,K., Hora,D.Jr., Cunningham,P., Wunderler,D.L., McManus,O.B., Slaughter,R., Bugianesi,R.,

Felix,J., Garcia,M., Williamson,J., Kaczorowski,G., Sigal,N.H., Springer,M.S., Feeney,W. (1997). Blockade of the voltage-gated potassium channel $K_{v1.3}$ inhibits immune responses in vivo. *J. Immunol.* **158:** 5120-5128.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22250	ORIG-1	ACORDA THERAPEUTICS INC	FAMPRIDINE TABLETS

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/s/

RICHARD A HOUGHTLING
12/22/2009

LOIS M FREED
12/23/2009
Please see memo for comments.

MEMO TO FILE

NDA 22-250 Fampridine for walking ability in multiple sclerosis patients.

Review of amendment 034.

Reviewer: Richard A. Houghtling, Ph.D.
Supervisor: Lois M. Freed, Ph.D.
Project Manager: James H. Reese, Ph.D.
Submit Date: October 28, 2009
FDA Received Date: October 28, 2009
Sponsor: Acorda Therapeutics
Agent: Brian A. Walter, Ph.D.
Drug: 4-Aminopyridine, Fampridine
Formulation: Fampridine-SR
Category: Analeptic, Potassium channel antagonist

History

This memo documents Acorda's response to a request for nonclinical information letter sent on October 23, 2009. Two questions were asked regarding a statement made in the metabolism report (8210924) submitted to the NDA on September 18, 2009. The statement in question was the following:

> "Following this study, a quantitative GLP validated method will be developed to quantify the (b) (4) plasma concentration as a function of dose and dosing time."

Question 1: When will the GLP (b) (4) metabolite study be submitted?

Acorda Response:

> Acorda identified the (b) (4) in mouse and rat plasma retains that were obtained from GLP toxicokinetic (TK) studies (7338-103 and 7338-104, respectively). These data were summarized in a metabolism report (8210924) and submitted to the NDA on September 18, 2009 (Sequence 0029). This metabolism study followed good scientific practices commonly used for metabolism studies (non-GLP) and was performed at (b) (4) It is our understanding that GLP studies are not generally required for characterization of metabolites.

Question 2: Did you measure this metabolite in human plasma?

Acorda Response:

> No studies have been performed to determine whether (b) (4) is a metabolite in humans.

Summary and Recommendations

Unfortunately, Acorda's response to the first question does not address the question asked and therefore a clarification is necessary.

The following clarification was submitted to the Project Manager on Friday November 13, 2009 for Acorda:

In our October 23, 2009 information request letter, we asked for the status of a GLP study of the metabolite, (b) (4). This request was based on the following statement made in your Drug Metabolism Report 8210924, submitted on September 18, 2009:

> "Following this study, a quantitative GLP validated method will be developed to quantify the (b) (4) plasma concentration as a function of dose and dosing time."

The results of DMR 8210924 were stated to be preliminary.

In your recent communication of October 28, 2009, you discussed DMR 8210924, but did not provide an answer to our inquiry. Therefore, we again ask that you provide us with the status of the planned GLP study of (b) (4)

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22250	ORIG-1	ACORDA THERAPEUTICS INC	FAMPRIDINE TABLETS

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/s/

RICHARD A HOUGHTLING
11/16/2009

LOIS M FREED
11/16/2009

MEMO TO FILE

NDA 22-250 Fampridine for Walking Ability in Multiple Sclerosis patients

Review of Amendment 0009.

Reviewer: Richard A. Houghtling, Ph.D.
Supervisor: Lois M. Freed, Ph.D.
Project Manager: James H. Reese, Ph.D.
Submit Date: 15 May 2009
FDA Received Date: 15 May 2009
Sponsor: Acorda Therapeutics
Agent: Brian A. Walter, Ph.D.
Drug: Fampridine, 4-Aminopyridine
Formulation: Fampridine-SR
Category: Analeptic, Potassium channel antagonist

This memo documents a response from Acorda regarding a request for nonclinical information sent in a letter dated 12 May 2009.

Question 1: **Study M-2001-03 is missing Figure 3-1 (see p.9).**

Acorda Response: Figure 3-1 was missing from Study M-2001-03 entitled "Gender Differences in the Pharmacokinetics of 4-Aminopyridine in Sprague-Dawley Rats" provided in the NDA.

Acorda contacted the original author [redacted] (b) (4), currently at GlaxoSmithKline), who provided missing chromatograms. Study M-2001-03 pdf page 9 has been updated to provide the missing HPLC chromatograms.

Question 2: **Study 2001-04, in the Appendix 1, Chart 3: Rat 3 AMITRIP is missing.**

Acorda Response: Chart 3: Rat 3 AMITRIP, the concentration-time graph, is provided in the original study report in the NDA, and can be found in Study M-2001-04 pdf page 16.

Question 3: **In the Pivotal Rat Study (684-010), Table 9 is incomplete. In the PDF file, page 72 (corresponding to Table 9, marked on the right with 68) is MISSING. In its place is a duplicate of page 70 (marked on the right with 66).**

Acorda Response: Study 684-010, titled "26-Week Dietary Toxicity Study in Rats," has a duplicate page in Table 9 of the report. Acorda has replaced the duplicate page with the missing report page 68 (report pdf page 72) and an updated Study 684-010 is provided in Sequence 0009.

Question 4: **Study 8ACORP1 Final Report is missing signatures and dates from the Study Director and the Quality Control Personnel.**

Acorda Response: The e-signature of the Study Director was provided in the last page of 8ACORP1. Acorda has obtained the e-signature of the Director of Quality Assurance Unit and VP, Scientific Operations and this is provided in the updated study report on pdf page 43.

Question 5: **Study (b) (4)-2620-105, the data summary table and individual animal data for the ophthalmoscopy performed in this study are missing.**

Acorda Response: In the study protocol, on pdf page 671, a table of ophthalmoscopic data is listed as one of the tables to be included the final report; however, as noted in the final report for Study (b) (4)2620-105, on pdf page 32, there did not appear to be any ophthalmoscopic abnormalities found during the study. On pdf page 38, the veterinarian explained the lack of ophthalmoscopic data in his one-page signed "Opthalmoscopic Report." We do not believe, therefore, that any ophthalmoscopy was performed and thus neither a data summary table nor individual data are available.

Summary and Recommendations

The responses provided by the sponsor are adequate and the additional updated studies are available for review.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
`IDA-22250	ORIG-1	ACORDA THERAPEUTICS INC	FAMPRIDINE TABLETS

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/s/

RICHARD A HOUGHTLING
11/18/2009

LOIS M FREED
11/18/2009