CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202276Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on N202-276 avanafil
From A. Jacobs, AD 3/6/12

1. I concur that there are no pharm/tox related approval issues.

2. I concur that the pregnancy category should be C.

3. I have made various substantive and editorial suggestions for the NDA review. I have discussed these with the primary reviewer and the supervisor, and they will be addressed as appropriate.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ABIGAIL C JACOBS
03/26/2012

FOOD AND DRUG ADMINISTRATION
SUPERVISORY MEMO

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	March 21, 2012
Reviewer:	Lynnda Reid, Ph.D. Supervisory Pharmacologist
NDA #/date:	202-276, June 30, 2011
Sponsor:	VIVUS, Inc.
Drug Product:	Avanafil (TA-1790)
Indication:	Treatment of Erectile Dysfunction

Background: Avanafil is a PDE5 inhibitor indicated for the treatment of erectile dysfunction (ED). The recommended dose of 100 mg can be taken as needed approximately 30 minutes before desired sexual activity. The dose may be decreased to 50 mg or increased to a maximum recommended dose (MRHD) of 200 mg. Recommended use should not exceed once per 24 hours.

The sponsor submitted a complete nonclinical package that included general and safety pharmacology and pharmacokinetics/ADME assessments; single- and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproductive and developmental toxicology studies; and a single-dose phototoxicity study.

Pharmacology: The physiologic mechanism of erection of the penis involves release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. NO then activates the enzyme guanylate cyclase, which results in increased levels of cGMP, producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. Avanafil has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of NO by inhibiting PDE5, which is responsible for degradation of cGMP in the corpus cavernosum. When sexual stimulation causes local release of NO, inhibition of PDE5 by avanafil causes increased levels of cGMP in the corpus cavernosum, resulting in smooth muscle relaxation and inflow of blood to the corpus cavernosum.

General Toxicology: Toxicology studies were conducted via oral gavage in CD-1 mice, CD rats, and NZW rabbits; or via capsules in Beagle dogs. Repeat-dose toxicology studies were conducted in mice and rats for up to 2 years, and in dogs for up to 9 months.

In single-dose studies, no significant morbidity was observed at oral doses up to 2000 mg/kg in mice, rats, and dogs; or following intravenous doses up to 40 mg/kg in mice and rats. In repeat-dose studies, dosing was limited by CNS-related adverse effects. Clinical signs such as ataxia, tremor, convulsion, prostration, hypoactivity, and/or recumbency preceded death in mice and pregnant rats, or led to suspension of dosing in dogs at exposures approximately 5-8 times greater than the mean C_{max} levels at the MRHD.

Cardiovascular effects observed in dogs following single-dose and subchronic administration included decreased blood pressure and increased heart rate associated with prolonged QT interval. At 100 mg/kg, a decrease in the R wave, a potential decrease in the T wave, and an apparent notch on the T wave were observed; and some avanafil-treated animals had slight (≥160 beats/min) to severe (≥200 beats/min) tachycardia. Microscopic changes in the heart included arteritis in a branch of the cardiac extramural coronary artery or vascular inflammation. The no observed adverse effect level (NOAEL) in the 9-month dog study resulted in exposures approximately 3 times greater than the systemic exposure in men at the MRHD; the lowest exposure level at which effects were observed (LOAEL) was 9 times greater than the systemic exposure in men at the MRHD.

Treatment-related findings in the liver were observed in all animal species tested at exposures 8-20 times the MRHD. Increased total bilirubin correlated with microscopic findings of chronic inflammation, segmental arteritis, and hepatocyte necrosis was noted at 2-4 weeks but was not progressive following chronic administration. In dogs, there were no significant findings in the liver at doses up to the highest dose tested, 100 mg/kg/day, following chronic administration. The NOAEL for the liver findings in dogs, male rats, female rats, and mice corresponded to exposures approximately 9, 1, 12, and 11 times greater, respectively, than the systemic exposure in men at the MRHD.

Avanafil was associated with altered hematology parameters and histopathological findings in the immune system. Increased reticulocytes, decreased RBC parameters, and increased WBC counts were seen as early as 1 week in rats and dogs. In rats, changes in RBC and WBC parameters were still present at the end of the 4-week recovery period following 6-month dosing. In humans, increased infections (e.g., upper respiratory tract infection, nasopharyngitis) were among the most frequently reported adverse events in patients taking avanafil in multiple-dose PRN studies. The NOAEL for the hematologic and immune system findings in male dogs, rats, and mice were

approximately 3, 1, and 5 times greater, respectively, than the systemic exposure in men at the MRHD.

Genotoxicity: Based on weight of evidence, avanafil was not considered to be genotoxic. Seven in vitro and in vivo genotoxicity studies were performed. Avanafil tested negative in two Ames assays, two chromosome aberration tests using CHL/IU and CHO cells, and in the in vivo mouse micronucleus and the rat UDS assays. Equivocal results were only observed in mouse lymphoma assay using L5178Y/TK+/- cells where a positive response was reported at 4 hours but not at 24 hours.

Carcinogenicity: Avanafil was not carcinogenic as tested in the two-year rat and mouse carcinogenicity studies. The highest doses used in the two-year studies resulted in systemic exposures approximately 8 times higher in male rats and 11 times higher in male mice than those observed clinically following use of 200 mg avanafil.

Reproductive toxicology: Based on animal data, avanafil is predicted to have a low risk for major developmental abnormalities in humans. For labeling, the recommended Pregnancy Category is C.

No teratogenic effects were observed at exposures up to approximately 30 times the MRHD in rats and 6-fold the MRHD in rabbits. Nonteratogenic effects consisting of decreased body weights in rats and increased late resorptions in rabbits were only observed at maternally toxic doses. In a pre- and post-natal development study in rats, offspring growth and maturation were reduced when maternal rats were exposed to a dose producing exposures approximately 17 times the human exposure. There was no effect on reproductive performance of the maternal rats or offspring, or on the behavior of the offspring at up to the highest dose tested. The NOAEL for developmental toxicity (100 mg/kg/day) was only approximately 2 times greater than the systemic exposure in men at the MRHD.

Avanafil-treated rats had reduced fertility in both males and females at exposures approximately 11- and 30-times, respectively, the exposure (AUC) in men at the MRHD. Changes in reproductive parameters in male rats included no or reduced sperm motility and increased abnormal sperm morphology (broken sperm with detached heads). Adverse effects on sperm were reversible at the end of a 9-week drug-free period. The NOAEL in rats is comparable to exposures in men at the MRHD.

Summary: The toxicity profile for avanafil is similar to that observed for other PDE5 inhibitors. The most concerning adverse nonclinical findings were CNS toxicity and impaired fertility, which occurred at low exposure multiples compared to clinical exposures. Signs of CNS toxicity were not reported in clinical studies

and potential adverse effects on fertility were not assessed following repeat exposures.

Although exposure multiples based on AUC or Cmax are low when comparing the animal NOAEL and LOAEL to exposures in men at the MRHD, these represent conservative estimates. Safety margins are expected to be much greater based on the following factors:

- Exposure multiples were calculated using bound plus unbound avanafil plasma levels. Only free, unbound avanafil is pharmacologically active. The concentration of free avanafil is higher in animals compared to humans: in vitro binding was 91% in rats, 93% in dogs and 99% in men.
- Animals were dosed daily for 6-9 months providing continuous exposure to avanafil. The frequency of exposures in men is expected to be much less when used only on an as needed basis, allowing for recovery for any potential adverse effects in targeted tissues.

Outstanding nonclinical issues: Based on adverse effects on spermatogenesis and fertility in animals, a post-marketing clinical study will be requested to further evaluate potential effects on spermatogenesis in men following repeat-dose administration. Labeling will indicate that the effect of avanafil on human spermatogenesis is unknown.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Yangmee Shin, that nonclinical data support approval of avanafil at doses up to 200 mg, to be used on an as needed basis for the treatment of erectile dysfunction.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LYNNDA L REID
03/26/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202-276 **Applicant:** VIVUS, Inc **Stamp Date:** 6/29/11

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

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		Most of the toxicology studies including the chronic toxicity in rats, carcinogenicity, and the reproductive and developmental toxicity studies were conducted using formulations different from the to-be-marketed formulation. The 9-month toxicity study in dogs was conducted with the to-be-marketed formulation.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		The study report for the return-to-fertility will be submitted during the mid-cycle of the NDA as agreed at the pre-NDA meeting.

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		The exposure multiples for the carcinogenicity studies were extrapolated on a mg/m^2 basis due to the lack of protein binding data for mice.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		Impurity (b) (4) was evaluated with in vitro genotoxicity studies.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

The Division reminds the sponsor that the reversibility study on fertility and sperm parameters in rats is submitted no later than the mid-cycle of the NDA review.

Yangmee Shin, Ph.D. 7/20/2011

Reviewing Pharmacologist/Toxicologist Date

Lynnda Reid, Ph.D.

Team Leader/Supervisor Date

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

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

YANGMEE SHIN
03/21/2012
amended with minor corrections

LYNNDA L REID
03/22/2012

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 202-276
Supporting document/s:	\\CDSESUB1\EVSPROD\NDA202276\202276.ENX
Applicant's letter date:	June 29, 2011
CDER stamp date:	June 30, 2011
Product:	[TRADENAME] (avanafil, TA-1790)
Indication:	Treatment of erectile dysfunction
Applicant:	VIVUS, Inc.
Review Division:	Division of Reproductive and Urologic Products
Reviewer:	Yangmee Shin, Ph.D.
Supervisor/Team Leader:	Lynnda Reid, Ph.D.
Office Director:	Julie Beitz, M.D.
Project Manager:	Eufrecina DeGuia

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202-276 are owned by VIVUS.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Avanafil is a PDE5 inhibitor indicated for the treatment of erectile dysfunction (ED) at the proposed recommended dose of 100 mg a day taken as needed approximately 30 minutes before sexual activity. The dose may be increased to a maximum recommended human dose (MRHD) of 200 mg or decreased to 50 mg with the maximum recommended dosing frequency of not more than once per day.

1.2 Brief Discussion of Nonclinical Findings

Standard nonclinical safety studies were provided including general and safety pharmacology; pharmacokinetics (PK); absorption, distribution, metabolism and excretion (ADME); general toxicology; genotoxicity; carcinogenicity; and reproductive and developmental toxicology studies.

Avanafil inhibited PDE5 in canine lungs (IC_{50}=4.2-5.2 nM) and human platelets (IC_{50}=8.9 nM) with in vitro potency similar to sildenafil (IC_{50}=1.6 nM for canine lungs, 4.3 nM for human platelets). Avanafil displayed high selectivity for PDE5 versus PDE6 (~100-fold) and other PDEs (>1000-fold). However, the IC_{50} value for PDE6 (630 nM) is only approximately 10-fold greater than the C_{max} for unbound avanafil in humans taking 200 mg (~26 ng/mL, 54 nM), suggesting the potential for avanafil to interfere in retinal phototransduction.

As expected from the pharmacologic activity of a PDE5 inhibitor, avanafil induced vasodilation in vitro and in vivo. Other cardiovascular effects included increased heart rate (HR), reduced blood pressures (BP), and prolonged QT interval. In toxicology studies, histopathological findings in the heart (e.g., fibrosis, thrombus, inflammation) were noted in avanafil-treated animals. Avanafil also inhibited HERG channel (IC_{50}=15.8 μM), action potential duration (APD), and Na (IC_{50} >50 μM) and L-type Ca (IC_{50} >30 μM) channel activities in vitro, suggesting that avanafil has mixed ion channel effects with a large safety margin (>290-fold unbound C_{max} at 200 mg).

Avanafil was rapidly absorbed and cleared systemically, primarily via fecal elimination, in both animals and humans. In rats, the highest tissue concentrations were present in the GI tract, liver, and kidney at approximately 20 times the levels in blood. The lowest levels were observed in the brain, spinal cord, eye, and testis. In vitro, avanafil was extensively metabolized primarily by CYP3A4, with a minor contribution by CYP2C. The metabolic profile was qualitatively similar in male rats, male dogs, and men. The major circulating metabolites in men were M16 (open pyrrolidine ring carboxylic acid isomers) and M4 (hydroxyl metabolite).

Dose-limiting CNS-related adverse effects were observed in avanafil-treated animals. In particular, clinical signs such as ataxia, tremor, convulsion, prostration, hypoactivity, and/or recumbency, led to mortality in mice and pregnant rats, or suspension of dosing in dogs at exposures approximately at 5-8 fold greater than the mean C_{max} levels at the

MRHD. The presence of the neurological signs following repeat-dosing and the lack of the effects in single-dose studies at up to 2000 mg/kg/day are indicative of delayed onset for CNS-related symptoms. The NOAEL (600 mg/kg/day in mice, 300 mg/kg/day in pregnant rats, 60 mg/kg/day in dogs) represents exposures levels approximately 3-5 fold higher than the mean C_{max} (~2600 ng/mL) at the MRHD in humans.

Avanafil was not considered to be genotoxic based on weight of evidence from in vitro and in vivo genotoxicity tests. Avanafil tested negative in two Ames assays, two chromosome aberration tests using CHL/IU and CHO cells, and in vivo in the mouse micronucleus and the rat UDS assays.

In 2-year carcinogenicity studies, there were no positive tumor findings in mice or rats at up to the highest doses tested (approximately 11-, 8- and 34-fold the MRHD based on AUC for mice, male rats and female rats, respectively).

Avanafil-treated rats had reduced fertility in both males and females at approximately 11- and 30-fold the AUC, respectively, at the MRHD. Changes in reproductive parameters included no or reduced sperm motility, altered estrous cycles, and increased abnormal sperm morphology (broken sperm with detached heads). Adverse effects on sperm were reversible at the end of a 9-week drug-free period. Based on the animal findings and the conflicting results from single-dose clinical studies, a follow-up clinical study will be requested to further evaluate potential effects on spermatogenesis in men following repeat-dose administration.

No teratogenic effects were observed at exposures up to approximately 30-fold the MRHD in rats and 6-fold the MRHD in rabbits. In rats, nonteratogenic effects observed at these exposures included decreased fetal body weights in the presence of maternal toxicity (e.g., central nervous system-related clinical signs and mortality, reduced body weights and body weight gains). In rabbits, increased postimplantation loss, which correlated with increased late resorptions, was noted in the presence of reduced maternal body weight and body weight gain. In a pre- and post-natal development study, offspring growth and maturation were reduced when maternal rats were exposed to a dose producing exposures approximately 17 times the human exposure.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, the nonclinical data submitted support the approval of avanafil for the treatment of erectile dysfunction at the proposed doses.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Recommended labeling for relevant nonclinical sections (see annotated label in Appendix):

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

[TRADENAME] is a phosphodiesterase 5 (PDE5) inhibitor indicated for the treatment of erectile dysfunction.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

[TRADENAME] is a phosphodiesterase 5 (PDE5) inhibitor indicated for the treatment of erectile dysfunction.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

[TRADENAME] is not indicated for use in women. There are no adequate and well-controlled studies of STENDRA in pregnant women.

Fetal Risk Summary

Based on animal data, [TRADENAME] is predicted to have a low risk for major developmental abnormalities in humans.

Animal Data

In pregnant rats administered 100, 300, or 1000 mg/kg/day avanafil from gestation days 6 to 17, no evidence of teratogenicity, embryotoxicity, or fetotoxicity was observed at exposures up to approximately 8 times the Maximum Recommended Human Dose (MRHD) of 200 mg based on AUCs for total avanafil (protein bound plus free avanafil). At the maternally toxic dose (1000 mg/kg/day), a dose producing exposures approximately 30 times the MRHD on an AUC basis, decreased fetal body weight occurred with no signs of teratogenicity. In pregnant rabbits administered 30, 60, 120, or 240 mg/kg/day avanafil from gestation days 6 to 18, no teratogenicity was observed at exposures up to approximately 6 times the human exposure at the MRHD based on AUC. At the high dose associated with maternally-reduced body weights, increased postimplantation loss was observed consistent with increased late resorptions.

In a pre- and post-natal development study in rats given 100, 300, or 600 mg/kg/day avanafil on gestation days 6 through lactation day 20, offspring growth and maturation were reduced when maternal rats were given avanafil doses greater than or equal to 300 mg/kg resulting in exposures greater than or equal to 17 times the human exposure. There was no effect on reproductive performance of the maternal rats or offspring, or on the behavior of the offspring at up to the highest dose tested. The no

observed adverse effect level (NOAEL) for developmental toxicity (100 mg/kg/day) was approximately 2-fold greater than the systemic exposure in humans at the MRHD.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The physiologic mechanism of erection of the penis involves release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. NO then activates the enzyme guanylate cyclase, which results in increased levels of cGMP, producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. Avanafil has no direct relaxant effect on isolated human corpus cavernosum, but enhances the effect of NO by inhibiting PDE5, which is responsible for degradation of cGMP in the corpus cavernosum. (b) (4)

Studies *in vitro* have shown that avanafil is selective for PDE5. Its effect is more potent on PDE5 than on other known phosphodiesterases (greater than 100-fold for PDE6; greater than 1,000-fold for PDE4, PDE8 and PDE10; greater than 5,000-fold for PDE2 and PDE7; greater than 10,000-fold for PDE1, PDE3, PDE9, and PDE11). Avanafil is greater than 100-fold more potent for PDE5 than PDE6, which is found in the retina and is responsible for phototransduction (b) (4)

In addition to human corpus cavernosum smooth muscle, PDE5 is also found in other tissues including platelets, vascular and visceral smooth muscle skeletal muscle, brain, heart, liver, kidney, lung, pancreas, prostate, bladder, testis, and seminal vesicle. The inhibition of PDE5 in these tissues by avanafil may be the basis for the enhanced platelet antiaggregatory activity of NO observed *in vitro* and peripheral vaso-dilatation *in vivo*.

12.2 Pharmacodynamics

(b) (4)

12.3 Pharmacokinetics

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

Carcinogenesis

Avanafil was not carcinogenic to CD-1 mice when administered daily at doses of 100, 200, or 600 mg/kg/day orally by gavage for at least 98 weeks (approximately 11 times the MRHD on an AUC basis) or to Sprague Dawley rats when administered daily at doses of 100, 300, or 1000 mg/kg/day orally by gavage for at least 100 weeks

(b) (4)

Mutagenesis

Avanafil was not genotoxic in a series of tests. Avanafil was not mutagenic in Ames assays. Avanafil was not clastogenic in chromosome aberration assays using Chinese hamster ovary and lung cells, or in vivo in the mouse micronucleus assay. Avanafil did not affect DNA repair when tested in the rat unscheduled DNA synthesis assay.

Impairment of Fertility

In a rat fertility and early embryonic development study administered 100, 300, or 1000 mg/kg/day for 28 days prior to paring and continued until euthanasia for males, and 14 days prior to pairing to gestation day 7 for females, a decrease in fertility, no or reduced sperm motility, altered estrous cycles, and an increased percentage of abnormal sperm (broken sperm with detached heads) occurred at exposures in males approximately 11 times the human exposure at a dose of 200 mg. The altered sperm effects were reversible at the end of a 9-week drug-free period. Systemic exposure at the NOAEL (300 mg/kg/day) was comparable to the human AUC at the MRHD of 200 mg.

13.2 Animal Toxicology and/or Pharmacology

Repeated oral administration of avanafil in multiple species resulted in signs of centrally-mediated toxicity in animals including ataxia, tremor, convulsion, hypoactivity, recumbency and/or prostration at doses resulting in exposures approximately 5-8 times the MRHD based on C_{max} and 8-30 times the MRHD based on AUC.

2 Drug Information

2.1 Drug

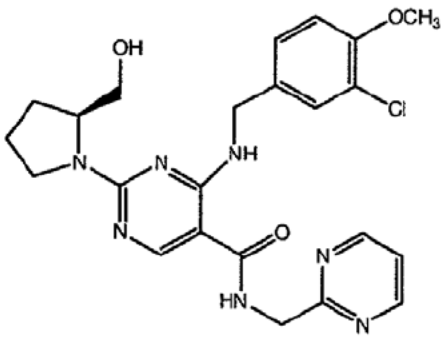
CAS Registry Number: 330784-47-9

Generic Name: Avanafil

Code Name: TA-1790

Chemical Name: (*S*)-4-[(3-Chloro-4-methoxybenzyl)amino]-2-(2-(hydroxymethyl)-1-pyrrolidinyl)-*N*-(2-pyrimidinylmethyl)-5-pyrimidinecarboxamide
Molecular Formula/Molecular Weight: $C_{23}H_{26}ClN_7O_3$/483.95

Structure or Biochemical Description



Pharmacologic Class: PDE5 inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 51,235; DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4); DMF (b) (4) DMF (b) (4)

2.3 Drug Formulation

Oral, oval, pale yellow, immediate release tablet at 50 mg, 100 mg, and 200 mg strengths

The following table describes the composition of the avanafil drug product.

			50 mg Tablets		100 mg Tablets		200 mg Tablets	
Component	**Reference to Quality Standard**	**Function**	**mg**	**%**	**mg**	**%**	**mg**	**%**
Avanafil	In-house Standard	Active Ingredient	(b) (4)					
Mannitol	USP							
Fumaric Acid	NF							
Hydroxypropylcellulose	NF							
Low substituted Hydroxypropylcellulose	NF							
(b) (4) Calcium Carbonate	USP							
Magnesium Stearate	NF							
Yellow Ferric Oxide	NF							
Total Mass (mg/tablet)	--							

(b) (4)

2.4 Comments on Novel Excipients

All inactive ingredients and excipients are found in previously approved drug products.

2.5 Comments on Impurities/Degradants of Concern

The impurity (b) (4) was assessed with in vitro genotoxicity studies, and tested negative. The specification for unspecified process impurities and degradation products are acceptable (See Chemistry Review for details).

2.6 Proposed Clinical Population and Dosing Regimen

Avanafil is indicated for the ED at the recommended starting dose of 100 mg. Avanafil should be taken orally as needed approximately 30 minutes before sexual activity. Based on individual efficacy and tolerability, the dose may be increased to a maximum dose of 200 mg or decreased to 50 mg with the maximum recommended dosing frequency of once per day. Sexual stimulation is required for a response to treatment. Avanafil may be taken with or without food.

2.7 Regulatory Background

VIVUS is submitting an electronic NDA for the treatment of erectile dysfunction (ED). Clinical trials were conducted and supporting nonclinical data were reviewed under IND 51,235. A pre-NDA meeting was held on October 20, 2011, to discuss the NDA submission for avanafil immediate release tablets under 505(b)(1).

3 Studies Submitted

3.1 Studies Reviewed

A full nonclinical program on avanafil was conducted and submitted. These included general and safety pharmacology, pharmacokinetics/ADME, single- and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproductive and developmental toxicology, and a single-dose phototoxicity studies. The following nonclinical studies included in this review:

Pharmacology:

- Inhibitory Effects of TA-1790 (Avanafil), Sildenafil, Vardenafil, and Tadalafil on Eleven PDE Isozymes
- Inhibitory Effects of TA-1790, A PDE5 Inhibitor, on PDE5 and PDE4 from Canine Lung and PDE3 from Canine Heart
- Inhibitory Effects of TA-1790 and Sildenafil on PDE5 from Human Platelets
- Effect of TA-1790 (PDE5 Inhibitor) on the Relaxation Induced by Electrical Field Stimulation in the Isolated Rabbit Corpus Cavernosum
- Potentiating effects of TA-1790 on the Penile Tumescence in the Anesthetized Rabbits
- Effects of Intravenous Administration with TA-1790 on the Penile Tumescence in the Anesthetized Dogs
- Effects of Intraduodenal Treatment with TA-1790 on the Penile Tumescence in the Anesthetized Dogs
- Potentiating effects of intravenous TA-1790 on penile tumescence in anesthetized Monkeys

Safety Pharmacology:

- Effects of TA-1790 on Cloned hERG Channels Expressed in Mammalian Cells
- Effect of TA-1790 on Human Cardiac I_{Na} (hHNa) Current Expressed in Mammalian Cells
- Effect of TA-1790 on the Native Cardiac L-type Calcium Current ($I_{Ca,\,L}$) in Guinea Pig Cardiomyocytes
- Vasodilator Activity of TA-1790 in the Isolated Rat Aorta
- Vasorelaxing Effects of TA-1790 in the Endothelium-Denuded Rat Thoracic Aorta
- Effects of TA-1790 on Ca^{2+} Induced Contractions in K^{+}-depolarized Rat Aorta without Endothelium
- Effect of TA-1790 on Cardiac Action Potential Configuration
- Effect of TA-1790 on Action Potentials in Isolated Cardiac Purkinje Fibers
- Safety Pharmacology Study of TA-1790 in Dogs-Effects on the Cardiovascular and Respiratory Systems
- Hemodynamic Effects of Intravenously Infused TA-1790 in Anesthetized Dogs
- Hemodynamic Effects of Intravenously Infused TA-1790 in the Anesthetized Open-Chest Dogs
- Effects of TA-1790 on General Activity and Behavior in Mice
- Central Nervous System: Effect of TA-1790 on Spontaneous Locomotor Activity Count in Mice
- Central Nervous System: Effect of TA-1790 on Pentobarbital-Induced Sleep in Mice
- Central Nervous System: Effect of TA-1790 on Maximal Electroshock-Induced Convulsion in Mice
- Central Nervous System: Effect of TA-1790 on Pentylentetrazol-Induced Convulsion and Death in Mice
- Central Nervous System: Proconvulsant Effect of TA-1790 in Mice Given Subliminal Electroshock
- Central Nervous System: Proconvulsant Effect of TA-1790 in Mice Subliminal Dose of Pentylenetetrazol
- Central Nervous System: Effect of TA-1790 on Acetic Acid-Induced Writhing in Mice
- Safety Pharmacology Study of TA-1790 in Rats: Effects on the Central Nervous System
- Central Nervous System: Effect of TA-1790 on Body Temperature in Rats
  Effects of TA-1790, Sildenafil, and E4021 on Electroretinogram of Isolated Rabbit Retina
- Safety Pharmacology Study of TA-1790 in Dogs- Effects on Eye Function
- Effects of Intraduodenal Administration of TA-1790 on 30Hz Flicker-Induced ERG in Anesthetized Dogs
- Autonomic Nervous System and Smooth Muscle: Effect of TA-1790 on Agonist-Induced Contraction of Isolated Guinea Pig Ileum
- Autonomic Nervous System and Smooth Muscle: Effect of TA-1790 on Spontaneous Contraction of Isolated Rabbit Jejunum
- Gastrointestinal System: Effect of TA-1790 on Gastric Emptying in Mice
- Gastrointestinal System: Effect of TA-1790 on Small Intestinal Transit in Mice
- General Pharmacological Study of TA-1790: Effect on Gastric Acid Secretion in Rats
- Water and Electrolyte Metabolism: Effect of TA-1790 on Urine Volume and Electrolyte Excretion in Saline-Loaded Rats
- Effects of TA-1790 and Sildenafil on Adenosine Responses in the Isolated Rat Vas Deferens
- Effect of TA-1790 on Platelet Aggregation in Human Platelets
- Effects of Blood Coagulation and Fibrinolysis Systems in Rats after Oral Administration Once Daily for 5 Consecutive Days
- Effects of TA-1790 on Nitric Oxide Synthase Activity and Radioligand Binding Assays

PK/ADME/TK:

- Biopharmaceutics Classification System (BCS) Permeability Evaluation for Avanafil
- The Time Course of Plasma Concentration of Radioactivity after Oral and Intravenous Administration of ^{14}C-TA-1790 to Male Rats
- Time Course of Unchanged TA-1790 in Plasma after Oral or Intravenous Administration of TA-1790 to the Rats

- Pharmacokinetics of Avanafil and its Two Metabolites (M4 and M16 Isomers) in Fasted Male Sprague-Dawley Rats Following Single Oral Doses of Avanafil
- Toxicokinetic Study by Single Oral administration of TA-1790 in Rats
- A Two-week Repeated Oral Dose Toxicity Study of TA-1790 in Rats
- TA-1790: Oral Toxicokinetic Study in Male and Pregnant Female Rats in Support of Toxicity Studies
- TA-1790: Oral Toxicokinetic Study in Male and Pregnant Female Rabbits in Support of an Embryo-Fetal Developmental Toxicity Study
- Time Courses of Plasma Concentration of Unchanged TA-1790 After Single Oral and Intravenous Administration of TA-1790 to Male Dogs
- Absorption and Excretion of ^{14}C-TA-1790 in the Dogs after Oral and Intravenous Administration
- Two-week Repeated Oral Dose Toxicity Study of TA-1790 in Dogs
- Absorption and Excretion of ^{14}C-TA-1790 in the Monkeys after oral and Intravenous Administration
- Absorption Sites from the Gastrointestinal Tract of ^{14}C-TA-1790 in Rats
- Binding of ^{14}C-TA-1790 to Plasma Proteins in Vitro
- Protein Binding Studies for Avanafil Metabolites, M4 and M16 in Rat, Dog, Rabbit Plasma and Avanafil in Rabbit Plasma Samples
- Plasma Protein Binding of Avanafil in Young and Elderly Healthy Male Subjects from Study TA-014
- Plasma Protein Binding of Avanafil and Its Metabolites M4 and M16 in Subjects with Hepatic Impairment and in Healthy Control Male Subjects from Study TA-012
- Plasma Protein Binding of Avanafil and Its Metabolites M4 and M16 in Subjects with Renal Impairment and in Healthy Control Male Subjects from Study TA-013
- Tissue Distribution by Quantitative whole-Body Autoradioluminography after Oral and Intravenous Administration of ^{14}C-TA-1790 to Male Albino Rats
- Tissue Distribution by Quantitative whole-Body Autoradioluminography after Oral and Intravenous Administration of ^{14}C-TA-1790 to Male Pigmented Rats
- Time Courses of Concentration in Eyeball after Intravenous Administration of ^{14}C-TA-1790 or Sildenafil to Male Pigmented Rats
- Interspecies Differences in Metabolic Stability of TA-1790 among the Male Rat, Dog and Humans Liver Microsomes
- Identification of Metabolites of TA-1790 Formed by hepatic Microsomes and Hepatocytes
- Inference of the Cytochrome P450 Isoforms Involved in the Metabolism of TA-1790 in Human Liver Microsomes- Inference based on the study with cDNA-expressed human cytochrome P450 isoforms
- Inference of the Cytochrome P450 Isoform Involved in the Metabolism of TA-1790 in Human Liver Microsomes- Inference based on an immunoinhibition study
- Search for Radioactive Metabolites in the Plasma after Oral Administration of ^{14}C-TA-1790 to the Rat
- Excretion, Mass Balance, and Metabolite Profiling of Radioactivity in Intact and Bile-duct Cannulated Sprague-Dawley Rats after an Oral Dose of [^{14}C]-TA-1790
- Excretion, Mass Balance, and Metabolite Profiling of Radioactivity in Beagle Dogs after an Oral Dose of [^{14}C]-TA-1790
- In Vitro Inhibitory Effects of TA-1790 on Human Cytochrome and Implications for Metabolic Drug Interactions
- ADME-TOX Module 2 CYP Inhibition: Study of M4, M16 and M27
- Evaluation of the Potential for Induction of CYP1A2, CYP2B6, and CYP3A4 Activities in Cultured Human Hepatocytes by Avanafil
- The Cumulative Excretion of Radioactivity in the Urine, Feces and Expired Air after Oral and Intravenous Administration of ^{14}C-TA-1790 to Rats
- The Radioactivity Excretion Rate into the Bile after Oral and Intravenous Administration of ^{14}C-TA-1790 to Rats
- Enterohepatic Circulation of ^{14}C-TA-1790 in Rats

- MDR1-MDCK and MDCK-Wt Bidirectional Permeability for Avanafil as a Potential P-glycoprotein Substrate or Inhibitor

General Toxicology:

- Single Oral Dose Toxicity Study of TA-1790 in Mice
- Single Intravenous Dose Toxicity Study of TA-1790 in Mice
- Single Oral Dose Toxicity Study of TA-1790 in Rats
- Toxicokinetic Study by Single Oral administration of TA-1790 in Rats
- Single Intravenous Dose Toxicity Study of TA-1790 in Rats
- Single Oral Dose Toxicity Study of TA-1790 in Dogs (Increasing-Dose Study)
- Single Dose Oral Toxicokinetic Study of TA-1790 in Dogs
- A Two-Week Repeated Oral Dose Toxicity Study of TA-1790 in Rats
- Two-Week Oral Dose Toxicity Study of TA-1790 in Dogs
- 28-Day Oral Dose Toxicity Study with TA-1790 in Male Rats with a 1-Week Recovery
- 28-Day Oral Dose Toxicity Study with TA-1790 in Male Beagle Dogs with a 2-Week Recovery
- 13-Week Oral Gavage Preliminary Carcinogenicity and Toxicokinetic Study with TA-1790 in Mice
- 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with TA-1790 in Rats with a 13-Week Interim Sacrifice and a 4-Week Recovery Period
- 9-Month Oral Chronic Toxicity Study with TA-1790 in Male Dogs with a 4-Week Recovery Period

Genetic Toxicology:

- Reverse Mutation Test of TA-1790 in Bacteria
- In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)
- Bacterial Reverse Mutation Assay
- Bacterial Reverse Mutation Study of TA-1790 Impurity (b) (4)
- Chromosome Aberration Test of TA-1790 in Cultured Mammalian Cells
- In Vitro Mammalian Chromosome Aberration Test
- Chromosome Aberration Study of TA-1790 Impurity (b) (4) in Cultured Mammalian Cells
- In Vitro Mammalian Cell Gene Mutation Test
- In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)
- Unscheduled DNA Synthesis Test with Mammalian Liver Cells

Carcinogenicity:

- A 2-Year Carcinogenicity Study of TA-1790 in Mice
- A 2-Year Carcinogenicity Study of TA-1790 in Rats

Reproductive and Developmental Toxicology:

- TA-1790: A Study of Fertility and Early Embryonic Development to Implantation in Rats
- TA-1790: An Oral Study of Reversibility of Effects on Fertility and Early Embryonic Development in Male Rats
- Oral Embryo-Fetal Toxicity Study in Rats
- An Oral Range-Finding Study for Effects on Embryo-Fetal Development in Rabbits
- Study for Effects on Pre- and Post-natal Development, including Maternal Function in Rats following Oral Administration

Special Toxicology:

- Single Dosage Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of TA-1790 on Eyes and Skin in Pigmented Rats

3.2 Studies Not Reviewed

The following non-GLP studies are not included within the NDA review, but are summarized in the **Integrated Summary and Safety Evaluation**.

- A One-Week Oral Repeated Dose Toxicity Study of TA-1790 in Rats
- A One-Week Oral Repeated Dose Toxicity Study of TA-1790 in Dogs

3.3 Previous Reviews Referenced

IND 51,235

4 Pharmacology

4.1 Primary Pharmacology

Avanafil is a potent PDE5 inhibitor in canine lungs (IC_{50}=4.2-5.2 nM) and human platelets (IC_{50}=8.9 nM). Avanafil displayed approximately 100-fold selectivity for PDE5 versus PDE6; 1,000-fold for PDE4, PDE8A, and PDE10A; 5,000-fold for PDE2 and PDE7B; and 10,000-fold for PDE1, PDE3, PDE9A, and PDE11A, isolated from various sources (see Table 1 below). The IC_{50} value for PDE6 (630 nM) is approximately 10-fold above unbound C_{max} in humans taking 200 mg (~26 ng/mL, 54 nM).

Table 1 Inhibitory Effects of Avanafil, Sildenafil, Vardenafil and Tadalafil on PDE Isozymes and Selectivity versus PDE5

		PDE1	PDE2	PDE3	PDE4	PDE5	PDE6	PDE7B	PDE8A	PDE9A	PDE10A	PDE11A
		Rat heart	Bovine adrenal	Canine heart	Canine lung	Canine lung	Bovine retina	Recomb.	Recomb.	Recomb.	Rat striatum	Recomb.
TA-1790	IC_{50} (nM)	53000	51000	>100000	5700	5.2	630	27000	12000	>100000	6200	>100000
	Selectivity vs PDE5	10192	9808	>19231	1096	1	121	5192	2308	>19231	1192	>19231
Sildenafil	IC_{50} (nM)	600	63000	26000	5000	1.6	25	22000	>100000	3600	5400	7800
	Selectivity vs PDE5	375	39375	16250	3125	1	16	13750	>62500	2250	3375	4875
Vardenafil	IC_{50} (nM)	85	23000	2200	1200	0.084	1.8	1500	84000	1400	1500	500
	Selectivity vs PDE5	1012	273810	26190	14286	1	21	17857	1000000	16667	17857	5952
Tadalafil	IC_{50} (nM)	42000	>100000	>100000	59000	4.0	2200	>100000	>100000	>100000	35000	100
	Selectivity vs PDE5	10500	>25000	>25000	14750	1	550	>25000	>25000	>25000	8750	25

The human circulating metabolites M4, M16, and M27 were found to inhibit human platelet PDE5 with higher IC_{50} values than avanafil (51, 4100, and 4500 nM, respectively). Considering the low plasma concentrations of the major circulating metabolites M4 (~23%) and M16 (~29%) of the parent drug and the low in vitro inhibitory potency for PDE5 (<18%) of avanafil, the metabolites are unlikely to produce a major contribution to the therapeutic activity of avanafil (e.g., M4 accounts for 4% of total pharmacologic activity).

In isolated corpus cavernosum from New Zealand White (NZW) rabbits, avanafil potentiated electrical field stimulation (EFS)-induced relaxation in a concentration-dependent manner at 0.003-0.3 μM, similar to sildenafil. In vivo, avanafil and sildenafil both produced a dose-dependent potentiation of penile tumescence at 10-1000 μg/kg intravenously administered to either anesthetized NZW rabbits or cynomolgus monkeys. When administered intraduodenally to dogs, avanafil and sildenafil potentiated dose-

dependent penile tumescence with ED_{200} of 37.5 µg/kg (59.6 ng/mL, 0.12 µM) and 34.6 µg/kg (38.8 ng/mL, 0.082 µM), respectively, at doses of 3-300 µg/kg.

4.2 Secondary Pharmacology

The sponsor stated that no secondary pharmacodynamics studies were performed. However, considering the widespread distribution of the PDE5 in addition to human corpus cavernosum including platelets, vascular and visceral smooth muscle skeletal muscle, brain, eye, heart, liver, kidney, lung, pancreas, *etc*, inhibition of the enzyme is expected to be implicated in many physiological and pathological processes in undesired targets. Some of these endpoints were assessed in safety pharmacology studies.

Various receptors and ion channels were exposed to avanafil to characterize the selectivity for potential molecular targets using radioligand binding assays. Avanafil did not substantially displace radioligand binding to any of the receptors and ion channels tested at concentrations up to 10 µM, while sildenafil citrate inhibited binding to A_1, A_{2A}, A_{2B} and α_1 receptors in a concentration-dependent manner at 1 and 10 µM (see Table below).

Table 2 Effects of Avanafil and Sildenafil in NOS and Receptors/Ion Channel Binding Assays

Enzyme or Receptor	Source	TA-1790		Sildenafil	
		1 µM	10 µM	1 µM	10 µM
Constitutive Nitric Oxide Synthase	Rat cerebellum	6	15	2	5
Inducible Nitric Oxide Synthase	Mouse macrophages	1	13	12	8
Adenosine A_1	Human recombinant	8	19	73	94
Adenosine A_{2A}	Human recombinant	-5	9	82	90
Adenosine A_{2B}	Human recombinant	1	21	39	70
Adrenergic α_1, Non-selective	Rat brain	7	17	25	57
Adrenergic α_2, Non-selective	Rat cortex	4	7	1	-2
Adrenergic β_1	Human recombinant	3	-1	10	4
Adrenergic β_2	Human recombinant	2	4	2	2
Adrenergic β_3	Human recombinant	19	35	5	7
Atrial Natriuretic Factor (ANF)	Guinea pig adrenal gland	-3	-4	-3	-1
Bradykinin B_2	Human recombinant	-1	1	-3	0
Calcium channel Type L, Benzothiazepine	Rat cerebral cortex	7	3	14	17
Calcium channel Type L, Dihydropyridine	Rat cerebral cortex	4	10	12	35
Calcium channel Type L, Phenylalkylamine	Rat brain	-6	7	12	35
Calcium channel Type N	Rat brain frontal lobe	3	5	3	4
Dopamine D_1	Human recombinant	-7	-11	12	-3
Dopamine D_{2L}	Human recombinant	1	-13	6	-4
Histamine H_1, Central	Guinea pig brain	10	12	10	14
Muscarinic, Non-Selective, Central	Rat cortex	9	19	11	17
Opiate, Non-Selective	Rat brain	14	6	15	13
Serotonin 5-HT_1, Non-Selective	Rat brain cortex	-7	-11	-8	-12
Serotonin 5-HT_2	Rat brain	6	10	4	11
Vasopression V_{1A}	Human recombinant	0	9	2	0

4.3 Safety Pharmacology

Respiratory and Cardiovascular Effects:

- Avanafil inhibited hERG current in a concentration-dependent manner with IC_{50} of 15.8 µM, which corresponded to exposures approximately 290-fold above the unbound C_{max} (~26 ng/mL, 0.054 µM) in human subjects after the maximum recommended single oral dose at 200 mg.
- Avanafil produced a concentration-dependent inhibition of hHNa current (IC_{50} >50 µM) and L-type calcium channels (IC_{50} >30 µM) expressed in guinea pig ventricular myocytes.
- Avanafil at concentrations up to 100 µM produced a concentration-dependent decrease in the duration of the action potential (APD) in canine Purkinje fibers.
- Both avanafil and sildenafil produced a concentration-dependent vasodilation in endothelium-intact (0.0001-10 µM) and in denuded (0.0001-100 µM) rat aorta.
- Both avanafil and sildenafil also showed altered hemodynamic and ECG effects (e.g., decreased systolic blood pressure, mean arterial blood pressure, diastolic blood pressure, pulmonary arterial pressure, vascular resistance of coronary artery, vascular resistance of common coronary artery and total peripheral

resistance; increased heart rate and cardiac output; prolonged QT interval) in anesthetized (1-300 µg/kg/min, intravenous) and/or conscious dogs (30 mg/kg, oral, ~4-fold above unbound C_{max} ~0.114 µg/mL, 0.2 µM).
- Avanafil had potentiating effects on nitroglycerin-induced hypotension in anesthetized dogs at ≥0.1 mg/kg.

Central Nervous System Effects:
- Avanafil decreased spontaneous locomotor activity at an oral dose of 1000 mg/kg in mice and rats, increased sleeping time at 300 mg/kg in mice.

Gastrointestinal Effects:
- Avanafil had a statistically-significant inhibition of acetylcholine-, histamine-, and serotonin-induced contractions in isolated guinea pig ileum, and the amplitude of spontaneous contractions in isolated rabbit jejunum at 10 µM.
- Avanafil significantly decreased gastric secretion in rats at 300 mg/kg.

Genitourinary and Hemostasis:
- Decreased urine volume and electrolyte excretion were noted from 10 mg/kg avanafil.
- Avanafil potentiated the anti-aggregation action of SNP in human platelet-rich plasma at 10 µM.

Ocular Effects:
- Avanafil produced a concentration-dependent increase in b-wave amplitude at 1-30 µM, but to a lesser extent than sildenafil.

Abuse and Dependence: Not provided

The sponsor stated that no adverse events of drug abuse were reported during the clinical development program. The sponsor further stated that there was essentially a "withdrawal period" after every dose recorded in the clinical development program, and there were no withdrawal issues associated with avanafil during the clinical development program, given that avanafil is intended to be used on an as needed basis. The potential for misuse and abuse (e.g., development of tolerance, dose-increment, drug-seeking behavior) following long-term use of PDE5 inhibitors is currently unknown. PDE5 inhibitors may be different from other drugs of abuse in that the increasing use of PDE5 inhibitors among relatively healthy men associated with alcohol or other drug use may promote psychological dependence on the drug as PDE5 inhibitors become widely available and are perceived as relatively safe drugs. This issue could be revisited as more PDE5 inhibitors are being developed for daily administration.

The following table summarizes the results from the safety pharmacology studies.

Study Type	Species	Route	Dose	Main Results	
				Avanafil	Sildenafil
Hemodynamic & respiratory systems	Anesthetized dog, ♂ (non-GLP)	IV	0, 1, 10, 100, 300 µg/kg/min	↓MAP at ≥1 µg/kg/min	↓MAP at ≥1 µg/kg/min; ↑VBF & ↓VRVA at ≥100 µg/kg/min
	Anesthetized open-chest dog, ♂ (non-GLP)	IV	0, 1, 10, 100, 300 µg/kg/min	↓MAP/PAP/VRCA/VRCCA at ≥1 µg/kg/min; ↑CBF at ≥100 µg/kg/min; ↑CO & ↓TPR at ≥100 µg/kg/min	↓MAP/VRCA/VRCCA & ↑CCBF at ≥1 µg/kg/min; ↑HR at ≥100 µg/kg/min; ↑CO & ↓TPR at ≥100 µg/kg/min
	Conscious dog, ♂/♀ (GLP)	Oral	0, 3, 10, 30 mg/kg (escalating)	↓SBP/MAP/DBP; ↑HR & ↑QT interval at 30 mg/kg (unbound C_{max}~0.114 µg/mL, ~0.2 µM)	-
	Anesthetized dog, ♂ (non-GLP)	ID	0, 0.1, 1 mg/kg	↑Nitroglycerin-induced hypotension at ≥0.1 mg/kg	↑Nitroglycerin-induced hypotension at ≥0.1 mg/kg
	Guinea pig papillary muscle, ♂ (non-GLP)	In vitro	1, 10 µM	No effect on APD	-
	HEK cells (GLP)	In vitro	1, 10, 30,100 µM	Concentration-dependent ↓hERG current (IC_{50}=15.8 µM)	-
	Human heart (non-GLP)	In vitro	10, 30, 50 µM	Concentration-dependent ↓hHNa current (IC_{50}>50 µM)	-
	Guinea pig ventricular myocytes (non-GLP)	In vitro	3, 10, 30 µM	Concentration-dependent ↓$I_{ca,L}$ current (IC_{50}>30 µM)	-
	Dog Purkinje fiber (GLP)	In vitro	1, 10, 100 µM	Concentration-dependent ↓APD_{90}	-
	Rat aorta, ♂ (non-GLP)	In vitro	0.0001-100 µM	Vasodilatation in biphasic pattern (intact & denuded)	Vasodilatation in biphasic pattern (intact & denuded)
CNS & general behavior	Rat, ♂/♀ (GLP)	Oral	0, 30, 180, 1000 mg/kg	↓Spontaneous locomotor activity at 1000 mg/kg (unbound C_{max}~1.2-2.7 µg/mL, ~2.4-5.54 µM)	-
	Rat, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on rectal temperature	-
	Mouse, ♂ (non-GLP)	Oral	0, 10, 30, 100, 300 & 1000 mg/kg	↓Spontaneous locomotor activity at 1000 mg/kg	-
	Mouse, ♂ (non- GLP)	Oral	0, 100, 300 mg/kg	No significant effect on locomotor behavior	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	↑Pentobarbital-induced sleeping time at 300 mg/kg	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on electroshock-induced convulsions	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on pentylenetetrazol-induced convulsions	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on subliminal electroshock procedure	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on subliminal pentylenetetrazol procedure	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No antinociceptive effect on acetic acid-induced writhing test	-

Eye	Rabbit (non-GLP)	In vitro	0.3, 1, 3, 10, 30 μM	Concentration-dependent ↑b-wave amplitude at ≥1 μM	Concentration-dependent ↑b-wave amplitude at ≥1 μM; ↑a- & b-wave implicit time at ≥1 μM; ↓a-wave amplitude at ≥10 μM
	Anesthetized dog, ♂ (non-GLP)	ID	0, 10 & 30 mg/kg	No significant effect on ERG	Delayed time to peak of positive wave at ≥1 mg/kg
	Conscious dog (GLP)	Oral	0, 10, 30 & 100 mg/kg	No significant effect on after dark & light adaptation (unbound C_{max}~0.58 μg/mL, ~1.2 μM)	-
Renal system	Rat, ♂ (non-GLP)	Oral	0, 1, 3, 10, 30 mg/kg	↓Urine volume/Na^+/Cl^- excretion at ≥10mg/kg	-
	Rat (non-GLP)	In vitro	0.3, 1, 3 μM	No effect on EFS-induced tension suppression by a A1/A2 receptor agonist (NECA)	Concentration-dependent reversal of NECA-induced tension suppression at ≥0.1 μM
GI tract	Rat, ♂ (non-GLP)	ID	0, 100, 300 mg/kg	↓Gastric secretion at 300 mg/kg in pylorus-ligated rats	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on gastric emptying	-
	Mouse, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg	No significant effect on small intestinal transit	-
	Guinea pig, ♂ (non-GLP)	In vitro	0, 1, 10 μM	↓Ach, histamine & serotonin-induced contraction of isolated ileum at 10 μM	-
	Rabbit, ♂ (non-GLP)	In vitro	0, 1, 10 μM	↓Amplitude of isolated jejunum at 10 μM	-
Hemostasis	Rat, ♂ (non-GLP)	Oral	0, 100, 300 mg/kg (5-day MD)	No significant effect on HT/PT/APTT/Fg/ELT	-
	Human platelets (M) (non-GLP)	In vitro	0.1, 1, 10 μM	↑Anti-aggregatory action of SNP at 10 μM (IC_{50}=3.1 μM)	↑Anti-aggregatory action of SNP at ≥0.1 μM (IC_{50}=1.5-2.5 μM)

SBP=systolic blood pressure; MAP=mean arterial blood pressure; DBP=diastolic blood pressure; HR=heart rate; PAP=pulmonary arterial pressure; VRVA=vascular resistance of vertebral artery blood flow; VBF=vertebral arterial blood flow; VRCA=vascular resistance of coronary artery; VRCCA=vascular resistance of common coronary artery; CCBF=common carotid blood flow; CO=cardiac output; TPR=total peripheral resistance; HT=hematocrit; PT= prothrombin time; APTT=activated partial thromboplastin time; Fg= plasma fibrinogen concentration; ELT=euglobulin clot lysis time
-; not available

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption: Absorption of avanafil was rapid in rat and dog after oral administration with T_{max} of 0.5 and 0.7 hour, and $T_{1/2}$ of 0.9 and 1.3 hours, respectively. With repeated dosing, prolonged absorption was noted mostly at high doses. In fasted dogs, earlier T_{max}, and lower C_{max} and AUC values were achieved than in the fed animals. Avanafil was also rapidly absorbed in humans following oral administration and reached T_{max} of 30 to 45 minutes in the fasted state, but T_{max} was delayed by 1.25 hours after a high-fat meal diet. Avanafil terminal $T_{1/2}$ varied across studies ranging from 1 to 8 hours, probably due to different analytical methods employed. In bile duct-cannulated rats following oral administration, approximately 78% and 3% of the administered radioactivity was recovered from the bile and urine, respectively, within 24 hours, suggesting that at least 81% of avanafil was absorbed from the GI tract in the rat, and

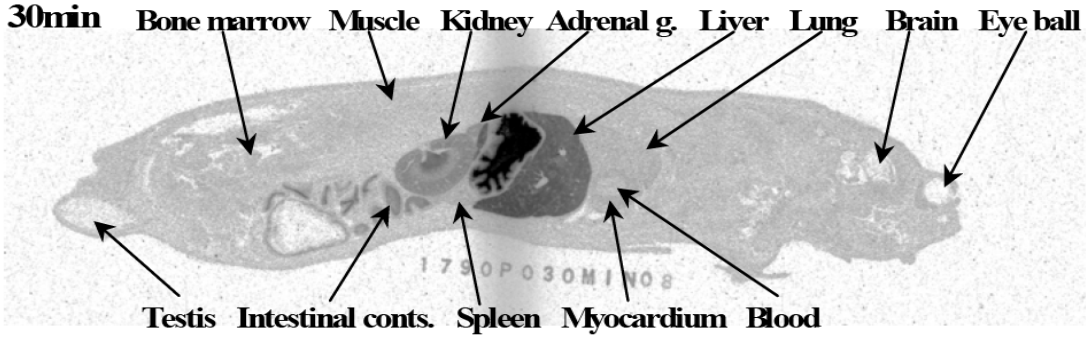
biliary elimination is an important pathway for the excretion of avanafil. The oral bioavailability of avanafil was estimated to be 1.5% in rats (possibly due to high first pass metabolism), 35-40% in dogs and 15% in monkeys, due to high first pass metabolism.

Table 3 PK Parameters of Avanafil Following a Single Oral Dose in the Rat, Dog and Monkeys

Species (sex, n)	Dose/Route (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}inf}$ (ng•h/mL)	CL (mL/min/kg)	$t_{½}$ (h)	F (%)
Rat (M, 4)	3 / IV	-	-	518	97.3	0.87	-
Rat (M, 4)	3 / PO	7.0	0.50	7.9	-	0.85	1.5
Dog (M, 4)	1 / IV	-	-	869	19.2	2.5	-
Dog (M, 4)	1 / PO	120	0.69	257	-	1.3	30
Monkey (M, 3)	1 / IV	-	-	1,432	11.9	1.8	-
Monkey (M, 3)	1 / PO	113	2.0	212	-	0.82	15

Distribution: The highest concentrations of radioactivity were found in GI contents (>30-fold of blood), liver (~30-fold of blood) and kidney (~18-fold of blood in pelvis; ~6-fold of blood in cortex and medulla) at 0.5 hour post-dose following oral administration of avanafil using whole body autoradiography. High levels (~1.2–2.1 times the blood concentration) were also found in the hypophysis, harderian gland, salivary gland, cardiac muscle, lung, spleen, pancreas, brown fat, and bone marrow. Less than 0.2 times of the blood concentration was seen in the brain, spinal cord, eyeball and testis. By 24 hours, the highest concentrations of radioactivity were still detected in the liver and GI tract although most of the radioactivity was excreted from the body.

Figure 1 Distribution of Radioactivity after Oral Administration of ^{14}C-Avanafil to Male Albino Rats using Whole Body Autoradioluminography



In pigmented male Long-Evans rats, the highest levels of radioactivity were seen in the melanin-containing tissues such as the uveal tract of the eyes and hair follicles after oral dosing. Radioactivity was still detectable in the uveal pigment of the eyes at 168 hours after dosing, suggesting that avanafil and/or its metabolites have an affinity for melanin.

The binding of avanafil to plasma protein, predominantly to albumin of rat (0.3/1/3 µg/mL), dog (0.3/1/3 µg/mL), rabbit (0.8/8 µg/mL), and human (0.3/1/3 µg/mL) in vitro were 91%, 93%, 96%, and 99%, respectively. M4 and M16 were less bound to plasma protein than avanafil in all species with 76%, 80%, 88%, and 97% binding for M4, respectively; and 52%, 36%, 82%, and 84% binding for M16, respectively, at concentration ranges from 0.3 to 15 µg/mL.

Metabolism: Avanafil displayed interspecies differences in metabolic stability in liver microsomal incubations in that avanafil was metabolized approximately 8 and 21 times faster by rats than by humans and dogs, respectively. In rat plasma, the major metabolites identified, representing ≥10% of total radioactivity, were monohydroxy avanafil (M5 and M9), despyrrolidine avanafil (M27), carboxylic acid avanafil (M10), and hydroxy M22 (M40). In the dog, avanafil was mainly monohydroxylated (M4, M5 or M9), oxidized (M10), and underwent pyrrolidine ring opening reaction following the formation of M9 (M16 and M22) and demethylation (M2). In humans, 2 major circulating metabolites M16 (open pyrrolidine ring carboxylic acid metabolite) and M4 (hydroxyl metabolite) represented 8-10% of the total radioactivity or 23-29% of unchanged avanafil (see Table below). These metabolites were a mixture of isomers which were not separated and eluted as a single peak. In vitro metabolism studies indicated that avanafil was extensively metabolized by P450 isoforms, predominantly by CYP3A4, with a minor contribution by CYP2C.

Avanafil was extensively metabolized in rats, particularly in males, with 2-5 fold greater concentrations of M4 than the parent in plasma, probably resulting from sexual dimorphism of CYP3A4. In pregnant rats, avanafil was not as extensively metabolized as that in male rats based on the observation that the mean exposures to M4 and M16 in pregnant female rats were about 10% and 3%, respectively, that of avanafil, regardless of avanafil dose, formulation, or gestation days.

Table 4 In Vivo Species Comparison of Avanafil Metabolite Profile

Species	Sample	Sampling Time	% Dose	% of Total Radioactivity in Sample					
				Parent	M4	M9	M10	M16	M27
Male Sprague-Dawley Rats	Plasma	0-6 h	NA	17.4	4	11.6	10 [a]	5 [b]	9.6
	Urine	0-24 h	4.5	ND-2.3	5.2-10 [c]	ND	ND-2.4	14-18	ND-1.6
	Bile	0-24 h	61.0	1.2-3.8	ND-19 [c]	ND	13-39	ND-8	ND
	Feces	0-24 h	26.4	27-37	8.9-18 [c]	ND	10-11	14-15	ND
Male Beagle Dogs	Plasma	0.5 & 2 h	NA	56-70	2.4-3.5	2.4-2.5	ND	1.1-2.4	ND
	Urine	0-24 h	1.4	ND-3.8	ND	ND	8.7-9.4	18-20	ND
	Feces	0-48 h	85.8	41-83	ND-9.2 [d]	ND	4.4-12	7.5-24	ND
Male Human Subjects	Plasma	0-12 h	NA	36.7	8.4	2.3	2.3	10.6	3.8
	Urine	0-24 h	19.4	ND-1.8	ND	ND-2.6	8.2-12	47-64	ND
	Feces	0-120 h	58.0	ND-10.4	ND-6.6 [c]	ND-2.5	12-23	14-37	ND

M4 and M9: monohydroxy metabolites of avanafil; M10: avanafil carboxylic acid metabolite; M16: TA-1790 open pyrrolidine ring carboxylic acid metabolite; M27= avanavil despyrrolidine metabolite

[a]M40 (hydroxy M22) coeluted with M10 in pooled rat plasma

[b]M14 (glucuronide of *O*-demethylated avanafil), M17 (hydroxylated avanafil carboxylic acid), and M21 (oxidation of M16) coeluted with M16 in pooled rat plasma

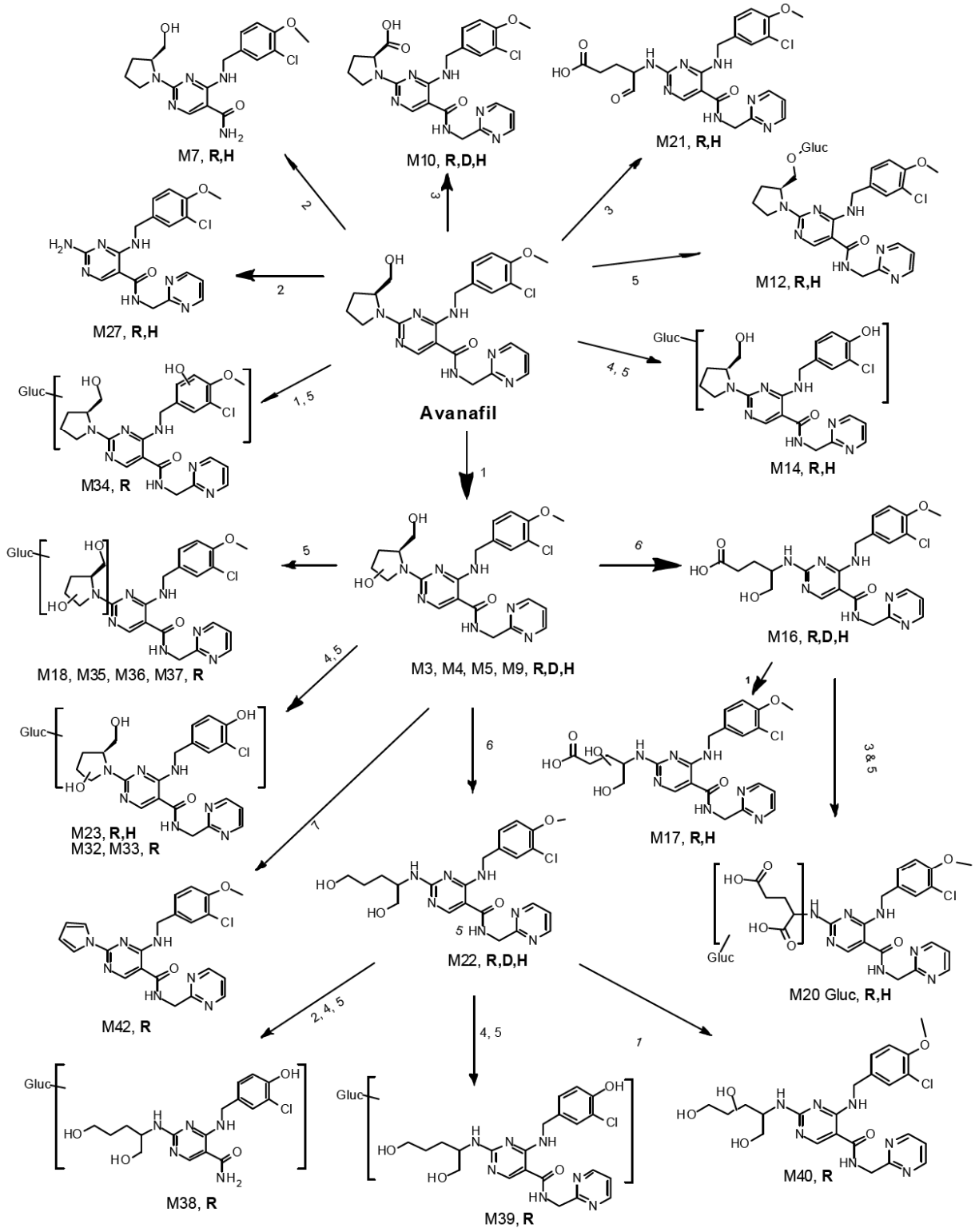
[c]Coeluted with another mono-hydroxylated metabolite M5

[d]Coeluted with M5 and M2 (*O*-demethylated avanafil)

NA: Not Assessed

ND: Not Detected

Figure 2 Proposed Metabolic Pathways of Avanafil



R=present in rats; D=present in dogs; H=present in humans; 1=hydroxylation; 2=N-dealkylation; 3=oxidation; 4=demethylation; 5=glucuronide conjugation; 6=pyrrolidine ring opening via oxidative cleavage; 7=dehydrogenation

Excretion: In rats, the majority of radioactivity was excreted primarily into the feces via the bile (>90% of the dose) and only partly into the urine (3.8-8.3%) within 48 hours following oral and intravenous administration routes. Avanafil was also mainly eliminated in the feces (most likely via bile), with ~92%, ~81% and ~63% of total radioactive dose eliminated in feces in dogs, monkeys and humans, respectively. Intraduodenally injection of radioactive bile collected from rats following oral administration of [^{14}C]-avanafil to another bile-duct cannulated rats, demonstrated enterohepatic circulation of avanafil and/or its metabolites. The extent of reabsorption was estimated to be at least 34.5% of the intraduodenally administered dose.

Drug-Drug Interaction: Avanafil was a weak substrate of human P-glycoprotein in Caco-2, MDR1, and WT cells (R_E=1.8) at 10 μM.

Avanafil inhibited CYP enzymes in human liver microsomes such as CYP3A4 (IC_{50}=34.5 μM), CYP2C8 (IC_{50}=15.2 μM), CYP2C9 (IC_{50}=11.1 μM), CYP2C19 (IC_{50}=28.9 μM), CYP1A1/2 (IC_{50}>100 μM), CYP2D6 (IC_{50}=88.5 μM), CYP2A6 (IC_{50}>100 μM), CYP2E1 (IC_{50}>100 μM) and CYP2B6 (IC_{50}>100 μM) with IC_{50} values ranging from 11 μM to greater than 100 μM. The three metabolites of avanafil (M4, M16, and M27) at concentrations (10 μM) ≥10-fold the maximal plasma avanafil concentrations at 200 mg weakly inhibited CYP1A, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 activities, suggesting that avanafil and its metabolites possess a potential for drug-drug interactions. Avanafil also caused an induction of CYP1A2, CYP2B6, and CYP3A4 activities in primary human hepatocytes at 50 μM with the enzyme activity increase among 3 donors ranging from 1-128%, 48-274% and -6.5-231%, respectively.

5.2 Toxicokinetics

Toxicokinetics were assessed from a 13-week toxicology study in mice, 1- and 2-week toxicology studies in the rat and dog, 4-week and 6-month toxicology studies in the rat, and 9-month toxicology study in the dog. Additional toxicokinetic studies were conducted in order to assess the exposure to the metabolites M4 and M16 following 2-week repeated oral administration of avanafil in male rats and pregnant rats and rabbits. In the repeat-dose studies with rats of 4-week duration and longer, PK parameters were not calculated due to a small number of plasma samples. Mean C_{max} and AUC values for avanafil and/or metabolites (M4, M16) generally increased in a dose-related manner at up to the highest doses tested in mice and dogs (2000 mg/kg for the mouse and 100 mg/kg for the dog). In rats, systemic exposure to parent and metabolites was supra dose-proportional with some accumulation following repeat-dosing. Female rats had higher systemic exposure to avanafil than male rats, while no marked gender-related differences were observed in the mouse and dog. Avanafil exposure was lower in pregnant female rats than in non-pregnant females: ~23% reduction in C_{max} and 18-36% reduction in AUC.

6 General Toxicology

6.1 Single-Dose Toxicity

No mortality was observed at up to the highest dose tested in mice and rats: 2000 mg/kg following oral dosing or 40 mg/kg following intravenous administration.

In an escalating oral dose study in dogs at up to 2000 mg/kg tested, all animals survived. Observed clinical signs included emesis in males at ≥300 mg/kg and in female at all doses, hyperemia of the conjunctiva and/or oral mucosa at ≥300 mg/kg, and salivation at 2000 mg/kg in both sexes. Decreased BP in all treated groups and increased HR in HD females were reported. Slight increase in triglycerides (TG) was observed in females at ≥900 mg/kg. At necropsy, a gallbladder cyst and hematomas in the adipose tissue were reported in one male, and enlarged thyroid and adipose tissue hematomas were observed in the female associated with microscopic findings of vacuolization of the renal glomerulus and adipose tissue hemorrhage, however, these changes were not considered to be toxicologically significant by the sponsor.

In a study (non-GLP) to evaluate the effect of feeding or fasting on the toxicity of single oral doses of avanafil in male dogs, fewer clinical signs and toxicity were noted in fasted animals (e.g., emesis at ≥30 mg/kg; watery stool, decreased activity, conjunctival congestion and tachycardia at 300 mg/kg) as compared to fed animals (e.g., emesis at 10 and 100 mg/kg; tachycardia at ≥30 mg/kg; decreased BP and decreased activity at 100 mg/kg; drowsiness and ptosis at 300 mg/kg), possibly due to lower systemic exposure.

The following table summarizes the major observations made in single-dose toxicity studies.

Species	Route	Doses, mg/kg	LD_{50}, mg/kg	Major Findings
Mouse, ♂♀ (ICR) 5/sex/group	Oral	0, 500, 1000, 2000	>2000	No mortality/clinical signs/gross findings ↓Food intake (10%) on Day14 in HD♀
	IV	0,10, 20 & 40	>40	No mortality/clinical signs Subcutaneous hemorrhage of upper eyelid/eye lid moisture/ swelling in 1 LD male
Rat, ♂♀ (SD) 6/sex/group	Oral	0, 500, 1000, 2000	>2000	No mortality/gross findings Loose stool in 1 MD & 1 HD♂
	IV	0, 10, 20, 40	>40	No mortality/clinical signs/gross findings
Dog (beagle)	Oral	0, 30, 100, 300, 900, 2000 (escalating dose on D0, 4, 7, 11, 14 & 18), 2♂/1♀	>2000	No mortality Vomiting at ≥300 mg/kg♂ & ≥30 mg/kg♀; mucosal hyperemia at ≥300 mg/kg; salivation at 2000 mg/kg ↓SBP/DBP/MAP & ↑HR at ≥30 mg/kg, ↑QTc at 2000 mg/kg Renal glomerular vacuolization & adipose tissue hemorrhage
		10, 30, 100, 300 (fasted & fed), 3♂/group	>300	No mortality -Fasted: emesis at ≥30 mg/kg; watery stool, ↓activity, conjunctival congestion & tachycardia at 300 mg/kg; -Fed: emesis at 10 & 100 mg/kg; tachycardia at ≥30 mg/kg; ↓BP & ↓activity at 100 mg/kg; drowsiness & ptosis at 300 mg/kg

6.2 Repeat-Dose Toxicity

Study title: A Two-Week Repeated Oral Dose Toxicity Study of TA-1790 in Rats

Study no.:	10-AVANAFIL-TOX-08
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/29/2000 (receipt of animals)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #00010, 99.6%

Key Study Findings

- Clinical signs: salivation at ≥300 mg/kg/day
- Hematology: increased reticulocytes (up to 28%) and WBC parameters (up to 60%) at 1000 mg/kg/day
- Liver: dose-related increase in absolute liver weights (up to 25%) and hepatic enzyme (aminopyrine demethylase and aniline hydroxylase) activities (up to 60%) at ≥100 mg/kg/day associated with altered clinical chemistry parameters (dose-related decrease in LDH up to 19% and TG in males up to 60% at ≥100 mg/kg/day; increased ALT up to 60% and albumin up to 9% in males at 1000 mg/kg/day)
- Kidney: increased BUN (up to 24%) and CPK (up to 14%) in females at 1000 mg/kg/day
- Lung: alveolar infiltration in females at 1000 mg/kg/day.
- NOAEL=100 mg/kg/day (~0.1-fold for males and ~1-fold for females the AUC at MRHD)
- No MTD achieved

Methods

Doses:	0, 100, 300, 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution/0.5% carmellose sodium containing 0.1% hydrogenated castor oil (HCO)-60
Species/Strain:	Rat/Crj:CD®(SD)IGS from (b) (4)
Number/Sex/Group:	10/sex/group
Age:	6 Weeks at dosing initiation
Weight:	189-217 g for males; 143-169 g for females
Satellite groups:	3/sex/group for TK
Unique study design:	N/A
Deviation from study protocol:	Not significant

The following table summarizes the noteworthy observations made in rats at 2 weeks.

Doses, mg/kg / Observations	0		100		300		1000	
	10M	10F	10M	10F	10M	10F	10M	10F
Clinical signs,								
Salivation					5	2	9	4
Eye discharge								1
Water intake, Week 2, n=8/sex/group	30.0	30.8	**35.6**	28.1	**35.8**	28.1	**38.2****	25.8
Clinical chemistry,								
ALT, IU/L	28.9	21.7	27.9	19.9	29.0	21.4	**36.1****	**34.7****
LDH, IU/L	157	104	**136**	89	**133**	97	**127**	117
CPK, IU/L	236.4	118.2	172.8**	110.4	198.7	113.2	191.3	**134.3**
Ca^{2+}, mg/dL	8.99	9.53	**9.34****	9.61	**9.43****	9.71	**9.62****	9.59
Inorganic phosphorus , mg/dL	8.09	7.35	7.84	**7.89**	8.10	**8.17**	**8.58**	**8.37***
Albumin, g/dL	2.51	2.78	2.60	2.79	2.59	2.79	**2.74****	2.83
A/G	0.92	0.97	**0.94**	0.97	**0.97**	0.98	**1.06****	**1.04****
BUN, mg/dL	13.4	14.3	13.8	13.5	13.5	15.0	13.6	**17.8****
Triglyceride, mg/dL	49.3	16.3	**36.8**	14.7	**32.5***	17.1	**19.0****	14.3
Free fatty acid , mg/dL	0.62	0.58	0.47**	0.53	0.52	0.49	**0.45****	0.50
Hematology,								
Reticulocytes, %	4.0	2.5	3.9	3.2*	4.2	3.0	4.4	**3.2***
WBC, 10^3/µL	7.98	4.55	6.26	**5.14**	7.07	**5.64**	**10.72***	**7.36****
Eosinophils, 10^3/µL	0.04	0.06	0.04	0.04	0.04	0.06	0.03	**0.03***
Neutophils, 10^3/µL	0.86	0.59	0.67	0.57	0.58	0.58	**1.07**	**0.78**
Lymphocytes, 10^3/µL	6.93	3.82	5.39	**4.44**	6.29	**4.92**	**9.33***	**6.40****
Monocytes, 10^3/µL	0.13	0.08	0.15	0.08	0.13	0.08	**0.26****	**0.14****
Prothrombin time, sec	18.0	14.6	18.6	14.0	**15.8**	14.4	**14.4****	14.7
Activated partial thromboplastin time, sec	17.1	13.6	17.6	13.2	**16.7**	13.7	**15.0***	14.3
Urinalysis, n=8/sex								
Volume, mL/day	10.2	12.1	**14.9**	11.9	**15.6**	12.1	**15.1**	12.1
Na, mEq/day	1.57	1.30	1.69	**1.22**	1.86*	**1.16**	1.47	**1.04**
Organ weights, g								
Liver	8.44	5.89	**9.38***	**6.22**	**9.53***	**6.78****	**10.57****	**7.5****
Lung	1.11	0.97	1.23	1.00	1.25*	1.08*	**1.24***	0.98
Thymus	0.56	0.51	0.63	0.57	0.64	0.53	**0.49**	**0.44**
Ovary (R), mg	-	57.9	-	56.6	-	66.5	-	**51.0**
Liver biochemistry, n=6/sex								
Aniline hydroxylase, nmol/mg protein/min	0.797	0.743	**0.897**	0.714	**1.000****	**0.846**	**1.139****	**0.979***
Aminopyrine demethylase, nmol/mg protein/min	3.561	3.068	3.483	**3.240**	**3.954**	**3.448**	**5.595****	**5.009****
Cytochrome P450, nmol/mg protein	0.587	0.483	**0.618**	0.499	**0.616**	0.390	**0.648**	0.426
Histopathology,								
Lung, alveolar infiltration, foam cell			-	-	-	-		2
Thyroid gland, ultimobranchial rest	1	1	-	-	-	-	5	2
Toxicokinetics$^{\#}$, 3/sex/timepoint								
$AUC_{0\text{-}24hr}$ (µg·hr/mL) Day 0			1.63	9.30	9.44	89.47	62.45	294.26
Day 16			1.12	12.35	7.62	99.20	64.79	269.38
C_{max} (µg/mL) Day 0			0.52	4.15	2.04	8.11	5.01	15.65
Day 16			0.47	4.50	1.06	9.18	6.37	18.88
T_{max} (hr) Day 0			0.5	1.0	0.5	6.0	6.0	10.0
Day 16			0.5	0.5	2.0	6.0	6.0	3.0
$t_{1/2}$ (hr) Day 0			ND	ND	1.7	ND	ND	ND
Day 16			ND	ND	3.4	ND	ND	2.8

Significantly different from the control at $p<0.05^{*}$ or $p<0.01^{**}$
-; not available
ND=not determined
$^{\#}$LLOQ=20 ng/mL

Study title: Two-Week Oral Dose Toxicity Study of TA-1790 in Dogs

Study no.:	10-AVANAFIL-TOX-09
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/24/2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 00010, 99.4%

Key Study Findings

- Clinical signs: emesis (males) at ≥10 mg/kg/day; watery diarrhea (male), shivering (female), eye discharge (female), and inanimation at 100 mg/kg/day
- CVS: increase in HR and a decrease in SBP, MBP, and DBP at 2 or 6 hours post-dosing at 1000 mg/kg (one female with severe tachycardia on Day 7)
- Hematology: decreased RBC (males), increased WBC parameters (up to 60%), and increased reticulocytes (up to 70%) at ≥30 mg/kg/day
- Liver: increased hepatic drug-metabolizing enzyme activities (up to 60%) and clinical chemistry parameters (decreased TG and total cholesterol up to 50% and phospholipid up to 20%; increased total bilirubin up to 150% correlated with yellowish color of the serum) at ≥30 mg/kg/day
- NOAEL=10 mg/kg/day (~1-fold the AUC at MRHD)
- No MTD reached

Methods

Doses:	0, 10, 30, 100 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	N/A
Formulation/Vehicle:	Capsule/lactose
Species/Strain:	Dog/beagle purchased from (b) (4)
Number/Sex/Group:	3/sex/group
Age:	7 Months at arrival
Weight:	9.7-11.9 kg for males, 9.7-10.9 kg for females
Satellite groups:	None
Unique study design:	N/A
Deviation from study protocol:	Not significant

The following table summarizes the noteworthy observations made in dogs at 2 weeks.

Doses, mg/kg / Observations	0		10		30		100	
	3M	3F	3M	3F	3M	3F	3M	3F
Clinical signs,								
Inanimation							1	1
Emesis			1		1	1	1	3
Mucous feces							1	
Watery diarrhea							1	
Shivering								1
Eye discharge								1
Body weights, Day 14, kg	10.73	10.07	10.70	9.70	10.10	10.03	**10.57**	**9.80**
Food consumption, Day 13, g/day	300	300	287.7	157.7	300	300	**260.7**	**232**
Electrocardiography, Day 12-14, 2-hr								
Respiratory rate, breath/min	22.7	33.3	34.7	**31.7**	40.7	**27.7**	23.3	**23.7**
HR, beats/min	124.7	108.7	158.0	**126.3**	**136.0**	**142.7**	**154.0**	**135.3**
SBP, mmHg	176.3	181.0	203.3	169.3	173.7	203.3	**155.7**	**165.0**
MBP, mmHg	114.3	128.3	131.7	122.7	113.3	141.0	**108.7**	**112.3**
DBP, mmHg	83.0	101.7	95.7	98.7	83.0	109.3	85.0	**86.0**
Clinical chemistry, Day 14								
LDH, IU/L	101.3	77.0	**74.7**	88.3	**70.7**	79.3	**76.0**	**71.0**
ALP, IU/L	161.7	154.3	**155.7**	**124.3**	**129.7**	**118.7**	**126.3**	**128.7**
CPK, IU/L	139.77	92.43	107.4	93.13	**115.67**	120.80	**111.53**	102.53
Triglyceride, mg/dL	16.07	21.10	17.60	**20.03**	**11.67**	**13.80**	**8.70**	**10.87**
Total cholesterol	125.47	126.07	111.5	168.10	115.83	128.20	**95.93**	123.33
Phospholipid, mg/dL	280.0	290.3	**265.0**	365.3	**269.3**	**286.3**	**220.0**	**284.7**
Creatinine, mg/dL	0.660	0.690	0.690	0.680	**0.703**	0.667	**0.747**	**0.753**
Total bilirubin, mg/dL	0.10	0.10	0.10	0.11	**0.12**	**0.13**	**0.16**	**0.25**
Hematology, Day 14								
RBC, 10^6/µL	7.257	6.230	7.000	6.760	**6.583**	6.223	**6.150***	6.350
Hematocrit, %	47.80	41.77	46.43	45.37	**44.63**	42.03	**41.57**	42.67
Platelets, 10^3/µL	301.0	356.0	360.7	327.7	354.7	338.3	**439.3***	**493.0**
H-reticulocytes#, %	1.93	1.80	3.90	2.33	**2.70**	**3.30**	**3.30**	**2.67**
Neutrophils, 10^3/µL	6.773	6.303	8.617	5.953	7.943	**10.263**	7.710	**7.230**
Eosinophils, 10^3/µL	0.300	0.327	**0.420**	0.337	**0.467**	**0.590**	**0.500**	**0.403**
Lymphocytes, 10^3/µL	2.810	3.273	3.604	**3.810**	3.230	**4.010**	**3.657**	**3.933**
Urinalysis, Day 13								
Volume, mL/day	169.0	162.0	**108.0**	134.7	**118.7**	204.7	**129.3**	**135.0**
Na, mEq/day	9.24	11.41	5.91	8.92	8.30	12.08	**6.63**	**3.57***
K, mEq/day	31.11	36.35	19.98	23.82	32.94	38.99	**23.50**	**27.79**
Cl, mEQ/day	18.77	24.05	11.78	12.00	20.71	23.33	17.82	**15.01**
Organ weights, g								
Lung	80.27	77.20	81.70	67.67	77.80	71.70	79.60	**64.33**
Thymus	5.07	8.57	6.33	7.57	6.67	8.27	5.60	**7.00**
Ovary (R), mg	-	587.0	-	640.7	-	550.7	-	**970.0**
Uterus	-	7.67	-	**13.93**	-	**12.40**	-	**11.90**
Prostate	5.93	-	5.10	-	5.93	-	**6.77**	-
Thyroid (R), mg	414.0	550.0	398.7	437.7	406.7	508.0	**542.7**	**420.3**
Liver biochemistry,								
Aniline hydroxylase, nmol/mg protein/min	0.247	0.245	0.194	**0.332**	0.224	**0.307**	**0.346**	**0.393**
Aminopyrine demethylase, nmol/mg protein/min	3.139	3.611	3.073	3.230	**3.677**	**4.173**	**4.978**	**4.960**
Cytochrome P450, nmol/mg protein	0.246	0.246	0.212	**0.316**	**0.268**	**0.304**	**0.346**	**0.321**
Gross pathology,								
Duodenum/Jejenum/Ileum, brown content							1	1
Cecum/Colon/Rectum, brown content								1
Ovary, left cystic ovarian bursa								1
Adrenal gland, left nodule					1			1
Colon, red/multiple nodule					1			1

Observations \ Doses, mg/kg	0		10		30		100	
	3M	3F	3M	3F	3M	3F	3M	3F
Histopathology,								
Kidney, interstitial mononuclear cell accumulation, focal						1	1	
collecting duct pigment deposit, hemosiderin								1
papillary duct cellular cast, scattered								1
Prostate gland, interstitial mononuclear cell infiltr, focal		-	1	-		-	1	-
Spleen, capsule hemorrhage, focal		1					1	
Skin, hair follicle cellular infiltration, focal							1	
Submandibular gland, cellular infiltration , focal				1			1	1
Pons, perivascular neutrophil accumulation, focal							1	
Lung, Alveolar microgranuloma				1		2		1
Liver, perivascular mononuclear cell accumulation				1				1
Vagina, muscular layer cyst	-		-		-		-	1
Thymus, involution								1
Mammary gland, interstitial hemorrhage				1		1		2
Mesencephalon, perivascular mononuclear cell, focal								1
Ganglion, stellate, interstitial lymphocyte infiltration, focal								1
Pituitary, anterior lobe, acidophilic cell hyperplasia				1				1
Toxicokinetics[@], 3/sex/timepoint								
$AUC_{0\text{-}24hr}$ (µg·hr/mL) Day 0			5.25	6.07	33.91	30.52	104.57	89.30
Day 6			5.05	8.99	36.08	24.98	194.85	141.48
Day 13			5.53	9.55	29.76	41.58	162.74	227.48
C_{max} (µg/mL) Day 0			1.01	1.27	3.07	3.82	7.46	6.90
Day 6			1.25	2.10	3.93	3.38	18.36	15.96
Day 13			1.36	2.01	4.19	4.79	16.26	20.58
T_{max} (hr) Day 0			1.7	1.7	6.0	3.3	10.0	7.0
Day 6			1.0	1.7	6.0	5.0	5.0	5.0
Day 13			1.7	2.7	4.0	5.0	5.0	4.0
$t_{1/2}$ (hr) Day 0			2.2	3.4	ND	4.2	14.3	4.7
Day 6			1.8	2.5	5.2	4.3	2.9	5.3
Day 13			1.6	2.2	2.9	3.9	4.6	4.4

[#]H-reticuloytes; Reticulocyte ratio with high nucleic acid concentration
[@]LLOQ=0.02 µg/mL, LOD=0.051 µg/mL
Significantly different from the control at $p<0.05^{*}$ or $p<0.01^{**}$
-; not available
ND=not determined

Study title: 28-Day Oral Dose Toxicity Study with TA-1790 in Male Rats with a 1-Week Recovery

Study no.:	6584-136
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/12/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #Oct2901 (89.3%), Oct2901-001 (104%), Oct3001-001 (89.3%)

Key Study Findings

- Hematology: dose-related increase in WBC parameters (up to 25%) at ≥300 mg/kg/day (non-reversible at the end of recovery period)
- Liver: reduced glucose (up to 11%), decreased TG (up to 33%, non-reversible at the end of recovery period), increased albumin (up to 7%), elevated A/G ratio (up to 17%), and increased total bilirubin (up to 100%, non-reversible at the end of recovery period) at 1000 mg/kg
- Kidney: increased urine volume (up to 70%), lower glucose and urine specific gravity at 1000 mg/kg/day
- NOAEL=100 mg/kg/day (~0.1-fold for males and ~1-fold for females the AUC at MRHD)
- No MTD achieved

Methods

Doses:	0, 100, 300, 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Suspension/sodium carboxymethylcellulose, polysorbate 80, purified water, xanthan gum, microcrystalline cellulose, sucrose, sodium benzoate, disodium dentate, citric acid monohydrate and 10 N sodium hydroxide
Species/Strain:	Rat/Crl:CD®(SD)IGS BR from (b) (4)
Number/Sex/Group:	15 Males/group (10/group for main, 5/group for recovery)
Age:	6 Weeks at dosing initiation
Weight:	180-221 g
Satellite groups:	None
Unique study design:	N/A
Deviation from study protocol:	Not significant

The following table summarizes the noteworthy observations made in the 4-week rat study.

Dose, mg/kg / **Observations**	**0** 15M	**100** 15M	**300** 15M	**1000** 15M
Body weights, g (recovery)	368 (382)	370 (409)	364 (416)	**354 (367)**
Body weight gains, g, Weeks 1-5 (recovery)	165 (19)	169 (26)	163 (23)	**155 (17)**
Hematology, Week 4 (Week 5 for recovery)				
WBC, E3/µL	8.6 (8.5)	8.2 (8.4)	**9.2 (9.4)**	**10.6* (10.8)**
Lymphocytes, E3/µL	7.3 (6.8)	7.0 (7.4)	**7.8 (7.9)**	**9.1* (8.9)**
Clinical chemistry, Week 4 (Week 5 for recovery)				
Glucose, mg/dL	97	91	**90**	**86***
Albumin, g/dL	4.2	4.3	4.3	**4.5***
A/G	1.8	1.8	1.9	**2.1***
Total bilirubin, mg/dL	0.1 (0.1)	0.1 (0.2)	0.1 (0.2)	**0.2* (0.2)**
TG, mg/dL	60 (49)	**52 (51)**	**53 (49)**	**40 (28)**
Urinalysis, Week 4				
Urine volume, mL	17.2	14.2	**19.0**	**29.3***
Organ weights, absolute, g, n=10/group				
Liver	11.187	10.635	10.809	**13.088***
Salivary gland, mandibular	0.703	**0.669**	**0.637**	**0.627**
Thymus	0.500	**0.499**	**0.497**	**0.417**
Gross pathology, n=10/group				
Mandibular lymph node, diffusely red, bilateral				1
Histopathology, n=10/group				
Pituitary, hypertrophy				1
Lung, macrophages, alveolar		2	1	1
mineralization, vessel				1
Liver, arteritis, segmental				1
Rectum, circulating lymphocytes, increased		1		1
Lymph node, mandibular, hemorrhage, acute, n=1				1
Toxicokinetics#, 3-15/timepoint				
C, µg/mL, Day 1, 0.5 hr		0,43	0.76	0.92
24 hr		BLD	BLD	BLD
Day 28, 0.5 hr		0.62	0.73	0.90
24 hr		BLD	BLD	BLD

Numbers in parentheses represent values at the end of recovery period.
Significantly different from the control at $p<0.05^{*}$ or $p<0.01^{**}$
#BLD=below detection limit (LLOQ=11 µg/mL , LOD=0.25 µg/mL for 100 & 300 mg/kg, 0.5 µg/mL for 1000 mg/kg)

Study title: 28-Day Oral Dose Toxicity Study with TA-1790 in Male Beagle Dogs with a 2-Week Recovery

Study no.:	6584-131
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/10/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #XA118A (75 mg), #XA118B (150 mg), #XA119 (150 mg), 98.3-106%

Key Study Findings

- Clinical signs: ataxia, hypoactivity, recumbency, and retching at 100 mg/kg/day on Day 4; body tremors on Day 26 in one male at 75 mg/kg/day; non-formed feces at ≥10 mg/kg/day; snorting behavior at ≥30 mg/kg/day (Day 9 at 30 mg/kg/day, Days 20-26 at ≥30 mg/kg/day)
- Liver: increased total bilirubin (up to 50%) and chronic inflammation in the liver at 100 mg/kg/day
- Kidney: renal chronic inflammation in one male at 100/75 mg/kg/day
- Thymus: increased lymphocyte depletion associated with decreased weights at ≥30 mg/kg/day
- Epididymides: Hypospermia in one male each at ≥30 mg/kg/day
- NOAEL=10 mg/kg/day (~1-fold the AUC at MRHD)
- MTD=100 mg/kg/day (based on the CNS clinical signs)

Methods

Doses:	0, 10, 30, 100/75 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	N/A
Formulation/Vehicle:	Capsule/mannitol, fumaric acid, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, calcium carbonate, and magnesium stearate
Species/Strain:	Male dog/beagle from (b) (4)
Number/Sex/Group:	10 Males/group (7/group for main, 3/group for recovery)
Age:	13-17 Months at treatment initiation
Weight:	7.7-13.7 kg
Satellite groups:	None
Unique study design:	The high-dose group (100 mg/kg) was not dosed on Days 5 and 6 and was reduced to 75 mg/kg/day beginning on Day 7 due to toxicity.
Deviation from study protocol:	Not significant

The following table summarizes the noteworthy observations made in the 4-week dog study.

Observations \ Dose, mg/kg	0 10M	10 10M	30 10M	100/75* 10M
Clinical signs,				
Ataxia				1
Tremors, body, entire				1
Snorting through nose			1	5
Hypoactive				3
Recumbent, lateral				1
sternal				1
Retching				2
Vomiting, containing food		2		1
Salivation, excessive				1
Non-formed feces		1	2	2
Body weights, Week 4, kg (recovery)	11.8 (12.3)	11.6 (12.8)	11.6 (11.9)	**10.8* (11.4)**
Body weight change, Weeks 1-4, kg	0.5	0.3	0.3	**-0.5**
Food consumption, Week 4, g, n=10	2144	**2139**	**2116**	**2051**
Clinical chemistry, Week 4				
Total bilirubin, mg/dL	0.2	0.2	0.2	**0.3***
Hematology, Week 4				
Activated partial thromboplastin time	11.8	12.1	12.2	**12.8***
Gross pathology, n=7 (n=3 for recovery)				
Skin, alopecia, focal				1
Histopathology, n=7 (n=3 for recovery)				
Bone marrow, sternum, hypercellularity	1			2
Lung, infiltrate, macrophage, alveolar				1
inflammation, chronic (recovery)		(1)	(1)	1(1)
Kidney, inflammation, chronic				1
Liver, inflammation, chronic				2
Thymus, depletion, lymphocytic, diffuse, multifocal		1	5	4
Lymph node, mesenteric, hemorrhage, multifocal				1
infiltrate, macrophage, pigmented				1
Thyroid, cyst, ultimobranchial	1	2		3
Adrenal cortex, cortical tissue, extracapsular (recovery)		1	(1)	1(1)
Skin, erosion				1
inflammation, chronic, active				1
Epididymides, hypospermia			1	1
Toxicokinetics#, 3-15/timepoint				
C, µg/mL, Day 1, 0.5 hr		0.3	0.88	0.24
24 hr		0.06	0.12	2.5
Day 28 , 0.5 hr		0.07	0.31	0.77
24 hr		0.05	0.13	0.55

*Animals were not dosed on Days 5 & 6 due to clinical signs of ataxia, hypoactivity, recumbency and retching, and doses were reduced to 75 mg/kg on Day 7

Numbers in parentheses represent values at the end of recovery period.

Significantly different from the control at $p<0.05^{*}$ or $p<0.01^{**}$

#LLOQ=0.226 µg/mL , LOD=0.02 µg/mL

Study title: 13-Week Oral Gavage Preliminary Carcinogenicity and Toxicokinetic Study with TA-1790 in Mice

Study no.:	6584-150
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/21/2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #0646-18 (103%), #0845-18 (105%), #0846-19 (96.1%), #0845-19 (102%), #0846-20 (106%), #0845-20 (101%)

Key Study Findings

- Mortality: found dead or sacrificed in moribund condition of 2000 mg/kg/day animals attributable to clinical signs (hunched/thin appearance, cold to touch, head tremors, convulsions, irregular/labored/audible respiration, yellow hair coat, blue skin at ventral abdomen and/or hypoactivity)
- Clinical signs: hunched/thin appearance, rough/yellow hair coat (perineal area), and cold to touch (tail) in surviving animals at 2000/1000 mg/kg/day
- Liver: hepatocellular centrilobular hypertrophy (minimal) associated with increased weights in males at ≥600 mg/kg/day and in females at 2000/1000 mg/kg/day
- Kidney: minimal mineralization in males at ≥600 mg/kg
- Heart: focal/multifocal fibrosis (minimal) at 2000/1000 mg/kg/day; mineralization (minimal) in males at ≥600 mg/kg/day; lymphohistiocytic infiltrates in males at 2000/1000 mg/kg/day; brown pigment in males at ≥600 mg/kg/day and in females at 2000/1000 mg/kg/day
- NOAEL=200 mg/kg/day (~1-2 fold the AUC at MRHD)
- MTD=1000 mg/kg/day (based on the CNS signs and cardiac findings)

Methods	
Doses:	0, 200, 600, 2000/1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Suspension/sodium carboxymethylcellulose, polysorbate 80, purified water, xanthan gum, microcrystalline cellulose, sucrose, sodium benzoate, disodium edetate, citric acid monohydrate & 10 N sodium hydroxide
Species/Strain:	Mouse/Crl:CD®(ICR) BR from (b) (4)
Number/Sex/Group:	10/sex/group
Age:	7 Weeks at dosing initiation
Weight:	26.8-37.9 g for males, 20.9-29.3 g for females
Satellite groups:	2-5/sex/timepoint for TK

Unique study design: N/A
Deviation from study protocol: Not significant

Observations and Results

Mortality: Twice daily

- Found dead or sacrificed moribund: 1 male at 600 mg/kg on Day 10 (gavage error based on the esophageal perforation), and 2 males and 2 females on Days 42-49 at 2000 mg/kg (clinical signs prior to death included convulsions, hunched posture, thin appearance, audible/irregular/labored respiration, rough hair coat, head tremors, hypoactivity, yellow hair coat in perineal area, cold to touch, and/or blue skin in ventral abdominal)

Clinical Signs: Once daily for cage-side observations; Pre-study & weekly thereafter for detailed exam

- Rough or yellow hair coat at ≥200 mg/kg;
- Blue haircoat (in males), hunched/thin appearance and cold to touch in surviving animals at 2000/1000 mg/kg

Body Weights: Pre-study, 1st day & weekly thereafter

- Decrease (up to 13%) in mean body weights on Days 36 through 57 and mean body weight gains (87%) for Days 1 to 50 in males at 2000 mg/kg compared to controls, which was recovered following reduction of the dose to 1000 mg/kg

Feed Consumption: Weekly

- Decrease (up to 9%) in food intake in males at 2000 mg/kg, which returned to control levels at 1000 mg/kg

Ophthalmoscopy: Not performed

ECG: Not performed

Hematology: Day 93

- Unremarkable

Clinical Chemistry: Day 93

- Unremarkable

Urinalysis: Not provided

Gross Pathology:

- Unremarkable

Organ Weights: Day 93 Day 93 (adrenal glands, brain, heart, kidneys, liver with gallbladder, lungs, ovaries, prostate, mandibular salivary glands, seminal vesicles, spleen, testes with epididymis, thymus, thyroids with parathyroid, uterus)

- Dose-related decrease (up to 16%) in absolute salivary gland weights in females at ≥LD
- Dose-dependent increase (up to 21%) in absolute liver/gallbladder weights at HD

Histopathology: Day 93

Adequate Battery: Yes

Peer Review: No

Histological Findings:

- Heart: minimal focal/multifocal mineralization in males at ≥MD; minimal lymphohistiocytic infiltrates in females at ≥MD and in HD males; minimal golden brown granular pigment in males at ≥MD and in HD females; minimal focal/multifocal interstitial fibrosis at HD
- Liver: centrilobular hypertrophy in males at ≥MD & HD females; hepatocyte necrosis in one HD male; acute inflammation at HD in the liver
- Kidney: minimal mineralization in males at ≥MD and in a HD female; minimal to slight lymphohistiocytic infiltrate at HD

Special Evaluation: None

Toxicokinetics: 1, 2, 6, & 24 hours post-dose on Day 1; pre-dose & 1, 2, 6, & 24 hours post-dose on Day 91 (via cardiac puncture)

- Supra-proportional increase in exposure between LD and MD in males, and dose-related increase in females

Dosing Solution Analysis: Samples were collected from the placebo and test article formulations (10 mL each) used for Week 1, once every 4 weeks thereafter, and following the last dose administered. Dose preparations were shaken for at least 30 seconds before sample collection. Samples were stored in a refrigerator, set to maintain 2 to 8°C, protected from light until packed on frozen cold packs and shipped by overnight courier to (b) (4) for analysis.

- Concentrations ranged between 94.1-112%

The following table summarizes the noteworthy observations made in the 13-week mouse study.

Observations \ Dose, mg/kg	0		200		600		2000/1000[#]	
	10M	10F	10M	10F	10M	10F	10M	10F
Mortality[#]					1		2	2
Clinical signs,								
Appearance, hunched							2	3
thin								3
Behavior, hypoactive							2	
convulsions								1
tremors, head							1	
Respiration, irregular/labored/audible							1	2
Skin, blue, ventral-abdominal							2	
rough hair coat			1		1		10	6
yellow hair coat, perineal area			1		1		5	
Cold to touch, tail or entire body							3	
Body weights, g, Day 43	36.9	28.7	37.1	29.2	35.3	30.2	**32.4**[*]	29.1
Body weight gains, g, Days 1-50	4.7	4.1	5.5	5.0	**4.3**	5.8	**0.6**[*]	**3.0**
Food consumption, g, Days 43-50	40.1	35.9	38.1	38.0	38.1	40.2	**37.5**	37.5
Organ weights, absolute, g								
Salivary gland, mandibular	0.336	0.255	0.325	**0.231**	0.342	**0.218**[*]	0.316	**0.214**[*]
Liver/gallbladder	1.976	1.613	**2.111**	**1.653**	**2.220**	**1.771**	**2.394**[*]	**1.957**[*]
Gross pathology,								
Ovary, cyst(s)				1				2
Histopathology,								
Kidney, cast, proteinaceous	3	2		1			3	4
infiltrate, lymphohistiocytic	8	6		1			9	8
mineralization					1		2	1
Liver, hypertrophy, hepatocellular, centrilobular					2		4	3
hepatocyte necrosis, individual							1	
inflammation, acute			1				1	1
Heart, fibrosis, focal/multifocal							5	5
mineralization, focal/multifocal					1		2	
infiltrate, lymphohistiocytic						2	4	1
pigment, brown					2		2	6
Lacrimal gland, infiltrate, lymphohistiocytic	5	2					8	1
Urinary bladder, infiltrate, lymphohistiocytic							2	
Prostate, infiltrate, lymphohistiocytic	3	-					5	-
Toxicokinetics[@], 2-5/sex/timepoint								
$AUC_{0\text{-}24hr}$, µg·hr/mL, Day 1			6.50	3.99	39.1	67.7	104	99.9
Day 91			5.51	14.6	88.1	80.2	64.3	142
C_{max}, µg/mL Day 1			2.88	1.94	6.48	5.95	7.45	12.7
Day 91			2.03	3.64	7.71	13.5	13.0	17.0
T_{max}, hr Day 1			1.0	1.0	1.0	2.0	6.0	2.0
Day 91			1.0	1.0	1.0	1.0	1.0	1.0
$T_{1/2}$, hr Day 1			ND	ND	3.7	ND	ND	5.9
Day 91			ND	2.4	12.8	3.4	2.8	ND

[#]Dosing at 2000 mg/kg was suspended from Day 47 through Day 56 due to excessive toxicity, and resumed on Day 57 at 1000 mg/kg.
-; not available
ND=not determined
Significantly different from the control at $p<0.05$[*]
[@]LLOQ=0.506 µg/mL

Study title: 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with TA-1790 in Rats with a 13-Week Interim Sacrifice and a 4-Week Recovery Period

Study no.:	6584-149
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/2/2002 (animal arrival)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #003010, 95.4-110%

Key Study Findings

- Spleen: increased incidence and/or severity (minimal to severe) of hematopoiesis associated with dose-related increase in spleen weights and altered hematology parameters (non-reversible decrease in RBC parameters and increase in WBC parameters) at ≥100 mg/kg
- Liver: increased incidence and/or severity (minimal to slight) of centrilobular hypertrophy at ≥300 mg/kg in both sexes, and hepatocyte necrosis in HD females associated with increased weights and ALP, and decreased TG (males)
- Thyroid: increased incidence and severity (minimal to severe) of follicular cell hypertrophy with decreased colloid at 1000 mg/kg
- Heart: increased incidence of degeneration/necrosis in 1000 mg/kg females (partially-reversible)
- Pancreas: minimal to slight acinar cell atrophy in 1000 mg/kg males (partially-reversible)
- Prostate: increased incidence of chronic-active inflammation at 1000 mg/kg
- Uterus: increased incidence of dilatation at 1000 mg/kg (partially-reversible)
- NOAEL<100 mg/kg (<0.1-fold for males and <1-fold for females the AUC at MRHD)
- MTD=1000 mg/kg (based on the incidence and severity of the microscopic legions in the heart, liver, and the spleen)

Methods

Doses:	0, 100, 300, 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Suspension/corn syrup, sodium carboxymethylcellulose, polysorbate 80, purified water, xanthan gum, microcrystalline cellulose, sucrose, sodium benzoate, disodium edetate, citric acid monohydrate and 10 N sodium hydroxide
Species/Strain:	Rat/Crl:CD®(SD)IGS BR from (b) (4)
Number/Sex/Group:	25/sex/group (10/sex/group for interim sacrifice; 5/sex/group for recovery)

Age:	6 Weeks at dosing initiation
Weight:	151-224 g for males; 129-183 g for females
Satellite groups:	5/sex/group for TK
Unique study design:	N/A
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

- Sacrificed in extremis on Week 26 in 1 MD male due to an apparent hindlimb injury and hematopoietic neoplasia in multiple tissues

Clinical Signs: Twice daily for cage-side observations; Pre-study & weekly thereafter for detailed exam

- Clear oral discharge at ≥MD
- Yellow hair coat in females at ≥MD and a HD male
- Red oral discharge in HD females

Body Weights: Pre-study, 1st day & weekly for Weeks 1-14; once every 4 weeks thereafter; & Weeks 27 & 31

- Dose-related reduction in mean body weights in males in all treated groups and in females at ≥MD up to 6% compared to controls at the end of the study; dose-related reduction in males at ≥MD up to 11% and in females in all treated groups up to 21% compared to controls during the recovery period
- Dose-dependent decrease in mean overall body weight gains up to 10% in both sexes compared to controls at the end of the study; decreased mean body weight gains in females in all treated groups up to 61% compared to controls at the end of the recovery period

Feed Consumption: Weekly for Weeks 1-13; once every 4 weeks thereafter; & Weeks 26 & 30

- Increase in mean food intake in both sexes up to 8-14% at ≥MD compared to controls at the end of the study

Ophthalmoscopy: Pre-study & Weeks 13 & 26 for opthalmoscopy & ERG

- Unremarkable

ECG: Not performed

Hematology: Weeks 14 (interim sacrifice); 27 (terminal sacrifice) & 31 (recovery sacrifice)

- Dose-related decrease in RBC in males at ≥MD (up to 10%) and in HD females (~6%) compared to controls (non-reversible during the recovery period)
- Dose-related decrease in hemoglobin (up to 6%) and hematocrit (up to 4%) in females in all treated groups and in HD males (6%) compared to controls (non-reversible during the recovery period)

- Dose-related increase in MCV (up to 6%), MCH (up to 4%), and lymphocyte (up to 75%) in males at ≥LD and in HD females (~60% for WBC; ~50% for lymphocytes) compared to controls (non-reversible MCV and MCH during the recovery period)
- Increased reticulocytes at HD up to 28%
- Dose-related increase in WBC (up to 63%) at ≥MD in both sexes compared to controls
- Decrease in eosinophils at HD (~50%) in both sexes compared to controls

Clinical Chemistry: Weeks 14 (interim sacrifice); 27 (terminal sacrifice) & 31 (recovery sacrifice)

- Dose-related increase in A/G ratio in males (up to 21%) in all treated groups compared to controls
- Dose-related decrease in TG (up to 72%) in males in all treated groups compared to controls
- Increased ALP (up to 14-50%) at ≥MD compared to controls
- Decrease in globulin (due to α1-globulin) up to 15% in HD males compared to controls

Urinalysis: Weeks 14 (interim sacrifice); 27 (terminal sacrifice) & 31 (recovery sacrifice)

- Increase in volume (up to 110-129%) in all treated groups compared to controls
- Decreased Na (up to 28-45%) and K (up to 32-38%) at ≥MD compared to controls
- Increased Cl in HD males (200%) and females in all treated groups (up to 40%) compared to controls
- Dose-related increase in Cl excretion (up to 240%) in males in all treated groups and in HD females (53%) compared to controls

Gross Pathology: Weeks 14 (interim sacrifice), 27 (terminal sacrifice) & 31 (recovery sacrifice)

- Unremarkable

Organ Weights: Weeks 14 (interim sacrifice), 27 (terminal sacrifice), & 31 (recovery sacrifice): adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, mandibular salivary glands, seminal vesicles, spleen, testes, epididymides, thymus, thyroid with parathyroid, uterus

- Dose-dependent increase in absolute liver weights (up to 28-59%) in all treated groups compared to controls
- Dose-dependent increase in absolute spleen weights in males (up to 25%) at ≥MD and in females (up to 44%) in all treated groups compared to controls
- Dose-dependent increase in absolute kidney (up to 15%), lung (up to 17%) and heart (up to 16%) weights in males in all treated groups and in HD females compared to controls
- Dose-related increase in absolute adrenal weights in females (up to 46%) in all treated groups compared to controls

Histopathology: Weeks 14 (interim sacrifice), 27 (terminal sacrifice) & 31 (recovery sacrifice)

Adequate Battery: yes (no pathology report submitted)

Peer Review: no

Histological Findings:

- Spleen: increased incidence and/or severity (minimal to severe) of hematopoiesis in all treated groups
- Liver: increased incidence and severity (minimal to slight) of centrilobular hypertrophy at ≥MD**;** increased incidence of necrosis in hepatocyte necrosis in HD females
- Thyroid: increased incidence and severity (minimal to severe) of follicular cell hypertrophy with decreased colloid at HD
- Heart: increased incidence of degeneration/necrosis in HD females
- Pancreas: minimal to slight acinar cell atrophy in HD males (partially-reversible)
- Prostate: increased incidence of chronic-active inflammation at HD
- Uterus: increased incidence of dilatation at HD (partially-reversible)

Special Evaluation: none

Toxicokinetics: Day 1 at 1 & 24 hours post-dose; once pre-dose; & 1 & 24 hours postdose during Weeks 13 & 26 (blood from jugular vein)

- Increase in plasma concentrations with dose levels being higher in females than in males; accumulation upon repeat-dosing
- PK parameters not calculated due to insufficient data (only 2-3 sampling times on each sampling day) and detection limits (below the lower limit of quantitation of 253 ng/mL)

Dosing Solution Analysis: Samples of the placebo and test article formulations used for Weeks 1, 5, 9, 13, 17, 21, 25, and 27, and following the last dose administered (Day 184) were collected and shipped to (b) (4) for analysis. In addition, one vial of the 30 mg/mL preparation was collected from the shipment received at (b) (4) on 1/2/2003 because of a delay in the receipt of the last container of this preparation, and was shipped to (b) (4) for analysis of linearity and system suitability.

- Concentrations ranged between 99.2-120%

The following table summarizes the noteworthy observations made in the 6-month toxicology study in rats.

Dose, mg/kg / Observations	0		100		300		1000	
	25M	25F	25M	25F	25M	25F	25M	25F
Mortality, Day 181					1[#]			
Clinical signs,								
Discharge, clear-oral					1	3	9	15
red-oral								2
Skin & pelage, yellow hair coat, perineal area						2	1	4
Body weights, g, Week 27 (main), n=14-15/grp	635	335	**618**	336	**615**	**323**	**598**	**316**
Week 31 (recovery), n=5/group	655	393	674	**378**	**644**	**356**	**585**	**309**
Body weight gains, g, Weeks 1-27, n=14-15/grp	448	181	**429**	181	**418**	**170**	**408**	**162**
Weeks 27-31, n=5/group	24	38	19	**32**	22	**12***	**39**	**15***
Food intake, g Week 26	185	130	180	127	**191**	**132**	**200**	**148**
Hematology, Week 27/31, n=9-10/group								
RBC, E6/µL, main	8.21	7.40	8.21	7.57	**7.92**	7.44	**7.41***	**6.93***
recovery	8.75	7.51	**8.40**	7.45	**8.23**	7.79	**8.04**	**6.99**
Hemoglobin, g/dL, main	14.5	14.5	14.8	**14.3**	14.3	**14.2**	**13.6**	**13.7***
recovery	15.5	14.8	15.0	14.5	15.1	14.9	15.2	**13.9**
HCT, g, main	43.1	42.4	44.4	**42.1**	**42.8**	**42.0**	**41.4**	**40.7**
recovery	46.1	43.2	**44.4**	42.6	**44.6**	43.8	**44.6**	**40.8**
MCV, fL, main	52.7	57.3	**54.0**	55.7	**54.2**	56.5	**55.9***	58.9
recovery	52.8	57.5	**53.0**	57.1	**54.3**	56.2	**55.5***	58.4
MCH, pg, main	17.7	19.6	**18.0**	19.0	**18.2**	19.0*	**18.4**	19.8
recovery	17.7	19.7	**17.9**	19.5	**18.4**	19.1	**18.9***	19.8
Reticulocyte, E3/µL	137	129	152	121	143	120	**167**	**165**
WBC, E3/µL	6.9	4.1	6.6	3.8	**8.0**	**4.4**	**9.8***	**6.7***
Lymphocyte, E3/µL	4.4	3.0	**4.7**	2.6	**5.1**	**3.3**	**7.7***	**4.5***
Eosinophil, %	2	2	2	2	2	2	**1***	**1**
Clinical chemistry, Week 27, n=9-10/group								
A/G	1.4	1.8	**1.5**	1.9	**1.5**	2.0	**1.7***	1.7
TG, mg/dL	121[b]	50	**82**	48	**51***	51	**34***	52
ALP, IU/L	77	32	69	32	**89**	**36**	**88**	**48**
Globulin, g/dL	3.3	3.1	3.0	2.9	3.1	2.9	**2.8**	3.0
Urinalysis, Week 27, n=9-10/group								
Volume, mL	9	6.5	**12.6**	**9.5**	**15.0**	**16.7**	**18.9**	**14.9***
Na, mmol/L	40	49	40	**27***	**27**	**18***	**29**	**27***
K, mmol/L	136	106	124	**80**	**79**	**70**	**93**	**66**
Cl, mmol/L	8	25	**10**	**14**	**10**	**6***	**24**	**15**
Cl excretion, mmol	0.07	0.15	**0.09**	0.14	**0.15**	0.06	**0.24***	**0.23**
Organ weight, g, Week 27, n=9-10/group								
Liver	15.9	8.26	**16.21**	**8.67**	**16.90**	**9.44***	**20.38***	**13.16***
Kidney	3.64	2.15	**3.67**	2.23	**4.06**	2.19	**4.06**	**2.48***
Spleen	0.96	0.57	0.91	**0.58**	**1.05**	**0.60**	**1.20***	**0.83***
Lung	1.88	1.41	**1.93**	1.53	**2.08***	1.44	**2.18***	**1.59***
Heart	1.78	1.17	**1.85**	1.19	**2.06**	1.15	**2.08***	**1.34**
Adrenal	0.06	0.07	0.06	**0.08**	0.07	**0.09**	0.07	**0.10***
Gross pathology, Week 27, n=9-10/group								
Liver, dark focus, area								1
Uterus, lumen, fluid	-		-	2	-		-	1
Histopathology, Week 27, n=9-10/group								
Liver, necrosis, individual hepatocyte	5		4	1	4		3	3
hypertrophy, hepatocellular, centrilobular	1				9	8	10	9
Thyroid, hypertrophy, decreased colloid, follicular	7	3					10	9(1)
Heart, degeneration/necrosis	7	1(1)					6(1)	5(1)
Pancreas, atrophy, acinar							2(1)	
Prostate, inflammation, chronic-active	1	-		-	1	-	3	-
Uterus, dilatation	-	1	-	2	-		-	3(2)
Spleen, hematopoiesis, extramedullary, increased	6	1	7	3	9	3	9	8

Toxicokinetics[@], 5/timepoint/group							
C, µg/mL, Day 1, 1 hr		0.816	3.10	1.67	7.61	1.07	4.41
24 hrs		BLC	BLC	BLC	BLC	BLC	BLC
Week 13, 1 hr		0.460	4.36	0.759	9.71	1.34	7.42
24 hrs		BLC	BLC	BLC	BLC	BLC	BLC
Week 26, 1 hr		0.609	5.31	1.38	20.0	4.15	16.5
24 hrs		BLC	BLC	BLC	BLC	0.0241	0.648

[#]Sacrificed in moribund on Week 26 (Day 181) due to an apparent hindlimb injury & hematopoietic neoplasia in multiple tissues
[@]BLC=below the calibration limit (LLOQ=253 ng/mL)
Numbers in parentheses represent recovery animals.
Statistically significant at p=0.05[*]
-; not available

Study title: 9-Month Oral Chronic Toxicity Study with TA-1790 in Male Dogs with a 4-Week Recovery Period

Study no.: 6584-142
Study report location: Module 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/16/2002 (animal arrival)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: TA-1790, XA126A/XA126B, 95.4-110%

Key Study Findings

- Clinical signs: Increased incidence of cagesores at ≥30 mg/kg
- Kidney: increased tubular regeneration in all treated groups (partially-reversible at 60 mg/kg)
- Heart: moderate vascular inflammation in one male at 60 mg/kg
- Lung: hyperplasia and alveolar macrophage infiltrates at 60 mg/kg (partially-reversible)
- Thyroid: cyst/lymphohistiocytic infiltrate at ≥30 mg/kg (non-reversible)
- Prostate: lymphohistiocytic infiltrate in all treated groups (partially-reversible at ≥30 mg/kg)
- NOAEL=10 mg/kg/day (~1-fold the AUC at MRHD)
- No MTD reached

Methods

Doses: 0, 10, 30, 60 mg/kg/day
Frequency of dosing: Once daily
Route of administration: Oral
Dose volume: N/A
Formulation/Vehicle: Capsule/mannitol, fumaric acid, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, calcium carbonate, magnesium stearate
Species/Strain: Dog/purebred beagle from (b) (4)
Number/Sex/Group: 5 Males/group for main; 3 males/group for recovery)
Age: 9-10 Months at dosing initiation

Weight:	7.7-12.5 kg
Satellite groups:	None
Unique study design:	N/A
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

None

Clinical Signs: Twice daily

- Increased incidence of discolored/liquid/mucoid feces in all treated groups
- Red genital discharge in males in all treated groupsduring semen collection
- Increased incidence of cagesores at ≥MD (e.g., interdigital furunculosis/cellulitis/abscess, superficial dermatitis treated systemically with Clavamox)
- Retching in one HD animal

Body Weights: Weekly during Weeks –1 to 14, & Weeks 18, 22, 26, 30, 34, 38, 40, 42, & 44

- Dose-related decrease in mean body weights (up to 10%) and body weight gains (no gains at HD) in all treated groupscompared to controls

Feed Consumption: Weekly during Weeks -1 to 13; & Weeks 17, 21, 25, 29, 33, 37, 39, & 41

- Unremarkable

Ophthalmoscopy: Once prior to initiation of treatment & Weeks 12, 25, 38, & 43 for opthalmoscopy; Once prior to initiation of treatment & Weeks 13, 26, 39, & 44 for ERG

- Unremarkable

ECG: Once prior to initiation of treatment & Weeks 12, 25, 38, & 43 (once) at pre-dose, 1 & 3 hrs post-dose

- Unremarkable (data not provided)

Hematology: Once prior to initiation of treatment & Weeks 14, 27, 40, & 44

- Increased reticulocytes (up to 42%) at ≥MD and lymphocytes (up to 19%)

Clinical Chemistry: Once prior to initiation of treatment & Weeks 14, 27, 40, & 44

- Dose-related decrease in inorganic phosphorus (IP) up to 17% in all treated groups compared to controls

Urinalysis: Once prior to initiation of treatment & Weeks 14, 27, 40, & 44

- Lower urine pH at HD
- Reduced excretion of urine Na (up to 37%), K (up to 69%), and Cl (up to 40%) at ≥MD compared to controls

Gross Pathology: Week 40 or 44

- Unremarkable

Organ Weights: Week 40 or 44: adrenal glands, brain, heart, kidneys, liver with gallbladder, lung, pituitary gland, prostate, mandibular salivary glands, spleen, testes, epididymides, thymus, thyroids with parathyroid

- Decreased thyroid/parathyroid weights (~26%) at HD compared to controls

Histopathology: Week 40 or 44

Adequate Battery: yes

Peer Review: no

Histological Findings:

- Kidney: increased tubular regeneration in all treated groups (partially-reversible at HD)
- Heart: vascular inflammation with moderate severity in 1 HD male
- Lung: hyperplasia and alveolar macrophage infiltrates at HD (partially-reversible)
- Thyroid: cyst/lymphohistiocytic infiltrate at ≥30 mg/kg (non-reversible)
- Prostate: lymphohistiocytic infiltrate at ≥10 mg/kg (partially-reversible at ≥30 mg/kg)

Special Evaluation: Weeks 12, 25, 38 & 42 for sperm evaluations

- No significant effect on sperm motility, concentration and morphology compared to controls in sperm analysis

Toxicokinetics: Day 1 & Weeks 13, 26, & 39 at 1, 2, 4, 8, & 24 hours post-dose (blood from jugular vein)

- Dose-proportional increase in AUC and C_{max} over dose levels with T_{max} of 2.4-6 hours

Dosing Solution Analysis: Samples were collected from each lot of the control (90 and 180 capsules) and test article (150 and 300 capsules) following the last dose and were shipped to the (b) (4) for analysis.

- Concentrations ranged between 66.2-102% for 50 mg, and 85.5-104% for 100 mg capsules

The following table summarizes the noteworthy observations made in the 9-month toxicology study in dogs.

Dose, mg/kg / **Observations**	**0** 8M	**10** 8M	**30** 8M	**60** 8M
Clinical signs,				
Appearance, cagesore	2	2	5	4
Behavior, retching				1
Discharge, red, penile area during semen collection		1	3	3
Excretion, discolored/mucoid/non-formed		2	5	4
Body weight, kg, Week 40	11.3	**10.9**	**10.6**	**10.2**
Body weight gain, kg, Weeks 1-40	0.7	**0.6**	**0.4**	**0.0***
Hematology, Week 40				
MCV, fL	63.5	62.5	62.9	**65.9***
MCH, pg	22.0	21.8	21.9	**23.1***
Reticulocytes, E3/UL	53	49	**75**	**71**
Lymphocytes, E3/UL	1.6	**1.8**	**1.9**	**1.9**
Clinical chemistry, Week 40				
IP, mg/dL	3.0	**2.8**	**2.5**	**2.5**
Urinalysis, Week 40				
pH	8.1	7.3*	7.8	**7.3***
Na exc, mmol	4.65	3.29	**1.22**	**2.95**
K exc, mmol	23.45	22.15	**13.20**	**16.20**
Cl exc, mmol	14.14	14.80	**7.72**	**8.42**
Organ weights, Week 40, g				
Thyroid/parathyroid	0.931	0.781	0.826	**0.685**
Histopathology, n=5/group (n=3 for recovery)				
Lung, hyperplasia, type II pneumocyte	2	3	1(1)	4(1)
infiltrate, macrophage, alveolar	2	1	2(1)	3(1)
Kidney, regeneration, tubular, increased		2	1	1(1)
mineralization, tubular, increased	1	1(1)	2	2(1)
glomerulosclerosis				1
Heart, inflammation, vascular				1
Thyroid, cyst			2(1)	1(2)
infiltrate, lymphohistiocytic			1	1(1)
Prostate, infiltrate, lymphohistiocytic	(1)	2	1(1)	2(1)
Toxicokinetics#, 8/timepoint				
$AUC_{0\text{-}24hr}$, µg·hr/mL Day 1		14.0	34.3	106
Week 13		5.97	29.9	92.7
Week 26		6.40	47.7	126
Week 39		5.85	24.1	74.4
C_{max}, µg/mL Day 1		2.14	4.95	10.20
Week 13		1.15	4.29	9.99
Week 26		1.08	6.08	14.10
Week 39		1.19	3.04	7.80
T_{max}, hr Day 1		3.3	3.8	5.0
Week 13		4.0	3.5	4.5
Week 26		2.4	3.3	4.0
Week 39		3.0	4.0	6.0

Numbers in parentheses represent recovery animals.
Significantly different from controls at p=0.05*
#LLOQ=0.1264 µg/mL

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Reverse Mutation Test of TA-1790 in Bacteria

Study no.:	10-AVANAFIL-TOX-11
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 21, 2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 00010, 99.4%

Key Study Findings

- No increase in the number of revertant colonies of the test strains either in the presence or absence of S9 mix under the condition of the study

Methods

Strains:	*Escherichia coli* WP2 *uvrA*; *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537
Concentrations in definitive study:	0, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate
Basis of concentration selection:	5000 µg/plate
Negative control:	DMSO
Positive control:	-S9: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for WP2 uvrA, TA98 and TA100; sodium azide for TA1535; 9-aminoacridine for TA1537 +S9: 2-aminoanthracene
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	Test article and controls in the presence or absence of S9 mix were mixed with bacterial suspension, and incubated for 20 min at 37°C (pre-incubation method). The top agar was added and poured onto the minimal glucose agar plate, and were incubated for 48 hrs at 37°C. The number of His^+ and Trp^+ revertant colonies was counted using a dissection microscope or naked eye.

Study Validity

- The number of spontaneous revertant colonies on the solvent control plates is similar to that of the historical data from the testing facility.
- The number of revertant colonies is considerably larger (>2-fold) in the positive control groups than in the solvent control groups.
- Both in the presence and absence of S9 mix, the test substance is considered to be mutagenic when it shows a positive dose-response relationship where the number of revertant colonies is more than 2-fold that at the solvent control level.

Results

- No increase in the number of revertant colonies of the test strains either with or without S9 mix
- Precipitation:
  - at ≥1250 µg/plate in all strains with and without S9 mix in the 1st (pilot) assay conducted at 0, 4.88, 19.53, 78.13, 312.5, 1250 and 5000 µg/plate
  - at ≥625 µg/plate in all strains with and without S9 mix in the 2nd (main) assay conducted at 0, 78.13, 312.5, 625, 1250, 2500 and 5000 µg/plate
- Reproducibility confirmed in the 3rd assay
- No information on test conditions (e.g., criteria for validity, historical control data, *etc*)

Study title: Bacterial Reverse Mutation Assay

Study no.:	AA56WS.503.BTL
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 21, 2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 60004·02·00l-R, 98.5%

Key Study Findings

- No increase in the number of revertant colonies of the test strains either in the presence or absence of S9 mix under the condition of the study

Methods

Strains:	*Escherichia coli* WP2 *uvrA*; *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537
Concentrations in definitive study:	0, 15, 50, 150, 500, 1500, 5000 µg/plate
Basis of concentration selection:	5000 µg/plate
Negative control:	DMSO
Positive control:	-S9: methyl methanesulfonate for WP2 uvrA; 2-nitrofluorene for TA98; sodium azide for TA100 and TA1535;

	9-aminoacridine for TA1537 +S9: 2-aminoanthracene
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	S9 or Sham mix, tester strain and vehicle or test article dilution were added to molten selective top agar at 45±2°C, and the mixture was overlaid onto the surface of minimal bottom agar (plate incorporation method). The plates were inverted and incubated for approximately 48 to 72 hours at 37±2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted. Cytotoxicity was evaluated by using a dissecting microscope. Precipitate was evaluated by visual examination. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

Study Validity

- All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation *(rfa)* and the deletion in the *uvrB* gene.
- Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- All WP2 *uvrA* cultures must demonstrate the deletion in the *uvrA* gene.
- All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2 *uvrA,* 10-60
- Tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/mL.
- The mean of each positive control must exhibit at least a 3-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of 3 non-toxic dose levels is required to evaluate assay data.
- A dose level is considered toxic if one or both of the following criteria are met: (1) >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value accompanied by an abrupt dose-dependent drop in the revertant count and (2) At least a moderate reduction in the background lawn.

Results

- No increase in the number of revertant colonies of the test strains either with or without S9 mix in the mutagenicity assays
- Precipitation:
    - at ≥1800 µg/plate in all strains with and without S9 mix in an initial assay (data not provided) and a repeat assay conducted at 0, 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg/plate

  - at ≥500 µg/plate in all strains with and without S9 mix in a confirmatory assays (TA100 was retested due to tester strain culture contamination)
- Cytotoxicity:
  - at ≥1800 µg/plate in the initial assay
  - at 5000 µg/plate in some strains (e.g., TA-1537, WP2 *uvr*A) with and without S9 mix in the repeat assay
- Study met the validity criteria

Study title: Bacterial Reverse Mutation Study of TA-1790 Impurity (b) (4)

Study no.:	B080856
Study report location:	Module 4.2.3.7.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 6, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, E1556101A, 98.5%

Key Study Findings

- No increase in the number of revertant colonies either in the presence or absence of S9 mix under the condition of the study

Methods

Strains:	*Escherichia coli* WP2 *uvrA*; *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537
Concentrations in definitive study:	0, 156, 313, 625, 1250, 2500, 5000 µg/plate
Basis of concentration selection:	5000 µg/plate
Negative control:	DMSO
Positive control:	-S9: 2-(2-furyl)-3-(5-nitro-2-furyl) Acrylamide for WP2 uvrA, TA98 and TA100; sodium azide for TA1535; 9-aminoacridine for TA1537 +S9: 2-aminoanthracene
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	Test article in the presence or absence of S9 mix was mixed with bacterial suspension, and incubated for 20 min at 37°C (pre-incubation method). The top agar was added and poured onto the minimal glucose agar plate, and were incubated for 48 hrs at 37°C. Revertant colonies were counted with an automatic colony analyzer or manually. Plates were checked for precipitation with unaided eyes.

Study Validity

- The negative control values are within the acceptable ranges calculated based on the historical data.
- The positive control substances increase the number of revertant colonies evidently in the respective tester strains, and the values are within the proper ranges calculated based on the historical data.
- There are 4 or more doses giving no microbial toxicity and at least 5 analyzable doses.
- There is no bacterial or fungous contamination in the sterility test.
- There is no loss in the test plates for colony counting due to contamination or other unexpected situations.

Results

- No significant increase (<2-fold) in number of revertant colonies of the test-article group compared to the corresponding negative control values both in the presence and absence of S9 mix with reproducibility
- Precipitation:
  - at ≥78.1 µg/plate in all strains without S9 mix and ≥313 µg/plate with S9 mix in a preliminary test conducted at 0, 1.22, 4.88, 19.5, 78.1, 313, 1250 and 5000 µg/plate
  - at ≥156 µg/plate in all strains with and without S9 mix in a main test
- Study met the validity criteria

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosome Aberration Test of TA-1790 in Cultured Mammalian Cells

Study no.:	10-AVANAFIL-TOX-10
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 17, 2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 00010, 99.3%

Key Study Findings

- No increase in the frequency of cells with structural abnormalities or polyploids either in the presence or absence of S9 mix in any treatment groups under the condition of the study

Methods

Cell line:	CHL/IU cells
Concentrations in definitive study:	-S9: 0, 27.5, 55, 110 µg/mL for 6 hrs; 15, 30, 60 µg/mL for 24 hrs

	+S9: 0, 40, 80, 160 µg/mL for 6 hrs
Basis of concentration selection:	50% Cell growth
Negative control:	DMSO
Positive control:	-S9: mitomycin C; +S9: cyclophosphamide
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	After cells (2.5×10^5) were seeded onto a tissue culture dish and incubated for 24 hrs, the cells were exposed to the test substance or controls for 6 or 24 hrs with or without S9. For the 6-hr groups, the cell monolayer was washed, and cultured for a further 18 hours in fresh medium. Colcemid solution was added to the culture, and cells were treated for 2 hrs toward the end of incubation. The cells were treated with 0.25% trypsin, removed, resuspended in medium, and centrifuged at 4°C (1300 rpm for 5 min). The cells were, then, treated with KCl hypotonic solution at room temperature for 15 min, fixed, and harvested by centrifugation. The cell fraction was suspended in a small amount of Carnoy's fixative. Structural and numerical chromosome abnormalities of 200 metaphase cells (100 cells from each duplicate culture) for each dose were recorded using the Chromosome Aberration Test system.

Study Validity

- Positive controls show increased frequency of cells with structural abnormalities (20.5-28%).
- The frequencies of cells with structural abnormalities and polyploids of the untreated and solvent control culture in CHL/IU cells do not exceed 3%.

Results

- No increase in the frequency of cells with structural abnormalities or polyploids with or without S9 mix in any treatment groups
- Precipitation in a dose-finding study:
  - at ≥180 µg/mL for 6-hour group at –S9 conducted at 0, 10, 20, 30, 60, 90, 120, 150, 180 and 210 µg/mL
  - at 210 µg/mL for 6-hour group at +S9 conducted at 0, 10, 20, 30, 60, 90, 120, 150, 180 and 210 µg/mL
  - at 180 µg/mL for 24-hour group at -S9 conducted at 0, 5, 10, 20, 30, 60, 90, 120, 150 and 180 µg/mL
- Relative cell growth:
  - Dose-dependent suppression in all treatment groups with IC_{50} of 100.76 µg/mL for 6-hour group at –S9; 155.44 µg/mL for 6-hour group at +S9; and 57.05 µg/mL for 24-hour group at –S9 in the dose-finding study

  - o Dose-dependent suppression of cell growth in all treatment groups with 29.5% for 6-hour group at –S9, 39.5% for 6-hour group at +S9, and 45% for
    24-hour group at –S9 at the highest concentrations in a main assay
- No information on test conditions (e.g., pH, osmolality, karyotype features, historical control data, *etc*)

Study title: In Vitro Mammalian Chromosome Aberration Test

Study no.:	AA56WS.331.BTL
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 9, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 00010, 98.5%

Key Study Findings

- No increase in the frequency of cells with structural abnormalities with or without S9 mix in any treatment groups under the condition of the study

Methods

Cell line:	CHO cells
Concentrations in definitive study:	-S9; 0, 5, 10, 20, 40, 60, 80 µg/mL for 4 hrs 0, 2.5, 5, 10, 20, 40, 60 µg/mL for 20 hrs +S9; 0, 2.5, 5, 10, 20, 40, 60 µg/mL for 4 hrs
Basis of concentration selection:	50% reduction in mitotic index for –S9 at 4 hr; 50% reduction in cell growth for +S9 at 4 hrs & -S9 at 20 hrs
Negative control:	DMSO
Positive control:	-S9: mitomycin C; +S9: cyclophosphamide
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	After cells (2.5×10^5) were seeded on a flask and incubated for 16-24 hrs, the cells were exposed to the test substance or controls for 4 or 24 hrs with or without S9. For the 4-hr groups, the cells were washed, and cultured for a further 20 hrs in fresh medium. Colcemid solution was added to the culture 2 hrs prior to the cell harvest, and metaphase cells were harvested by trypsinization, collected, resuspended in medium, and centrifuged again. The supernatant was removed, and fixed in Carnoy's fixative, and harvested by centrifugation. Structural and numerical chromosome abnormalities of 200 metaphase

cells (100 cells per duplicate flask) for each dose were recorded using a microscope stage.

Study Validity

- Positive controls show statistically significant (p=0.05) increase in frequency of cells with structural abnormalities relative to solvent controls.
- The frequencies of cells with structural abnormalities in the solvent control are within the range of the historical control.

Results

- No increase in the frequency of cells with structural abnormalities with or without S9 mix in any treatment groups
- Statistically significant increase in numerical aberrations at ≥40 µg/mL at +S9 for 4-hour group (7%), but within the historical solvent control range (0-11%)
- Precipitation:
  - at ≥145.2 µg/mL in the absence and presence of S9 at 0, 0.484, 1.452, 4.84, 14.52, 48.4, 145.2, 484, 1452 and 4840 µg/mL in a preliminary toxicity study
- Relative cell growth and mitotic index:
  - ≥50% cell growth inhibition at ≥145.2 µg/mL at 4 hours without S9, ≥48.4 µg/mL at 4 hours with S9 and 20 hours without S9 in an initial chromosome aberration assay (terminated at harvest time due to contamination)
  - Cell growth inhibition of 47% and mitotic index inhibition of 56% at –S9 for 4-hour group; 50% and 7% at +S9 for 4-hour group; and 54% and 36% at –S9 for 24-hour group, respectively, at the highest concentrations in a repeat chromosome aberration assay
- Study met the validity criteria

Study title: Chromosome Aberration Study of TA-1790 Impurity (b) (4) in Cultured Mammalian Cells

Study no.:	B080857
Study report location:	Module 4.2.3.7.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 25, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, E1556101A, 98.5%

Key Study Findings

- No increase in structural or numerical aberrations with or without S9 mix under the condition of the study

Methods

Cell line:	CHL/IU cells
Concentrations in definitive study:	-S9: 0, 31.3, 62.5, 125, 250, 500, 1000, 1250, 1500, 1750, 2000 µg/mL for 6 hrs; 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/mL for 24 hrs +S9: 0, 31.3, 62.5, 125, 250, 500, 1000, 1250, 1500, 1750, 2000 µg/mL for 6 hrs
Basis of concentration selection:	50% cell growth inhibition
Negative control:	DMSO
Positive control:	-S9: mitomycin C; +S9: benzo[a]pyrene
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	After cells (4×10^5 cells/mL) were seeded in a plastic plate and incubated for 3 days, the cells were exposed to the test substance or controls for 6 or 24 hrs with or without S9. For the 6-hr groups, the cells were washed with medium, and cultured for a further 18 hrs in fresh medium. All plates were examined macroscopically for precipitation of the test substance in the culture medium at the beginning and end of the treatment. Colcemid was added to the plates 2 hrs before the end of culture. The cells were, then, treated with 0.25% trypsin, harvested, and centrifuged at 1000 rpm for 5 min. The cells were treated with KCl hypotonic solution at 37°C for 15 min, and fixed. The number of metaphase cells was counted by 500 cells per plate (1000 cells per concentration), and 100 cells per plate (200 cells per concentration) were analyzed.

Study Validity

- The number of observed metaphase cells satisfies the criterion for the negative control, positive control and ≥3 concentrations of the test substance.
- Incidences of structurally and numerically aberrant cells in the negative control group are within the range of the background data in the testing facility.
- Incidence of the structurally aberrant cells in the positive control group is ≥10%.

Results

- No increase in the frequency of structurally or numerically aberrant cells with or without S9 mix in any treatment groups
- Precipitation:
  - at ≥313 µg/mL at the beginning and the end of the treatment in the absence and presence of S9 conducted at 0, 78.1, 156, 313, 625, 1250 and 5000 µg/mL in a cell growth inhibition test

  - at ≥125 µg/mL at the beginning and the end of the treatment at 6 hours and ≥200 µg/mL at 24 hours in a chromosomal aberration test
- Relative cell growth:
  - Dose-related cell growth inhibition up to 2500 µg/mL with IC_{50} of 1706.1 µg/mL at 6 hours without S9; 1199.9 µg/mL at 6 hours with S9; 486.5 µg/mL at 24 hours without S9 in the preliminary assay
- Relative mitotic index:
  - 80% inhibition at 1250 µg/mL for 6-hour groups; 73% at 700 µg/mL for 24-hour group at the highest concentrations in the chromosome aberration assay (plateau transition after the doses)
- Study met the validity criteria

Study title: In Vitro Mammalian Cell Gene Mutation Test

Study no:	AA56WS.704.BTL
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 21, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #60004-02-001, #60004-02-001-R, 98.5%

Key Study Findings

- Negative without activation with a 4-hour exposure, equivocal without activation with a 24-hour exposure, and positive with S9 activation for 4 hours under the conditions of the study

Methods

Cell line:	L5178Y/$TK^{+/-}$ mouse lymphoma cells
Concentrations in definitive study:	-S9; 0, 50, 75, 100, 125, 150 µg/mL for 4 hrs 0, 10, 25, 50, 75, 100 µg/mL for 24 hrs +S9; 0, 10, 25, 50, 75, 100 µg/mL for 4 hrs
Basis of concentration selection:	relative suspension growth
Negative control:	DMSO
Positive control:	-S9: methyl methanesulfonate; +S9: 7,12-Dimethyl-benz(a)anthracene
Formulation/Vehicle:	Solution/DMSO
Incubation & sampling time:	Cells were incubated with solvent or the test article for 4 hours in the presence or absence of S9 and for 24 hours in the absence of S9 at 37±1°C, washed, resuspended and counted using an electronic cell counter at $3x10^5$ cells/mL. For expression of the mutant phenotype, the cultures were treated for 24 & 48 hours, plated with and

without the selective agent TFT for 10-14 days.

Study Validity

- The spontaneous mutant frequency of the solvent control cultures is within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group is greater than 50%.
- At least one concentration of each positive control exhibits mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level.
- The positive control MMS exhibits the expected increase in small colonies.
- A minimum of four analyzable concentrations with mutant frequency data will be required. Ideally, the highest concentration should produce at least 80% toxicity (no more than 20% survival). In the case of a test article with a steep toxicity curve (no concentrations with 10-20% survival), the results may be considered acceptable if a dose spacing of ≤2-fold is used and the highest dose tested showed <20% survival or total kill.

Results

- Mutant frequency:
    - 4 Cloned cultures between 55 and 99 mutants/10^6 cells, and 2 cultures with dose-dependent increase of 100 mutants/10^6 cells over the solvent control in the presence of S9 at 4 hours (79-105% RTG)
    - 3 Cloned cultures between 55 and 99 mutants/10^6 cells in the absence of S9 at 24 hours (40-100% RTG)
- Colony size distributions: Increase in the frequency of small, medium and large TFT-resistant colonies for the S9-activated cultures treated with 50, 75 and 100 µg/mL compared to solvent controls over a range from 0.2 to 1.1 mm in diameters (expected increase in small colonies consistent with damage to multiple loci on chromosome 11 in addition to functional loss of the TK locus)
- Precipitation:
    - Visible precipitate at ≥150 µg/mL at 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500 and 750 µg/mL in a preliminary assay
    - Visible precipitate at ≥100 µg/mL for –S9 (32-66% RSG) and for +S9 (94-100% RSG at 4 hours; 65-106% RSG at 24 hours) in a repeat mutagenicity assay (first assay failed due to high solvent control mutant frequencies without S9)
- Study met the validity criteria

The following table summarizes the cloning data in the absence or presence of S9.

-S9 at 4 hours

Treatment (µg/mL)	RCG (%)	RSG (%)	RTG (%)	Mutant Frequency (per 10^6 cells)	Induced Mutant Frequency (per 10^6 cells)
DMSO		-		24 47	
MMS, 10 20	65 20	56 33	36 7	370 624	334 588
Avanafil, 50	120 116	58 64	69 75	47 39	11 4
75	109 82	66 53	72 44	20 38	-15 2
100#	125 97	47 52	58 51	28 22	-8 -14
125#	111 100	44 45	49 45	35 27	-1 -8
150#	104 107	32 45	33 48	59 33	24 -3
200#+		44 32			

#Precipitating dose
% RCG (relative cloning growth) = (average viable count of treated culture / average viable count of solvent control} x 100
% RSG (Relative Suspension Growth) = (total treatment suspension growth / average solvent control total suspension growth) x 100
% RTG (Relative Total growth) = (% suspension x % cloning growth) / 100
Mutant frequency = (average number TFT colonies / average number viable count colonies) x 200
Induced mutant frequency = mutant frequency - average mutant frequency of solvent controls
+ - Not chosen for cloning due to precipitate

-S9 at 24 hours

Treatment (µg/mL)	RCG (%)	RSG (%)	RTG (%)	Mutant Frequency (per 10^6 cells)	Induced Mutant Frequency (per 10^6 cells)
DMSO				61 48	
MMS, 2.5 5	75 58	96 64	72 38	139 354	84 299
Avanafil, 10	94 69	106 104	100 72	110 113	**55** **58**
25	87 82	86 96	75 79	106 112	51 **57**
50	74 71	76 74	57 53	72 57	18 3
75	98 108	70 67	68 70	55 33	1 -21
100#	95 61	65 66	62 40	61 67	6 13
125#+		59 26			

#Precipitating dose
% RCG (relative cloning growth) = (average viable count of treated culture / average viable count of solvent control} x 100
% RSG (Relative Suspension Growth) = (total treatment suspension growth / average solvent control total suspension growth) x 100
% RTG (Relative Total growth) = (% suspension x % cloning growth) / 100
Mutant frequency = (average number TFT colonies / average number viable count colonies) x 200
Induced mutant frequency = mutant frequency - average mutant frequency of solvent controls
+ - Not chosen for cloning due to precipitate

+S9 at 4 hours

Treatment (µg/mL)	RCG (%)	RSG (%)	RTG (%)	Mutant Frequency (per 10^6 cells)	Induced Mutant Frequency (per 10^6 cells)
DMSO				38 32	
DMBA, 3 4	88 73	81 81	71 59	201 237	166 201
Avanafil, 10	85 80	99 99	84 79	106 91	**71** **56**
25	93 95	94 100	88 94	78 78	43 43
50	95 107	99 99	95 105	58 69	23 34
75	100 97	100 98	100 95	97 132	**62** **97**
100[#]	98 91	97 95	95 86	164 218	**129** **183**
125[#+]		0 0			

[#]Precipitating dose
% RCG (relative cloning growth) = (average viable count of treated culture / average viable count of solvent control} x 100
% RSG (Relative Suspension Growth) = (total treatment suspension growth / average solvent control total suspension growth) x 100
% RTG (Relative Total growth) = (% suspension x % cloning growth) / 100
Mutant frequency = (average number TFT colonies / average number viable count colonies) x 200
Induced mutant frequency = mutant frequency - average mutant frequency of solvent controls
[+] - Not chosen for cloning due to precipitate

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mammalian Erythrocyte Micronucleus Test

Study no:	AA56WS.123 .BTL
Study report location:	Module 4.2.3.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 9, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #60004-02-001, #60004-02-001-R, 98.5%

Key Study Findings

- No induction of a significant increase in micronucleated polychromatic erythrocytes in either male or female ICR mice at doses up to 2000 mg/kg under the conditions of the study

Methods

Doses in definitive study:	0, 500, 1000, 2000 mg/kg
Frequency of dosing:	Test article or control was administered by a single injection, and bone marrow cells were taken from femurs after 24 or 48 hrs (vehicle & HD only).
Route of administration:	Intraperitoneal injection

Dose volume:	20 mL/kg
Formulation/Vehicle:	Suspension/corn oil
Species/Strain:	Mouse/ICR
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	MTD
Negative control:	Corn oil
Positive control:	Cyclophosphamide monohydrate (50 mg/kg)

Study Validity

- The mean incidence of micronucleated polychromatic erythrocytes does not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control.
- The incidence of micronucleated polychromatic erythrocytes in the positive control group is significantly increased relative to the vehicle control group.

Results

- No significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow after a single intraperitoneal administration of avanafil at doses up to 2000 mg/kg
- Dose-dependent reduction of 9 to 30% in the ratio of polychromatic erythrocytes to total erythrocytes, indicative of direct bone marrow toxicity of the drug
- Toxicity:
    - No mortality up to 2000 mg/kg tested; piloerection in all treated males and in 2000 mg/kg females; lethargy in males at ≥100 mg/kg and in HD females in a pilot study
    - Lethargy and piloerection in all treated animals in a definitive study
- Study met the validity criteria

7.4 Other Genetic Toxicity Studies

Study title: Unscheduled DNA Synthesis Test with Mammalian Liver Cells

Study no:	AA56WS.381.BTL
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 6, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #0727-20 (purity unknown)

Key Study Findings

- No induction of a significant increase in the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the negative control) in hepatocytes isolated from treated rats under the conditions of the study

Methods

Doses in definitive study:	0, 500, 1000, 200 mg/kg
Frequency of dosing:	Test article or control was administered via oral gavage, and primary hepatocytes were isolated 2-4 hours or 12-16 hours post-exposure.
Route of administration:	Oral gavage
Dose volume:	5, 10, 20 mL/kg
Formulation/Vehicle:	Suspension/corn syrup, sodium carboxymethylcellulose, polysorbate 80, purified water, xanthan gum, microcrystalline cellulose, sucrose, sodium benzoate, disodium edetate, citric acid monohydrate, 10N sodium hydroxide
Species/Strain:	Male rat/Sprague Dawley
Number/Sex/Group:	3 males/group
Satellite groups:	None
Basis of dose selection:	200 mg/kg
Negative control:	Placebo formulation
Positive control:	Dimethylnitrosamine (35 mg/kg)

Study Validity

- The proportion of cells in repair in the negative control group is less than 15% and the mean net nuclear grain count of the negative control must be <1.
- The mean net nuclear grain count of the positive control group is at least 5 counts over that of the negative control group.

Results

- Means of the net nuclear grain counts:
  - -0.9, -0.2 and -0.1 for 500, 1000 and 2000 mg/kg, respectively, using primary hepatocytes isolated 2 to 4 hours post-exposure
  - -0.8, -0.1 and -0.5 for 500, 1000 and 2000 mg/kg, respectively, using primary hepatocytes isolated 12 to 16 hours post-exposure
- No significant increase in the mean net nuclear counts compared to those from the negative control group
- No mortality or clinical signs up to 2000 mg/kg tested
- No information on test conditions (e.g., cell viability, evidence for systemic exposure)
- Study met the validity criteria

8 Carcinogenicity

Study title: A 2-Year Carcinogenicity Study of TA-1790 in Mice

Study no.:	1060-005
Study report location:	Module 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 16, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP303020, 98.89%
CAC concurrence:	Yes (December 16, 2003)

Key Study Findings

- Negative for both sexes tested at up to an MTD

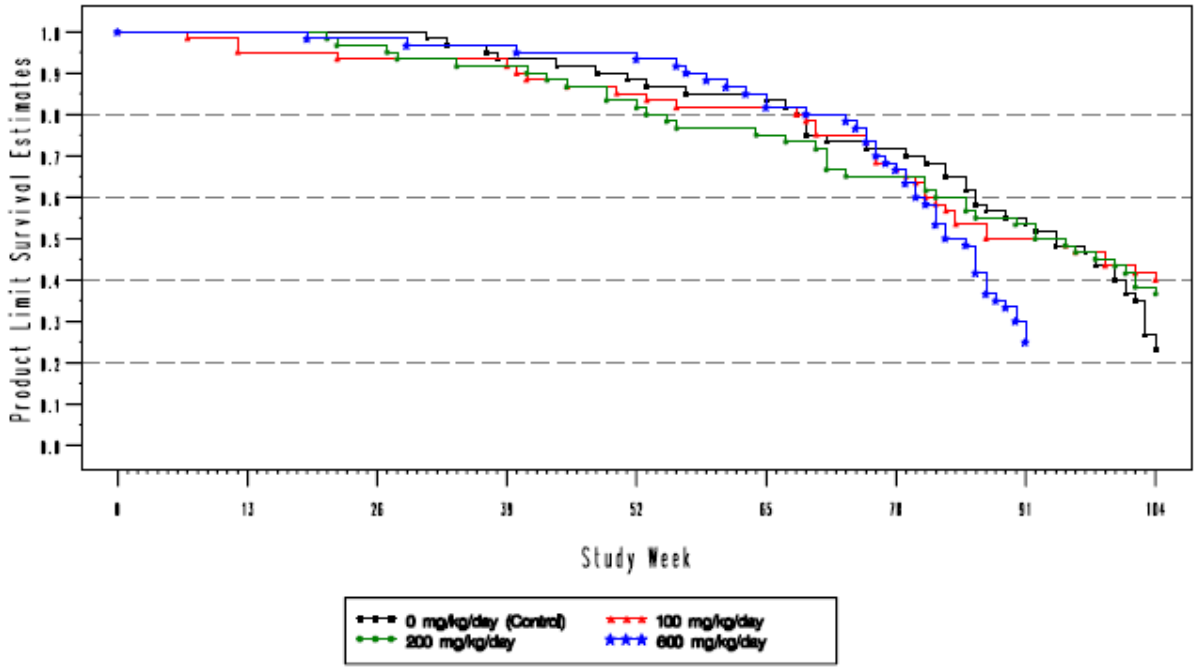
Adequacy of Carcinogenicity Study:

The Exec CAC noted that the study was adequately performed. However, the vehicle used in the main study (solution in 0.5% carmellose sodium solution containing 0.1% HCO-60 in purified water), differed from that in the dose-ranging study (suspension in corn syrup, sodium carboxymethylcellulose, polysorbate 80, purified water, xanthan gum, microcrystalline cellulose, sucrose, sodium benzoate, disodium edetate, citric acid monohydrate and 10N sodium hydroxide), which is suboptimal. The HD (600 mg/kg, $AUC_{0\text{-}24h}$~80000-88000 ng·hr/mL) produced approximately 11-fold the MRHD (200 mg, $AUC_{0\text{-}24h}$~8000 ng·hr/mL) on an AUC basis.

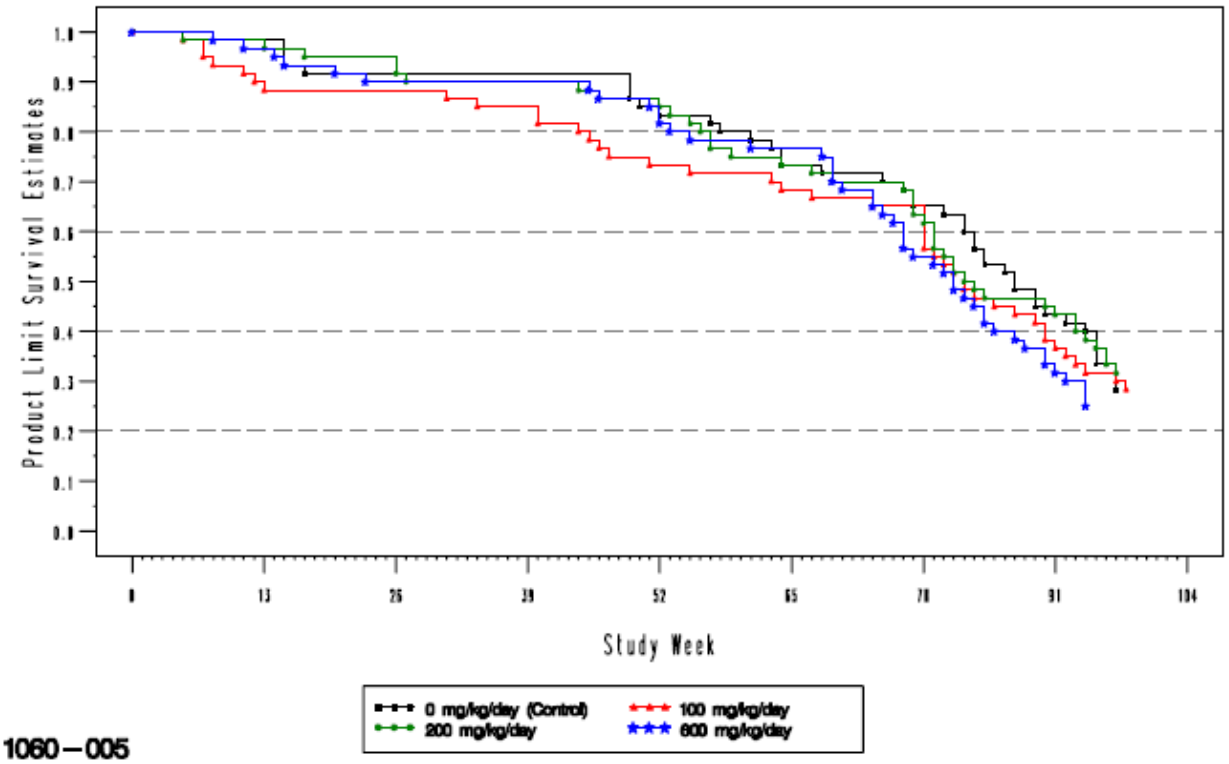
Survival for males and females at HD (600 mg/kg/day) was comparable to controls for the first 18 months of study, but was lower during the following 3 to 4 months of study. There were no significant changes in body weights compared to controls throughout the study. The HD was terminated early during Week 92 for males and Week 94 for females (concurred by the Exec CAC). All remaining male and female groups were terminated during Week 104 and Week 98, respectively. Treatment was stopped early in Week 91 for HD males and females due to increased morbidity and mortality. The survival at the terminal necropsy was 30.0%, 40.0%, 30.0%, and 26.7% in males, and 28.3%, 35.0%, 38.3%, and 23.3% in females at 0, 100, 300, and 1000 mg/kg/day, respectively. The cumulative survival estimates were 53.3%, 50.0%, 53.3% and 25.0% in males, and 43.3%, 36.7%, 43.3% and 31.7% in females, with a statistically significance ($p<0.05$) in overall treatment-related effect, and treatment effect between control and high dose groups on the survival of male mice. The cause of deaths was not determined for most of the animals found dead or euthanized *in extremis*.

The following sponsor's figures illustrate the survival rate estimated for mice.

Mean Survival Estimate of Male Mice



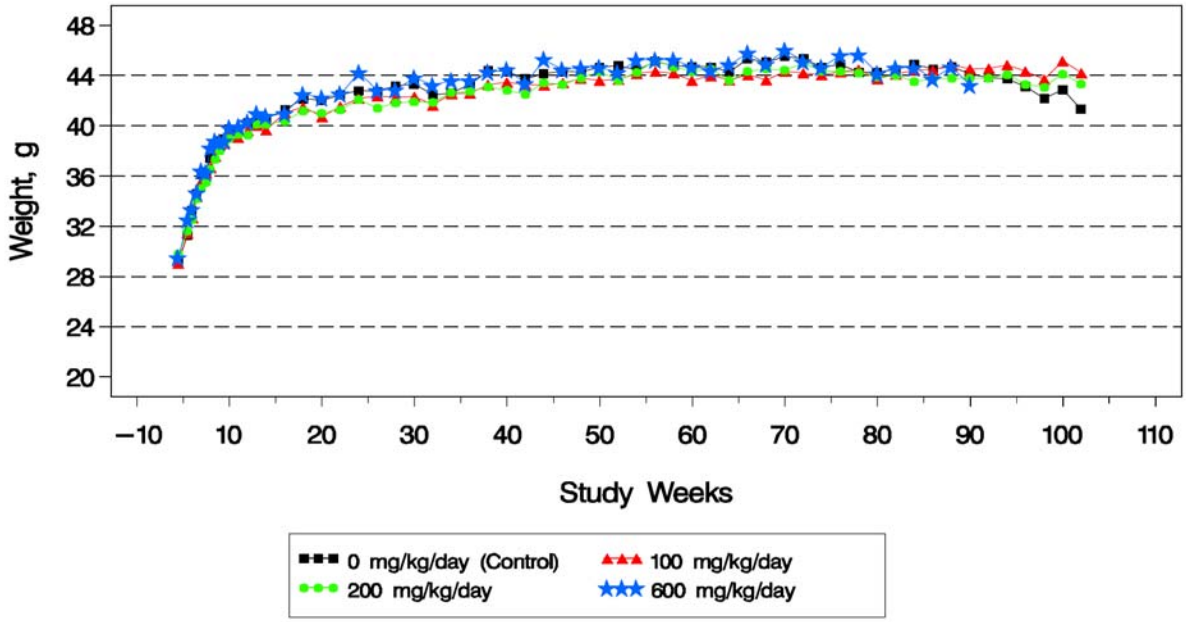
Mean Survival Estimate of Female Mice



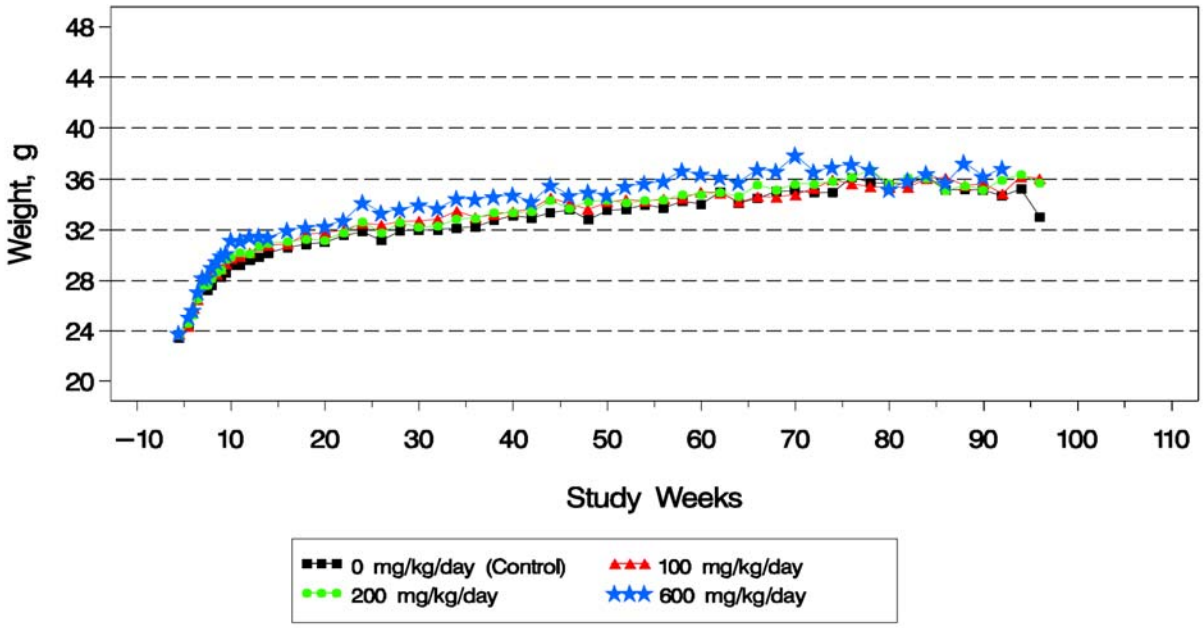
1060–005

The following sponsor's figures illustrate body weights for male and female mice.

Mean Body Weights of Male Mice



Mean Body Weights of Female Mice



Appropriateness of Test Models:

It appears that the use of the test model is appropriate based on the totality of the information provided (e.g., genotoxicity, dosing regimen, route of administration). PK parameters were only provided from the 13-week dose range-finding study utilizing a different vehicle, and metabolite profiles were not provided for mice.

Evaluation of Tumor Findings:

Per the Exec CAC, no neoplasms were clearly drug-related. The increased incidences of malignant hepatocellular carcinoma in males (0, 2, 2, and 4 at 0, 100, 200, and 600 mg/kg/day, respectively) and benign bronchiolar alveolar adenoma in the lung (6, 8, 9, and 15 at 0, 100, 200, and 600 mg/kg/day, respectively) were not statistically significant. The increased tumor incidence in male mice (4, 6.6%) was slightly higher than the historical background incidence range (0-3, 0-5% in 2004-2006) from the conducting laboratory. Mice with the hepatocellular carcinoma died prematurely, possibly indicating tumor occurrence with reduced latency. Two mice that had hepatocellular carcinoma metastasized to lungs. None of other tumor incidences reported in treated groups including subcapsular cell adenoma and pheochromocytoma in adrenal glands, abdominal cavity carcinoma, adenoma and adenocarcinoma in mammary gland, cystadenoma in ovaries, mesenchymal and transitional cell papilloma tumors in urinary bladder, and fibroma and leiomyosarcoma in uterus/cervix were statistically significant.

Methods

Doses:	0, 100, 200, 600 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10, 6, 6.67, 10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Sodium Solution containing 0.1% hydrogenated castor oil-60 in deionized water (Formulation A)
Basis of dose selection:	MTD (mortality & cardiac toxicity)
Species/Strain:	Mouse/Crl:CD-1®(ICR)BR from (b) (4)
Number/Sex/Group:	60/sex/group
Age:	6 Weeks at receipt
Animal housing:	Individually
Paradigm for dietary restriction:	*ad libitum*, except during designated periods
Dual control employed:	No
Interim sacrifice:	Dosing was stopped prior to Week 91 for both males and females at HD, and the HD group was terminated early during Week 92 for males and Week 94 for females due to reduced survival rate below 15. All remaining female groups were terminated early during Week 98 (concurred by the Exec CAC).
Satellite groups:	None
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily during Weeks 1 through 52, and 3 times daily thereafter

- Decreased survival in males and females at HD (with unknown cause of death) compared to controls during the following 3 to 4 months of study, with survival rate at the terminal necropsy of 53%, 50%, 53%, and 25% in males, and 42%, 33%, 40%, and 30% in females at 0, 100, 200, and 600 mg/kg/day, respectively

Clinical Signs: Twice daily during Weeks 1 through 52, and 3 times daily thereafter for cageside observations; weekly for a detailed clinical examination

- Increased incidence of impaired righting reflex, distended abdomen, extended penis, discolored hair, and cold to touch skin in HD males

Body Weights: 2 Days after receipt, prior to randomization, the day prior to test article administration, weekly for the first 14 weeks, & once every 2 weeks thereafter

- Unremarkable

Feed Consumption: Weekly during the first 14 weeks, & once every 2 weeks thereafter

- Unremarkable

Clinical Pathology: At study termination on surviving animals & euthanized *in extremis* on all animals for hematology

- Increased leukocytes (~200%), neutrophils (~130%), lymphocytes (~240%), monocytes (~490%), eosinophils (~120%), basophils (~80%), and large unstained cells (~410%) in HD females compared to controls

Gross Pathology: Necropsies performed at the start of the next working day after refrigeration overnight for animals that were found dead after regular working hours; euthanized on Day 638 for HD males, & Day 655 for HD females; on Day 680 for all remaining females

- Increased incidence of distended urinary bladder in HD males

Histopathology

Peer Review: No

Neoplastic findings:

- Non-statistically significant increase in incidence of malignant hepatocellular carcinoma in liver in males with 0, 2, 2, and 4 tumors at 0, 100, 200, and 600 mg/kg/day

The following table summarizes the survival rate and the incidence of neoplastic findings in mice.

Dose, mg/kg / Observations	0		100		200		600	
	60M	60F	60M	60F	60M	60F	60M	60F
Survival rate[@], %, weeks 0-104	53.3[#]	43.3	50.0	36.7	53.3	43.3	25.0	31.7
Neoplastic lesions, unscheduled+scheduled necropsy								
Adrenal glands, adenoma, subcapsular cell, benign, primary			2		1		1	1
pheochromocytoma, malignant, primary							1	
Cavity, abdominal, carcinoma, malignant, primary								1
Harderian glands, adenoma, benign, primary	4		4		4	2	5	1
adenocarcinoma, malignant, primary							1	
Heart, pheochromocytoma, malignant, secondary							1	
Kidneys, carcinoma, bronchiolar alveolar, malignant, secondary							1	
Liver, adenoma, hepatocellular, benign, primary	7	1	7		6	1	7	2
carcinoma, hepatocellular, malignant, primary			2		2		4	1
ito cell tumor, benign, primary							1	
Lung, adenoma, bronchiolar alveolar, benign, primary	6	7	8	4	9	8	15	10
carcinoma, bronchiolar alveolar, malignant, primary	5	1	4	2	5	1	3	
carcinoma, hepatocellular, malignant, secondary							2	1
Mammary gland, adenocarcinoma, malignant, primary	-		-	1	-	1	-	1
adenoma, benign, primary	-		-		-		-	1
Ovaries, cystadenoma, benign, primary	-		-	1	-	2	-	1
Skin, fibrous histiocytoma, malignant, primary				1				1
Urinary bladder, mesenchymal tumor, benign, primary				1			1	
papilloma, transitional cell, benign, primary							1	
Uterus/Cervix, fibroma, benign, primary	-		-		-	2	-	1
leiomyosarcoma, malignant, primary	-		-	2	-		-	2

[@]Males and females at 600 mg/kg were euthanized on Day 638 and Day 655, respectively, due to survival rate below 15. All remaining females were euthanized on Day 680 due to reduced survival falling to 17 in control group.
Statistically significant from controls at p=0.05 by Kaplan-Meier (trend[#]) or log-rank (pair-wise[*]) test based on the sponsor's analysis
-; not available

Non Neoplastic findings:

- Increased incidence and/or severity of cardiomyopathy and thrombosis at HD
- Increased incidence and severity of alveolar histiocytosis at HD
- Increased incidence of ureter and urinary bladder dilatation in HD males

The following table summarizes noteworthy non-neoplastic findings with avanafil in 2-year mouse study.

Dose, mg/kg / Observations	0		100		200		600	
	60M	60F	60M	60F	60M	60F	60M	60F
Clinical signs,								
Righting reflex, impaired		2	1	1		3	4	1
Abdomen, distended	11	9	15	14	7	9	27	10
Penis, extended	7		1				14	
Hair, discolored	3	3	4	3		2	11	1
Skin, cold to touch	14	19	14	17	18	14	23	13
Hematology, $10^3/\mu L$								
Leukocytes[#]	8.96	3.41	6.57	4.06	9.48	4.06	8.28	**10.51****
Neutrophils[#]	3.357	2.07	2.554	1.911	4.628	2.105	4.541	**4.788**
Lymphocytes[#]	5.082	1.188	3.583	1.951	4.283	1.748	3.253*	**4.034****
Monocytes	0.226	0.067	0.125*	0.076	0.16	0.061	0.183	**0.396****
Eosinophils	0.182	0.061	0.172	0.081	0.227	0.105	0.177	**0.136**
Basophils	0.009	0.005	0.01	0.012	0.009	0.013	0.023	**0.009**
Large unstained cells[#]	0.098	0.023	0.113	0.025	0.172	0.027	0.106**	**0.972**
Gross pathology,								
Urinary bladder, distended with urine	$14^{2/3/4}$		$8^{2/3/4}$		$9^{2/3/4}$	2^{2}	$27^{2/3/4}$	
Histopathology[@], unscheduled+scheduled								
Heart, cardiomyopathy	$34^{1/2}$	13^{1}	$15^{1/2}$	$9^{1/2}$	$14^{1/2}$	$9^{1/2}$	$48^{1/2/3}$	$21^{1/2}$
thrombus	$3^{3/4}$	1^{3}	$2^{3/4}$	$2^{1/2}$		1^{4}	$10^{1/2/3/4}$	$5^{1/2/3}$
Lung, histiocytosis, alveolar	$11^{1/2/3}$	$11^{1/2/4}$	$7^{1/2/3/4}$	$15^{1/2/3/4}$	$9^{1/2/3}$	$12^{1/2/3/4}$	$20^{1/2/3/4}$	$20^{1/2/3/4}$
Spleen, lymphoid, depletion	3^{2}	$2^{1/2}$	$4^{1/2}$	6^{1}	2^{2}	3^{1}	2^{2}	$6^{1/2}$
necrosis	1^{2}	$2^{1/2}$	$2^{1/2}$	$5^{1/2}$	2^{1}	$2^{1/2}$	$2^{1/2}$	$7^{1/2}$
Ureters, dilatation	$2^{3/4}$		$5^{2/3/4}$		$2^{1/4}$		$6^{1/2/3/4}$	
Urinary bladder, dilatation	$11^{2/3/4}$		$5^{3/4}$		$8^{2/3}$	1^{3}	$20^{2/3/4}$	

[#]One female at 600 mg/kg (#9475) exhibited a marked elevation in some hematology parameters at study termination
[@]Severity grade 1; minimal, 2; mild, 3; moderate, 4; severe
Statistically significant from controls at p=0.05* or p=0.01*

Toxicokinetics: Not provided

TK analysis was conducted in the 13-week dose range-finding study, however, a different vehicle was used for that study.

Dosing Solution Analysis: Samples were collected from the LD and HD formulations prior to initiation of test article administration and during Week 92 for homogeneity. Samples (10 mL/sample) of the prepared formulations mixed for the pretest homogeneity analysis were stored refrigerated for up to 21 days, and were collected after 7 and 21 days for stability analysis. Samples of the dosing formulations at each concentration were also collected weekly during Weeks 1 through 4, then every 4 weeks for the remainder of the study, except for Week 92, and stored refrigerated for concentration analysis.

- Homogeneous for dosing formulations prepared in Weeks 1 and 92 with mean recovery between 90.4% and 101.6%;
- Stable for 7 and 21 days with mean recovery between 90.4% and 101.6%;
- Mean recovery between 83.0% and 112.5% for concentrations of 10, 30, and 100 mg/mL for Weeks 1 through 104 except in Week 8 at 100 mg/mL where the mean concentration was about 40% lower than expected

Study title: A 2-Year Carcinogenicity Study of TA-1790 in Rats

Study no.:	1060-004
Study report location:	Module 4.2.3.4.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 16, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP303020, 98.89%
CAC concurrence:	Yes (December 16, 2003)

Key Study Findings

- Negative for both sexes tested up to an MTD

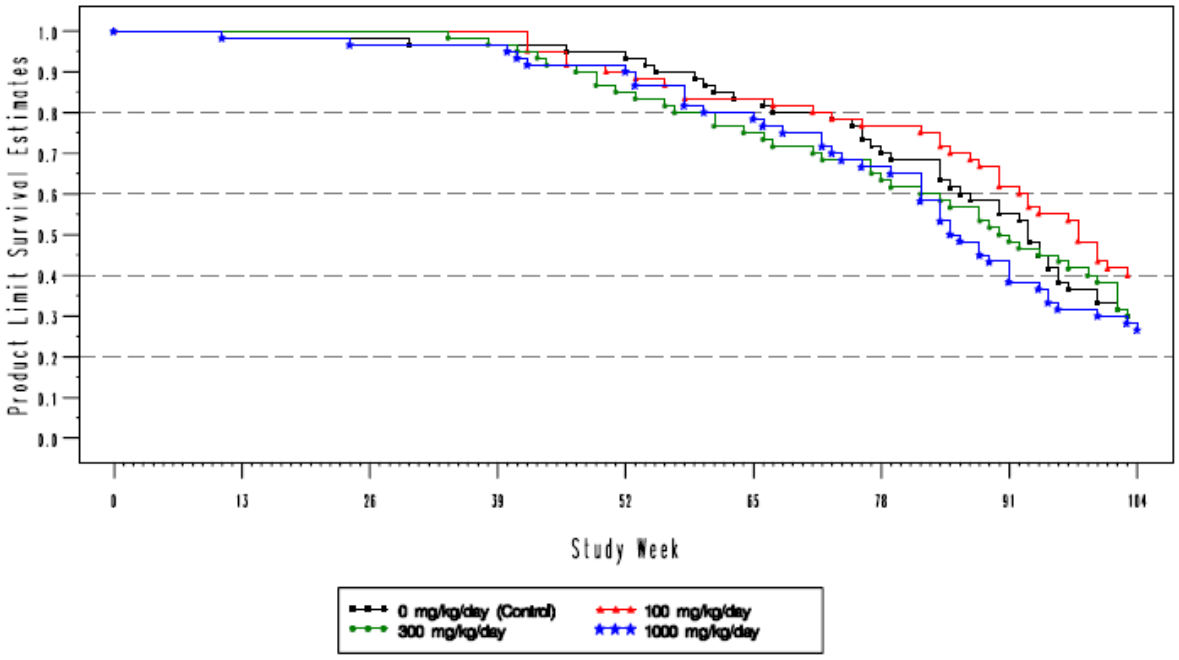
Adequacy of Carcinogenicity Study:

The Exec CAC noted that the study was adequately performed. However, the vehicle used in the main study (solution in 0.5% carmellose sodium solution containing 0.1% HCO-60 in purified water), differed from that in the dose-ranging study (suspension in corn syrup, sodium carboxymethylcellulose, polysorbate 80, purified water, xanthan gum, microcrystalline cellulose, sucrose, sodium benzoate, disodium edetate, citric acid monohydrate and 10N sodium hydroxide), which is suboptimal.

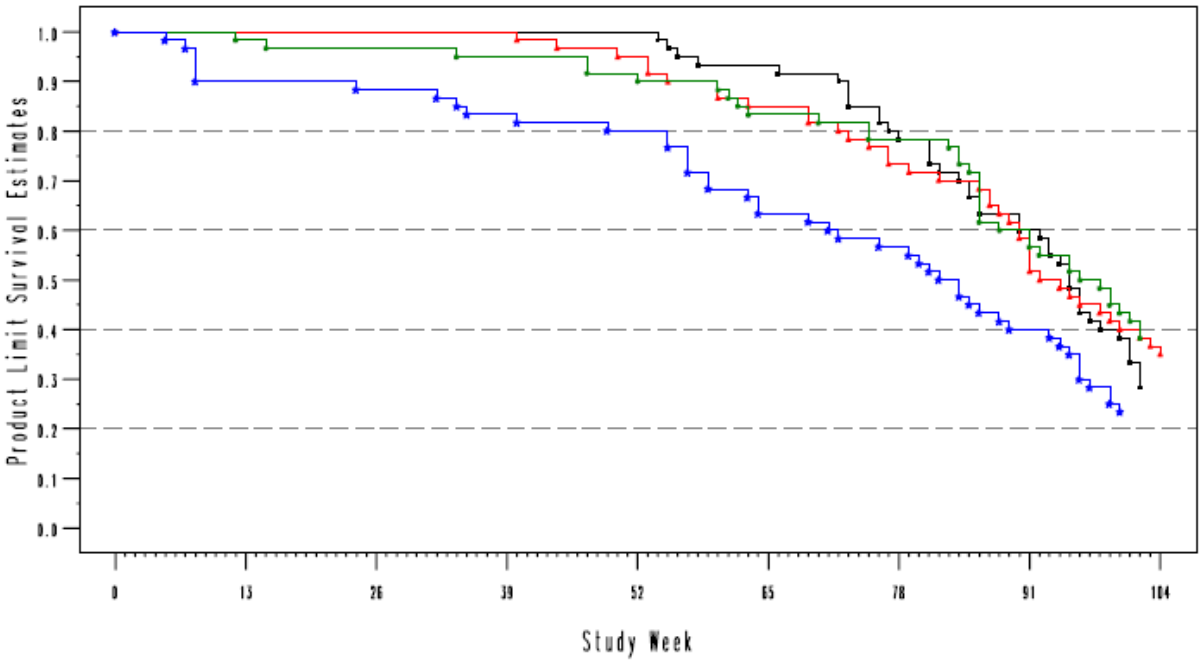
Survival for males at 1000 mg/kg/day was comparable to controls for the first 18 months of study, but was lower during the last 6 months of study. Survival for females at 1000 mg/kg/day was lower after the second month of study compared to controls and continued lower throughout the study. The HD females were terminated in Week 100 due to reduced survival below 15 animals. A statistically significant dose-response relationship and differences between the control and the HD groups in survival were observed in female rats (see Statistical review). The survival at the terminal necropsy was 30.0%, 40.0%, 30.0%, and 26.7% in males, and 28.3%, 35.0%, 38.3%, and 23.3% in females at 0, 100, 300, and 1000 mg/kg/day, respectively. The cumulative survival estimates were 30.0%, 40.0%, 30.0%, and 26.7% in males, and 28.3%, 35.0%, 38.3%, and 40.0% with a statistically significance ($p<0.05$) in overall dose-response effect and treatment effect between control and high-dose group on the survival of female rats. Decreased body weights were observed in HD animals at up to 8% in males and 23% in females compared to controls at the end of the study. A specific cause for the decreased survivability in HD females was not determined. It appears that the HD females exceeded an MTD based on the overall survival curve and the body weights.

The following sponsor's figures illustrate the survival rate estimated for rats.

Mean Survival Estimate of Male Rats



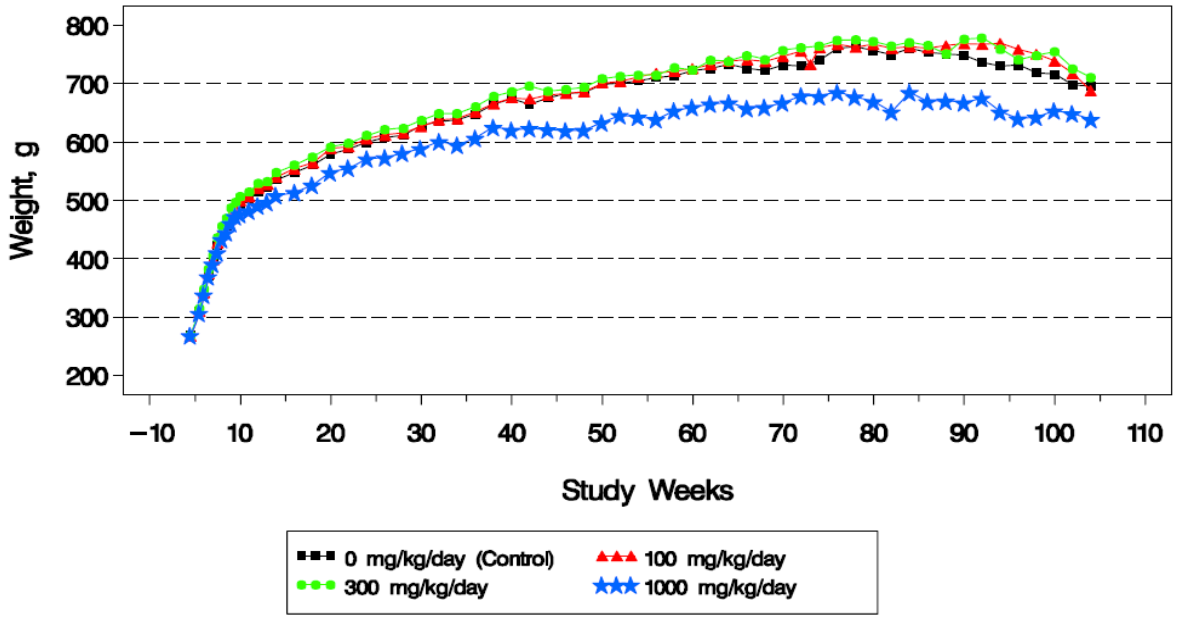
Mean Survival Estimate of Female Rats



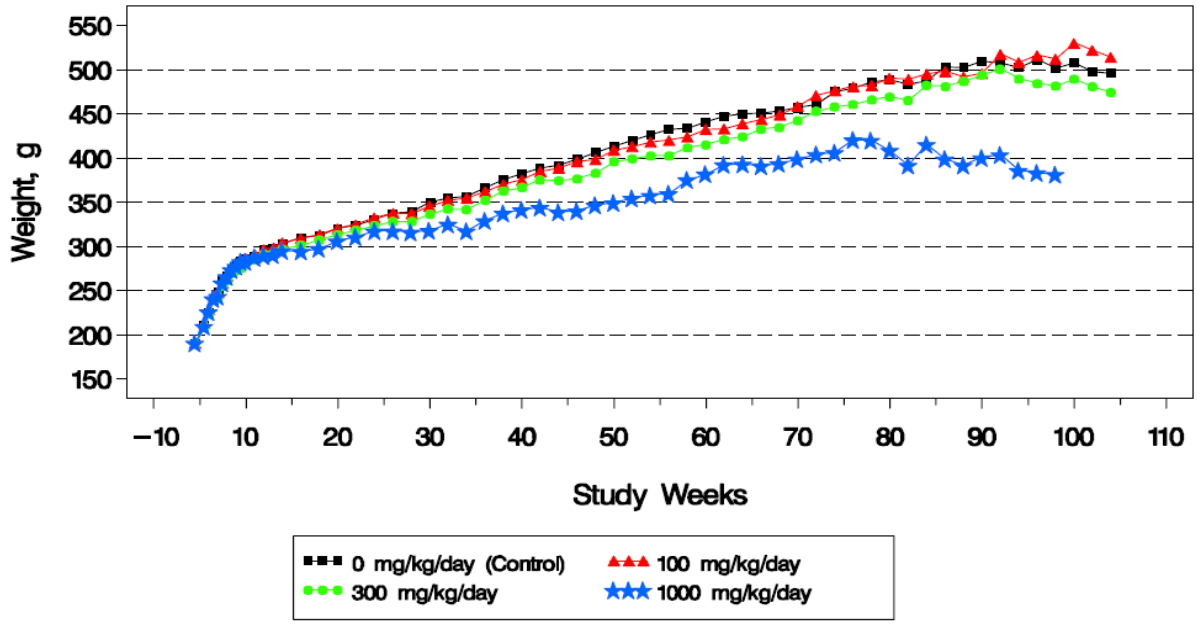
1060–004

The following sponsor's figures illustrate body weights for male and female rats.

Mean Body Weights of Male Rats



Mean Body Weights of Female rats



Appropriateness of Test Models:

The choice of the 2-year study is considered appropriate with respect to animal strain, dosing frequency, and the route of administration. Avanafil was extensively metabolized in rats, particularly in males. Plasma concentrations of the parent drug were 2-5 fold lower than the metabolite M4 based on the AUC levels obtained from a 2-week TK bridging study. On the other hand, M4 was approximately 0.4-fold the parent drug in male human subjects. The HD (1000 mg/kg) is approximately 8- and 34-fold above the exposure at the MRHD (total $AUC_{0\text{-}24h}$~8 μg·hr/mL) based on plasma concentrations of the parent drug ($AUC_{0\text{-}24h}$~65 μg·hr/mL for males, ~290 μg·hr/mL for females) from the 2-week toxicity study.

Evaluation of Tumor Findings:

Per the Exex CAC, there were no neoplasms that were clearely drug-related. Increased incidence of benign hepatocellular adenoma was observed in males with 0, 2, 0, and 5 tumors at 0, 100, 300, and 1000 mg/kg/day, respectively. The incidence of hepatocellular adenoma gave p values of 0.006 by Lin and Rahman's criteria for dose-response relationship and 0.022 by Haseman's criteria for pairwise comparisons based on the Agency's statistical analysis. The sponsor's statistical analysis shows p values of 0.018 and 0.021 for the Peto's test (survival adjusted), 0.026 and 0.023 by the Cochran-Armitage test (survival unadjusted) and 0.057 for the Fisher's exact test (survival unadjusted). The increased liver adenomas were statistically significant in both dose-response (p=0.006) and pair-wise (p=0.022) comparisons when considered as a rare tumor based on <1% of spontaneous incidence in concurrent control group. The increased tumor incidence in male rats (5, 8.3%) was slightly higher than the historical background incidence range (0-1, 0-1.7% in 2004- 2007) from the conducting laboratory. The sponsor stated that all five rats with the liver tumors survived to scheduled sacrifice, and no evidence of progression of lesions to malignant tumors was found. The increased incidence of focal cystic degeneration and eosinophilic focus in the liver may be represented as progenitor lesions or markers for the hepatic carcinogenic effects, suggesting that such degenerative and inflammatory changes may be associated with enhanced hepatocyte proliferation. The increased incidence of the liver tumors could be due to a greater biotransformation capability of males than in females. The Exec CAC noted that the incidence was not remarkable, given the relatively high historical background rate noted for the hepatocellular adenomas in male rodents via oral gavage. The sporadic increase in tumor incidences including the hibernoma, schwannoma, basal cell lip carcinoma, cholangioma in liver, acinar cell adenoma in pancreas, adenoma in parathyroid glands, malignant rhabdomyosarcoma in skin subcutis, seminoma in testes, follicular cell adenoma in thyroid gland, mesothelioma in mesentery/peritoneum, mesothelioma in ovaries, thymoma in thymus, adenoma and stromal sarcoma, and vagina polyp was not statistically significant.

The numerical increase in hepatocellular tumors (adenomas and/or carcinomas) was also observed in male mice (CD-1) and rats (CD) treated with another PDE5 inhibitor, tadalafil (Cialis) although the findings were not statistically significant. Nitroglycerin, for which pharmacological action is attributable to its metabolic reduction to NO, has also

shown a dose-related increase in hepatocellular carcinomas in both sexes of rats (CD) receiving up to 434 mg/kg/day as reflected in current labels.

The following table summarizes the non-neoplastic and neoplastic findings in the liver.

Dose, mg/kg / Observations	0		100		300		1000	
	60M	60F	60M	60F	60M	60F	60M	60F
Enlarged	0	1^{2}	1^{4}	$4^{2/4}$	1^{2}	0	$4^{2/3}$	1^{2}
Degeneration, cystic, focal	6^{1}	$2^{1/2}$	8^{1}	0	10^{1}	0	16^{1}	2^{1}
Focus of cellular alteration, eosinophilic	0	3^{2}	4^{1}	$3^{1/2}$	1^{1}	1^{1}	$6^{1/2}$	$3^{1/2}$
Adenoma, hepatocellular, benign, total	0#	0	2	0	0	1	5	0
unscheduled	0	0	1	0	0	0	3	0
scheduled	0	0	1	0	0	1	2	0
Carcinoma, hepatocelluar, malignant, total	0	0	0	0	0	0	0	0
Adenomas+Carcinomas	0	0	2	0	0	1	5*	0

Severity grade 1; minimal, 2; mild, 3; moderate, 4; severe
#p=0.006 by Lin and Rahman's criteria and *p=0.022 by Haseman's criteria based on Agency's analysis

Methods

Doses:	0, 100, 300, 1000 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10, 6, 6.67, 10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Sodium Solution containing 0.1% HCO-60 in deionized water (Formulation A)
Basis of dose selection:	MTD (mortality & cardiac toxicity)
Species/Strain:	Rat/CD®[Crl:(SD)IGS BR] from (b) (4)
Number/Sex/Group:	60/sex/group
Age:	6 Weeks at receipt
Animal housing:	Individually
Paradigm for dietary restriction:	*ad libitum*
Dual control employed:	No
Interim sacrifice:	The test article was administered once a day for 104 consecutive weeks except for females at 1000 mg/kg/day that were terminated prematurely during Week 100 due to reduced survival rate below 15.
Satellite groups:	None
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily for the 1st 12 months, & 3 times daily thereafter

- Lower survival rate at HD for males during most of the last 6 months of study and for females after the 2nd month of study compared to controls, and continued lower survival rates throughout the study, resulting in terminated early in Week 100 for females

- Survival at the terminal necropsy was 30.0%, 40.0%, 30.0%, and 26.7% in males and 40.0%, 42.0%, 45.0%, and 25.0% in females in the 0, 100, 300, and 1000 mg/kg/day groups, respectively

Clinical Signs: Twice daily during Weeks 1 through 52, & 3 times daily thereafter for cageside observations; weekly for a detailed clinical examination

- Increased incidence of salivation, sparse hair (females) and scabbed area (males) in all treated groups
- Increased incidence of abrasions in males at ≥MD
- Increased incidence of red material around mouth, discolored hair, and distended abdomen in HD females

Body Weights: 2 Days after receipt, prior to randomization, the day prior to test article administration, weekly for the first 14 weeks, & once every 2 weeks thereafter

- Lower body weights at HD compared to controls throughout the study with -8% in males and -23% in females at the end of the study

Feed Consumption: Weekly during the first 14 weeks, & once every 2 weeks thereafter

- Unremarkable

Clinical Pathology: At study termination on surviving animals and animals euthanized *in extremis* on all animals for hematology

- Dose-related decrease in neutrophils in males in all treated groups (up to 39%) and in HD females (~23%) compared to controls
- Dose-related increase in lymphocytes (up to 44%) and basophils (up to 33%) in females in all treated groups compared to controls
- Decrease in eosinophils at HD (17-33%)

Gross Pathology: Necropsies performed at the start of the next working day after refrigeration overnight for animals that were found dead after regular working hours; euthanized on Day 638 for HD males, & Day 655 for HD females; on Day 680 for all remaining females

- Increased incidence and severity of enlarged liver in HD males

Histopathology

Peer Review: No

Neoplastic findings:

- Statistically significant increase in benign hepatocellular adenoma in males of 0, 2, 0, and 5 tumors at 0, 100, 300, and 1000 mg/kg/day, respectively, associated with increased incidences of focal cystic degeneration and eosinophilic focus of cellular alteration (p=0.006 by Lin and Rahman trend test and p=0.022 by Haseman's criteria)

The following table summarizes the survival rate and the incidence of neoplastic findings in rats.

Observations \ Dose, mg/kg	0		100		300		1000	
	60M	60F	60M	60F	60M	60F	60M	60F
Survival rate[@a], %, weeks 0-105	30.0	28.3[#]	40.0	35.0	30.0	38.3	26.7	40.0[*]
Neoplastic lesions, unscheduled+scheduled necropsy								
Adipose tissue, brown, hibernoma, benign, primary							1	
malignant, primary			1	1			1	
Adrenal glands, pheochromocytoma, benign, primary	10	1	7	5	8	1	4	
malignant, primary			1		2	1	2	
Heart, schwannoma, benign, primary							1	
Lip, carcinoma, basal cell, malignant, primary							1	
Liver, adenoma[b], hepatocellular, benign, primary			2			1	5	
cholangioma, benign, primary							1	
Lung, carcinoma, c-cell, malignant, secondary							1	
hibernoma, malignant, secondary			1				1	
LN, mandibular, carcinoma, c-cell, malignant, secondary							1	
mesenteric, mesothelioma, malignant, secondary								1
Pancreas, adenoma, acinar cell, benign, primary							1	
Parathyroid glands, adenoma, benign, primary	1					1	3	2
Skin, subcutis, rhabdomyosarcoma, malignant, primary							1	
sarcoma, undifferentiated, malignant, primary						1		1
Testes, seminoma, benign, primary		-		-		-	1	-
Thyroid gland, adenoma, follicular cell, benign, primary			1	1			1	2
carcinoma, follicular cell, malignant, primary	1		1					
Mammary gland, adenocarcinoma, malignant, primary		5		2		8		5
fibroadenoma, benign, primary		28	1	23		18		15
Mesentery/peritoneum, mesothelioma, malignant, primary					1			1
Ovaries, mesothelioma, malignant, secondary	-		-		-		-	1
Pituitary gland, adenoma, pars distalis, benign, primary	31	49	28	39	20	39	28	29
carcinoma, pars distalis, malignant, primary						1		
Thymus gland, thymoma, benign, primary								1
Uterus/Cervix, adenoma, benign, primary	-		-		-		-	1
sarcoma, stromal, malignant, primary	-		-		-		-	1
Vagina, polyp, benign, primary	-		-		-		-	1

[@]Females at 1000 mg/kg were euthanized prematurely during Week 100 due to reduced survival rate below 15.
[a]Statistically significant from controls at p=0.05 by Kaplan-Meier (trend[#]) or log-rank (pair-wise[*]) test based on the sponsor's analysis
[b]Statistically significant from controls at p=0.006 by Lin and Rahman's criteria and p=0.022 using the Haseman's criteria based on Agency's analysis
-; not available

Non Neoplastic Findings:

- Liver: Increased incidence of cystic degeneration in males in all treated groups; eosinophilic focus in HD males

The following table summarizes the noteworthy non-neoplastic findings with avanafil in 2-year rats.

Dose, mg/kg / Observations	0		100		300		1000	
	60M	60F	60M	60F	60M	60F	60M	60F
Clinical signs,								
Salivation		1	2	4	15	16	24	19
Abdomen, distended		2		1		3		7
Material around mouth, red	1	4	2	4	2	2	5	13
Abrasions	1	2	1	4	4	9	8	
Hair discolored, red	1	1		1			1	13
Hair sparse	5	7	6	8	3	13	7	23
Scabbed area	6	3	22	9	25	14	22	6
Body weights, g, weeks 98/104	695.8	501.4	688.5	512.1	710.9	481.5	**639.0**	**381.4****
Body weight change, %			-1	+3.7	+2.2	-4.3	**-8.2**	**-23.1**
Hematology, $10^3/\mu L$								
Neutrophils	5.396	3.535	**4.716**	3.756	**3.852**	3.501	**3.307***	**2.725**
Lymphocytes	7.434	4.623	7.501	**4.923**	6.876	**5.81**	7.035	**6.644***
Eosinophils	0.147	0.13	0.136	0.146	0.154	0.139	**0.122**	**0.087**
Basophils	0.048	0.038	0.050	**0.040**	0.046	**0.044**	0.051	**0.068****
Gross pathology,								
Liver, enlarged		1^{2}	1^{4}	$4^{2/4}$	1^{2}		$4^{2/3}$	1^{2}
Lung, consolidated		1^{3}	1^{2}	1^{2}	1^{3}		1^{3}	$4^{2/3}$
discoloration, red	$7^{2/3/4}$	$3^{1/3}$	$8^{2/3}$	$5^{2/3}$	$6^{2/3}$	2^{2}	$7^{2/3}$	$8^{2/3}$
Spleen, enlarged	$2^{2/3}$	1^{3}	$3^{1/2}$		$2^{1/2}$	$4^{1/2}$	1^{2}	3^{2}
Trachea, fluid	1^{3}		$4^{2/3}$	3^{2}	$2^{2/3}$		1^{3}	$5^{2/3}$
Histopathology$^{\#}$**,** unscheduled+scheduled necropsy								
Bone marrow, sternum, depletion	3^{2}	$12^{2/3}$	2^{2}	$8^{1/2/3}$	1^{3}	2^{2}	$7^{1/2}$	2^{2}
Epididymides, oligospermia/germ cell debris, bilateral	$6^{1/3/4}$	-	$2^{2/3}$	-	2	-	$10^{1/2/3/4}$	-
Kidneys, hydronephrosis, bilateral			2^{2}	1^{1}	1^{3}		$3^{1/2}$	
infiltration, lymphocytic	4^{1}	1^{1}	$3^{1/2}$		1^{1}	3^{1}	4^{1}	4^{1}
Liver, degeneration, cystic, focal	6^{1}	$2^{1/2}$	8^{1}		10^{1}		16^{1}	2^{1}
focus of cellular alteration, eosinophilic		3^{2}	4^{1}	$3^{1/2}$	1^{1}	1^{1}	$6^{1/2}$	$3^{1/2}$
hyperplasia, bile duct	$33^{1/2/3}$	$14^{1/2}$	$29^{1/2}$	$13^{1/2}$	$20^{1/2/3}$	$6^{1/2}$	$10^{1/2}$	8^{1}
LN, mesenteric, erythrocytosis/erythrophagocytosis, sinus	1^{2}	2^{2}	1^{3}	$4^{1/2}$	2^{2}	4^{2}	$7^{1/2}$	$4^{1/3}$
Spleen, depletion, lymphoid	$4^{2/3}$	$8^{2/3/4}$	$5^{2/3}$	$5^{2/3}$	$6^{1/2/3}$	2^{3}	$10^{1/2/3/4}$	$12^{1/2/3/4}$
Testes, degeneration/atrophy, seminiferous tubules, bilater	$4^{2/3/4}$	-	$2^{2/3}$	-	4^{4}	-	$10^{1/2/3/4}$	-
Thyroid gland, hyperplasia, follicular cell	1^{2}		1^{2}	1^{1}		2^{2}	1^{2}	$3^{2/3}$

$^{\#}$Severity grade 1; minimal, 2; mild, 3; moderate, 4; severe
Statistically significant from controls at p=0.05* or p=0.01**
-; not available

Toxicokinetics: Not provided

TK parameters were not provided in the 26-week dose range-finding study. TK parameters from the 2-week study are available, however, a different vehicle was used which may have affected exposures in the 2-year study.

Dosing Solution Analysis: Samples were collected from LD and HD formulations prior to initiation of test article administration and during Week 92 for homogeneity. Samples (10 mL/sample) mixed for the pretest homogeneity analysis were stored refrigerated for up to 21 days, and were collected after 7 and 21 days for stability analysis. Samples at each concentration were also collected weekly during Weeks 1 through 4, then every 4 weeks for the remainder of the study, except for Week 92, and stored refrigerated for concentration analysis.

- Homogeneous for dosing formulations prepared in Weeks 1 and 92 with mean recovery between 90.4% and 101.6%
- Stable for 7 and 21 days with mean recovery between 90.4% and 101.6%
- Mean recovery between 83.0% and 112.5% for concentrations of 10, 30, and 100 mg/mL for Weeks 1 through 104 with except in Week 8 at 100 mg/mL where the mean concentration was about 40% lower than expected

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: TA-1790: A Study of Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	1060-008
Study report location:	Module 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 20, 2006 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #17AP303020, 98.89%

Key Study Findings

- Mortality: one female at 1000 mg/kg euthanized *in extremis* on Day 19 (noted with prior to necropsy clinical signs including decreased activity, yellow discolored hair in the anogenital region, red material around eye and mouth, and salivation)
- Clinical signs: salivation, red material around mouth/nose (males), sparse hair, and hunched posture (males) at ≥300 mg/kg
- Body weights: lower mean body weights (up to 10%) and body weight gains (up to 90%) in males and females (up to 69%) at 1000 mg/kg compared to controls throughout the treatment period
- Altered reproductive parameters at 1000 mg/kg (outside the historical background range):
  - Longer mean estrous cycle length and number of estrous cycles
  - Lower number of females with viable embryos
  - Lower pregnancy and fertility/fecundity indices
  - Decreased number of males impregnating a female and decreased copulatory interval
  - No or decreased sperm motility or increased abnormal sperm
- NOAEL=300 mg/kg for reproductive performance, fertility, and parental toxicity (~1-fold for males and ~12-fold for females the AUC at MRHD)

Methods

Doses:	0, 100, 300, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% HCO-60
Species/Strain:	Rat/CD® [Crl:CD®(SD)] from (b) (4)
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	The vehicle or test article was administered 28 days prior to pairing (1:1 for the maximum mating period of 21 days) and continued until euthanasia for males (64-70 days), and14 days prior to pairing through mating to gestation days (GD) 7 for females (29-43 days). Females were examined daily by vaginal lavage to establish estrous cyclicity. Positive evidence of copulation (GD 0) was established by daily inspection for a copulatory plug or vaginal smear for sperm.
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

- One female at 1000 mg/kg euthanized *in extremis* in on treatment day 19 with unknown cause of morbidity; clinical signs noted prior to sacrifice including decreased activity, yellow discolored hair in the anogenital region, red material around eye and mouth and salivation

Clinical Signs: Twice daily for cageside observations; & daily during treatment (& GD 13 for mated females) for detailed examination

- Salivation, red material around mouth and nose, sparse hair, and hunched posture in males at ≥MD
- Salivation in HD females during the gestation period

Body Weight: Twice weekly during treatment until euthanasia for males; twice weekly prior to and during cohabitation for females; GD 0, 4, 7, 10, and 13 for mated females

- Lower mean body weights (up to 10%) and body weight gains (up to 90%) in HD males compared to controls throughout the treatment period
- Lower mean body weights (up to 9%) and weight gains (up to 69%) in HD females compared to controls during premating days and gestation days

Feed Consumption: Weekly

- Decrease in food intake during premating days 1-8 (up to 13%) and GD 0-4 (up to 20%) in HD females compared to controls

Toxicokinetics: At 0.5, 1, 2, 4, 7, 12, and 24 hours post-dose on Days 1 & 14 for 300 and 1000 mg/kg for males in a bridging study (non-GLP)

- More than dose-proportional increase in AUC and C_{max} for avanafil and M4 at 300 and 1000 mg/kg tested

Dosing Solution Analysis: Samples were collected from the LD and HD formulations during Week 1 and 3 for homogeneity analysis. Samples (5 mL each) of the prepared formulations mixed for Week 1 were stored refrigerated for up to 21 days, and were collected after 7 and 21 days for the stability analysis. Samples of the dosing formulations at each concentration were also collected during Weeks 1, 3 and 10, and stored refrigerated for concentration analysis.

- Homogeneous for dosing formulations prepared in Weeks 1 and 3 with mean recovery of 97% and 107%
- Stable for 7 and 21 days with mean recovery between 95.3% and 98.7%
- Mean recovery between 90.3% and 110.3% for concentrations of 0, 10, 30, and 100 mg/mL for Weeks 1, 3 and 10

Necropsy: GD 13

- Unremarkable

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.): GD 13

- Longer mean estrous cycle length (~24%) and lower mean number of estrous cycles (~24%) in HD females compared to controls
- Decreased number of females with viable embryos (21 out of 25), decreased number of pregnant females (21 out of 25), decreased pregnancy and fertility/fecundity indices (84%) at HD compared to controls (outside the historical background range)
- Decreased number of males impregnating a female (21 out of 25) at HD compared to controls
- Reduced copulatory interval (~32%) in HD males compared to controls
- Increased pre-implantation loss at HD
- Sperm parameters:
    - No (5 out of 25) or decreased (mean 50.8%) sperm motility at HD compared to the mean historical control (90.1%) or concurrent control (85.8%)
    - Lower total sperm concentration (~10%) compared to concurrent controls at HD (within the range of historical control data)
    - Increased abnormal sperm (mostly separation of the sperm tail from the head) at HD (mean 36.58%) compared to concurrent controls (6.42%) or the mean historical controls (3.14%)

The following table summarizes the noteworthy observations made in the fertility and early embryonic study in rats.

Dose, mg/kg / Observations	0		100		300		1000	
	25M	25F	25M	25F	25M	25F	25M	25F
Mortality, day 19								1[#]
Clinical signs, (GD)								
Salivation					9	2(1)	19	1(9)
Material around mouth/nose, red			1		2		4	
Posture, hunched					2		5	
Hair sparse, abdominal/forefoot/hind limb/lumbar	4		8	1(1)	5	3(2)	9	5(3)
Body weights, g								
Premating, day 12	325.5	216.2	324.6	215.6	324.7	215.0	**315.4**	**208.8**
day 26	391.0	-	389.9	-	390.1	-	**370.3***	-
Pairing, day 47	451.2	-	**454.4**	-	**449.2**	-	**423.2**	-
Postmating, day 68	500.9	-	491.1	-	501.7	-	**452.2****	-
Gestation, day 4	-	252.5	-	251.5	-	252.9	-	**230.8****
Body weight gains, g								
Premating, days 5-8	15.5	4.9	14.4	5.2	16.4	5.7	**13.6**	**1.5***
days 1-15	80.2	29.0	78.5	30.0	79.6	29.9	**70.5**	**21.7***
days 1-29	137.4	-	138.2	-	135.8	-	**111.7***	-
Pairing, days 47-50	7.7	-	5.5	-	9.4	-	**0.9****	-
Postmating, days 64-68	9.6	-	**7.9**	-	**7.1**	-	**2.5****	-
Gestation, day 0-4	-	26.7	-	**24.1**	-	**22.6***	-	**11.9****
Food consumption, g/animal/day								
Premating, days 1-8	-	17.1	-	17.2	-	16.8	-	**14.9****
Gestation, days 0-4	-	22.5	-	22.4	-	22.5	-	**17.9****
Reproductive parameters,								
Estrous cycling, mean cycle length, days	-	4.5	-	4.6	-	4.7	-	**5.6****
number of cycles	-	2.1	-	2.2	-	2.2	-	**1.6***
Number of pregnant females	-	25	-	24	-	25	-	**21**
Fertility index, %	100	100	96	96	100	100	**84**	**84**
Fecundity index, %	100	100	96	96	100	100	**84**	**84**
Pregnancy index, %	-	100	-	96	-	100	-	**84**
Number of males impregnating a female	25	-	24	-	25	-	**21**	-
Number of females with viable embryos	-	25	-	24	-	25	-	**21**
Copulatory interval, days	3.1	-	3.6	-	4.6	-	**2.4**	-
Preimplantation loss, %	-	6.27	-	4.04	-	6.85	-	**10.30**
Sperm analysis,								
Sperm motility, %	85.8	-	86.8	-	85.8	-	**50.8****	-
Total sperm concentration/cauda epididymis x 10^8	3.595	-	3.536	-	3.644	-	**3.236**	-
Sperm concentration/g cauda epididymis x 10^8	12.5	-	12.446	-	12.356	-	**11.732**	-
Abnormal sperm, %	6.42	-	4.74	-	5.38	-	**36.58****	-

[#]Euthanized *in extremis* with unknown cause (clinical signs prior to necropsy including decreased activity, yellow discolored hair in the anogenital region, red material around eye and mouth and salivation)
-; not available
Significantly different from controls at $p=0.05^{*}$ or $p=0.01^{**}$

The following table summarizes the TK parameters assessed in a 2-week bridging study in male rats (3/timepoint/group).

Toxicokinetics@ \ Dose, mg/kg		0		100		300		1000	
		25M	25F	25M	25F	25M	25F	25M	25F
Avanafil									
AUC_{0-24hr}, ng·hr/mL	Day 1	-	-	-	-	3900	-	33200	-
	Day 14	-	-	-	-	4570	-	85000	-
C_{max}, ng/mL	Day 1	-	-	-	-	1250	-	2420	-
	Day 14	-	-	-	-	1210	-	5920	-
T_{max}, hr	Day 1	-	-	-	-	0.5	-	7	-
	Day 14	-	-	-	-	0.5	-	7	-
$t_{1/2}$, hr	Day 1	-	-	-	-	nc	-	nc	-
	Day 14	-	-	-	-	nc	-	nc	-
M4									
AUC_{0-24hr}, ng·hr/mL	Day 1	-	-	-	-	14200	-	162000	-
	Day 14	-	-	-	-	14300	-	218000	-
C_{max}, ng/mL	Day 1	-	-	-	-	2960	-	12800	-
	Day 14	-	-	-	-	2770	-	15200	-
T_{max}, hr	Day 1	-	-	-	-	0.5	-	7	-
	Day 14	-	-	-	-	7	-	7	-
$t_{1/2}$, hr	Day 1	-	-	-	-	nc	-	nc	-
	Day 14	-	-	-	-	nc	-	nc	-
M16									
AUC_{0-24hr}, ng·hr/mL	Day 1	-	-	-	-	715	-	2400	-
	Day 14	-	-	-	-	904	-	7300	-
C_{max}, ng/mL	Day 1	-	-	-	-	246	-	289	-
	Day 14	-	-	-	-	243	-	770	-
T_{max}, hr	Day 1	-	-	-	-	0.5	-	0.5	-
	Day 14	-	-	-	-	0.5	-	0.5	-
$t_{1/2}$, hr	Day 1	-	-	-	-	nc	-	3.82	-
	Day 14	-	-	-	-	nc	-	10.8	-

@AUC_{0-t} = AUC_{0-7} for all values for 300 mg/kg/day due to BLQ at 24 hr; AUC_{0-t} = AUC_{0-24} for all values for 1,000 mg/kg/day
LLOQ=20 ng/mL for avanafil; LLOQ=5 ng/mL for M4 & M16
nc = no reliable value could be calculated for $t_{1/2}$
-; not available

Study title: TA-1790: An Oral Study of Reversibility of Effects on Fertility and Early Embryonic Development in Male Rats

Study no.:	1060-050
Study report location:	Module 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 27, 2010 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, #17AP303020, 98.8%

Key Study Findings

- Mortality: one male at 1000 mg/kg euthanized *in extremis* on Day 22 (noted with a left hind limb that was swollen with impaired function and discolored purple on Day 20)
- Clinical signs: salivation in treated animals (one male in both main and recovery period)

- Body weights: lower mean body weights (3-9%) and body weight gains (up to 15% on Days 1-64) compared to controls throughout the treatment and recovery period (partial recovery for body weights and full recovery for body weight gains)
- Altered sperm parameters at 1000 mg/kg/day:
  - No (2 out of 10) or lower sperm motility compared to concurrent control or historical control data; reversible at the end of recovery period
  - Altered sperm morphology (broken sperm with detached heads) compared to concurrent control or historical control data; reversible at the end of recovery period

Methods

Doses:	0, 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% HCO-60
Species/Strain:	Rat/CD® [Crl:CD®(SD)] from (b) (4)
Number/Sex/Group:	10 males/group; 32 females/group
Satellite groups:	22 males/group
Study design:	The vehicle or test article was administered 28 days prior to pairing for males and continued through the mating (14 days for the 1st mating & 7 days for the 2nd mating for females with no evidence of mating) and post-mating period until euthanasia in total of 9 weeks.
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

- One male at 1000 mg/kg euthanized *in extremis* on Day 22; left hind limb that was swollen with impaired function and discolored purple on Day 20

Clinical Signs: Once daily for detailed examination; weekly during the recovery period

- Salivation (one male in both main and recovery period)

Body Weight: Day 1 & twice weekly at 3-4 day intervals during 9-week treatment period; once weekly during 9-week recovery period

- Lower mean body weights (3-9%) and body weight gains (up to 15% on Days 1-64) compared to controls throughout the treatment and recovery period (partial recovery for body weights and full recovery for body weight gains)

Feed Consumption: Weekly prior to dosing & after completion of mating period until euthanasia

- Unremarkable

Estrous Cycle: Daily for 2 days prior to pairing beginning on Day -1 until evidence of copulation

- Unremarkable

Necropsy: GD 13

- Unremarkable

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.): GD 13

- 93.5% (2 nonpregnant) fertility or fecundity indices within the range (84-100%; average 94.2%) of historical control data from the conducting laboratory

Sperm analysis: At necropsy & end of recovery period

- No (2 out of 10) or lower (average 73.8%) sperm motility compared to concurrent controls (average 94.5%; 90-99%) or historical control data (average 90.1%; 81.4-97.5%); reversible at the end of recovery period
- Altered sperm morphology (broken sperm with detached heads) with a mean of 26% compared to concurrent controls (average 3.35%; 1.5-7.5%) or historical control data (average 3.14%; 0.6-6.3%); reversible at the end of recovery period

The following table summarizes the noteworthy observations made in the reversibility study in rats.

Dose, mg/kg / Observations	0		1000	
	32M	32F	32M	32F
Mortality, day 22			1[#]	
Clinical signs, (recovery)				
Salivation			21 (1)	-
Hair sparse, abdominal/anogenital	1	-	4	-
Body weights, g				
Premating, day 4	294.6	-	**285.1****	-
day 25	400.1	-	**379.1****	-
Pairing, day 29	416.2	-	**395.5***	-
day 39	451.5	-	**421.6****	-
Postmating, day 43	460.3	-	**427.1****	-
day 71	537.9	-	**487.3****	-
day 126	615.4	-	**590.2**	-
Body weight gains, g				
Premating, days 1-4	21.0	-	**13.2****	-
days 1-29	142.5	-	**124.1***	-
Pairing, days 29-32	6.3	-	**0.7****	-
days 39-43	8.8	-	**5.5***	-
Postmating, days 46-50	13.3	-	**8.7****	-
days 1-64	247.3	-	**211.5****	-
days 64-126	89.5	-	**114.0****	-
Food consumption, g/animal/day				
Premating, days 1-8	28.9	-	**26.8****	-
Gross pathology				
Thymus, focus/foci, mild/moderate		-	2	-
Reproductive parameters,				
Fertility index, %	100	-	93.5	-
Fecundity index, %	100	-	93.5	-
Number of females not pregnant	-	0	-	3
Number of females pregnant	-	32	-	30
Number of females with viable embryos	-	31	-	28
Number of pregnant females with confirmed mating date	-	1	-	2
Corpora lutea, number/animal	-	15.2	-	14.0*
Preimplantation loss, %/animal	-	8.36	-	4.38
Sperm analysis, n=10/group				
Sperm motility, %	94.5	-	**73.8**	-
Total sperm concentration/cauda epididymis x 10^8	3.204	-	2.898	-
Sperm concentration/g cauda epididymis x 10^8	10.059	-	9.652	-
Abnormal sperm, %	3.35	-	**26.00***	-

[#]Euthanized *in extremis* in one male at 1000 mg/kg on day 22 (noted with a left hind limb that was swollen with impaired function and discolored purple on Day 20)
Numbers in parentheses represent animals with effects at the end of recovery period.
-; not available
Significantly different from controls at p=0.05* or p=0.01**

Dosing Solution Analysis: Samples (1 mL each) were collected during Week 1 for homogeneity (1000 mg/kg) and concentration (0 and 1000 mg/kg) analyses. Stability when stored refrigerated (2 to 8°C) was not done because the result at the concentration used on study for 14 days has been established previously.

- Homogeneous with mean recovery of 100.5%
- Mean recovery between 98.6% and 100.9%

9.2 Embryonic Fetal Development

Study title: Oral Embryo-Fetal Toxicity Study in Rats

Study no.:	1060-011
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 20, 2006 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP303020, 98.89%

Key Study Findings

Maternal toxicity:

- Mortality:
  - Found dead: 1 female at 100 mg/kg on Day 14, and 3 females at 1000 mg/kg on Days 8-10
  - Euthanized *in extremis*: 3 females at 1000 mg/kg on Days 8-12; clinical signs noted were decreased activity, lacrimation, agonal changes including ataxia, prostration, tremors, low carriage, red/black material around eyes/mouth, skin cold to touch, eyelid partially/completely closed, and difficult breathing before death
- Clinical signs: decreased activity, salivation, prostration, vulva discharge, sparse hair, difficult breathing, and lacrimation at 1000 mg/kg
- Body weights: lower mean body weights (up to 11% on GD 20) and body weight gains (up to 90% on GD 6-9) associated with reduced food intake at ≥300 mg/kg compared to controls during the entire treatment period
- Gross pathology: red/black foci in the glandular/nonglandular stomach, green/red fluid in the urinary bladder in morbid dams or dams euthanized *in extremis* at 1000 mg/kg
- Cesarean section: lower gravid uterine weights (~10%), adjusted maternal body weights (~9%), and body weight gains (~34%) at 1000 mg/kg compared to controls
- NOAEL=300 mg/kg for maternal toxicity (~8-fold the AUC at MRHD)

Fetal toxicity:

- Cesarean section: total (early) litter resorption in 1 female that was found dead and 1 surviving female pregnant by stain at 1000 mg/kg
- Fetal weights: lower fetal body weights (~12%) at 1000 mg/kg compared to controls
- NOAEL=300 mg/kg for developmental toxicity (~8-fold the AUC at MRHD)

Methods

Doses:	0, 100, 300, 1000 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg/dose
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% HCO-60 (Formulation A)
Species/Strain:	Rat/CD® [Crl:CD®(SD)] from (b) (4)
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	The vehicle or test article was administered to the time-mated females daily from GD 6 to 17.
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

- Found dead:
  - 3 HD females on Days 8, 9 and 10
  - 1 LD female on Days 14
  - 1 Control female on Day 13 due to dosing error
- Euthanized *in extremis*:
  - 3 HD females on Days 8, 9 and 12
  - Clinical signs noted prior to sacrifice included decreased activity, lacrimation, agonal changes including ataxia, prostration, tremors, low carriage, red/black material around eyes/mouth, skin cold to touch, eyelid partially/completely closed and difficult breathing before death

Clinical Signs: Twice daily for cageside observations; daily from GD 6 to GD 20 for detailed observations

- Agonal changes including ataxia, prostration, tremors, low carriage, red/black material around eyes/mouth, skin cold to touch, eyelid partially/completely closed, and difficult breathing in animals dying or euthanized *in extremis* at HD
- Decreased activity, salivation, prostration, vulva discharge, sparse hair, difficult breathing, and lacrimation in surviving animals at HD

Body Weight: GD 0, 6, 9, 12, 15, 18, & 20

- Lower body weights (up to 11% on GD 20) and body weight gains (up to 90% on GD 6-9) at ≥MD compared to controls during the entire treatment period

Feed Consumption: GD 0, 6, 9, 12, 15, 18, & 20

- Lower food intake at ≥MD compared to controls during the entire treatment period and from GD 0 to 20 (15%)

Toxicokinetics: 0.5, 1, 2, 4, 7 & 24 hours post-dose on GD 6 and 17 for 100 and 300 mg/kg in a bridging study (non-GLP)

- Dose-proportional increase in AUC and C_{max} for avanafil, M4 and M16 at 100 and 300 mg/kg
- 1.5-2 fold increase in AUC for avanafil, M4 and M16 with multiple dosing at 300 mg/kg

Necropsy: GD 20

- Red/black foci in the glandular/nonglandular stomach, green/red fluid in the urinary bladder in animals dying or euthanized *in extremis*

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): GD 20

- Total (early) resorptions in 1 HD female that found dead, and in 1 HD surviving female pregnant by stain
- Lower gravid uterine weights (~10%), adjusted body weights (~9%) and body weight gains (~34%) at HD compared to controls

Offspring (Malformations, Variations, etc.): GD 20

- Lower fetal body weights (~12%) at HD compared to controls
- Increased rudimentary rib skeletal variation at HD (66.7% compared to 29.2% in concurrent controls or 60.9% in historical background data)

The following table summarizes the noteworthy observations made in pregnant rats.

Dose, mg/kg Observations	0 25F	100 25F	300 25F	1000 25F
Mortality[#], GD 8-14	1	1		6
Clinical signs,				
Decreased activity				5
Ataxia				1
Prostration				2
Salivation				2
Tremors				1
Low carriage				1
Discharge, vulva, red/brown				2
Lacrimation, eye, left/right				4
Material around eyes, left/right, red				2
mouth, red				1
nose, black/red				4
Eyelid partially/completely closed				1
Hair discolored, yellow, anogenital		1	5	1
sparse, abdominal/forelimb/hindlimb/lumbar/sacral	1	1	2	4
Cold to touch, skin				1
Breathing difficult				2
Body weights, GD 20, g	364.8	366.4	358.3	**329.4****
Body weight gains, g				
GD 6-9	10.0	10.0	**6.5**	**-1.0****
GD 9-12	13.7	12.2	12.3	**6.5****
GD 12-15	20.5	21.3	19.9	**9.2***
GD 15-18	33.1	34.4	32.1	**24.2***
GD 6-18	77.4	77.8	**70.8**	**39.4****
GD 6-20	111.1	112.1	**103.6**	**73.2****
GD 0-20	154.4	155.3	**147.3**	**114.9****
Food consumption, g/animal/day,				
GD 6-9	22.09	**21.41**	**20.43**	**14.98****
GD 9-12	24.03	**23.16**	**23.09**	**17.61****
GD 12-15	24.15	24.24	**22.53**	**16.43****
GD 15-18	27.07	27.15	**26.39**	**23.44***
GD 6-18	24.33	**24.11**	**23.11**	**18.11****
GD 6-20	24.63	**24.48**	**23.61**	**19.59****
GD 0-20	23.28	**23.03**	**22.39**	**19.68****
Macroscopic findings,				
Stomach, glandular, foci, black/red, mild/moderate				1
thickened, mild				1
nonglandular, foci, black				1
Urinary bladder, fluid, green/red, minimal/mild				5
Cesarean section, GD 20				
Number of females with viable fetuses	24	24	25	**18**
pregnant by stain				**1**
with all resorptions				**1**
Gravid uterine weights, g	67.3	69.7	66.5	**60.7**
Final body weights, g	364.8	366.4	358.3	**331.9**
Adjusted body weights, g	297.6	296.7	291.8	**271.2****
Adjusted weight gains, g	87.1	**85.7**	**80.8**	**57.8****

[#]Found dead in 1 control female on day 13 (dosing error); found dead in 1 female at 100 mg/kg on day 14 & 3 females at 1000 mg/kg on days 8, 9 and 10; euthanized *in extremis* in 3 females on days 8, 9 and 12 with unknown cause of deaths
-; not available
Significantly different from controls at p=0.05* or p=0.01**

The following table summarizes the TK parameters assessed in a bridging study, in which avanafil was administered to female rats from GD 6 to 17 (3/timepoint/group).

Toxicokinetics[@] \ Dose, mg/kg		0 25F	100 25F	300 25F	1000 25F
Avanafil					
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	12700	40400	-
	GD 17	-	10200	63000	-
C_{max}, ng/mL	GD 6	-	5220	12000	-
	GD 17	-	3450	7050	-
T_{max}, hr	GD 6	-	1	0.5	-
	GD 17	-	0.5	4	-
$t_{1/2}$, hr	GD 6	-	1.99	nc	-
	GD 17	-	nc	3.13	-
M4					
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	1610	4060	-
	GD 17	-	1370	8470	-
C_{max}, ng/mL	GD 6	-	585	926	-
	GD 17	-	382	898	-
T_{max}, hr	GD 6	-	1	0.5	-
	GD 17	-	1	4	-
$t_{1/2}$, hr	GD 6	-	nc	nc	-
	GD 17	-	nc	3.65	-
M16					
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	348	864	-
	GD 17	-	315	1400	-
C_{max}, ng/mL	GD 6	-	147	378	-
	GD 17	-	115	138	-
T_{max}, hr	GD 6	-	1	0.5	-
	GD 17	-	1	1	-
$t_{1/2}$, hr	GD 6	-	nc	nc	-
	GD 17	-	nc	3.49	-

[@]$AUC_{0\text{-}t}$ = $AUC_{0\text{-}7}$ for all values for GD 6 & 17 for 300 mg/kg/day & for GD 6 for 300 mg/kg/day due to BLQ at 24 hr; $AUC_{0\text{-}t}$=$AUC_{0\text{-}24}$ for all values for GD 17 or 300 mg/kg/day (LLOQ=20 ng/mL for avanafil; LLOQ=5 ng/mL for M4 & M16)
nc = no reliable value could be calculated for $t_{1/2}$
-; not available

The following table summarizes the noteworthy observations made in rat fetuses.

Observations \ Dose, mg/kg	0 24M	0 24F	100 24M	100 24F	300 25M	300 24F	1000 18M	1000 18F
Body weights, litter, g	4.14	3.90	4.02	3.79	4.11	3.92	**3.67****	**3.42****
Malformations (M)/variations (V), litter	n=24		n=24		n=25		n=18	
Visceral, (M) thoracic cavity, aortic arch, right sided							1 (5.6)	
innominate artery, absent							1 (5.6)	
(V) thoracic cavity, aortic arch, retroesophageal							1 (5.6)	
Skeletal (V) ribs, rudimentary, #litters (%)	7 (29.2)		11 (45.8)		12 (48.0)		12 (66.7)	
(V) sternum, (V) entire, not ossified							1 (5.6)	
(V) cervical vertebra, neural arch, incomplete ossified							1 (5.6)	

Significantly different from controls at p=0.01**

Dosing Solution Analysis: Samples at LD and HD for the GD 2 dosing formulations were collected prior to initiation of drug administration for homogeneity analysis. Samples (5 mL each) of the prepared formulations mixed for Week 1 were stored refrigerated for up to 21 days, and were collected after 7 and 21 days for stability

analysis. Samples of the dosing formulations at each concentration were also collected from the 1st and last weeks, & stored refrigerated for concentration analysis.

- Homogeneous for dosing formulations prepared with mean recovery of 95.3% and 102.4%
- Stable for 7 and 21 days with mean recovery between 95.3% and 98.7%
- Mean recovery between 90.3% and 110.3% for concentrations of 0, 10, 30, and 100 mg/mL for Weeks 1 and 2

Study title: An Oral Range-Finding Study for Effects on Embryo-Fetal Development in Rabbits

Study no.:	1060-009
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/2/2006 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP303020, 98.89%

Key Study Findings

Maternal toxicity:

- Mortality:
  - Found dead: 2 females at 500 mg/kg on GD 17 and 18, and 3 females at 1000 mg/kg on GD 14, 19 and 20; unknown cause of deaths
  - Abortion: 1 female at 500 mg/kg that aborted on GD 27 was sacrificed
- Clinical signs: few/absent feces and sparse hair at ≥125 mg/kg, thin appearance at ≥250 mg/kg, decreased activity and red material in the pan beneath the cage and at ≥500 mg/kg
- Body weights: dose-related decrease in mean body weights (up to 21%) and body weight gains (up to 98%) associated with reduced food intake (up to 76%) during treatment and post-treatment interval at ≥125 mg/kg
- Cesarean section:
  - Decreased pregnancy index at ≥500 mg/kg
  - Decreased mean gravid uterine weights (up to 39%) at ≥500 mg/kg;
  - Dose-dependent decrease in adjusted body weights (up to 9%) and body weight gains (up to 11%) at 125-500 mg/kg

Fetal toxicity:

- Cesarean section:
  - Increased post-implantation loss and total number of resorptions at ≥500 mg/kg
  - Total littler (early) resorptions in 2 females at 1000 mg/kg/day
  - Decreased litter size and viable fetuses at 1000 mg/kg/day
- Fetal weights: lower mean fetal litter weights (up uo 19%) at all doses compared to controls

Methods

Doses:	0, 125, 250, 500, 1000 mg/kg
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg/dose
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% HCO-60 (Formulation A)
Species/Strain:	New Zealand White Rabbit/Hra:(NZW)SPF from (b) (4)
Number/Sex/Group:	6 Time-mated females/group
Satellite groups:	None
Study design:	The vehicle or test article was administered to the time-mated females daily from GD 6 to 18.
Deviation from study protocol:	Not significant

The following table summarizes the noteworthy observations made in the dose range-finding embryo-fetal toxicity study in rabbits.

Dams:

Observations \ Dose, mg/kg	0 6F	125 6F	250 6F	500 6F	1000 6F
Mortality[#], found dead, GD 14-20				2	3
abortion, GD 27				1	
Clinical signs,					
Decreased activity				1	2
Feces, few/absent/mucoid/soft		1	4	6	6
Material in pan/bedding, red				4	3
Hair, discolored, red, anogenital/hind limb				1	1
sparse, anogenital/forelimb/hindlimb/axillary/inguinal		1	1	1	3
Thin, appearance			1	5	4
Skin, cold to touch					1
Body weights, kg					
GD 21	3.56	**3.477**	**3.332**	**3.05***	**2.94****
29	3.637	**3.62**	**3.505**	**3.345**	**3.263**
Body weight gains, kg					
GD 6-19	0.165	**0.063**	**-0.108****	**-0.473****	**-0.607****
0-29	0.418	**0.393**	**0.283**	**0.045**	**-0.01**
Food consumption, g/animal/day					
GD 6-19	137.4	**122.6**	**80.6****	**26.3****	**30.7****
0-29	130.0	**120.2**	**103.8**	**72.6****	**98.2**
Macroscopic findings,					
Skin, subacute, hemorrhage, minimal		1	1	1	1
Lung, discoloration, red, moderate					1
Stomach, foci, red, minimal					1
Vagina, fluid, black, mild					1
Cesarean section, GD 29	n=6	n=6	n=6	n=2	n=1
Number of not pregnant				1	2
Pregnancy index, %	100	100	100	83.3	66.7
Number of died pregnant				2	1
abortions				1	
females with all resorptions					2
females with viable fetuses	6	6	6	2	1
Corpora lutea, number/animal	9.3	9.7	**10.2**	**10.5**	**11.0**
Preimplantation loss, %/animal	12.27	1.67	10.66	4.17	**54.55**
Viable fetuses, number/animal	8.2	9.3	9.2	9.0	**1.3***
Postimplantation loss, % implants/animal	3.18	1.52	1.52	**9.09**	**73.33**
Litter size, number/animal	8.2	9.3	9.2	9.0	**1.3***
Resorptions, total, number/animal	0.3	0.2	0.2	**1.0**	**5.7**
early, number/animal	0.2	0.0	0.0	0.0	**5.7**
late, number/animal	0.2	0.2	0.2	**1.0**	0.0
Gravid uterine weight, kg	0.467	0.514	0.492	**0.451**	**0.283**
Final body weight, kg	3.637	**3.62**	**3.505**	**3.345**	**3.43**
Adjusted final body weight, kg	3.169	**3.106**	**3.013**	**2.894**	3.147
Adjusted weight change, kg	-0.049	**-0.12**	**-0.208**	**-0.406**	-0.043

[#]Found dead in 2 females at 500 mg/kg on GD 17 & 18, and 3 females at 1000 mg/kg on GD 14, 19 & 20; sacrificed in 1 female that aborted at 500 mg/kg on GD 27

Significantly different from controls at p=0.05* or p=0.01**

Fetuses:

Observations \ Dose, mg/kg	0 6F	125 5F	250 6F	500 2F	1000 1F
Body weights, litter, g	42.57	**39.21**	**39.93**	**34.53**	47.28

Study title: Study for Effects on Embryo-Fetal Development in Rabbits

Study no.:	1060-010
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 8, 2006 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP303020, 98.89%

Key Study Findings

Maternal toxicity:

- Abortion: 1 female at 240 mg/kg aborted on GD 24 and was sacrificed
- Clinical signs: increased incidence of few/absent/mucoid/soft feces in treated groups
- Body weights: lower mean body weights (up to 5%) and body weight gains (up to 92% on GD 13-16) associated with lower food intake at 240 mg/kg compared controls
- NOAEL=120 mg/kg for maternal toxicity (~2-fold the AUC at MRHD)

Fetal toxicity:

- Cesarean section: Increased postimplantation loss and total number of resorptions (total early resorptions in 1 female at 60 mg/kg/day and late resoprtions in 1 female at 240 mg/kg/day) resulting in a reduced number of viable fetuses
- NOAEL=120 mg/kg for developmental toxicity (~2-fold the AUC at MRHD)

Methods

Doses:	0, 30, 60, 120, 240 mg/kg
Frequency of dosing:	Once daily
Dose volume:	4 mL/kg/dose
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% HCO-60 (Formulation A)
Species/Strain:	New Zealand White Rabbit/Hra:(NZW)SPF from (b) (4)
Number/Sex/Group:	23 Time-mated females/group
Satellite groups:	None
Study design:	The vehicle or test article was administered to the time-mated females daily from GD 6 to 18.
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

- Sacrificed in 1 female at 240 mg/kg that aborted on GD 24

Clinical Signs: Twice daily for cageside observations; daily from GD 6 to GD 29 for detailed observations

- Increased incidence of abnormal feces at ≥30 mg/kg
- Difficult breathing in one HD female

Body Weight: GD 0, 6, 10, 13, 16, 19, 21, 25 & 29

- Lower mean body weights from GD 10 throughout the study (up to 5%) and lower body weight gains on GD 6-10, 10-13, 13-16, 16-19, and 6-19 compared to controls at 240 mg/kg (up to 92% on GD 13-16)

Feed Consumption: GD 0, 6, 10, 13, 16, 19, 21, 25, & 29

- Lower mean food intake (up to 35% on GD 13-16) compared to controls at 240 mg/kg during GD intervals 6-10, 10-13, 13-16, 16-19, and 6-19

Toxicokinetics: at 0.5, 1, 2, 4, 7, 12, & 24 hr post-dose on GD 6 & GD 18 for 120 and 240 mg/kg (non-GLP) in a bridging study

- More than dose-proportional increase in AUC and C_{max} at 120 and 240 mg/kg
- Minimal accumulation for avanafil, M4, and M16 with multiple dosing

Necropsy: GD 29

- Unremarkable

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): GD 29

- Total litter resorptions: early resorptions in 1 female at 60 mg/kg (within historical control background range) and late resorptions in 1 female at 240 mg/kg/day (above historical control background range)
- Increased postimplantation loss at 240 mg/kg/day (within historical control background range)
- Decreased viable fetuses and litter size at 240 mg/kg/day (within historical control background range)

Offspring (Malformations, Variations, etc.): GD 29

- Decrease in mean fetal weights (~7%) for males at 240 mg/kg compared to controls due to one fetus (#306)
- External malformations in a single fetus (#6) from a single litter (#307) at 240 mg/kg/day included anencephaly (absent head), anophthalmia (absent eyes), agnathia (absent jaws) and aglossia (absent tongue) (not listed in historical control data provided from the conducting laboratory)

- Increased visceral variations of extra subclavian artery in thoracic cavity at ≥60 mg/kg/day (not listed in historical control data provided from the conducting laboratory)

The following table summarizes the noteworthy observations made in pregnant rabbits.

Dose, mg/kg / Observations	0 23F	30 23F	60 23F	120 23F	240 23F
Mortality[#], GD 24/26	1				1
Clinical signs,					
Feces, few/absent/mucoid/soft	3	4	5	7	9
Material in pan/bedding, red			1		2
Thin appearance			1		1
Hair discolored, red, anogenital/tail					1
Breathing difficult					1
Body weights, kg					
GD 10	3.525	3.516	3.497	3.497	**3.480**
13	3.572	3.570	3.534	3.553	**3.492**
16	3.620	3.611	3.563	3.602	**3.496**
19	3.675	3.638	3.590	3.615	**3.473***
21	3.712	3.682	3.640	3.668	**3.520**
25	3.777	3.730	3.705	3.763	**3.676**
29	3.809	3.770	3.743	3.827	**3.739**
Body weight gains, kg					
GD 6-10	0.045	**0.036**	**-0.01**	**-0.030***	**-0.060****
10-13	0.047	0.054	0.038	0.056	**0.011**
13-16	0.048	0.041	0.029	0.049	**0.004**
16-19	0.054	**0.027**	**0.027**	**0.013**	**-0.023****
6-19	0.195	**0.159**	**0.084**	**0.088**	**-0.068****
0-29	0.472	0.397	0.398	0.487	**0.358**
Food consumption, g/animal/day					
GD 6-10	147.3	142.8	144.5	141.4	**123.3***
10-13	132.8	132.8	**131.3**	**122.0**	**97.7***
13-16	120.7	**115.7**	**114.1**	**110.0**	**78.2***
16-19	143.2	**123.8**	**122.1**	**116.4**	**81.7****
19-21	136.0	128.6	131.4	130.3	**105.3**
6-19	136.9	**129.8**	**129.3**	**123.9**	**97.4****
0-29	126.5	122.7	124.7	**122.5**	**115.8**
Macroscopic findings,					
Cavity, thoracic, adhesion/edema/red fluid/foreign material		1			3
Heart, fluid, clear, moderate					1
Skin, subcutis, foreign material, moderate					2
Uterus/Cervix, fluid, red, moderate					1
Cesarean section, GD 29					
Number of abortions					1
Viable fetuses, number/animal	8.5	8.9	7.7	9.4	**7.0**
Postimplantation loss, % implants/animal	2.76	5.01	11.41	3.24	**11.89**
Litter size, number/animal	8.5	8.9	7.7	9.4	**7.0**
Resorptions, total, number/animal	0.2	0.5	0.9	0.3	**1.1**
early, number/animal	0.2	0.3	0.5	0.3	0.4
late, number/animal	0.0	0.1	0.4	0.0	**0.7***

[#]Found dead in 1 control female on GD 26 with unknown cause of death; sacrificed in 1 female at 240 mg/kg that aborted on GD 24
Significantly different from controls at p=0.05* or p=0.01**

The following table summarizes the TK parameters assessed in a bridging study, in which avanafil was administered to female rabbits from GD6 to 18 (3/timepoint/group).

Toxicokinetics[@] \ Dose, mg/kg		0 23F	30 23F	60 23F	120 23F	240 23F
Avanafil						
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	-	-	14900	52300
	GD 18	-	-	-	18300	45300
C_{max}, ng/mL	GD 6	-	-	-	6450	14700
	GD 18	-	-	-	7590	10900
T_{max}, hr	GD 6	-	-	-	1.20	1.00
	GD 18	-	-	-	1.00	1.30
$t_{1/2}$, hr	GD 6	-	-	-	1.62	1.32
	GD 18	-	-	-	1.32	2.49
M4						
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	-	-	4590	16100
	GD 18	-	-	-	6740	16300
C_{max}, ng/mL	GD 6	-	-	-	1810	3830
	GD 18	-	-	-	2490	3530
T_{max}, hr	GD 6	-	-	-	1.20	1.20
	GD 18	-	-	-	1.10	1.30
$t_{1/2}$, hr	GD 6	-	-	-	1.84	1.12
	GD 18	-	-	-	1.12	2.69
M16						
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	-	-	20700	76800
	GD 18	-	-	-	23200	53700
C_{max}, ng/mL	GD 6	-	-	-	9690	24100
	GD 18	-	-	-	11300	16400
T_{max}, hr	GD 6	-	-	-	1.00	1.20
	GD 18	-	-	-	0.80	1.30
$t_{1/2}$, hr	GD 6	-	-	-	2.06	2.14
	GD 18	-	-	-	1.82	4.23

[@]LLOQ=20 ng/mL for avanafil; LLOQ=5 ng/mL for M4 & M16
-; not available

The following table summarizes the noteworthy observations made in rabbit fetuses.

Observations \ Dose, mg/kg	0 19M 21F	30 21M 20F	60 21M 22F	120 23M 23F	240 20M 20F
Body weights, litter, g	44.1 42.1	40.8 40.5	42.6 41.4	41.6 40.3	**40.8**** 41.5
Malformations (M)/variations (V), litters (%)	n=21	n=21	n=22	n=23	n=22
External (M) head#, entire, absent					1 (4.5)
(M) eye(s) #, anophthalmia					1 (4.5)
(M) jaws#, agnathia					1 (4.5)
(M) tongue#, absent					1 (4.5)
Visceral, thoracic cavity (V) subclavian artery, extra			1 (4.5)	2 (8.7)	2 (9.1)
(V) ventricle, absent					1 (4.5)
(M) ductus arteriosus, absent					1 (4.5)
constricted					1 (4.5)
Skeletal (M) skull, basisphenoid#, misshapen					1 (4.5)
(M) frontal bone#, absent					1 (4.5)
(M) jugal#, misshapen					1 (4.5)
(M) mandible#, fused					1 (4.5)
(M) misshapen					1 (4.5)
(M) maxilla#, misshapen				1 (4.3)	1 (4.5)
(M) nasal bone#, misshapen				1 (4.3)	1 (4.5)
(M) fused					1 (4.5)
(M) orbital cavity#, absent					1 (4.5)
(M) occipital bone#, absent					1 (4.5)
(M) palatine#, absent					1 (4.5)
(M) parietal bone#, fused					1 (4.5)
(M) misshapen					1 (4.5)
(M) premaxilla#, fused					1 (4.5)
(M) misshapen					1 (4.5)
(M) squamosal#, misshapen					1 (4.5)
(M) tympanic ring#, absent					1 (4.5)
(V) hind limb, talus, not ossified					1 (4.5)
(M) rib, rib, branched		1 (4.8)			1 (4.5)
(V) sternum, sternebra, additional ossification cntr		1 (4.5)		2(8.7)	1 (4.5)
(V) misshapen					1 (4.5)
(M) thoracic vertebra#, neural arch, extra			1 (4.5)		1 (4.5)
(M) misshapen					1 (4.5)

#A single fetus (#6) in a litter (#307) had multiple malformations.
Significantly different from controls at p=0.05* or p=0.01**

Review Comments: It is unclear to the reviewer that the altered caesarian section data at HD are incidental findings despite that the values, except for the late resorption, were within historical background range from the conducting laboratory. Mean male fetal weights were decreased (~7%) at HD compared to controls due to one fetus. Some litters had variations and malformations that were not listed in historical control data provided from the conducting laboratory. One or two litters each at ≥60 mg/kg/day had visceral variations in thoracic cavity (extra subclavian artery). A single fetus from a single litter at HD had multiple external malformations including anencephaly (absent head), anophthalmia (absent eyes), agnathia (absent jaws), aglossia (absent tongue). However, the relationship to drug is unclear, considering that the incidences were low and isolated to one or two fetuses in a single litter.

Dosing Solution Analysis: Samples at 30 and 240 mg/kg/day from Week 1 preparation were collected for homogeneity analysis. Samples (5 mL each) of the prepared formulations mixed during the last week of dosing were stored for 0, 7 and 21

days at room temperature for stability analysis. Samples of the dosing formulations at each concentration were also collected from the 1st and last weeks for concentration analysis.

- Homogeneous for dosing formulations with mean recovery of 94.4% and 99.5%
- Stable for 7 and 21 days with mean recovery between 98.1% and 106.2%
- Mean recovery between 94.7% and 103.0% for concentrations of 0, 7.5, 15, 30 and 60 mg/mL

9.3 Prenatal and Postnatal Development

Study title: Study for Effects on Pre- and Post-natal Development, including Maternal Function in Rats following Oral Administration

Study no.:	1060-035
Study report location:	Module 4.2.3.5.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 13, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP440020, 100.4%

Key Study Findings

F0 females:

- Clinical signs: salivation at ≥300 mg/kg
- Body weights: lower mean body weights (up to 4%) and body weight gains (up to 52% on GD 6-10) and mean food intake (up to 20% on GD 6-10) compared to controls at ≥300 mg/kg during gestation and lactation periods
- NOAEL=300 mg/kg for maternal toxicity (~17-fold the AUC at MRHD)

F1 generation:

- Mortality: 1 F1 female at 600 mg/kg died 14 days into the premating growth period (noted with bilateral enlarged kidneys, moderately distended ureters and a moderately distended urinary bladder at necropsy)
- Fetal weights: lower pup body weights from weaning throughout the start of F1 post-mating at 100 mg/kg/day (up to 5%); from pre-weaning Day 7 throughout F1 post-mating and throughout PND 56 in males (up to 8%) and until PND 35 in females (up to 6%) at 300 mg/kg; and at birth and throughout the study in males (up to 11%) and in females (up to 12%) throughout lactation, at weaning, and through PND 35 at 600 mg/kg/day
- Sexual maturation: delayed preputial separation (1.3 days) or vaginal opening (1.2 days) at 1000 mg/kg/day
- NOAEL= 600 mg/kg for reproductive performance for F0 and F1, and F1 Behavior (~25-fold the AUC at MRHD)
  100 mg/kg for F1 pup growth (~2-fold the AUC at MRHD)

Methods

Doses:	0, 100, 300, 600 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg/dose
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% Cremophor® RH 40 in deionized water (Formulation B)
Species/Strain:	Rat/CD® [Crl:CD®(SD)] from (b) (4)
Number/Sex/Group:	25 time-mated females (P0)/group
Satellite groups:	None
Study design:	Test article or vehicle administration for the P0 females began on GD 6 and continued throughout lactation day (LD) 20. F1 pups were selected at least 80 days of age for breeding.
Deviation from study protocol:	Not significant

Observations and Results

F0 Dams:

Mortality: Twice daily

- None

Clinical Signs: Twice daily for cageside observations; daily from GD 6 to LD 20 for detailed observations

- Salivation at ≥MD
- Increased incidence of red material around nose in females in all treated groups during GD 6-22

Body Weight: GD 0, 6, 10, 14, 17 & 20, & LD 0, 4, 7, 10, 14, 17, & 21

- Lower mean body weights from GD 14 through LD 17 (up to 4%) and body weight gains over GD 6-10 (~52%), GD 17-20 (~9%), GD 6-20 (~15%), GD 0-20 (~10%), LD 0-4 (~45%) and LD 10-14 (~18%) at ≥MD (up to 52%) in comparison to controls

Feed Consumption: GD 0, 6, 10, 14, 17 & 20, & LD 0, 4, 7, 10, 14, 17, & 21

- Lower mean food intake compared to controls at ≥MD over GD 6-10 (~20%), 17-20, 6-20 (~8%) and 0-20 (~6%), and over LD 10-14 (~8%), 14-17 (~12%), 17-21 (~8%) and 0-21 (~7%)

Necropsy: LD 21

- Unremarkable

Uterine Content: Twice daily for signs of parturition

- Increased stillborn pups (2-3.47%/litter) in all treated groups (historical background rate 0-2.34%/litter)

Toxicokinetics: GD 6 and 20 for 100, 300, and 600 mg/kg in a bridging study (non-GLP)

- More than dose-proportional increase in AUC at 100, 300, and 600 mg/kg for avanafil, M4 and M16; less-than dose-proportional increase in C_{max} at 100, 300, and 600 mg/kg
- 1.5-4 Fold increase in AUC and C_{max} (M4 and M16 at 600 mg/kg) for avanafil, M4 and M16 with multiple dosing at 300 and 600 mg/kg

The following table summarizes the noteworthy observations made in F0 dams administered from GD 6 to 18.

F0

Dose, mg/kg Observations	0 25F	100 25F	300 25F	600 25F
Clinical signs, GD 6-22/LD0-20				
Salivation			2/2	6/7
Material around nose, red	0/3	2/2	5/1	5/1
Body weights, g				
GD 20	367.1	370.0	**362.6**	**352.1**
LD 7	311.9	**311.1**	**303.9**	**297.4***
Body weight gains, g				
GD 6-10	18.8	19.2	**15.9**	**9.0****
6-20	115.6	118.6	**110.4**	**98.2****
0-20	159.7	160.8	**154.5**	**143.1****
LD 0-4	14.5	**13.9**	**9.0**	**8.0**
10-14	6.0	2.5	5.6	**4.9**
Food consumption, g/animal/day				
GD 6-10	20.5	**20.3**	**18.9**	**16.5****
17-20	25.5	25.8	**25.7**	**23.7***
6-20	23.1	23.0	**22.5**	**21.2****
0-20	21.6	21.5	**21.2**	**20.4***
LD 10-14	60.1	**58.1**	**57.5**	**55.5***
14-17	64.8	**60.3**	**57.8***	**57.3***
17-21	74.3	**72.3**	**69.1***	**68.4***
0-21	55.3	**54.3**	**52.2**	**51.4***
Parturition,				
Stillborn index, %/litter	0.62	**3.47**	**3.38**	**2.00**

Significantly different from controls at p=0.05 or p=0.01

The following table summarizes the TK parameters assessed in a bridging study, in which avanafil was administered to female rats from GD 6 to 20.

Toxicokinetics[#] \ Dose, mg/kg		0 25F	100 25F	300 25F	600 25F
Avanafil					
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	12600	38900	132000
	GD 20	-	14000	139000	199000
C_{max}, ng/mL	GD 6	-	5940	14200	16800
	GD 20	-	3710	9800	15000
T_{max}, hr	GD 6	-	0.5	1	1
	GD 20	-	1	7	4
$t_{1/2}$, hr	GD 6	-	nc	nc	nc
	GD 20	-	nc	nc	3.42
M4					
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	1440	3760	15500
	GD 20	-	1510	13800	30000
C_{max}, ng/mL	GD 6	-	562	1190	1050
	GD 20	-	388	924	2170
T_{max}, hr	GD 6	-	0.5	1	1
	GD 20	-	1	7	7
$t_{1/2}$, hr	GD 6	-	nc	nc	nc
	GD 120	-	nc	nc	nc
M16					
$AUC_{0\text{-}24hr}$, ng·hr/mL	GD 6	-	418	830	2640
	GD 20	-	445	2070	4590
C_{max}, ng/mL	GD 6	-	199	283	299
	GD 20	-	128	185	434
T_{max}, hr	GD 6	-	0.5	1	1
	GD 20	-	1	1	0.5
$t_{1/2}$, hr	GD 6	-	nc	nc	nc
	GD 20	-	nc	5.46	5.82

[#]$AUC_{0\text{-}t}$ = $AUC_{0\text{-}7}$ for all values for GD & GD20 for 100 mg/kg/day & for GD 6 for 300 mg/kg/day due to BLQ at 24 hr; $AUC_{0\text{-}t}$=$AUC_{0\text{-}24}$ for all values for GD 20 for 300 mg/kg/day & for both days for 600 mg/kg/day (3/timepoint/group)
LLOQ=20 ng/mL for avanafil; LLOQ=5 ng/mL for M4 & M16
nc = no reliable value could be calculated for $t_{1/2}$
-; not available

F1 Generation:

Pup Survival: Daily

- Unremarkable

Pup Clinical Signs: LD 0, 4, 7, 14, & 21

- Unremarkable

Pup Body Weight: LD 0, 4, 7, 14, & 21

- Lower mean pup body weights compared to controls at F1 pup weaning (LD21) at LD (up to 3%)
- Lower mean pup body weights compared to controls at MD (up to 8%) from pre-weaning (LD 7) through PND 28

- Lower mean pup body weights compared to controls at HD (up to 13%) at birth, through lactation to weaning, and on PND 28

Pup Necropsy: PND 28

- Hydrocephaly in one HD male

F1 Sexual Maturation: PND 28 onwards for vaginal opening (females); PND 35 onwards for preputial separation (males)

- Delayed preputial separation (1.3 days) or vaginal opening (1.2 days) at HD

F1 Behavior & Physical Development: LD 2 for static righting reflex & pinna detachment; LD 11 for cliff aversion; LD13 for eye opening; LD16 for air drop righting reflex; PND 22 for auditory response; PND 35 for motor activity; PND 70-80 for step-through passive avoidance test

- Reduced number of HD females with passive avoidance

F1 Mortality: Twice daily

- One HD female died 14 days into the premating growth period (bilateral enlarged kidneys, moderately distended ureters and a moderately distended urinary bladder at necropsy noted)

F1 Clinical Signs: Twice daily for overt changes in appearance & behavior; weekly for detailed observations

- Unremarkable

F1 Body Weights: Weekly from PND 28 for males; weekly until positive evidence of copulation & GD 0, 7, 10 & 13 for females

- Lower mean body weights (~5%) compared to controls for the LD group at premating (females) or until postmating (males) periods
- Lower mean body weights compared to controls at premating Weeks 1-8, at paring Weeks 9-11 and post-mating Weeks 12-13 for males (up to 8%), and for females from premating Weeks 1 to 5 (up to 6%) at MD
- Lower mean body weights in comparison to controls throughout the study for males (up to 11%), and for females for the 1st 3 premating weeks and then remained lower for the next 7 premating weeks (up to 12%) at HD
- Dose-related decrease in body weight gains in females in all treated groups during GD 0-7 (up to 6%) and GD 10-13 (up to 19%) compared to controls

F1 Reproduction: PND 80

- Unremarkable

F1 Uterine Examinations: GD 13

- Unremarkable

F1 Necropsy: GD 13

- Brain deformity/malformation in 1 HD female

- Enlarged kidneys, a moderately distended ureters and urinary bladder in 1 HD female died

The following table summarizes the noteworthy observations made in the F1 generation in rats.

Pups:

Dose, mg/kg / Observations	0		100		300		600	
	25M	25F	25M	25F	25M	25F	25M	25F
Body weights, g								
Day 0	7.09	6.74	7.19	6.92	7.29	6.86	**6.78**	**6.37***
Day 4 Preculling	11.79	11.37	11.81	11.39	11.85	11.24	**10.67****	**10.11****
Day 4 Postculling	11.81	11.38	11.84	11.35	11.86	11.23	**10.66****	**10.16****
Day 7	18.86	18.18	19.06	18.12	**18.62**	**17.75**	**16.87****	**16.05****
Day 14	37.61	36.54	37.72	36.42	**36.34**	**35.19**	**33.94****	**33.03****
Day 21 Weaning	61.53	59.49	**59.39**	**57.60**	**56.64****	**54.60****	**53.60****	**51.89****
Day 28 Postweaning	103.93	95.28	**101.69**	**92.04**	**98.18****	**89.24****	**92.67****	**85.17****
Sexual maturation,								
Vaginal opening, days	-	32.2	-	32.1	-	**32.8**	-	**33.5***
#pups passing	-	23	-	25	-	19	-	22
Preputial separation, days	43.0	-	43.0	-	**43.8**	-	**44.2**	-
#pups passing	25	-	25	-	25	-	25	-
Necropsy, PND 28	n=73	n=75	n=68	n=79	n=73	n=75	n=70	n=70
Brain, hydrocephaly				1			1	

-; not available
Significantly different from controls at p=0.05* or p=0.01**

F1:

Dose, mg/kg / Observations	0		100		300		600	
	25M	25F	25M	25F	25M	25F	25M	25F
Mortality[a]								1
Behavioral observations, PND 70-80								
Passive avoidance, passive	24	23	24	19	21	21	24	**17**
non-passive	1	2	1	6	4	4	1	7
Body weights, g								
Pre-mating, Week 1	143.4	123.5	**138.6**	**123.2**	**133.0***	**115.8***	**127.3****	**111.1****
Pairing, Week 9	535.9	335.8	**522.3**	330.2	**521.7**	338.3	**490.4****	**284.1**
Post-mating, Week 12	606.6		589.9		592.5		**560.2****	
Body weight gains, g		n=18		n=20		n=21		n=22
GD 0-7	-	39.2	-	**38.5**	-	**36.9**	-	**35.9**
10-13	-	17.8	-	**16.1**	-	**15.8**	-	**14.4**
Necropsy, GD 13								
Brain, deformity/malformation								1
Kidneys, enlarged, moderate								1
Ureters, distended with fluid								1
Urinary bladder, distended with urine, moderate								1

[a]One female died 14 days into the premating growth period (bilateral enlarged kidneys, moderately distended ureters and a moderately distended urinary bladder)
-; not available
Significantly different from controls at p=0.05* or p=0.01**

F_2 Generation: Not provided

Dosing Solution Analysis: Samples at 100 & 600 mg/kg/day from Week 1 preparation were collected for homogeneity analysis. Samples of the prepared formulations mixed during the last week of dosing were stored for 0, 7 and 21 days at room temperature for stability analysis. Samples of the dosing formulations at each concentration were also collected from the Weeks 1, 2, 3 and 4 for concentration analysis.

- Homogeneous for dosing formulations prepared with mean recovery of 90%
- Stable for 7 and 21 days; accurately prepared
- Mean recovery between 88.7% and 100.9% for concentrations of 0, 10, 30 and 60 mg/mL

10 Special Toxicology Studies

Study title: Single Dosage Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of TA-1790 on Eyes and Skin in Pigmented Rats

Study no.:	MID00025
Study report location:	Module 4.2.3.7.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 12, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TA-1790, 17AP440030, 100.4%

Key Study Findings

Avanafil:

- Minimal neutrophilic infiltrations in the corneal stroma and the bulbar conjunctiva, and focal retinal necrosis

8-MOP:

- Skin reactions in the lightly and darkly-pigmented skin sites (erythema) and ocular responses (e.g., minimal to moderate periorbital edema/vacuolation, diffuse corneal edema and corneal ulcer; minimal to mild focal necrosis in lens; minimal to moderate mixed inflammatory cell infiltrations of the bulbar conjunctiva)

Methods

Doses:	0, 100, 300, 1000 mg/kg for avanafil; 50 mg/kg for 8-MOP
Frequency of dosing:	Single
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% Carmellose Na solution containing 0.1% Cremophor® RH 40 in deionized water (Formulation B)
Species/Strain:	Male pigmented rat/Crl:LE (Long Evans) from (b) (4)

(b) (4)

Number/Sex/Group:	5 Males/group for avanafil; 3 males for 8-MOP
Age:	66 days at arrival
Weight:	350-383 g at study assignment
Satellite groups:	None
Unique study design:	Prior to UVR exposure, rats were anesthetized via intramuscular injection of ketamine/xylazine, and then positioned on plastic trays with laboratory tape. The head of each rat was elevated in order to expose the eyes to UVR intensity comparable to that of the skin sites. UVR exposure began 30±5 minutes after completion of administration for avanafil and 60±10 minutes after completion of administration for a comparator control 8-methoxypsoralen (8-MOP). The rats were placed 1.2 meters from the UVR source at the time of exposure. An instrumental UVR exposure dose equivalent to 0.5 minimal erythema dose (a UVR dose adequate to elicit a barely perceptible response in skin) was delivered to each rat over a period of 30 minutes. Rats were individually examined 1, 2 and 3 days after UVR exposure for viability, clinical and skin reaction observations, body weights, UVR exposure, ophthalmology, and histopathology.
Deviation from study protocol:	Not significant

Observations and Results

Mortality: Twice daily

- None

Clinical Signs: During acclimation, prior to/after formulation administration, 30±5 minutes and 4 hours±30 minutes after the completion of UVR exposure for general appearance &/or signs of skin responses at the site of UVR exposure, as well as 1, 2, & 3 days after UVR exposure

Avanafil:

- Scab(s) of the right eyelid in 1 HD rat

8-MOP:

- Chromodacryorrhea in 1 rat (1 eye)
- Sparse periorbital hair coat

Skin Reactions:

Avanafil:

- Unremarkable

8-MOP:

- Erythema grade 1 (light redness) in 2 rats (3 occurrences) and erythema grade 2 (distinct redness) in 1 rat (1 occurrence) in the lightly-pigmented skin
- Erythema grade 1 in 1 of 3 rats (1 occurrence) in the darkly-pigmented skin sites

Body Weights: Weekly during the acclimation period, on the day of formulation administration, on each day of observation, & prior to sacrifice

Avanafil:

- Reduced mean body weights (up to 5% on Day 4) and mean body weight gains (up to 65% during Days 1-4) for the entire observation period in all treated groups

8-MOP:

- Reduced mean body weights (~4%) and group mean body weight gains (~76%) during Days 1-4

Ophthalmoscopy: Before assignment to study & 3 days after UVR exposure

Avanafil:

- Peripheral retinopathy in 2 rats (2 eyes) at MD

8-MOP:

- Diffuse corneal edema in 3 rats
- Corneal ulcer in 2 rats (2 eyes)
- Eye lids half-closed in 1 of 3 rats (2 eyes)

Histopathology: Following completion of the final ophthalmological examination for evaluation of cornea, lens, bulbar conjunctiva (when present), vitreous and aqueous chambers, optic nerve (when present), retina, sclera, iris, ciliary body, and choroids

Avanafil:

- Minimal unilateral neutrophilic infiltrations in the corneal stroma in 1 vehicle (left eye), 1 MD (right eye) and 2 HD (both eyes) animals
- Minimal neutrophilic infiltrations in the bulbar conjunctiva of 1 control and 2 LD rats
- Minimal focal retinal necrosis in 2 MD (both eyes) and 1 HD (left eye) animals

8-MOP:

- Moderate corneal stromal edema and mild infiltrations of neutrophils into the corneal stroma in all rats
- Minimal or mild intercellular edema, mild focal hyperplasia, minimal to moderate vacuolation, and minimal or mild focal necrosis in the corneal epithelium in 2-3 rats
- Minimal focal hyperplasia and subcapsular necrosis of the lens in the right eye of 1 rat
- Minimal to moderate mixed inflammatory cell infiltrations of the bulbar conjunctiva in both eyes of all rats

Special Evaluation: None

The following table summarizes the noteworthy observations made in the phototoxicity study in pigmented rats.

Dose, mg/kg / **Observations**	**0** 5M	**100** 5M	**300** 5M	**1000** 5M	**8-MOP, 50** 3M
Clinical signs,					
Periorbital scab, right eye				1	
Chromodacryorrhea					1
Periorbital edema					2
Sparse hair coat, periorbital	1	1	1		1
Skin reaction,					
Erythema, lightly pigmented site					3
darkly pigmented site					1
Ophthalmology,					
Corneal edema, diffuse					3
Peripheral retinopathy			2		
Corneal ulcer					2
Lids half closed					1
Body weights, Day 2, g	469.6	**456.0**	**460.0**	**444.4**	**450.7**
Body weight gains, Days 1-4, g	+8.6	**-1.6**	**-1.2**	**-4.6**	**-2.7**
Histopathology,					
Cornea, edema, stroma, left					3
right					3
intercellular, epithelium, left					2
hyperplasia, epithelium, focal, left					2
right					2
infiltration, neutrophils, stroma, left	1			1	3
right			1	1	3
necrosis, epithelium, focal, right					2
vacuolation, epithelium, left					3
right					2
Lens, hyperplasia, epithelium, focal, right					1
necrosis, subcapsular, focal, right					1
Retina, necrosis, focal, left			1	1	
right			1		
Bulbar conjunctiva, infiltration, mixed cell, left					3
right					3
neutrophilic, left	1	2			

Stability and Homogeneity: Samples (2 mL each) for 100 & 1000 mg/kg were collected for homogeneity analysis. Duplicate samples of the dosing formulations at each concentration were also collected for concentration analysis. Stability not determined.

- Homogeneous for dosing formulations prepared with relative standard deviation of 0.898 and 0.13%
- Mean recovery at 102.8-105.2% for dose concentrations

11 Integrated Summary and Safety Evaluation

Avanafil (TA-1790) is a phosphodiesterase 5 (PDE5) inhibitor indicated for the treatment of erectile dysfunction (ED). Proposed clinical doses are 50, 100 and 200 mg taken on an as needed bases (PRN), but no more often than once per 24 hours.

In support of the NDA, the sponsor conducted 23 clinical studies including 3 Phase 3 studies, 3 Phase 2 studies and 17 Phase 1 studies in healthy males or mild to moderate ED patients. Exploratory doses ranged from 12.5 mg to 800 mg with a maximum duration of 52 weeks (50, 100, and 200 mg). Approximately 1900 men were administered avanafil including 860 patients exposed for at least 3 months, 490 patients exposed for at least 6 months, and 150 patients treated for at least 12 months. The most frequently reported adverse events associated with avanafil treatment included headache, flushing, nasal congestion, nasopharyngitis, influenza, upper respiratory tract infection, and back pain.

The nonclinical program for avanafil includes general and safety pharmacology, PK and ADME, general toxicology, genotoxicity, reproductive and developmental toxicology, and carcinogenicity studies. The sponsor also submitted a single-dose phototoxicity study.

Pharmacology: Avanafil potentiated EFS-induced relaxation in the isolated rabbit corpus cavernosum in a concentration-dependent manner (0.003-0.3 μM). Dose-dependent pelvic nerve stimulated penile tumescence was also observed in rabbit, dog, and monkey ED models following intravenous or intraduodenal administration (10-1000 μg/kg). The inhibitory potency of avanafil on PDE5 (IC_{50}=4.2-5.2 nM in canine lungs, IC_{50}=8.9 nM in human platelets) was comparable to sildenafil (IC_{50}=1.6 and 4.3 nM, respectively). Avanafil displayed high selectivity for PDE5 versus PDE6 (>100-fold) and for other PDEs (>1000-fold). The IC_{50} value for PDE6 (630 nM) is approximately 10-fold greater than the C_{max} for unbound avanafil in humans taking 200 mg avanafil (~26 ng/mL, 0.054 μM), suggesting a possible role for avanafil in the phototransduction cascade in rods and cones that may have pathological consequences on the transmission and regulation of the human light response. Two major human circulating metabolites (M16 and M4) were found to inhibit human platelet PDE5 with higher IC_{50} values (51 and 4100 nM, respectively) compared to the parent drug. Considering the low exposure to M4 and M16 (20-30% of parent) and the >10-fold lower activity of M4 and M16 for PDE5 compared to avanafil, the metabolites are unlikely to significantly contribute to the pharmacodynamic or toxicologic effect of avanafil.

No secondary pharmacodynamics studies were provided. However, inhibition of PDE5 in various tissues and organs including platelets, lung, heart, liver, brain, kidney visceral/vascular/skeletal smooth muscle, and testes, could lead to effects in undesired targets related to the increased bioavailability of nitric oxide (NO) and increased cGMP levels in sites where PDE5 actively hydrolyzes cGMP.

Safety Pharmacology: Safety pharmacology evaluations included core battery and supplemental (eye, GI tract) studies. Avanafil decreased spontaneous locomotor activity

at 1000 mg/kg in rats and mice, and prolonged phenobarbital-induced sleeping time in mice at 300 mg/kg. Avanafil decreased blood pressure (BP), produced variable changes in R and T waves, increased heart rate (HR) and cardiac output, and prolonged QT interval in conscious and anesthetized dogs. Avanafil also potentiated nitroglycerin-induced hypotension in anesthetized dogs. In vitro, avanafil inhibited hERG channel (IC_{50}=15.8 μM), action potential duration, and Na (IC_{50} >50 μM) and L-type Ca (IC_{50} >30 μM) channel activities, suggesting that avanafil may block Ca and Na channels as well as K channels similar to other cardiac drugs. The vasodilator activity of avanafil seen in isolated aorta models (e.g., inhibition of calcium-induced contractions) suggests that avanafil produces a calcium channel antagonist-like vasorelaxation. Avanafil decreased gastric secretion after a single intraduodenal administration of 300 mg/kg, and decreased urine volume and electrolyte excretion at oral doses ≥10 mg/kg in the rat. Other effects included ocular effects, i.e., concentration-dependent increase in b-wave amplitude at 1-30 μM, and potentiation of the anti-aggregation action of SNP on human platelets at 10 μM. The hemodynamic, GI and genitourinary effects were observed at <10-fold the C_{max} of unbound avanafil at the MRHD, while all other effects, were observed at ≥20-fold the C_{max} of unbound avanafil at the MRHD.

Avanafil has not been systematically studied in animals to assess its potential for abuse, tolerance or physical dependence. No in vitro binding studies were performed to determine the pharmacological site of action of the parent drug and its active metabolites.

ADME/PK/TK: The ADME profile is qualitatively similar between animals tested and men. Avanafil is rapidly absorbed and removed from the plasma in the rat, dog, and monkey following a single oral dose, but T_{max} and $T_{1/2}$ are delayed with repeated dosing. The oral bioavailability of avanafil was only 1.5% in rats compared to 35-40% in dogs and 15% in monkeys. Bioavailability in men is unknown. Median T_{max} is 30 to 45 minutes and the mean terminal $T_{1/2}$ 5 to 8 hours in men. The mean $T_{1/2}$ varied between 1 to 8 hours in men dosed at 200 mg across the PK studies. The sponsor stated that the longer $T_{1/2}$ seen in the recent studies compared to previous studies is likely to be due to the more sensitive bioanalytical method used. Steady state PK parameters were not assessed following repeat-dosing of 200 mg *QD*. However, following the initiation of 200 mg *BID* dosing (TA-07) steady state was reached within 24 hours. In rats, avanafil distributed rapidly to tissues with the highest levels in the GI tract, liver, and kidney at 0.5 hour post-dose, and the lowest levels in the brain, spinal cord, eye, and testis. Avanafil showed high affinity for melanin in the uveal tract and hair follicles with half-lives of 15 and 315 hours, respectively. Avanafil was not phototoxic in male pigmented rats following a single dose. Avanafil was primarily excreted in the feces, mostly likely via bile in animals and humans.

Avanafil was extensively metabolized in animals and humans. In vitro studies showed that metabolism was primarily by CYP3A4, with a minor contribution by CYP2C. The major circulating metabolites in men, M16 (open pyrrolidine ring carboxylic acid isomers) and M4 (hydroxyl metabolite), account for 10.6% and 8.4% of the total

radioactivity. These metabolites were also detected in male rats and male dogs. Metabolite profile was not determined for mice. Sexual dimorphism was noted in rats.

Limited TK was assessed in repeat-dose toxicology studies. In the repeat-dose studies in rats of 4 week duration and longer, PK parameters were not calculated due to a small number of plasma samples. Separate non-GLP TK studies were conducted in male rats, pregnant rats (GD 6-17), and pregnant rabbits (GD 6-20) following 2-week treatments with avanafil during the late stage of drug development. Exposure to metabolites was not, however, evaluated in dogs or mice. The sponsor stated that metabolites were not assessed in dogs because avanafil was metabolically more stable in the dog.

General Toxicology: Toxicology studies were conducted via oral gavage in CD-1 mice, CD rats, and NZW rabbits; or via capsules in Beagle dogs. It should be noted that PK parameters were generally not assessed in the toxicology studies, and different formulations were used across the studies in rats and mice. Most of the toxicology studies, including chronic toxicity in rats, carcinogenicity and reproductive and developmental toxicology studies, were conducted using formulations different from the to-be-marketed formulation. Only the 9-month toxicity study in dogs was conducted with the to-be-marketed formulation. The sponsor stated that the difference in formulations administered is unlikely to have any adverse impact on the safety data collected during the course of the program, considering that the materials tested in the nonclinical studies were representative of drug product used in the clinical program with respect to impurities and degradants.

In single-dose studies, no significant morbidity was observed at oral doses up to 2000 mg/kg in mice, rats, and dogs; and intravenous doses at up to 40 mg/kg in mice and rats. Repeat-dose oral toxicology studies were conducted in mice and rats for up to 2 years (oral gavage), and in dogs for up to 9 months. The highest doses tested for the longest duration are summarized in the following table:

Species	Dose (mg/kg)	Duration	AUC_{0-24}* (µg·hr/mL)	Exposure Multiple compared to MRHD#
Rat	1000	2 years	~60♂-270♀	~8♂-34♀ fold
Dog	60	9 months	~70	~9 fold
Mouse	600	2 years	~90	~11 fold

*Unbound+bound
#Mean AUC_{0-24h}~8 µg·hr/mL for avanafil given 200 mg in healthy males

Adverse effects observed in avanafil-treated animals at exposures which exceeded those at the MRHD included the following:

- **CNS**: ataxia, convulsion, tremor, hypoactivity, recumbency, prostration
- **CVS**: fibrosis, thrombus, inflammation, degeneration, necrosis, decreased BP, increased HR and tachycardia, QT interval prolongation
- **GI tract**: vomiting, loose stool, distended abdomen
- **Liver**: hepatocyte necrosis, hepatocellular hypertrophy, increased bilirubin, increased hepatic drug-metabolizing enzymes, decreased triglyceride

- **Thyroid**: follicular cell hypertrophy/hyperplasia, lymphohistiocytic infiltrate
- **Kidney**: hydronephrosis, tubular regeneration
- **Respiratory system**: irregular/labored/audible respiration, alveolar histiocytosis/infiltrate, hyperplasia
- **Hematopoietic and Immune systems**: increased WBC parameters and reticulocytes, decreased RBC parameters, splenic hematopoiesis/lymphoid depletion
- **Male Reproductive system**: altered sperm parameters (decreased/no motility, abnormal sperm), lymphohistiocytic infiltrates in prostate, testicular degeneration/atrophy

The following are the toxicologically significant effects noted in avanafil-treated animals.

CNS: CNS-related adverse effects manifested as clinical signs. In particular, some of the findings (e.g., ataxia, tremor, convulsion, hypoactivity, recumbency, prostration) preceded mortality in mice and pregnant rats at high doses, or termination of dosing in dogs. In mice administered 2000 mg/kg/day (~5-7 fold the C_{max} at the MRHD), CNS-related clinical signs included head tremor in one male on Day 43; convulsions in one female on Day 49, and irregular/labored/audible respiration on Day 45 in one male and two females on Days 42, 44 and 49. Clinical signs observed in pregnant rats administered 1000 mg/kg/day (~7 fold the C_{max} at the MRHD) that were found dead or enthanized *in extremis* included ataxia (GD 9), decreased activity (GD 8/9, GD 11/12), prostration (GD 8/12), difficult breathing (GD 8/12), and tremors (GD 12). In dogs, repeat doses higher than 75 mg/kg/day could not be achieved due to dose-limiting clinical signs. One dog at 75 mg/kg/day had entire body tremors on Day 26. At 100 mg/kg/day, animals exhibited ataxia, hypoactivity, recumbency, and/or retching on Day 4. Snorting behavior was noted on Day 9 at 75 mg/kg/day, and between Days 20 and 26 for animals at 30 and 75 mg/kg/day. The sponsor did not provide detailed information on the onset, duration, or reversibility of neurological effects. However, the lack of these signs in safety pharmacology studies in animals dosed subacutely up to 1000 mg/kg/day and single doses up to 2000 mg/kg/day suggests a delayed onset for CNS toxicity. The NOAEL (C_{max}~7-14 µg·hr/mL) is approximately 3-5 fold the C_{max} for the MRHD of 200 mg in humans (~2.6 µg/mL). These clinical signs were not apparent in animals treated with other PDE5 inhibitors. In humans, seizures have been reported as postmarketing events with previously marketed PDE5 inhibitors. According to the Clinical Reviewer Dr. Guodong Fang, there were no CNS signs observed in avanafil-treated patients during the clinical trials.

Published studies suggest a possible role for NO-cGMP in PDE5-induced CNS effects. NO functions both as an anticonvulsant and a proconvulsant by modulating the seizure threshold depending on the type of seizure, source of NO, dose, and the type of neurotransmitters involved (Pharmacol Rep 62:383, 2010; Epilepsia 51:1552, 2010; Eur J Pharmacol 617:79, 2009; Eur J Pharmacol 587:129, 2008; Br J Pharmacol 147:935, 2006; Neurosci Lett 376:116, 2005). In a recent study, rats pretreated with sildenafil or tadalafil at therapeutic doses and then exposed to hyperbaric oxygen had significantly faster and greater increases in cerebral blood flow and shortened convulsive latency (J

Appl Physiol 106:1234, 2009), suggesting that PDE5 inhibitors may perturb the cerebrovascular vasoconstrictive response, a mechanism to protect neurons from increased blood oxygen tension. EEG abnormalities were reported in ED patients given tadalafil at the time of maximum plasma concentrations (Neurol Res 31:313, 2009), indicative of potential neurological adverse events mediated by the PDE5 inhibitor.

CVS: Altered hemodynamics and ECG effects were noted in dogs in single-dose and short-term studies. Cardiovascular changes included decreased BP and increased HR associated with prolonged QT interval. Some avanafil-treated animals had slight (≥160 beats/min) to severe (≥200 beats/min) tachycardia. At the high-dose (100 mg/kg), a decrease in the R wave, a potential decrease in the T wave, and an apparent notch on the T wave were noted in dogs. There were no remarkable hemodynamic findings in the chronic dog study at doses up to 60 mg/kg/day.

Microscopic changes in the heart included arteritis in a branch of the cardiac extramural coronary artery or vascular inflammation. In rats, cardiac degeneration/necrosis was noted in females at 1000 mg/kg/day after 6 months, but was not progressive following 2 years of treatment. In mice, minimal signs of focal/multifocal fibrosis, mineralization, lymphohistiocytic infiltrates, and brown pigment were observed in the heart at the highest dose administered (1000 mg/kg/day) for 3 months. After treatment for 2 years, increased severity of thrombus (minimal to severe) and cardiomyopathy (minimal to moderate) were seen at 600 mg/kg, suggesting that the cardiac toxicity was aggravated with prolonged treatment in mice.

In humans, there were cases of hemodynamic changes and cardiac events (e.g., dizziness, abnormal ECG, hypotension, hypertension, syncope, acute myocaridial infarction) associated with avanafil treatment during clinical trials. The NOAEL for the cardiac findings corresponded to exposures approximately 3 fold, 8-11 fold, 34 fold, and 2 fold the anticipated human exposure ($AUC_{0\text{-}24h}$~8 μg·hr/mL) at the MRHD of 200 mg in dogs (~24 μg·hr/mL), male rats (~65-85 μg·hr/mL), female rats (~270 μg·hr/mL), and mice (~6-15 μg·hr/mL), respectively.

Liver: Treatment-related findings in the liver were observed in all animal species tested. Increases in liver weights and/or hepatic drug metabolizing enzyme activity (e.g., aminopyrine demethylase, aniline hydroxylase), and reduced clinical chemistry parameters (e.g., LDH, TG, total cholesterol, total protein, albumin, HDL) were noted in rats and dogs as early as 1 week. Increased total bilirubin correlated with microscopic findings of chronic inflammation, segmental arteritis, and hepatocyte necrosis was noted at 2-4 weeks. Increased incidence and/or severity of focal cystic degeneration and eosinophilic focus were seen in male rats at the highest dose after 2-year treatment. Altered clinical chemistry parameters, increased liver weights, and centrilobular hypertrophy may be the result of avanafil-induced hepatic enzyme induction in rats. In dogs, there were no significant findings in the liver at doses up to the highest dose tested, 60 mg/kg/day, following chronic administration. The NOAEL for the liver findings corresponded to exposures approximately 9-fold, 1-fold, 12-fold and 11-fold the anticipated human exposure ($AUC_{0\text{-}24h}$~8 μg·hr/mL) at the MRHD in dogs (~70

μg·hr/mL), male rats (8 μg·hr/mL), female rats (~99 μg·hr/mL), and mice (~88 μg·hr/mL), respectively.

Hematopoietic and Immune Systems: Avanafil was associated with altered hematology parameters and histopathological findings in the immune system. Increased reticulocytes, decreased RBC parameters, and increased WBC counts were seen as early as 1 week in rats and dogs. In rats, changes in RBC and WBC parameters were still present at the end of the 4-week recovery period following 6-month dosing. At 6 months, dose-related increases in extramedullary hematopoiesis in the spleen were noted. In dogs, dose-related increases in both incidence and severity (minimal to severe) of multifocal and diffuse lymphocyte depletion, associated with dose-related reduction in thymus weights, were found at 1 month. However, thymic effects not noted in the 9-month dog study conducted with comparable doses, suggesting an adapted response following chronic exposures. The anemic response may have resulted from chronic inflammation. The hematotoxicity seen in avanafil-treated animals appears to be a secondary effect rather than a direct toxic effect although a role of the PDE5 inhibitor in hematopoietic tissues cannot be excluded based on the findings that PDE5 inhibitors such as vardenafil (Eur Urol 51:1411, 2007; Int J Impot Res 17:377, 2005) and tadalafil (Int J Impot res 18: 484, 2006) increase circulating bone marrow-derived endothelial progenitor cells in humans (Trends Mol Med 10:421, 2004; Nat Med 9:1370, 2003). In humans, increased infections (e.g., upper respiratory tract infection, nasopharyngitis) were among the most frequently reported adverse events in patients taking avanafil in multiple-dose PRN studies. The NOAEL for the hematologic and immune system findings corresponded to exposures approximately 3-6 fold, 1 fold, 12 fold, 5-11 fold, and 0.5-2 fold the anticipated human exposure ($AUC_{0\text{-}24h}$~8 μg·hr/mL) at the MRHD in dogs (~24-48 μg·hr/mL), male rats (~7 μg·hr/mL), female rats (~90 μg·hr/mL), male mice (~39-88 μg·hr/mL), and female mice (~4-15 μg·hr/mL), respectively.

Male reproductive organs: Morphological changes in male reproductive organs (e.g., testicular degeneration/atrophy, oligospermia) were not remarkable compared to other PDE5 inhibitors. In dogs, arteritis in arterioles in the epididymis was noted in one animal receiving 100 mg/kg/day of avanafil for 1 week. Hypospermia was noted in one dog each at 30 and 100/75 mg/kg/day in the 4-week study, however, this may be due to immaturity. In rats, increased incidences of bilateral oligospermia/germ cell debris in epididymides and testicular degeneration/atrophy were noted at HD (1000 mg/kg/day) in the 2-year study.

In a fertility study, altered sperm parameters were noted in avanafil-treated male rats at exposures approximately 11-fold the human exposure. These included markedly reduced sperm motility (average 51%) or no (5 out of 25) motility compared to concurrent controls (average 86%), suggesting potential detrimental effects on male reproductive function. Avanafil-treated rats also had a marked increase in incidence of broken sperm with detached heads (average 37%) compared to controls (average 6.4%), suggesting that avanafil may cause spermatogenic dysfunction. In a follow-up study evaluating reversibility of effects on fertility and sperm parameters in male rats treated for 9 weeks followed by a 9-week recovery period, a period encompassing the

full duration of the spermatogenic cycle in rats, abnormal sperm morphology noted at the end of treatment returned to normal during the recovery period. The NOAEL (AUC_{0-24h}~5 µg·hr/mL) is comparable to the MRHD (AUC_{0-24h}~8 µg·hr/mL).

In humans, 2 single-dose studies (TA-014 and TA-021) assessed sperm function. Two subjects (out of 18) had reductions in sperm count (12-19 million/mL) and progressive motility (24-34%) at 1 hour on Day 1 following a 200 mg dose (TA-014). However, there were no significant effects on sperm parameters in a single-dose follow-up study (TA-021). Avanafil and its metabolites distributed into seminal fluid with the geometric mean seminal fluid/plasma concentration ratios for avanafil, M4, and M16 of approximately 0.06/0.07, 0.70/0.83 and 0.37/0.74, respectively, following plasma (0.75/1 hour post-dose) and semen (0.75-1.5/1 hour post-dose) collections on Days 1 and/or 5 from young male subjects. PDE5 is expressed in testicular seminiferous tubules and in epididymides in humans (Mol Cell Endocrinol *in press*; BMC Pharmacology 11S:23, 2011; Hum Reprod 26:1450, 2011). In men, altered sperm motility and viability have been reported following treatment with other PDE5 inhibitors (Curr Pharm Des 15:3506, 2009; Asian J Androl 10:115, 2008; Fertil Steril 88:860, 2007; Fertil Steril 87:1064, 2007; Am J Obstet Gynecol 182:1013, 2000).

Genotoxicity:
Avanafil was not considered to be genotoxic based on weight of evidence from 7 different in vitro and in vivo genotoxicity tests. Avanafil tested negative in two Ames assays and two chromosome aberration tests using CHL/IU and CHO cells, and in the in vivo mouse micronucleus and the rat UDS assays. Avanafil increased forward mutations at a 4-hour exposure with metabolic activation at concentrations, including at which precipitation occurred, in the mouse lymphoma assay using L5178Y/TK+/- cells.

Carcinogenicity: In 2-year carcinogenicity studies, there were no positive tumor findings in mice and rats by CDER criteria at up to the highest doses tested (approximately 11-, 8- and 34-fold the maximum recommended human dose based on AUC for mice, male rats and female rats, respectively). The slight numerical increases in hepatocellular tumors in male rats and mice were not considered to be clearly drug-related due to the high background rate in historical controls.

Conclusion: From a nonclinical perspective, avanafil does not appear to have more benefit or risk than any of the previously approved PDE5 inhibitors. Avanafil-related adverse effects in animals were similar to adverse effects observed with other PDE5 inhibitors.

The following table summarizes exposure multiples at the NOAEL and the LOAEL for adverse findings in the major toxicology studies compared to the anticipated human exposure at the MRHD. It should be noted that exposure multiples for general toxicity were calculated based on the PK data obtained from either short-term or 2-week bridging studies using different formulations due to the lack of TK data collected in the toxicology studies. In addition, the exposure multiples were calculated using AUC values for total (bound plus unbound) exposure for the following reasons:

1) In vitro concentration ranges used for the protein binding do not cover the entire range of concentrations achieved in vivo in animals and humans;
2) Protein binding is high in all species tested including humans and the unbound fraction of drug is greater in animals than in humans; and
3) Protein binding data were not obtained from mice.

Plasma concentrations for the active circulating metabolites M16 and M4 were not incorporated into the exposure multiples presented due to the lack of data in dogs and mice. Systemic exposures in humans at the MRHD (200 mg) are approximately 8000 ng·hr/mL for $AUC_{0\text{-}24h}$ and 2600 ng/mL for C_{max} based on the results from overall human PK studies, rather than the PK parameters from a single study (TA-011) used by the sponsor: 4690 ng·hr/mL and 1880 ng/mL, respectively.

Table 5 Summary of Exposure Multiples for the Major Findings Observed in Animals at the NOAEL and the LOAEL Compared to Humans Based on AUC

Study Type	Target Organ Toxicity	Dose, mg/kg		Exposure Multiple*	
		NOAEL	LOAEL	NOAEL	LOAEL
General toxicity	CNS (tremor, convulsion, ataxia, hypoactivity, prostration, recumbency)	Dog: 60♂	100/75♂	9-16♂	20-28♂
		Pregnant rat: 300	1000	5-8	30
		Mouse: 600	2000/1000	11	8-18
	CVS (fibrosis, thrombus, inflammation, cardiomyopathy)	Dog: 30♂	60♂	3♂	9♂
		Rat#@: 1000	-	8-11♂, 34♀	-
		Mouse: 200	600	2	11
	Respiratory tract (irregular/labored/audible respiration, alveolar histiocytosis/macrophage infiltrate, lung hyperplasia)	Dog: 30♂	60♂	3♂	9♂
		Mouse: 200	600	2	11
	Hematotoxicity (↓RBC, ↑WBC, ↑reticulocytes, lymphoid depletion)	Dog: 30♂	60♂	3-6♂	9-16♂
		Rat#: 300	1000	1♂, 12♀	8♂, 34♀
		Mouse: 600♂ 200♀	2000/1000♂ 600♀	5-11♂ 0.5-2♀	10
	Liver (hepatocyte necrosis, hepatocellular hypertrophy, eosinophilic focus, ↑bilirubin, ↓triglyceride, ↑liver weights)	Dog: 60♂	100/75♂	9♂	20♂
		Rat#@: 300	1000	1♂ 12♀	8♂ 34♀
		Mouse: 600	2000/1000	11	8-18
	Kidney (hydronephrosis, tubular regeneration)	Dog: 10♂	30♂	1♂	3♂
		Rat#@: 300	1000	1♂ 12♀	8♂ 34♀
	Thyroid (cyst/lymphohistocystic infiltrate, follicular hypertrophy, hyperplasia)	Dog: 10♂	30♂	1♂	3♂
		Rat#@: 300	1000	1♂ 12♀	8♂ 34♀
	Male reproductive system (testicular degeneration/atrophy, lymphohistiocytic infiltrates in prostate)	Dog: 30♂	60♂	3♂	9♂
		Rat#@: 300♂	1000♂	1♂	8-11♂
		Mouse: 600♂	2000/1000♂	11♂	8♂
Reproductive & Developmental toxicity	Fertility (↓pregnancy/fertility/fecundity, ↑estrous cycle length, low/no sperm motility, ↑abnormal sperm)	Rat#@: 300	1000	1♂ 12♀	8♂ 30♀
	Maternal toxicity (mortality, clinical signs, ↓body weights, ↓gravid uterine weights)	Rat@: 300	1000	8	30
		Rabbit@: 120	240	2	6
	Fetal development (postimplantation loss, resorptions, ↓litter size, ↓fetal weights)	Rat@: 300	1000	8	30
		Rabbit@: 120	240	2	6
	Prenatal/postnatal development (↓pup/F1 weights, ↓sexual maturation)	Rat@: 100	300	2	17

*Mean $AUC_{0\text{-}24h}$~8 µg·hr/mL given 200 mg in healthy males
#Plasma AUC for rats based on 2-week exposure levels using a different formulation due to the lack of TK data in the corresponding studies
@Plasma AUC for rats based on a 2-week bridging study.
-: not available

12 Appendix/Attachments

12.1 Annotated Labeling

The following annotated labeling is the Division's recommendations to the sponsor's proposed labeling. The revisions are limited to sections where the context has been altered (in red) or deleted (in strikethrough).

(b) (4)

3 Page(s) of Draft Labeling have been Withheld in full as b4 (CCI/ TS) immediately following this page

12.2 Table 6 Summary of the Major Findings in Repeat-Dose Toxicology Studies with Avanafil

Study Type	Species	Route	Dose, mg/kg	Major Findings
1 Week (non-GLP)	Rat	Oral gavage	0, 30, 100, 300	Salivation at HD; ↑a- & b-wave amplitudes at HD♀ ↑Liver weights♀/drug metabolizing enzymes at ≥MD♂♀ ↓LDH/TG at ≥LD♂ & HD♀; ↑ALP/Cl/K at ≥LD♂ ↑WBC/reticulocytes at ≥LD; Sinusoidal cortex-medullary dilatation in adrenal gland at HD NOAEL=100 mg/kg/day
	Dog♂	Oral gavage	0, 10, 30, 100	↓Activity, deep sedation, recumbent position, staggering walk, hypothermia, ptosis, miosis, conjunctival hyperemia, abnormal feces, ↓body temperature, & respiration rate at HD ↑HR/↓MBP at ≥LD, tachycardia at ≥MD ↓ERG-wave amplitude ratios at ≥MD; ↑a- & b-wave latency at ≥MD ↓R wave/↓T wave at HD ↓RBC at ≥MD; ↓TG at ≥MD; ↓TCHO/TP/albumin/Ca/K/IP/HDL at HD ↑ALT/ glucose at HD; Arteritis in coronary artery & epididymis at HD Renal collecting tubule calcification at HD Spermatic granuloma in epididymis at HD NOAEL=10 mg/kg/day
2 Weeks (GLP)	Rat	Oral gavage	0, 100, 300, 1000	Salivation at HD; ↑water intake/urine volume at ≥LD ↓RBC & ↑WBC/reticulocytes at HD ↑ALT/albumin at HD; ↓TG/LDH at ≥LD♂; Ca♂/IP♀ at ≥LD; ↑BUN/CPK at HD♀ ↑Liver weights/drug metabolizing enzymes at ≥LD Lung alveolar infiltration at HD♀ NOAEL=100 mg/kg/day
	Dog	Oral capsule	0, 10, 30, 100	Vomiting at ≥LD; loose stool/shivering♀/inanimation/watery diarrhea♀/eye discharge♀ at HD ↑HR (tachycardia)/↓SBP/DBP/MAP at HD ↓RBC♂/↑WBC at HD; ↓TG/TCHO♂/PL♂ at ≥MD; ↑TB/hepatic enzymes at ≥MD NOAEL=10 mg/kg/day
1 Month (GLP)	Rat♂	Oral gavage	0, 100, 300, 1000	↑WBC at ≥MD (non-reversible); ↓G/TG at HD, ↑A/TB (non-reversible) at HD, ↑A/G ratio at HD; ↑urine volume at HD, ↓urine G/SG at HD NOAEL=100 mg/kg/day
	Dog♂	Oral capsule	0, 10, 30, 100/75	HD reduced to 75 mg/kg on D5/6 due to severe clinical signs (ataxia, hypoactivity, recumbency, retching); non-formed feces at ≥LD; snorting behavior at ≥MD; body tremor at HD ↑TB & chronic inflammation in liver at HD Thymus lymphocyte depletion at ≥MD; Hypospermia at ≥MD NOAEL=10 mg/kg
3 Months (GLP)	Mouse	Oral gavage	0, 200, 600, 2000/1000	Mortality at 2000 mg/kg/day; dosing suspended on D47-56 & reduced on D57 to 1000 mg/kg due to excessive toxicity (hunched/thin appearance, rough/yellow hair coat, cold to touch, irregular/labored/audible respiration, tremor/convulsion, hypoactivity, ↓BWG) Renal mineralization at HD♂ ↑Liver weights/hepatocellular hypertrophy at ≥MD♂ & at HD♀; hepatocyte necrosis/inflammation at HD Cardiac fibrosis (HD), mineralization (≥MD♂) & lymphohistiocytic infiltrate (HD♂) NOAEL=200 mg/kg/day

6 Months (GLP)	Rat	Oral gavage	0, 100, 300, 1000	Sacrificed in extremis in 1♂ at MD due to an apparent hindlimb injury & hematopoietic neoplasia in multiple organs ↓TG♂/↑liver weights/hepatocyte necrosis♀/hepatocellular hypertrophy at ≥MD; Thyroid follicular cell hypertrophy at HD ↑Spleen weights/extramedullary hematopoiesis & ↓RBC &↑MCV/MCH/reticulocytes/WBC at ≥LD♂ or at HD♀ Cardiac degeneration/necrosis♀ at HD Acinar cell atrophy in pancreas at HD (partially reversible); Chronic-active inflammation in prostate at HD Uterine dilatation at HD (partially reversible) NOAEL<100 mg/kg
9 Months (GLP)	Dog♂	Oral capsule	0, 10, 30, 60	Abnormal feces at ≥LD; ↑cage sores at ≥MD Lung hyperplasia/alveolar macrophage at HD Renal tubular regeneration at ≥LD (partially reversible at HD) Cardiac inflammation at HD Lung hyperplasia/alveolar macrophage infiltrates at HD (partially reversible) Lymphohistiocytic infiltrate in thyroid at ≥MD (non-reversible) & prostate at ≥LD (partially reversible) NOAEL=10 mg/kg

12.3 Table 7 Summary of the Major Findings in Reproductive and Developmental Toxicology Studies with Avanafil

Study Type	Species	Route	Dose, mg/kg	Major Findings
Fertility & early embryonic development (GLP)	Rat	Oral	0, 100, 300, 1000	Euthanized *in extremis* in one female at HD on D19 (noted with clinical signs prior to necropsy with ↓activity, yellow discolored hair in anogenital region, red material around eye and mouth & salivation) Salivation, red material around mouth/nose♂, hunched posture♂ & sparse hair in abdomen/forefoot/hindlimb at ≥MD; ↓BW/BWG & food intake at HD; ↑Estrous cycle length/↓#estrous cylcles; ↓#females with viable embryos; ↓pregnancy/fertility/fecundity indices, ↓copulatory interval, ↓sperm motility; ↑abnormal (broken) sperm at HD NOAEL=300 mg/kg
Reversibility on fertility & embryonic development	Rat	Oral	0, 1000	Euthanized *in extremis* in 1 HD male on D22 with a swollen left hind limb with impaired function and discolored purple; Salivation; ↓BW/BWG throughout the treatment and recovery period; ↓Sperm motility; ↑abnormal sperm (reversible at 9-week recovery period)
Embryonic fetal development (GLP)	Rat	Oral	0, 100, 300, 1000	Found dead or euthanized *in extremis* in HD dams (↓activity, lacrimation, ataxia, prostration, tremors, low carriage, red/black material around eyes/mouth, skin cold to touch, eyelid partially/completely closed, difficult breathing, red/black foci in glandular/nonglandular stomach & green/red fluid in the urinary bladder); ↓Activity, salivation, prostration, vulvar discharge, sparse hair, difficult breathing & lacrimation at HD; ↓BW/BWG & food intake in dams at HD; All resorptions in one dying HD female & pregnant by stain in 1HD surviving female; ↓#Viable fetuses/all resorptions at HD; ↓Gravid uterine weights/adjusted maternal BW/BWG at HD; ↓Fetal weights at HD; NOAEL=300 mg/kg for maternal & developmental toxicity
	Rabbit	Oral	0, 30, 60, 120, 240	Sacrificed in 1 HD female that aborted on GD24; Irregular feces at ≥LD; difficult breathing in HD dam; ↓BW/BWG & food intake at HD; ↑Postimplantation loss/total (late) resorptions at HD; ↓Viable fetuses at HD; NOAEL=120 mg/kg for maternal & developmental toxicity
Prenatal & postnatal development (GLP)	Rat	Oral	0, 100, 300, 600	Salivation at ≥MD; ↓BW/BWG & food intake at ≥MD; ↓pup/F1 BW at ≥LD; Mortality in one HD F1 female 14 days post-weaning (noted with bilateral enlarged kidneys, distended ureters and distended urinary bladder at necropsy); ↓Sexual maturation (1.3 days for vaginal opening & 1.2 days for preputial separation) at HD NOAEL=300 mg/kg/day for maternal toxicity; 600 mg/kg/day for reproductive performance for F1 & F1; 100 mg/kg/day for F1 pup growth

12.4 Executive CAC Minutes for Protocols and Final Studies

Executive CAC
Date of Meeting: December 16, 2003
Mouse/Rat Carcinogenicity Dose-Selection Protocol

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Robert Osterberg, Ph.D., HFD-520, Alternate Member
Laurie Mcleod-Flynn, Ph.D., HFD-580, Acting Team Leader
Yangmee Shin, Ph.D., HFD-580, Presenting Reviewer

Author of Draft: Yangmee Shin

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-year carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from Agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #51,235
Drug Name: TA-1790
Sponsor: VIVUS

Background:
TA-1790 is a phosphodiesterase 5 inhibitor for the treatment of male erectile dysfunction (b) (4) The drug was negative in an *in vitro* Ames test, two chromosome aberration assays (CHL/IU cells and CHO cells), and an *in vivo* mouse micronucleus assay and UDS assay. The drug was positive in mouse lymphoma gene mutation test with S9 at 4 hrs, negative without S9 at 4 hrs and equivocal without S9 at 24 hrs. The sponsor is proposing 2-year bioassays by oral gavage administration in mice and rats based on a 3-month study for mice and a 6-month study for rats. The dose range-finding studies were conducted using a drug suspension, which gives lower systemic exposure than using a drug solution. Definitive metabolic profiles in animals and humans have not been provided.

Mouse Dose Selection:
The sponsor proposed doses of (b) (4) in both sexes of CD-1 mice based on MTD (target organ toxicity). A 3-month oral gavage study was conducted at doses of 0, 200, 600 and 2000/1000 mg/kg. The high dose of 2000 mg/kg was reduced to 1000 mg/kg on Day 57 due to excessive toxicity resulting in mortality of 2 males and 2 females. Major target organs were the liver and the heart including focal/multifocal cardiac fibrosis seen from 1000 mg/kg dose. Plasma exposures (based on AUC) at the proposed high dose is expected to be comparable to the anticipated human exposure at the maximum proposed human dose of (b) (4)

Rat Dose Selection:
The sponsor proposed doses of (b) (4) in both sexes of SD rats based on MTD (target organ toxicity). A 6-month oral gavage study was conducted at doses of 0, 100, 300 and 1000 mg/kg. The sponsor stated that the incidence and severity of the effects in the liver, thyroid, heart and spleen could increase over a longer period of time. However, the high dose of 1000 mg/kg reached MTD based on decrease in mean body weight gain (9-10%). Plasma exposures (based on AUC) at the proposed high dose is expected to be comparable to or lower than the anticipated human exposure at the maximum proposed human dose of (b) (4)

Executive CAC Recommendations and Conclusions:

Mouse:

- The Committee did not concur with the proposed [redacted] (b) (4)
- The Committee recommended doses of 0, 100, 200 and 600 mg/kg based on the deaths seen at 2000 mg/kg and the cardiac fibrosis seen at 2000/1000 mg/kg in the 3-month studies.

Rats:

- The Committee did not concur with the proposed [redacted] (b) (4)
- The Committee recommended doses of 0, 100, 300 and 1000 mg/kg based on a 9-10% decrement in in body weight gain) at 1000 mg/kg in the 6-month studies.

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances:
(a) For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
(b) For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
(c) For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma, etc., see McConnell et al., JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level.
(d) For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

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

cc:\
/Division File, HFD-580
/Laurie Mcleod, HFD-580
/Yangmee Shin, HFD-580
/Eufrecina Deguia, HFD-580
/Adele Seifried, HFD-024

Executive CAC
Date of Meeting: March 10, 2009
Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Barry Rosloff, Ph.D., DPP, Alternate Member
Lynnda Reid, Ph.D., DRUP, Supervisor
Yangmee Shin, Ph.D., DRUP, Presenting Reviewer

Author of Draft: Yangmee Shin

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

IND 51,235
Drug Name: Avanafil (TA-1790)
Sponsor: VIVUS, Inc.

Background:
VIVUS is developing avanafil, a phosphodiesterase 5 inhibitor, for oral treatment of erectile dysfunction. Avanafil tested negative in the in vitro Ames test, two chromosome aberration assays (CHL/IU cells and CHO cells), and the in vivo mouse micronucleus assay and UDS assay, but was positive in the mouse lymphoma gene mutation test at 4 hrs with S9. Dose selection for the 2-year carcinogenicity studies was based on an MTD in both mice and rats. In vivo metabolic profile of avanafil in humans and animal species has not been provided.

Rat Carcinogenicity Study:
The rat study was conducted in Sprague Dawley rats [Crl:CD® (SD)IGS BR] given in 0.5% carmellose sodium solution containing 0.1% HCO-60 (a polyoxyethylene castor oil derivative) in purified water by oral gavage. The high-dose female group was terminated in Week 100 due to reduced survival below 15. The survival rate at the terminal necropsy was 30%, 40%, 30%, and 27% in males, and 28%, 35%, 38%, and 23% in females at 0, 100, 300 and 1000 mg/kg/day, respectively. A specific cause for the decreased survivability in high-dose females was not determined.

Benign hepatocellular adenoma in liver was observed in males with 0/60, /60, 0/60 and 5/60 at 0, 100, 300 and 1000 mg/kg/day, respectively, associated with increased incidences of focal cystic degeneration and eosinophilic focus of cellular alteration. However, none of the increased tumor incidences in treated groups was statistically significant by CDER criteria. There were no hepatocellular carcinomas in any group. Systemic exposure at high dose ($AUC_{0\text{-}24h}$~62000-294000 ng•hr/mL) was approximately 7-32 fold above the exposure in humans given the MRHD of 200 mg twice a day 12 hours apart ($AUC_{0\text{-}24h}$~9000 ng•hr/mL) based on the 2-week study using the same formulation.

Mouse Carcinogenicity Study:
The mouse study was conducted in CD-1 mice [Crl:CD-1® (ICR)BR] given in 0.5% carmellose sodium solution containing 0.1% HCO-60 in purified water by oral gavage. Treatment was stopped early in Week 91 for high-dose males and females, and the high dose groups were terminated early during Week 92 for males and Week 94 for females. All remaining male and female groups were terminated during Week 104 and Week 98, respectively. The survival rate at the terminal necropsy was 23%, 40%, 37%, and 25% in males, and 28%, 28%, 32%, and 25% in females at 0, 100, 200 and 600 mg/kg/day, respectively. A specific cause for the decreased survivability was not determined. However, higher numbers of the high-dose males died due to liver tumors compared to control mice (7 *vs.* 2). Hepatocellular carcinomas in liver were observed in males with 0/60, 2/60, 2/60 and 4/60 at 0, 100, 200 and 600 mg/kg/day. None of the increased tumor incidences observed in treated groups was statistically significant by CDER criteria. Systemic exposure at high dose ($AUC_{0\text{-}24h}$~80000-88000 ng•hr/mL) was approximately 8.8-9.7 fold above the exposure in humans given the MRHD at a steady state ($AUC_{0\text{-}24h}$~9000 ng•hr/mL) based on the 3-month study using a different formulation, so a direct comparison of the systemic exposure may not be appropriate.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee concurred that the study was adequate, noting prior Exec CAC dose concurrence. However, the Committee noted that the vehicle used, differed from that in the dose-ranging study, which is suboptimal.

- The Committee concurred that there were no clearly drug--related neoplasms.

Mouse:

- The Committee concurred that the study was adequate, noting prior Exec CAC dose concurrence. However, the Committee noted that the vehicle used, differed from that in the dose-ranging study, which is suboptimal.

- The Committee concurred that there were no clearly drug-related neoplasms.

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

cc:\
/Division File, DRUP
/Lynnda Reid, DRUP
/Yangmee Shin, DRUP
/Eufrecina Deguia, DRUP
/Adele Seifried, OND IO

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

YANGMEE SHIN
03/21/2012

LYNNDA L REID
03/21/2012