CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202155Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 202155
Submission date: 9/28/2011
Drug: apixaban
Sponsor: Bristol-Myers Squibb Company and Pfizer
Indication: Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation

Reviewing Division: Division of Cardiovascular and Renal Products

Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for apixaban to be sufficient to support the proposed use.

The applicant and reviewer proposed pregnancy category B for the labeling. This appears appropriate as no fetal toxicity or malformations were observed in rats, mice and rabbits.

Apixaban was evaluated for carcinogenicity in 2-year rat and mouse studies. The Executive Carcinogenicity Assessment Committee concluded that these studies were adequate and there were no clearly drug-related tumors in either study.

Testicular degeneration was noted in male rats treated with apixaban for 3 months beginning on postnatal day 4. The incidence was 33% at the highest dose of 600 mg/kg, which was higher than concurrent and historical controls. The severity at this dose was moderate to marked. The findings appeared reversible after a recovery period of 35 days and no impairment of mating or reproductive parameters was noted. Based on levels of unbound drug, there appears to be no margin between the NOAEL and the human exposure for this finding. The pharm/tox reviewer recommended that an additional juvenile animal study be conducted to determine if there is a critical period for toxicity before any pediatric trials are conducted.

Conclusions:
I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA. No additional nonclinical studies are recommended at this time. The proposed Established Pharmacologic Class for apixaban is "factor Xa inhibitor". This is appropriate because it is consistent with other moieties of this class. I agree with the division pharm/tox recommendations on labeling.

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

/s/

PAUL C BROWN
06/21/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202,155
Supporting document/s:	Electronic Document Room (EDR)
Applicant's letter date:	09/28/11, 09/29/10 (Nonclinical)
CDER stamp date:	09/28/11, 09/19/10 (Nonclinical)
Product:	Apixaban
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Bristol-Myers Squibb Company and Pfizer
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	Patricia P. Harlow, Ph.D.
Supervisor/Team Leader:	Thomas Papoian, Ph.D., D.A.B.T.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Alison Blaus

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202,155 are owned by Bristol-Myers Squibb Company and Pfizer or are data for which Bristol-Myers Squibb Company and Pfizer has obtained a written right of reference. Any information or data necessary for approval of NDA 202,155 that Bristol-Myers Squibb Company and Pfizer do not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202,155.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Apixaban, an inhibitor of the coagulation Factor Xa (FXa), is being developed as an antithrombotic/anticoagulant agent (b) (4). Under NDA 202,155, apixaban is proposed for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. The dosing regimen in the Phase 3 trial was 5 mg of apixaban twice a day in patients with normal renal function.

1.2 Brief Discussion of Nonclinical Findings

This addendum to the previous nonclinical review filed on 2/21/12 is limited to documenting recommendations for the nonclinical portions of the label.

1.3 Recommendations

1.3.1 Approvability

NDA 202,155 for apixaban is approvable from a pharmacology and toxicology perspective for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation.

1.3.2 Additional Non Clinical Recommendations

The nonclinical addendum dated 4/13/2012 recommended that the label should indicate that apixaban is dialyzable.

1.3.3 Labeling

Sponsor's proposal:

8.1 Pregnancy

Pregnancy Category B

(b) (4)

Reviewer's recommendation:

8.1 Pregnancy

Pregnancy Category B

There are no adequate and well-controlled studies in pregnant women. Treatment during pregnancy may induce pregnancy-related hemorrhage and/or emergent delivery for which a reversal agent is not available.

Demonstrated fetal exposure to apixaban during treatment of pregnant rats, rabbits and mice after implantation until the end of gestation did not induce fetal malformations or fetal toxicity. No maternal or fetal deaths were attributed to bleeding. Increased incidence of maternal bleeding was observed in rats, rabbits and mice at maternal exposures that were 4, 1, and 19 times, respectively, the human exposure of unbound drug, based on AUC comparisons at the maximum recommended human dose (MRHD) of 10 mg (5 mg twice daily). ELIQUIS should be used during pregnancy only if the potential benefit justifies the potential risk to mother and fetus.

The sponsor did not propose wording for Section 8.2

Reviewer's recommendation:

8.2 Labor and Delivery

Safety and effectiveness of apixaban during labor and delivery have not been studied in clinical trials. Consider the risks of bleeding and of stroke in using apixaban in this setting [see Warnings and Precautions (5.2)].

Treatment of pregnant rats from implantation (gestation Day 7) to weaning (lactation Day 21) with apixaban at a dose of 1000 mg/kg (about 5 times the human exposure) did not result in death of offspring or mother rats during labor in association with uterine bleeding. However, increased incidences of bleeding signs, primarily during gestation, occurred at apixaban doses of ≥25 mg/kg, a dose corresponding to ≥1.3 times the human exposure.

Sponsor's proposal:

8.3 Nursing Mothers

(b) (4)

Reviewer's recommendation:

8.3 Nursing Mothers

It is unknown whether apixaban or its metabolites are excreted in human milk. Data in rats indicate a high excretion of apixaban in milk (12% of the maternal dose). A risk to newborns and infants cannot be excluded.

A decision must be made either to discontinue breastfeeding or to discontinue/abstain from ELIQUIS therapy.

Sponsor's proposal:

12 Clinical Pharmacology

12.1 Mechanism of Action

(b) (4)

Reviewer's recommendation:

Apixaban is an oral, reversible, and selective active site inhibitor of FXa. It does not require antithrombin III for antithrombotic activity. Apixaban inhibits free and clot-bound FXa, and prothrombinase activity. Apixaban has no direct effect on platelet aggregation, but indirectly inhibits platelet aggregation induced by thrombin. By inhibiting FXa, apixaban decreases thrombin generation and thrombus development. Preclinical studies of apixaban in animal models have demonstrated antithrombotic efficacy in the prevention of arterial and venous thrombosis.

Sponsor's proposal:

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4)

Reviewer's recommendation:

Apixaban was not carcinogenic when administered to mice and rats for up to 2 years. The systemic exposures (AUCs) of unbound apixaban in male and female mice at the highest doses tested (1500 and 3000 mg/kg/day) were 9 and 20-times, respectively, the human exposure of unbound drug at the MRHD of 10 mg/day. Systemic exposures of unbound apixaban in male and female rats at the highest dose tested (600 mg/kg/day) were 2- and 4-times, respectively, the human exposure.

Apixaban was not mutagenic in the bacterial reverse mutation (Ames) assay, and not clastogenic in Chinese hamster ovary cells *in vitro*, in a 1-month *in vivo*/*in vitro* cytogenetics study in rat peripheral blood lymphocytes, or in a rat micronucleus study *in vivo*.

Apixaban had no effect on fertility in male or female rats when given at doses up to 600 mg/kg/day, a dose resulting in exposure levels that are 2 and 4-times, respectively, the human exposure.

Apixaban administered to female rats at doses up to 1000 mg/kg/day from implantation through the end of lactation produced no adverse findings in male offspring (F_1 generation) at doses up to 1000 mg/kg/day, a dose resulting in exposure that is 5-times the human exposure. Adverse effects in the F_1-generation female offspring were limited to decreased mating and fertility indices at 1000 mg/kg/day, a dose resulting in exposure that is 5-times the human exposure.

Sponsor's proposal:

(b) (4)

Reviewer's recommendation:

Section 13.2 was omitted in the dabigatran and rivaroxaban labels and should be omitted from the apixaban label.

2 Drug Information

2.1 Drug

CAS Registry Number: 503612-47-3

Generic Name: Apixaban

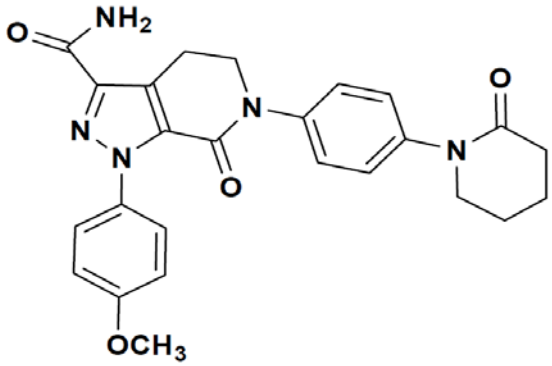
Code Names: BMS-562247, DPC-AG0023

Chemical Name: 1-(4-Methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide

Molecular Formula/Molecular Weight: $C_{25}H_{25}N_5O_4$/459.50

Structure:

Figure 1: Structure of Apixaban



Pharmacologic Class: Factor Xa inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

(b) (4)

IND 68598, DCRP (11/09/2006)

2.3 Drug Formulation

Apixaban is formulated for oral administration as immediate release, film-coated tablets containing either 2.5 or 5 mg of active compound. The tablets also contain anhydrous lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulfate, and magnesium stearate. The film coating for the 2.5 mg tablet is (b) (4) which contains hypromellose (b) (4) lactose monohydrate, titanium dioxide, triacetin, and iron oxide yellow. The film coating for the 5 mg tablet is (b) (4)

(b) (4), which contains hypromellose (b) (4) lactose monohydrate, titanium dioxide, triacetin, and iron oxide red.

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of apixaban tablets.

2.5 Comments on Impurities/Degradants of Concern

A detailed review of the apixaban impurities was filed in DARRTS on October 31, 2011.

2.6 Proposed Clinical Population and Dosing Regimen

Apixaban is being developed for the prevention and treatment of multiple thrombosis-mediated conditions. Under NDA 202,155, apixaban is proposed for the prevention of stroke or systemic embolism associated with atrial fibrillation. The dosing regimen in the Phase 3 trial was 5 mg of apixaban once a day in atrial fibrillation patients with normal renal function.

2.7 Regulatory Background

Apixaban submissions have been reviewed (b) (4)

Documents originally submitted to NDA 202,155 on 09/29/2010 included nonclinical study reports. Additional submissions were made to NDA 202,155 on 11/03/2010 and 08/18/2011 prior to the PDUFA submission on 09/28/2011.

Table 1: Apixaban Regulatory Submissions

Application	Division	Initial date	Indication
(b) (4)			
IND 68598	DCRP	11/09/2006	Prevention of thromboembolic events in atrial fibrillation patients
(b) (4)			
NDA 202,155	DCRP	09/28/2011	Prevention of stroke or systemic associated with atrial fibrillation

(b) (4),
DCRP Division of Cardiovascular and Renal Products

3 Studies Submitted

3.1 Studies Reviewed

None.

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Please refer to the nonclinical review of NDA 202,155 by P. Harlow filed on 02/21/2012.

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

/s/

PATRICIA P HARLOW
05/16/2012

THOMAS PAPOIAN
05/16/2012
I concur.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202,155
Supporting document/s:	Electronic Document Room (EDR)
Applicant's letter date:	09/28/11, 09/29/10 (Nonclinical)
CDER stamp date:	09/28/11, 09/19/10 (Nonclinical)
Product:	Apixaban
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Bristol-Myers Squibb Company and Pfizer
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	Patricia P. Harlow, Ph.D.
Supervisor/Team Leader:	Thomas Papoian, Ph.D., D.A.B.T.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Alison Blaus

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202,155 are owned by Bristol-Myers Squibb Company and Pfizer or are data for which Bristol-Myers Squibb Company and Pfizer has obtained a written right of reference. Any information or data necessary for approval of NDA 202,155 that Bristol-Myers Squibb Company and Pfizer do not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202,155.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Apixaban, an inhibitor of the coagulation Factor Xa (FXa), is being developed as an antithrombotic/anticoagulant agent (b) (4). Under NDA 202,155, apixaban is proposed for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. The dosing regimen in the Phase 3 trial was 5 mg of apixaban twice a day in patients with normal renal function.

1.2 Brief Discussion of Nonclinical Findings

This document is an addendum to the previous nonclinical review filed on 2/21/12. The two studies reviewed in this document (b) (4) could not be located in the NDA 202,155 submission.

As a follow-on study to the previous pharmacokinetic interaction study using activated charcoal, dogs received five treatments in a Latin-square design. The treatments were apixaban (5 mg/kg) alone, apixaban followed by oral administration of a low dose of activated charcoal (250 mg/kg) at 3 hours after the apixaban dose, apixaban followed by oral administration of a low dose of activated charcoal at 5 hours after the apixaban dose, apixaban followed by oral administration of a low dose of activated charcoal at 3 and 5 hours after the apixaban dose, or apixaban followed by oral administration of a high dose of activated charcoal (2500 mg/kg) at 3 hours after the apixaban dose. The greatest reduction of apixaban $AUC_{(0-24)}$ was 45.7% for the high dose charcoal treatment at 3 h post apixaban dose. Neither the low dose nor the high dose treatment significantly decreased Cmax values. However, the high dose charcoal treatment significantly increased the clearance as well as decreased the plasma trough concentration and mean residual time. This additional study in dogs provides further support that activated charcoal may be useful in cases of apixaban overdose.

The second study examined the effect of hemodialysis on circulating concentrations of apixaban after oral or intravenous administration to four fasted male beagle dogs. The dogs received the following four sequential treatments separated by at least 48 hours: oral apixaban (5 mg/kg) without hemodialysis, intravenous apixaban (1 mg/kg) without hemodialysis, intravenous apixaban (1 mg/kg) with hemodialysis, and oral apixaban (5 mg/kg) with hemodialysis. Over the total 4 hour dialysis period, approximately 19% and 5.5% of the apixaban dose was recovered in the dialysate after intravenous and oral dosing, respectively. Although the overall $AUC_{(0-24hr)}$ of apixaban was not reduced, the $AUC_{(0-4hr)}$ was reduced 19.8% and 6.5% during dialysis following intravenous and oral apixaban administration, respectively. The mean plasma apixaban Cmax concentrations for the four dogs were reduced 24% and 12% during dialysis after intravenous and oral administration, respectively. These results indicate that apixaban is dialyzable and dosage adjustments may be necessary for patients taking apixaban while undergoing dialysis treatments.

1.3 Recommendations

1.3.1 Approvability

NDA 202,155 for apixaban is approvable from a pharmacology and toxicology perspective for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation.

1.3.2 Additional Non Clinical Recommendations

The sponsor should be encouraged to conduct a study in patients undergoing dialysis. The label should indicate that apixaban is dialyzable.

1.3.3 Labeling

Section 2.2 indicates that [redacted] (b) (4) The sponsor should be encouraged to conduct a study with rivaroxaban in patients undergoing dialysis. The label should indicate that "A preclinical study in dogs demonstrated that apixaban was found in the dialysate after oral and intravenous administration of apixaban administration.

Section 10 already indicates that [redacted] (b) (4) No change is necessary.

2 Drug Information

2.1 Drug

CAS Registry Number: 503612-47-3

Generic Name: Apixaban

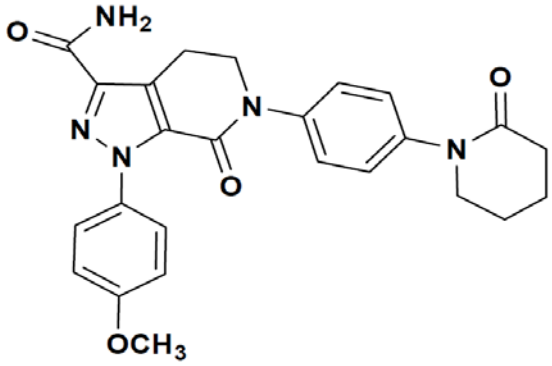
Code Names: BMS-562247, DPC-AG0023

Chemical Name: 1-(4-Methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide

Molecular Formula/Molecular Weight: $C_{25}H_{25}N_5O_4$/459.50

Structure:

Figure 1: Structure of Apixaban



Pharmacologic Class: Factor Xa inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

(b) (4)

IND 68598, DCRP (11/09/2006)

2.3 Drug Formulation

Apixaban is formulated for oral administration as immediate release, film-coated tablets containing either 2.5 or 5 mg of active compound. The tablets also contain anhydrous lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulfate, and magnesium stearate. The film coating for the 2.5 mg tablet is (b) (4), which contains hypromellose (b) (4), lactose monohydrate, titanium dioxide, triacetin, and iron oxide yellow. The film coating for the 5 mg tablet is (b) (4) which contains hypromellose (b) (4) lactose monohydrate, titanium dioxide, triacetin, and iron oxide red.

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of apixaban tablets.

2.5 Comments on Impurities/Degradants of Concern

A detailed review of the apixaban impurities was filed in DARRTS on October 31, 2011.

2.6 Proposed Clinical Population and Dosing Regimen

Apixaban is being developed for the prevention and treatment of multiple thrombosis-mediated conditions. Under NDA 202,155, apixaban is proposed for the prevention of

stroke or systemic embolism associated with atrial fibrillation. The dosing regimen in the Phase 3 trial was 5 mg of apixaban once a day in atrial fibrillation patients with normal renal function.

2.7 Regulatory Background

Apixaban submissions have been reviewed (b) (4) . Documents originally submitted to NDA 202,155 on 09/29/2010 included nonclinical study reports. Additional submissions were made to NDA 202,155 on 11/03/2010 and 08/18/2011 prior to the PDUFA submission on 09/28/2011.

Table 1: Apixaban Regulatory Submissions

Application	Division	Initial date	Indication
(b) (4)			
IND 68598	DCRP	11/09/2006	Prevention of thromboembolic events in atrial fibrillation patients
(b) (4)			
NDA 202,155	DCRP	09/28/2011	Prevention of stroke or systemic associated with atrial fibrillation

(b) (4), DCRP Division of Cardiovascular and Renal Products

3 Studies Submitted

3.1 Studies Reviewed

The two studies listed in Table 2 are reviewed in this document and were not previously reviewed. These studies (b) (4) could not be located in the NDA 202,155 submission. However, one other study (930039361) in supporting document 294 had been submitted to NDA 202,155 and was reviewed in the previous nonclinical review of NDA 202,155 (2/21/12).

Table 2: List of Studies Reviewed

Document Number	Study Title
930046725	Effects of Activated Charcoal Administration on Pharmacokinetics of Apixaban (BMS-562247) Following Oral Administration in Male Dogs (follow-on)
930046779	Effect of Hemodialysis on Circulating Concentrations of Apixaban (BMS-562247) following oral and Intravenous Dosing in Male Dogs

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Nonclinical review of NDA 202,155 by P. Harlow filed on 02/21/2012 was referenced.

4 Pharmacology

No additional pharmacology study was submitted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

In the previously reviewed Study 930039361, pharmacokinetics of apixaban were determined in four male beagle dogs after oral administration of 5 mg/kg apixaban in four sequential treatments that were separated by at least three days. Apixaban alone was administered during the first treatment period and apixaban followed by oral administration of activated charcoal (250 mg/kg) at 0.25, 1, and 3 hours after the apixaban dose was administered in the subsequent three treatment periods. The results indicated that all activated charcoal treatments decreased apixaban AUC values; however treatment at 3 hours after apixaban administration had the greatest effect of 37%, 42% and 51% for $AUC_{(0-24)}$, $AUC_{(2-24)}$, and $AUC_{(4-24)}$, respectively (Table 3).

Table 3: Reviewer's Summary - Results from Study 930039361

Parameter/Treatment	Mean ±SD (Range)	
	Time of charcoal treatment after apixaban dose	
	T1: No charcoal	T4: 3 hour
AUC $_{(0-24)}$, µg•h/mL	78.6 ± 7.0	49.2 ± 1.8*
% AUC reduction	0	37.0%
Cmax, µg/mL	9.35 ± 1.33	8.07 ± 0.80
Tmax, h	4 ± 0	2.5 ± 1
C24, µg/mL	0.47 ± 0.32	0.06 ± 0.03*
MRT, h	8.54 ± 2.31	5.28 ± 0.63
T1/2, h	5.69 ± 2.16	3.03 ± 0.24
CL/F, mL/h/kg	60.1 ± 15.6	100.9 ± 4.1*
* $p<0.05$		

Study title: Effects of Activated Charcoal Administration on Pharmacokinetics of Apixaban (BMS-562247) Following Oral Administration in Male Dogs (follow-on)

Study no.: 930046725 (Document 930046725)

Conducting laboratory and location: (b) (4)

Drug, lot #, and % purity: Apixaban (BMS-562247), lot 7A28071, purity 99.6%

The pharmacokinetics of apixaban (BMS-562247) was determined in five fasted male beagle dogs after oral administration of 5 mg/kg apixaban in 0.5% Tween 80 in Labrafil suspension in five sequential treatments that were separated by at least three days. In a Latin-square design, the treatments were apixaban alone, apixaban followed by oral administration of a low dose of activated charcoal (250 mg/kg) at 3 hours after the apixaban dose, apixaban followed by oral administration of a low dose of activated charcoal at 5 hours after the apixaban dose, apixaban followed by oral administration of a low dose of activated charcoal at 3 and 5 hours after the apixaban dose, or apixaban followed by oral administration of a high dose of activated charcoal (2500 mg/kg) at 3 hours after the apixaban dose. Blood was collected at 0.5, 1, 2, 4, 6, 8, 12, 14, 20, and 24 hours after apixaban administration. After preparation of plasma, apixaban concentrations were determined by a valid LC/MS/MS method. If the animal vomited, the vomit was also collected for analysis.

The low dose activated charcoal treatment decreased plasma apixaban $AUC_{(0-24)}$ values by 15.5%, 6.9%, and 21.5% when administered at 3 h, at 5 h, and at both 3 and 5 h, respectively, post apixaban dose (Table 4). However, the greatest reduction of apixaban $AUC_{(0-24)}$ was 45.7% for the high dose charcoal treatment at 3 h post apixaban dose. Neither the low dose nor the high dose treatment significantly decreased Cmax values. However, the high dose charcoal treatment significantly increased the clearance (CL/F) as well as decreased the plasma trough concentration (C24) and mean residual time (MRT).

The sponsor maintains that these results are consistent with the first charcoal study. However, the reviewer notes that the low dose charcoal treatment at 3 hr post apixaban dose was less effective in the current study than in the previous study. For example, the low dose activated charcoal treatment at 3 hr decreased apixaban $AUC_{(0-24)}$ values by 15.5% in the current study and by 37% in the previous study (Table 3).

Although this study suggests that activated charcoal may be used to treat an apixaban overdose in humans, the reviewer notes that 20-fold higher doses of apixaban (100 mg/kg) were used in the chronic dog toxicity study. However, the $AUC_{(0-24)}$ (97 µg•h/mL) and Cmax (10.8 µg•h/mL) values in the absence of charcoal treatment in this study are 31- and 54-fold the total human $AUC_{(0-24)}$ (3.1 µg•h/mL) and Cmax (0.2 µg/mL) (clinical pharmacology report CV185046) in patients with atrial fibrillation.

Table 4: Reviewer's Summary – Results from Study 930046725 – Effect of Activated Charcoal on Apixaban Exposure

Parameter/ Treatment		Mean ±SD			
		Charcoal treatment, time after apixaban dose			
	T1: No charcoal (Apixaban alone)	T2: 250 mg charcoal at 3 hr post dose	T3: 250 mg charcoal at 5 hr post dose	T4: 250 mg charcoal at 3 and 5 hr post dose	T5: 2500 mg charcoal at 3 hr post dose
AUC $_{(0\text{-}24)}$, µg•h/mL	97.0 ±23.6	82.0 ± 10.5	90.3 ±19.7	76.1 ± 8.3	52.6 ± 4.7*
% AUC reduction	0	15.5	6.9	21.5	45.7
Cmax, µg/mL	10.8 ± 1.9	10.9 ± 1.2	11.1 ± 2.6	11.0 ± 0.9	9.96 ± 1.5
Tmax, h	4.5 ± 1.3	2.8 ± 0.5	3.5 ± 0.7	3.2 ± 0.8	2.6 ± 0.5
C24, µg/mL	0.29 ± 0.13	0.29± 0.13	0.24 ± 0.11	0.18 ± 0.14	0.07 ± 0.06*
MRT, h	7.71 ± 0.5	6.81 ± 0.8	6.71 ± 0.9	6.25 ± 0.76	4.82 ± 0.6*
T1/2, h	3.17 ± 0.6	4.13 ± 1.0	3.34 ± 0.6	3.15 ± 0.6	3.01 ± 0.6
CL/F, mL/h/kg	47.4 ± 8.6	60.4 ± 7.5	57.3 ± 15.2	65.6 ± 8.0	95.0 ± 9.4*
Animals vomiting	#2, #3 (3 times), #4	#1		#1, #4, #5	#1, #4, #5
Total vomit as % dose	22%, 3.3%, 8.3%	4.4%		4.4%, 0.2%, 1%	9.5%, NC, NC

* p<0.05, NC = Not enough to collect

Study title: Effect of Hemodialysis on Circulating Concentrations of Apixaban (BMS-562247) following oral and Intravenous Dosing in Male Dogs

Study no.: 930046779 (Document 930046779)

Conducting laboratory and location: (b) (4)

Drug, lot #, and % purity: Apixaban (BMS-562247), lot 7A28071, purity 99.6%

This study examined the effect of hemodialysis on circulating concentrations of apixaban (BMS-562247) after oral or intravenous administration to four fasted male beagle dogs. The dogs received the following four sequential treatments with apixaban separated by at least 48 hours: oral without hemodialysis, intravenous without hemodialysis, intravenous with hemodialysis, and oral PK with hemodialysis, respectively. The oral gavage treatment was 5 mg/kg in 0.5% Tween 80 in Labrafil suspension followed by a 10 mL flush of water. The intravenous treatment was 1 mg/kg in 35% 2-hydroxypropyl-beta-cyclodextrin 10 mM phosphate, pH 7.0 by bolus injection followed by 0.5 mL flush of 0.9% NaCl. Dialysis parameters were 50 mL/min blood flow and 300 mL/min dialysate flow in the opposite direction on a Fresenius 2008H Hemodialysis System started approximately 5 min after administration of the apixaban dose. Blood was collected from a port immediately before the dialysis filter at 0.166, 0.25, 0.5, 1, 2, 3, 4, 8, 12, 16, and 24 hr and at 0.166, 0.25, 0.5, 1, 2, and 3 hr from the port immediately after the dialysis filter. Plasma was prepared and apixaban concentrations were determined by a valid LC/MS/MS method.

Over the total 4 hour dialysis period, approximately 19% and 5.5% of the apixaban dose was recovered in the dialysate after intravenous and oral dosing, respectively (Table 5). Although the overall $AUC_{(0\text{-}24hr)}$ of apixaban was not reduced, the $AUC_{(0\text{-}4hr)}$ was reduced 19.8% and 6.5% during dialysis following intravenous and oral apixaban administration, respectively. The mean plasma apixaban Cmax concentrations for the four dogs were reduced 24% and 12% during dialysis after intravenous and oral administration of apixaban. These decreases for Cmax during dialysis after intravenous and oral administration of apixaban were 36% and 19% when the values for the one dog exhibiting an increased Cmax value following dialysis were omitted (Figure 2). These results indicate that apixaban is dialyzable and dosage adjustments may be necessary for patients taking apixaban while undergoing dialysis treatments.

Table 5: Sponsor's Tables from Study 930046779

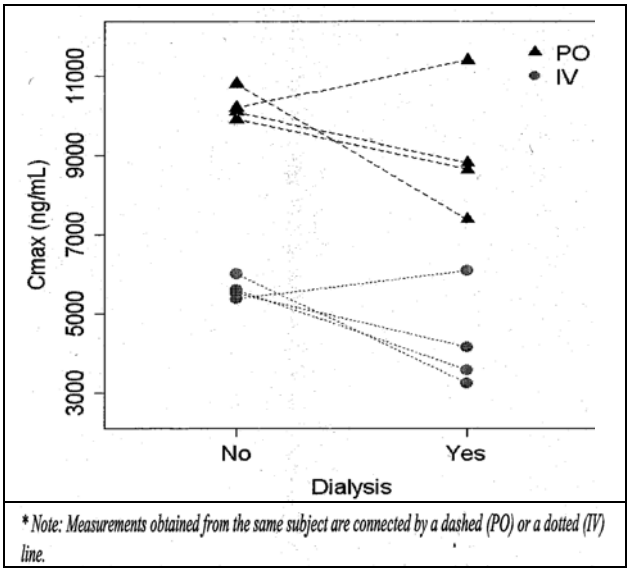
Dialysate

	Time or animal number	IV	PO
Concentration (ng/mL)	0-1 h	43.8 ± 9.7	13.4 ± 11.4
	1-2 h	29.1 ± 8.7	41.1 ± 12.5
	2-3 h	20.7 ± 3.8	60.1 ± 13.0
	3-4 h	16.9 ± 3.4	60.1 ± 9.3
Amount recovered during 0-4 h (µg)	# 201	2284	3244
	# 202	1704	2106
	# 203	2349	3083
	# 204	1611	3179
Total amount recovered (µg)		1987 ± 461	2902 ± 535

Plasma pharmacokinetics

PK Parameters	PO	PO Dialysis	IV	IV Dialysis
Cmax (µg/mL)	10.3 ± 0.39	9.05 ± 1.69	5.64 ± 0.27	4.29 ± 1.26
Tmax (h)	3.75	3.25	0.25	0.35
AUC0-4 (µg•h/mL)	25.3 ± 3.9	20.3 ± 6.3	12.3 ± 0.5	11.5 ± 1.5
AUC0-24 (µg•h/mL)	82.3 ± 8.6	83.2 ± 21.3	19.3 ± 2.2	20.9 ± 2.5
T1/2 (h)	5.3 ± 3.9	5.9 ± 2.4	4.3 ± 0.8	3.9 ± 0.5

Figure 2: Sponsor's Figure from Study 930046779



** Note: Measurements obtained from the same subject are connected by a dashed (PO) or a dotted (IV) line.*

5.2 Toxicokinetics

No additional toxicokinetic study was submitted.

6 General Toxicology

No additional toxicology study was submitted.

7 Genetic Toxicology

No additional genetic toxicology study was submitted.

8 Carcinogenicity

No additional carcinogenicity study was submitted.

9 Reproductive and Developmental Toxicology

No additional reproductive and developmental toxicology study was submitted.

10 Special Toxicology Studies

No additional special toxicology study was submitted.

11 Integrated Summary and Safety Evaluation

The two studies reviewed in this document do not alter the safety assessment provided in the previous nonclinical review of NDA 202,155 by P. Harlow filed on 02/21/2012.

The additional pharmacokinetic interaction study in dogs provides further support that activated charcoal may be useful in cases of apixaban overdose.

The second study examined the effect of hemodialysis on circulating concentrations of apixaban (BMS-562247) after oral or intravenous administration to dogs. The results indicate that apixaban is dialyzable and dosage adjustments may be necessary for patients taking apixaban while undergoing dialysis treatments. The sponsor should be encouraged to conduct a study in patients undergoing dialysis. The label should indicate that apixaban is dialyzable.

12 Appendix/Attachments

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

/s/

PATRICIA P HARLOW
04/13/2012

THOMAS PAPOIAN
04/13/2012
I concur.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202155
Supporting document/s:	Electronic Document Room (EDR)
Applicant's letter date:	09/28/11, 09/29/10, 12/02/11, 10/28/11
CDER stamp date:	09/28/11, 09/19/10, 12/02/11, 10/28/11
Product:	Apixaban
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Bristol-Myers Squibb Company and Pfizer
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	Patricia P. Harlow, Ph.D.
Supervisor/Team Leader:	Thomas Papoian, Ph.D., D.A.B.T.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Alison Blaus

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202155 are owned by Bristol-Myers Squibb Company and Pfizer or are data for which Bristol-Myers Squibb Company and Pfizer has obtained a written right of reference. Any information or data necessary for approval of NDA 202155 that Bristol-Myers Squibb Company and Pfizer do not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202155.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Apixaban, an inhibitor of the coagulation Factor Xa (FXa), is being developed as an antithrombotic/anticoagulant agent [redacted] (b) (4). Under NDA 202,155, apixaban is proposed for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. The dosing regimen in the Phase 3 trial was 5 mg of apixaban twice a day in patients with normal renal function.

1.2 Brief Discussion of Nonclinical Findings

Apixaban (BMS-562247) directly inhibits the coagulation Factor Xa (FXa) in the absence of antithrombin III with an inhibition constant (Ki) in the nanomolar range. As a result, apixaban decreases the conversion of prothrombin to proteolytically active thrombin thereby decreasing thrombin-mediated activation of both coagulation and platelet aggregation. In purified in vitro systems, apixaban inhibits FXa with high affinity and selectivity compared with related proteases involved in coagulation, fibrinolysis, and digestion. Addition of apixaban to normal human plasma caused concentration-dependent prolongations in standard coagulation assays. Apixaban also inhibited prothrombinase-bound and clot-bound FXa activity. Although apixaban did not inhibit platelet aggregation induced by adenosine diphosphate, α-thrombin, thrombin receptor-activating peptide (SFLLRN-NH2) or collagen, apixaban inhibited tissue factor-induced platelet aggregation.

Apixaban dose-dependently inhibited both arterial and venous thrombosis after intravenous administration in rabbits, rats, and dogs. In rats and rabbits, doses of apixaban that produced antithrombotic efficacy were lower than doses that increased bleeding times after standardized incisions. Addition of apixaban to aspirin or clopidogrel treatment increased blood flow and reduced thrombus weights in a rabbit arterial thrombosis model.

Apixaban did not significantly affect the central nervous, cardiovascular, respiratory, or renal systems based on safety pharmacology evaluations conducted as part of the repeated dose studies in rats and dogs. In vitro studies showed that neither apixaban nor O-desmethyl apixaban sulfate significantly inhibited peak tail hERG currents or significantly altered action potential duration in rabbit Purkinje fibers. Studies in conscious dogs after either oral or intravenous administration of apixabn identified no significant changes in hemodynamic or ECG parameters, including QTc.

Pharmacokinetic studies conducted in rats, dogs and chimpanzees showed rapid absorption of orally administered apixaban in these species. Apixaban bioavailability, volume of distribution, clearance, and half-life varied somewhat among species; however, the fraction not bound to serum protein varied 10-fold among species.

In vivo studies in rats, dogs and humans indicated preferential distribution of apixaban into plasma. Tissue distribution studies in rats using radio-labeled apixaban showed that the gastrointestinal tract (stomach, small intestine, large intestine, cecum), thyroid, urinary bladder, adrenal glands, liver, and kidneys were exposed to the highest

concentrations of apixaban, while the brain, heart and bone marrow were exposed to the lowest concentrations. However, radioactivity was still present in the eyes of pigmented rats at 168 hours after dosing. Elimination half-life estimates for apixaban-equivalents were less than 5 hours for adrenal glands, blood, plasma, and testes, but greater than 60 hours for the eyes, bone marrow, and cecum. In an acceptable neutral red uptake phototoxicity assay using mouse fibroblasts (Balb/c 3T3), apixaban exhibited no phototoxic potential.

In rats, dogs, rabbits and mice, excretion occurred primarily through the feces as unchanged apixaban. An intravenous study in rats indicated that unchanged apixaban appeared to be directly excreted into the gastrointestinal tract. Urinary excretion of orally administered apixaban was higher in humans than in animals, although most excretion was fecal in all species, including humans.

Apixaban is excreted into the milk of rats primarily as the parent apixaban. Concentrations of apixaban in milk were higher than concentrations in blood and plasma at all time points. The milk:plasma concentration ratio was 30 over the 24 hour collection period. Assuming a milk flow of 2.75 mL/hr in Sprague Dawley rats, a relatively high value of approximately 12% of the maternal dose is excreted into the milk over a 24 hour period.

Evaluation of metabolites in animals and man indicated that metabolism of apixaban primarily involves O-desmethylation and hydroxylation, followed by sulfation or glucuronidation with sulfation predominating in humans. Although apixaban was the predominant radioactive component in plasma samples from humans, rats, mice and dogs, O-desmethyl apixaban glucuronide was the predominant component in rabbit plasma.

The most predominant circulating human metabolite was O-desmethyl apixaban sulfate, which represented 24% to 27% of the total apixaban exposure. Rats and dogs had low circulating levels of O-desmethyl apixaban sulfate and its precursor, O-desmethyl apixaban. However, the total exposure to O-desmethyl apixaban sulfate and O-desmethyl apixaban in dogs was higher than the exposure in man. Therefore, O-desmethyl apixaban sulfate was considered toxicologically qualified.

In vitro studies using mouse, rat, dog, monkey, and human hepatocytes showed low rates of apixaban metabolism with O-desmethyl apixaban representing <1.5% of the total radioactivity. Incubation of [^{14}C]-apixaban with human intestinal microsomes, human liver microsomes or rat liver S9 produced low amounts of O-desmethyl apixaban and two hydroxy apixaban metabolites, but not O-desmethyl apixaban sulfate.

In vitro studies using inhibitors of sulfotransferases (SULT) indicated that SULT1Al is involved in the formation of O-desmethyl apixaban sulfate. A study using human cDNA-expressed sulfotransfereases showed that only SULT1Al*2 and SULT1A2*1 formed O-desmethyl apixaban sulfate with the rate for SULT1Al*2 seven-fold higher than that for SULT1A2*1.

Additional in vitro studies using a panel of recombinant human cDNA-expressed cytochrome P450 isoforms indicated that CYP1A2, 2J2, and 3A4 formed higher amounts of O-desmethyl apixaban (M2) and CYP3A4 and 3A5 formed higher amounts

of hydroxy apixaban (M4) and 3-hydroxy apixaban (M7) than other P450 enzymes. Incubation of [^{14}C]-apixaban with human liver microsomes in the presence or absence of CYP isoform-selective inhibitors indicated that the formation of M2, M4, and M7 was significantly decreased by CYP3A4 inhibitors (ketoconazole and troleandomycin) and partially inhibited by a CYP1A2 inhibitor (furafylline). The correlation between the formation rates of M2, M4, and M7 and activities of ten P450 enzymes were evaluated using a panel of human liver microsomes from 16 donors. Overall, the studies indicate that the apixaban metabolites M2, M4, and M7 are primarily produced by CYP3A4 with some contribution of CYP1A2 for formation of M2.

An in vitro study using cultured human hepatocytes indicated that apixaban is not a significant inducer of CYP1A2, CYP2B6 or CYP3A4/5 activity or mRNA expression. Using human liver microsomes and CYP probe substrates, the IC_{50} values for apixaban inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP3A4 and CYP2C19 were greater than 20 µM.

Apixaban is a substrate for P-glycoprotein (P-gp) and the multidrug transport protein mouse breast cancer resistance protein (BCRP). In vitro studies with several types of cultured cells indicated the absorption of apixaban may be affected by P-gp inhibitors. However, the inability of apixaban to inhibit P-gp suggests that apixaban will not alter the absorption of drugs that are P-gp substrates. In vitro studies indicated greater inhibition of apixaban transport by strong inhibitors of both CYP3A4 and P-gp (ketoconazole) than with co-administration with an inhibitor of P-gp alone (diltazem). In Madin-Darbin canine kidney (MDCKII) over-expressing the multidrug transport protein mouse breast cancer resistance protein (MDCKII-BCRP) apixaban efflux was completely inhibited by Ko134, a known BCRP inhibitor.

In a pharmokinetic interaction study, dogs received apixaban alone during the first treatment period. In the subsequent three treatment periods, apixaban administration was followed by oral administration of activated charcoal (250 mg/kg) at 0.25, 1, and 3 hours after the apixaban dose . Although all activated charcoal treatments decreased apixaban $AUC_{(0\text{-}24h)}$ values, treatment with activated charcoal at 3 hours after apixaban administration produced the greatest decrease of 37%.

Single dose toxicology studies were conducted in mice and rats. Although the oral LD_{50} for apixaban was greater than 4000 mg/kg in both species, the lowest lethal intravenous doses were 50 and 25 mg/kg in mice and rats, respectively.

The longest chronic repeat dose toxicology studies were 6 and 12 duration in rats and dogs, respectively. Compound-related findings (prolongation of coagulation parameters, evidence of bleeding, and effects on red cell parameters) were related to the pharmacological activity of apixaban. Transient effects on serum potassium values were noted in both rats and dogs. The NOAELs of 600 mg/kg/day in rats and 100 mg/kg/day in dogs correspond to exposure ratios of 3.5-4 and 20-28 times, respectively, the human exposure of unbound apixaban at a dose of 5 mg twice a day.

In two valid and acceptable assays, apixaban did not induce excess reverse mutations in five recommended bacterial strains in the Ames assay in the absence and presence of metabolic activation. In one valid and acceptable assay, apixaban did not increase excess micronucleus formation in rats after three oral doses of 2000 mg/kg/day, the limit

dose for the assay. Apixaban did not induce excess chromosomal aberrations in Chinese hamster ovary cells in vitro in either the presence or absence of metabolic activation at a concentration that produced acceptable toxicity.

Although O-desmethyl apixaban sulfate was not detected after incubation of [^{14}C]-apixaban with rat liver S9, O-desmethyl apixaban and two hydroxy apixaban metabolites were formed. The genotoxicity of O-desmethyl apixaban and two hydroxy apixaban metabolites were evaluated in the in vitro bacterial reversion and mammalian chromosomal aberration assays. Separate genotoxicity testing of O-desmethyl apixaban sulfate was not considered necessary, because 1) adequate levels of the O-desmethyl apixaban were formed with rat liver S9 and 2) O-desmethyl apixaban sulfate is a phenol sulfate conjugate with no pharmacological activity and no structural alerts.

In an acceptable carcinogenicity study, CD-1 male and female mice received oral dietary doses of apixaban for up to 105 and 97-100 weeks, respectively. The incidences of a few tumors were increased in the higher dose groups compared to those in the control groups. However, none of these tumors had a p-value that attained the significance level of $p < 0.005$ required for a common tumor to be considered positive. Therefore, no statistically significant neoplastic findings were related to apixaban treatment under the conditions of this study. At the maximum dosages of 1500 mg/kg/day in males and 3000 mg/kg/day in females during week 25, the exposure ratios were 8.8 and 20.1 times, respectively, the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations.

In an acceptable carcinogenicity study, Sprague Dawley rats received oral dietary doses of apixaban for up to 104 weeks. The incidences of a few tumors were increased in the higher dose groups compared to those in the control groups. However, none of these tumors had a p-value that attained the significance level required for the tumor to be considered positive. Therefore, no statistically significant neoplastic findings were related to apixaban treatment under the conditions of this study. At the maximum dosage of 600 mg/kg/day in males and females during Week 52, the exposure ratios were 2 and 4 times, respectively, the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations.

In an acceptable oral fertility and early embryo development study, male and female rats were treated with 0, 50, 200 and 600 mg/kg/d. No drug-related effects were observed on estrous cycling, mating or fertility in treated males or treated females, although body weight gain decreased slightly in the high dose males. The NOAEL for fertility, maternal toxicity and embryo toxicity was 600 mg/kg. The NOAEL for paternal toxicity was 200 mg/kg. At the NOAEL dosages of 200 and 600 mg/kg in males and females, the exposure ratios were 2.7 and 4.2 fold the human exposure at a dose of 5 mg BID based on unbound apixaban concentrations.

In an acceptable oral embryo-fetal development study in rats, the NOAEL for maternal toxicity was 1000 mg/kg/day, because of a decrease in mean body weight gain and the significantly increased incidence of signs of vaginal bleeding at 3000 mg/kg/day. The NOAELs for fetal toxicity and malformation were 3000 mg/kg/day. At the NOAEL dosages of 1000 and 3000 mg/kg/day, the exposure ratios were 4.2 times the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations. In

a separate toxicokinetic experiment on gestation day 15, fetal exposures to apixaban at 2 to 8 hours after dosing were 5.1 to 11.7% of maternal exposures of females dosed at 3000 mg/kg.

In an acceptable oral embryo-fetal development study in mice, the NOAEL for maternal toxicity was 1500 mg/kg/day, although the incidence of clinical signs of bleeding increased slightly with dose. The NOAELs for fetal toxicity and malformation were 1500 mg/kg/day. The toxicokinetic evaluation indicated that mean maternal exposures were similar at all doses, indicating saturation of exposure. At the NOAEL dosage of 1500 mg/kg, the exposure ratio of unbound apixaban concentrations was 19 times the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations.

In an acceptable embryo-fetal development study in rabbits, the maximum possible dose that could be orally administered was 1500 mg/kg. The NOAEL for maternal toxicity was 1500 mg/kg. The NOAELs for fetal toxicity and malformations were 1500 mg/kg. At the dosage of 1500 mg/kg, the exposure ratio was only 0.33 times the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations.

In an adequate embryo-fetal development study, apixaban was administered at 0, 1.25, 2.5 and 5.0 mg/kg intravenously once daily from gestation day 7 to 19 to New Zealand White rabbits that were sacrificed on gestation day 29. A subsequent study evaluated maternal and fetal toxicokinetics on GD 19 in rabbits receiving only the high dose of 5 mg/kg. The NOAELs of 5 mg/kg for maternal toxicity, fetal toxicity and malformation corresponded to $AUC_{(0\text{-}24h)}$ exposures of 0.95 µg.hr/mL in the main study, and 2.6-3.2 µg.hr/mL in the extension study, which evaluated timepoints earlier than 30 minutes. The exposure ratios at 5 mg/kg in the two toxicokinetic assessments were 0.93 and 2.9 times, respectively, the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations.

Results of a dose finding prenatal/postnatal development study in rats indicated that dosing in the main study did not need to be interrupted during parturition. In the main study, pregnant rats received a daily dose of apixaban by gavage from gestation day 6 to lactation day 20. In blood samples collected on lactation day 6 at 4 hours after dosing with 25, 200 and 1000 mg/kg, prothrombin times (PT) were prolonged 1.65, 2.25, and 2.64 fold, respectively, compared to the control means. No drug related mortality of F0 dams occurred, although the incidence of bleeding signs, primarily during gestation, increased in the treated groups with the incidence similar in the mid and high dose groups. The NOAEL for F0 maternal effects, F1 pre- and peri-natal toxicity, F1 postnatal development, F1 male mating/fertility and F2 pre-natal fetal toxicity was 1000 mg/kg/day. The NOAEL for F1 female mating/fertility was 200 mg/kg based on decreased mating index at 1000 mg/kg that was slightly below the historical range. At the NOAEL dosages of 200 and 1500 mg/kg, the exposure ratios were 4.9 and 5.4 times, respectively, the human exposure at a dose of 5 mg twice a day based on unbound apixaban concentrations.

In the main juvenile toxicology study in rats, oral doses of 0, 10, 50, or 600 mg/kg/day apixaban were administered daily for 3 months to four sets of Sprague-Dawley rats beginning at postnatal day 4. The Set 1 animals were necropsied at the end of dosing. The Set 2 animals were used to evaluate reproductive effects after the end of treatment

and then were necropsied on postnatal day 120-130 after a 1 month recovery period. The Set 3 and Set 4 animals were used to evaluate toxicokinetics on postnatal day 10 and coagulation on postnatal day 21, respectively. At the end of treatment mean body weights and body weight gains were decreased in a dose-dependent manner relative to the vehicle control group in the males, but not the females. Except for the expected prolongation of coagulation times, the only potential drug-related adverse effect was the increased incidence of unilateral or bilateral degeneration of the testicular seminiferous tubules in the high dose Set 1 males that were necropsied at the end-of dosing. The incidence of testicular degeneration was 33%, a value above the maximum of 26.7% in the historical control data. Although this finding appeared reversible in the recovery group, the reviewer believes that the testicular finding is drug-related, because of the severity of the testicular finding and the correlation with hypospermia in the epididymides. Mean apixaban $AUC_{(0\text{-}24h)}$ and Cmax values on postnatal day 21 were 1.6-2.9 and 1.9-3.8 fold higher, respectively, than those on postnatal day 87. These increased apixaban plasma concetrations on postnatal day 21 correlated with the increased prothrombin time values on postnatal day 21 compared to those on postnatal day 87. The plasma concentrations at 2 or 4 hr post dose on postnatal day 10 were 1.9 to 6.6 fold higher than the plasma concentrations at the respective timepoints on postnatal day 21. These increases in the main study are consistent with similar increases in apixaban plasma concentrations on postnatal day 10 compared to those on postnatal day 21 in the juvenile dose-range finding study.

1.3 Recommendations

1.3.1 Approvability

NDA 202,155 for apixaban is approvable from a pharmacology and toxicology perspective for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation. Most of the toxicities identified in the non-clinical studies are either attributable to the pharmacodynamic effect of apixaban or satisfactory safety margins have been demonstrated relative to human therapeutic exposures. Although no animal deaths occurred during parturition in the pre/postnatal development study, the label needs to warn women of child-bearing potential of the high risks for bleeding during labor and delivery

1.3.2 Additional Non Clinical Recommendations

Because of the severity of the testicular degeneration in the juvenile study, the reviewer recommends that an additional juvenile animal study be conducted to determine if a critical period for toxicity can be identified before any pediatric studies are conducted in humans.

1.3.3 Labeling

A separate labeling review will be written after discussions with the reviewers in the Division of Hematology Products.

2 Drug Information

2.1 Drug

CAS Registry Number: 503612-47-3

Generic Name: Apixaban

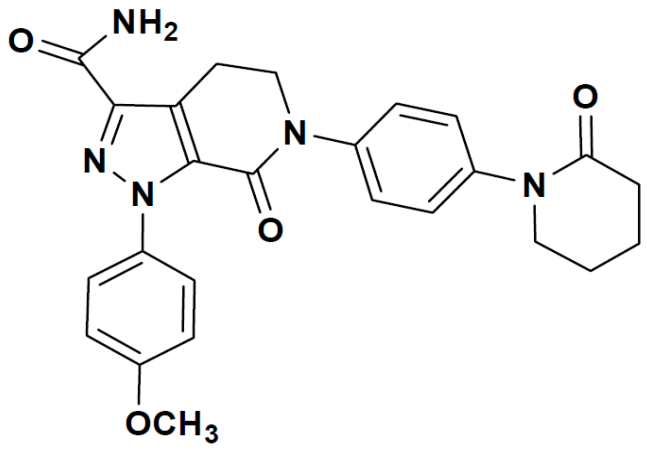
Code Names: BMS-562247, DPC-AG0023

Chemical Name: 1-(4-Methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide

Molecular Formula/Molecular Weight: $C_{25}H_{25}N_5O_4$/459.50

Structure:

Figure 1: Structure of Apixaban



Pharmacologic Class: Factor Xa inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

(b) (4) IND 68598

2.3 Drug Formulation

Apixaban is formulated for oral administration as immediate release, film-coated tablets containing either 2.5 or 5 mg of active compound. The tablets also contain anhydrous

lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulfate, and magnesium stearate. The film coating for the 2.5 mg tablet is [redacted], which contains hypromellose [redacted], lactose monohydrate, titanium dioxide, triacetin, and iron oxide yellow. The film coating for the 5 mg tablet is [redacted], which contains hypromellose [redacted], lactose monohydrate, titanium dioxide, triacetin, and iron oxide red.

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of apixaban tablets.

2.5 Comments on Impurities/Degradants of Concern

A detailed review of the apixaban impurities was filed in DARRTS on October 31, 2011. The paragraphs below summarize the findings.

The sponsor identified eight potential impurities in the apixaban drug substance by HPLC. In lots used for general toxicology studies, most of the impurities, except [redacted] and [redacted] were present at levels less than the specified level of [redacted]% . However, the exposure level of each impurity in animals was at least 90-fold higher than the maximum predicted exposure level in humans. Therefore, the eight apixaban impurities are considered qualified in terms of general toxicology.

Since the one lot of apixaban drug substance used for the apixaban Ames assay had very low levels of three impurities, the reviewer requested that the CDER Computational Toxicology Group evaluate all apixaban immpurities for genotoxicity. Although the Derek for Windows application did not identify structural alerts for any of the apixaban impurities, the FDA Computational Toxicology Group predicted [redacted] to be positive in the Ames assay and [redacted] to be positive in the micronucleus assay.

[redacted] was present at adequate levels in the Ames assay of the apixaban drug substance and the reliability of the positive prediction in the Ames assay by Computational Toxicology is considered weak. Although the sponsor synthesized impurity [redacted] and could potentially evaluate this impurity directly in an Ames assay, such an evaluation is not considered necessary.

Although [redacted] was not present at adequate concentrations in the in vivo micronucleus assay, chromosomal aberrations were evaluated ex vivo in a 1-month repeat dose study using an apixaban lot in which [redacted] was present at [redacted]%. The plasma in high dose rats contained [redacted] at 362-fold the maximum possible human exposure. Therefore, the clastogenic potential of [redacted] was adequately tested and an additional in vivo study for clastogenicity is not necessary.

The sponsor identified six compounds in the apixaban synthesis as genotoxic. The levels of these compounds are controlled [redacted] in the manufacturing process of apixaban. Spiking/purging studies confirmed that each of the genotoxic compounds was adequately purged and the level of each genotoxic compound in the final apixaban drug substance is <10 ppm. The maximum exposures of each compound as well as the total combined exposure of all six compounds to a patient taking the

maximum dose of 10 mg of apixaban per day are less than the Threshold of Toxicological Concern of 1.5 µg per day. Therefore, the levels of the identified genotoxic compounds are being adequately controlled.

2.6 Proposed Clinical Population and Dosing Regimen

Apixaban is being developed for the prevention and treatment of multiple thrombosis-mediated conditions. Under NDA 202,155, apixaban is proposed for the prevention of stroke or systemic embolism associated with atrial fibrillation. The dosing regimen in the Phase 3 trial was 5 mg of apixaban twice a day in atrial fibrillation patients with normal renal function.

2.7 Regulatory Background

Apixaban submissions have been reviewed (b) (4) Documents originally submitted to NDA 202155 on 09/29/2010 included nonclinical study reports. Additional submissions were made to NDA 202155 on 11/03/2010 and 08/18/2011 prior to the PDUFA submission on 09/28/2011.

Table 1: Apixaban IND and NDA Applications

Application	Division	Initial date	Indication
(b) (4)			
IND 68598	DCRP	11/09/2006	Prevention of thromboembolic events in atrial fibrillation patients
(b) (4)			
NDA 202155	DCRP	09/28/2011	Prevention of stroke or systemic associated with atrial fibrillation

(b) (4), DCRP Division of Cardiovascular and Renal Products

3 Studies Submitted

Appendix 1 contains a list of all study reports submitted under NDA 202155.

3.1 Studies Reviewed

The studies listed in Table 2 are reviewed in this document and were not previously reviewed.

Table 2: List of Studies Reviewed

Document Number	Study Title (Study Number)
Pharmacology	
930028740	Apixaban Inhibits Human Clot-Bound Factor Xa Activity in Vitro
930028741	Effects of Apixaban on Tissue-Factor Induced Human Platelet Aggregation In Vitro
Pharmacodynamic Drug Interactions	
930028739	Arterial Antithrombotic and Bleeding Time Effects of Apixaban in Combination with Antiplatelet Therapy in Rabbits
Pharmacodynamics/ADME	
Analytical Assay	
930033949	Method Validation Report for the Determination of BMS-562247 in Low Sample Volume Rat Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M)
930012207	Method Validation Report for The Determination of BMS-562247 in Mouse Plasma (K_2EDTA) Using LC-API/MS/MS (AR562F)
930015560	Method Validation Report for The Determination of BMS-562247 in Mouse Fetal Embryo Extract Using LC-API/MS/MS (AR562H)
930013967	Method Validation Report for The Determination of BMS-730823 in Rat Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M3)
930014148	Method Validation Report for The Determination of BMS-730823 in Dog Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M4)
930014144	Method Validation Report for The Determination of BMS-730823 in Mouse Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M5)
930013970	Method Validation Report for The Determination of BMS-730823 in Rabbit Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M6)
930013971	Method Validation Report for The Determination of BMS-730823 in Monkey Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M7)
930016662	BMS-562247: Intravenous Study of Embryo-Fetal Development in Rabbits – Toxicokinetics (DN05050)
930014442	Mass Balance of Radioactivity after Oral Administration of [^{14}C]BMS-562247 to Male CD-1 Mice (MBA00221)
930017359	Mass Balance of Radioactivity After Intravenous and Oral Administration of [^{14}C]BMS-562247 to Female New Zealand White Rabbits (MBA00222)
930037205	Assessment of Inhibition of Digoxin Efflux in LLC-PK_1 Cell Monolayers (300963740)
930037853	Evaluation of Common NSAIDs' Ability to Inhibit P-gp Efflux.
930024170	In Vitro Evaluation Of BMS-562247 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes (b) (4) 063004)
930039361	Effects of Activated Charcoal Administration on Pharmacokinetics of Apixaban (BMS-562247) Following Oral Administration in Male Dogs
Toxicology	
Repeated Dose Toxicology Studies	
930012967	BMS-562247: Six-Month Oral Toxicity Study in Rats (DN03118)†
Carcinogenicity	
930031442	BMS-562247: 104-Week Dietary Carcinogenicity Study in Mice (DN05068)
930031443	BMS-562247: 104-Week Dietary Carcinogenicity Study in Rats (DN05069)
Reproductive and Developmental Toxicology Studies	
930015215	BMS-562247: Thirteen-Day Intravenous Range-Finding Study in Pregnant Rabbits (DN05006)
930016662	BMS-562247: Intravenous Study of Embryo-Fetal Development in Rabbits (DN05050)

† Only partially reviewed previously

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Dr. Joseph, Dr. Honchel and the reviewer of the current document previously reviewed study reports (b) (4) 68598 as indicated in Table 3. Appendix 1 lists the specific study reports evaluated in each review.

Table 3: List of Previous Reviews References

Application	Supporting Document (SD)	Reviewer	Review Date
(b) (4)	SD 0, 6, 11, 14, 18, 20, 24, 27	D. Joseph	07/18/05
	SD 74, 89, 117, 135, 150	R. Honchel	03/31/09
	SD 264	R. Honchel	02/19/09
	SD 461	R. Honchel	08/31/09
	SD 472	R. Honchel	10/02/09
	SD 513	R. Honchel	03/19/10
	SD 476	R. Honchel	03/26/10
	SD 492	R. Honchel	03/30/10
	SD 0	P. Harlow	09/19/05
	SD 2, 3	P. Harlow	12/02/05
IND 68598	SD 0, 6	P. Harlow	03/21/07

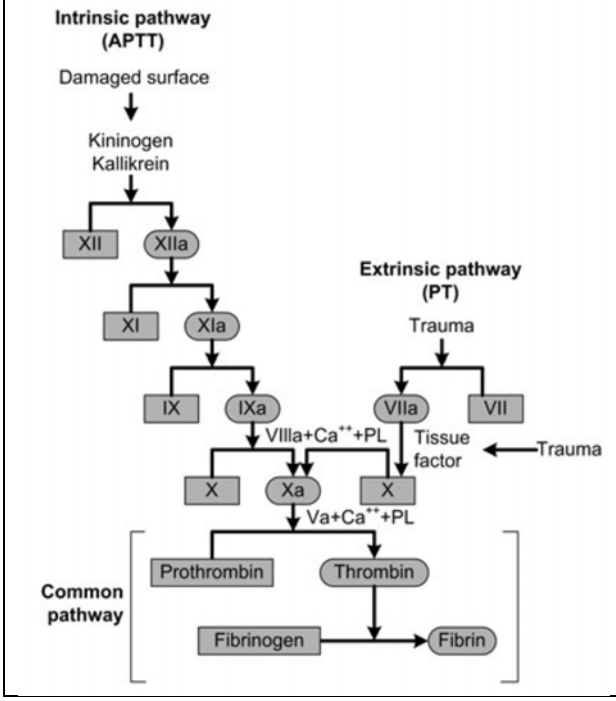
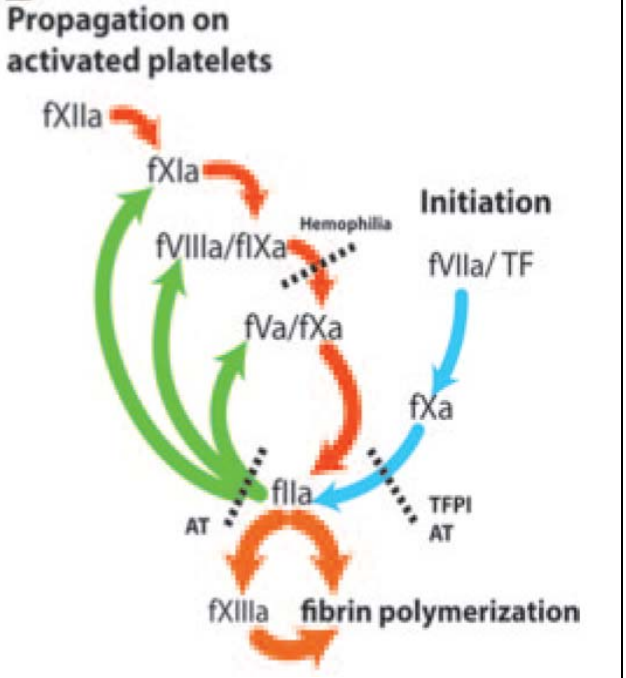
4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action:

Apixaban (BMS-562247) binds directly to and inhibits the activity of Factor Xa, the human plasma serine protease that converts prothrombin to thrombin, which is the serine protease that converts fibrinogen to fibrin in forming a blood clot. The inhibition of Factor Xa by apixaban is independent of other factors, particularly antithrombin III. Factor Xa plays a central role in coagulation as illustrated in either the older cascade model of coagulation (Figure 2A) or the contemporary model of coagulation involving initiation, amplification and propagation (Figure 2B). Since FXa can be generated by both the extrinsic and intrinsic pathways and one molecule of factor Xa can generate more than 1,000 thrombin molecules [Mann et al. 2003], Factor Xa is considered a good target enzyme for the inhibition of coagulation and the prevention of thrombosis.

Figure 2: Two models of coagulation

A: Cascade model (Adams and Bird, 2009)	B: Contemporary model (Tanaka et al. 2009)
	

Although some free Factor Xa (FXa) exists in plasma, FXa normally combines with FVa in a 1:1 complex in the presence of calcium on platelet phospholipid membrane surfaces at activation-dependent receptor sites for FVa or FXa (Fager 2010). Effector cell protease receptor-1 (EPR-1), a 65-kDa membrane receptor for FXa, is only one component of a complex platelet receptor for the prothrombinase complex (Bouchard et al. 1997; Tracy et al. 1992). The concentration of FXa is the rate-limiting component of prothrombinase complex formation and ultimately the generation of thrombin activity (Rand 1996). Prothrombinase is approximately 300,000-fold more active than FXa alone in catalyzing the conversion of prothrombin to thrombin [Nesheim, et al. 1979].

The literature indicates that FXa not only plays a critical role in coagulation and hemostasis, but it also mediates intracellular signaling by protease activated receptors (PAR), which require proteolytic cleavage for activation rather than ligand binding (Borensztajn et al. 2008). Four PAR receptors (PAR-1 to PAR-4) are currently known to be encoded in the mammalian genome (Traynelis and Trejo 2007). Although soluble FXa activates both PAR-1 and PAR-2, FXa in a ternary complex with TF–FVIIa primarily activates PAR-2 (Ruf et al. 2003; Feistritzer et al. 2005). The PAR-1 and PAR-2 receptors are expressed in a large variety of tissues and cells, including the lungs, the cardiovascular system, the epidermis, osteoblasts, the immune system, the kidney, the nervous system, the gastrointestinal tract, the pancreas and the liver (Macfarlane et al. 2001). FXa-dependent signaling can promote cell proliferation, cell migration and fibrosis as well as induce production of pro-inflammatory (Papapetropoulos et al 1998; Yamaguchi et al 1999), expression of adhesion molecules (Senden et al 1998), and tissue factor gene expression (Camerer et al 1999). Therefore, inhibition of FXa may have additional consequences beyond direct inhibition of clot formation.

Drug activity related to proposed indication:

In vitro studies

Apixaban binds to purified human FXa with a Ki at 25^0 of 0.075 nM (Table 4). Lineweaver-Burk analysis indicates apixaban is a competitive inhibitor of FXa relative to a tripeptide substrate. The X-ray crystal structure of apixaban in a complex with human FXa indicated that the apixaban structure is highly complementary to the FXa enzyme active site (Pinto et al. 2007). Therefore, the mechanism of apixaban inhibition of FXa is independent of anti-thrombin III and is unlike that of heparin or low molecular weight heparin. Kinetic analysis using purified human FXa indicated the inhibition of purified FXa by apixaban was rapid and reversible (Luettgen et al 2011). Apixaban also inhibited human FXa activity when present in the prothrombinase complex on washed human platelets or present in clots formed in human platelet-poor plasma.

Table 4: Reviewer's Summary – Inhibition of FXa by Apixaban

Free human FXa enzyme assay	Ki at 25º C Ki at 37º C	0.075 ± 0.003 nM 0.25 ± 0.011 nM
	Dissociation, t½ off	1.4-2.3 min
	k_{on} k_{off}	1.3-2.0 $X10^7$ M^{-1} s^{-1} 11.3 $X10^{-3}$ s^{-1}
	IC_{50}	0.7 nM
Prothrombinase bound FXa	Ki at 25º C	0.63 ± 0.06 nM
	Ki at 37º C	0.62 nM
	Dissociation, t½ off	1.2 min
Clot-bound human FXa, IC_{50} at 37º C		1.3 nM

In purified systems, apixaban inhibits FXa with high affinity and selectivity compared with related proteases involved in coagulation, fibrinolysis, and digestion (Table 5).

Table 5: Reviewer's Summary – Selectivity of Apixaban for FXa

Fold selectivity	Protease
>30,000	thrombin, chymotrysin, plasma kallikrein,
>100,000	Factor IXa, Factor VIIa, Factor XIa, urokinase
>200000	cathepsin B, cathepsin D, cathepsin G, cathepsin L, chymase, MT-SP1 Matriptas, neutrophil elastase, plasmin, trypsin
>300000	activated protein C, complement factor 1, tissue kallikrein-1, tissue plasminogen activator,

The following study reports were not previously reviwed.

Study title: Apixaban Inhibits Human Clot-Bound Factor Xa Activity In Vitro

Study no.: 930028740 (Document 930028740)
Conducting laboratory and location: Bristol Myers Squibb, Hopewell
Drug, lot #, and % purity: BMS-56224, lot not indicated

Clots made with human platelet-poor plasma were washed extensively to remove unbound FXa. The washed clots were incubated first with vehicle or various concentrations of apixaban and then with Factor Va and prothrombin. Prothrombin fragment 1+2 (F1+2) released into supernatant was measured as an indication of clot-

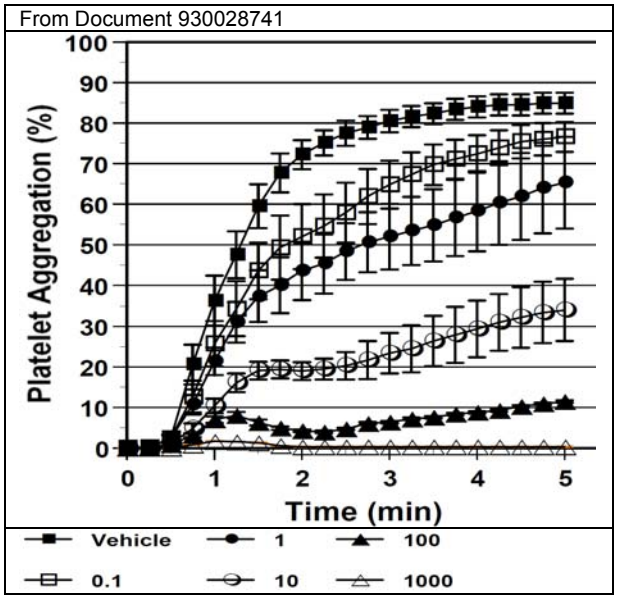
bound FXa activity using an ELISA. Apixaban inhibited clot-bound FX activity with IC_{50} of 1.3 nM at 37º C.

Study title: Effects of Apixaban on Tissue-Factor Induced Human Platelet Aggregation In Vitro

Study no.:	930028741 (Document 930028741)
Conducting laboratory and location:	Bristol Myers Squibb, Hopewell
Drug, lot #, and % purity:	BMS-56224, lot not indicated

Human platelet-rich plasma (PRP) was prepared from citrated human blood, which had been mixed with corn trypsin inhibitor to inhibit the contact factor pathway and with Pefabloc® FG to prevent fibrin polymerization. The PRP was then mixed with vehicle or increasing concentrations of apixaban followed by addition of human tissue factor (TF) with $CaCl_2$ to trigger platelet aggregation measured with an aggregometer. The vehicle-treated PRP (n=10 subjects) was aggregated 85±3% within 5 minutes. Apixaban inhibited TF-induced platelet aggregation with IC_{50} (nM) of 3.5 nM (Figure 3). However, apixaban did not directly inhibit platelet aggregation induced by adenosine diphosphate, α-thrombin, thrombin receptor-activating peptide (SFLLRN-NH2) or collagen. Therefore, apixaban indirectly inhibits platelet aggregation by reducing thrombin production.

Figure 3: Sponsor's Figure – Apixaban Inhibition of Tissue Factor-Induced Platelet Aggregation



Addition of apixaban to normal human plasma causes concentration-dependent prolongations in standard coagulation assays. Although interindividual variation was observed, generally an apixaban concentration of 3.6 μM was required to double the prothrombin time (PT), and a concentration of 7.4 μM was required to double the aPTT compared to control values (Wong et al 2011). In contrast, apixaban at concentrations up to 20 μM did not affect thrombin time.

In vivo studies

The sponsor's table below (Table 6) summarizes the antithrombotic and antihemostatic effects of apixaban as evaluated in anesthetized rats, rabbits, and dogs. The models included arterial-venous shunt thrombosis, tissue factor-stasis venous thrombosis, $FeCl_2$-induced vena cava thrombosis, $FeCl_2$-induced carotid artery thrombosis, electrically induced carotid arterial thrombosis and deep vein thrombosis (a thread-induced vena cava thrombosis model). Hemostasis was assessed in models of cuticle bleeding time, renal cortex bleeding time, and mesenteric bleeding time.

Apixaban dose-dependently inhibited both arterial and venous thrombosis after intravenous administration in rabbits, rats, and dogs. In rats, an apixaban plasma concentration of 5 μM resulted in a 50% inhibition of thrombus weight in the arterial-venous shunt model in rats, but only a 34% increase in bleeding time. In rabbits, a plasma concentration of apixaban that resulted in 80% inhibition of thrombosis in arterial-venous shunt and arterial models produced no increase in bleeding time. In dogs, a plasma concentration of apixaban that inhibited arterial-venous shunt thrombosis by 40% and doubled the time to occlusion produced only a 1.1 to 1.3-fold increase in relative prothrombin time, relative aPTT and relative bleeding time.

Table 6 : Sponsor's Summary - Apixaban Activity in Thrombosis Models

	Model	Endpoint	Results
Rat	AV shunt	Inhibition of thrombus weight	IC_{50} = 5.0 μM; free IC_{50} = 220 nM
FXa K_i = 1.3 nM Plasma protein binding = 95.6%	$FeCl_2$ induced venous thrombosis	Inhibition of thrombus weight	Est IC_{50} = 1.5 μM; free Est IC_{50} = 66 nM
	$FeCl_2$ induced arterial thrombosis	Inhibition of thrombus weight	Est IC_{50} = 2.0 μM; free Est IC_{50} = 89 nM
	Renal cortex bleeding time	Increase of template bleeding time	34% increase at 5 μM
Rabbit	AV shunt	Inhibition of thrombus weight	IC_{50} = 329 nM; free IC_{50} = 109 nM
FXa K_i = 0.16 nM Plasma protein binding = 66.9%	Electric current induced arterial thrombosis	Carotid artery blood flow	EC_{50} = 115 nM; free IC_{50} = 38 nM
	Deep venous thrombosis	Inhibition of clot growth	IC_{50} = 105 nM; free IC_{50} = 35 nM
	Rabbit cuticle bleeding	Prolongation of bleeding time	No increase at dose which inhibited AV shunt and arterial thrombosis up to 80%
Dog	AV shunt	Inhibition of thrombus weight	IC_{50} = 1.4 μM; free IC_{50} = 123 nM
FXa K_i = 1.8 nM Plasma protein binding = 91.2%	Electric current injury of femoral artery	Time to occlusion of femoral artery	EC_{2x} = 1.2 μM; free EC_{2x} = 106 nM

Documents 930028738, 930028749, 930028752

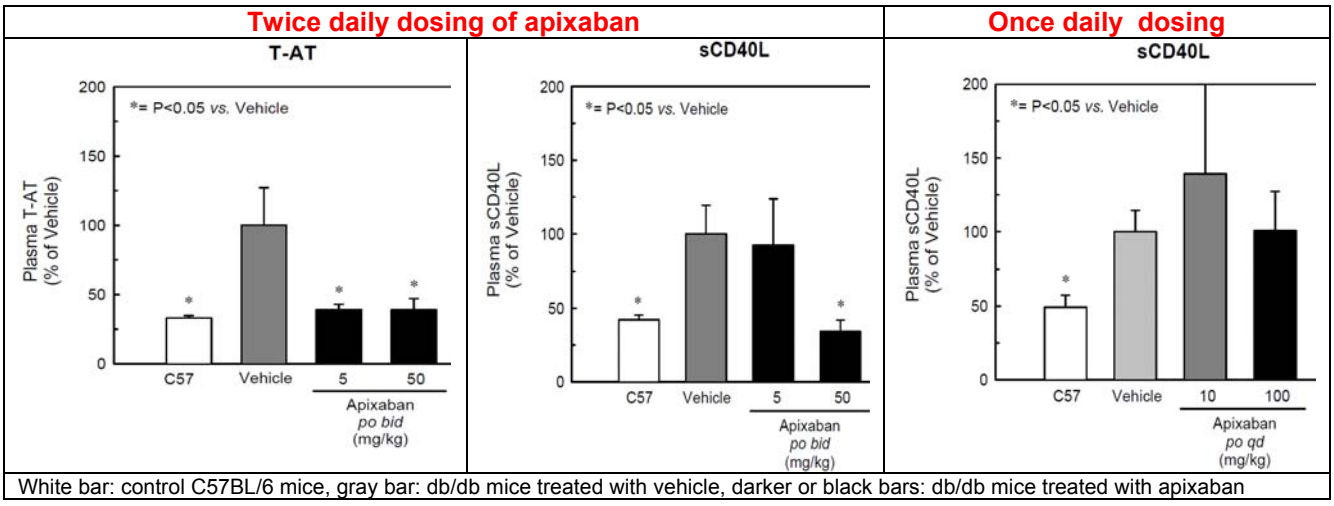
The following study report was not previously reviewed.

Study title: The Effects of Factor Xa Inhibitor, Apixaban, on Elevated Thrombotic Biomarkers in Diabetic/Obese Mice

Study no.: 930028753 (Document 930028753)
Conducting laboratory and location: Bristol Myers Squibb, Hopewell
Drug, lot #, and % purity: BMS-56224, lot not indicated

Elevated plasma levels of the thrombotic biomarkers thrombin-antithrombin III (T-AT) and soluble CD40 ligand (sCD40L) are found in diabetic db/db mice compared to the levels in normoglycemic C57BL/6 mice. Apixaban in vehicle (99.5% Labrafil/0.5% Tween 80) was administered orally at 5 and 50 mg/kg to db/db mice once or twice daily for three days. After dosing on the third day blood samples were collected and plasma prepared for measurement of T-AT and sCD40L by ELISA assays. Administration of apixaban twice daily at 50 mg/kg to db/db mice significantly decreased levels of T-AT and sCD40L compared to the levels in vehicle treated db/db mice. However, administration of apixaban at 5 mg/kg did not reduce the levels of sCD40L, but did reduce the levels of T-AT in db/db mice. Once daily dosing of apixaban was not effective in reducing the levels of these markers in db/db mice compared to twice daily dosing.

Figure 4: Sponsor's Figures – Effects of Apixaban on Plasma T-AT and sCD40L in db/db Mice



White bar: control C57BL/6 mice, gray bar: db/db mice treated with vehicle, darker or black bars: db/db mice treated with apixaban

4.2 Secondary Pharmacology

No significant binding or interaction (≥50%) of apixaban at ≤ 10 µM was observed on 63 receptors, ion channels, or enzymes. At 10 µM apixaban demonstrated only marginal inhibition (20 to 30%) at histamine H2 receptors (21%), muscarinic M1 (20%) and M2 (30%) receptors, NOS second messenger neuronal binding receptors (21%), platelet activating factor receptors (22%), neurokinin NK2 {NKA} receptors (21%), and non-selective vasoactive intestinal peptide receptors (20%).

4.3 Safety Pharmacology

The sponsor concluded that apixaban produced no significant effects on the central nervous, cardiovascular, respiratory, and renal systems based on safety pharmacology evaluations conducted as part of the repeated dose studies in rats and dogs. Apixaban at maximal concentrations of 30 µM did not significantly affect peak tail hERG currents or action potential duration in rabbit Purkinje fibers. Likewise, the M1 metabolite (O-desmethyl apixaban sulfate, BMS-730823) at maximal concentrations of 30 µM did not significantly affect peak tail hERG currents or action potential parameters in rabbit

Purkinje fibers. In conscious dogs after either oral or intravenous administration of apixaban, no significant changes were produced in hemodynamic or ECG parameters, including QTc, in two studies.

4.4 Pharmacodynamic Drug Interactions

Pharmacodynamic drug interactions of apixaban in combination with aspirin alone or aspirin plus clopidogrel were evaluated in the rabbit arterial thrombosis model discussed below.

Study title: Arterial Antithrombotic and Bleeding Time Effects of Apixaban in Combination with Antiplatelet Therapy in Rabbits

Study no.:	930028739 (Document 930028739)
Conducting laboratory and location:	Bristol Myers Squibb, Hopewell
Drug, lot #, and % purity:	BMS-56224, lot not indicated

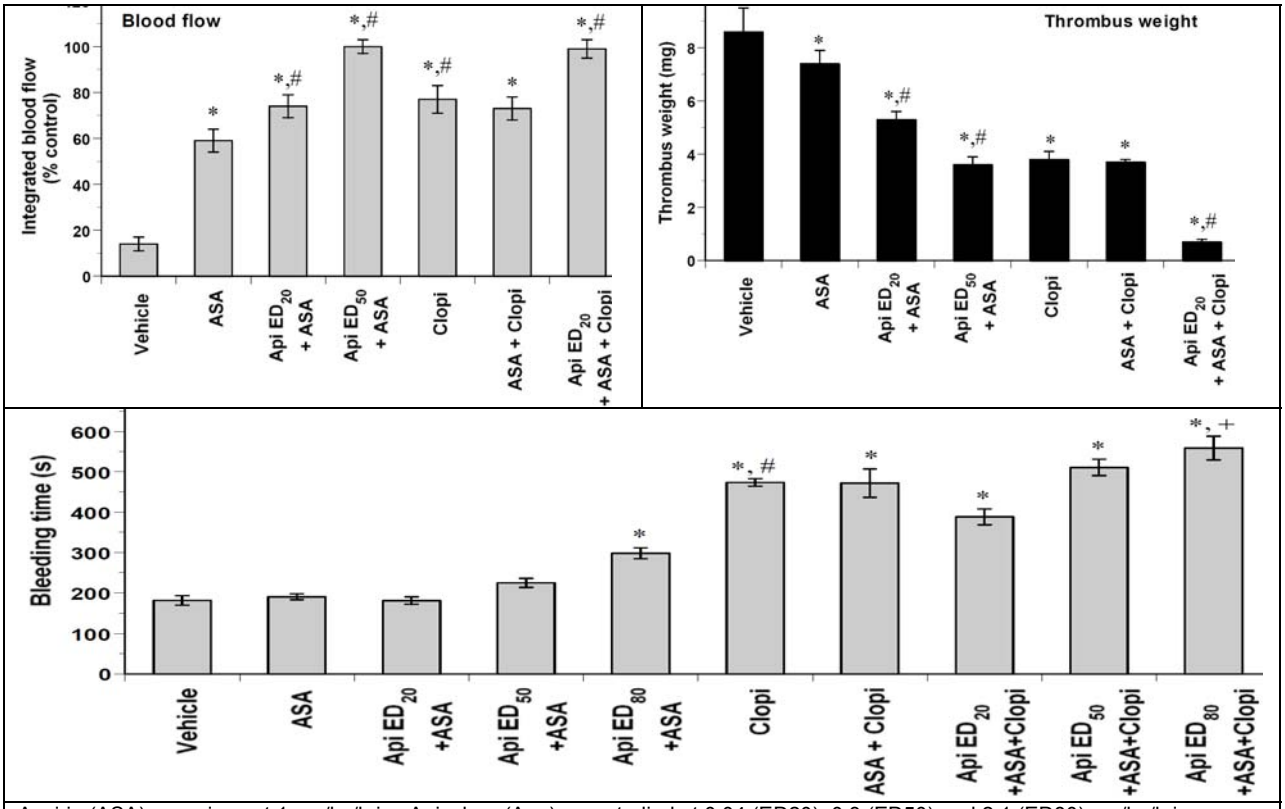
The effects of apixaban on antithrombotic efficacy and cuticle bleeding times were evaluated in the rabbit arterial thrombosis model (Wong et al. 2007) during treatment in combination with aspirin alone or aspirin plus clopidogrel. Male New Zealand White rabbits (n = 6 per group) received clopidogrel at 3 mg/kg/d or its vehicle (0.6% methylcellulose) orally once daily for three days. Following the third oral dose of clopidogrel or vehicle, rabbits were anesthetized, intravenous infusion of apixaban, aspirin, or the combination of apixaban and aspirin was initiated, and an electromagnetic flow probe was placed on a segment of an isolated carotid artery to monitor blood flow. Thrombosis induced by electrical stimulation of the carotid artery and carotid blood flow was measured continuously over a 90-min period to monitor thrombosis-induced occlusion. Integrated carotid blood flow was determined as area under the flow versus time curve. The apixaban intravenous doses of 0.04, 0.3 and 2.1 mg/kg/h represented the antithrombotic ED_{20}, ED_{50}, and ED_{80} doses, respectively, of apixaban alone for reduction of thrombus weight in this model (Wong et al 2008). Aspirin was used at 1 mg/kg/h, a dose previously shown to completely block ex vivo platelet aggregation response to arachidonic acid in rabbits.

The average carotid blood flow, thrombus weight and bleeding time in the vehicle-treated animals (controls) were 20±3 mL/min, 8.6±0.9 mg and 181±12 s, respectively, and blood flow gradually decreased with total occlusion of the artery in 35 minutes after electrical stimulation. Aspirin alone produced some improvement in the patency of the thrombotic artery and integrated blood flow with a slight reduction of thrombus formation in the absence of a significant increase in bleeding time (Figure 5). Addition of apixaban at ED_{20} and ED_{50} to aspirin produced a dose-dependent increase in integrated blood flow, and reduced thrombus weight without a significant increase in bleeding time compared with aspirin alone. However, bleeding time was significanly increased 1.6-fold when apixaban at ED_{80} was added to to aspirin compared with aspirin alone.

The addition of aspirin and clopidogrel produced no additional increase in the integrated blood flow or reduction of thrombus weight. However, addition of apixaban at ED_{20} to

the combination of aspirin and clopidogrel produced a higher integrated blood flow and a greater reduction of thrombus weight compared with the dual combination of aspirin and clopidogrel. Clopidogrel alone significantly increased bleeding time by 2.6-fold compared to vehicle. The addition of clopidogrel dose-dependently increased bleeding time in combination with aspirin plus apixaban at the ED_{20}, ED_{50}, and ED_{80}.

Figure 5: Sponsor's Figures - Effects on Blood Flow, Thrombus Weight and Bleeding Time



Aspirin (ASA) was given at 1 mg/kg/h i.v. Apixaban (Apu) was studied at 0.04 (ED20), 0.3 (ED50) and 2.1 (ED80) mg/kg/h i.v. Clopidogrel (Clopi) was dosed orally at 3 mg/kg/d. *P < 0.05, compared to aspirin; #P < 0.05, compared with the preceding group and +P < 0.05, compared to ASA plus Clopi. Means ± SE and n = 6 per group.

5 Pharmacokinetics/ADME/Toxicokinetics

Drs. Joseph, Honchel, and Harlow previously reviewed most of the studies describing the absorption, distribution, metabolism and excretion of apixaban. The sections below briefly summarize this information. A few studies not previously reviewed are reviewed below.

5.1 PK/ADME

Methods of analysis

Blood samples were collected with potassium EDTA as the anticoagulant.
Plasma or embryo extract was prepared and an internal standard was added. The liquid chromatography/tandem mass spectrometry (LC/MS/MS) method used acetonitrile extraction, while the LC-API/MS/MS (liquid chromatography atmospheric pressure

ionization tandem mass spectrometry) method used acetonitrile: methanol (3:1) extraction prior to analysis. The methods are summarized in Table 7.

Table 7: Reviewer's Summary – Methods of Analysis

Document/ analyte	Species/ sample	Volume µL	Detection	Internal standard	LLQ, ng/mL	ULQ, ng/mL	Precision (for LLQ)	Accuracy (for LLQ)
930033949 BMS-562247	Rat plasma	20	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤5% (≤20%)	≤9% (≤20%)
930008022 BMS-562247	Rat plasma	50	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤6% (≤20%)	≤3% (≤20%)
930008990 BMS-562247	Rabbit plasma	50	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤5% (≤20%)	≤3% (≤20%)
930012207 BMS-562247	Mouse plasma	50	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤6% (≤20%)	≤3% (≤20%)
930015560 BMS-562247	Mouse embryo extract	50	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤10% (≤20%)	≤14% (≤20%)
930013967 BMS-562247 [BMS-730823]	Rat plasma	50	LC-API/ MS/MS	BMS-562247-M4 (BMS-561389)	2 (5)	2000	≤5% (≤20%) [8%]	≤5% (≤20%) [3%]
930014148 BMS-562247 [BMS-730823]	Dog plasma	50	LC-API/ MS/MS	BMS-562247-M4 (BMS-561389)	2 (5)	2000	≤15% (≤20%)	≤15% (≤20%)
930008644 BMS-562247	Rat embryo extract	50	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤10% (≤20%)	≤8% (≤20%)
930011627 BMS-562247	Dog plasma	50	LC-API/ MS/MS	BMS-562247-M4	2	2000	≤5% (≤20%)	≤3% (≤20%)
930014144 BMS-562247 [BMS-730823]	Mouse plasma	50	LC-API/ MS/MS	BMS-562247-M4 (BMS-561389)	2 (5)	2000	≤3% (≤20%) [≤5%]	≤6% (≤20%) [≤10%]
930013970 BMS-562247 [BMS-730823]	Rabbit plasma	50	LC-API/ MS/MS	BMS-562247-M4 (BMS-561389)	2 (5)	2000	≤4% (≤20%) [9%]	≤7% (≤20%) [4%]
930013971 BMS-562247 [BMS-730823]	Monkey plasma	100	LC-API/ MS/MS	BMS-562247-M4 (BMS-561389)	2 (5)	2000	≤3% (≤20%) [7%]	≤11% (≤20%) [4%]
930002436 BMS-562247	Dog plasma	100	LC/ MS/MS	BMS-561389	5	1000	≤8%	≤6%
930002437 BMS-562247	Rat plasma	100	LC/ MS/MS	BMS-561389	5	1000	≤11%	≤10%

Absorption

In vitro

Apixaban is absorbed *in vitro* by isolated sections from all regions of rat intestine with the permeability coefficient highest for the jejunum and lowest for the duodenum and colon. In the jejunum, the serosal to mucosal drug permeability was greater than the mucosal to serosal permeability, indicating that apixaban is a substrate for one or more efflux pumps.

In Caco-2 human intestinal epithelial cell monolayers, which express a number of efflux and influx transporters, the apparent permeability coefficient of apixaban (20 to 200 µM) was 12 to 37 fold higher in the basal-to-apical direction than the apical-to-basal direction indicating that apixaban is a substrate for intestinal efflux pump(s). LY335979, a P-glycoprotein (P-gp) inhibitor, and probenecid, a selective multiple drug resistance (MDR) gene-associated protein inhibitor, incompletely decreased the apixaban efflux ratio in Caco-2 cultures. However, the known inhibitors of P-gp, cyclosporin A and ketoconazole, reduced the efflux ratio of apixaban by 84 and 93%, respectively, indicating that apixaban is a P-gp substrate. Since the efflux of digoxin, a known P-gp substrate, was completely inhibited by cyclosporin A and ketoconazole, the incomplete inhibition of apixaban efflux suggests that apixaban is also a substrate for transporters other than P-gp.

Using parental LLC-PK1 (porcine kidney-derived) cells and LLC-PK1 cells expressing the human P-gp protein, the efflux ratios of [^{14}C]-apixaban indicated active basal-to-apical transport across P-gp-transfected LLC-PK1 monolayers. Ketoconazole inhibited the efflux ratio of 5 and 50 µM apixaban in P-gp-expressing LLC-PK1 cells with IC_{50} values of 2.9 and 5.4 µM, respectively. In parental cells lacking human P-gp, efflux ratios of 1.4 to 4.4 were observed, suggesting non-P-gp-mediated transport of apixaban.

The transport of apixaban was not significantly altered in MDCK cells expressing OAT1 and in HEK-293 cells expressing human OATP1B1, OATP1B3, OATP2B1, OAT3 or OCT1 cDNA as single transporter compared to non-transfected cells. However, mean efflux ratios of [^{14}C]-apixaban were between 1 to 3 in parental cells Madin-Darbin canine kidney (MDCKII) cells and between 8 and 19 in MDCKII cells over-expressing the multidrug transport protein mouse breast cancer resistance protein (MDCKII-BCRP). Furthermore, the known BCRP inhibitor, Ko134, completely inhibited apixaban efflux indicating apixaban is a substrate for the BCRP transporter. In contrast, diltiazem and naproxen did not inhibit apixaban efflux in either parental or MDCKII-BCRP cells. Ketoconazole only partially inhibited apixaban transport by 33% in MDCKII-BCRP cells, but almost completely inhibited (91%) the efflux of apixaban in parental MDCKII cells. Since MDCKII cells express canine P-gp, the effects of ketoconazole in this study are consistent with apixaban being a substrate of P-gp as well as substrate of BCRP. Overall, apixaban is subject to both P-gp-mediated and non-P-gp-mediated efflux, which could limit in vivo absorption.

In vivo

Following single oral doses, apixaban had a mean bioavailability (F) of 34, 88, and 51 % in rats, dogs, and chimpanzees, respectively. The maximum concentration (Cmax) occurred at 0.5, 1 and 2 hours following oral administration in rats, dogs, and chimpanzees, respectively, indicating rapid absorption. Table 8 summarizes the apixaban pharmacokinetic parameters after single dose administration to rats, dogs and chimpanzees.

Table 8: Reviewer's Summary – Pharmacokinetic Parameters in Rats, Dogs and Chimpanzee

	Rat		Dog		Chimpanzee	
Document	930002493		930002493		930002493	
Route	IV	Oral	IV	Oral	IV	Oral
Dose, mg/kg	0.5	2.0	0.2	0.5	0.2	0.5
Cmax, µg/mL	NA	1.1	NA	1.1	NA	1.6
Tmax, hr.	NA	0.5	NA	1.0	NA	2.0
$AUC_{(0-24)}$, µg*hr/mL	2.0	2.7	3.9	8.0	10.1	13.6
Cl (mL/min/kg)	4.3	NA	0.87	NA	0.3	NA
Vss (L/kg)	0.34/0.28	NA	0.27/0.32	NA	0.17	NA
T½, hr	2.6/1.2	3.5/2.8	5.0	5.8	6.8	4.9
F (%)	NA	30/37	NA	60/115	NA	43/59
NA = not applicable						

Distribution

The volume of distribution, Vss, for apixaban in single dose pharmacokinetic studies was between 0.27 and 0.34 L/kg for rats and dogs. Since the standard value for the plasma volume is 0.05-0.2 L/kg, some distribution to the extracellular space occurs in rats and dogs. The volume of distribution for apixaban in chimpanzees was lower than in rats and dogs indicating more confinement to intravascular space.

The blood to plasma distribution of radioactive apixaban was 1.03 and 1.09 using blood from dogs and humans, respectively, in vitro. These values suggest a uniform distribution between plasma and red blood cells.

Although plasma protein binding of apixaban did not vary greatly between male and female rats, dogs and humans, protein binding did vary greatly among other species with the highest in rats (96%) and the lowest in mice (51-56%) (Table 9). As a result, exposure comparisons between species should be based on the unbound fraction of apixaban. The principal human serum binding protein was serum albumin.

Table 9: Reviewer's Summary - Apixaban Protein Binding

Species/Strain	Apixaban Concentration, µg/mL	Fraction bound, %	Fraction unbound, %
Rat	0.46-4.59	95.2-96.4	3.6-4.8
Mouse	45.9, 230	56, 51	44, 49
Rabbit	0.46	66.2	33.8
	1.38	62.3	37.7
	4.59	61.5	38.5
Dog	0.46-4.59	91.0-93.7	6.3-9
Cynomolgus monkey	0.46	57.6-58.2	41.8-42.4
	1.38	51.9-61.4	38.6-48.1
	4.59	61.2-63.5	36.5-38.8
Chimpanzee	0.46-4.59	94.3-95.1	4.9-5.7
Human	0.46	86.8-86.9	13.1-13.2
Human serum*	0.034-0.11		7.1
Human albumin		66	
Human α1-acid glycoprotein		9	
From Document 930002493 * From Document 930031025			

Tissue distribution studies were conducted in male pigmented Long-Evans rats and in male and female albino Sprague Dawley rats following oral administration of a single dose of [^{14}C]-apixaban. The former study detected radioactivity by tissue excision and liquid scintillation counting; the latter study detected radioactivity by quantitative whole body autoradiography. Although the dose of radioactivity was the same in both studies, the dose of apixaban was 4-fold higher in the former study than the latter study.

In the Long Evans rat, the mean apixaban-equivalent concentration-time profiles for blood and plasma were similar with maximum concentrations at 4 hours after dosing and background levels at 48 hours after dosing (Table 10). The mean blood: plasma concentration ratios of 0.45 to 0.7 suggest preferential partitioning of radioactive apixaban into plasma. These results are different than the in vitro results that suggest a uniform distribution between blood and plasma.

The mean concentration-time profiles for apixaban-equivalents in most tissues, except gastrointestinal tissues, were generally similar to the profile for plasma with a Tmax at 4 hours post dose. Most of the radioactive dose at each sampling time was associated with gastrointestinal tract contents. Tissues with the highest concentrations (Cmax) and/or exposure (AUC) were those of the gastrointestinal tract (stomach, small intestine, large intestine, cecum) followed by the thyroid, urinary bladder, adrenal glands, liver, and kidneys. The brain, heart and bone marrow had the lowest concentrations. Exposures in pigmented and non-pigmented skin were similar at 43 and 46 µg-eq*hr/gm. However, radioactivity was still present in the eyes of pigmented rats at 168 hours after dosing. Elimination half-life estimates for apixaban-equivalents were less than 5 hours for adrenal glands, blood, plasma, and testes (Table 11). The eyes, bone marrow, and cecum had the longest half-lives (>60 hours).

Tissue distribution and profiles of radioactivity in the albino Sprague Dawley rat was generally similar to that in the pigmented Long Evans rats. However, the Tmax was shorter (1 hour) and little radioactivity was observed beyond 8 hours post dose, except in the large and small intestine and the cecum

Table 10: Reviewer's Summary - Apixaban Tissue Distribution in Male Rats

	Radioactivity (µg equivalents/gm) in male rats										
Strain	Long-Evans pigmented rat							Sprague Dawley albino rat			
Document	930005742							930036905			
Dose	20 mg/kg, 5 µCi/mg, 100 µCi/kg							5 mg/kg, 20 µCi/mg, 100 µCi/kg			
Detection	Tissue excision, liquid scintillation counting							Quantitative whole body autoradiography			
Hours post dose	**1**	**4**	**12**	**24**	**48**	**98**	**168**	**1**	**4**	**8**	**24**
Adrenal	5.84	26.8	0.26	0.04	0	0	0	1.2	0.76	0.17	NS
Blood	2.32	2.35	0.16	0.022	0	0	0	1.18	0.60	0.08	NS
Brain	0.06	0.1	0	0	0	0	0	0.04	BQL	BQL	BQL
Cecum	1.75	5.7	6.52	2.25	2.08	0.42	0.39	1.09	0.45	5.25	0.1
Eyes	0.78	2.37	1.07	0.35	0.24	0.14	0.071	0.38	0.21	0.08	NS
Heart	0.04	0	0.03	0.05	0	0.04	0	0.70	0.45	0.08	NA
Intestine, large	3.25	5.2	13.0	1.93	0.85	0.30	0.21	0.73	0.64	0.12	0.30
Intestine, small	25.4	44.0	4.29	3.67	0.20	0.14	0.34	3.09	0.78	2.24	0.08
Kidney	3.17	7.33	0.34	0.12	0.02	0.013	0	1.72	1.36	0.39	BQL
Liver	8.94	16.0	0.92	0.28	0.07	0.05	0.036	4.03	3.75	0.53	BQL

	Radioactivity (µg equivalents/gm) in male rats										
Strain	Long-Evans pigmented rat							Sprague Dawley albino rat			
Document	930005742							930036905			
Dose	20 mg/kg, 5 µCi/mg, 100 µCi/kg							5 mg/kg, 20 µCi/mg, 100 µCi/kg			
Detection	Tissue excision, liquid scintillation counting							Quantitative whole body autoradiography			
Hours post dose	**1**	**4**	**12**	**24**	**48**	**98**	**168**	**1**	**4**	**8**	**24**
Non-Pigmented Skin	0.85	1.6	0.86	0.35	0.52	0.09	0	0.68	0.37	BQL	NS
Pigmented skin	0.69	1.0	0.50	0.45	0.52	0.17	0	-	-	-	-
Plasma	3.26	5.13	0.269	0.048	0	0	0	-	-	-	-
Skeletal muscle	0.59	0.9	0	0.06	0	0	0	0.43	0.27	0.06	BQL
Stomach	46.2	49.7	4.17	0.99	0.04	0.40	0.074	0.85	0.71	0.10	BQL
Testes	0.27	0.82	0.07	0.01	0	0	0	0.70	0.43	0.06	BQL
Thyroid	2.24	66.1	0.36	0	0.61	0	0	0.70	0.43	0.09	BQL
Urinary bladder	10.3	53.2	5.59	0.44	0.12	0.05	0.26	47.9	0.90	0.08	NS

NS = Not sampled, since not visualized on autoradioluminograph, considered as BQL. BQL = Value below the LLOQ
LLOQ = 0.00075540 µCi/g / 0.02024 µCi/µg = 0.037 µg equivalent / g tissue

Table 11: Sponsor's Summary - Apixaban Tissue Distribution in Pigmented Long Evans Rats

Matrix	C_{max} (µg-eq/g)	T_{max} (h)	T_{last} (h)	AUC_{last} (µg-eq·h/g)	AUC_{INF} (µg-eq·h/g)	$t_{1/2}$ (h)
Adrenal Glands	26.8	4	24	162	162	4.6
Blood	2.35	4	24	19.3	19.4	4.2
Bone (femur)	0.981	4	48	6.97	8.27	11.9
Bone Marrow (femur)	0.306	4	4	0.459	4.95[a]	64.1[a]
Brain	0.0988	4	4	0.260	NE	NE
Cecum	6.52	12	168	269	327	63.5
Eyes	2.37	4	168	51.0	57.6	64.1
Heart	0.0472	24	96	2.24	NE	NE
Intestine, Large	13.0	12	168	256	270	46.8
Intestine, Small	44.0	4	168	429	450	42.0
Kidneys	7.33	4	96	53.2	53.7	25.1
Liver	16.0	4	168	127	130	57.6
Lungs	12.9	4	96	79.7	79.7	14.5
Plasma	5.13	4	24	37.7	38.0	4.8
Skeletal Muscle (pectoral)	0.809	1	48	6.64	6.68	6.6
Skeletal Muscle (thigh)	0.903	4	24	6.25	NE	NE
Skin, Nonpigmented	1.56	4	96	46.0	50.0	30.0
Skin, Pigmented	1.01	4	96	42.9	55.4	52.4
Spleen	2.09	1	48	12.7	13.0	11.2
Stomach	49.7	4	168	453	457	38.5
Testes	0.823	4	24	5.85	5.92	4.6
Thyroid	66.1	4	48	379	388	9.96
Urinary Bladder	53.2	4	168	393	402	24.3

NE Not estimated.
a Calculated using elimination rate constant for eyes.

Radioactive apixaban was rapidly absorbed and detected in all fetal tissues evaluated by 0.5 hour post dose in Sprague Dawley pregnant female rats (Document 930036905). The highest concentrations in fetal blood and tissues were at 4 hour post-dose and concentrations rapidly declined thereafter (Table 12). Radioactive tissue concentrations in fetal tissues, except the amnion, were below the quantitation limit (BQL) by 24 hours post-dose. The fetal tissue levels were less than the fetal blood levels with the kidney having the highest concentration of apixaban radioactivity. The fetal blood levels of radioactivity were 22% and 39% of the corresponding maternal blood level at 1 and 4

hours post dose, respectively. However, this study did not determine whether the radioactivity was still in the form of apixaban. In the embryo-fetal development (EFD) studies in mice, rats and rabbits, the levels of apixaban were measured by LC/MS/MS (Table 13). The levels of apixaban in the embryo extract and the fetal: maternal ratios in the rat EFD study (Document 930009803) are comparable to the concentration of radioactivity in the fetal kidney in the tissue distribution study (Document 930036905). Although the levels of apixaban in rabbits after intravenous dosing were at least 10-fold lower than that in the rat, the levels of apixaban in fetal mice were similar to that in the rat. Thus, at least later during gestation, apixaban crosses the placenta and can be found in the fetus, particularly in mice and rats.

Table 12: Reviewer's Summary of Apixaban Tissue Distribution in Pregnant Female Rats and Fetuses

Study	Radioactivity (µg equivalents/gm)									Fetal:maternal ratio	
930036905	Non-pregnant female			Pregnant female			Fetus, GD 18				
Hours post dose	1	4	8	1	4	8	1	4	8	1	4
Blood	0.88	0.35	0.17	1.44	1.30	0.09	0.31	0.51	0.07	0.22	0.39
Brain	0.19	BQL	BQL	0.08	0.04	BQL	0.054	0.055	NS	0.68	1.38
Kidney (cortex)	1.36	1.21	0.42	2.03	1.93	0.21	0.20	0.46	NS	0.098	0.24
Liver	4.30	3.98	1.12	7.38	6.13	0.65	0.22	0.32	0.06	0.03	0.05
Cecum	0.80	9.64	18.7	0.88	5.05	10.1					
Small intestine	1.26	13.6	18.8	7.18	51.3	4.72					
Stomach	24.3	2.31	0.55	12.77	52.7	0.75					
Placenta							0.74	1.06	0.10		
Amnion							2.12	11.24	2.12		

NS = Not sampled, since not visualized on autoradioluminograph, considered as BQL
BQL = Value below the LLOQ (0.00075540 µCi/g / 0.02024 µCi/µg = 0.037 µg equivalent / g tissue)

Table 13: Reviewer's Summary – Distribution of Apixaban to the Fetus in Embryo-Fetal Development Studies

Species/strain/route/GD (Document number)	Dose, mg/kg	Maternal Plasma		Embryo/Fetal Extract		Ratio of embryo/fetal to maternal	
		Cmax	AUC	Cmax	AUC	Cmax	AUC
Mouse / CD-1/ oral/ GD 15 (930016586)	600	2.62	10.1	0.22		0.084	
	900	4.29	14.9	0.46		0.11	
	1500	3.70	17.1	0.41		0.11	
Rat/ SD/ oral/ GD 15 (930009803)	3000	7.22	36.4	0.625	2.59	0.087	0.071
Rabbit / NZW/ IV/ GD 19 (930016662) [Metabolite M1]	5	6.35 [0.008]	2.69 [0.0078]	0.056	0.028	0.009	0.01
Rabbit / NZW/ oral/ GD 19 (930016662)	1500	0.025	0.36	<0.003[†]		<0.08	

[†] Only 1 sample, collected at 2 hours after dosing, had a concentration (0.0029 µg/mL) above LLOQ,
GD = Gestation Day, M1 = O-desmethyl apixaban sulfate

Metabolism

Evaluation of metabolites in animals and man indicated that metabolism of apixaban (BMS-562247) primarily involves O-demethylation and hydroxylation, although other

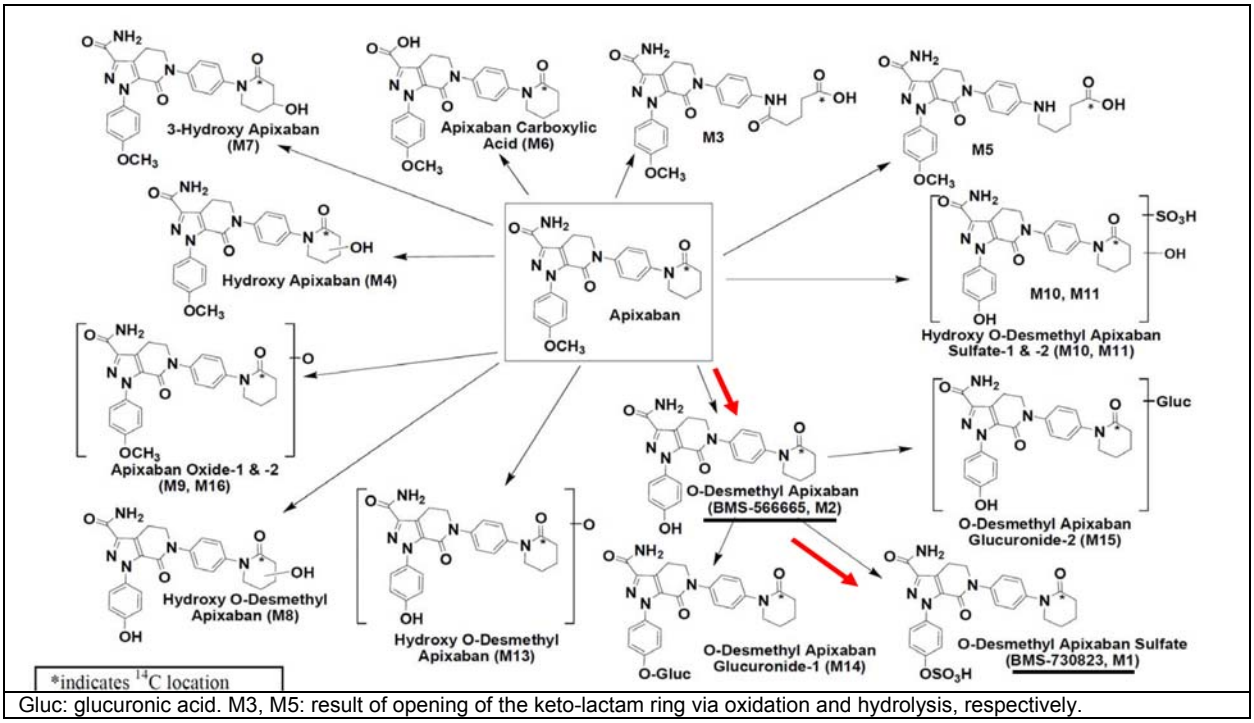
pathways such as oxidation, exist in some species. Conjugation reactions include glucuronidation and sulfation with the latter predominating in the human. The sponsor's proposed metabolic pathways for apixaban in humans and the species used for toxicology studies are shown in Figure 6 with the principal pathway in humans highlighted with red arrows. In humans, the principal pathway involves O-desmethylation of apixaban to O-desmethyl apixaban (M2, BMS-566665) followed by sulfation to O-desmethyl apixaban sulfate (M1, BMS-730823).

In humans, rats, mice and dogs, apixaban was the predominant radioactive component in plasma samples after administration of [^{14}C]-apixaban (Table 14). In rabbits, O-desmethyl apixaban glucuronide was the predominant radioactive component. In humans, the parent apixaban accounted for 84% of the plasma radioactivity at 6 hours after dosing and 53 to 59% of the plasma radioactivity 48 hours after dosing.

The most predominant circulating human metabolite was O-desmethyl apixaban sulfate (M1). Rats and dogs had low circulating levels (<1%) of O-desmethyl apixaban sulfate whereas rabbits had higher levels, particularly at 1 hour after dosing. In humans, the $AUC_{(0\text{-}48\ h)}$ for O-desmethyl apixaban sulfate was 24 to 27% of the $AUC_{(0\text{-}48\ h)}$ for the parent apixaban (M1).

Minor human plasma metabolites include O-desmethyl apixaban (M2), 3-hydroxy apixaban (M7), and hydroxylated O-desmethyl apixaban sulfate-1 (M10). O-desmethyl apixaban (M2) was found circulating in plasma of mice, rats, rabbits and dogs at levels higher than the levels in humans. The plasma of mice and rats also contained 3-hydroxy apixaban (M7). However, hydroxylated O-desmethyl apixaban sulfate-1 (M10) was not detected in plasma of mice, rats, rabbits or dogs.

Figure 6: Sponsor's Figure – Apixaban Metabolism



Gluc: glucuronic acid. M3, M5: result of opening of the keto-lactam ring via oxidation and hydrolysis, respectively.

Table 14: Reviewer's Summary – Apixaban Metabolites in Various Species

	Percentage of radioactive metabolites in plasma at the indicated hours post dose after an oral dose of [^{14}C]-apixaban to mice, rats, rabbits, dogs, and humans									
	Mouse		Rat		Rabbit		Dog		Human	
Metabolite	1	4	4	12	1	4	4	12	6	12
Apixaban	97.3	75.2	94.6	91.4	9.1	8.9	93.7	92.1	83.5	71.5
M1	N.D.	N.D.	0.4	0.9	23.4	4.2	0.2	0.4	14.9	27.5
M2	0.6	3.1	0.9	1.1	29.2	17.4	2.5	2.5	0.21	N.D.
M4	N.D.	N.D.	0.8	0.7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
M7	0.4	2.8	0.4	0.4	N.D.	N.D.	N.D.	N.D.	0.67	0.30
M10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.76	0.67
M14	0.4	2.4	N.D.	N.D.	37.7	53.3	N.D.	N.D.	N.D.	N.D.
Others	0.6	10.8	N.D.	N.D.	1.2	16.2	0.1	1	N.D.	N.D.

N.D., not detected, M1 = O-desmethyl apixaban sulfate (BMS-730823), M2 = O-desmethyl apixaban (BMS-566665), M4 = hydroxy apixaban, M7 = 3-hydroxy apixaban, M10 = Hydroxy O-Desmethyl Apixaban Sulfate-1, M14 = O-Desmethyl Apixaban Glucuronide-1

In vitro O-desmethyl apixaban sulfate (M1, BMS-730823), did not significantly inhibit FXa (Ki = 58 µM) compared to the inhibition by apixaban (BMS-562247) of FXa (Ki = 0.08 nM). O-desmethyl apixaban sulfate also did not significantly inhibit thrombin or trypsin (Ki >10 µM). Therefore, O-desmethyl apixaban sulfate does not contribute to the pharmacodynamic effect of apixaban. Additionally, O-desmethyl apixaban sulfate metabolite at 30 µM did not significantly affect peak tail hERG currents or action potential parameters in rabbit Purkinje fibers, although no positive control was run in parallel.

O-desmethyl apixaban sulfate metabolite is a phenol sulfate that is expected to be more stable than benzyl sulfate based on quantum calculations for heats of reaction. Therefore, O-desmethyl apixaban sulfate is unlikely to produce intermediates that react with DNA. The Executive CAC concluded the lack of O-desmethyl apixaban sulfate in mouse plasma and the low level of O-desmethyl apixaban sulfate in rat plasma did not preclude concurrence with the sponsor's carcinogenicity protocols.

Human exposure to O-desmethyl apixaban sulfate is 24 to 27% of the total apixaban exposure. In contrast, O-desmethyl apixaban sulfate is present at only 0.9 and 2.5% in rat and dog plasma, respectively. The reviewer consulted with the Pharmacokinetics Subcommittee to confirm that the toxicity of O-desmethyl apixaban sulfate did not need to be evaluated separately. The committee concluded that additional toxicity testing was not necessary since comparable AUC levels of O-desmethyl apixaban sulfate plus O-desmethyl apixaban were expected in the dog and in humans (Table 15). Additional genotoxicity testing was not considered necessary, because 1) adequate levels of the M2 metabolite were formed with rat liver S9 and 2) M1 is a phenol sulfate conjugate with no pharmacological activity and no structural alerts.

Table 15: Reviewer's Summary of Apixaban Metabolite Exposure

Metabolite	Name	6-month Rat	1-year Dog	Human
Parent	Apixaban			
	AUC of parent	35.5/34.4	99.4/137	3.1
	Unbound AUC of parent	1.42/1.62	8.35/11.5	0.409
M1	O-Desmethyl Apixaban Sulfate (BMS-730823)	0.9%	0.4%	27.5%
	Calculated AUC of M1, M/F	0.32/0.31	0.40/0.55	0.87
	Calculated unbound AUC of M2, M/F	0.013/0.014	0.033/0.046	0.11
M2	O-Desmethyl Apixaban (BMS-566665)	1.1%	2.5%	0.21%
	Calculated AUC of M2, M/F	0.39/0.38	2.5/3.4	0.007
	Calculated unbound AUC of M2, M/F	0.016/0.018	0.20/0.29	0.0009
	Total AUC of M1 + M2, M/F	0.71/0.69	2.9/3.95	0.9
	Unbound AUC of M1 + M2, M/F	0.029/0.032	0.23/0.34	0.11

In vitro metabolism

Incubation of [^{14}C]-apixaban with human intestinal microsomes and human liver microsomes from adults, children (1-6 years old) and infants produced low amounts of metabolites O-desmethyl apixaban (M2) , M4, and M7. However, O-desmethyl apixaban sulfate (M1) was not detected. Likewise, incubation of [^{14}C]-apixaban with rat liver S9 produced metabolites M2, M4, and M7 along with lower amounts of M6, M8, M9 and M13 (Document 930013242). Although M1 was not detected, these results indicate that the the genotoxicity of M2, M4, and M7 were evaluated at least at the higher concentrations of apixaban used in the in vitro bacterial reversion and mammalian chromosomal aberration assays (see Section 7).

Only when O-desmethyl apixaban (M2) was incubated in the presence of 3'-phosphoadenosine 5'-phosphosulfate was O-desmethyl apixaban sulfate (M1) detected in reactions with S9 preparations from various species (Document 930015043). However, the formation of M1 with S9 from monkey and human was 3-fold higher than S9 from dogs and 70-fold higher than with S9 from mouse, rat and rabbit. Inhibition of the human S9 reactions by quercetin and 2, 6-dichloro-4-nitrophenol, but not estrone, indicated that the sulfotransferase, SULT1AI, is involved in the formation of M1.

Of the five human cDNA-expressed sulfotransfereases (SULT) tested, only SULT1AI*2 and SULT1A2*1 exhibited significant formation of M1 (O-desmethyl apixaban sulfate). Since the rate of M1 formation was 7-fold higher with SULT1AI*2 than that with SULT1A2*1, SULT1AI plays the primary role in the sulfate conjugation of O-desmethyl apixaban in humans.

In vitro studies were conducted to identify the principal cytochrome P450 enzyme(s) involved in the biotransformation of apixaban (Document 930037129). The first study used incubation of [^{14}C]-apixaban with a panel of 13 recombinant human cDNA-expressed cytochrome P450 isoforms. CYP1A2, 2J2, and 3A4 showed higher formation of metabolite M2 than the other P450 enzymes. CYP3A4 and 3A5 showed the higher formation of M4 and M7 than other P450 enzymes. The other P450 enzymes tested (CYP2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, and 3A7) metabolized apixaban at very low rates, if at all.

Incubation of [^{14}C]-apixaban with human liver microsomes in the presence or absence of CYP isoform-selective inhibitors indicated that the formation of M2, M4, and M7 was significantly inhibited by CYP3A4 inhibitors ketoconazole and troleandomycin and partially inhibited by furafylline, a CYP1A2 inhibitor. The correlation between the formation rates of M2, M4, and M7 and activities of ten P450 enzymes were evaluated using a panel of human liver microsomes from 16 donors. The predetermined CYP3A4/5 activity showed the best correlations for formation of M2, M4, and M7 (r = 0.76, 0.90, and 0.96, respectively). Overall, the studies indicate that the apixaban metabolites M2, M4, and M7 are primarily produced by CYP3A4 with some contribution of CYP1A2 for formation of M2.

Excretion

In humans, excretion of orally administered apixaban involves both fecal and urinary routes (Table 16). However, excretion in dogs, rats, mice and rabbits is primarily through the fecal route. Following an IV dose of [^{14}C]-apixaban to rats, 52% of the bile total radioactivity or 12% of the dose was apixaban, indicating that biliary clearance of apixaban occurs (Table 17). However, following an oral dose of [^{14}C]-apixaban to bile duct-cannulated rats, only 2.6% of the dose was recovered in the bile, indicating biliary clearance was a minor apixaban elimination pathway.

Table 16: Reviewer's Summary – Excretion of Apixaban and Its Metabolites in Various Species

	Percentage of radioactive metabolites in urine and feces after an oral dose of [^{14}C]-apixaban to intact mice, rats, rabbits, dogs, and humans									
	Mouse		Rat		Rabbit		Dog		Human	
Document	930015646		930007004		930014907		930007005		930010261	
Metabolite	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
Apixaban	13.6	73.2	12.1	65.9	0.04	39.4	7.2	58.6	21.5	34.0
M1	<0.1	ND	<0.1	ND	0.1	0.4	<0.1	ND	1.6	1.2
M2	0.5	5.9	0.2	2.9	1.4	13.4	0.7	9.4	ND	12.2
Total	15.2	83.9	13.0	71.8	1.8	54.3	8.6	72.3	24.5	56.0

Table 17: Reviewer's Summary - Excretion in Rats

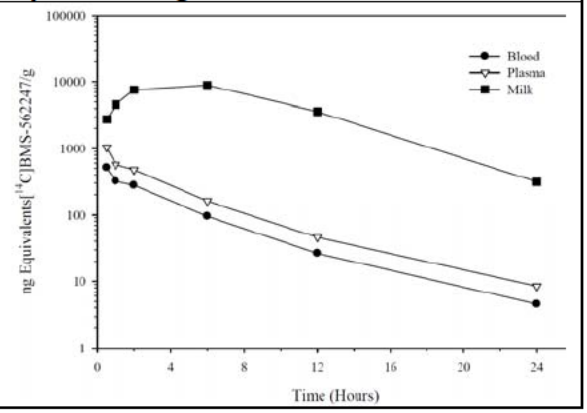
Document	930010961				930007004			
Route, dose	IV dose, 15 mg/kg				Oral dose, 30 mg/kg			
	Intact		BDC		Intact		BDC	
	Total	Parent	Total	Parent	Total	Parent	Total	Parent
Urine	20.7	19.0	46.6	43.3	13.4	12.1	10.5	9.4
Bile			23.0	12.0			2.6	0.5
Feces	12.7	7.9	24.9	21.8	74.0	65.9	69.8	64.6
Collection duration	0-24 hr		0-24 hr		0-168 hr		0-48 hr	
BDC = bile duct-cannulated								

Apixaban constituted at least 96% of the radioactivity in milk samples at all time points after oral administration of [^{14}C] apixaban (5 mg/kg) to female lactating Sprague-Dawley rats between Day 10 to 11 post partum (Document 93003700). The Cmax of apixaban in blood and plasma was 0.5 hour postdose, but the Cmax in milk was at 6 hours post dose (Table 18). After reaching Cmax, apixaban concentrations in milk, blood, and

plasma declined with a similar half-life. The mean blood:plasma concentration ratios ranged from 0.529 to 0.610 indicating apixaban is primarily in the plasma. At all time points concentrations of apixaban in milk were much higher than blood and plasma concentrations. The milk: plasma concentration ratios ranged from a low of 2.8 at 0.5 hour and increased to a high of 83.8 at 12 hours post dose with a ratio of 30 over the 24 hour collection period. Assuming a milk flow of 2.75 mL/hr (McGuire, 1995), approximately 12% of the maternal apixaban dose is excreted into the milk over a 24 hour period. If apixaban also concentrates in the milk of humans, infants that nurse a woman being treated with apixaban may be exposed to the drug.

Table 18: Reviewer's Summary - Apixaban Excretion in Rat Milk

Reviewer's Summary – Document 93003700					Sponsor's Figure – Document
	Cmax µg eq/g	AUC (0-24) µg eq*hr/g	Tmax	t½	
Blood	0.514	1.94	0.5	4.16	
Plasma	1.040	3.42	0.5	4.32	
Milk	8.920	103	6	3.67	
Blood/ plasma	0.49	0.57			
Blood/milk	8.59	30			



The following studies were not previously reviewed.

Study title: Mass Balance of Radioactivity after Oral Administration of [^{14}C]-BMS-562247 to Male CD-1 Mice

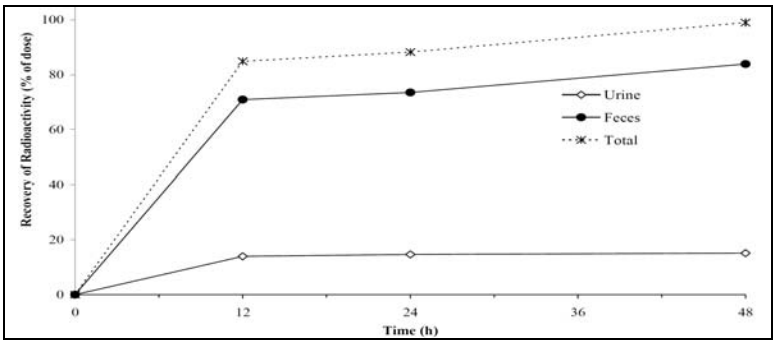
Study no.: MBA00221 (Document 930014442)
Conducting laboratory and location: (b) (4)
Drug, lot #, and % purity: [^{14}C]-BMS-562247-02, lot 002

The extent of excretion of total radioactivity was determined after oral administration of [^{14}C]-apixaban at 30 mg/kg in 0.5% (v/v) Tween 80 in Labrafil to fasted male CD-1 mice. Terminal blood samples were collected from five Group 1 animals/time point prior to dosing and at 1, 4, 12, 24, and 48 hours after dosing. After processing the blood for plasma, the plasma samples were analyzed for total radioactivity. The five animals in Group 2 were group housed in a metabolism cage for the separate collection of urine and feces, which were collected before dosing and over three intervals through 48 hours after dosing. A cage rinse was performed after the postdose collections through 48 hours postdose.

After an oral dose of [^{14}C]-apixaban, male CD-1 mice excreted 71% in feces and 14% in urine or a total of 85% of the dose within 12 hours. At 48 hours post dose, the total recoveries of radioactivity were 84% in feces and 15% in urine. The total recovery of the administered dose was 105% with 4.3% of the dose found in cage residue samples and

1.7% remaining in the carcasses. The Tmax was at 1 hour postdose, indicating rapid absorption of [^{14}C]-apixaban in mice. The highest mean concentrations of radioactivity in plasma (2890 ng equiv/g) rapidly decreased to below the quantifiable limits by 12 hours after dosing.

Figure 7: Sponsor's Figure – Apixaban Excretion in Mice



Study title: Mass Balance of Radioactivity After Intravenous and Oral Administration of [^{14}C]BMS-562247 to Female New Zealand White Rabbits (MBA00222)

Study no.: MBA00222 (Document 930017359)

Conducting laboratory and location: (b) (4)

Drug, lot #, and % purity: [^{14}C]-BMS-562247-02, lot 002

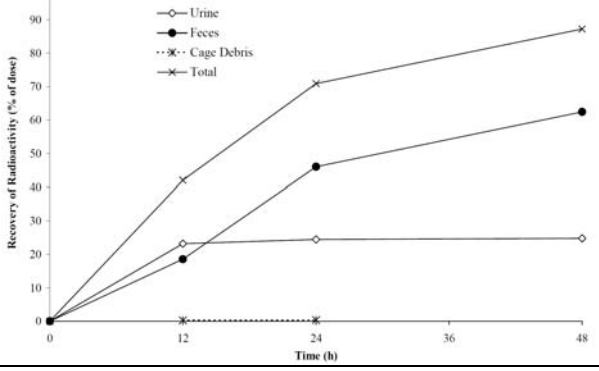
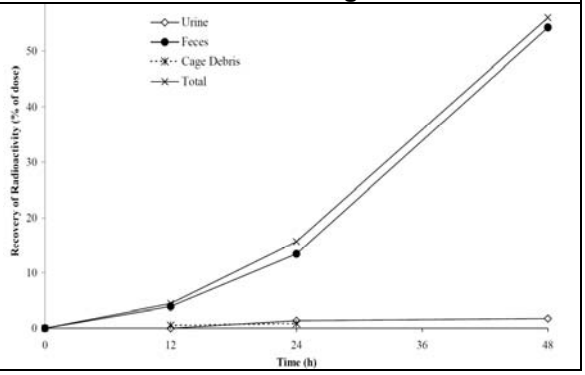
The excretion of total radioactivity was determined after intravenous and oral administration of [^{14}C]-apixaban to fasted female New Zealand White rabbits. One group of 3 female New Zealand White rabbits received a single intravenous bolus dose of 5 mg/kg [^{14}C]-apixaban in cyclodextrin buffer. The other group received a single oral gavage dose of 30 mg/kg of [^{14}C]-apixaban in 0.5% (v/v) Tween 80 in Labrafil®. Blood samples were collected from all animals prior to dosing and at 0.083, 0.25, 0.5, 1, 4, 12, 24, and 48 hours after dosing. After processing the blood for plasma, the plasma samples were analyzed for total radioactivity. The animals were housed individually in metabolism cages for the separate collection of urine and feces, which were collected before dosing and over intervals through 48 hours after dosing.

Following intravenous dosing of [^{14}C] apixaban to female New Zealand White rabbits, 62% of radioactivity was in the feces and 25% was in the urine with a total recovery of 88.00% of the administered dose. Cage residue samples only contained a combined total of 0.77% of the dose. The highest mean plasma concentration of radioactivity at 5 minutes postdose (8990 ng equiv/g) rapidly decreased to below the quantifiable limit (57.7 ng equiv/g) by 12 hour after dosing.

At 48 hours following oral dosing of [^{14}C]-apixaban, 54% of radioactivity was in the feces and 1.8% was in the urine. A combined total of 1% of the dose was found in cage residue samples. Only 57% of the administered dose was recovered following oral dosing of [^{14}C]-apixaban. The report attributed this low recovery to inadequate duration of sample collection; however, radioactivity remaining in the carcass, particularly the

intestinal tract, was not evaluated. Following oral dosing, only the 1 and 4 hour time points postdose had concentrations of radioactivity (314 and 404 ng equiv/g, respectively) above the quantifiable limit (202 ng equiv/g).

Figure 8: Sponsor's Figure - Excretion in Rabbits

Intravenous dosing	Oral dosing
	

Pharmacokinetic drug interactions

Using human liver microsomes the potential of apixaban to inhibit or induce eight cytochrome P450 (CYP) enzymes was evaluated using CYP probe substrates at concentrations approximately equal to their Km values (Document 930024170). The IC_{50} values for apixaban inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, and CYP3A4 were > 45 μM and the IC_{50} value for CYP2C19 was between 20 and 30 μM. As indicated in the review of Document 930024170 below, apixaban concentrations up to 20 μM did not significantly induce CYP1A2, CYP2B6 or CYP3A4/5 activity or mRNA expression. Since the mean peak apixaban plasma concentration in humans is 0.8 μM, these in vitro studies suggest that apixaban will have little effect on the metabolism of CYP substrates.

As discussed above under absorption, apixaban is a substrate of the P-gp transporter. Therefore, the absorption of apixaban may be affected by P-gp inhibitors. However, the inability of apixaban to inhibit P-gp suggests that apixaban will not alter the absorption of drugs that are P-gp substrates. In MDCKII cells or MDCKII cells expressing BCRP, diltiazem and naproxen did not inhibit apixaban efflux. However, ketoconazole only partially inhibited apixaban transport by 33% in MDCKII cells expressing BCRP, but almost completely inhibited (91%) the efflux of apixaban in MDCKII cells. Since MDCKII cells express canine P-gp, the effects of ketoconazole in this study are consistent with apixaban being a substrate of P-gp as well as BCRP. The efflux of digoxin, a P-gp substrate, was evaluated in P-gp-expressing Caco-2 and LLC-PK1 cell monolayers in the presence of diltiazem. In the two cell lines, diltiazem at 30 μM inhibited digoxin efflux by 33 and 47%, respectively, indicating that it is a P-gp inhibitor. Thus, a potential pharmacokinetic interaction between apixaban and diltiazem is possible. Indeed, a 40% increase in apixaban exposure was observed in humans when oral apixaban was coadministered with oral diltiazem (Document 930022706).

The bi-directional permeability of apixaban in Caco-2 cell monolayers (Document 930037717) was inhibited by ketoconazole (71%), but to a lesser extent by naproxen

(42%) and cyclosporin A (43%). When apixaban (10 mg) was co-administered with naproxen (500 mg) to healthy subjects, the mean apixaban exposure was increased by 54% relative to that observed following administration of apixaban alone (Document 930033529). However, a similar co-administration of ketoconazole with apixaban was associated with a 100% increase in apixaban exposure (Document 930019582). These results are consistent with a higher increase in apixaban exposure during co-administration with strong inhibitors of both CYP3A4 and P-gp than with co-administration with an inhibitor of P-gp alone.

The following study was not previously reviewed.

Study title: In Vitro Evaluation Of BMS-562247 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes

Study no.:	(b) (4) 063004 (Document 930024170)
Conducting laboratory and location:	(b) (4)
Drug, lot #, and % purity:	BMS-562247, lot 5L00821, purity 99.9%

The effect of apixaban on the activity and mRNA expression of three cytochrome P450 enzymes (CYP1A2, CYP2B6, and CYP3A4/5) was evaluated in primary cultures of human hepatocytes from three donors. The hepatocytes were treated once daily for three consecutive days with vehicle (0.1% DMSO), apixaban at 0.2, 2.0 or 20 µM, or one of three known human CYP inducers (omeprazole, phenobarbital, or rifampin). After treatment, cells were evaluated for cytotoxicity based on leakage of lactate dehydrogenase. A portion of the cells were harvested and microsomes prepared for the evaluation of CYP1A2 (phenacetin O-dealkylation), CYP2B6 (bupropion hydroxylation) and CYP3A4/5 (testosterone 6β-hydroxylation) activity by HPLC/MS/MS. Another portion of cells was used to measure CYP1A2, CYP2B6 or CYP3A4 mRNA levels.

As expected, the positive controls (omeprazole, phenobarbital and rifampin) resulted in increases in CYP enzyme activity and in CYP mRNA levels (Table 19). Treatment with apixaban produced no measurable cytotoxicity based on lactate dehydrogenase release. All concentrations of apixaban did not significantly induce microsomal CYP1A2, CYP2B6 or CYP3A4/5 activity or levels of CYP1A2 mRNA. However, the highest concentration of apixaban (20 µM) resulted in statistically significant 1.8 and 2.8 fold increases in CYP2B6 and CYP3A4 mRNA levels, respectively. In contrast, phenobarbital increased CYP2B6 mRNA levels by 17-fold and rifampin increased CYP3A4 mRNA levels by 20-fold. In comparison apixaban is not considered a significant inducer of CYP1A2, CYP2B6 or CYP3A4/5 activity or mRNA expression.

Table 19: Reviewer's Compilation of Sponsor's Tables – Apixaban Induction of CYP Activity and CYP mRNA Levels

Treatment group	Concentration	CYP P450 Enzyme activity (Fold induction[a])			CYP P450 mRNA level (relative to GAPDH mRNA) (Fold induction)		
		Phenacetin O-dealkylation § (CYP1A2)	Bupropion hydroxylation § (CYP2B6)	Testosterone 6β-hydroxylation § (CYP3A4/5)	CYP1A2 §	CYP2B6	CYP3A4
DMSO	0.1%	1.00 ± 0.37	1.00 ± 0.30	1.00 ± 0.27	1.00 ± 0.63	1.00 ± 0.26	1.00 ± 0.85
BMS-562247	0.2 µM	0.973 ± 0.003	0.892 ± 0.049	0.984 ± 0.005	0.825 ± 0.150	0.947 ± 0.107	1.12 ± 0.34
BMS-562247	2.0 µM	0.934 ± 0.028 *	0.934 ± 0.044	0.907 ± 0.134	1.02 ± 0.14	1.11 ± 0.20	1.49 ± 0.41
BMS-562247	20 µM	0.928 ± 0.037 *	1.12 ± 0.20	1.23 ± 0.21	1.16 ± 0.15	1.83 ± 0.31 †	2.77 ± 0.52 †
Omeprazole	100 µM	37.4 ± 1.2	11.0 ± 10.9	2.50 ± 1.34	253 ± 193	NA	NA
Phenobarbital	750 µM	2.03 ± 0.44	22.0 ± 22.4	7.36 ± 3.99	NA	16.5 ± 18.4 *	NA
Rifampin	10 µM	2.19 ± 0.14	13.1 ± 6.8	8.97 ± 5.16	NA	NA	19.5 ± 8.2 *

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase (a housekeeping gene).
Values are the mean ± standard deviation of three human hepatocyte preparations:H656, H658 and H660.
NA: Not applicable, treatment group not analyzed for respective CYP mRNA.
Fold Inductions are rounded to three significant figures and standard deviation is rounded to the same degree of accuracy.
§ Significance found among treatment groups (where 0.1% DMSO is the vehicle control) according to Kruskal-Wallis One Way Analysis on Ranks ($p < 0.05$) but unable to specify the groups that statistically differ from the other groups according to Dunnett's test with positive controls.
† Statistically significant compared to control (0.1% DMSO) according to Dunnett's Test ($p < 0.05$) without positive controls.
* Statistically significant compared to control (0.1% DMSO) according to Dunnett's Test ($p < 0.05$) with positive controls.

Other Pharmacokinetic Studies

Study title: Effects of Activated Charcoal Administration on Pharmacokinetics of Apixaban (BMS-562247) Following Oral Administration in Male Dogs

Study no.: **930039361** (Document 930039361)

Conducting laboratory and location: (b) (4)

Drug, lot #, and % purity: BMS-562247, lot 7A28071, purity 99.6%

The pharmacokinetic parameters of apixaban (BMS-562247) were determined in four fasted male beagle dogs after oral administration of 5 mg/kg apixaban in 0.5% Tween 80 in Labrafil suspension in four sequential treatments that were separated by at least three days.

During the first treatment period, apixaban alone was administered. In the subsequent three treatment periods, activated charcoal (250 mg/kg) was orally administered at 0.25, 1, and 3 hours after the apixaban dose. Blood was collected at 0.5, 1, 2, 4, 6, 8, 12, 14, 20, and 24 hours after apixaban administration. After preparation of plasma, apixaban concentration was determined by a valid LC/MS/MS method.

Although all activated charcoal treatments decreased apixaban AUC values, treatment at 3 hours after apixaban administration had the greatest effect of 37%, 42% and 51% for $AUC_{(0-24)}$, $AUC_{(2-24)}$, and $AUC_{(4-24)}$, respectively (Table 20). Although the T1/2 and Cmax values decreased up to 47% and 14%, respectively, in the charcoal treated groups, the differences were not statistically significant. No correlation existed between low AUC or Cmax values and animals that vomited. The clearance (CL/F) increased,

mean residual time (MRT) decreased and Tmax values decreased following charcoal administration.

The plasma trough concentration (C24) was decreased by 59%, 70%, and 87% for the treatments at 0.25, 1 and 3 hours, respectively, after apixaban dosing. The sponsor proposes that activated charcoal administration decreased apixaban exposure by increasing apixaban elimination and decreasing apixaban absorption and reabsorption. Although this study suggests that activated charcoal may be used to treat an apixaban overdose in humans, the reviewer notes that 20-fold higher doses of apixaban (100 mg/kg) were used in the chronic dog toxicity study. However, the $AUC_{(0-24)}$ and Cmax values in the absence of charcoal treatment in this study are 25- and 46-fold the total human $AUC_{(0-24)}$ (3.1 µg•h/mL) and Cmax (0.2 µg/mL) (clinical pharmacology report CV185046).

Table 20: Reviewer's Summary – Activated Charcoal Effect on Apixaban Exposure

Parameter/Treatment		Mean ±SD (Range)		
		Time of charcoal treatment after apixaban dose		
	T1: No charcoal	T2: 0.25 hour	T3: 1 hour	T4: 3 hour
Clinical signs	-	#2 vomited twice	#4 vomited once	#2 vomited once
AUC $_{(0-24)}$, µg•h/mL	78.6 ± 7.0 (70.4-85.6)	59.5 ± 4.6* (53.4-63.6)	63.9 ± 5.4 (58.4-70.8)	49.2 ± 1.8* (46.8-50.8)
AUC $_{(2-24)}$, µg•h/mL	70.2 ± 8.2 (63.3-79.70%)	49.0 ± 6.4* (40.2- 53.8)	52.1 ± 7.1* (43.8-61.1)	40.7 ± 2.3* (38.8-44.0)
AUC $_{(4-24)}$, µg•h/mL	53.5 ± 9.8 (43.8-65.6)	33.3 ± 6.3* (25.1-39.2)	35.7 ± 6.2* (28.4-43.6)	26.3 ± 3.4* (23.3-31.1)
Cmax, µg/mL	9.35 ± 1.33 (8.14-10.9)	8.42 ± 0.82 (7.33-9.33)	8.99 ± 0.91 (8.24-10.3)	8.07 ± 0.80 (7.36-9.18)
Tmax, h	4 ± 0	2.5 ± 1 (2 – 4)	2.5 ± 1 (2 – 4)	2.5 ± 1 (2 – 4)
C24, µg/mL	0.47 ± 0.32 (0.26-0.94)	0.19± 0.12* (0.08-0.35)	0.14 ± 0.06* (0.07-0.22)	0.06 ± 0.03* (0.03-0.10)
MRT, h	8.54 ± 2.31	6.39 ± 1.09	5.93 ± 0.54	5.28 ± 0.63
T1/2, h	5.69 ± 2.16 (4.18-8.88)	4.24 ± 1.01 (3.36-5.21)	3.96 ± 0.91 (3.09-5.22)	3.03 ± 0.24 2.79-3.35
CL/F, mL/h/kg	60.1 ± 15.6 51.4-66.0	82.0 ± 6.9* 74.1-92.5	77.4 ± 11.2* 68.8-84.2	100.9 ± 4.1* 97.0-106.5
* p<0.05				

The NDA submission contained the following two studies that had not been previously reviewed. However, neither of the studies used apixaban.

Study title: Assessment of Inhibition of Digoxin Efflux in LLC-PK1 Cell Monolayers (300963740)

Study no.: **300963740** (Document 930037205)

Conducting laboratory and location: (b) (4)

This study did not evaluate an effect of apixaban. The study showed that pre-experimental trans-epithelial electrical resistance, post-experimental lucifer yellow A to B flux values, and digoxin polarization ratios in absence and presence of positive control inhibitors were consistent with a properly functioning MDR1-LLC-PK1 monolayer model.

Naproxen (at 3 mM and 6 mM), indomethacin (0.25 mM), sulindac (2.5 mM), meloxicam (0.5 mM), diclofenac (2 mM), and ibuprofen (1 mM) inhibited digoxin efflux by ≤ 25% in P-gp expressing LLC-PK1 cell monolayers. However, diltiazem (30 µM) inhibited digoxin efflux by 33%.

Study title: Evaluation of Common NSAIDs' Ability to Inhibit P-gp Efflux

Study no.: 930037853 (Document 930037853)
Conducting laboratory and location: Not specifically indicated

This study did not evaluate an effect of apixaban. The study evaluated a panel of non-steroidal anti-inflammatory drugs for their ability to inhibit digoxin efflux in a Caco-2 cell model. Ibuprofen, sulindac, diltiazam, fenoprofen, and naproxen inhibited digoxin efflux by greater than 40%.

5.2 Toxicokinetics

Toxicokinetics were generally presented as part of the toxicology study reviews. The following table (Table 21) is the sponsor's summary of AUC values in critical toxicology studies. The sponsor's AUC exposure multiples are not shown because they are based on total exposure and not unbound exposure.

Table 21: Sponsor's Summary of Toxicokinetics

Species	Study (Sampling time)	Dose (mg/kg)	AUC (µg•h/mL)[a]			
			Apixaban		*O*-Desmethyl Apixaban Sulfate	
			M	F	M	F
Mouse	105 week (Week 26)	150	2.8	5.2	nd	nd
		500	5.1	10.4	nd	nd
		1500/3000 (M/F)[c]	7.3	16.8	nd	nd
Rat	6 month (Week 26)	50	16.6	26.4	nd	nd
		200	21.6	27.2	nd	nd
		600[c]	35.5	34.4	nd	nd
Rat	104 week (Week 26)	50	13.4	22	0.24	nd
		200	20.3	32.3	0.28	0.22
		600[c]	20.3	35.5	0.36	0.22
Dog	12 month (Week 52)	10	71.8	40.8	nd	nd
		30	92.2	96.5	nd	nd
		100[c]	99.4	137	nd	nd
Rat fertility/early embryonic development	Day 15	50	12.8	23.5	nd	nd
		200	24.4	22.8	nd	nd
		600	27.6	36.3	nd	nd
Pregnant Mice	TK (GD15)	600		14.6		nd
		900		17.5		nd
		1500[c,d]		15.9		nd
Pregnant Rat	TK[g](GD15)	100	-	nd	-	nd
		300		nd		nd
		1000	-	nd	-	nd
		3000[c,d]	-	36.4	-	nd
Lactating Rat	Pre-and Post-natal Study (LD4)	25[f]		11.7		
		200		43.4		
		1000[e]		47.5		
Pregnant Rabbit	TK[g] (GD19)	60	-	nd	-	nd
		180	-	nd	-	nd
		500		nd		nd
		1500[c,d]	-	0.036	-	nd

Abbreviations: nd = not determined, GD = Gestation Day, LD = Lactation Day

[a] Calculated from time zero to the time of the last measurable plasma concentration,

[b] Human AUC0-24 at 10 mg (5 mg BID) is 3.1 µg•h/mL and 0.94 µg•h/mL for apixaban and *O*-desmethyl apixaban sulfate, respectively (CV185046). AUC multiple = animal AUC ÷ human AUC.

[c] NOAEL

[d] Fetal NOAEL

[e] NOAEL for maternal toxicity, F_1-generation behavioral performance, and F_1-generation male reproductive performance

[f] NOAEL for F_1-generation female reproduction

[g] Multiple based on a comparison of AUC0-8h in pregnant rat to AUC0-12 of 1.6 µg•h/mL at 10 mg (5 mg BID)

6 General Toxicology

6.1 Single-Dose Toxicity

Drs. Joseph and Honchel previously reviewed single dose toxicology studies in mice, rats, dogs and monkeys. The oral LD_{50} for apixaban was greater than 4000 mg/kg in rats and mice, greater than 1500 mg/kg in dogs, and greater than 300 mg/kg in

monkeys. However, intravenous doses of apixaban as low as 50 and 25 mg/kg produced lethality in mice and rats, respectively.

6.2 Repeat-Dose Toxicity

The full reviews of repeat dose toxicology studies in rats, mice and dogs from 2 to 52 weeks in duration by Drs. Joseph, Honchel, and Harlow can be found in DARRTS. Table 22 summarizes the toxicology studies in rats and dogs submitted to [redacted] (b) (4) NDA 202155.

Although none of these studies used the formulation of apixaban proposed for marketing [redacted] (b) (4) µm), the mouse and rat carcinogenicity studies used lots of drug substance meeting these criteria during the second year of the studies.

Compound-related findings (prolongation of coagulation parameters, evidence of bleeding, and effects on red cell parameters) were related to the pharmacological activity of apixaban. Transient effects on serum potassium values were noted in both rats and dogs.

Table 22: Reviewer's Summary - Repeat Dose Toxicology studies

Species	Duration, weeks	Doses, mg/kg	BMS-562247 lot, (Process) (b) (4)	Maximum AUC, µg*hr/mL	Comments	Document
Rat/HSD	2 (gavage)	0, 75, 150, 300		19.7	Increased PT and APTT, decreases in potassium	930001603
Rat/ Crl:CD (SD)	2 (dietary)	200, 600, 1500		M: 16.2 F: 22.8	Dose of 1500 mg/kg/day well tolerated	930031453
Rat/ HSD	2 (gavage)	0, 200, 600		M: 29.3 F: 43.4	Increased PT and APTT, increased spleen weight	930009629
Rat/ Crl:CD (SD)	2 (intravenous)	0, 0.4, 1.25, 4, 12.5		M: 26.9 F: 47.7	Decrease in body weight gain in high dose females renal histopathology due to vehicle	930010865
Rat/HSD	13 (gavage)	0, 75, 150, 300		M: 10.4 F: 12.1	Increased PT and APTT,	930005268
Rat/Crl:CD (SD)	13 (dietary)	0, 600, 1800, 2400		M: 27.0 F: 47.0	Decreased body weight gain in males at ≥1800 mg/kg, increased PT	930031531
Dog/Beagle	2 (gavage)	30, 100		M: 156 F: 132	Increased PT and APTT, increased lung inflammation	930006683
Dog/Beagle	2 (intravenous)	0, 0.4, 1.25, 4		M: 118 F: 132	Increased PT and APTT	930010669
Dog/Beagle	13 (gavage)	0, 5, 10, 20		M: 52.7 F: 46.0	Increased bleeding time ≥10mg/kg, increased PT, decreased red cell parameters and decreased serum K	930004221

Table 23 compares the results in the chronic rat and dog toxicology studies.

Table 23: Reviewer's Comparison of Rat and Dog Chronic Toxicity Studies

Parameter	Rat	Dog
Strain	SprageDawley (Crl:CD®(SD) IGS BR)	Beagle
Study duration	6 months	12 months
Document code	930012967	930012966
Study code	DN03118 (b) (4) No: 800546)	DN03117
Location	(b) (4)	
GLP/QA	Indicated	Indicated
Drug batch, purity	BMS-562247, lot # 3J69810, purity 99.5% (b) (4)	BMS-562247, lot # 3J69810, purity 99.5% (b) (4)
Formulation	Labrafil/0.5% Tween 80	suspension in Labrafil/0.5% Tween 80
Doses, mg/kg/day	0 (water), 0 (vehicle), 50, 200, 600	0 (vehicle), 10, 30, and 100
Route	Oral gavage	Oral in gelatin capsules
Number/sex/group	Main: 20, Recovery: 5	6/sex with 2/sex sacrificed at 26 weeks
Mortality	11/12 deaths attributed to blood sampling	No deaths
Adverse clinical signs	Salivation and red staining of fur	No drug-related clinical signs
Body weight	BW gains in the treated groups were attributed to the vehicle	No treatment related effect
Food intake	No treatment related effect	No treatment related effect
Ophthalmoscopy	No treatment related effect	No treatment related effect
Electrocardiography	Not monitored	No treatment related effect
Hematology	RBC counts and RBC-related parameters (hemoglobin and hematocrit) also decreased significantly. Statistically significant increases in mean platelet volume were evident in all female treated groups at 4, 13, and 26 weeks and in all male groups at 26 weeks.	Statistically significant changes in hemoglobin, hematocrit, and reticulocytes were occasionally observed
Coagulation	PT was prolonged 1.2-1.4 fold and 1.4-1.5 fold in males and females, respectively; aPTT was prolonged 1.4-1.5 fold and 1.5-1.7 fold in males and females when blood was collected 2-3 hours post dosing	Prolongation in PT (1.9- to 2.4-fold higher than pretest values) and aPTT (1.3- to 1.6-fold higher than pretest values) were observed in all treatment groups at 2 hours after dosing throughout the study.
Clinical chemistry	Increases in calcium and total protein at 26 weeks and decreased potassium at 4 weeks	Statistically significant changes in alkaline phosphatase, aspartate aminotransferase, sorbitol dehydrogenase, and triglycerides occasionally observed. However, these changes were considered incidental
Urinalysis	No treatment related effect	No treatment related effect
Macroscopic pathology	Increased incidence of thickening, raised areas, and/or depressed areas in the non-glandular region of the stomach in males	No treatment related effect

Parameter	Rat					Dog				
Organ weights	Statistically significant increases in heart and kidney relative weight were observed in the mid-dose female groups relative to the vehicle control group.					No treatment related effect				
Microscopic pathology	increased incidence of minimal or slight erythrophagocytosis/ hemosiderosis in the mesenteric lymph nodes ulceration/erosion and/or epithelial hyperplasia due to vehicle					No treatment related effect				
Toxicokinetics AUC (mg*hr/L)	Dose, mg/kg	Male		Female		Dose, mg/kg	Male		Female	
		D1	D181	D1	D181		D178	D360	D178	D360
	50	9.8	16.6	11.7	26.4	10	58.6	71.8	56.9	40.8
	200	12.5	21.6	16	36.6	30	75.1	92.2	80.0	96.5
	600	16.9	35.5	18.6	34.4	100	86.4	99.4	135.5	137
NOAEL	600 mg/kg					100 mg/kg				

Based on the NOAELs in the chronic toxicology studies, safety margins were calculated below based on total and unbound exposures (Table 24). The 20 to 28-fold safety margin from the chronic dog study is higher than the 3.5 to 4.0 safety margin in the chronic rat study.

Table 24: Safety Margins Based on Chronic Toxicology Studies

			Exposure at NOAEL		Safety Margin‡	
Study/ Species	Sex	NOAEL (mg/kg) M/F	Total AUC (mg*hr/L)	Unbound† AUC (mg*hr/L)	Based on Total AUC at NOAEL	Based on Unbound AUC
6 month - rat	M	600	35.5	1.42	11.5	**3.5**
	F	600	34.4	1.62	11.1	**4.0**
12 month - dog	M	100	99.4	8.35	32.0	**20.4**
	F	100	137.0	11.5	44.2	**28.1**

† Unbound fractions in humans, male rats, female rats, and dogs are 13.2%, 4.0%, 4.7%, and 8.4%, respectively.
‡ Comparison to human exposure at the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0\text{-}24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL.

The following study was previously only partially reviewed prior to the presentation of the protocol for the rat carcinogenicity study to the Executive Carcinogenicity Assessment Committee. The report submitted at that time was incomplete.

Study title: BMS-562247: Six-Month Oral Toxicity Study in Rats

Study no.: DN03118 ((b) (4) No: 800546)
Document: 930012967
Conducting laboratory and location: (b) (4)
Date of study initiation: December 16, 2003
GLP compliance: Indicated
QA statement: Present
Drug, lot #, and % purity: BMS-562247, Lot 3J69810, purity 100%

Key Study Findings

Male and female Sprague Dawley rats received daily oral doses of apixaban at 50, 200, and 600 mg/kg for 26 weeks. Two control groups received either the vehicle (Labrafil/0.5% Tween 80) or water. On days 90 and 181, the dose of 600 mg/kg corresponded to $AUC_{(0\text{-}24h)}$ values of 32.7 and 35.5 µg.hr/mL in males and 36.2 and 34.4 µg.hr/ml in females, respectively.

Relative to the water control group, findings attributed to the vehicle included salivation, increased body weight and body-weight gain, necropsy findings of thickened non-glandular stomach, and microscopic ulceration/erosion and/or epithelial hyperplasia with inflammation in the non-glandular stomach.

At 4 weeks, red blood cell counts (RBC) decreased significantly in the male and female treated groups relative to the vehicle control groups. Other RBC-related parameters (hemoglobin and hematocrit) also decreased significantly in males, but not in females. At 13 weeks, hemoglobin and hematocrit increased significantly only in the high dose females. These changes were not observed at the end of the study, although they are possibly related to the pharmacodynamic effect of apixaban. Platelet counts were not affected by treatment with apixaban. However, statistically significant increases in mean platelet volume relative to the vehicle control groups were evident in all female treated groups at 4, 13, and 26 weeks and in all male groups at 26 weeks.

Changes attributed to drug treatment included prolongation of coagulation times in blood samples collected 2-3 hours after dosing at 26 weeks relative to both control groups. PT was prolonged 1.2-1.4 fold and 1.4-1.5 fold in males and females, respectively; aPTT was prolonged 1.4-1.5 fold and 1.5-1.7 fold in males and females, respectively.

The sponsor did not attribute the changes in calcium, chloride, total protein and potassium to treatment with apixaban.

The incidence of erythrophagocytosis/hemosiderosis increased in the mesenteric lymph nodes of treated animals at all doses after 26 weeks. Following a 4-week recovery period the incidence of this finding was similar across all groups indicating that this finding was reversible.

Since both prolongation of clotting times and hemosiderosis are the result of the pharmacodynamic effect of apixaban, NOAEL in this study was considered to be 600 mg/kg/day.

Methods

Doses:	0 (water), 0 (vehicle), 50, 200, and 600 mg/kg
Frequency of dosing:	Daily for 26 weeks
Route of administration:	Oral gavage
Dose volume:	4 mL/kg
Formulation/Vehicle:	Labrafil/0.5% Tween 80
Species/Strain:	Rat/ Sprague Dawley (Crl:CD®(SD) IGS BR)
Number/Sex/Group:	Main: 20/sex/group; Recovery: 5/sex/group
Age:	6 weeks of age
Weight:	Males: 150 to 184 g, Females: 117 to 143 g.
Satellite groups:	None
Unique study design:	Not applicable
Deviation from study protocol:	Of the seven protocol deviations listed, the one potentially most likely to have affected interpretation was that the order of necropsy was changed from that indicated in the protocol. However, the report indicated that a similar proportion of animals from each group and sex were euthanized on any one day.

Observations and Results

Mortality

The animals were examined visually for mortality, morbidity twice daily throughout the study.

Mortality occurred in both control and treated groups as indicated in Table 25. The sponsor concluded the incidence of mortality was not related to apixaban treatment, but was related temporally to blood sampling.

Table 25: Reviewer's Summary of Mortality – Study DN03118

		Males		Females	
Group	**Dose, mg/kg**	**Deaths**	**Animal - day of death - comment**	**Deaths**	**Animal - day of death - comment**
1	0, vehicle	2	1011-Day 22- hemorrhage 1013-Day 22- hemorrhage	0	
2	0, water	0		4	2517-Day 23- hemorrhage 2521-Day 177- hemorrhage 2524-Day 177- hemorrhage 2525-Day 177- hemorrhage
3	50	0		1	3502-Day 86- skull perforation
4	200	1	**4012-Day 22- hemorrhage**	4	**4501-Day 25- hemorrhage** 4502-Day 23- hemorrhage 4522-Day 86- hemorrhage **4525-Day 86- hemorrhage**
5	600	0		2	5505-Day 87- hemorrhage 5511-Day 182- hemorrhage
Bold text indicates animals for whom hemorrhage was considered to be the cause of death					

Clinical Signs

Clinical signs were recorded daily. In addition, a detailed examination was performed at least once prior to the start of treatment and weekly throughout the treatment and recovery periods.

Since the finding of salivation was observed in the vehicle control groups and the apixaban treated groups, but not the water control groups, this observation is related to the vehicle (Table 26). The reviewer noted that the incidence of red staining of fur was increased in the male treated groups compared to the control groups. However, this increased incidence was not observed in females.

Table 26: Reviewer's Summary of Clinical Signs – Study DN03118

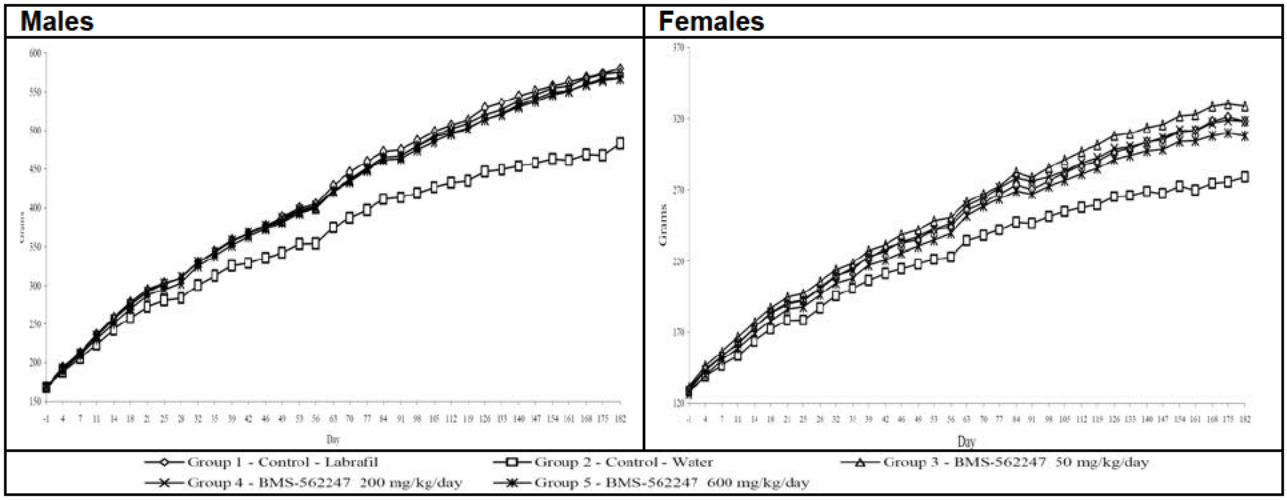
	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose	0, V	0, W	50	200	600	0, V	0, W	50	200	600
Observation										
Salivation	18	0	22	23	22	21	0	23	21	21
Fur staining, red, muzzle	5	3	10	20	17	15	9	13	13	15
Total fur staining, red	9	11	20	26	32	31	25	39	26	35
V = vehicle, W = water										

Body Weights

The animals in all groups were weighed on the day of randomization, twice weekly during Weeks 1 to 8, then weekly for the duration of the study and immediately before necropsy.

Compared to the vehicle control groups, no significant effects on body weight or body-weight gain were observed in the apixaban treated groups during either the treatment or recovery periods (Figure 9). However, compared to the water control groups, body weight and body-weight gain increased in both the vehicle control and apixaban treated groups. The body-weight gain increases of 26-30% and 20-31% in the treated male and female groups, respectively, were attributed to the vehicle (Labrafil/0.5% Tween 80).

Figure 9: Sponsor's Body Weight Graphs during Treatment – Study DN03118



Food Consumption

Individual food consumption for all animals was monitored daily by recording the number of pellets.

Compared to the vehicle control groups, no significant effects on food consumption were observed in the apixaban treated groups during either the treatment or recovery periods. However, food consumption occasionally (Days 21, 84, 175, and 182) decreased similarly up to 50% in both the vehicle control and apixaban treated groups compared to the water control groups on days associated with urine collection.

Ophthalmoscopy

All animals were examined once prior to the start of treatment, prior to dosing once during Weeks 13 and 26 of treatment and once during Week 30 (recovery Week 4). A board-certified veterinary ophthalmologist performed funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations after administration of 0.5% atropine sulfate.

No ocular changes were attributable to drug treatment.

Hematology

Blood samples for hematology were collected from a jugular vein of all animals during weeks 4, 13, 26, and of recovery animals during week 30. Hematology parameters included blood cell morphology, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and red cell distribution width (RDW), hematocrit, hemoglobin (Hb), mean platelet volume (MPV), platelet count, red blood cell count (RBC), reticulocyte count (absolute and percent), and white blood cell count (total, absolute and percent differential). The coagulation parameters of prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen were determined in blood samples collected at necropsy, which was performed 2-3 hours after dosing in week 26.

Compared to the vehicle control groups, the coagulation parameters aPTT and PT were prolonged in both male and female treated groups in blood samples collected 2-3 hours after dosing (Table 27). PT was prolonged 1.2-1.4 fold and 1.4-1.5 fold in males and females, respectively; aPTT was prolonged 1.4-1.5 fold and 1.5-1.7 fold in males and females, respectively. All individual animal PT and aPTT values for group 5 were above the maximum individual values in the vehicle control groups.

At 4 weeks, red blood cell counts (RBC) decreased significantly in the male and female treated groups relative to the vehicle control groups. Other RBC-related parameters (hemoglobin and hematocrit) also decreased significantly in males, but not in females. At 13 weeks, hemoglobin and hematocrit increased significantly in the high dose females. These changes, although related to the pharmacodynamic effect of apixaban, were no longer evident by the end of the study.

Mean platelet counts were not affected by treatment with apixaban. However, statistically significant increases in mean platelet volume were evident in all female treated groups at 4, 13, and 26 weeks and in all male groups at 26 weeks. The mean increases in the high dose groups were 10% and 7% in males and females,

respectively. The sponsor maintained these increases were not toxicologically significant, because the individual values generally fell within the range of values for the concurrent vehicle control group. The reviewer notes that some individual animals, particularly females, had values for mean platelet volume that were equal to or outside the extremes of concurrent vehicle control range. Recent literature indicates that increased platelet volume is associated with inflammation, thrombosis and increased cardiovascular disease risk (Vizioli et al. 2009, Chu et al 2010). However, blood for determination of mean platelet volume was anti-coagulated with EDTA, which can result in unreliable values for mean platelet volume (Bath and Butterworth 1996). Furthermore, mean platelet volume did not increase in the 3-month study.

Table 27: Reviewer's Summary - Selected Hematology Parameters - Study DN03118

Week	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose	0, V	0, W	50	200	600	0, V	0, W	50	200	600
RBC, 10^6/µL										
4	7.852 7.12- 8.41	7.770	**7.622***	**7.628***	**7.528*** 7.01 (3)- 8.26	7.855 7.44- 8.23	7.853	7.783	**7.526***	**7.520*** 6.96 (8)- 8.15
13	8.846	8.831	8.618	8.738	8.757	8.078	7.940	8.070	8.040	8.129
26	8.853	8.840	8.692	8.835	8.845	7.988	8.074	8.096	7.883	7.914
Hb, gm/L										
4	15.93	15.78	**15.50***	**15.54***	**15.38***	15.33	15.53	15.56	15.28	15.35
13	16.05	16.23	15.73	15.90	15.92	15.01	14.99	15.22	15.39	**15.44***
26	15.99	16.36	15.85	15.98	16.02	15.10	15.39	15.37	15.14	15.12
HT, %										
4	49.28	47.16	**46.39***	**46.42***	**46.14***	45.67	46.93	46.90	45.75	45.47
13	50.05	49.92	48.25	48.95	49.10	45.44	44.89	45.69	46.27	**46.89***
26	48.99	49.76	48.37	49.17	49.31	45.70	46.36	46.05	45.37	45.57
Platelet count, 10^3/µL										
4	1253	1220	1273	1212	1272	1206	1212	1225	1227	1179
13	1192	1168	1172	1094	1184	1125	1128	1137	1075	1114
26	1114	1102	1096	1042	1077	1019	1043	1013	986	1028
Mean platelet volume, fL										
4	8.63 7.7- 9.7	8.46 7.9- 9.0	8.56 8.2- 9.1	8.75 8.3- 10.5 (1)	8.77 8.3-9.2	9.73 8.8-10.7	10.05* 9.4-10.7	**10.18*** **9.7-** 10.9 (4)	**10.09*** **9.5-** 10.8 (3)	**10.27*** 9.5-11.5 (3)
13	8.58 8.0- 9.3	8.68 8.1- 9.7	8.86 8.4- 9.2	8.85 8.3- 11.3 (2)	**9.18*** 8.7-9.7 (9)	8.73 8.2-9.4	9.04* 8.4-10.3	**9.00*** 8.3-9.6 (3)	**9.11*** 8.8-9.6 (4)	**9.28*** 8.2-10.3 (9)
26	7.94 7.0- 10.6	7.85 7.3- 8.9	**8.45*** 7.9- 9.2	**8.57*** 7.7-9.5	**8.70*** 8.2-10.0	7.94 6.9-8.8	8.18 7.6-10.5	**8.43*** 7.7- 11.0 (5)	**8.37*** 7.0-9.1 (4)	**8.49*** 8.0-9.0 (5)
30 R	9.44 9.0- 10.1	9.72 9.4- 10.6	9.90 8.8- 10.9	10.04 9.5- 10.7	10.16 9.6-11.0 (2)	7.46 6.9-8.7	7.60 7.5-7.7	7.56 7.4-7.8	7.97 7.7-8.1	8.30 8.0-8.4
aPTT, sec										
26	22.16 20.2- 24.2	20.37	**31.00***	**33.12***	**32.63*** 25.2-42.5	19.23 16.5- 22.0	19.05	**28.13***	**28.86***	**32.42*** 22.5-42.5
PT, sec										
26	16.17 15.3- 17.0	17.08	**20.12***	**21.46***	**22.49*** 17.4-29.9	15.16 14.3- 16.2	**15.86**	**20.89***	**21.44***	**23.26*** 18.0-29.1

Week	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose	0, V	0, W	50	200	600	0, V	0, W	50	200	600

V = vehicle, W = water, * $p < 0.05$ relative to Group 1, vehicle control
The mean for each group is shown. The range of individual animal values is shown below the mean. The numbers in parentheses indicate the number of animals with values equal to or outside the extremes of individual values in the vehicle control group

Clinical Chemistry

Blood samples for clinical chemistry were collected from a jugular vein of all animals during weeks 4, 13, 26, and of recovery animals during week 30. Animals were fasted overnight prior to sampling. Clinical chemistry parameters included alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bicarbonate, blood urea nitrogen, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, sorbitol dehydrogenase, bilirubin (total, direct and indirect), total protein, and triglycerides. Globulin and A/G ratio were calculated.

The sponsor concluded that no clear effects of treatment on serum chemistry parameters were seen during the 26 weeks of treatment. Any changes were not considered drug related because they were either not consistently dose related or were within the normal reference range. However, the reviewer noted changes during treatment in chloride, calcium, potassium and total protein that deserve comment (Table 28).

Mean chloride levels were increased in the apixaban treated groups relative to the vehicle control groups. Although a clear dose relationship did not always exist for each timepoint, the values for some animals were equal to or above the maximum of the concurrent vehicle control range.

Mean calcium values were increased in a dose-dependent manner in males at 26 weeks and in females at 13 and 26 weeks. The values for some animals were equal to or above the maximum of the concurrent vehicle control range.

Although mean total protein values were not significantly changed at 4 and 13 weeks, the mean total protein values at 26 weeks increased dose-dependently in both male and female apixaban treated groups. The values for some animals were equal to or above the maximum of the concurrent vehicle control range.

Mean potassium values were decreased in both males and female treated groups after 4 weeks of treatment. Subsequently, the decreases were no longer dose-dependent and the mean potassium values in the male treated groups increased relative to that for the vehicle control group. However, decreased potassium values were noted in the 3 month oral gavage study, but not in the 3-month dietary study.

Table 28: Reviewer's Summary of Selected Clinical Chemistry Parameters – Study DN03118

Week	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose	0, V	0, W	50	200	600	0, V	0, W	50	200	600
Chloride, mEq/L										
4	100.9 96-107	104.0*	**103.7***	**103.4***	**103.3*** 98-106	105.5 102-112	107.4	105.1	105.6	**108.0*** 103-111
13	93.7 91-98	96.0	**95.6***	**96.4***	**96.3*** 92-100 (7 above)	97.3 94-100	98.0	97.5	**99.3***	98.5 95-102 (9 above)
26	99.7 97-103	101.4	100.8	100.8	100.8 96-105 (5 above)	97.4 92-102	98.7	99.3	99.1	**99.8*** 94-104 (6 above)
Calcium, mg/dL										
4	11.75	11.36*	**11.45**	**11.57**	11.63	11.39	11.51	11.23	11.25	11.37
13	11.66 10.9-12.4	11.55	11.24*	11.46	11.37 10.8-12.4	11.20 10.3-11.9	11.49	11.48	**11.60***	**11.68*** **11.3-12.4** **(7)**
26	11.71 11.3-12.6	11.96	11.98	**12.18***	**12.28*** 11.5-13.6 (7 above)	11.36 10.4-12.2	11.50	11.45	**11.70***	**11.91*** 11.2-12.8 (6 above)
Potassium, mEq/L										
4	6.06 5.29-6.94	5.80	5.79	**5.65***	**5.52*** 4.78 -7.1 (10 below)	5.61 4.80-6.11	6.00	5.60	5.45	5.46 4.85-6.21
13	6.23 5.35-7.62	6.13	5.72*	5.90	6.01 5.38-6.80	5.55 4.81-6.54	5.34	5.41	5.41	5.49 4.79 -6.16 (1 below)
26	5.35 4.70-6.20	6.03	5.74	**5.78***	**5.82*** 4.94-6.50 (2 above)	5.13 4.47-5.98	5.15	**4.82*** (1 below)	4.86	5.04 4.41-5.58
Total protein, gm/dL										
4	6.59	6.50	6.41	6.42	6.44	6.95	7.06	6.84	6.84	6.96
13	7.32	7.28	7.20	7.32	7.29	7.56	7.71	7.78	7.73	7.70
26	7.63 7.3-8.3	7.68	7.70	**7.93***	**7.96*** **7.4-8.7** **(4 above)**	8.37 7.4-9.3	8.42	8.54	8.54	8.70 7.6-9.7 (5 above)

V = vehicle, W = water, * $p < 0.05$ relative to Group 1, vehicle control
The mean for each group is shown. The range of individual animal values is shown below the mean. The numbers in parentheses indicate the number of animals with values equal to or outside the extremes of individual values in the vehicle control group

Urinalysis

During Weeks 4, 13, 26, and 30, urine samples were collected for 16-hours in metabolism cages from main study and recovery animals that were deprived of food, but had free access to water. Urinalysis parameters included bilirubin, blood, color, appearance, glucose, ketones, nitrite, pH, protein, specific gravity (qualitative analysis), urobilinogen, volume and microscopy of centrifuged sediment.

Compared to the vehicle control groups, no significant effects on urinalysis parameters were observed in the apixaban treated groups during either the treatment or recovery periods.

Gross Pathology

Animals were euthanized using exsanguination after isoflurane anesthesis and subjected to detailed necropsy. The whole or a sample of the tissues listed below (Table 29) from all animals was preserved in 10% neutral buffered formalin, except for the epididymides, eyes, optic nerves and testes, which were fixed in Zenker's fluid. Bone was decalcified prior to sectioning. Femoral bone marrow smears, were prepared, stained, and retained, but not evaluated.

Table 29: Reviewer's Summary of Tissues Preserved - Study DN03118

Abnormal tissues	Heart (with aorta)	Sciatic nerve
Adrenal glands	Ileum	Seminal vesicles
Aorta - thoracic	Jejunum	Skeletal muscle
**Bone and bone marrow (sternum)	Kidneys	Skin
	Lacrimal glands (extraorbital)	Spinal cord (cervical)
**Bone (femur)	Liver	Spleen
Brain (cerebellum, cerebrum, midbrain and medulla)	++Lungs	Stomach
	Lymph nodes (mandibular, mesenteric)	Tattoos[†]
Cecum	+Mammary gland	*Testes
Colon	*+Optic nerves	Thymus
Duodenum	Ovaries	+Thyroid with parathyroids
*Epididymides	Pancreas	Tongue
Esophagus	Pituitary	Trachea
*Eyes	Prostate	Urinary bladder
Femur	^Rectum	Uterus (horns, body, cervix)
Harderian gland	Salivary gland	Vagina

* Fixed in Zenker's fluid
** Bone decalcified prior to sectioning
+ Optic nerves, parathyroid glands and mammary gland were only examined histopathologically if present in routine sections of eyes, thyroid and skin, respectively.
++ Infused with neutral buffered 10% formalin
^ Tissue retained but not processed

Notable necropsy findings included an increased incidence of thickening, raised areas, and/or depressed areas in the non-glandular region of the stomach (Table 30). The incidence was similar in the treated groups and the vehicle control group in the females. However, the incidence was higher in the treated groups than in the vehicle control group in the males.

Table 30: Reviewer's Summary of Necropsy Findings - Study DN03118

	Males					Females-+				
Group	1	2	3	4	5	1	2	3	4	5
Dose	0, V	0, W	50	200	600	0, V	0, W	50	200	600
Observation No. examined	20	20	20	20	20	20	23	20	22	20
Stomach - thickening	1	1	5	4	4	4	1	5	4	3
Stomach – raised area	1	0	2	1	1	1	0	0	1	1
Stomach – depressed area	0	0	0	2	2	2	0	1	0	1

V = vehicle, W = water

Organ Weights

From each animal at necropsy, the following organs excised and weighed: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate (with seminal

vesicles), spleen, testes, thymus, thyroid (with parathyroid glands), and uterus (with cervix).

Some statistically significant organ-weight changes were observed in the treated groups and in the vehicle control groups compared to the water control group. Since the changes were similar in the treated groups and in the vehicle control groups, the sponsor concluded that no treatment related effects were observed on organ weights. However, the reviewer notes that statistically significant increases in heart and kidney weight relative to body weight were observed in the mid-dose female groups relative to the vehicle control group (Table 31). Although the increases in heart and kidney relative weight in the high-dose female groups were not statistically significant relative to the relative weights in the vehicle control group, the increases were similar in the mid- and high-dose groups. However, these findings did not correlate with any histopathology finding.

Table 31: Reviewer's Summary of Heart and Kidney Organ Weights - Study DN03118

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose	0, V	0, W	50	200	600	0, V	0, W	50	200	600
Heart										
Absolute	1.6089	1.4731	1.6169	1.5696	1.5760	1.0401	0.9894	1.0863	1.1010	1.0613
Relative[T]	0.2885	0.3354	0.2923	0.2903	0.2906	0.3474	0.3854	0.3523	0.3758*	0.3692
Kidney										
Absolute	3.0963	2.6216	3.0672	3.0171	3.0713	1.8543	1.8525	1.8839	1.9899	1.8701
Relative[T]	0.5554	0.5948	0.5540	0.5586	0.5652	0.6188	0.7208	0.6131	0.6767*	0.6520
* $p<0.05$ relative to vehicle control group, [T] Relative to body weight										

Histopathology

Adequate Battery

Tissue samples from all study animals were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Optic nerves, parathyroid glands and mammary gland were only examined histopathologically if present in routine sections of eyes, thyroid and skin, respectively. Table 29 lists the tissues collected at necropsy and examined microscopically from all animals in the control and high dose groups sacrificed at the end of the scheduled treatment and recovery periods. Only macroscopic changes and treatment related findings were examined for the low and mid dose groups.

Peer Review

A pathology peer review statement indicated that all tissues were reviewed for selected vehicle control (2/sex), water control (2/sex), and high dose groups (5/sex). The sections of stomach and mesenteric lymph node were reviewed for all animals.

Histological Findings

An increased incidence of minimal or slight erythrophagocytosis/hemosiderosis in the mesenteric lymph nodes was observed in the treated groups relative to both control groups (Table 32). However, the difference was greater between the treated groups and

the water control group than between the treated groups and the vehicle control group. The difference was greater between the male groups than between the female groups. Although the incidence or severity of this finding in treated groups was not dose related, this finding is likely to be related to the pharmacological effect of apixaban. However, no clear increase in hemorrhage was observed in the treated groups in any one tissue, for example the thymus, or across all tissues.

An apparent effect of the administration of the vehicle control was seen in the non-glandular stomach of rats in all groups administered the vehicle, with or without apixaban. In these groups a proportion of rats had rather pronounced changes consisting of minimal to moderate ulceration/erosion and/or epithelial hyperplasia, often with inflammation, and subepithelial edema. These changes correlated with the gross findings recorded in some animals at necropsy. Although the incidence of ulceration increased in treated males compared to both control groups, no increase in incidence was observed in females. No dose-related response was seen with apixaban treatment.

Table 32: Reviewer's Summary of Relevant Histology Findings - Study DN03118

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose, mg/kg	0, V	0, W	50	200	600	0, V	0, W	50	200	600
Lymph node, mesenteric	20	20	20	20	20	20	23	20	22	20
Erythrophagocytosis and/or hemosiderosis	5	3	15	14	14	9	3	12	16	12
Grade 1	5	3	15	14	13	8	3	12	16	12
Grade 2	-	-	-	-	1	1	-	-	-	-
Stomach	20	20	20	20	20	20	23	20	22	20
Hyperplasia, squamous mucosa	6	-	12	10	9	13	-	12	7	5
Grade 1	2	-	2	3	2	3	-	3	3	1
Grade 2	1	-	5	2	5	6	-	6	2	2
Grade 3	3	-	5	5	2	4	-	3	2	2
Ulceration/erosion, squamous mucosa	1	-	6	4	7	6	-	7	3	2
Grade 1	-	-	2	2	1	-	-	3	-	1
Grade 2	-	-	4	2	6	5	-	3	3	2
Grade 3	1	-	-	-	-	1	-	1	-	-
Thymus	20	20	4	4	20	20	23	3	10	20
Hemorrhage	7	6	4	3	6	7	11	3	8	8
* p<0.05 relative to vehicle control group										

Toxicokinetics

On Days 1, 90 and 181, blood samples were collected from a jugular vein of 3-4 animals/sex/treated group/time point at 1, 2, 4, 8, and 24 hours after dosing. Each treated animal was bled a maximum of 2 times on each day of collection. Control animals similarly bled at a convenient time so the same blood volume was collected. However, the blood from control animals was discarded without analysis. The blood samples were processed to plasma which was then assayed for apixaban using an internal standard with a validated LC/MS/MS method that had an analytical range of 2-2000 ng/mL.

Although exposures to apixaban increased with dose, the increases were less than dose proportional (Table 33). The high dose of 600 mg/kg was 12-fold the low dose of 50 mg/kg; however, the mean $AUC_{(0\text{-}24\ h)}$ for the high dose group was only 1.3 to 2.1 fold the mean $AUC_{(0\text{-}24\ h)}$, for the low dose group. No significant gender differences were

observed. On Day 90, the mean $AUC_{(0\text{-}24\ h)}$ values were 1.3- to 2.3-fold higher than the mean $AUC_{(0\text{-}24\ h)}$ values on Day 1, indicating some accumulation with repeated dosing. However, the mean $AUC_{(0\text{-}24\ h)}$ values on Day 90 and 181 were similar, suggesting that no further accumulation occurred after plasma levels reached steady-state. The Cmax values showed a less consistent increase with repeated dosing, possibly due to the generally high inter-animal variation (mean 45%, median 44%, and maximum 130%) in plasma concentration values.

Table 33: Reviewer's Summary of Toxicokinetic Parameters – Study DN03118

		Males			Females		
	Day	50	200	600	50	200	600
$AUC_{(0\text{-}24h)}$, µg•hr/mL (mean)	1	9.78	12.5	16.9	11.7	16	18.6
	90	20.7	16.7	32.7	22.2	25.2	36.2
	181	16.6	21.6	35.5	26.4	27.2	34.4
Mean Cmax, µg/mL (SD)	1	1.94 (0.37)	2.38 (0.34)	3.27 (1.02)	2.13 (0.57)	2.74 (0.57)	2.93 (0.3)
	90	2.95 (0.98)	2.26 (0.35)	3.63 (1.61)	2.81 (0.84)	3.26 (1.6)	4.82 (2.3)
	181	2.13 (1.06)	3.05 (0.97)	4.48 (0.46)	3.93 (3.21)	2.95 (1.43)	3.98 (1.84)

Dosing Solution Analysis

Measured concentrations of apixaban in the dose formulations deviated from expected concentrations by less than 8% (Table 34). The formulations were homogenous. Stability of the dosing formulation for 7 days was previously shown in the 3 month toxicology study in rats (DN02043).

Table 34: Reviewer's Summary of Formulation Analyses – Study DN03118

	Observed concentration, mg/mL											
Date	December 2003			January 2004			March 2004			June 2004		
Nominal	12.5	50	150	12.5	50	150	12.5	50	150	12.5	50	150
Bottom	12.9, 12.8	49.6, 49.9	151.9, 147.9	12.9, 13.0	50.9, 50.9	156.0, 155.5						
Middle	12.6, 12.5	50.7, 50.8	148.3, 148.6	12.4, 12.9	50.5, 50.4	154.7, 154.9	12.4, 12.3	49.1, 47.5	143.9, 145.6	11.6, 11.7	49.8, 48.1	147.1, 147.6
Top	12.6, 12.5	51.2, 51.1	150.0, 149.7	12.4, 12.7	50.5, 50.0	156.4, 156.8						

7 Genetic Toxicology

Apixaban was not mutagenic or clastogenic in several in vitro assays or clastogenic in vivo. The study reports for genetic toxicology studies for apixaban were previously reviewed as indicated in Table 35. The following paragraphs and Table 36 briefly summarize the genetic toxicology results for apixaban.

Table 35: Summary of Genetic Toxicology Study Reviews

Document number	Study number	Study	Lot BMS-562247	Reviewed	
				Under IND (b) (4)	Reviewer
920016149	DS02019	Exploratory Ames (Not GLP)	Not provided		D. Joseph
930002536	DS02121	Ames	2E55171		D. Joseph
930006384	DS03194	Cytogenetics in vitro	2F52153		D. Joseph
					P. Harlow
930002539	DS02117	Micronucleus in vivo	2E55171		D. Joseph
930015541	DS05177	Cytogenetics in vitro	4K83298		R. Honchel
930015561	DS05163	Cytogenetics in vivo/in vitro	4K83298		R. Honchel

Apixaban did not induce excess reverse mutations in five recommended bacterial strains in the absence or presence of metabolic activation (Study DS02121). Apixaban did not increase excess in vivo micronucleus formation in rats 24 hours after three oral doses of 2000 mg/kg/day, the limit dose for the assay (Study DS02117). Apixaban did not increase excess chromosomal aberrations in Chinese hamster ovary cells in vitro after a 4 hour exposure in either the presence or absence of metabolic activation at a concentration that produced acceptable toxicity (Study DS03194). However, after a 20 hour exposure in the absence of activation, apixaban induced a statistically significant dose-related increase in chromosomal aberrations, although the mean for the high dose was within the historical range of the negative controls. To clarify this equivocal result, the Division of Gastrointestinal and Coagulation Drug Products requested that the sponsor repeat the mammalian chromosomal aberration assay with a 20 hour exposure. The repeat study using a 20 hour exposure and a maximum concentration of 550 µg/mL based on precipitation produced no statistically significant increase in the frequency of cells with structural or numerical aberrations (DS05177).

Table 36: Reviewer's summary of genotoxicity studies with Apixaban (BMS-562247)

	In vitro			In vivo	
Study title	Ames Reverse- Mutation Study in Salmonella and Escherichia coli	Cytogenetics Study in Chinese Hamster Ovary Cells	BMS-562247: Cytogenetic Study in Chinese Hamster Ovary Cells	Oral Micronucleus Study in Male Rats	BMS-562247: 1-Month Oral In Vivo/In Vitro Cytogenetics Study in Rat Peripheral Blood Lymphocytes
Study code	DS02121	DS03194	DS05177	DS02117	DS05163
Document #	930002536	930006384	930015541	930002539	930015561
Conducting laboratory and location	Bristol-Myers Squibb, Syracuse, NY	(b) (4)		Bristol-Myers Squibb, Syracuse, NY	(b) (4)
Study initiation	7/15/02	9/17/03	11/14/05	7/29/02	10/26/05
GLP/QA	Yes/yes	Yes/yes	Yes/yes	Yes/yes	Yes/yes
Drug lot/purity	BMS-562247, batch 2E55171, purity 98.3%	BMS-562247, batch 2F52153, purity 98.9%	BMS-562247, Batch # 4K83298, purity 99.4%	BMS-562247, batch 2E55171, purity 98.3%	BMS-562247, Batch # 4K83298, purity 99.4%
Formulation or vehicle	DMSO	DMSO	DMSO	0.5% Tween-80 in Labrafil	0.5% Tween-80 in Labrafil
Metabolic activation	Yes, induced rat liver S9	Yes, induced rat liver S9	Assay only in absence of S9	Not applicable – *in vivo*	Not applicable – *in vivo*
Maximum dose	Assay 1, 5000 µg/plate Assay 2: 5000 µg/plate	Assay 1, 550 µg/mL ±S9 Assay 2: 550 µg/mL -S9	100, 400, and 550 µg/mL	Three doses of 500, 1000, 2000 mg/kg/day	0 (vehicle), 25, 200, or 600 mg/kg/day for 30 days
Appropriate replication	Assay 1: Duplicate plates Assay 2: Triplicate plates	Duplicate cultures for each dose in each assay	Duplicate cultures for each dose in each assay	5/sex/group in main study.	10/sex/group
Toxicity or exposure	Ppt and toxicity in both assays +/- S9 at 3000/2500 and 5000 µg/plate	Ppt at beginning of assay ≥ 400 µg/mL. Growth inhibition > 50% at 550 µg/mL	Ppt ≥ 600 µg/mL. Growth inhibition 45% at 550 µg/mL	No deaths, clinical signs or bone marrow toxicity. Previous TK study indicates saturation >300 mg/kg	No deaths, clinical signs of toxicity. In high dose Apixaban AUC: 20550 ng.hr/mL, M1 AUC 89 and 56 ng.hr/mL in males and females
Appropriate controls	Yes, with and without S9*	Yes, with and without S9	Yes, mitomycin C (MMC)	Yes, cyclophosphamide	Yes, cyclophosphamide
Study Comments	Five recommended strains. *Only 2-AA with S9, but commercial supplier tested S9 batch with benzo(a)pyrene)	Without S9: 4 hr exposure in one assay & 20 hr exposure in the other assay. With S9, 4 hr exposure in one assay.	Without S9: 20 hr exposure in one assay	Oral administration. Single timepoint (24 after last dose) Evaluated 2000 PCE/animal	24 hr after last oral dose blood collected for lymphocyte culture, fixation and chromosome evaluation
Study result	Negative	4 hr assays : Negative 20 hr. assay: Equivocal	Negative	Negative	Negative
Study evaluation	Both assays acceptable. Formulations within 7% of nominal	Assays are acceptable Formulation within 6% of nominal.	Assay is acceptable Formulation within 10% of nominal.	Acceptable Formulations within 7.4% of nominal	Acceptable Formulations within 9% of nominal

In clinical study CV185006, metabolites O-desmethyl apixaban sulfate (M1), O-desmethyl apixaban (M2), 3-hydroxy apixaban (M7), and hydroxylated O-desmethyl apixaban sulfate-1 (M10) were observed in plasma after oral administration of [^{14}C] apixaban to humans (Document 930010261). Metabolite M1 was of concern because it represented greater than 20% of the exposure of apixaban. After incubations of apixaban with rat liver S9 (Study 930013242) metabolites M2, M7, and hydroxylated O-desmethyl apixaban (M8) were present at 37%, 6.6%, and 4.0%, respectively, of the total radioactivity. However, metabolites M1 and M10 were not observed. Adequate amounts of M2, but not M1, are produced by rat liver microsomes. Therefore, M1 had not been adequately evaluated for genotoxicity. The sponsor argued that M1 is a phenol sulfate that is unlikely to form an intermediate that could react with DNA. Such reactive intermediates are formed from benzyl sulfates (Glatt 1997). The reviewer consulted with the Pharmacokinetics Subcommittee to confirm that the genotoxicity of M1 did not need to be evaluated separately. The committee concluded that additional genotoxicity testing was not considered necessary, because 1) adequate levels of the M2 metabolite were formed with rat liver S9 and 2) M1 is a phenol sulfate conjugate with no pharmacological activity and no structural alerts.

8 Carcinogenicity

Two year carcinogenicity studies were conducted in CD-1 mice and Sprague Dawley rats using multiple lots of apixaban. The complete reviews of these study reports are below.

Study title: BMS-562247: 104-Week Dietary Carcinogenicity Study in Mice

Document no./Study no.:	930031442/DN05068
Study report location:	EDR, Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dec. 22, 2005
GLP compliance:	Indicated
QA statement:	Present

Drug:	Lot used in weeks	Process	BMS-562247 Lot number	% Purity	Particle size D
(b) (4)	1-14	(b) (4)	4K85835	99.9%	(b) (4)
	15-30		4K83298	100.2%	
batches	31-40		4K86939	99.8%	
(b) (4)	41-56		4K89700	99.6%	
	57-102		5L00821	99.8%	
batches	103-105		6J12238	99.8%	
	(b) (4)				

CAC concurrence:	On November 15, 2005, the Executive CAC concurred with the sponsor's proposed doses of 0, 150, 500, and 1500 mg/kg/day I n diet (ad lib feeding) in males and 0, 150, 500, and 3000 mg/kg/day by diet (ad lib feeding) in females based on saturation of absorption with the qualification that the same form of apixaban be used in the cancer bioassay as was used in the 13-week toxicology studies (Appendix 2).
Protocol	

Study results: On November 29, 2011, the Executive CAC discussed the study results and concurred that the study was adequate and there were no clearly drug-related neoplasms. The Executive CAC meeting minutes are in Appendix 3.

Key Study Findings

Introduction

CD-1 male and female mice received oral dietary doses of apixaban for up to 105 and 97-100 weeks, respectively. At dosages of 150, 500, and 1500 mg/kg/day the mean $AUCs_{(0\text{-}24h)}$ in males during Week 25 were 2850, 5120, and 7330 ng.hr/mL. At dosages of 150, 500, and 3000 mg/kg/day the mean $AUCs_{(0\text{-}24h)}$ in females during Week 25 were 5180, 10400, and 16800 ng.hr/mL.

Summary of Non-neoplastic Findings

Although the sponsor concluded no non-neoplastic histology finding was related to apixaban treatment, the reviewer noted a few statistically significant findings, particularly in the high dose groups. The findings of extramedullary hematopoiesis in the liver in male mice and hemorrhage in the thymus of female mice are consistent with the pharmacodynamic effect of apixaban as a FXa inhibitor.

Adequacy of Carcinogenicity Study

The mouse carcinogenicity study for apixaban used the doses that were recommended by the Exec CAC based on saturation of exposure at the high-dose. The study length was acceptable since the male and female mice were treated for up to 104 weeks and 97-100 weeks, respectively. No statistically significant difference in mortality was observed between the combined control groups and the treated groups for either sex. No significant treatment-related effects were observed overall on food consumption or bodyweight, although the reviewer noted that the high dose female group gained approximately 10% less bodyweight than the control group from week 6 through 76. Therefore, the high dose selection in this study was appropriate.

One stipulation in the Exec CAC acceptance of the protocol was that the same form of apixaban be used in the cancer bioassay as was used in the 13-week preliminary studies. Although the cancer bioassay used multiple apixaban lots that differed in particle size distribution and process used for synthesis, the apixaban in all of these lots was (b) (4)
apixaban. Furthermore, the exposures to apixaban in the cancer bioassay were comparable to exposures in the preliminary mouse studies.

Appropriateness of Test Models

The Crl: CD-1™ (ICR) BR strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data have been established. Although the proposed metabolic pathway of apixaban in mice and man is generally similar involving O-desmethylation with hydroxylation and sulfation, the major human metabolite M-1 (O-desmethyl apixaban sulfate) is not detected in mouse plasma. However, in evaluating the protocol for the mouse carcinogenicity study the Exec CAC

concluded that the M1 metabolite, a phenolic sulfate conjugate, is more stable thermodynamically than benzylic and aniline sulfate esters and is considered less likely to form a reactive electrophile.

Summary of Tumor Findings

The incidences of a few tumors were increased in the higher dose groups compared to those in the control groups. In males, the incidence of Schwannoma (nerve sheath tumor) increased in the mandibular salivary gland; however, the p value for this tumor in the current study did not attain the significance level of $p < 0.025$ required for a rare tumor to be considered positive. The incidence of the combination of hemangiomas and hemangiosarcomas ($p = 0.0487$), uterine/cervical glandular polyps alone ($p = 0.0230$), and glandular polyps combined with adenocarcinomas ($p = 0.0081$) increased in females. However, none of these tumors had a p-value that attained the significance level of $p < 0.005$ required for a common tumor to be considered positive. Therefore, according to the criteria in current CDER guidance, no statistically significant neoplastic findings were related to apixaban treatment under the conditions of this study.

Evaluation of Tumor Findings

The FDA nonclinical and statistical reviewers concur with the sponsor that no significant evidence of neoplasia related to apixaban treatment was observed in CD-1 mice.

Methods

Doses:	0, 150, 500, and 1500 mg/kg/day in males and 0, 150, 500, and 3000 mg/kg/day in females
Frequency of dosing:	Daily in diet (ad lib feeding)
Dose volume:	Not applicable
Route of administration:	Oral (in the diet)
Formulation/Vehicle:	Diet
Basis of dose selection:	Saturation of absorption
Species/Strain:	Mouse/Crl:CD-1® (ICR) BR
Number/Sex/Group:	60 animals/sex/group
Age:	Approximately 6 weeks of age at study start
Animal housing:	Individual housing
Paradigm for dietary restriction:	Not applicable (ad lib feeding)
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes (4 groups of 25 animals/sex/group for TK and 1 animal/sex/group as sentinel animals)
Deviation from study protocol:	1. The original map showing the location of a palpable mass for female 1593 in the 600 mg/kg/day group was inadvertently discarded 2. The Helicobacter sp PCR assay was conducted under a non-GLP based quality system.

Observations and Results

Mortality

The animals were examined visually for mortality, injury, and morbidity twice daily during Weeks 1-52 and three times daily from week 53 to study termination.

The overall mortality in the female treated groups was higher than the mortality in the male treated groups. Consequently, all the female groups were necropsied prior to the protocol-specified termination week 105. The surviving female mice in the high-dose group and one control group (Control 2) exhibited similar survival (25%) and were sacrificed in weeks 97 and 98, respectively. All remaining female groups (Control 1, low dose and mid dose) were sacrificed during week 100. In contrast, all surviving males in all groups were sacrificed during week 105.

The sponsor's analysis indicated that the survival in the male and female treated groups was not statistically different from that of the combined control groups (Table 37). Although the survival was not statistically different between the two male control groups, the survival was statistically different between the two female control groups ($p = 0.033$). An explanation of this divergence in survival (42% versus 25%) was not provided. However, historical data (2003-2007) for other oral carcinogenicity studies using CD-1 mice indicate that survival in female control groups ranged from 23% to 36%.

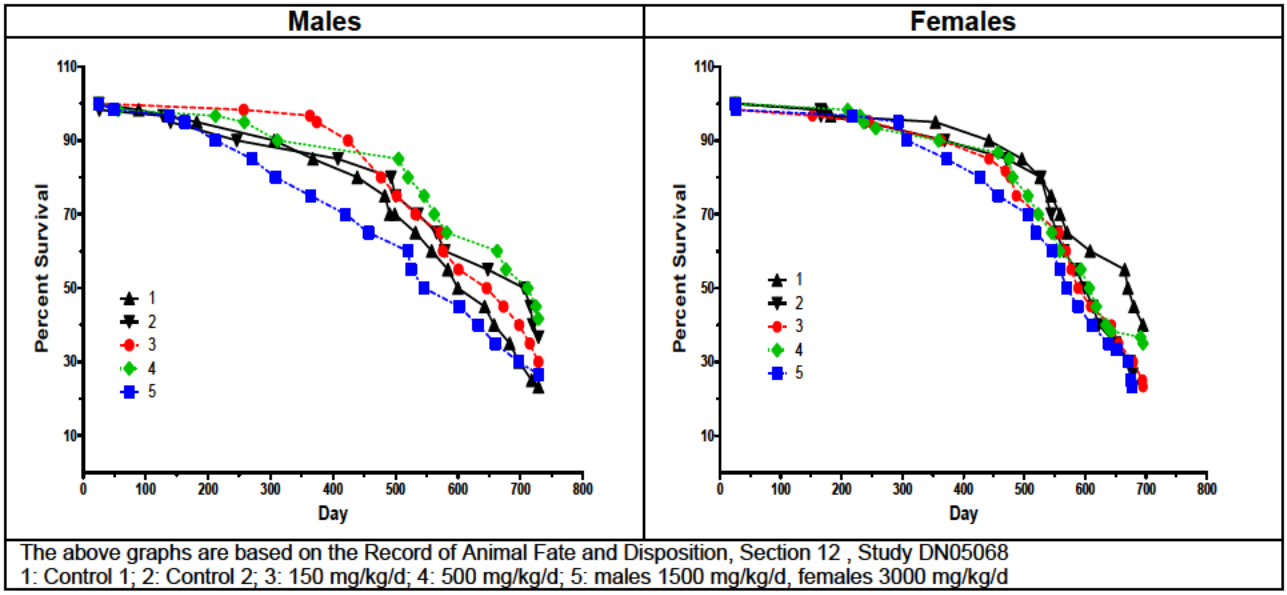
Table 37: Reviewer's Summary – Mortality in CD-1 Mice - Study DN05068

Main animals	Males					Females				
Dose, mg/kg	0	0	150	500	1500	0	0	150	500	3000
Group	1	2	3	4	5	1	2	3	4	5
Total number/group	60	60	60	60	60	60	60	60	60	60
Intercurrent deaths	45	37	20	26	17	35	45	45	38	45*
% mortality	75	62	67	57	72	58	75	75	63	75
Number surviving to terminal sacrifice	15	23	20	26	17	25	15	15	22	15
% survival	25	38	33	43	28	42	25	25	37	25
Mean Control % survival	31.5					33.5				
Sponsor's pairwise comparison, p value	0.108					0.033				
Sponsor's trend test, p value			0.84	0.10	0.32			0.27	0.96	0.10

* One death was considered accidental.

The sponsor's survival curves plotted the combined survival in the two control groups versus the survival in each treated group. The reviewer's graphs (Figure 10) below indicate survival in the control groups diverged beginning about week 82 in both males and females. The survival curves for both the male and female high dose groups are below the curves for either the control groups or the other treated groups. This is particularly evident between weeks 39 and 82 in males and between weeks 55 and 64 in females. However, the survival curves are not ordered by dose for either males or females.

Figure 10: Reviewer's Kaplan-Meier Curves for Mouse Carcinogenicity Study



The above graphs are based on the Record of Animal Fate and Disposition, Section 12 , Study DN05068
1: Control 1; 2: Control 2; 3: 150 mg/kg/d; 4: 500 mg/kg/d; 5: males 1500 mg/kg/d, females 3000 mg/kg/d

Lymphoid tumors in both sexes and liver tumors in males were the most common neoplastic causes of death. The most frequent non-neoplastic causes of death were amyloidosis in males and females, heart failure/atrial thrombi and urogenital inflammation/obstruction/calculi in males, and ovarian cysts/hemorrhage in females. No obvious cause of death was related to apixaban treatment.

Clinical Signs

Each main study animal received a detailed clinical examination once weekly. The observations included evaluation of the external appearance, general behavior, respiration, circulation, autonomic and nervous system effects as well as palpation of tissue masses. Sentinel animals were evaluated serologically for twelve mouse viruses and mycoplasma quarterly during the study and prior to study termination.

There was no increased incidence of specific clinical observation or mass finding that could be related to apixaban treatment. However, the sponsor did note the incidence of convulsions (Table 38) during the study, but maintained that the incidence was not dose-related and was similar to the laboratory historical incidence. Although the mean number of convulsions per animal was higher in the treated male groups, the sponsor indicated that the highest number of convulsions exhibited by a single control male and a single high-dose male was 25 and 23, respectively. Since the incidence of convulsions was higher during the last 20 weeks of the study, the sponsor attributed the convulsions to the age of the mice, and the sporadic handling in a dietary long-term study. In addition, convulsions in the male control group 2 occurred earlier during the study than the first convulsions in the treated groups.

Table 38: Reviewer's Summary – Convulsions in Study DN05068

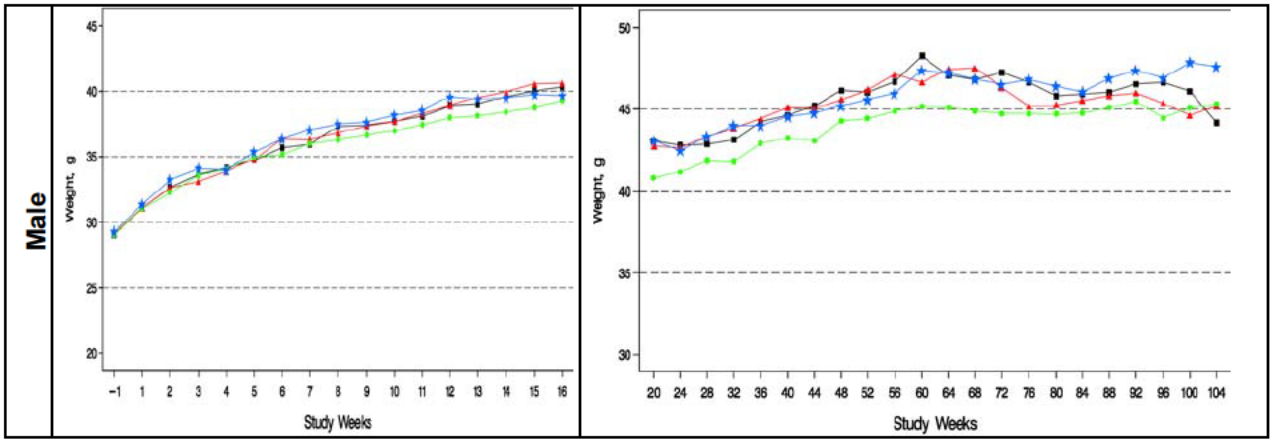
	Males					Females				
Incidence of convulsions	**Control 1**	**Control 2**	**150**	**500**	**1500**	**Control 1**	**Control 2**	**150**	**500**	**3000**
# animals	11	14	15	21	12	1	1	1	3	0
# episodes	32	60	142	243	114	5	12	3	10	0
Episode/animal	2.9	4.3	9.5	11.6	9.5	5	12	3	3.3	0
Week first observed	34	15	42	31	26	53	49	95	49	-

Body Weights

Body weights for all animals were measured on Days -13 and -3, once weekly during weeks 1 to 16, and once monthly thereafter.

The sponsor concluded that mean body weight values were not affected by apixaban treatment. In the males, the mean body weight for the mid-dose group (500 mg/kg/day) was consistently lower than that for the combined control groups with values at weeks 15, 20 and 60 being statistically significant (Figure 11). No dose-related trend was observed for mean body weight or mean body weight gain for the male-treated groups. However, mean body weights for the high dose females were significantly lower than those in the control group at weeks 14, 16, 48, 56, and 60. Although the mean body weights for the high dose females were consistently less than those in the control groups from week 6 through 84, no significant difference was observed at study end. ICH Guidance S1C indicates that the high dose in carcinogenicity studies should produce no more than a 10% decrease in body weight gain relative to that in the control group. Since the mean body weight gain for the high dose female group was approximately 10% less than those in the control groups (Table 39), the dosage of 3000 mg/kg was appropriate.

Figure 11: Sponsor's Body Weight Graphs – Study DN05068



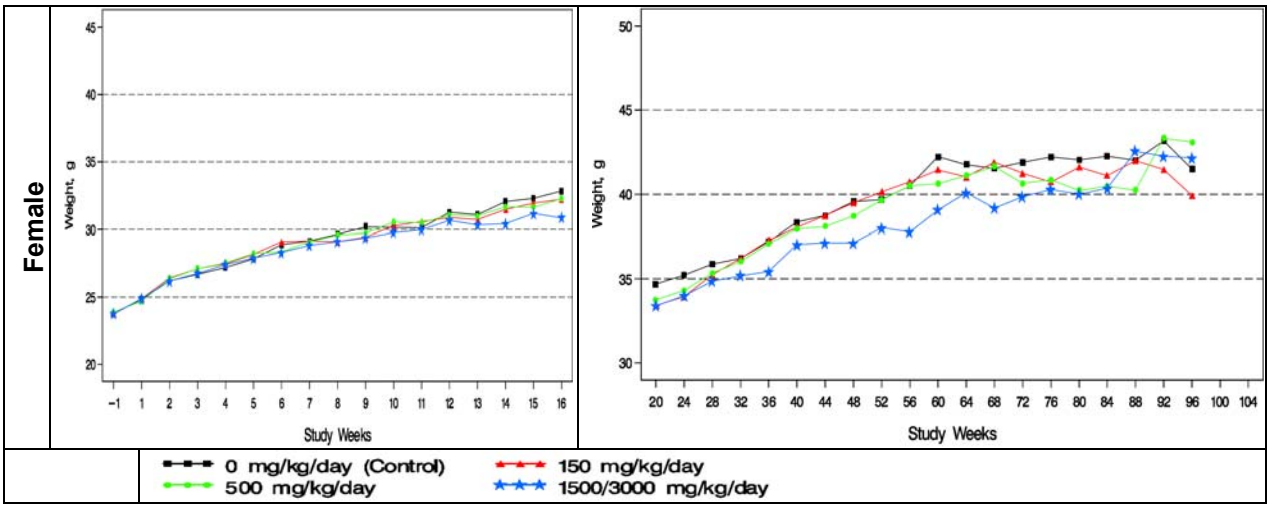


Table 39: Reviewer's Summary – Female Body Weight Gain – Study DN05068

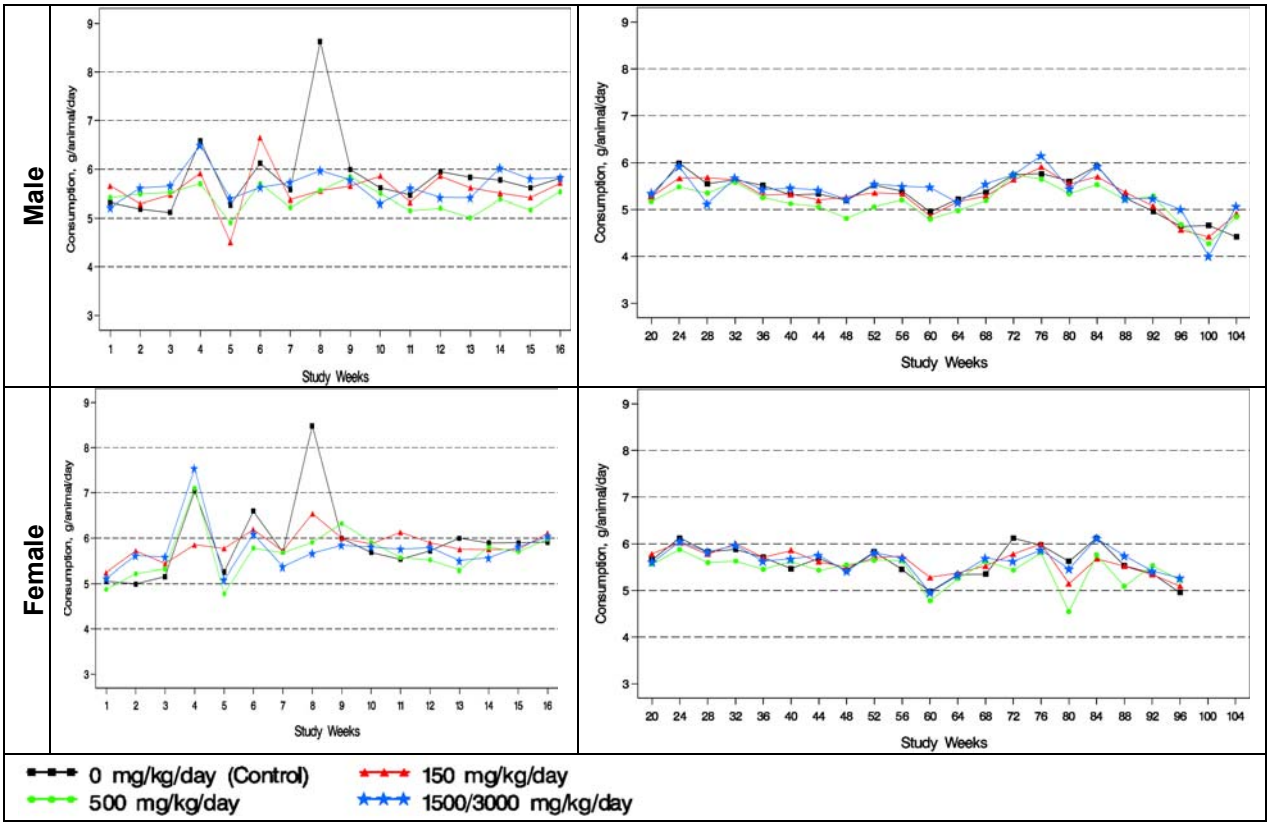
Dose	Control			150 mg/kg			500 mg/kg			3000 mg/kg			
Week	BW, gm	Gain, gm	N	BW, gm	Gain, gm	N	BW, gm	Gain, gm	N	BW, gm	Gain, gm	N	% of control gain
-1	23.75		120	23.72		60	23.89		60	23.75		60	
6	28.87	5.12	120	29.09	5.37	59	28.33	4.44	60	28.31	4.56	59	89.1
13	31.09	7.34	120	30.73	7.01	59	30.99	7.1	60	30.34	6.59	59	89.8
28	35.87	12.12	116	35.25	11.53	58	35.34	11.45	60	34.86	11.11	59	91.7
40	38.35	14.6	115	38.05	14.33	57	37.94	14.05	56	37.02	13.27	58	90.9
52	39.68	15.93	112	40.15	16.43	55	39.65	15.76	53	38.02	14.27	53	89.6
64	41.75	18.0	106	41.04	17.32	51	41.11	17.22	53	40.09	16.34	46	90.8
76	42.22	18.47	94	40.73	17.01	44	40.85	16.96	42	40.29	16.54	39	89.6
88	42.01	18.26	63	41.99	18.27	27	40.24	16.35	23	42.56	18.81	21	103.0
96	41.48	17.73	49	39.93	16.21	19	43.11	19.22	23	42.16	18.41	18	103.8

Food Consumption

Food consumption was determined for individual animals once weekly during weeks 1 to 16 and for 1 week at monthly intervals thereafter.

No clear apixaban-related effect was observed on mean food consumption values, except that the variation in food consumption was greater during the first 10 weeks of the study (Figure 12). In addition, food consumption in control males and control females during week 8 was higher than food consumption in apixaban-treated groups.

Figure 12: Sponsor's Figures – Food Consumption - Study DN05068



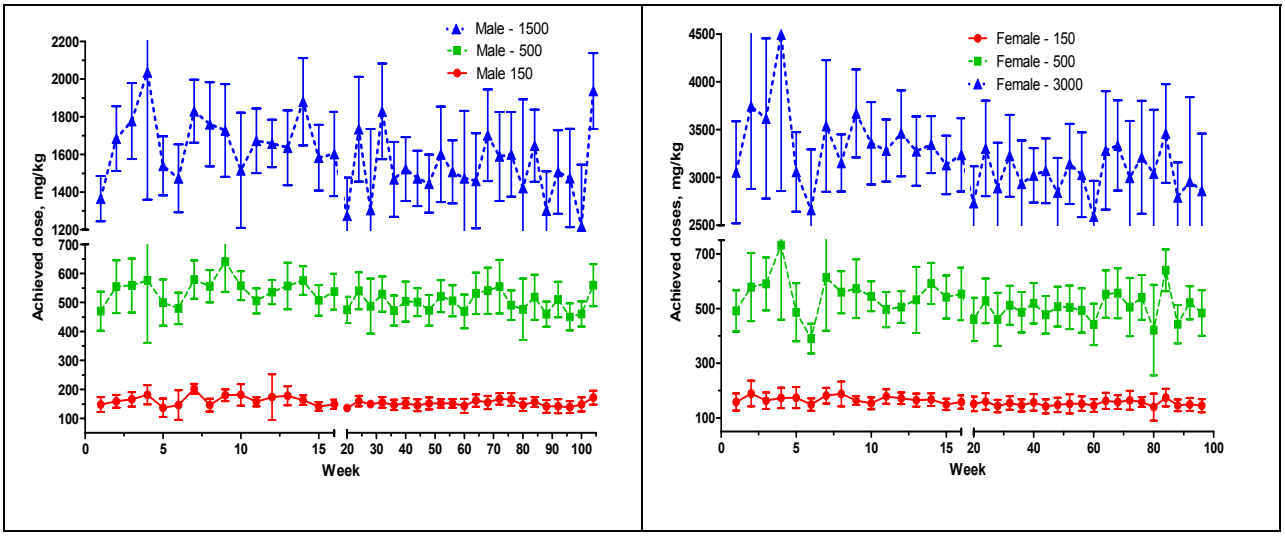
Based on body weight, diet admixture concentration, and food consumption, achieved doses were calculated once weekly through Week 16 and once monthly thereafter.

Overall, the calculated mean achieved doses for the entire study were 104% to 106% of intended doses, indicating that the mice were dosed as intended (Table 40). However, graphs (Figure 13) of the achieved doses during the study indicate the achieved doses were more variable during the first 8 weeks of the study when the mice were growing most rapidly.

Table 40: Reviewer's Summary – Achieved Doses - Study DN05068

Males				Females			
Achieved Dose, mg/kg	**150**	**500**	**1500**	Achieved Dose, mg/kg	**150**	**500**	**3000**
Mean	156.4	518.7	1584.6	Mean	159.2	523.2	3188.6
SD	14.8	42.3	185.4	SD	13.0	64.4	355.1
% expected	104.2	103.7	105.6	% expected	106.2	104.6	106.2
Maximum	202.0	640.8	2034.7	Maximum	189.3	732.3	4494.1
Minimum	136.1	450.1	1217	Minimum	139.7	390.3	2593.2

Figure 13: Reviewer's Graphs of Achieved Doses - Study DN05068



Gross Pathology

The surviving males in all main groups were sacrificed during week 105. In contrast, all the female main groups were necropsied prior to the protocol-specified termination week 105. The surviving female mice in the high-dose group and one control group (Control 2) were sacrificed in weeks 97 and 98, respectively. All remaining female groups (Control 1, low dose and mid dose) were sacrificed during week 100. Animals found dead during the study were necropsied at the earliest opportunity. The animals were subjected to systematic examination and the organs listed in Table 41 were fixed in 10% neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using a modified Davidson's fixative (Creasy and Jonnassen 1999). The urinary bladder and lungs were initially inflated with 10% neutral buffered formalin prior to further fixation by immersion.

Table 41: List of Tissues Collected

Adrenal glands	Jejunum	Skeletal muscle (diaphragm)
Aorta	Kidneys	Skeletal muscle (quadriceps femoris)
Bone with marrow (femur and sternum) (Bone marrow smear†*)	Liver[#]	Skin (dorsal thorax)
	Lungs	Spinal cord (cervical, thoracic, lumbar)
Brain (cerebrum, mid-brain, cerebellum, and medulla/pons)	Lymph nodes (mandibular, mesenteric and regional where applicable)	Spleen
		Stomach (glandular and nonglandular)
Cecum	Mammary gland	
Colon	Nasal turbinates[‡]	Testes
Duodenum	Ovaries	Thymus
Epididymides	Pancreas	Thyroid/parathyroids
Esophagus	Pituitary	Tissue masses
Eyes with optic nerve	Prostate	Tongue
Gall bladder[#]	Rectum[‡]	Trachea
Gross lesions	Salivary glands (mandibular)	Urinary bladder
Harderian glands	Sciatic nerve	Uterus with cervix
Heart	Seminal vesicles	Vagina
Ileum		

†Bone marrow smears were collected at the scheduled necropsy and held.
‡Collected and preserved, not microscopically examined. # Gallbladder was opened and the contents examined.

The incidences of macroscopic findings or masses were similar in all dose groups, and therefore, not related to apixaban treatment in either males or females that died on study or at the scheduled end of the treatment period. Calculi were found in the gallbladder of 15 animals, but the sponsor concluded this was not a apixaban-related effect, since the appearance and incidence of the calculi were similar in all dose groups.

Histopathology

After fixation, samples of protocol-designated tissues from all main study animals were processed to hematoxylin and eosin-stained paraffin sections. One veterinary pathologist examined the slides for male animals and another pathologist examined the slides for female animals.

Peer Review

The sponsor's pathologist evaluated all tissues sections from 10 animals per sex from the control and high dose groups in a peer review of the histopathology. In addition, the sponsor's pathologist evaluated sections of neoplastic lesions and spleen from all animals and sections of the ovary and uterus from all females.

Neoplastic

The incidences of the most notable tumors in the mouse carcinogenicity study are summarized in Table 42 below. The statistical evaluations by the sponsor are in Appendix 4 and Appendix 5. Historical control data provided by the sponsor are in Appendix 6. The reader is also referred to the statistical review by Dr. Matthew Jackson.

The sponsor's statistical evaluation of the tumors in male mice indicated only one tumor had a p-value less than 0.05. The incidence of Schwannoma (nerve sheath tumor) in the mandibular salivary gland of high-dose males was 3.3%, and attained a p-value of 0.0269 in the Peto-Pike trend analysis. The (b) (4) listing (2005) of spontaneous tumors in CD-1 mice indicates an overall incidence of 0.16% for nerve sheath tumors (benign and malignant combined) in the skin of male mice. Nerve sheath tumors in the salivary gland were not listed. Although nerve sheath tumors in the salivary gland could be considered a rare tumor, the p value for this tumor in the current study did not attain the significance level of $p < 0.025$ required for a rare tumor to be considered positive.

The sponsor's statistical evaluation of the tumors in female mice indicated several tumors had p-values less than 0.05 for the Peto-Pike trend analysis. These tumors included the combination of hemangiomas and hemangiosarcomas ($p = 0.0487$) and uterine/cervical glandular polyps alone ($p = 0.0230$) and glandular polyps combined with adenocarcinomas ($p = 0.0081$). Although the incidence of uterine endometrial polyps in high dose female mice when combined with uterine adenocarcinoma approached the critical statistical significance of 0.005 for a common tumor, the incidences of uterine endometrial polyps (8.3%) and adenocarcinoma (1.67%) in the current study are within the conducting laboratory's historical ranges for this mouse strain (2 - 20% and 0 – 3.3%, respectively). No tumor in the current study had a p-value that attained the significance level of $p < 0.005$ required for a common tumor to be considered positive. Furthermore, the incidence of hyperplasia in the uterus with cervix was similar across control and treated groups. Therefore, according to the criteria in current CDER

guidance, no statistically significant neoplastic findings were related to apixaban treatment under the conditions of this study.

Table 42: Reviewer's Summary – Notable Neoplastic Findings - Study DN05068

Mouse Carcinogenicity Study Neoplastic findings All animals		BMS-562247 Dose level (mg/kg/day)							
		Male				Female			
		0	150	500	1500	0	150	500	3000
Organ/Tissue Finding	#/group	120	60	60	60	120	60	60	60
Salivary gland, mandibular	#	120	60	60	**60**	120	60	60	60
Schwannoma	#	0	0	0	**2**	0	0	0	0
	%	0	0	0	**3.33**	0	0	0	0
Peto-Pike trend test, p-value					**0.027**				
Schwannoma, epididymides	#	120	60	60	60	-	-	-	-
	#	0	0	1	0				
	%	0	0	1.67	0				
Peto-Pike trend test, p-value				0.424					
Schwannoma, skin	#	120	60	60	60	120	60	60	60
	#	0	0	0	0	1	0	0	0
	%	0	0	0	0	0.83	0	0	0
Multi-centric neoplasm	#	120	60	60	60	120	60	60	60
Hemangioma, benign	#	1	1	0	2	6	1	0	3
	%	0.83	1.67	0	3.33	5.0	1.67	0	5.0
Peto-Pike trend test, p-value					0.108				0.281
Hemangiosarcoma, malignant	#	7	1	2	3	3	2	4	4
	%	5.83	1.67	3.33	5.0	2.5	3.33	6.67	6.67
Peto-Pike trend test, p-value					0.436				0.062
Hemangioma or Hemangiosarcoma	#	8	2	2	5	9	3	4	**7**
	%	6.67	3.33	3.33	8.3	7.5	5.0	6.67	**11.67**
Peto-Pike trend test, p-value					0.196				**0.0487**
Uterus with cervix	#	-	-	-	-	120	60	60	60
Adenocarcinoma	#					0	0	1	1
	%					0	0	1.67	1.67
Peto-Pike trend test, p-value									0.094
Polyp, endometrial glandular	#					4	0	1	**5**
	%					3.3	0	1.67	**8.3**
Peto-Pike trend test, p-value									**0.026**
Polyp, stromal	#					1	3	1	1
	%					0.83	5.0	1.67	1.67
Peto-Pike trend test, p-value									0.431
Adenocarcinoma or Polyp, glandular	#					4	0	2	**6**
	%					3.3	0	3.33	**10.0**
Peto-Pike trend test, p-value									**0.0081**
Hyperplasia, cervical endometrial	#					5	4	0	4
	%					4.2	6.67	0	6.67
Hyperplasia, cystic endometrial	#					102	48	47	51
	%					85.0	80.0	78.3	85.0
Hyperplasia, stromal	#					1	2	0	1
	%					0.83	3.33	0	1.67

* P value by Peto-Pike trend test, Historical controls at (b) (4): uterine endometrial polyps 2-20%; uterine adenocarcinoma 0-3.3%; uterine endometrial polyps or uterine adenocarcinoma 2-21.5%

Non Neoplastic

The sponsor concluded no non-neoplastic histology finding was related to apixaban treatment. However, a few statistically significant findings, particularly in the high dose groups were noted by the reviewer (Table 43). The incidence of liver necrosis, particularly centrilobular hepatocyte necrosis, was increased in high-dose female, but not male, mice. However, the severity of necrosis was similar to or less than the severity in the control group. The incidence of lymphocyte hyperplasia in the mandibular lymph node was increased in the low and high dose male groups; however, a clear dose relationship was lacking. An increased incidence of extramedullary hematopoiesis in the spleens of male and female mice was noted at all dose levels relative to the incidence in the controls, although a clear relationship to dose was lacking. The increased incidence of extramedullary hematopoiesis in the liver was dose-related in male, but not female, mice. The incidence of hemorrhage in the ovaries of apixaban treated female mice was slightly increased at all dose levels relative to the incidence in the controls, although a clear relationship to dose was lacking. In contrast, the incidence of hemorrhage in the thymus of high dose female mice was clearly elevated relative to the incidence in control females. The findings of extramedullary hematopoiesis and hemorrhage are consistent with the pharmacodynamic effect of apixaban as a FXa inhibitor.

Table 43: Reviewer's Summary – Non-neoplastic Findings - Study DN05068

Mouse Carcinogenicity Study		BMS-562247 Dose level (mg/kg/day)							
Non-neoplastic findings		Male				Female			
All animals		0	150	500	1500	0	150	500	3000
Organ/Tissue Finding #/group		120	60	60	60	120	60	60	60
Liver	#	120	60	60	60	120	60	60	60
Hematopoiesis,	#	2	3	3	**8‡**	6	6	**11‡**	3
extramedullary	%	1.7	5	5	**13.3**	5	10	18.3	5
Necrosis, hepatocytes,	#	4	2	0	1	5	3	0	**9†**
centrilobular	%	3.3	3.3	0	1.7	4.2	5	0	**15**
Necrosis, focal	#	4	5	4	4	5	4	5	5
	%	3.3	8.3	6.6	6.6	4.2	6.6	8.3	8.3
Necrosis, individual	#	0	0	0	2	2	2	1	1
hepatocyte	%	0	0	0	3.3	1.7	3.3	1.7	1.7
Any liver necrosis	#	8	7	4	7	12	8*	6	15
	%	6.6	11.7	6.6	11.7	10	13.3	10	**25**
Spleen	#	117	60	60	60	118	60	60	60
Hematopoiesis,	#	71	46	44	39	79	50	46	43
extramedullary	%	60.6	76.6	73.3	65	66.9	83.3	76.6	71.6
Lymph node, mandibular	#	110	59	58	55	114	57	59	56
Lymphocyte/plasmacyte	#	0	**3†**	0	**7‡**	5	1	1	0
hyperplasia	%	0	**5.1**	0	**12.7**	4.4	1.7	1.7	0
Any lymph node	#	110	60	60	60	118	60	60	60
Lymphocyte/plasmacyte	#	1	3	0	**8**	5	3	1	1
hyperplasia	%	0.91	5	0	**13.3**	4.2	5	1.6	1.6

Mouse Carcinogenicity Study Non-neoplastic findings		BMS-562247 Dose level (mg/kg/day)							
			Male				Female		
	All animals	0	150	500	1500	0	150	500	3000
Organ/Tissue Finding	#/group	120	60	60	60	120	60	60	60
Hemorrhage									
Ovaries	#	-	-	-	-	120	60	60	60
Hemorrhage	#	-	-	-	-	46	27	25	24
	%	-	-	-	-	38.3	45	41.6	40
Thymus	#	87	49	50	45	112	58	58	56
Hemorrhage	#	1	0	0	0	1	0	0	**5†**
	%	1.1	0	0	0	0.9	0	0	**8.9**
Any organ	#	120	60	60	60	120	60	60	60
Hemorrhage	#	28	13	12	8	50	28	27	31
	%	23.3	21.6	20	13.3	41.6	46.6	45	**51.6**

* One female had two diagnoses in liver † $p < 0.05$ ‡ $p<0.01$ using one-sided Exact fisher test

Toxicokinetics

At 1, 2, 4, 8, and 24 hours after the start of the dark cycle, blood samples were collected from five toxicokinetic animals per timepoint on Day 176 from apixaban-treated mice or on Day 180 from control mice for determination of plasma apixaban concentrations by a validated liquid chromatography/tandem mass spectrometry method with a lower limit of quantification (LLOQ) of 2 ng/mL. In calculating AUC values, the sponsor assumed that the toxicokinetics of apixaban were at steady state by Day 176 of the study and used the measured value at 24-hours on Day 176 for both the 0-hour and the 24-hour concentration value.

The plasma samples from two control female mice had apixaban concentrations above the LLOQ and were re-analyzed. Female 2705 (1 hr post dark cycle start) had an initial value of 4.03 ng/mL and re-analysis values of 3.78 and 3.82 ng/mL. Female 2717 (8 hr post dark cycle start) had an initial value of 2.56 ng/mL and re-analysis values of 2.80 and 13.8 ng/mL. The concentration in the plasma sample from mouse 2705 was less than 2% of the mean trough concentration at 24 hr in female mice in the low dose (150 mg/kg) group on Day 176. Although these findings do not significantly impact the toxicokinetic results, they were not thoroughly investigated or explained. Considering that analysis of the formulation indicated no apixaban in the basal diet, the source of this contamination is unclear.

Although the apixaban plasma concentrations in individual mice varied up to 7.5-fold within a group time-point, the mean apixaban plasma concentrations varied no more than 2.5-fold, indicating continuous exposure to apixaban (Figure 14). Systemic apixaban exposures ($AUC_{(0\text{-}24h)}$) after 176 days of dietary administration of apixaban increased with dose, but the increases were less than dose proportional (Table 19). In the low and mid dose groups, the systemic exposures in females were 1.8 to 2.0 fold higher than those in males. In contrast, the exposures in the high dose males and females were almost proportional (2.3-fold) to the dose administered. The total exposures (AUC) to apixaban in the high dose male and female mice on Day 176 were 2.4 and 5.4 times, respectively, the total exposure in humans treated with the recommended human dose of 5 mg twice a day. However, the difference in protein

binding of mouse and human serum increases the ratio of unbound exposure to 8.8 and 20.1 for high dose male and female mice, respectively (Table 44).

Figure 14: Reviewers Graphs - Apixaban Plasma Concentrations - Study DN05068

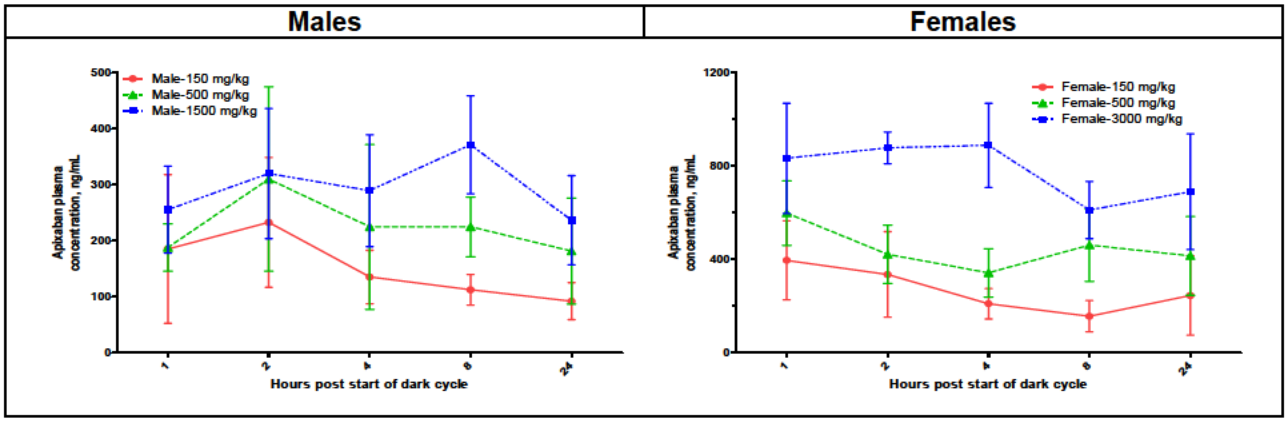


Table 44: Reviewer's Summary - Mouse/Human Exposure Ratios - Study DN05068

		Males			Females		
	Dose (mg/kg)	**150**	**500**	**1500**	**150**	**500**	**3000**
Total C_{max}	ng/mL	233	310	372	396	599	889
Total $AUC_{(0-24)}$	ng*hr/mL	2850	5120	7330	5180	10400	16800
Total mouse/total human AUC ratio		0.9	1.65	2.36	1.67	3.35	5.4
Unbound C_{max}	ng/mL	114	152	182	194	294	436
Unbound $AUC_{(0-24)}$	ng*hr/mL	1396	2509	3592	2538	5096	8232
Unbound mouse/unbound human AUC ratio		3.4	6.1	8.8	6.2	12.5	20.1

At the RHD of 10 mg (5 mg BID) for AF, the apixaban total $AUC_{(0-24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). Using % unbound apixaban as 49% for mouse and 13.2% for human. The human unbound AUC is 0.409 µg•h/mL

The Executive CAC concurred with the sponsor's protocols for the carcinogenicity studies with the stipulation that the same form of apixaban be used in the cancer bioassay as was used in the 13-week toxicology studies. The lot of apixaban used in the 3-month study was Lot 4K85835. However, the cancer bioassay used multiple apixaban lots that differed in particle size distribution and process used for synthesis. Of the (b) (4) used during development, (b) (4) was chosen for commercial development. (b) (4) was used in the 13-week study and during weeks 1-14 of the carcinogenicity study. During weeks 15 through 56, other (b) (4) lots of apixaban, were used. These lots (4K83298, 4K86939, and 4K89700) were produced using (b) (4) From week 57 through study termination, (b) (4) lots of apixaban produced by (b) (4) were used. However, analysis by powder X-ray diffraction indicated tha (b) (4) of apixaban.

Exposures to apixaban in the cancer bioassay were determined during week 25 when Lot 4K83298, (b) (4) lot produced with (b) (4)

[redacted], was used. Comparison of the exposures (AUC) in the cancer bioassay to the exposures in the preliminary mouse studies (Table 45) indicates comparable exposures, particularly at the highest dosages.

Table 45: Reviewer's Comparison of Exposures in Mouse Studies

		AUC (µg.hr/mL)			
	Dose,	Prior studies		Carcinogenicity study (week 25)	
Prior Studies	mg/kg/d	Male	Female	Male	Female
	150			2.85	5.18
Study DN04059 (2-week diet) Lot [redacted]	300	4.89	5.2		
	500			5.12	10.4
	600	4.53	8.06		
	1500	6.71	9.95	7.33	
Study DN04099 (3-month diet), Lot [redacted]	1500	9.6	13.6		
	3000	9.3	18.2		16.8
	4500	10.4	18.5		
	6000	10.3	19.7		
[redacted]				Study DN05068, During week 25, Lot [redacted] was used	

Dosing Solution Analysis

Table 46 shows the mean recovery of 9 replicates (3 replicates each from top, middle, bottom) as well as the minimum and maximum recovery for admixtures analyzed during the study. The range of mean recovery for all diet admixtures analyzed was 94.3% to 104.4% of nominal. No BMS-562247 was detected in the basal diet. The admixtures were considered homogenous since the minimum and maximum recoveries were 88.5% and 107.2%, respectively, of nominal. Analysis of samples stored at room temperature indicated that apixaban was stable in diet admixture for at least 15 days.

Table 46: Reviewer's Summary of Apixaban Recovery from Admixtures

Week	Dose, mg/kg	Males			Females		
		150	**500**	**1500**	**150**	**500**	**3000**
1	Mean	100.4	95.7	94.4	101.8	94.9	**94.3**
	Maximum	97.4	92.5	92.9	97.1	**88.5**	92.5
	Minimum	102.5	101.3	97.0	111.0	101.5	97.2
13	Mean	103.9	99.6	101.0	102	95.8	98.0
	Maximum	100.6	96.6	96.8	100.0	94.6	94.6
	Minimum	107.2	102.9	103.8	104.4	100.7	100.2
25/26	Mean	99.4	97.9	102.4	99.4	97.9	97.1
	Maximum	96.0	95.7	98.3	97.1	89.0	94.9
	Minimum	105.4	101.5	106.4	102	105.1	102.4
39/40	Mean	100.4	100.4	100.8	99.5	99.7	99.3
	Maximum	99.6	95.7	98.9	98.0	95.0	97.3
	Minimum	102.0	105.8	102.5	103.3	103.0	100.7
51/52	Mean	98.0	**104.4**	98.0	97.1	99.0	102.7
	Maximum	94.8	102.2	95.3	95.5	96.5	101.9
	Minimum	100.4	**107.2**	106.7	99.3	104.4	104.6
65	Mean	100.9	99.3	98.4	101.2	100.7	100.6
	Maximum	98.8	95.9	95.9	97.0	99.3	98.8
	Minimum	102.2	100.1	100.1	105.2	102.8	101.7
77/78	Mean	99.0	102.4	98.7	99.0	101.6	103.1
	Maximum	97.9	100.4	97.6	98.1	100.6	100.6
	Minimum	100.1	107.0	99.4	100.1	103.1	108.6
91/92	Mean	100.2	99.4	98.6	100.9	98.9	99.4
	Maximum	98.9	97.7	98.1	98.6	96.5	98.6
	Minimum	101.9	102.8	99.7	106.5	100.8	100.6
103/104	Mean	99.2	98.5	98.9			
	Maximum	96.3	97.1	97.5			
	Minimum	102.7	103.8	103.7			

Three replicates were analyzed from top, middle and bottom. The mean, minimum and maximum of the nine samples are presented.

Study title: BMS-562247: 104-Week Dietary Carcinogenicity Study in Rats

/Document no./Study no.:	930031443/DN05069
Study report location:	EDR, Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 21, 2006
GLP compliance:	Indicated
QA statement:	Present

Drug:	Lot used in weeks	Process	BMS-562247 Lot number	Purity	Particle size $D_{10, 50, 90}$
(b) (4)	1-9	(b) (4)	4K85835	99.9%	(b) (4)
	10-26		4K83298	100.2%	
batches	27-35		4K86939	99.8%	
	36-51		4K89700	99.6%	
(b) (4)	52-91		5L00821	99.8%	
batches	92-105		6J12238	99.8%	
	(b) (4)				

CAC concurrence protocol:	On November 15, 2005, the Executive CAC concurred with the sponsor's proposed doses of 0, 50, 200, and 600 mg/kg/day by diet (ad lib feeding) based on saturation of absorption with the qualification that the same form of apixaban be used in the cancer bioassay as was used in the 13-week toxicology studies. The Executive CAC meeting minutes are in Appendix 2.
CAC concurrence study results:	On November 29, 2011, the Executive CAC discussed the study results and concurred that the study was adequate and there were no clearly drug-related neoplasms. The Executive CAC meeting minutes are in Appendix 3.

Key Study Findings

Introduction

Sprague Dawley rats received oral dietary doses of apixaban for up to 104 weeks. At dosages of 0, 50, 200, and 600 mg/kg/day, the mean $AUCs_{(0\text{-}24h)}$ were 8.67, 16.0, and 19.1 µg*hr/mL in males and 11.9, 23.3, and 31.3 µg*hr/mL in females, respectively, on day 356 of treatment.

Summary of Non-neoplastic Findings

Many of the statistically significant non-neoplastic findings did not exhibit a clear dose relationship. Although the sponsor attributed some statistically significant findings to normal biological variation, findings, such as increased extramedullary hematopoiesis, increased pigment, and decreased thrombosis are consistent with the pharmacodynamic effect of apixaban as a FXa inhibitor.

Adequacy of Carcinogenicity Study

The rat carcinogenicity study used the doses (0, 50, 200, and 600 mg/kg/d) that were recommended by the Executive CAC. The study length was acceptable since the rats

were treated for up to 104 weeks. No treatment-related effect on mortality was observed.

No significant treatment-related effects were observed overall on food consumption or bodyweight. However, the reviewer noted that mean body weight and body weight gain decreased up to 10% and 15%, respectively, in the high dose male group from weeks 60 to 104.

One stipulation in the Executive CAC acceptance of the protocol was that the same form of apixaban be used in the cancer bioassay as was used in the 13-week preliminary studies. Although the cancer bioassay used multiple apixaban lots that differed in particle size distribution and process used for synthesis, the apixaban in all of these lots was (b) (4) of apixaban. Furthermore, the exposures to apixaban in the cancer bioassay were comparable to exposures in the preliminary rat studies.

Appropriateness of Test Model

The Sprague Dawley rat strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data are available. Although the proposed metabolic pathway of apixaban in rat and man is generally similar involving O-desmethylation with hydroxylation and sulfation, the levels of the major human metabolite M-1 (O-desmethyl apixaban sulfate) are very low in rat plasma (<2% of parent) compared to the levels in humans (> 27% of parent). However, in evaluating the protocol for the rat carcinogenicity study the Exec CAC concluded that the M1 metabolite, a phenolic sulfate conjugate, is more stable thermodynamically than benzylic and aniline sulfate esters and is considered less likely to form a reactive electrophile.

Summary of Tumor Findings

The incidence of malignant lymphoma, a common tumor, was increased in the high dose males ($p_t = 0.037$) and females ($p_t = 0.032$). However, the p value for this tumor in the trend test did not attain the significance level of $p_t < 0.005$ required for a common tumor to be considered positive. The only tumor with a p-value < 0.05 in the pairwise Exact Fisher test was adrenal pheochromocytoma in the low dose male group. However, this tumor did not exhibit a positive dose response relationship or a p-value of < 0.005 in the trend test. Therefore, according to criteria in current CDER guidance, no statistically significant neoplastic findings were related to apixaban treatment under the conditions of this study.

Evaluation of Tumor Findings

The FDA nonclinical and statistical reviewers concur with the sponsor that no significant evidence of neoplasia related to apixaban treatment was observed in Sprague Dawley rats.

Methods

Doses:	0, 50, 200, and 600 mg/kg/day by diet. Individual doses were based on estimating the mean body weight and food consumption/sex/ group from the most recent values recorded with the exception of Weeks 1 and 2 for which individual doses were based on data from a previous study ((b) (4) Study 455-066).
Frequency of dosing:	Daily (ad lib feeding)
Dose volume:	Not applicable – based on diet preparation according to weight of food consumed
Route of administration:	Oral through the diet
Formulation/Vehicle:	In the diet
Basis of dose selection:	Saturation of absorption
Species/Strain:	Rat (Crl:CD®[SD])
Number/Sex/Group:	60 rats/sex/group
Age:	6-7 weeks of age
Animal housing:	1 rat/stainless steel wire mesh cage
Paradigm for dietary restriction:	Not applicable – ad lib diet
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes, for toxicokinetics (9 rats/sex for control, low, mid and high dose groups) and sentinel animals (1 rat/sex/group)
Deviation from study protocol:	Although deviations from the protocol occurred, the study director concluded these deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

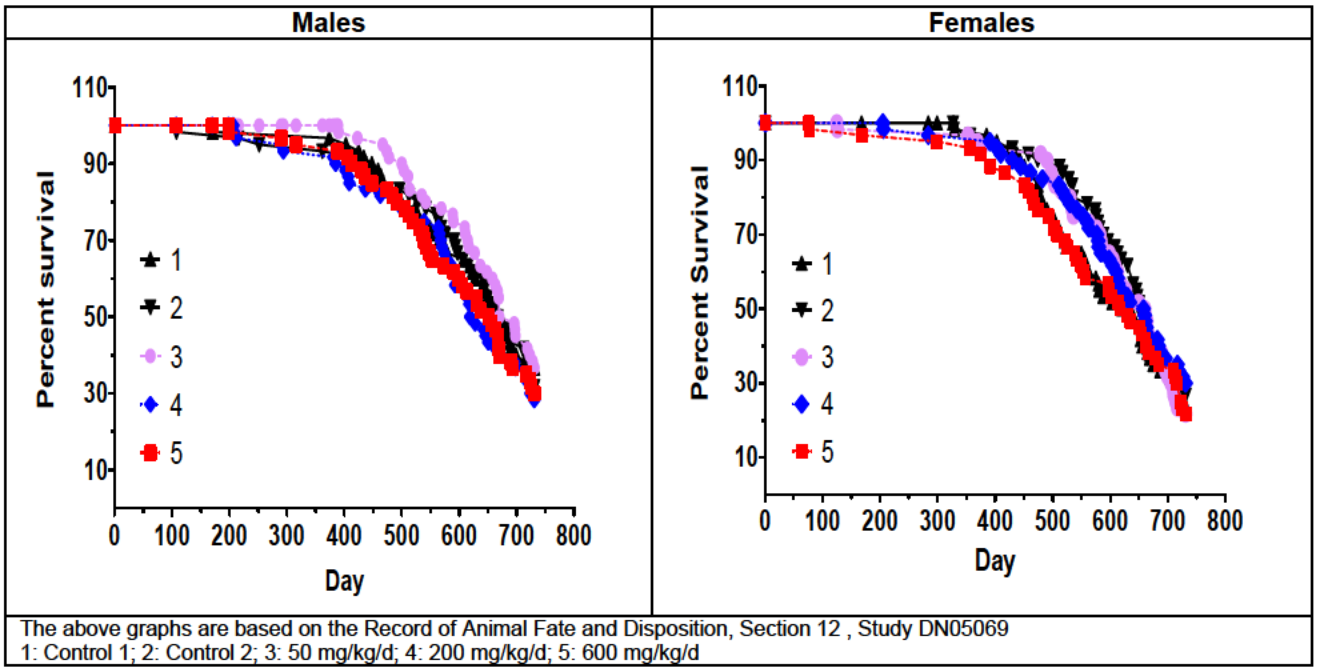
The animals were examined visually for mortality, morbidity injury and availability of food and water twice daily during weeks 1-52. Beginning week 53, a third mortality check was conducted.

The overall mortality rates were not significantly different between control and apixaban-treated male and female rats. Overall, 36% of control males, 33% of treated males, 27% of control females and 26% of treated females survived until the end of the study (Table 47, Figure 15).

Table 47: Reviewer's Summary of Mortality in Study DN05069

Main animals	Males					Females				
Dose, mg/kg	0	0	50	200	600	0	0	50	200	600
Group	1	2	3	4	5	1	2	3	4	5
Total number/group	60	60	60	60	60	60	60	60	60	60
Number surviving to terminal sacrifice	23	20	23	18	19	15	17	13	19	14
% survival	38	33	37	30	32	25	28	22	32	23
Intercurrent deaths	37	40	37	42	41	45	43	47	41	46
% mortality	62	67	62	70	68	75	72	78	68	76

Figure 15: Reviewer's Kaplan-Meier Curves - Rat Carcinogenicity Study DN05069



The above graphs are based on the Record of Animal Fate and Disposition, Section 12 , Study DN05069
1: Control 1; 2: Control 2; 3: 50 mg/kg/d; 4: 200 mg/kg/d; 5: 600 mg/kg/d

The pathologist noted that the most common causes of unscheduled deaths in males and females were pituitary gland tumors (adenomas and carcinomas), which were present at similar incidences in control and treated animals.

Clinical Signs

Detailed clinical examinations, including palpation of tissue masses, were made once weekly in all groups during treatment.

Although the sponsor concluded that the clinical observations were sporadic in nature, the reviewer noted the incidence of impaired limb function was increased in the apixaban-treated male and female groups, but not in a dose-dependent manner (Table 48). The incidence of red discolored skin increased in the high dose females, but not the high dose males.

Table 48: Reviewer's Summary – Clinical signs – Study DN05069

	Males					Females				
Dose, mg/kg	**0**	**0**	**50**	**200**	**600**	**0**	**0**	**50**	**200**	**600**
Limb function impaired	43/3	52/7	219/12	121/11	115/5	15/2	11/4	152/7	22/5	95/8
Skin discolored red	2/1	18/3	3/2	0/0	1/1	0/0	0/0	1/1	2/2	36/3

Body Weights

The animals in all groups were weighed on the day after receipt, prior to randomization, weekly during Weeks 1 to 16, then every 4 weeks for the duration of the study and immediately before necropsy.

Apixaban treatment had minimal effect on mean body weight and body weight gain in the female groups (Figure 16, Table 49). Although apixaban treatment had minimal effect on values for mean body weight and body weight gain in the male groups through week 56, mean body weight and body weight gain decreased up to 10% and 15%, respectively, in the high dose male group from weeks 60 to 104. However, a clear dose response relationship is lacking.

Figure 16: Sponsor's Body Weight Graphs - Rat Carcinogenicity - Study DN05069

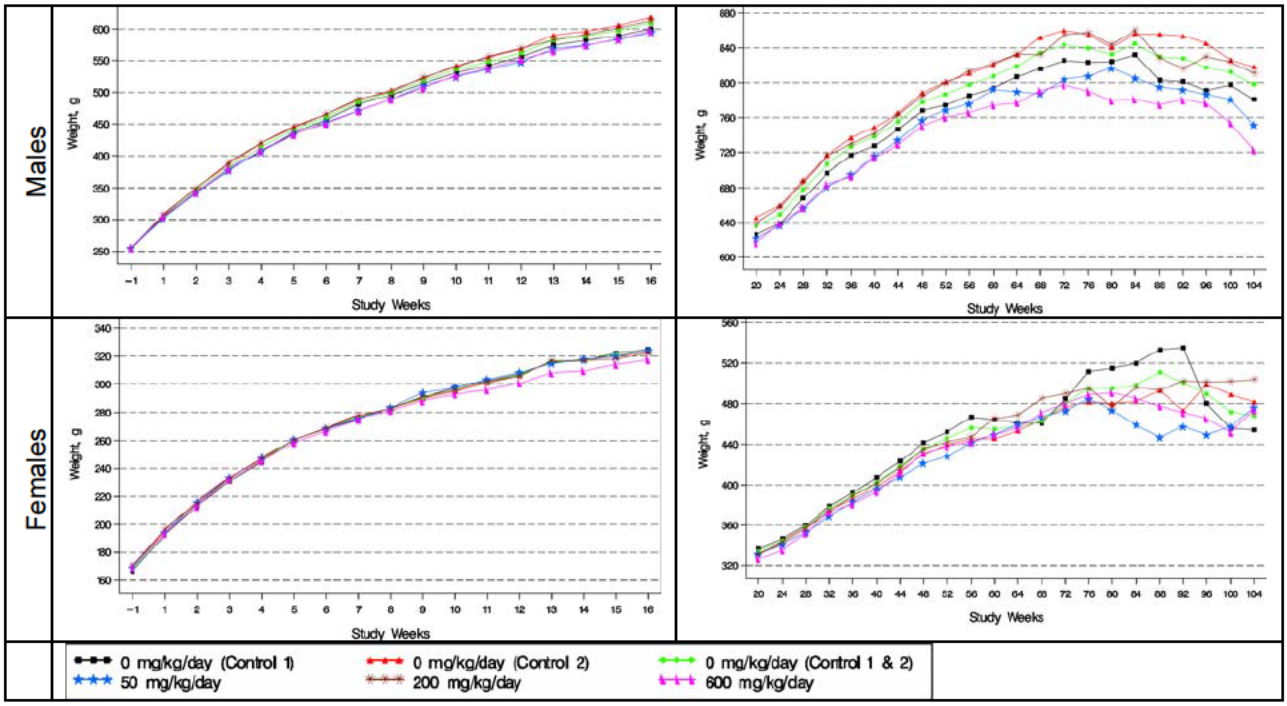


Table 49: Reviewer's Summary - Body Weight/Body Weight Gain - Study DN05069

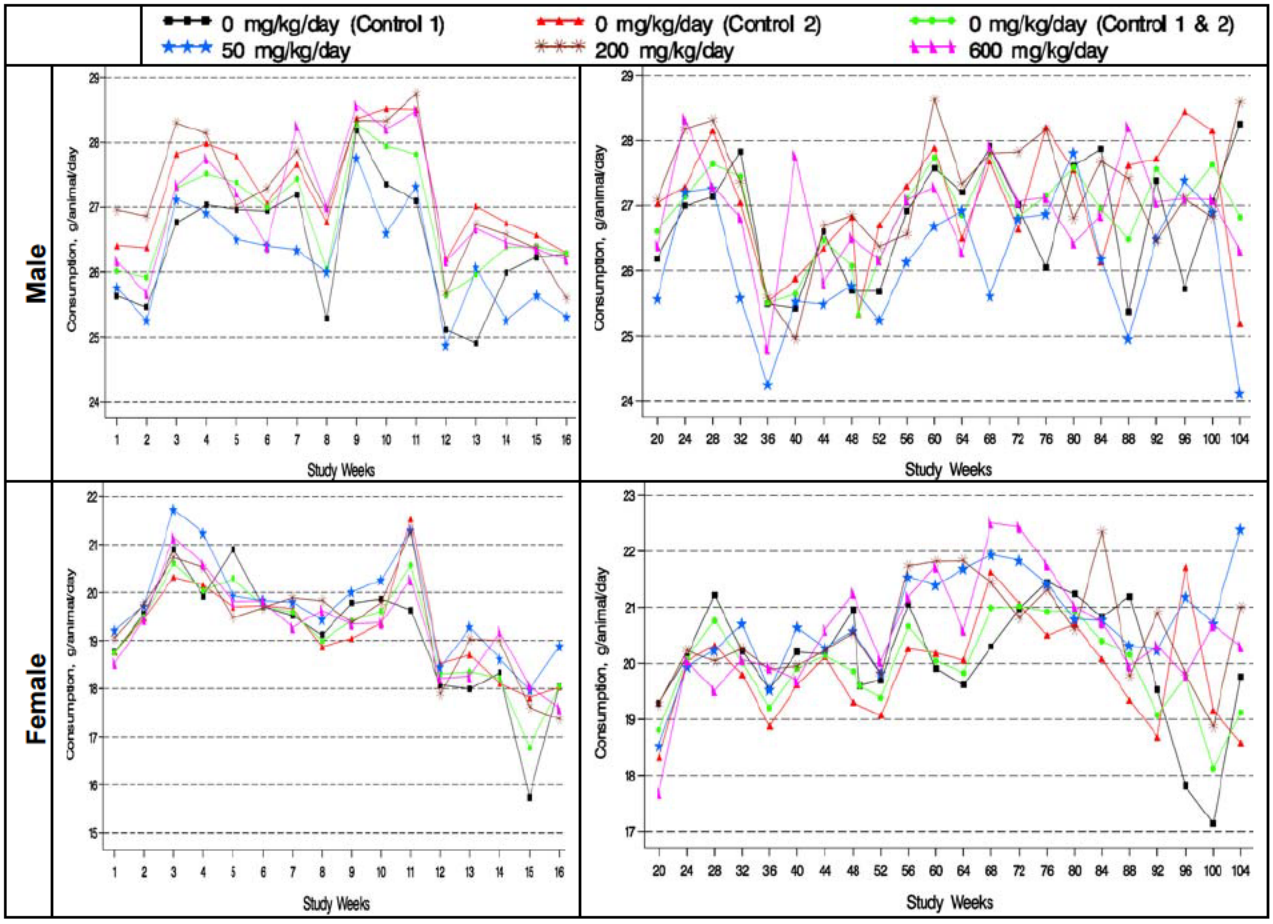
Group/ Week		Mean body weight, gm						Mean body weight gain, gm					
		1	2	Mean 1+2	3	4	5 (% 1+2 mean)	1	2	Mean 1+2	3	4	5 (% 1+2 mean)
Males	1	303.9	307.0	305.4	301.6	308.0	305.1						
	13	573.9	589.9	581.9	568.4	582.2	565.7 (97.2)	270	282.9	276.5	266.8	274.2	260.6 (94.2)
	24	638.7	659.6	649.1	636.2	657.5	638.8 (98.4)	334.8	352.6	343.7	334.6	349.5	333.7 (97.0)
	40	727.4	748.7	737.8	715.6	741.4	714.1 (96.8)	423.5	441.7	432.4	414	433.4	409 (94.6)
	52	774.3	801.1	787.3	768.5	799.6	760.6 (96.6)	470.4	494.1	481.9	466.9	491.6	455.5 (94.5)
	64	806.7	832.5	819.3	789.9	833.0	776.7 (94.8)	502.8	525.5	513.9	488.3	525	471.6 (91.8)
	76	823.8	855.4	839.8	807.8	858.0	790.4 (94.1)	519.9	548.4	534.4	506.2	550	485.3 (90.8)
	92	801.3	853.9	828.0	792.1	816.7	780.5 (94.2)	497.4	546.9	522.6	490.5	508.7	475.4 (90.9)
	104	780.7	818.2	798.1	751.5	811.1	722.7 (90.6)	476.8	511.2	492.7	449.9	503.1	417.6 (84.8)
Females	1	192.2	195.7	194	194	196.5	193.8						
	13	315.6	316.4	316	314.8	316.8	308.4 (97.6)	123.4	120.7	122.1	120.8	120.3	114.6
	24	346.6	342.6	344.6	341.4	343.9	336 (97.5)	154.4	146.9	150.65	147.4	147.4	142.2 (93.9)
	40	407.4	397.8	402.6	396.1	401.9	393.2 (97.6)	215.2	202.1	208.65	202.1	205.4	199.4 (94.4)
	52	452	439.7	445.9	428.3	442.2	438.1 (98.2)	259.8	244	251.9	234.3	245.7	244.3 (95.5)
	64	460.7	453.1	456.8	459.7	468.7	456 (99.8)	268.5	257.4	262.95	265.7	272.2	262.2 (97.0)
	76	512	481.6	494.9	484.7	495.3	488.8 (98.8)	319.8	285.9	302.85	290.7	298.8	295 (99.7)
	92	535.3	473.4	501.3	457.2	502.6	471.4 (94.0)	343.1	277.7	310.4	263.2	306.1	277.6 (97.4)
	104	454.1	481.9	468.4	476.1	503.9	474 (101.2)	261.9	286.2	274.05	282.1	307.4	280.2 (89.4)

Food Consumption

Food consumption was measured and recorded for all animals weekly during Weeks 1 to 16, then every 4 weeks for the duration of the study.

Apixaban did not affect mean food consumption values in a dose-related manner over the duration of the study (Figure 17). The majority of statistically significant differences in food consumption were in the low dose groups.

Figure 17: Sponsor's Figures – Food Consumption - Study DN05069



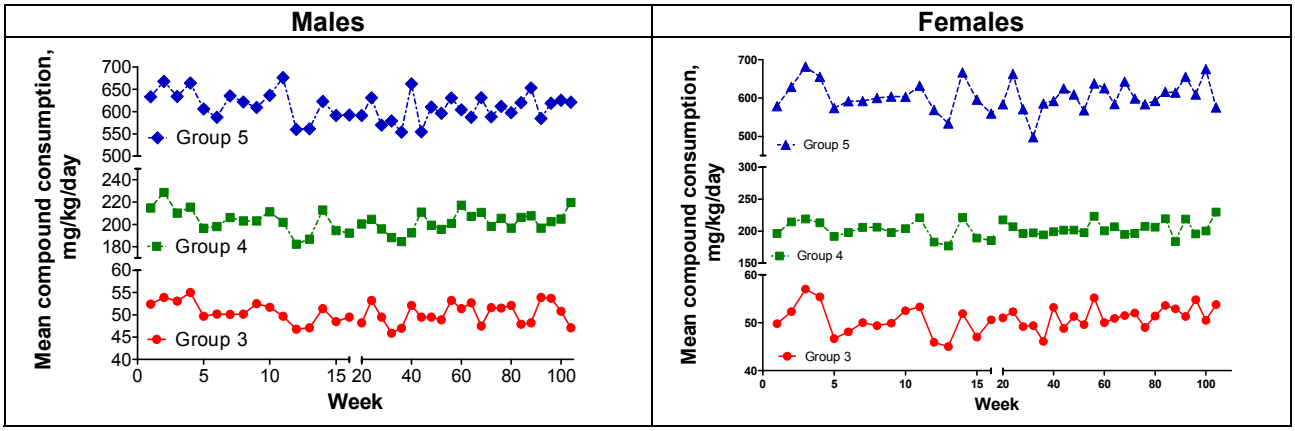
Achieved doses, based on body weight, diet admixture concentrations, and food consumption, were calculated once weekly through Week 16 and once monthly thereafter.

All weekly calculated average achieved doses at all doses for males and at the low and mid doses for females were within acceptance criteria (± 15% of intended doses) (Table 50, Figure 18). For the high dose females, the achieved doses were 83% to 114% of intended dose. The low value of 83% was a single event occurring for Week 32.

Table 50: Reviewer's Summary of Achieved Doses - Study DN05069

Males				Females			
Achieved Dose, mg/kg	**50**	**200**	**600**	**Achieved Dose, mg/kg**	**50**	**200**	**600**
Mean	50.45	202.7	611.1	Mean	50.86	203.2	604.8
SD	2.36	10.0	31.6	SD	2.73	12.4	38.7
% expected	110.9	101.4	101.8	% expected	101.7	101.6	100.8
Maximum	55	228.7	676.3	Maximum	57	229.9	682.2
Minimum	45.9	182.2	553.8	Minimum	45	176.8	498.3

Figure 18: Reviewer's Graphs of Achieved Doses - Study DN05069



Gross Pathology

The surviving animals in all main groups were sacrificed during week 105. Animals found dead or euthanized during the study were necropsied at the earliest opportunity. The animals were subjected to systematic examination and the organs listed in Table 51 were fixed in 10% neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using a modified Davidson's fixative (Creasy and Jonnassen 1999). The urinary bladder and lungs were initially inflated with 10% neutral buffered formalin prior to further fixation by immersion.

Table 51: List of Tissues Collected - Study DN05069

Adrenal glands	Liver[#]	
Aorta	Lungs	Skin (dorsal thorax)
Bone with marrow (femur and sternum) (Bone marrow smear[†*])	Lymph nodes (mandibular, mesenteric and regional where applicable)	Spinal cord (cervical, thoracic, lumbar)
Brain (cerebrum, mid-brain, cerebellum, and medulla/pons)	Mammary gland	Spleen
Cecum	Nasal turbinates[‡]	Stomach (glandular (fundic and pyloric) and nonglandular)
Colon	Ovaries	Testes
Duodenum	Pancreas	Thymus
Epididymides	Pituitary	Thyroid with parathyroids
Esophagus	Prostate	Tissue masses with regional lymph node
Eyes with optic nerve	Rectum[‡]	Tongue
Gross lesions	Salivary glands (mandibular)	Trachea
Harderian glands	Sciatic nerve	Urinary bladder
Heart	Seminal vesicles	Uterus with cervix
Ileum	Skeletal muscle (diaphragm)	Vagina
Jejunum	Skeletal muscle (quadriceps femoris)	Zymbal's gland
Kidneys		

The incidences of macroscopic pathology findings in males and females were generally similar across control and treated groups and were considered unrelated to treatment with apixaban. The pathologist noted enlarged brains in three high dose females, but concluded the finding was not drug related upon microscopic examination, because this change was attributed to ventricular dilatation secondary to lymphoma in one female or pituitary adenoma in the other females.

Histopathology

After fixation, samples of protocol-designated tissues from all main study animals were processed to hematoxylin and eosin-stained paraffin sections. Although a (b) (4) veterinary pathologist conducted the microscopic evaluation of early decedents, the sponsor subsequently decided to have a sponsor's pathologist conduct the microscopic evaluation for this study.

Peer Review

A second sponsor's pathologist conducted a peer review of all tissues from 34 control males, 19 control females, 21 high dose males, and 15 high dose females. In addition, the second pathologist reviewed the bone marrow, thyroid gland, and parathyroid gland from all animals and the mammary gland from all females. The review also included all histiocytic sarcomas and lymphomas (systemic neoplasms) as well as selected neoplasms from the adrenal gland, pituitary gland, thymus, uterus, and cervix.

Neoplastic

The incidences of the most notable tumors in the rat carcinogenicity study are summarized in Table 52 below. The statistical evaluations of the sponsor are in Appendix 7 and Appendix 8. Historical control data provided by the sponsor are in Appendix 9. The reader is also referred to the statistical review by Dr. Matthew Jackson.

The incidence of malignant lymphoma, a common tumor, was increased in the high dose males (p_t = 0.037) and females (p_t = 0.032). However, the p value for this tumor in the trend test did not attain the significance level of p_t < 0.005 required for a common tumor to be considered positive. Additionally, the incidences of malignant lymphoma for males (5.0%) and females (6.7%) in the current study are within or close to the conducting laboratory's range of concurrent and historical values for this rat strain (2 - 20% and 0 – 3.3%, respectively).

The only tumor with a p-value < 0.05 in the pairwise Exact Fisher test was adrenal pheochromocytoma in the low dose male group. However, this tumor did not exhibit a positive dose response relationship or a p-value of < 0.005 in the trend test.

Therefore, according to the criteria in current CDER guidance, no statistically significant neoplastic findings were related to apixaban treatment under the conditions of this study.

Table 52: Reviewer's Summary – Neoplastic Findings - Study DN05069

Rat Carcinogenicity Study		BMS-562247 Dose level (mg/kg/day)							
Neoplastic findings		Male				Female			
All animals		0	50	200	600	0	50	200	600
Organ/Tissue Finding	#/group	120	60	60	60	120	60	60	60
Multi-centric neoplasm	#	120	60	60	60	120	60	60	60
Lymphoma, malignant	#	1	1	0	**3**	2 (2, 0)†	1	2	4
	%	0.83	1.67	0	**5.0**	1.67 (3.3, 0)†	1.67	3.33	**6.67**
Peto-Pike trend test, p-value					**0.037**				**0.032**

Rat Carcinogenicity Study Neoplastic findings		BMS-562247 Dose level (mg/kg/day)							
		Male				Female			
All animals		0	50	200	600	0	50	200	600
Organ/Tissue Finding #/group		120	60	60	60	120	60	60	60
Adrenal gland	#	120	60	60	60	120	60	60	60
Pheochromocytoma	#	9	**12***	5	4	3	3	0	2
	%	7.5	**20.0**	8.3	6.6	2.5	5.0	0	3.33
Peto-Pike trend test, p-value					0.745				0.430
Pheochromocytoma, complex	#	0	0	0	0	0	0	0	1
	%	0	0	0	0	0	0	0	1.67
Pheochromocytoma, combined	#	9	12	5	4	3	3	0	3
	%	7.5	20.0	8.3	6.6	2.5	5.0	0	5.0

* One-Sided Exact Fisher Test, $p \leq 0.05$, † Values for individual control groups

Non Neoplastic

The sponsor concluded that no drug-related non-neoplastic change was observed at any dose in the rat carcinogenicity study. The reviewer notes some statistically significant findings in Table 53.

Some findings, such as increased incidence of hyperplasia in the Harderian gland of males and increased incidence of atrophy in the Harderian gland of females, did not exhibit a clear dose relationship. Although the higher incidence of bone marrow hyperplasia within the femur and sternum in high dose females exhibits some dose relationship, the sponsor attributed this finding to normal biologic variation. In contrast, the mid-dose males have a decreased incidence of bone marrow hyperplasia within the femur and sternum.

An increase in the incidence of extramedullary hematopoiesis in the adrenal cortex in females was observed at all doses. The sponsor attributed this finding to normal biological variation because corresponding increases in extramedullary hematopoiesis were not observed in other tissues in these animals (eg, spleen and liver). However, some treated females did exhibit increased incidences of extramedullary hematopoiesis in the spleen and liver. The incidence of chronic thrombosis in the adrenal gland was decreased in the treated female groups. In addition, the incidence of pigment in the pancreas increased in treated male groups and pigmented macrophage accumulation increased in the mandibular lymph node of high dose males. Increased extramedullary hematopoiesis, increased pigment, and decreased thrombosis are consistent with the pharmacodynamic effect of apixaban as a FXa inhibitor.

Table 53: Reviewer's Summary – Non-Neoplastic Findings - Study DN05069

Rat Carcinogenicity Study Non-neoplastic findings		BMS-562247 Dose level (mg/kg/day)							
		Male				Female			
	All animals	0	50	200	600	0	50	200	600
Organ/Tissue Finding	#/group	120	60	60	60	120	60	60	60
Number of animals examined		120	60	60	60	120	60	60	60
Adrenal gland, hyperplasia, medullary cell	#	32	17	13	9	15	9	**18‡**	13
	%	26.6	28.3	21.6	15.0	12.5	15.0	**30.0**	21.6
Adrenal gland, hematopoiesis, extramedullary	#	4	2	3	3	7	**9†**	7	8
	%	3.3	3.3	5.0	5.0	5.8	**15.0**	11.7	13.3
Adrenal gland, thrombosis, chronic	#	3	0	2	0	23	**5†**	7	**3‡**
	%	2.5	0	3.3	0	19.2	8.3	11.6	**5.0**
Bone marrow, femur, hyperplasia	#	76	36	**24†**	35	77	42	44	**47†**
	%	63.3	60	**40**	58.3	64.2	70	73	**78.3**
Bone marrow, sternum, hyperplasia	#	77	41	**27†**	36	62	37	36	**46‡**
	%	64.2	68	**45**	60	51.6	61.6	60	**76.6**
Harderian gland, glandular epithelium hyperplasia	#	5	**13‡**	**8†**	4	9	2	1	3
	%	4.2	**21.6**	**13.3**	6.6	7.5	3.3	1.7	5.0
Harderian gland, atrophy	#	36	12	12	21	60	31	**47‡**	34
	%	30	20	20	35	50	51.6	**78.3**	56.6
Liver, hematopoiesis, extramedullary	#	25	12	19	16	33	**27†**	22	16
	%	20.8	20	31.6	26.6	27.5	**45**	36.7	26.6
Lung, bronchioalveolar cell hyperplasia	#	0	1	**5‡**	1	3	1	1	3
	%	0	1.7	**8.3**	1.7	2.5	1.7	1.7	5.0
Mandibular lymph node, pigmented macrophage accumulation	#	74	36	33	**48‡**	104	48	52	51
	%	61.6	60	55	**80**	86.6	80	86	85
Pancreas, pigment	#	84	**51†**	46	49	88	45	41	38
	%	70	**85**	76.6	81.6	73.3	75	68.3	63.3
Pancreas, basophilic focus	#	12	12	**13†**	3	18	10	5	10
	%	10	20	**21.6**	5	15	16.6	8.3	16.6
Spleen, hematopoiesis, extramedullary	#	65	33	34	33	58	29	**41‡**	33
	%	54.1	55	56.6	55	48.3	48.3	**68.3**	55

† $p < 0.05$ ‡ $p<0.01$ using one-sided Exact Fisher test

Toxicokinetics

At 1, 2, 4, 8, and 24 hours after the start of the dark cycle, blood samples were collected from four toxicokinetic (TK) animals treated with apixaban (BMS-562247) per timepoint on days 175, 356, and 384 for determination of plasma concentrations of apixaban and O-desmethyl apixaban sulfate (BMS-730823) by validated LC/MS/MS methods with a lower limit of quantification of 2 ng/mL. Blood was similarly collected from control TK animals on Days 178, 361, and 389. In calculating AUC values, the sponsor assumed that the toxicokinetics of apixaban were at steady state by day 175 of the study and used the measured value at 24-hours on day 176 for both the 0-hour and the 24-hour concentration value. The additional collection on day 384 was for comparison of the exposure of [redacted] (b) (4) apixaban (Lot 5L00821) used beginning in week 52 to exposure of apixaban (Lot 4K89700) used prior to week 52.

Apixaban at concentrations that ranged from 2 to 6 ng/mL was detected in 8 of 124 plasma samples from control rats given basal diet. The source of the contamination was not identified. Because the highest concentration (6 ng/mL) of these sporadic findings

was less than 2 % of mean concentrations in the low dose group, the sponsor concluded these events did not impact the study results.

Exposures (AUC) to apixaban generally increased with dose. However, the exposures were not dose proportional with only 2.2 and 2.7 fold increases in exposure at the high dose (600 mg/kg) in males and females, respectively, compared to the exposure at the low dose (50 mg/kg) (Table 54). Exposures to apixaban in females were 1.3 to 1.8 fold higher than exposures in males. Exposures to apixaban were generally similar on Days 175 and 356, indicating little accumulation beyond week 25. Exposures to apixaban were 1.1 to 1.8 fold higher during week 54 when a [redacted] (b) (4) lot (5L00821) was used compared to the exposures when [redacted] (b) (4) lots (4K83298 and 4K89700) were used. The difference in exposure was larger at the low dose (1.6, 1.8) compared to the high dose (1.06, 1.1). The exposures (AUC) to apixaban in high dose males and female rats on day 356 and 384 were similar to the exposures at 600 mg/kg in the 3-month preliminary dietary rat study. The total exposures (AUC) to apixaban in high dose males and female rats on day 356 were 6.2- and 10.1-fold the total exposure in humans treated with the recommended human dose of 5 mg twice a day. However, the difference in protein binding of rat (95.3-96%) and human (86.8%) serum decreases the ratio of unbound exposure to 1.9-2.0 and 3.6-4.0 for high dose male and female rats, respectively (Table 55).

Exposure to O-desmethylapixaban sulfate (M1, BMS-730823) represented 1.4 to 1.8% and 0.6 to 0.7% of the exposure to the parent apixaban in males and females, respectively. Exposure to BMS-730823 was 1.3 to 1.9 fold higher in males than the exposure in females on days 356 and 384.

Table 54: Reviewer's Summary – Toxicokinetics - Study DN05069

				Males			Females		
Analyte	**Day/Lot**	**Dose (mg/kg)**		**50**	**200**	**600**	**50**	**200**	**600**
BMS-562247 (apixaban)	Day 175/ 4K83298	C_{max}	µg/mL	0.44	0.77	0.80	0.51	0.94	1.26
		$AUC_{(0-24)}$	µg*hr/mL	8.51	16.2	16.6	10.9	20.9	29.2
	Day 356/ 4K89700	C_{max}	µg/mL	0.39	0.75	1.01	0.54	1.05	1.37
		$AUC_{(0-24)}$	µg*hr/mL	8.67	16.0	19.1	11.9	24.3	31.3
	Day 384/ 5L00821	C_{max}	µg/mL	0.64	0.99	1.03	1.14	1.41	1.67
		$AUC_{(0-24)}$	µg*hr/mL	13.4	20.3	20.3	22.0	32.3	35.5
BMS-7308 23	Day 175	$AUC_{(0-24)}$	µg*hr/mL	0.18	0.20	0.25	NC	0.048	0.049
	Day 356	$AUC_{(0-24)}$	µg*hr/mL	0.22	0.24	0.36	NC	0.14	0.19
	Day 384	$AUC_{(0-24)}$	µg*hr/mL	0.24	0.28	0.36	NC	0.22	0.22
3-month dietary study			**Dose**	**600**	1800	2400	**600**	1800	2400
Study DN04100 Lot 4K85835	BMS-562247	C_{max}	µg/mL	1.16	1.36	1.41	1.84	1.77	2.52
		$AUC_{(0-24)}$	µg*hr/mL	22.1	24.8	27.0	36.9	35.0	47.0
	BMS-730823	C_{max}	µg/mL	0.013	0.018	0.017	0.007	0.007	0.013
		$AUC_{(0-24)}$	µg*hr/mL	0.27	0.35	0.33	0.15	0.05	0.18

Table 55: Reviewer's Summary of Exposures in Study DN05069

BMS-562247 (apixaban)			Males			Females		
Day/Lot	Dose (mg/kg)		50	200	600	50	200	600
Day 175/ (b) (4)	Total $AUC_{(0-24)}$	µg*hr/mL	8.51	16.2	16.6	10.9	20.9	29.2
	Unbound AUC	µg*hr/mL	0.34	0.65	0.66	0.51	0.98	1.37
	Ratio of rat unbound AUC/ unbound human AUC		0.83	1.58	1.61	1.25	2.4	3.35
(b) (4)	Total $AUC_{(0-24)}$	µg*hr/mL	8.67	16.0	19.1	11.9	24.3	31.3
	Unbound AUC	µg*hr/mL	0.35	0.64	0.76	0.56	1.14	1.47
	Ratio of rat unbound AUC/ unbound human AUC		0.86	1.56	1.86	1.36	2.8	3.6
384/ (b) (4)	Total $AUC_{(0-24)}$	µg*hr/mL	13.4	20.3	20.3	22.0	32.3	35.5
	Unbound AUC	µg*hr/mL	0.54	0,81	0.81	1.03	1,52	1,66
	Ratio of rat unbound AUC/ unbound human AUC		1.32	1.98	1.98	2.5	3.7	4.0

At the RHD of 10 mg (5 mg BID) for AF, the apixaban total $AUC_{(0-24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). Using % unbound apixaban as 4.0% and 4.7% for male and female rats, respectively, and 13.2% for human. The human unbound AUC is 0.409 µg•h/mL

Dosing Formulation Analysis

The admixtures were considered homogenous since the minimum and maximum recoveries for individual replicates were 84.8% and 112%, respectively, of nominal (Table 56). All diet admixtures were within the range of mean recovery and were 94.2% to 107.3% of nominal (Table 57). Analysis of samples stored at room temperature indicated that apixaban was stable in diet admixture at both low and high concentrations for at least 15 days. No apixaban was detected in the basal diet.

Table 56: Reviewer's Summary of Homogeneity Analysis - Study DN05069

Week	Dose, mg/kg	Males			Females		
		50	200	600	50	200	600
1	Mean	97.2	96.0	97.1	95.3	93.5	94.2
	Minimum	101.6	97.9	**112**	96.8	96.1	94.9
	Maximum	94.1	93.8	**84.8**	91.6	91.5	92.3
10	Mean	98.1	99.8	99.2	99.9	98.0	98.1
	Minimum	102.7	112.4	101	101.9	100.7	101.2
	Maximum	96.0	96.3	99.4	97.9	94.1	95.0
26	Mean	100.7	104.8	99.6	99.6	103.6	104.4
	Minimum	104.8	111.3	102.4	104.9	105.2	108.6
	Maximum	98.2	100.9	97.0	96.1	101.7	101.4
36	Mean	97.9	99.3	102.4	98.2	102.7	102.2
	Minimum	100.6	100.8	105.8	101.8	103.8	106.3
	Maximum	95.9	97.0	100.4	96.2	101.1	100.1

Three replicates were analyzed from top, middle and bottom. The mean, minimum and maximum of the nine samples are presented.

Table 57: Reviewer's Summary of Apixaban Recovery in Admixtures

Week	Dose, mg/kg	Males			Females		
		50	200	600	50	200	600
1	Mean	99.6	97.3	102.1	100.0	**94.2**	99.1
	Minimum	98.1 101.1	96.8	96.5	99.6	93.3	98.6
	Maximum		97.9	107.2	100.3	95.5	99.6
14	Mean	98.4	101.5	104.1	102.7	102.5	106.1
	Minimum	96.9	100.0	103.4	102.0	101.8	105.0
	Maximum	100.2	102.8	104.9	103.1	103.1	107.3
39	Mean	97.0	98.5	96.3	99.9	103.6	96.5
	Minimum	96.5	98.9	95.5	99.6	102.8	95.4
	Maximum	99.5	97.7	96.8	100.3	104.5	97.4
52	Mean	99.6	101.9	97.8	103.7	106.1	98.8
	Minimum	98.6	99.4	94.3	102.0	105.7	98.0
	Maximum	100.3	103.2	99.7	105.7	106.4	100.4
65	Mean	101.5	100.3	104.3	99.8	101.9	101.3
	Minimum	101.2	99.9	103.3	99.0	100.1	100.4
	Maximum	101.9	100.5	105.2	100.3	103.3	102.9
78	Mean	98.7	99.7	97.7	99.1	101.7	100.6
	Minimum	98.1	98.6	95.9	97.8	**93.2**	99.7
	Maximum	99.4	100.7	99.1	99.9	**111.1**	101.3
91	Mean	101.0	103.2	105.7	101.0	100.9	**107.3**
	Minimum	99.2	100.9	102.8	100.1	100.4	105.7
	Maximum	102.1	106.2	107.6	101.9	101.7	110.5
104	Mean	102.1	100.8	101.8	101.3	103.4	101.6
	Minimum	101.8	100.1	101.3	100.9	102.3	101.3
	Maximum	102.3	101.4	102.4	102.0	105.0	102.3

Three replicates were analyzed. The mean, minimum and maximum of the three samples are presented.

9 Reproductive and Developmental Toxicology

Study reports for the reproductive and developmental studies using oral gavage administration (Fertility and Early Embryo Development [FEED] in rats, Embryo-Fetal Development [EFD] in rats, rabbits and mice, Pre-/Postnatal Development [PPND] in rats, and juvenile toxicity in rats) were previously reviewed under INDs (b) (4) and (b) (4) (Table 58). The full reviews of these studies summarized in Table 59 can be found in DARRTS. The NDA submission included an EFD study in rabbits using intravenous administration. This study is reviewed in section 9.2.

Table 58: Overview of Reproductive and Developmental Studies

Document number	Study number	Study	BMS-562247 Lot number	Reviewed under	
				(b) (4)	
930016567	DN05056	Rat FEED	4K8535	R. Honchel	
930009803	DN03042	Rat EFD	3E68243		P. Harlow
930011664	DN03045	Rabbit EFD	3J69810 and 4B8161	R. Honchel	
930021450	DN06023	Mouse EFD	5B06549	R. Honchel	
930036745	DN08001	Rat PPND	5L00821	R. Honchel	
930035423	DN09014	Rat – Juvenile Toxicity	7A28071	R. Honchel	
930016662	DN05050	Rabbit EFD - IV	4K85835	Not previously reviewed	

Table 59: Reviewer's Summary of Previously Reviewed Reproductive and Developmental Studies

Study	FEED - Rat	EFD - Rat	EFD - Mouse	EFD - Rabbit	PPND - Rat
Species	Rat	Rat	Mouse	Rabbit	Rat
Document no.	930016567	930009803	930021450	930011664	930036745
Study code	DN05056	DN03042	DN06023	DN03045	DN08001
Drug lot/purity	4K8535, 99.9%	3E68243, 98.8%	5B06549, 99.7%	3J69810 and 4B81615, 99%	5L00821, 99.8%
Conducting lab and location	(b) (4)				
Study dates	June - Sept 2005	May –June 2003 (TK-2004)	April – May 2006	Dec 2003	Jan 2008- July 2008
GLP/QA	Yes	Yes	Yes	Yes	
Vehicle	99.5% Labrafil M-1944 CS/0.5% Tween 80),	99.5% Labrafil M-1944 CS/ 0.5% Tween 80	99.5% Labrafil/0.5% Tween 80	0.5% Methocel® A4M and 1% Tween® 80	0.5% Tween 80: 99.5% Labrafil
Strain	Sprague Dawley	Sprague Dawley	CD-1 mice	New Zealand white	Sprague Dawley
Number/group	25/sex/group (10/sex for TK and 10/sex for coagulation)	25 female/group	25/sex/group (10/sex for TK and 10/sex for coagulation)	20 female/group (30 for High Dose TK)	25 female/group
Doses, mg/kg	0, 50, 200, 600	0, 100, 300, 1000, 3000	0, 600, 900, 1500	0, 60, 180, 500, 1500	0, 25, 200, 1000
Route	Oral gavage	Oral gavage	Oral gavage	Oral gavage	oral
Treatment duration for males (M) and females (F)	M: daily from 14 d prior to mating for a total of 28 d. F: daily for 15 d prior to mating through GD 7	F: daily from GD 6 through GD15	F: daily from GD 6 through GD15	F: GD 6 through GD 19	F_0: GD 6 to PND 20
Cesarean section day	Gestation day (GD) 16	GD 20	GD 18	GD 29	F0: PND 21 F1: PND 21
Study acceptability	Litter numbers/ group acceptable. High dose adequate, because PT prolonged and HD males decreased BW gain (15%)	Litter numbers /group acceptable. High dose acceptable - BW gain and food consumption in HD was 7 and 10% less than control	Litter number/group acceptable. HD was half of the high dose of 3000 mg/kg in the carcinogen-icity study, but exposure (AUC) values were similar for all doses.	Litter number/group acceptable. High dose was maximum possible dose based on the maximum gavage volume (10 mL/kg) that can be administered and the maximum concentration of apixaban (150 mg/mL) that can be formulated in vehicle	Litter number/group acceptable. High dose was maximum poss ble dose based on the maximum gavage volume (6.7 mL/kg) that can be administered and the maximum concentration of apixaban (150 mg/mL) that can be formulated in vehicle
Comments: Parental	Mortality attributed to dosing errors or accident - M: 2 in 600 mg/kg group; F: 1 at 0 mg/kg. Body weight gain in high dose M decreased 15%	I death at 1000 mg/kg, but not considered drug-related. Increased peri-vaginal bleed-ing in all dose groups, but highest at 3000.	Mortality attributed to dosing errors or accident - 1 each in saline control, vehicle control, low, mid doses, & 2 at high dose	One doe in each of the 180- and 1500-mg/kg/day groups aborted on GDs 18 and 27, respectively – Not drug related	1 control dam with dystocia was euthanized on LD0. 1 high dose dam cannibalized all her pups. Increased signs of bleeding in mid & high dose in gestation, but not parturition
Comments: Embryo/fetal/ offspring	No drug-related effects	Pre-implantation loss slightly higher for two highest doses, but considered comparable across dose groups	No adverse drug-related effects. All fetal alterations noted were unrelated to drug treatment	No adverse drug-related effects. All fetal alterations noted were unrelated to drug treatment	No adverse drug-related effects on # pups/litter, sex ratio, survival or body weight. Slight decrease in mating & fertility in high dose F1 female, but not F1 males

Study	FEED - Rat	EFD - Rat	EFD - Mouse	EFD - Rabbit	PPND - Rat
Comments: Other findings:	No drug-related effects on estrous cycling, mating or fertility in treated M or F. Increased PT time all dose groups	No drug-related change in reproductive parameters or fetal development. Fetal exposure 6-9% of maternal	No drug-related change in reproductive parameters or fetal develop-ment. Increased PT time all dose groups	No drug-related change in reproductive parameters or fetal development. No fetal exposure detected	No maternal deaths due to excess bleeding. No malformed pups in drug-treated groups. Increased PT time in F0 dams all dose groups.
Parental NOAEL, mg/kg	M: 200 F: 600	1000	1500	1500	F0: 1000
Offspring NOAEL, mg/kg	Embryo toxicity: 600 Fertility: 600	Fetal toxicity & malformation: 3000	Fetal toxicity & malformation: 1500	Fetal toxicity & malformation: 1500	F1 pre-natal, peri-natal & post-natal development: 1000. F1 mating and fertility: F – 200; M – 1000 F2 prenatal: 1000
Doses, mg/kg	0, 50, 200, 600	0, 100, 300, 1000, 3000	0, 600, 900, 1500	0, 60, 180, 500, 1500	0, 25, 200, 1000
Ratio unbound exposure at NOAEL to human	Male: 2.4 Female, embryo toxicity and fertility: 4.2	Maternal: 4.2 Fetal toxicity & malformation: 4.2	Maternal, fetal toxicity & malformation: 19.1	Maternal, fetal toxicity & malformation: 0.33	F0, F1 toxicity & development, F1 male fertility, F2 toxicity: 5.4 F1 female fertility: 4.9

In the following sections, the reviewer summarizes for each previously reviewed reproductive and developmental study those parameters that affect the determination of the reviewer's NOAELs above. The intravenous EFD study in rabbits was not previously reviewed and is reviewed below in section 9.2.

9.1 Fertility and Early Embryonic Development

In an acceptable fertility and early embryo development study, male and female rats were treated with 0, 50, 200 and 600 mg/kg/d. The NOAEL for paternal and maternal toxicity was 200 and 600 mg/kg, respectively, because body weight gain decreased slightly in the high dose males (Table 60). All treated groups had significant increases in prothrombin time in blood collected 2 hours after dosing. No drug-related effects were observed on estrous cycling, mating or fertility in treated males or treated females. The NOAEL for fertility and embryo toxicity is 600 mg/kg.

Table 60: Reviewer's Summary Rat FEED – Document 930016567

Rat FEED	Dose (mg/kg/d)				
Study DN05056	**0**	**50**	**200**	**600**	**Historical***
Body weight gain: M, D 1-36	85.8	73.5	81.4	72.5	
F, D 0-16	81.0	87.4	84.9	80.4	
Prothrombin time, sec M	16.87	23.58*	26.38*	25.44*	
F	18.19	26.65*	31.76*	33.86*	
Treated males with untreated females					
Males mated	25	25	25	25	
Days in cohabitation	1.8	2.6	1.8	2.0	
Females pregnant	24	23	22	25	
Fertility index	96	92	88	100	
Corpora lutea /litter	15.7	16.0	15.8	14.8	
Implantations/litter	14.9	15.4	15.3	14.0	
Pre-implantation loss/litter	0.047	0.03	0.031	0.055	
Post-implantation loss/litter	0.05	0.013	0.032	0.042	
Resorption/litter	0.8	0.2	0.5	0.5	
# litters with any resorption (%)	12 (50)	5 (21.7)	7 (31.8)	10 (40)	
Live fetuses/litter	14.1	15.2	14.8	13.5	

Rat FEED	Dose (mg/kg/d)				
Study DN05056	**0**	**50**	**200**	**600**	**Historical***
Females with viable fetus (%)	24 (100)	23 (100)	22 (100)	25 (100)	
Treated females with untreated males					
Females mated	24 [a]	25	25	25	29 studies
Days in cohabitation (Range)	2.3 (1-7)	3.2 (1-4)	3.0 (1-13)	2.9 (1-18)	
Females re-mated	0	0	0	1	
Females pregnant	22	24	25	23	
Mating (fertility) Index	91.7	96	100	92	95.6 (87.5-100)
Corpora lutea/litter	15.0 (10-17)	16.5 (11-25)	16.6 (13-20)	15.9 (11-20)	16.4 (14.6-18.3)
Implantations/litter	14.2 (5-17)	15.7 (12-18)	15.7(13-18)	15.5 (10-20)	15.0 (13.5-16.0)
Females with viable fetus	22	24	25	23	
Females with no live fetus	0	0	0	0	0.01[b] (0-1)
Females with normal placentae	22 (100)	24 (100)	25 (100)	23 (100)	
Viable fetuses/litter	13.3 (1-17)	15.2 (7-16)	15.2 (6-17)	15.1 (0-16)	14.4 (13.0-15.7)
Pre-implantation loss/litter	0.056	0.042	0.048	0.025	0.0224 (0.01-0.049)
Resorptions/litter (range)	0.9 (0-4)	0.4 (0-2)	0.6 (0-2)	0.4 (0-2)	0.6 (0.2-1.2)
# litters with any resorption (%)	11 (50)	9 (37.5)	11 (44)	9 (39.1)	
apixaban M $AUC_{(0-24)}$[1], ng*hr/mL F		12800 23500	24400 22800	27600 36300	

* Historical studies used necropsy on GD 20, not GD 16. [a] Excludes female 603, who was found dead on Day 12 presumably from an intubation accident, [b] 2/205 litters

Table 61 below compares the exposures of rats at the NOAEL dose and at the dose at which toxicity was observed in the FEED study with exposures in patients with atrial fibrillation receiving 5 mg of apixaban twice a day.

Table 61: Comparison of Rat FEED Exposures to Human Exposures

Rat FEED		Comparisons to human exposure							
Study DN05056		**Based on NOAEL dose**				**Based on toxic dose**			
	NOAEL, mg/kg	**Total**		**Unbound**		**Total**		**Unbound**	
		AUC	**Fold**	**AUC**	**Fold**	**AUC**	**Fold**	**AUC**	**Fold**
Parental M	200	M: 24.4	7.9	M: 1.0	2.4	M: 27.6	8.9	M: 1.1	2.7
F	600	F: 36.3	11.7	F: 1.7	4.2	F: 36.3	11.7	F: 1.7	4.2
Fetal toxicity	600	36.3	11.7	1.7	4.2	36.3	11.7	1.7	4.2
Fertility	600	36.3	11.7	1.7	4.2	36.3	11.7	1.7	4.2

The % unbound apixaban was 4.0% and 4.7% for male and female rats, respectively, and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0-24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL

9.2 Embryonic Fetal Development

Rat

In an acceptable embryo-fetal development study in rats, the NOAEL for maternal toxicity was 1000 mg/kg, because of a decrease in mean body weight gain and the significantly increased incidence of signs of vaginal bleeding at 3000 mg/kg (Table 62). Pre-implantation loss increased slightly in the two highest dose groups. However, the sponsor did not consider this increase significant, because dosing did not begin until GD 6 presumably after implantation and the number of live fetuses was not affected. Therefore, the NOAEL for fetal toxicity was 3000 mg/kg. The only fetal alteration that increased with dose was the presence of a cervical rib at the 7th cervical vertebrae. However, the increase was not statistically significant and the incidence (8.0%) is within the historical range for this finding (2005-2007 (b) (4) Historical Control Data for

Crl:CD(SD) rats, range 0-9.1%). Since the percentage of fetuses with any malformation in all dose groups was within the concurrent control range, the NOAEL for malformations was 3000 mg/kg. In a separate toxicokinetic experiment on GD 15, fetal exposures at 2 to 8 hours after dosing were 5.1 to 11.7% of maternal exposures of females dosed at 3000 mg/kg.

Table 62: Reviewer's Summary Rat EFD – Document 930009803

Rat EFD	Dose , mg/kg/d				
Study DN03042	**0**	**100**	**300**	**1000**	**3000**
Body weight gain: F, GD 0-20	146.4	143.2	140.0	137.2	135.8
Females mated	25	25	25	25	25
Females with implantations	24	24	24	23	25
Females evaluated	24	24	24	22 [a]	25
Females with red peri-vaginal substance	1	6	8*	5	**16****
Females with normal placenta	24	23 [b]	24	22	25
Females with viable fetuses	24	24	24	22	25
Corpora lutea/litter	17.2	16.7	16.1	17.4	17.5
Implantation sites/litter	15.3	15.0	14.8	14.5	14.8
Pre-implantation loss, #/litter	0.10	0.095	0.076	0.156	0.15
Pre-implantation loss ≥3 (Max.)	7 (7)	7 (5)	5 (5)	11 (8)	10 (11)
Early resorption/litter	0.6	0.7	0.1	0.4	0.4
Late resorption/litter	0.2	0.0	0.0	0.0	0.0
Total resorption/litter	0.8	0.8	0.1	0.4	0.4
% resorbed /litter	4.9	4.9	0.8	2.3	3.9
# litter with any resorption	9	10	3	8	9
# litter with resorption ≥2	4	4	0	0	2
Dead fetuses/litter	0	0	0	0	0
Live fetuses/litter	14.6	14.3	14.7	14.2	14.4
% males	52.3	46.6	46.4	47.6	49.0
Fetal weight, gm	3.70	3.78	3.74	3.75	3.70
# fetuses with any alteration (%)	26 (7.4)	16 (4.7)	16 (4.5)	25 (8.0)	25 (6.9)
# litter with any alteration (%)	12 (50.0)	11 (45.8)	9 (37.5)	12 (54.5)	14 (56.0)
Presence of cervical rib at the 7th cervical vertebrae, #/litter (%)	1 (4.2)	1 (4.2)	0 (0)	1 (4.5)	2 (8.0)
$AUC_{(0-8)}$, ng*hr/mL, maternal					36400
$AUC_{(0-8)}$, ng*hr/mL, fetal					2590

[a] One female euthanized on GD 15, [b] One female had a fused placentae, * Statistically significant, p<0.05, ** Statistically significant, p<0.01

Table 63 below compares the exposures of rats at the NOAEL dose and at the dose at which toxicity was observed in the EFD study with exposures in patients with atrial fibrillation receiving 5 mg of apixaban twice a day.

Table 63: Comparison of Rat EFD Exposures to Human Exposures

Rat EFD		Comparisons to human exposure							
Study - DN03042		**Based on NOAEL dose**				**Based on toxic dose**			
	NOAEL	**Total**		**Unbound**		**Total**		**Unbound**	
	mg/kg	**AUC**	**Fold**	**AUC**	**Fold**	**AUC**	**Fold**	**AUC**	**Fold**
Maternal	1000	36.3[†]	11.7	1.7	4.2	36.4	11.7	1.7	4.2
Fetal toxicity	3000	36.4	11.7	1.7	4.2	36.4	11.7	1.7	4.2
Malformation	3000	36.4	11.7	1.7	4.2	36.4	11.7	1.7	4.2

The % unbound apixaban was 4.7% for female rats, respectively, and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0-24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL. [†] Since exposure was obtained only for the 3000 mg/kg dose, an estimate of the exposure at 1000 mg/kg was obtained from the rat FEED study in which the highest dosage was 600 mg/kg.

Mouse

In an acceptable embryo-fetal development study in mice, the NOAEL for maternal toxicity was 1500 mg/kg, although the incidence of clinical signs of bleeding increased slightly with dose (Table 64). All treated groups had significant increases in prothrombin time in blood collected 1 hour after dosing. Pre-implantation loss decreased significantly in the vehicle control group and the mid and high dose groups compared to the saline control group. However, this finding was not considered toxicologically significant because 1) dosing did not begin until GD 6 presumably after implantation, 2) the finding was not adverse, 3) the finding did not exhibit a dose-relationship, and 4) the number of live fetuses/litter was not significantly affected. The values for fetal parameters, such as number of resorptions, number of live fetuses, sex ratio and fetal body weight, for all groups were within the historical ranges reported in the 2003-2007 (b) (4) Historical Control Data for Crl:CD1(ICR) mice. The NOAEL for fetal toxicity was 1500 mg/kg. The percentage of fetuses with any alteration for all treated groups was within or below the concurrent control range. No individual alteration exhibited a statistically significant dose-dependent increase in incidence. The mean litter incidence of the presence of cervical ribs was higher in the high dose group (80%) than the mean incidence in either the saline (60.9%) or vehicle (54.5%) control groups. However, this incidence is within the historical control range for this alteration (26.3 – 85.7%) reported in the 2003-2007 (b) (4) Historical Control Data for Crl:CD1(ICR) mice. Therefore, the NOAEL for alterations was 1500 mg/kg. The toxicokinetic evaluation indicated that mean maternal exposures were similar at all doses, indicating saturation of exposure. However, the reviewer noted considerable inter-animal variation in plasma concentrations up to 4 hours post dose and a higher mean Cmax for the high dose group.

Table 64: Reviewer's Summary – Mouse EFD – Document 930021450

Mouse EFD	Dose, mg/kg				
Study DN06023	**0 (saline)**	**0 (vehicle)**	**600**	**900**	**1500**
Body weight gain kg, GD 6-18	25.4 g	26.2	27.4	26.2	26.4
Food consumed, GD 6-18	7.0	6.3	6.5	6.4	6.6
Prothrombin time, sec	8.94	9.97	23.0*	22.2*	19.5*
Females mated	26	26	27	26	26
Females with implantations	24	23	25	24	23
Female deaths	1	1	2	1	2
Females delivered (excluded) [a]	0	0	0	1	1
Females aborted	0	0	0	0	0
Females evaluated	23	22	24	22	20
Females with red peri-vaginal substance	0	0	1	2	3
Females with total resorption	0	0	0	0	0
Females with any resorption	10	10	13	13	8
Females with live fetuses (%)	23 (100)	22 (100)	24 (100)	22 (100)	20 (100)
Females with normal placenta	22 [b]	22	24	22	20
Corpora lutea/litter	13.1	12.6	13.3	13.0	12.7
Implantations/litter	12.1	12.4	12.8	12.7	12.4
Pre-implantation loss, #/litter	0.073	0.013**	0.038	0.019**	0.027*
Early resorption/litter	0.5	0.4	0.5	0.8	0.4
Late resorption/litter	0.1	0.1	0.0	0.0	0.2
Total post-implantation loss (resorption), #/litter	0.6	0.5	0.5	0.8	0.5

Mouse EFD	Dose, mg/kg				
Study DN06023	0 (saline)	0 (vehicle)	600	900	1500
# litter with any resorption	10	10	13	13	8
Dead fetuses/litter	0	0	0	0	0
Live fetuses/litter	11.5	11.9	12.2	11.9	11.8
% males	51.6	54.2	49.8	52.7	49.1
Fetal weight, gm	1.35	1.34	1.36	1.34	1.33
# fetuses with any alteration (%)	56 (21.1)	42 (16.0)	49 (16.7)	36 (13.7)*	45 (19.0)
# litter with alterations (%)	18 (76.3)	19 (86.4)	19(79.2)	16 (72.7)	17 (85.0)
Cervical vertebrae, cervical rib present at 7th cervical vertebrae					
Litter incidence, # (%)	14 (60.9)	12 (54.5)	12 (50.0)	12 (54.5)	16 (80)
$AUC_{(0-24)}$, ng*hr/mL, maternal			14600	17500	15900
Mean Cmax, ng/ml			3228	2542	4015
[a] Females that delivered on GD 18 were excluded. [b] One female had fused placentae, * p<0.05, ** p<0.01					

Table 65 below compares the exposures of patients with atrial fibrillation and exposures of mice at the NOAEL dose and the dose at which toxicity was observed in the EFD study.

Table 65: Comparison of Mouse EFD Exposures to Human Exposures

Mouse EFD		Comparisons to human exposure							
Study DN06023		Based on NOAEL dose				Based on toxic dose			
	NOAEL	Total		Unbound		Total		Unbound	
	mg/kg	AUC	Fold	AUC	Fold	AUC	Fold	AUC	Fold
Maternal	1500	15.9	5.1	7.8	19.1	15.9	5.1	7.8	19.1
Fetal toxicity	1500	15.9	5.1	7.8	19.1	15.9	5.1	7.8	19.1
Malformation	1500	15.9	5.1	7.8	19.1	15.9	5.1	7.8	19.1
The % unbound apixaban was 49% for mice and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0-24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL									

Rabbit

In an acceptable embryo-fetal development study in rabbits, the maximum feasible dose that could be orally administered was 1500 mg/kg. No drug related effect was observed on maternal toxicity (mortality, body weight, food consumption) and the NOAEL for maternal toxicity was 1500 mg/kg (Table 66). The fetal external and soft tissue alterations were observed only in single fetuses. Although fetal skeletal alterations were occasionally observed in more than one fetus per group, no dose-dependent increase in incidence was observed. The NOAEL for malformations and fetal toxicity is 1500 mg/kg. At the high dose, the mean maternal exposure was 355.3 ng*hr/mL and the Cmax was 25.4 ng/mL. The mean fetal exposure was less than the detection limit of 2 ng/mL; however, the fetal plasma concentration was 2.9 ng/mL at 2 hours post dose in one TK animal whose plasma concentration was 7.6 ng/mL.

Table 66: Reviewer's Summary Rabbit EFD - Document 930011664

Rabbit EFD	Dose, mg/kg				
Study DN03045	0	60	180	500	1500
Body weight gain kg, GD 7-20	0.25 kg	0.25	0.28	0.28	0.26
Females mated	20	20	20	20	20
Female deaths	0	0	0	0	0
Females with implantations	20	19	18	18	17
Females delivered on GD 29	0	0	1	0	0
Females aborted	0	0	1	0	1
Females evaluated	20	19	16	18	16

Rabbit EFD	Dose, mg/kg				
Study DN03045	**0**	**60**	**180**	**500**	**1500**
Females with red substance perivaginal or in cage	0	1	0	1	1
Females with total resorption	0	0	0	0	0
Females with any resorption	5	10	5	6	8
Females with live fetuses (%)	20 (100)	19 (100)	16 (100)	18 (100)	16 (100)
Females with normal placenta	20	19	16	18	16
Corpora lutea/litter (range)	8.4 (6-13)	9.1 (6-12)	9.2 (7-12)	8.6 (6-13)	8.8 (4-15)
Implantations/litter (range)	7.8 (5-13)	9.1 (6-12)	9.1 (6-12)	8.2 (4-12)	8.6 (4-14)
Pre-implantation loss, #/litter	0.078	0.0	0.015	0.037	0.01
Early resorption/litter	0.35	0.47	0.13	0.28	0.56
Late resorption/litter	0.05	0.47	0.5	0.11	0.06
Total post-implantation loss (resorption), #/litter	0.4	0.9	0.6	0.4	0.6
# litter with any resorption	5	10	6	6	8
Dead fetuses/litter	0	0	0.1	0	0
Live fetuses/litter (range)	7.4 (2-12)	8.2 (4-11)	8.5 (6-11)	7.9 (4-11)	8.0 (4-13)
% males	55.9 [a]	53.2	43.6 [b]	50.6	52.9 [c]
Fetal weight, gm	47.3	44.3	44.0	43.8	44.4
# fetuses with any alteration (%)	21 (14.3)	18 (11.6)	21 (15.6)	17 (11.9)	25 (19.5)
# litter with alterations (%)	12 (60)	11 (57.9)	12 (75)	8 (44.4)	13 (81.2)
$AUC_{(0-24)}$, ng*hr/mL, maternal					355.3
[a] One female had no female fetus, [b] One female had no male fetus, [c] One female had no female fetus d					

Table 67 below compares the exposures of patients with atrial fibrillation and exposures of rabbits at the NOAEL dose and the dose at which toxicity was observed in the EFD study.

Table 67: Comparison of Exposures in the Rabbit Oral EFD Study to Human Exposures

Rabbit EFD - Oral		Comparisons to human exposure							
Study DN03045		**Based on NOAEL dose**				**Based on toxic dose**			
	NOAEL	**Total**		**Unbound**		**Total**		**Unbound**	
	mg/kg	**AUC**	**Fold**	**AUC**	**Fold**	**AUC**	**Fold**	**AUC**	**Fold**
Maternal	1500	0.36	0.12	0.136	0.33	0.36	0.12	0.136	0.33
Fetal toxicity	1500	0.36	0.12	0.136	0.33	0.36	0.12	0.136	0.33
Malformation	1500	0.36	0.12	0.136	0.33	0.36	0.12	0.136	0.33
The % unbound apixaban was 37.7% for rabbits and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0-24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL									

The following rabbit EFD development study was not previously reviewed.

Study title: BMS-562247: Intravenous Study of Embryo-Fetal Development in Rabbits

Study no.:	DN05050 (Document 930016662)
Study report location:	EDR Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Coagulation & TK – June 15, 2005 Maternal toxicity & EFD – July 3, 2005 Maternal & fetal TK – January 4, 2006
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BMS-56224, lot 4K85835, purity 99.9%

Key Study Findings

In an adequate embryo-fetal development study, BMS-56224 was administered at 0, 1.25, 2.5 and 5.0 mg/kg intravenously once daily from gestation day 7 to 19 to New Zealand White rabbits that were sacrificed on gestation day 29. A subsequent study evaluated maternal and fetal toxicokinetics on GD 19 in rabbits receiving only the high dose of 5 mg/kg.

No drug-related maternal mortality or abortion occurred. No drug-related effect was observed on body weight, body weight gain, or food consumption. The increased incidence of purple discoloration at injection sites was related to the pharmacodynamic effect of BMS-56224. Similarly, treatment with BMS-56224 resulted in increased prothombin values in blood samples collected 1 hour after dosing on GD 15.

The values for cesarean section parameters (corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions) were similar for all control and treated groups. Since no drug-related changes were observed in any maternal or litter parameters, the maternal NOAEL was 5 mg/kg. The number of live fetuses/litter, the ratio of males to females, and mean fetal body weights were not affected by apixaban treatment. No external, visceral or skeletal fetal alteration was considered drug-related. The NOAELs of 5 mg/kg for fetal toxicity and malformation corresponded to $AUC_{(0\text{-}24h)}$ exposures of 0.95 µg.hr/mL in the main study, and 2.6-3.2 µg.hr/mL in the extension study, which evaluated timepoints earlier than 30 minutes. The exposure to the metabolite, BMS-730823, was less than 1% of the parent apixaban in the high dose rabbits. The mean $AUC_{(0\text{-}24h)}$ value for apixaban in fetal cord plasma was 1.0% of the mean maternal $AUC_{(0\text{-}24h)}$ values, indicating that a low amount of apixaban crossed the placenta.

Methods

Doses:	0, 1.25, 2.5, 5.0 mg/kg The high dose of 5 mg/kg/day is the highest dose that can be given intravenously as a bolus injection, based on a maximum intravenous dose volume of 2 mL/kg and the maximum concentration of 2.5 mg/mL apixaban that can be formulated in the vehicle.
Frequency of dosing:	Daily from GD 7 to 19

Dose volume: 0.5, 1, 2 mL/kg as indicated in study design
Route of administration: Intravenous bolus injection
Formulation/Vehicle: 35% Hydroxypropyl-ß-cyclodextrin (HPBCD) in 10 mM phosphate buffer, pH 7.0,
Species/Strain: Rabbit, New Zealand White (Hra:(NZW)SPF)
Number/Sex/Group: Coagulation & TK – 5 females/group
Maternal toxicity & EFD – 22 females/group
Maternal & fetal TK – 30 females total
Satellite groups: Coagulation and TK
Study designs: **Main Study:**
The EFD, coagulation and initial TK evaluations were included in the main design below.

Group	# assigned Main	TK	Dose, mg/kg/d	Dose vol. ml/kg
1	22	5	0 (saline)	2
2	22	5	0 (vehicle)	0.5
3	22	5	0 (vehicle)	1
4	22	5	0 (vehicle)	2
5	22	5	1.25	0.5
6	22	5	2.5	1
7	22	5	5.0	2

Extension Study:
Maternal and fetal TK on GD 19 in rabbits receiving only the high dose were evaluated in a separate study

Part 1: Maternal TK – 15 rabbits

Rabbits assigned	Minutes Post dose on GD 19 5	10	20	30	60	120	180
First 5	X			X			X
Second 5		X			X		
Third 5			X			X	

Part 2: Fetal and maternal TK – 15 rabbits Rabbits were euthanized and maternal and fetal blood was collected

Time post dose	10 min	30 min	60 min
First 5 rabbits	X		
Second 5 rabbits		X	
Third 5 rabbits			X

Deviations from study protocol:
1. Clinical observations were not recorded on GD2 for TK groups.
2. An extra blood sample was collected from a group 4 rabbit prior to scheduled timepoint.
3. On GD 10, BMS-56224, instead of vehicle, was administered to two group 4 rabbits. However, the incorrectly dosed rabbits were replaced.
4. Postdose clinical observations for 15 rabbits were performed outside protocol specified range.
5. Additional clinical observations were collected on GD 15 and 19 for the coagulation and TK animals.
6. During acclimation, clinical observations were not performed on 4 rabbits.
7. Clinical observations were not performed on 1 high dose rabbit on GD 14.
8. Soft tissue evaluation of the head was not performed on 1 fetus from a group 2 rabbit.

Observations and Results

Mortality

The animals were examined visually for mortality, injury, and morbidity twice daily.

No drug-related maternal mortality or abortion occurred. However, on GD 25 one mid-dose rabbit (group 6) had an abortion that was not considered drug-related, because no abortions occurred in the high dose groups in the current study or the range-finding study.

Clinical Signs

Clinical observations were recorded once daily from arrival at the laboratory to GD 6, twice daily (prior to and at 1 hour post dose) on GD 7 to 19, and once daily from GD 20 to 29.

The incidence of purple discoloration at injection sites increased in all apixaban-treated groups (5, 6, and 7) compared to their respective control groups (Table 68). No dose-dependence was observed. The finding was attributed to route of administration (injection) in the presence of apixaban, a FXa inhibitor.

Table 68: Reviewer's Summary of Maternal Incidence of Purple Discoloration at Injection Sites – Study DN05050 - Rabbit EFD – IV dosing

Group	1	2	5	3	6	4	7
Dose, mg/kg	0 (saline)	0 (vehicle)	1.25	0 (vehicle)	2.5	0 (vehicle)	5.0
# rabbits/group	22	22	22	22	22	22	22
# observations	11	10	85	13	101^	8	91
# rabbits	4	5	16	11	19^	5	17
^ Includes observations in the rabbit (2422) that aborted and was euthanized on GD 25							

Body Weight

Body weights were recorded on GD 0 by the vendor, on the day of arrival at the laboratory, and then daily through GD 29.

Treatment with apixaban did not decrease bodyweight or bodyweight gain (Table 69).

Table 69: Reviewer's Summary – Bodyweight Gain - Study DN05050

Rabbit EFD IV dosing - Body weight gain, kg								
Group		1	2	5	3	6	4	7
Dose, mg/kg		0 (saline)	0 (vehicle)	1.25	0 (vehicle)	2.5	0 (vehicle)	5.0
GD 7-20	Mean	0.3	0.25	0.28	0.27	0.32	0.3	0.3
	SD	± 0.05	± 0.09	± 0.12	± 0.09	± 0.07	± 0.14	± 0.06
GD 7-29	Mean	0.5	0.43	0.44	0.48	0.48	0.48	0.47
	SD	± 0.11	± 0.16	± 0.21	± 0.09	± 0.14	± 0.15	± 0.11

Food Consumption

Food consumption values were recorded daily after arrival at the laboratory.

Treatment with apixaban did not decrease food consumption relative to the volume-matched vehicle controls (Table 70). Significantly higher food consumption values were observed in the high dose group compared to values in the respective vehicle control groups. However, food consumption values in the high dose group were similar to values in the saline control group.

Table 70: Reviewer's Summary – Food Consumption - Study DN05050

Rabbit EFD – IV dosing - Maternal food consumed, gm/d								
	Group	1	2	5	3	6	4	7
	Dose, mg/kg	0 (saline)	0 (vehicle)	1.25	0 (vehicle)	2.5	0 (vehicle)	5.0
GD 7-20	Mean	171.1	160.6	164.2	163.1	164.2	155.5	169.7*
	SD	14.2	21.8	17.4	18.7	30.2	27	14.2
GD 7-29	Mean	157.9	148.4	153.4	153.9	153.8	147.6	156.8
	SD	17.3	20.4	19.2	17.2	21.5	22.1	15.8
* Significantly different from group 4 value, $p < 0.05$								

Coagulation

Blood samples were collected from (non-fasted) satellite rabbits on GD 6 prior to dosing and on GD 15 at 1 hour after dosing. Plasma was prepared and used for the determination of prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen.

Treatment with apixaban resulted in a dose-dependent increase in PT values on GD 15 relative to pre-dose PT values on GD 6 (Table 71). However, the PT values for the vehicle control groups were similar to the values for the saline control group on both collection days.

Table 71: Reviewer's Summary – Prothrombin Times - Study DN05050

		Mean PT, sec (SD)		Mean Ratio GD15/ GD6	Minimum Ratio	Maximum ratio
Group	Dose, mg/kg	GD 6	GD 15			
1	0 (saline)	7.02 (0.37)	6.92 0.11	0.99 (0.06)	0.91	1.06
2	0 (vehicle 0.5 mL)	6.83 (0.33)	6.80 (0.26)	1.00 (0.02)	0.99	1.02
5	1.25	6.58 (0.13	7.40 (0.25)	1.14 (0.05)	1.07	1.20
3	0 (vehicle 1 mL)	6.56 (0.23)	6.88 (0.43	1.05 (0.05)	1.01	1.12
6	2.5	6.54 (0.21)	7.94 (0.21	1.22 (0.06)	1.12	1.28
4	0 (vehicle 2 mL)	6.58 (0.15)	6.83 (0.19)	1.05 (0.03)	1.02	1.09
7	5	6.72 (0.29)	10.03 (1.17)	1.48 (0.21)	1.2	1.70

In contrast to the PT values, the aPTT and fibrinogen values were highly variable and did not display clear relationships to treatment with apixaban (Table 72).

Table 72: Reviewer's Summary – Activated Partial Thromboplastin Times (aPTT) - Study DN05050

Group	Dose, mg/kg	Mean aPTT, sec (SD)		Ratio aPTT GD15/ GD6		Mean fibrinogen (fib), mg/dL (SD)		Ratio fib GD15/ GD6	
		GD 6	GD 15	Min	Max	GD 6	GD 15	Min	Max
1	0	57.6 (26.0)	60.8 (9.6)	0.66	1.74	264 (92)	288 (48)	0.80	2.49
2	0	43.7 (8.7)	61.4 (25.1)	1.30	1.64	295 (80	289 (72)	0.66	0.89
5	1.25	76.1 (28.5	49.7 (10.3)	0.35	1.16	458 (276)	308 (39)	0.39	0.93
3	0	51.9 (25.5)	36.9 (4.8)	0.43	1.1	321 (62)	289 (78)	0.56	1.02
6	2.5	47.3 (12.9)	56.0 (15.0)	0.59	2.0	276 (62)	340 (59)	1.05	1.66
4	0	55.4 (7.4)	34.7 (15.2)	0.31	1.10	305 (48)	296 (42)	0.92	1.0
7	5	36.8 (3.5)	63.1 (25.3)	1.18	2.46	245 (44)	299 (32)	1.07	1.43

Toxicokinetics

In the main study, blood from the medial auricular artery was collected from 5 non-fasted, main study TK rabbits/timepoint on GD 19 at 0.5, 1, 2, 4, 8 and 24 hours after dose administration. Plasma was prepared and analyzed for apixaban and O-desmethyl apixaban sulfate using validated LC/MS/MS methods with lower limits of quantification (LLOQ) of 2 ng/mL and 5 ng/mL, respectively.

Although the Cmax and $AUC_{(0\text{-}24h)}$ of apixaban increased with dose in the main study, the increases in Cmax and AUC were slightly more than dose-proportional with a 4.9 and 5.8-fold increase, respectively, between the low and high doses (Table 73). No apixaban was detected in samples from rabbits that received saline or vehicle. The concentration of O-desmethyl apixaban sulfate (BMS-730823) was above the LLOQ in 1:5 mid-dose rabbits and 4:5 high dose rabbits. The mean maternal Cmax and $AUC_{(0\text{-}24h)}$ of BMS-730823 was less than 1% of the Cmax and AUC of the parent apixaban in the high dose rabbits.

Table 73: Reviewer's Summary – Toxicokinetics Main Study - Study DN05050

	BMS-562247, mean (SD)				BMS-730823, mean (SD)			
Dose	Cmax	Range	AUC	Range	Cmax	Range	AUC	Range
1.25	261 (78)	164-334	164 (49)	101-211	<LLOQ	NA	NC	NA
2.5	566 (98)	411-680	393 (82)	269-488	<LLOQ	<LLOQ, 7.5	NC	<LLOQ - 7.5
5	1280 (266)	831-1530	954 (212)	579-1090	11.2 (5.0)	8.1-18.7^	4.4 (4.4)	2.0-11

^ 1/5 animals had Cmax < LLOQ (5 ng/mL), NA = Not applicable, NC = Not calculated, Cmax = ng/mL; AUC = ng.hr/mL

In a separate extension study, maternal and fetal exposures were determined in rabbits treated at the high dose of 5 mg/kg/day. In Part 1, blood samples were collected from 15 non-fasted rabbits at 5, 10, 20, 30, 60, 120, and 180 minutes after dosing on GD 19. In Part 2, both maternal and fetal exposures were determined in a second set of 15 rabbits treated at the high dose of 5 mg/kg/day. On GD 19, five non-fasted rabbits per timepoint were euthanized at 10, 30, and 60 minutes after dose administration. Immediately following euthanasia, maternal blood was collected from the inferior vena cava. The uterus was excised and fetal cord blood samples were collected from each fetal/placental unit and were pooled by litter.

Inclusion of timepoints at 5, 10, and 20 minutes in Part 1 of the extension study (Table 74) resulted in 6.9 and 3.4-fold higher maternal Cmax and $AUC_{(0\text{-}24h)}$ values, respectively, for apixaban relative to the initial main study evaluation. The mean maternal Cmax and $AUC_{(0\text{-}24h)}$ values for apixaban in Part 2 were 0.72 and 0.84 times the mean maternal Cmax and AUC values, respectively, for apixaban in Part 1 of the extension study. The mean Cmax and $AUC_{(0\text{-}24h)}$ values for apixaban in the fetal cord plasma were 0.88% and 1.0% of the mean maternal Cmax and AUC values, indicating that a low amount of apixaban crossed the placenta into the rabbit fetus. The finding of concentrations of BMS-730823 in the fetal cord plasma below the limit of detection was not unexpected considering that the Cmax of BMS-730823 in maternal plasma was less than 1% the Cmax of apixaban.

Table 74: Reviewer's Summary – Toxicokinetics – Extension Study - Study DN05050

	BMS-562247, mean (SD)			BMS-730823, mean (SD)		
Sample	Cmax, ng/mL	Cmax Range	AUC†	Cmax, ng/mL	Cmax Range	AUC†
Extension Part 1 (Timepoints 5, 10, 20, 30, 60, 120 and 180 minutes post dose)						
Maternal TK	8788 (817)	7550-9590	3210	13 (6)	8-19^	10.6
Extension Part 2 (Timepoints 10, 30, 60 min post dose)						
Fetal plasma	55.6 (26)	32-100	27.5	<LLOQ	NA	NC
Maternal plasma	6346 (1667)	3530-6850	2690	8 (4)	5-14	7.8
Main Study TK (from Table 73) (Timepoints 0.5, 1, 2, 4, 8 and 24 hrs post dose)						
Maternal TK	1280 (266)	831-1530	954	11.2 (5.0)	8.1-18.7^	4.4

†AUC = ng*hr/mL, ^ 2/5 animals had Cmax for BMS-730823 < LLOQ (5 ng/mL), NA = Not applicable, NC = Not calculated

Dosing Solution Analysis

Dosing solutions of apixaban in vehicle (35% HPBCD) were prepared at least once every 7 days. Apixaban concentration was measured in the formulations prepared for the first and last weeks of dosing in the main study and the first week of dosing in the extension study. All aliquots had apixaban concentrations within 9% of the intended concentration. The analyses also demonstrated the absence of apixaban in vehicle and saline control solutions.

Table 75: Reviewer's Summary of Dosing Solution Analyses - Study DN05050

Sample Date	Component	Expected BMS-56224, mg/mL	Observed BMS-56224, mg/mL	Mean, mg/ml	% recovery
6/14/2005	vehicle	0	0	-	
6/15/2005	Test article	2.5	2.46, 2.43	2.44	97.6
7/14/2005	saline	0	0	-	
7/14/2005	vehicle	0	0	-	
7/15/2005	Test article	2.5	2.42, 2.42	2.42	96.8
1/4/2006	Test article	2.5	2.46, 2.72	2.59	103.6

Necropsy

On GD 29, all surviving rabbits were euthanized, caesarean-sectioned, and subjected to a gross necropsy of the thoracic, abdominal, and pelvic viscera.

All rabbits, except one, appeared normal at necropsy. The finding of a misshapen right uterine horn in one high dose rabbit was considered not drug-related, because this finding has been observed previously in control animals at the conducting laboratory.

Cesarean Section Data

The number and distribution of corpora lutea were recorded. After removal of the uterus of each rabbit, it was examined for pregnancy, number and distribution of implantation sites, live and dead fetuses, and early and late resorptions. The size, color, and shape of placentae were determined.

The values for cesarean section parameters (corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions) were similar for all control and treated groups (Table 76). The values for all groups are within the ranges reported in the 2005-2007 (b) (4) Historical Control Data for New Zealand White Hra:(NZW)SPF rabbits in 53 full studies involving 1103 rabbits, 1022 of whom were pregnant at sectioning. The placentae for all animals were considered normal. Since no drug-related changes were observed in any maternal or litter parameters, the maternal NOAEL was the high dose of 5 mg/kg.

Offspring Data

Each fetus was removed from the uterus and placed in an individual container, which was labeled with the study number, litter number, and uterine distribution. Each fetus was subsequently weighed and examined for gross lesions. After euthanization, live fetuses were euthanized and examined internally to identify sex. Internal organs were evaluated in all fetuses by dissection. The brain was examined in situ after a single cross-section was made between the parietal and the frontal bones. Gross lesions were retained in 10% neutral buffered formalin. All fetuses were examined for skeletal alterations after staining with alizarin red S.

The number of live fetuses/litter, the ratio of males to females, and mean fetal body weights were not affected by apixaban treatment (Table 77). The values for all groups are within the ranges reported in the 2005-2007 (b) (4) Historical Control Data for New Zealand White Hra:(NZW)SPF rabbits.

The over-all fetal and litter incidences of fetuses with any alteration in the treated groups were equal to or less than the incidences in the control groups (Table 78). External and visceral alterations occurred in single fetuses, except for circumcorneal hemorrhage in the eyes (2 fetuses in control group 2) and absence of intermediate lobe of the lung (2 fetuses in control group 1). The visceral alterations of interventricular septal defect, enlarged heart, and distended aorta occurred in one fetus in the high dose group. The fetal and litter incidences of each of these defects (0.6 and 4.8%, respectively) are within the conducting laboratory's historical control ranges collected in 62 studies using New Zealand White Hra:(NZW)SPF rabbits from 2003 to 2005 (interventricular septal defect - 0 to 0.7% (0 to 5.6%); enlarged heart - 0 to 0.7% (0 to 5.6%); and distended/enlarged aorta - 0 to 0.8% (0 to 5.9%) as well as the ranges reported in the 2005-2007 (b) (4) Historical Control Data for New Zealand White Hra:(NZW)SPF rabbits. Although the incidences of some skeletal alterations were higher than the incidences of external and visceral alterations, the incidences in apixaban-treated

groups were equal to or less than the incidences in the control groups, except for the incidences of two skeletal alterations, partially fused nasals in the skull and small arch in thoracic vertebrae. The litter incidences of the former alteration in the low and high dose groups (4.5% and 4.8%, respectively) and the later alteration (4.8%) are within the historical control range reported in the 2005-2007 (b) (4) Historical Control Data for New Zealand White Hra:(NZW)SPF rabbits of 0-5.9% and 0-5.3%, respectively. Since no external, visceral or skeletal fetal alteration was considered drug-related, the NOAEL was 5 mg/kg.

Table 76: Compilation of Sponsor's Tables - Cesarean Section Data

DOSE GROUP DOSE (MG/KG/DAY)a VOLUME (ML/KG)		I 0 (SALINE CONTROL) 2	II 0 (VEHICLE) 0.5	V 1.25 0.5	III 0 (VEHICLE) 1	VI 2.5 1	IV 0 (VEHICLE) 2	VII 5.0 2
RABBITS TESTED	N	22	22	22	22	22	22	22
PREGNANT	N(%)	18(81.8)	21(95.4)	22(100.0)	22(100.0)	21(95.4)	22(100.0)	21(95.4)
ABORTED AND EUTHANATIZED	N(%)	0 (0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	18	21	22	22	20	22	21
CORPORA LUTEA	MEAN±S.D.	8.4 ± 1.6	8.1 ± 2.0	8.4 ± 1.6	8.6 ± 1.9	9.0 ± 1.7	9.0 ± 1.6	8.8 ± 1.7 [20]b
IMPLANTATIONS	MEAN±S.D.	7.9 ± 2.0	7.7 ± 2.1	8.3 ± 1.6	8.1 ± 1.6	8.6 ± 1.9	8.7 ± 1.7	8.4 ± 2.2
PREIMPLANTATION LOSS	MEAN±S.D.	5.3 ± 14.9	4.7 ± 7.2	1.1 ± 3.7*	4.9 ± 9.4	4.7 ± 10.7	2.7 ± 5.2	4.8 ± 13.7 [20]b
POSTIMPLANTATION LOSS	MEAN±S.D.	2.2 ± 5.1	8.2 ± 15.5	4.3 ± 6.6	1.7 ± 4.5	3.9 ± 6.2	2.7 ± 7.5	2.7 ± 6.2
LITTER SIZES	MEAN±S.D.	7.8 ± 2.0	7.0 ± 2.3	7.9 ± 1.6	8.0 ± 1.3	8.2 ± 2.0	8.5 ± 1.8	8.2 ± 2.1
LIVE FETUSES	N	140	148	174	175	165	187	172
	MEAN±S.D.	7.8 ± 2.0	7.0 ± 2.3	7.9 ± 1.6	8.0 ± 1.3	8.2 ± 2.0	8.5 ± 1.8	8.2 ± 2.1
DEAD FETUSES	N	0	0	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.7 ± 1.4	0.4 ± 0.6	0.2 ± 0.5	0.3 ± 0.5	0.2 ± 0.6	0.2 ± 0.5
LATE RESORPTIONS	N	3	3	6	3	2	2	3
	MEAN±S.D.	0.2 ± 0.4	0.1 ± 0.4	0.3 ± 0.6	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.4	0.1 ± 0.5
EARLY RESORPTIONS	N	0	11	2	1	4	3	2
	MEAN±S.D.	0.0 ± 0.0	0.5 ± 1.4	0.1 ± 0.3	0.0 ± 0.2	0.2 ± 0.4	0.1 ± 0.5	0.1 ± 0.3
DOES WITH ANY RESORPTIONS	N(%)	3(16.7)	8(38.1)	7(31.8)	3(13.6)	6(30.0)	3(13.6)	4(19.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	18(100.0)	21(100.0)	22(100.0)	22(100.0)	20(100.0)	22(100.0)	21(100.0)
DOES WITH NORMAL PLACENTAE	N(%)	18(100.0)	21(100.0)	22(100.0)	22(100.0)	20(100.0)	22(100.0)	21(100.0)

PREIMPLANTATION LOSS = (NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS)/NUMBER OF CORPORA LUTEA X 100
POSTIMPLANTATION LOSS = [(DEAD + RESORBED CONCEPTUSES)/IMPLANTATIONS] X 100
[ ] = NUMBER OF VALUES AVERAGED
a. Days 7 through 19 of gestation.
b. Excludes doe 2436 for which corpora lutea number appeared incorrectly recorded.
* Significantly different from the Group II value (p≤0.05); analyses restricted to Groups II and V.

Table 77: Compilation of Sponsor's Tables – Offspring Parameters -

DOSE GROUP		I	II	V	III	VI	IV	VII
DOSE (MG/KG/DAY)a		0 (SALINE CONTROL)	0 (VEHICLE)	1.25	0 (VEHICLE)	2.5	0 (VEHICLE)	5.0
VOLUME (ML/KG)		2	0.5	0.5	1	1	2	2
LITTERS WITH ONE OR MORE LIVE FETUSES	N	18	21	22	22	20	22	21
IMPLANTATIONS	MEAN±S.D.	7.9 ± 2.0	7.7 ± 2.1	8.3 ± 1.6	8.1 ± 1.6	8.6 ± 1.9	8.7 ± 1.7	8.4 ± 2.2
LIVE FETUSES	N	140	148	174	175	165	187	172
	MEAN±S.D.	7.8 ± 2.0	7.0 ± 2.3	7.9 ± 1.6	8.0 ± 1.3	8.2 ± 2.0	8.5 ± 1.8	8.2 ± 2.1
LIVE MALE FETUSES	N	62	74	93	83	85	95	88
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.2 ± 21.3	51.9 ± 16.8	54.1 ± 22.1	47.5 ± 22.3	50.7 ± 17.9	50.8 ± 15.6	52.4 ± 19.4
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	44.02 ± 4.95	44.68 ± 5.74	44.07 ± 4.99	44.23 ± 3.81	43.61 ± 4.81	42.83 ± 4.69	43.07 ± 3.90
MALE FETUSES	MEAN±S.D.	44.72 ± 5.40	45.98 ± 4.87	44.68 ± 5.67	43.93 ± 4.22 [21]b	43.87 ± 5.04	43.21 ± 4.87	43.87 ± 4.24
FEMALE FETUSES	MEAN±S.D.	42.62 ± 4.45 [17]c	43.08 ± 6.82 [20]c	43.93 ± 5.24 [21]d	44.34 ± 3.85 [21]e	44.23 ± 5.09	42.85 ± 5.26	42.13 ± 4.29 [20]f
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	2.2 ± 5.1	8.2 ± 15.5	4.3 ± 6.6	1.7 ± 4.5	3.9 ± 6.2	2.7 ± 7.5	2.7 ± 6.2

[ ] = NUMBER OF VALUES AVERAGED
a. Days 7 through 19 of gestation.
b. Litter 2347 had no male fetuses.
c. Litter 2309 had no female fetuses.
d. Litter 2333 had no female fetuses.
e. Litter 2363 had no female fetuses.

[ ] = NUMBER OF VALUES AVERAGED
a. Days 7 through 19 of gestation.
b. Litter 2347 had no male fetuses.
c. Litter 2333 had no female fetuses.
d. Litter 2405 had no female fetuses.
e. Litter 2363 had no female fetuses.
f. Litter 2452 had no female fetuses.

Table 78: Compilation of Sponsor's Tables - Fetal Alterations

DOSE GROUP		I	II	V	III	VI	IV	VII
DOSE (MG/KG/DAY)a		0 (SALINE CONTROL)	0 (VEHICLE)	1.25	0 (VEHICLE)	2.5	0 (VEHICLE)	5.0
VOLUME (ML/KG)		2	0.5	0.5	1	1	2	2
LITTERS EVALUATED	N	18	21	22	22	20	22	21
FETUSES EVALUATED	N	140	148b	174	175	165	187	172
LIVE	N	140	148	174	175	165	187	172
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	13(72.2)	13(61.9)	10(45.4)	15(68.2)	12(60.0)	16(72.7)	12(57.1)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	19(13.6)	22(14.9)	22(12.6)	26(14.8)	19(11.5)	27(14.4)	20(11.6)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	15.8 ± 15.7	16.5 ± 18.1	12.4 ± 15.4	14.9 ± 14.1	12.5 ± 14.9	14.4 ± 12.4	11.0 ± 12.5
Heart: large and interventricular septal defect; vessels: aorta distended								
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)c
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
Lungs: intermediate lobe absent								
LITTER INCIDENCE	N(%)	2(11.1)	0(0.0)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	1(4.8)c
FETAL INCIDENCE	N(%)	2(1.4)	0(0.0)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	1(0.6)
Skull: nasals, fused (partially)								
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
Cervical vertebrae: cervical rib present at 7th cervical vertebra								
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)	0(0.0)	0(0.0)	1(0.6)
Thoracic vertebrae: hemivertebra								
LITTER INCIDENCE	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	1(0.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
Thoracic vertebrae: arch, small								
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.6)

b. Soft tissue evaluation of the head was inadvertently not performed for fetus 2333-4. c. The alterations in Fetus 2441-4 included the indicated heart defects, thoracic vertebrae hemivertebra and thoracic vertebrae small arch, and the absence of an intermediate lobe of the lung.

Table 79 compares the exposures of rabbits at the NOAEL dose and at the dose at which toxicity was observed in the IV EFD study with exposures in patients with atrial fibrillation receiving 5 mg of apixaban twice a day.

Table 79: Comparison of Rabbit Exposures in the IV EFD Study to Human Exposures

Rabbit EFD - IV Study DN03045		Comparisons to human exposure							
		Based on NOAEL dose				Based on toxic dose			
	NOAEL	Total		Unbound		Total		Unbound	
	mg/kg	AUC	Fold	AUC	Fold	AUC	Fold	AUC	Fold
Maternal	5	1.0 (3.2†)	0.33 (1.0†)	0.38 (1.2†)	0.93 (2.9†)	1.0 (3.2†)	0.33 (1.0†)	0.38 (1.2†)	0.93 (2.9†)
Fetal toxicity	5	1.0 (3.2†)	0.33 (1.0†)	0.38 (1.2†)	0.93 (2.9†)	1.0 (3.2†)	0.33 (1.0†)	0.38 (1.2†)	0.93 (2.9†)
Malformation	5	1.0 (3.2†)	0.33 (1.0†)	0.38 (1.2†)	0.93 (2.9†)	1.0 (3.2†)	0.33 (1.0†)	0.38 (1.2†)	0.93 (2.9†)

The % unbound apixaban was 37.7% rabbits and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0\text{-}24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL.
† In the TK extension study in which earlier timepoints were collected, rabbits receiving 5 mg/kg had a mean AUC of 3.2 µg•h/mL.

9.3 Prenatal and Postnatal Development

In a dose range-finding study (DN0735), the sponsor specifically evaluated effects of 600 and 1000 mg/kg apixaban (Lot 5L00821) on parturition by evaluating the incidence of bleeding signs in the high dose group exposed to continuous daily dosing from gestation day (GD) 6 to lactation day (LD) 7 to the incidence of bleeding signs in a high dose group whose dosing was interrupted during parturition (GD 21 and LD 1). The overall incidence of bleeding signs increased slightly in both high dose groups compared to the incidence in the control and low dose groups (Table 80). The incidence of peri-vaginal bleeding signs was slightly increased in the high dose group that was dosed during parturition compared to the group that was not dosed during parturition. However, since no death occurred in any group, the sponsor concluded that dosing in the main study did not need to be interrupted during parturition.

Table 80: Reviewer's Summary - Bleeding Signs – Study DN0735

Red substance observed	Dose, mg/kg Location	0	600	1000	1000 - interrupted
Time period	Rats/group	10	10	10	10
During gestation	Peri-natal/oral	4	6	7	5
	Peri-vaginal	0	0	0	0
Parturition (LD 0, 1)	Peri-natal/oral	1	0	1	1
	Peri-vaginal	0	0	3	1
During lactation (LD 2 – 7, 8	Peri-natal/oral	0	2	1	2
	Peri-vaginal	0	0	1	2
Total incidence of bleeding signs		5	8	13	11

In the main prenatal/postnatal development study (Study DN08001), pregnant rats (25/group) received a daily dose of apixaban (Lot 5L00821) by gavage from GD 6 to lactation day (LD) Day 20. In blood samples collected on LD 6 at 4 hours after dosing with 0, 25, 200 and 1000 mg/kg, prothrombin times (PT) were prolonged 1.65, 2.25, and

2.64 fold, respectively, compared to the means in the control group. No drug related mortality of F0 dams occurred, although the incidence of bleeding signs, primarily during gestation, increased in the treated groups with the incidence similar in the mid and high dose groups (Table 81). No adverse effect in F0 dams was observed on body weight, body weight gain, food consumption, and maternal performance (length of gestation, duration of parturition, number of implantations, number of live pups, dead pups or malformed pups at birth, sex ratio (% males/females), live birth index, pregnancy rate, gestation index, placentae, or maternal behavior. The sponsor's analysis excluded a control female (1518), who was euthanized because of dystocia, but included the high dose female (F4524), who cannibalized all her pups. This analysis indicated a statistically significant increase in the number of dead pups in the high dose group. If both these females are either excluded or included, no significant difference in the number of dead pups is observed. Furthermore, this finding was not considered drug-related, because no effect was observed on the number of live pups/litter and the life birth index. Therefore, the NOAEL for maternal effects in F0 dams was 1000 mg/kg/day.

Table 81: Reviewer's Summary - Pre-/Peri-natal Development – Study DN08001

Rat PPND – DN08001		Dose, mg/kg			
Parameter	**Historical max.**	**0**	**25**	**200**	**1000**
F0 Generation					
F0 females mated		25	25	25	25
Females pregnant		24	25	25	25
Fertility index, %		96	100	100	100
Implantations/litter		13.6	13.8	14.4	13.6
F0 Dams with any prenatal loss		10	11	11	9
Prenatal loss/litter		0.71	0.76	0.76	0.56
F0 Dam deaths during gestation		0	0	0	0
F0 Prothrombin time, mean (sec)		14.24	23.46	32.06*	37.55*
F0 Dams with any bleeding signs (peri-oral/nasal or peri-vaginal) during					
gestation (GD 6-GD 20 parturition (GD 21, LD 0, LD 1) lactation (LD 2-20) Total		4 0 0 3	12 2 4 18	21 1 1 23	23 1 2 26
Dams delivering pups		23	25	25	25
Dams with viable pups		23	25	25	25
Gestation index, %		100	100	100	100
Gestation duration, days (Range)		21.9 (21-22)	21.8 (21-22)	21.7 (21-23)	21.9 (21-23)
Live F1 pups/litter – LD 0 (Range)		12.8 (6-16)	12.9 (6-17)	13.2 (2[T]-17)	12.8 (9-15)
Live birth index, %		94.5	93.9	91.5	91.4
Dams with only dead pups		0	0	0	0
Dams with any dead pup on LD 0 (total # of pups) [Dam #, # of pups] {^if F1518 is excluded}		2 {1 ^} (19, {5}) [F1509: 5, F1518: 14]	1 (3) [F2522: 3]	4 (11) [F3505: 1. F3509: 8, F3523: 1, F3525: 1]	4 (16) [F4502: 1, F4505: 1, F4514: 1, F4524: 13]
# dead F1 pups/litter LD 0		0.82 {0.2}	0.12	0.44	0.64
F0 Dams dying/euthanized, LD 0-4 (F0#, LD)		1 (1518, LD 0, dystocia)	0	0	1 (4524, LD 0, all pups cannibalized)
F0 Dams dying post LD 4		0	0	0	1 (4519, LD 13 – dosing error)
Dams with pups deaths LD 1-4 (# dams, # pups)		2, 2 (F1502: 1 F1509: 1)	3, 3 (F2512: 1, F2515: 1, F2524: 1)	4, 4 (F3504: 1, F3506: 1, F3507: 1, F3513: 1)	3, 5 (F4501: 1, F4508: 2, F4510: 2)
F0 Dams rearing F1 pups to LD 4		22	25	25	24
Rearing index, %		95.6	100	100	96.0
Malformed F1 pups		1 (F1536)	0	0	0

* $p < 0.05$, [T] F3509 had 2 males

Post-natal viability, survival, body weights, clinical signs, and physical development of F1 pups were not affected by drug treatment of the F0 dams (Table 82). The neurological results in the locomotor activity or water maze behavioral response studies were not affected by drug treatment. However, the acoustic startle study showed that the mean maximum startle voltage and the mean average startle voltage were significantly higher (58% and 60%, respectively) for F1 males in the high dose group compared to F1 males in the control group. Acoustic startle parameters in the female groups were not affected by treatment. The sponsor maintained that these effects were not drug-related because 1) the other startle parameters, startle at start voltage and time to maximum startle, were not affected, 2) no effects were observed in the other behavioral tests, and 3) the mid-dose group showed no significant effect even though exposures of F0 dams to apixaban were similar in both the mid- and high dose groups. However, the reviewer noted that in the dose-finding study the exposure for the 600 mg/kg group (36300 ng*hr/mL) was lower than the exposure in the main study for the 200 mg/kg group (43400 ng*hr/mL) and the mean values for maximum startle voltage and the average startle voltage exhibited a dose relationship. Although the mean values for maximum startle voltage and average startle voltage in the F1 males from F0 dams in the mid-dose group are not statistically different from the values in F1 males from F0 dams in the control group, the values are increased compared to those for F1 males from dams in the control and low dose groups.

To evaluate whether the changes in maximum startle voltage and the average startle voltage can be attributed to drug treatment, the reviewer examined the individual trial values for all male groups. One F1 male (422) from a high dose dam had markedly high anomalous individual trial values for maximum startle voltage (6772) and average startle voltage (1542) compared to the maximum individual trial values in the control (1685 and 536, respectively) and low dose (1787 and 350, respectively) groups. If the statistical Q test (Dean and Dixon, 1951) is applied, these anomalous individual trial values for male 422 can be rejected. Recalculation of the time block mean for the nine remaining trial values (Trials 2-10) for male 422 results in a time block mean of 1236 for the maximum startle voltage and of 242 for the average startle voltage. Since these values are similar to the corresponding values for the F1 males from mid-dose dams, the mean values for maximum startle voltage and average startle voltage for the F1 males from high dose dams should no longer be statistically different from the values in the control group. Furthermore, the sponsor provided historical control data indicating the maximum time block mean and the maximum individual trial values for maximum startle voltage and average startle voltage for male 422 are clearly above the respective maximum historical values. Therefore, the reviewer concludes the changes in maximum startle voltage and the average startle voltage in the high dose group can be attributed to aberrant values in one trial for one animal and not to drug treatment.

Table 82: Reviewer's Summary – F1 Development Parameters – Study DN08001

Rat PPND – DN08001		Dose, mg/kg			
Parameter	**Historical max.**	**0**	**25**	**200**	**1000**
F1 Generation – Pre-Weaning					
Live F1 pups/litter on LD 4		12.7 (6-16)	12.8 (6-17)	13.0 (2-17)	12.6 (9-15)
% F1 pup survival LD 1-4		98.84	99.23	98.83	98.33
% male – LD 0		55.6	49.7	53.0	53.7
F1 Body Weight on LD 1/litter		6.95 (0.37)	6.99 (0.47)	6.88 (0.47)	6.95 (0.55)
F1 Body Weight on LD 4/litter (SD)		12.25 (1.03)	12.16 (1.18)	11.78 (1.38)	12.29 (1.07)
Acoustic startle in F1 males– Trials 1-50 ([T] After exclusion of aberrant value for M422)					
Maximum startle (voltage)					
Group mean (SD)	387.0	243.2 (116.0)	263.9 (137.2)	332.1 (203.1)	**384.2*** (227.5)
Maximum time block mean for individual males	1337.1	746	776	1134	**1789** (M422,1236[T]) (1066, M425)
Maximum individual trial value for individual males	6221	1685	1787	3438	**6772** (M422) (2769, M413)
Linear time contrast	-525.8	-311.3	-327.9	-439.7	**-653.9***
Average startle (voltage)					
Group mean (SD)	81.9	51.0 (25.9)	53.8 (25.1)	69.2 (43.4)	**81.4*** (49.0)
Maximum time block mean for individual males	261.7	161.4	152.6	262.5	**372.4** (M422, 242[T]) (245, M416)
Maximum individual trial value for individual males	1199	536	350	903	**1542** (M422) (521, M416)
Linear time contrast	-119.3	-65.3	-72.7	-97.3	**-143.7***
Time of maximum startle (msec), mean (SD)		30.1 (4.2)	29.4 (4.3)	29.4 (3.5)	28.5 (4.3)
Startle at start voltage (v), mean (SD)		7.6 (3.0)	7.8 (2.9)	7.7 (2.8)	7.7 (2.3)

In an evaluation of F1 reproductive performance, no drug-related effect was observed on number of corpora lutea, implantation sites, live/dead embryos, sex ratio, pre- and post-implantation losses, resorptions, placentae or gravid uterus weight in F1 females mated with F1 males. Statistically significant increases in left seminal vesicle absolute and relative weight in the F1 males from the mid- and high dose groups were not considered drug-related, because the increases in relative weights were not dose-related.

However, a dose-related decrease was observed in mean mating index (Table 83) and fertility index when F1 males were mated with F1 females, although the values for the F1 animals in the mid and high dose groups are not significantly different statistically from the control values. Furthermore, these values are close to or within historical control ranges (75% to 100% for mating index; 62.5% to 100% for fertility index in the 1996-200 (b) (4) Historical Control Data (Montreal) for Sprague Dawley rats). The sponsor concluded the decreased mating and fertility indices in the mid and high dose groups were drug-related because the exposures to apixaban in the F0 dams were similar in both groups. In contrast, no effect was observed on mating index and fertility index when the same F1 males were mated with naïve females, indicating that the decrease in reproductive performance could be attributed to F1 females. Because additional F1 females were no longer available, mating F1 females with naïve males could not be conducted. The sponsor concluded the NOAEL for mating and fertility was 1000 mg/kg/day for F1 males and 25 mg/kg/day for F1 females.

Table 83: Reviewer's Summary - F1 Reproductive Parameters - Study DN08001

Rat PPND – DN08001		Dose, mg/kg			
Parameter	**Historical max.**	**0**	**25**	**200**	**1000**
F1 Reproductive parameters (Historical: 75% to 100% for mating index; 62.5% to 100% for fertility index)					
Mating of F1 males and F1 females					
Number mated		23	25	24 (23)	**23**
F1 females mating		22	23	19	17
Combined F1 mating index, %		95.7	92.0	79.2 (82.6)	**73.9**
F1 females pregnant		22	20	19	**15**
Combined F1 fertility index, %		95.7	80.0	79.2 (82.6)	65.2 (8)
F1 females failing to mate, [female #]		1 [169]	2 [251, 255]	5 [351, **362**, 365, 370, 372]	6 [454, 455, 458, 459, 471, 475]
F1 females failing to mate and losing BW immediately prior to or during mating (% of those failing to mate) [female #]		1 (100%) [169]	2 (100%) [251, 255]	4 (80%) [**362**, 365, 370, 372]	3 (50%) [454, 459, 471]
F1 females that mated and lost BW immediately prior to mating (% of those mating) [female #]		2 (9.1%) [156, 169, 172]	3 (13.0%) [256, 257, 260]	3 (15.8%) [366, 374, 375]	3 (17.6%) [456, 459, 470]
Mating of F1 males and naïve females					
F1 Male mating index, %		95.5	100	92.0	100
F1 Male fertility index, %		95.5	92.0	88.0	100

The reviewer noted the incidence of dark areas or foci on the thymus in F1 females that failed to mate was 60% and 67% in the mid and high dose groups, whereas the incidence of dark areas or foci on the thymus in F1 females that successfully mated was 6% to 11% in mid and high dose groups (Table 84). However, only the F0 females were dosed and the F1 animals were separated from the dams on LD 21. Since the reproductive performance evaluation of the F1 animals was initiated on LD 87-94, it is unlikely that apixaban was still present in these animals and contributed to the higher incidence of dark thymic foci. Whether this damage to the thymus contributed to the decreased mating and fertility in these females is unclear.

Table 84: Reviewer's Summary – F1 Female Macroscopic Findings

Rat PPND – DN08001		Dose, mg/kg			
Parameter	**Historical max.**	**0**	**25**	**200**	**1000**
F1 females failing to mate [female #]		1 [169]	2 [251, 255]	5 [351, **362**, 365, 370, 372]	6 (454, 455, 458, 459, 471, 475)
Thymus – Dark foci or mottled		0	2 (100%) [251, 255]	3 (60%) [**362**, 370, 372]	4 (67%) [454, 455, 458, 459]
F1 females mating		22	23	19	17
Thymus – Dark foci, dark area or discoloration		7 (31.8%)	3 (13%)	2 (10.5%)	1 (5.9%)

The reviewer noted that 10 out of the 14 females that failed to mate lost body weight immediately prior to or during mating. In contrast, only 10 of the 81 females that mated lost body weight immediately prior to or during mating. Furthermore, the reviewer notes that F362 in the mid-dose group (Group 3) was noted as having broken teeth (D24-D52) and a malocclusion (D 73-87). This clinical finding likely contributed to the more than 11% decrease in body weight on PND 84 and more than 13% decrease in bodyweight gain for PND 24-84 with a greater decrease from PND 59-84 in this female (Table 85).

The mean food consumption from PND 28-84 was 23.1 and 23.6 gm/animal/day in the control and mid-dose groups, respectively, and 20.3 gm/day for F362. A decrease in food consumption to 20 gm/day for F362 by itself is not expected to affect mating and fertility based on the study of Terry et al (2005), in which female rats receiving 20, 15, and 10 gm/day had a 6, 16, and 29% reduction in body weight compared to an ad lib control group during a 15 day pre-mating period. Only the 10 gm/day group had a significant reduction in copulation and pregnancy rates to 62% and 50%, respectively. However, the 15 gm/day group had significant reductions in the number of corpora lutea, implantations, and viable fetuses along with an increase in pre-implantation loss and the percentage of females with prolonged diestrus. Since F362 experienced a 11 and 15% reduction in body weight relative to the mean body weight for the control and mid-dose groups, a reduction in her ability to mate and become pregnant was not expected based on the decreased weight gain alone. However, F362 also was found to have a mottled thymus at necropsy and a body weight loss during the mating period. Since these factors may have also contributed to F362's health status, stress level, and ability to mate, the reviewer believes that F362 should be excluded from the analysis of reproductive performance. Exclusion of F362 results in increases to the mating and fertility indices to 82.6%, a value clearly within the historical range.

Table 85: Reviewer's Summary - F1 Female Body Weight Post-Weaning

Rat PPND – DN08001						Dose, mg/kg			
Parameter		Historical max.		0		25		200	1000
		Mean [individual] body weight on						Mean [individual] bodyweight gain	
Group	Dose	PND 24	PND 49	PND 59	PND 70	PND 80	PND 84	Days 24-84	Days 59-84
1	0	77.1	220.3	257.8	281.8	305.5	312.5	235.4	54.7
2	25	75.2	218.4	256.7	284.8	310.8	320.6	245.4	63.9
3	200	75.8	223.0	263.4	289.6	318.3	326.5	250.7	63.1
4	1000	76.5	227.7	268.7	296.7	323.6	332.0	255.5	63.3
F362	200	[74]	[213]	[235]	[249]	[276]	**[277] (-11.4%[1], -15.2%[2])**	**[203] (-13.7%[1], -19%[2])**	**[42] (-23.2%[1], -33.4%[2])**

[1] Relative to mean for control Group 1, [2] Relative to mean for Group 3

The sponsor cited the similar exposure in the mid- and high dose F0 females as the primary reason for concluding that the decreased mating and fertility in both the mid- and high dose groups are drug-related. On PND 4, the $AUC_{(0\text{-}24h)}$ values in the toxicokinetic females receiving 200 and 1000 mg/kg from GD 6 through PND 4 were 43.4 and 47.5 µg*hr/mL, respectively, in the main study (Table 86). However, the reviewer noted a 4 and 6-fold variation in the plasma concentration in individual toxicokinetic animals at 2-4 hours after dosing. The dose range-finding PPND study (DN07035) using the same lot of apixaban resulted $AUC_{(0\text{-}24h)}$ values of 36.3 and 46.7 µg*hr/mL for females receiving 600 and 1000 mg/kg from GD 6 through PND 5, 6, or 7. Although the plasma concentrations at 2-4 hours after dosing also varied 4 and 7 fold, the mean Cmax and the maximum individual plasma concentrations in females dosed at 600 mg/kg in the dose-finding study were less than the mean Cmax and the maximum individual plasma concentrations in females dosed at 200 mg/kg in the main study. Comparison with the toxicokinetic parameters obtained in the 6-month rat toxicology study indicates that the $AUC_{(0\text{-}24h)}$ and Cmax values obtained in the dose range-finding PPND study at 600 mg/kg are consistent with those in the 6-month study; however, the

$AUC_{(0\text{-}24h)}$ and Cmax values in the main PPND study at 200 mg/kg are higher than those in the 6-month study. Given the variation and discrepancies, the reviewer believes the main F0 females dosed at 200 mg/kg could have had a lower exposure to apixaban than is indicated from the $AUC_{(0\text{-}24h)}$ value obtained for the toxicokinetic F0 females. The reviewer's NOAEL for F1 female fertility is 200 mg/kg and the toxic dose is 1000 mg/kg.

Table 86: Reviewer's Comparison of TK Parameters in Studies DN08001, DN07035, and DN03118

TK parameters – Study DN08001 Main PPND study		**Dose mg/kg**				
TK parameters on PND 4 in F0 dams		**25**	**50**	**200**	**600**	**1000**
Main study	$AUC_{(0\text{-}24)}$, µg*hr/mL	11.7		43.4		47.5
	Mean Cmax, µg/mL	1.51		4.90		4.94
Individual range at 2-4 hr post dose		1.05-1.79		1.54-6.13		1.73-10.1
TK parameters – Study DN07035 Dose-finding PPND study		Note: GLP was not completely followed - no dose formulation analysis, no QA				
TK parameters on PND 6 in F0 dams					**600**	**1000**
Dose – finding study	$AUC_{(0\text{-}24)}$, µg*hr/mL				36.3	46.7
	Mean Cmax, µg/mL				3.33	4.19
Individual range at 2-4 hr post dose					0.615-4.66	1.5-6.36

TK parameters – Study DN03118 6 month toxicology study - females				**50**	**200**	**600**	
$AUC_{(0\text{-}24h)}$, µg•hr/mL (mean)	1			11.7	16	18.6	
	90			22.2	25.2	36.2	
	181			26.4	27.2	34.4	
Cmax, µg/mL (mean, SD)	1	Mean SD Range		2.13 0.57 1.48-2.85	2.74 0.57 2.13-3.42	2.93 0.3 2.66-3.25	
	90	Mean SD Range		2.81 0.84 1.92-3.94	3.26 1.6 2.16-5.61	4.82 2.3 1.37-6.08	
	181	Mean SD Range		3.93 3.21 0.68-7.10	2.95 1.43 1.30-3.81	3.98 1.84 1.69-6.20	

In the F1 females that did become pregnant, the number of live F2 fetuses, the sex ratio, and mean fetal litter weights were similar in all groups indicating the absence of a drug-related effect for F2 parameters. One fetus in the low dose group and two fetuses in the high dose groups had malformations; however, no individual malformation was in all these fetuses and the incidence rates are within that reported by [redacted] (b) (4) Therefore, the NOAEL for F2 pups was 1000 mg/kg/day.

Table 87 below compares the exposures of rats at the NOAEL dose and at the dose at which toxicity was observed in the PPND study with exposures in patients with atrial fibrillation receiving 5 mg of apixaban twice a day. Given the uncertainty of the exposure at 200 mg/kg, the exposure ratio for the NOAEL for mating /fertility in F1 females is a maximum value that could be inflated by more than 50%.

Table 87: Comparison of Rat Exposures in the PPND Study to Human Exposure

Rat PPND Study DN0801		Comparisons to human exposure							
		Based on NOAEL dose				Based on toxic dose			
	NOAEL, mg/kg	Total		Unbound		Total		Unbound	
		AUC	Fold	AUC	Fold	AUC	Fold	AUC	Fold
F0 maternal	1000	47.5	15.3	2.2	5.4	>47.5	>15.3	>2.2	>5.4
F1 pre- and peri-natal fetal toxicity	1000	47.5	15.3	2.2	5.4	>47.5	>15.3	>2.2	>5.4
F1 postnatal development	1000	47.5	15.3	2.2	5.4	>47.5	>15.3	>2.2	>5.4
F1 Mating /Fertility:	F: 200 M: 1000	43.4 47.5	14.0 15.0	2.0 2.2	4.9 5.4	47.5 >47.5	15.3 >15.3	2.2 >2.2	5.4 >5.4
F2 pre-natal fetal toxicity	1000	47.5	15.3	2.2	5.4	>47.5	>15.3	>2.2	>5.4

The % unbound apixaban was 4.7% for female rats and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0\text{-}24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL

9.4 Juvenile Toxicology and Development

Dr. Ronald Honchel previously reviewed the dose range finding study (DN08064) and the definitive juvenile toxicology study (DN09014) conducted using apixaban. In Study DN08064, doses of 0 (vehicle), 25, 200, or 1000 mg/kg/day apixaban (BMS-562247) were administered daily via gavage to Sprague-Dawley rats from postnatal days (PND) 4 to 22. In Study DN09014, doses of 0 (vehicle), 10, 50, or 600 mg/kg/day apixaban (BMS-562247) were administered daily via gavage to four sets of Sprague-Dawley rats from PND 4 through PND 89-94. Figure 18 shows the evaluations conducted for each of the four sets of animals per dose group. The Set 1 animals were necropsied at the end of dosing. The Set 2 animals were used to evaluate reproductive effects after the end of treatment and then were necropsied on PND 120-13 after the 1 month recovery period. An overall summary of Study DN09014 is provided in Table 88.

Figure 19: Reviewer's Diagram of Juvenile Toxicity Study

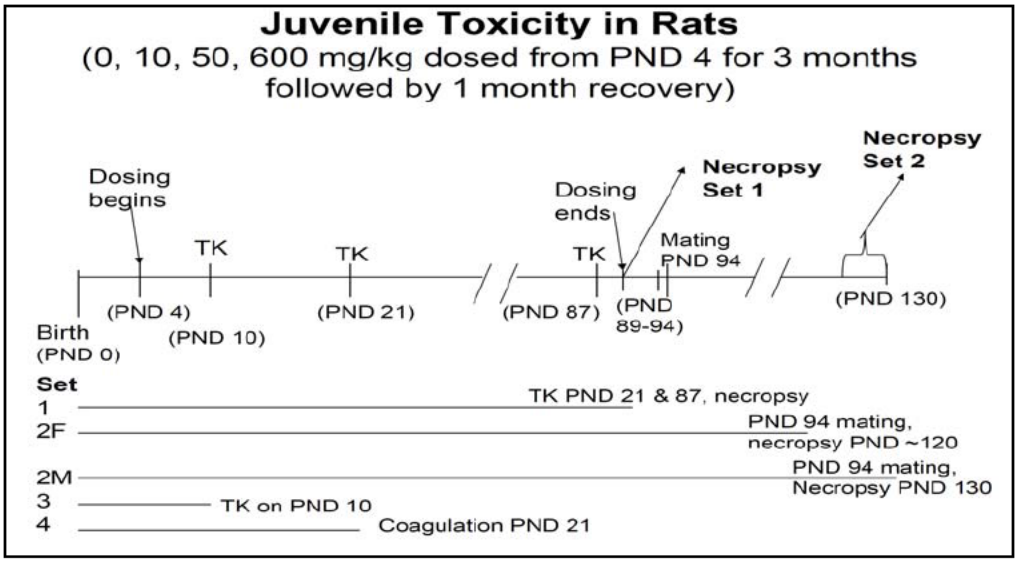


Table 88: Reviewer's Overview Summary of Juvenile Toxicity Study DN09014

Study title	**Apixaban (BMS-562247): Three-Month Oral Developmental Toxicity Study in Juvenile Rats with 1-Month Recovery**	
Document no.	930040744	
Study code	DN09014	
Drug lot/purity	7A28071, 99.7%	
Conducting lab and location	Bristol Myers Squibb, New Brunswick, NJ	
Study dates	June - Sept 2005	
GLP/QA	Yes/Yes	
Vehicle	99.5% Labrafil M-1944 CS/0.5% Tween 80),	
Species/Strain	Rat/Sprague Dawley	
Number/sex/group	Subset 1: 9-11/sex/group (end of dosing necropsy PND 87-94, and TK on PND 21 and 87). Subset 2: 10-12/sex/group (end of recovery necropsy PND 120-130, mating and fertility of treated animals with untreated animals of opposite gender on PND 94) Subset 3: 4/sex/group for groups 2-5 (toxicokinetics PND 10) Subset 4: 7-8/sex/group for groups 2-5 (coagulation PND 21)	
Doses, mg/kg	0 (water), 0 (vehicle), 10, 50, 600	
Route	Oral gavage	
Treatment duration	PND 4-94	
Cesarean section day	Gestation day (GD) 15 for untreated females that had mated with treated males	
Study acceptability	Numbers of animals/sex/group were minimal (10/sex), but acceptable. High dose was adequate, because PT was prolonged 1.3-2.2 fold in males and females and body weight gain decreased 7% in high dose males compared to vehicle controls	
Comments:	**Treated males**	**Treated females**
F1 Toxicology (Set 1)	Decrease in overall body weight of 6.7% in and in bodyweight gain of 6.9% high dose male group relative to vehicle control. Dose dependent increase in PT. At the end of dosing the incidence and severity of testicular degeneration/atrophy increased in high dose group (33.3%) that partially correlated with decreased testes weight and necropsy observations of decreased testicular size. These same males had hypospermia in the epididymides. However, after 1 month recovery, the finding present in both vehicle and treated groups was decreased in severity. These recovery males could impregnate untreated females.	Body weight in treated females was not significantly decreased. PT increased in all treated groups with 2.1 fold increase observed in high dose females.
F1 reproductive performance (Set 2)	In mating of treated males with untreated females, mating and fertility indices were not affected by treatment. Caesarean section parameters were not significantly affected by treatment.	In mating treated females with untreated males, mating and fertility indices were not affected by treatment. Live birth parameters were not significantly affected by treatment.
F2 offspring	Number of live F2 fetuses similar in all groups.	Number of live pups and their body weight were not affected by treatment
F1 Parental NOAEL, mg/kg	Males: 50 mg/kg for toxicology 600 mg/kg for mating and fertility	Females: 600 mg/kg for toxicology 600 mg/kg for mating and fertility
F2 Offspring NOAEL, mg/kg	600 mg/kg	600 mg/kg

Due to the pharmacodynamic effect of apixaban, prolongations of coagulation parameters were expected. At the end of dosing, PT values at 2-4 hours after dosing were prolonged 1.3, 1.5, and 1.7 fold in Set 1 animals dosed at 10, 50 and 600 mg/kg, respectively (Table 89). The values of 1.7 and 1.8 fold for PT at 600 mg/kg in this study are slightly higher than the values for PT of 1.4 fold and 1.5 fold in males and females, respectively, dosed at 600 mg/kg in the 6-month toxicology study (DN03118). However, on PND 21, PT values at 2-4 hours after dosing were prolonged 1.5, 1.8, and 2.1 fold in Set 4 animals dosed at 10, 50 and 600 mg/kg, respectively. The higher fold prolongation on PND 21 compared to PND 87 is consistent with the higher plasma concentration observed on PND 21 compared to PND 87 (see discussion below). In the dose-finding study (DN08064), prothrombin times were still slightly prolonged 1.27 and 1.15-fold in

males and females, respectively, despite the collection of blood for clinical pathology on PND 23 at least 24 hours after the last dose on PND 22.

Table 89: Reviewer's Summary – Coagulation Parameters – Studies DN09014 and DN08064

Rat Juvenile Toxicity		Dose, mg/kg/d				
Study DN09014		**0 (water)**	**0 (vehicle)**	**10**	**50**	**600**
Coagulation parameters in F1 animals (M, F) – 2-4 hours after dosing						
Prothrombin time, sec	PND 21		15.7, 15.7	**23.5*, 23.7***	**30.0*, 28.2***	**34.8*, 32.2***
	Fold vehicle control			1.5, 1.5	1.9. 1.8	2.2, 2.1
	PND 87±1	16.3, 15.9	15.3, 15.1	**20.0*, 21.3***	**23.1*, 23.7***	**26.4*, 27.4***
	Fold vehicle control			1.3, 1.4	1.5, 1,6	1.7, 1.8
aPPT, sec	PND 21		14.8, 12.2	15.5, **15.2***	**18.0*, 16.6***	**19.4*, 19.2***
	PND 87±1	16.5, 14.6	14.8, 14.1	15.6, **17.1***	**18.6***, 15.3	**19.3*, 17.7***
Fibrinogen, mg/dL	PND 21		180, 182	**203***, 203	**204***, 206	**205***, 201
	PND 87±1	305, 213	299, 248	311, 222	329, 224	327, 245
Dose finding study (DN08064)		**Dose, mg/kg**	**0**	**25**	**200**	**1000**
Coagulation parameters in F1 animals (M, F) – 24 hours after dosing last dose on PND 22						
Prothrombin time, sec	PND 22		18.8, 17.1	18.1, 17.9	18.8, 17.7	23.4, 19.6*
	Fold vehicle control			0.96, 1.05	1.0. 1.04	1.24, 1.15

The incidence of salivation was slightly increased in the treated groups relative to the incidence in the vehicle control group. At the end of treatment mean body weights and body weight gains were decreased in a dose-dependent manner relative to the vehicle control group in the F1 males, but not the F1 females (Table 90). Except for the expected prolongation of coagulation times discussed above, the only potential drug-related adverse effect was the microscopic finding of unilateral or bilateral degeneration of the testicular seminiferous tubules in one low dose and three high dose Set 1 males that were necropsied at the end-of dosing. The sponsor did not consider these findings apixaban-related because:

1) *"testicular degeneration is often observed as a background lesion in toxicity studies and was noted in a single control rat from this study;*
2) *retrospective evaluation of control Sprague-Dawley rats in 15 former general toxicology studies conducted at Bristol- Myers Squibb from 2005 to 2008, which were age-matched to the current study and thus were considered developmentally comparable indicated that up to 3 control rats/study spontaneously developed unilateral or bilateral testicular degeneration with similar severity."*

Upon request, the sponsor provided a table of historical data (Table 91). The mean age of the rats was 13 weeks (range 9-20 weeks) and the overall incidence of testicular degeneration was 12% with a range 5.6-26.7%. However, the severity of the testicular degeneration found in each study was not reported. The reviewer examined the three other rat toxicology studies submitted in NDA 202155 in which histopathology of the testes was performed (Table 92). The overall incidence of testicular degeneration or atrophy was 12.5%; however, the severity was only graded as minimal (1) and no adverse findings were reported for the epididymides. The control incidences of testicular degeneration reported in the submitted studies are higher than those reported by Lee et

al (1993) for young control Sprague Dawley rats in oral (2.5%) and inhalation (9.4%) toxicity studies.

In the juvenile Set 1 males the incidence of testicular degeneration was 33% in the high dose group. This incidence is higher than the maximum observed in the historical control data. The severity was graded as moderate in two males and marked in one male in the high dose Set 1 group. The severity of testicular degeneration in the high dose Set 1 males is higher than in the control groups in other submitted rat toxicology studies. In addition, moderate or marked hypospermia was observed in the epididymides of the same Set 1 males diagnosed with testicular degeneration. Furthermore, the testes weight relative to body weight was reduced in three of the same Set 1 males diagnosed with testicular degeneration.

In the Set 2 groups, one vehicle control male and one male in each of the treated groups were diagnosed with testicular degeneration after a one month recovery period (Table 90). However, the severity was graded as minimal. These males were mated with naïve females beginning on PND 94 and impregnated these females in ≤4 days

Because of the severity of the testicular finding and correlation with hypospermia in the epididymides and relative testes weight, the reviewer believes that the testicular finding is drug-related. However, the biological basis for this effect is unclear.

Table 90: Reviewer's Summary of Critical F1 Parameters from Study DN09014

Rat Juvenile Toxicity		**Dose, mg/kg/d**				
Study DN09014		**0 (water)**	**0 (vehicle)**	**10**	**50**	**600**
F1 Mortality	M	0	3	2	1	1
	F	0	2	1	2	1
Incidence of salivation (# animals)	M	0 (0)	16 (11)	24 (12)	25 (14)	20 (10)
	F	0 (0)	6 (4)	20 (10)	13 (10)	12 (9)
Body weight F1 male, gm (* p<0.05; ** p < 0.01 relative to vehicle control)						
Day 3		10.0	10.2	10.1	10.2	10.0
Day 17		42.4**	47.1	47.2	47.2	44.2*
Day 87		535.2	559.2	549.2	534.8	521.3
BW gain, Days 0-87		525.2	549.0	539.1	524.6	511.3
Body weight F1 female, gm						
Day 3		9.4	9.5	9.3	9.3	9.4
Day 17		40.8*	44.6	44.3	44.5	44.0
Day 87		284.3	313.2	296.6	298.9	302.4
Histopathology						
Set 1 F1 males – End of dosing (necropsied on PND 91)						
Testes, # examined		9	10	9	9	9
Degeneration, total incidence (%)		0 (0)	0 (0)	1 (11.1)	0 (0)	3 (33.3)
Severity (male #)	mild	-	-	1 (3104)	-	-
	moderate	-	-	-	-	2 (5102, 5110)
	marked	-	-	-	-	1 (5103)
Epididymides, # examined		9	10	2	1	10
Hypospermia, total incidence (%)		0	0	1 (11.1)	0	3 (30)
Severity (male #)	moderate	-	-	1 (3104)	-	2 (5110, 5102)
	marked	-	-	-	-	1 (5103)

Rat Juvenile Toxicity	Dose, mg/kg/d				
Study DN09014	**0 (water)**	**0 (vehicle)**	**10**	**50**	**600**
Testes weight relative to body weight, group mean	0.688	0.682	0.678	0.697	0.710
Male #, individual relative weight			3104, 0.218		5102, 0.49 5110, (0.41)[§] 5103, 0.741
Set 2 F1 males - End of recovery (necropsied PND 126-127)					
Testes, # examined	10	10	9	10	10
Degeneration, total incidence (%)	0	1 (10)	1 (11.1)	1 (10)	1 (10)
Severity minimal	-	1	1	1	1
Male # (fertility)	-	(2119 P)	(3118 P)	(4117 P)	(5112 P)
		4D	2D	3D	4D

Degeneration = degeneration of seminiferous tubule (diffuse, focal, or multifocal/unilateral or bilateral), P = male was fertile and impregnated naïve female. Historical control incidence of testicular degeneration in studies 2005-2008, see Table X, the mean incidence was 12% (range 5.6-26.7%). [§] Excluded from mean, because the left testes was absent (value in parentheses is twice the relative weight for right testes)

Table 91: Sponsor's Table: Control incidence of testicular degeneration/atrophy in Sprague-Dawley rat toxicity studies run at Bristol-Myers Squibb between 2005 and 2008 -

Study duration	Rat age at necropsy	Total no. of control male rats	No. affected control rats	Overall control incidence
3 months	19 weeks	16	2	12.5%
3 months	20 weeks	15	3	20%
1 month	13 weeks	10	1	10%
1 month	14 weeks	36	2	5.6%
1 month	13 weeks	15	1	6.7%
1 month	13 weeks	15	1	6.7%
1 month	13 weeks	15	1	6.7%
1 month	15 weeks	15	3	20%
2 weeks	9 weeks	6	1	16.7%
2 weeks	10-11 weeks	6	1	16.7%
2 weeks	10-11 weeks	6	1	16.7%
2 weeks	10-12 weeks	6	1	16.7%
2 weeks	12 weeks	10	1	10%
2 weeks	12-13 weeks	15	1	6.7%
2 weeks	12 weeks	15	4	26.7%

Provided in sequence 15 on 12/2/2011as an addendum to DN09014

Table 92 : Reviewer's Summary - Testicular Findings in Control Groups from Other Rat Toxicology Studies Submitted in NDA 202155

						Incidence (%) in Control Group of		
Study number	**Study date**	**Site**	**Duration, weeks**	**Age, weeks**	**Number/ group**	**Testicular degeneration or atrophy**	**Severity**	**Epididymides hypospermia**
DN02043	2003	BMS	13	18	10	1 (10%)	1	0
DN03118	2005	(b) (4)	26	32	20	Vehicle: 3 (15%) Water: 1 (5%)	1 1	0
DN04100	2009	(b) (4)	13	18	10	0	0	0

In the definitive study (Study DN09014) treated males and treated F1 females in Set 2 were co-inhabited with naive males and females, respectively, beginning PND 94 in order to evaluate F1 fertility and early embryonic development of F2 offspring. No drug-related change was observed in any maternal or litter parameter at caesarean-sectioning in untreated female rats mated with treated male rats at any dose tested. Values for implantations, litter sizes, viable conceptuses, and resorptions were comparable for all groups. After mating of treated females with untreated males, gestation and natural delivery, no effect of apixaban treatment with apixaban was observed on parturition, length of gestation, maternal performance, pup viability, pup survival, pup weight, sex ratio, or pup gross external observations.

Table 93: Reviewer's summary – F1 Reproductive Parameters

Rat Juvenile Toxicity	**Dose, mg/kg/d**				
Study DN09014	**0 (water)**	**0 (vehicle)**	**10**	**50**	**600**
Treated Set 2 F1 Females with untreated males (mating followed end of dosing, natural delivery of F2 pups)					
Mating index, %	100	100	90	100	100
Fertility index, %	90	90	100	100	100
F1 dams with stillborn pups (%)	0 (0)	1 (11.1)	0 (0)	0 (0)	1 (10)
F1 dams with all pups dying LD 1-4	0	0	0	0	0
F1 dams with all pups dying LD 5-6	0	0	0	0	1
Day 0, # F2 pups/liter, (M, F)	6.9, 7.2	6.4, 6.8	5.4, 6.3	6.1, 8.0	7.9, 7.0
Day 0 F2 Pup weights, (M, F)	7.1, 6.6	7.3, 6.9	7.7, 7.3	7.3, 6.9	7.4, 7.0
Day 4 F2 Pup weights, (M, F)	9.9, 9.1	10.7, 10.0	11.2, 10.8	10.1, 9.7	9.9, 10.1
Treated Set 2 F1 Males with untreated females (mating followed end of dosing, Cesarean section on GD 15)					
Number of mating pairs	10	10	9	10	10
Mating index, %	100	90	100	100	100
Fertility index, %	100	100	100	100	100
Corpora lutea (CL)/litter (SD) [Historical 14.1-18.0] Range	14.9 12 – 17	16.0 13 – 18	15.0 12 – 17	15.1 12 – 17	14.6 4 – 20
Implantation sites/litter (SD) ^ If female 042 (only 4 CL) excluded [Historical 11.4-17.0] Range	14.8 (1.6) 12 – 17	15.9 (1.6) 13 – 18	14.9 (1.5) 12 – 17	14.5 (1.8) 11 – 17	14.2 (4.7) 15.4^ 3 (10^) – 19
% Pre-implantation loss/litter (SD) ^ If female 042 (only 4 CL) excluded Range	0.7 (2.2) 0-7.1	0.6 (1.9) 0-5.6	0.7 (2.2) 0-6.7	4.1 (6.8) 0-21.4	4.7 (8.9) 2.4^ 0-25 (16.7^)
# litters with pre-implantation loss # with % pre-implantation loss > 10%	1 0	1 0	1 0	4 1	3 2 (1^)
% Post-implantation loss/litter Range	6.4 0-12.5	9.2 0-47.1	4.6 0-14.3	6.4 0-20	3.7 0-10.5
% litter with any resorption	70	55.6	44.4	60	50
Live F2 embryos/litter (SD) ^ If female 042 (only 4 CL) excluded	13.8 (1.1)	14.3 (2.4)	14.2 (1.8)	13.6 (2.1)	13.6 (4.4) (14.8^)
^ If female 042, who only had 4 CL, excluded – Female 042 was mated to male 5112					

On PND 21 and 87±1, systemic apixaban exposures ($AUC_{(0\text{-}24h)}$) increased in F1 animals; however, the increases were less than dose proportional (Table 94). Mean Tmax ranged from 1.0 to 4.0 hours post dose. On PND 10, mean plasma apixaban concentrations at 2 and 4 hours post dose increased in a dose-related manner between 10 and 50 mg/kg, and were similar or marginally increased between 50 and 600 mg/kg/day. Exposures in males on PND 21 and 87 were generally similar to those in females.

Mean apixaban $AUC_{(0\text{-}24h)}$ and Cmax values on PND 21 were 1.6-2.9 and 1.9-3.8 fold higher, respectively, than those on PND 87. As previously discussed, PT values on PND 21 were higher than those on PND 87. The plasma concentrations at 2 or 4 hr post dose on PND 10 were 1.9 to 6.6 fold higher than the plasma concentrations at the respective timepoints on PND 21. The reviewer notes that similar increases in apixaban plasma concentrations on PND 10 compared to PND 21 were observed in the juvenile dose-range finding study (DN08064) (Table 94, Figure 20). Although PT values were not obtained on PND 10, higher PT values on PND 10 may be expected based on higher plasma concentrations of apixaban. Therefore, caution is recommended in any future clinical trials, particularly in infants and very yound children.

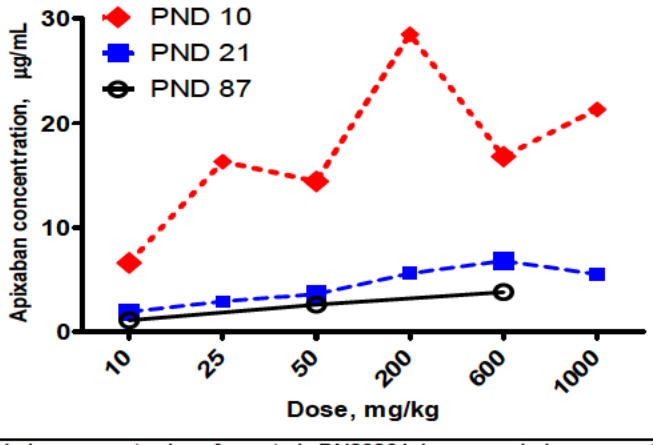
The reason for the decrease in apixaban exposures with age is unclear; however, but may be related to developmental changes as the animal ages. Since Duanmu et al 2006 showed that SULT1A1 expression did not differ significantly among human subjects of 0-1 year of age compared to subjects greater then 1 year of age, changes in sulfotransferase activity are unlikely to be involved in the increased apixaban exposure early in development. The sponsor proposed that the decreases in $AUC_{(0\text{-}24h)}$ and Cmax, and increase in Tmax with age in both male and female rats, across all doses, are consistent with a possible decrease in the rate of absorption during development. This proposal is consistent with marked developmental increases in Pgp expression in both the central nervous system and gastrointestinal tract, but not the liver or kidney, of FVB mice from birth through adulthood (Mahmood et al 2001; Watchko et al 2001).

Table 94: Reviewer's summary – TK Parameters in Juvenile Studies

Rat Juvenile Toxicity		**Dose, mg/kg/d**			
Study DN09014	**0 (water)**	**0 (vehicle)**	**10**	**50**	**600**
Toxicokinetic parameters – Study DN09014					
Plasma concentration, µg/mL, (M, F)	PND 10 – 2 hr post dose (M, F)		5.6, 7.1	14.1, 13.3	20.1, 15.5
	Fold PND 21		2.9, 3.7	2.9, 3.0	2.2, 1.9
	PND 10 – 4 hr post dose (M, F)		7.2, 6.5	16.3, 13.8	16.0, 15.5
	Fold PND 21		4.8, 3.0	5.6, 6.6	3.3, 3.1
	PND 21 – 2 hr post dose (M, F)		1.9, 1.9	4.9, 4.5	9.1, 8.1
	Fold PND 87		2.1, 1.5	1.6, 1.4	2.9, 1.7
	PND 21 – 4 hr post dose (M, F)		1.5, 2.2	2.9, 2.1	4.8, 5.0
	Fold PND 87		1.5, 1.8	1.3, 1.1	1.4, 1.4
	PND 87 – 2 hr post dose (M, F)		0.9, 1.3	3.0, 3.3	3.1, 4.9
	PND 87 – 4 hr post dose (M, F)		1.0, 1.2	2.2, 2.0	3.4, 3.7
AUC (0-24 hr), µg.hr/mL)	PND 21 (M, F)		14.5, 21.6	34.4, 29.2	69.5, 88.1
	PND 87 (M, F)		7.9, 9.5	16.8, 18.5	24.1, 29.9

Toxicokinetic parameters - Dose finding study, DN08064 (last dose PND 22)		**Dose, mg/kg**			
		0	**25**	**200**	**1000**
Plasma concentration (µg/mL, M, F)	PND 10 – 2 hr		15.0, 17.0	25.7, 23.1	20.2, 23.7
	Fold PND 21		4.4, 6.0	4.0, 3.6	3.1, 3.0
	PND 10 – 4 hr		13.5, 19.7	35.5, 29.5	20.6, 20.8
	Fold PND 21		5.4, 7.3	8.8, 5.3	4.7, 6.5
	PND 21 – 2 hr		3.4, 2.8	6.4, 6.4	6.6, 7.9
	PND 21 – 4 hr		2.5, 2.7	4.0, 5.6	4.4, 3.2
AUC, µg.hr/mL, PND 21 (M,F)			23.1, 21.9	50.9, 54.0	57.4, 67.6

Figure 20: Reviewer's Summary Plots of Mean Plasma Concentration in Juvenile Studies DN08064 and DN09014



Smaller symbols represent values from study DN08064; larger symbols represent values from study DN09014. The mean concentration at 2 and 4 hours post dose in males and females is plotted relative to the dose administered.

Table 95 below compares exposures of juvenile rats on PND 87 at the NOAEL dose and the dose at which toxicity was observed in the juvenile study with the exposures of adult patients with atrial fibrillation receiving 5 mg of apixaban twice a day. Given the uncertainty of the therapeutic dose and the unbound concentration of apixaban in children of any age, the exposure comparison below is only for the adolescent/young adult animal. Comparisons based on the unbound $AUC_{(0\text{-}24h)}$ at the NOAEL dose are 3.4 fold for all parameters in females. However, comparison in the males are 2.4 fold for F1 postnatal development, F1 mating/fertility, and F2 embryo development and 1.6 fold for F1 male toxicity based on the testicular degeneration. It is important to reiterate that the NOAEL for F1 mating/fertility of 600 mg/kg is based on the results in the separate set of recovery animals who were no longer being dosed.

Table 95: Comparison of Juvenile Rat Exposure on PND 87 to Human Exposures

Rat Juvenile Toxicity Study DN09014		Comparisons to human exposure							
		Based on NOAEL dose				Based on toxic dose			
	NOAEL, mg/kg	Total		Unbound		Total		Unbound	
		AUC	Fold	AUC	Fold	AUC	Fold	AUC	Fold
F1 postnatal development	600	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4
F1 toxicity	M: 50 F:600	M: 16.8 F: 29.9	M: 5.4 F: 9.6	M: 0.67 F:1.4	M: 1.6 F: 3.4	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4
F1 Mating /Fertility:	M: 600* F: 600	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4
F2 embryo development	600	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4	M: 24.9 F: 29.9	M: 8 F: 9.6	M: 1.0 F:1.4	M: 2.4 F: 3.4

The % unbound apixaban was 4.0% and 4.7% for male and female rats, respectively, and 13.2% for humans. At the RHD of 10 mg (5 mg BID) for AF, the apixaban total human $AUC_{(0\text{-}24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL.
* Note this NOAEL is based on mating after the termination of dosing.

10 Special Toxicology Studies

Local tolerance
No separate local tolerance study was submitted. The 2-week studies in rats and dogs indicated microscopic and macroscpic changes at the injection sites, primarily caused by the vehicle (35% hydroxypropyl-β-cyclodextrin (HPβCD) in 10 mM sodium phosphate, pH 7.0).

Hemolytic potential
In rabbit, rat, dog, and human whole blood exhibited a low hemolytic potential in an in vitro hemolysis assay (Document 930010276). The highest concentration of BMS-562247 at which no significant hemolysis occurred was 0.63 mg/mL for human erythrocytes and 0.31 mg/mL for rat, rabbit and dog erythrocytes.

Phototoxicity
The phototoxicity potential of apixaban was assessed in a neutral red uptake phototoxicity assay using mouse fibroblasts (Balb/c 3T3) (Document 930021524). Apixaban at concentrations up to 35 µg/mL did not elicit cytotoxicity to permit determination of IC_{50} values either in the presence or absence of ultraviolet-A light. Since a photoirritancy factor (PIF) could not be calculated, a PIF value of *1 was assigned. The calculated mean phototoxic effect (MPE) indexes were less than 0, indicating that apixaban has no phototoxic potential. In contrast, the positive control (75 µg/ml chlorpromazine) was phototoxic with a PIF of 32.

11 Integrated Summary and Safety Evaluation

Because of its central role in blood coagulation, the coagulation serine protease, FXa, is a major target for inhibition by therapeutic drugs for use in thromboembolic diseases. Apixaban (BMS-562247), an orally active non-peptide inhibitor of FXa, is approvable for the proposed indication from a pharmacology and toxicology perspective, because apixaban was shown to be efficacious in animal models of thrombosis and most of the toxicities observed in the nonclinical studies submitted are attributable to its pharmacodynamic or supra-pharmacodynamic (bleeding) effects. These effects included hemorrhage, extramedullary hematopoiesis, pigment deposition and secondary effects on red cell parameters. However, a few issues deserve discussion.

During the development of apixaban, the sponsor changed the process used to prepare the drug substance. (b) (4) as determined by powder X-ray

diffraction. Furthermore, the assays for apixaban, which are HPLC/MS/MS assays, were highly similar for all drug formulations. Although the different formulations have a different profile of impurities, the toxicology studies conducted with any (b) (4) formulations are acceptable in support of the NDA for the commercial formulation of drug substance.

Another issue that will affect revisions to the label is the need to express animal exposure ratios to the human exposure based on the unbound concentration. As summarized in Table 96, the Ki of apixaban for FXa from the different species used for toxicology studies varies more than 22 fold. The concentration of apixaban needed to double the prothrombin time varies by 3.4 fold. However, the binding of apixban to serum proteins varies by more than 10 fold. After correcting for protein binding, the concentration of apixaban needed to double the prothrombin time varies by 2.3 fold

Table 96: Reviewer's Summary – In Vitro Parameters Across Species

Parameter	Human	Rabbit	Rat	Dog	Mouse
FXa, Ki at 25°C, nM	0.08	0.16	1.4	1.8	ND
Concentration needed for 2-fold increase of PT, μM†	3.6	2.3	7.9	6.7	ND
Plasma protein fraction unbound	13.2%	37.7%	4.7%	8.4%	49%
Unbound concentration needed for 2-fold increase of PT, μM	0.47	0.86	0.37	0.56	ND
* From Wong et al. J. Thrombosis Thrombolysis 2011					

All of the previously presented animal to human exposure ratios for the chronic toxicology studies, reproductive and developmental toxicology studies and the carcinogenicity studies are summarized in Table 97. Using exposures based on the total concentration of apixaban, the exposure ratios agree with the values proposed by the sponsor in the draft label. However, the large differences in protein binding indicate that use of the unbound concentration of apixaban is more appropriate. Furthermore, use of the unbound concentrations would be consistent with the label for a previously approved FXa inhibitor, rivaroxaban.

The sponsor used a value of 3.1 μg•h/mL for the human $AUC_{(0\text{-}24h)}$, based on a clinical pharmacology report (CV185046, Document 930025972) of a multiple dose study evaluating the pharmacokinetics of apixaban in healthy male Japanese subjects. The mean $AUC_{(0\text{-}24h)}$ for a 5 mg apixaban dose twice a day was 2.88 μg•h/mL and the maximum $AUC_{(0\text{-}24h)}$ was 3.39 μg•h/mL. It is not clear how the sponsor arrived at the value of 3.1 μg•h/mL. However, according to the clinical pharmacology reviewer, Dr. Ju-Ping Lai, the median $AUC_{(0\text{-}24h)}$ for the atrial fibribrillation patients in ARISTOTLE was a higher value of 3.6 μg•h/mL. Therefore, the safety margins calculated below should be considered maximum values relative to the results of the ARISTOTLE trial. These safety margins are likely inflated by 15%.

Table 97: Summary of Animal to Human Exposure Ratios

			Exposure at NOAEL		Safety Margin‡	
Study/ Species	Sex	NOAEL (mg/kg)	Total AUC (mg*hr/L)	Unbound† AUC (mg*hr/L)	Based on Total AUC at NOAEL	Based on Unbound AUC
General toxicology – chronic studies						
6 month - rat	M	600	35.5	1.42	11.5	**3.5**
	F	600	34.4	1.62	11.1	**4.0**
12 month - dog	M	100	99.4	8.35	32.0	**20.4**
	F	100	137.0	11.5	44.2	**28.1**
Reproductive and Developmental Toxicology						
FEED – Rat Fertilization to implant	Paternal	200	24.4	1.0	7.9	2.4
	Maternal	600	36.3	1.7	11.7	4.2
	Embryo toxicity	600	36.3	1.7	11.7	4.2
	Fertility	600	36.3	1.7	11.7	4.2
EFD – Rat Implantation to GD 15	Maternal	1000	36.3	1.7	11.7	4.2
	Fetal - toxicity	3000	36.4	1.7	11.7	4.2
	Malformations	3000	36.4	1.7	11.7	4.2
EFD – Oral Rabbit Implantation to GD 20	Maternal	1500	0.36	0.14	0.12	0.33
	Fetal - toxicity	1500	0.36	0.14	0.12	0.33
	Malformations	1500	0.36	0.14	0.12	0.33
EFD – IV Rabbit Implantation to GD 20	Maternal	5	1.0 (3.2)§	0.38 (1.2)	0.33 (1.0)	0.93 (2.9)
	Fetal - toxicity	5	1.0 (3.2)	0.38 (1.2)	0.33 (1.0)	0.93 (2.9)
	Malformations	5	1.0 (3.2)	0.38 (1.2)	0.33 (1.0)	0.93 (2.9)
EFD – Mouse Implantation to GD 15	Maternal	1500	15.9	7.8	5.1	19.1
	Fetal - toxicity	1500	15.9	7.8	5.1	19.1
	Malformations	1500	15.9	7.8	5.1	19.1
Pre/Post-natal – Rat Implantation to weaning	F0 (maternal)	1000	47.5	2.2	15.3	5.4
	F1 (perinatal)	1000	47.5	2.2	15.3	5.4
	F1 (postnatal)	1000	47.5	2.2	15.3	5.4
	F1 fertility	**F: 200**	**43.4**	**2.0**	**14.0**	**4.9**
		M:1000	47.5	2.2	15.3	5.4
	F2 fetal	1000	47.5	2.2	15.3	5.4
Carcinogenicity						
Rat	Tumors	600	16.3-20.3	0.66-0.81	5.4-6.5	1.6-2.0
		600	29.2-35.5	1.37-1.66	9.4-11.4	3.4-4.0
Mouse	Tumors	1500	7.33	3.59	2.4	8.8
		3000	16.8	8.23	5.4	20.1

† Using % unbound apixaban as 4.0% for male rats, 4.7% for female rats, 37.7% for rabbits 49% for mouse, 8.4% for dogs, and 13.2% for human.
‡ At the RHD of 10 mg (5 mg BID) for AF, the apixaban total $AUC_{(0\text{-}24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046). The human unbound AUC is 0.409 µg•h/mL
§ In the rabbit EFD study using IV administration, exposure was determined initially using timepoints more appropriate for oral dosing. In an extension study using the same intravenous doses as in the main study, exposures were determined using timepoints more appropriate for intravenous dosing. These values are presented in parentheses.

Administration of apixaban did not result in malformation in rats, rabbits and mice. Furthermore, no significant embryo/fetal/offspring toxicity was observed in the FEED study and the EFD studies in rats, rabbits and mice. The NOAELs for embryo/fetal toxicity in the FEED study and the EFD study in rats were 600 mg/kg and 3000 mg/kg, respectively. These NOAELs corresponded to safety margins for embryo/fetal/offspring

toxicity of 4.2 and 4.2 fold, respectively, based on unbound exposure comparisons to the steady state unbound $AUC_{(0\text{-}24h)}$ of about 0.409 mg*hr/L for the 5 mg apixaban dose twice a day in patients with atrial fibrillation. Although the NOAEL for embryo/fetal toxicity in the oral EFD study in rabbits was 1500 mg/kg, total exposure in rabbits was lower than the total exposure in humans and no safety margin was achieved based on unbound exposure. To increase the exposure in rabbits, the sponsor conducted another EFD study in rabbits using intravenous administration of apixaban. In this intravenous rabbit EFD study, exposure was determined initially using timepoints more appropriate for oral dosing. In a subsequent toxicokinetic extension study using the same intravenous doses as in the main study, exposures (values in parentheses) were obtained using timepoints more appropriate for intravenous dosing. Using the initial $AUC_{(0\text{-}24h)}$ values, the unbound exposures of apixaban in rabbits were just under the unbound exposure in humans. Using the extension $AUC_{(0\text{-}24h)}$ values, the unbound exposures of apixaban in rabbits were 3 fold the unbound exposure in humans. In addition, the sponsor conducted yet another EFD study using mice. Again, no malformation or embryo/fetal/offspring toxicity was observed. The NOAEL of 1500 mg/kg for embryo/fetal toxicity in the oral EFD study in mice resulted in a exposure ratio of 5 fold the human exposure based on total concentrations of apixaban. After correction for protein binding, the safety margin in mice was 19 fold the human exposure based on unbound concentrations.

Prior to the definitive PPND study, the sponsor determined that interruption of dosing during parturition was not necessary, because no death occurred in any group in the dose range-finding study. However, the incidence of peri-vaginal bleeding signs was slightly increased in the high dose group that was dosed during parturition compared to the group that was not dosed during parturition. In the definitive study, dosing was not interrupted during parturition. No apixaban treated dam was found dead or euthanized because of bleeding during parturition. However, the incidence of bleeding signs increased in the mid and high dose groups during gestation. Some pups did die in all groups; however, the number of dead pups per litter and the number of live pups/litter were similar across all groups. No adverse effect was observed on F0 maternal parameters, F1 peri-natal toxicity or F1 postnatal development. The NOAEL was 1000 mg/kg corresponding an unbound exposure ratio of 5.4 fold compared to the unbound human exposure.

However, when F1 males were mated with F1 females, a dose-related decrease was observed in mean mating index and fertility index. Although the values for the F1 animals in the mid and high dose groups are not significantly different statistically from the control values and are are close to or within historical control ranges, the sponsor concluded the decreased mating and fertility indices were drug-related. Additional matings of the same F1 males with naïve females indicated that the decrease in reproductive performance could be attributed to F1 females. The sponsor concluded the NOAEL for mating and fertility was 1000 mg/kg/day for F1 males and 25 mg/kg/day for F1 females. However, as discussed in Section 9.3, the reviewer's NOAEL is 200 mg/kg and the toxic dose is 1000 mg/kg. As discussed in section 9.3, the exposure of the F0 dams at 200 mg/kg in the PPND study appears elevated relative the exposures in the dose range-finding study. Therefore, the reviewer recommends that the label refer to the exposure ratio at the toxic dose of 1000 mg/kg in discussion of the mating and

fertility findings in the F1 females. The F0 exposure at 1000 mg/kg corresponds to an unbound exposure ratio of 5.4 fold compared to the unbound human exposure. In the F1 females that did become pregnant, the number of live F2 fetuses, the sex ratio, and mean fetal litter weights were similar in all groups indicating the absence of a drug-related effect for F2 parameters. Therefore, the NOAEL for F2 pups was 1000 mg/kg/day, corresponding to an unbound exposure ratio of 5.4 fold compared to the unbound human exposure.

However, comparisons based solely on total exposure do not fully describe the drug treatment of the animals in the reproductive toxicology studies. Table 98 compares the plasma concentrations of apixaban in three EFD studies relative to the mean Cmax concentration in patients with atrial fibrillation. In the rat EFD study, the plasma concentrations of apixaban during the first 8 hours after dosing at the NOAEL dose of 3000 mg/kg in the dams were 10 to 13 times the mean human Cmax unbound plasma concentration. In the rabbit EFD study using intravenous administration, the plasma concentrations of apixaban during the first hour after dosing at the NOAEL dose of 5 mg/kg in the dams were 7.6 to 128 times the mean human Cmax unbound plasma concentrations. In the mouse EFD study, the plasma concentrations of apixaban during the first 8 hours after dosing at the NOAEL dose of 3000 mg/kg in the dams were 7.8 to 119 times the mean human Cmax unbound plasma concentration.Thus, the animals in the rat, rabbit, and mouse EFD studies were subjected to supra-therapeutic levels of apixaban for at least 33%, 4% and 33%, respectively, of each day following dosing. Given these very high unbound concentration multiples, it is surprising that more adverse events associated with the pharmacodynamic effect of apixaban, such as occult or overt bleeding, did not occur.

Table 98: Reviewer's Comparison of Plasma Concentrations in the EFD Studies

	Rat EFD (3000 mg/kg) after dosing on GD 15			**Rabbit IV EFD (5 mg/kg) after dosing on GD 19**			**Mouse EFD (1500 mg/kg) after dosing on GD**		
	Document 930009803			Document 930016662			Document 930021450		
Time post dose, hrs.	Plasma conc., ng/mL	Unbound conc., ng/mL	Unbound conc. relative to human Cmax	Plasma conc., ng/mL	Unbound conc., ng/mL	Unbound conc. relative to human Cmax	Plasma conc., ng/mL	Unbound conc., ng/mL	Unbound conc. relative to human Cmax
0.083				8788	3313	128			
0.166				5354	2018	78			
0.333				3604	1359	52			
0.5				2292	864	33	2530	1240	47.7
1				522	197	7.6	4015	1967	75.6
2	6249	293	11.3	49	18	0.7	2312	3100	119
4	5917	278	10.7				1278	626	24
6	5558	261	10						
8	7224	340	13.1				416	204	7.8
24							17	8	0.3

† Using % unbound apixaban as 4.7% for female rats, 37.7% for rabbits 49% for mouse, and 13.2% for human.
‡ At the RHD of 10 mg (5 mg BID) for AF, the apixaban total $AUC_{(0\text{-}24\ h)}$ value is 3.1 µg•h/mL (Clinical pharmacology report CV185046) and the Cmax is about 200 ng/mL. The human unbound AUC is 0.409 µg•h/mL and the unbound Cmax is 26 ng/mL

FX is known to be important in embryo/fetal development based on the embryo lethality of FX-deficient mice. Transgenic mouse embryos made genetically deficient in FX die either mid-gestation (11.5 days post coitum) or immediately after birth (Dewerchin et al. 2000). A similar early lethality may be associated with human FX deficiency, because no individual human has ever been identified that completely lack FX (Tai et al. 2007). The FX-deficient mice display a lethal phenotype similar to that observed for prothrombin (FII) and PAR-1-deficient mice. Transgenic mouse embryos made genetically deficient in prothrombin die either mid-gestation (9.5 days post coitum) or immediately after birth (Sun et al. 1998; Xue 1998). Likewise, approximately 50% of PAR-1-deficient mouse embryos die at midgestation days (9-10 post coitum) (Connolly et al. 1996).

The sponsor demonstrated the presence of apixaban in fetal plasma or tissue by several methods. In the whole body autoradiography study in pregnant albino rats on GD 18, radioactive apixaban was rapidly absorbed and detected in all fetal tissues evaluated from 0.5 to 8 hours post dose to Sprague Dawley pregnant female rats (Document 930036905). However, this study did not determine whether the radioactivity was still in the form of apixaban. In the EFD studies in mice, rats and rabbits, the levels of apixaban in maternal plasma and in fetal extracts or fetal blood were measured by LC/MS/MS. The levels of apixaban in the embryo extract and the fetal:maternal ratio in the rat EFD study (930009803) are comparable to the concentrations of radioactivity in the fetal kidney in the tissue distribution study (930036905). Thus, at least later during gestation, apixaban crosses the placenta and can be found in the fetus, particularly in mice and rats.

Overall, the submitted reproductive and developmental toxicicology studies indicate that apixaban is not a teratogen or embryo/fetal toxicant even though supra-pharmacodynamic plasma concetrations that could induce bleeding were present in rats, rabbits and mice. Although some evidence of bleeding was observed in these studies, no maternal deaths occurred as a result of bleeding. The current draft label for apixaban [redacted (b) (4)]. However, the use of apixaban in treating thromboembolic disorders during pregnancy may be acceptable, despite the potential bleeding risks to the mother and offspring as long as the label clearly indicates the risk. It would helpful to have actual data concerning women who received apixaban during pregnancy in the label.

During human labor and delivery, the need to reverse bleeding is sometimes necessary. Section 5.1 of the label indicates that in the event of bleeding complications, apixaban should be discontinued and appropriate treatment initiated. [redacted (b) (4)] administration of recombinant factor VIIa are specifically mentions. No pre-clinical study used administration of FVIIa [redacted (b) (4)] to reverse the pharmacodynamic action of apixaban. However, as indicated in Section 10 of the label, [redacted (b) (4)]. Although the administration of activated charcoal only partially (37%) reduced the total exposure (AUC) in dogs, the $AUC_{(0-24)}$ and Cmax values in the absence of charcoal treatment in this study are 25- and 46-fold the human $AUC_{(0-24)}$ (3.1 µg•h/mL and Cmax (0.2 µg/mL),

respectively, for total apixaban exposure following the recommended human dosage of 5 mg twice a day. Additional work needs to be conducted to find the appropriate agent and conditions that would reverse bleeding in humans, particularly in the peri-natal period.

The pilot and main juvenile studies in rats with apixaban covered the period of PND 4-22 and PND 4-90, respectively, corresponding to the human age ranges of neonate to toddler and neonate to young adult, respectively. Several findings require discussion.

Due to the pharmacodynamic effect of apixaban, prolongations of coagulation parameters were expected. In the juvenile studies, blood collection for monitoring coagulation parameters took place at an appropriate time after dose administration on PND 21 and 87, because apixaban dosing prolonged coagulation parameters. At the end of dosing, PT values at 2-4 hours after dosing were prolonged 1.7 and 1.8 fold in Set 1 males and females dosed at 600 mg/kg, respectively. The values of 1.7 and 1.8 fold for PT in this study are slightly higher than the values for PT of 1.4 and 1.5 fold in males and females, respectively, dosed at 600 mg/kg in the 6-month toxicology study (DN03118). However, on PND 21, PT values were prolonged 2.3 and 2.1 fold in the Set 4 males and females dosed at 600 mg/kg, respectively. The higher fold prolongation of PT on PND 21 compared to PND 87 is consistent with the higher plasma concentration observed on PND 21 compared to PND 87. Mean apixaban $AUC_{(0\text{-}24h)}$ and Cmax values on PND 21 at 600 mg/kg were 2.9 fold and 1.7-2.9 fold higher, respectively than those on PND 87. However, on PND 10 the plasma concentrations at 2 or 4 hr post dose at 600 mg/kg were 3.1-3.3 fold higher than the plasma concentrations at the respective timepoints on PND 21. Similar increases in apixaban plasma concentration on PND 10 compared to PND 21 were observed in the juvenile dose-range finding study (DN08064) (Figure 20). Although PT values were not obtained on PND 10, higher PT values on PND 10 may be expected based on higher plasma concentrations of apixaban.

The reason for the decrease in apixaban exposures in older animals is unclear; however, but may be related to developmental changes as the animal ages. Changes in sulfotransferase activity are unlikely to be involved in the increased apixaban exposure, at least in humans, since SULT1A1 expression was similar among human subjects either less than or greater than 1 year of age (Duanmu et al 2006). The elevated exposure may be due to a combination of immature renal development in the rat (Zoetis and Hunt, 2003) and decreased expression of CYP P450 3A4 (Asaoka et al. 2009, de Zwart et al. 2004, de Zwart et al. 2008) in the younger animals. Additionally, apixaban is a P-gp substrate. Pgp expression undergoes marked developmental increases in both the central nervous system and gastrointestinal tract, but not the liver or kidney, of FVB mice from birth through adulthood (Mahmood et al 2001; Watchko et al 2001). In addition, the levels of many coagulation factors, including FX, in the neonate at birth are about 40% of the adult levels (Andrew et al. 1987, Hassan et al. 1990). Any pediatric studies in very young humans will need to be designed with caution to determine the appropriate levels of apixaban for a therapeutic effect in neonates and infants undergoing rapid changes in their hemostatic, renal and metabolic systems.

A toxicity in the juvenile males that was not previously observed in adults is testicular degeneration. In the juvenile Set 1 males the incidence of testicular degeneration was 33%, an incidence that is higher than the maximum (26.7%) observed in the historical

control data. Furthermore, the moderate or marked severity of testicular degeneration was higher than the minimal severity observed in control animals. The testicular degeneration in the three high dose males correlated with moderate or marked hypospermia in the epididymides and partially correlated with reduced testes weight relative to body weight the same males. The sponsor argues that this finding should be considered biological variation because one vehicle control male and one male in each of the treated groups were diagnosed with testicular degeneration after a one month recovery period. These males were mated with naïve females beginning on PND 94 and were able to impregnate the females in ≤4 days. However, the severity of testicular degeneration in the recovery males on PND 130 was minimal. The reviewer believes that the testicular finding is drug-related and is of concern, because of the severity of the testicular finding in the high dose Set 1 males and its correlation with hypospermia in the epididymides. Humans have a low fertility relative to animal models, particularly rats, and consequently are more susceptible to adverse effects on gonadal function (Working, 1988).

The sponsor also argues that in the PPND study no findings of testicular degeneration were observed in the F1 males who were likely to have received apixaban through their mother's milk. The sponsor did demonstrate that approximately 12% of a maternal apixaban dose is excreted into rat milk on PND 10. A dam receiving the high dose of 1000 mg/kg in the PPND study would transfer 42 mg of apixaban to her eight pups through the milk. Each pup would receive an apixaban dose of 175 mg/kg on PND 10 with higher and lower doses prior to and after PND 10. However, in the PPND study, apixaban concentrations and coagulation parameters were not measured in the pups. No decrease was observed in the F1 testes weight relative to body weight and no gross pathology finding indicative of testicular degeneration was observed in any F1 group in the PPND study. Although no microscopic examination of the testes was conducted, the high dose F1 males in the PPND study were able to impreganate naïve females. These results provide some assurance that the mid dose of 200 mg/kg in the juvenile study is a NOAEL for the testicular toxicity if the critical period is prior to PND 21.

The biological basis for the testicular effect is unclear. However, the neonatal period has been shown to be critical for the development of normal spermatogenesis in the adult. The size of the population of Sertoli cells at the end of the perinatal period determines the final testicular size and the daily sperm production in sexually mature animals (França et al 2000, Orth et al 1988). Treatment of rats during the critical period of testis development PND 1-20 can change the proliferation of Sertoli cells and ultimately sperm production in the adult. For example, neonatal hypothyroidism causes delayed Sertoli cell maturation and impaired germ cell development in rats treated with propylthiouracil (de França et al 1995; Simorangkiret al 1997). In contrast, neonatal treatment with naloxone increased the number of Sertoli cells and sperm production in the adult (da Silva et al 2006). Since FXa-dependent signaling can promote cell proliferation (Marfarlane et al. 2001, Borensztajn et al 2008), signaling via FXa may be important during Sertoli cell proliferation in the neonatal period or during an alternative stage in testicular development.

Given the uncertainty of the therapeutic dose and the unbound concentration of apixaban in children of any age, an exposure comparison can only be made for the

adolescent/young adult animal. Comparisons based on the unbound $AUC_{(0\text{-}24h)}$ at the NOAEL dose are 3.4 fold for all parameters in females. However, comparison in the males are 2.4-fold for F1 postnatal development, F1 mating/fertility, and F2 embryo development and 1.6-fold for F1 male toxicity based on the testicular degeneration. It is important to reiterate that the NOAEL for F1 mating/fertility of 600 mg/kg is based on the results in the separate set of recovery animals who were no longer being dosed at the time of mating.

The current draft label notes that the (b) (4) The reviewer notes that in pigmented rats the mean concentration-time profile for apixaban-equivalents in the eye was generally similar to the profile for plasma with a Tmax at 4 hours post dose. However, exposure was still present in the eyes of pigmented rats at 168 hours after dosing at a level that was higher than that in the liver. The eyes had one of the longest tissue half-life for apixaban of 64 hours. The total AUC in the eye of 51 µg equivalents*hr/gm was higher than that in plasma (37.7 µg equivalents*hr/gm) and similar to that in the kidney (53 µg equivalents*hr/gm). The finding that exposure was similar in pigmented and non-pigmented skin suggests that the retention of apixaban in the eye is not related to melanin binding.

12 Appendix/Attachments

Appendix 1: NDA 202155 – Study Reports Submitted

Appendix 2: Executive CAC Meeting Minutes - November 15, 2005

Appendix 3: Executive CAC Meeting Minutes – November 29, 2011

Appendix 4: Sponsor's Summary of Tumors in Male Mice – Study DN05068

Appendix 5: Sponsor's Summary of Tumors in Female Mice – Study DN05068

Appendix 6: Sponsor's Summary of Mouse Historical Control Data - Study DN05068

Appendix 7: Sponsor's Summary of Tumors in Male Rats - Study DN05069

Appendix 8: Sponsor's Summary of Tumors in Female Rats - Study DN05069

Appendix 9: Sponsor's Summary of Historical Control Data in Rats - Study DN05069

Appendix 10: References

Appendix 1: NDA 202155 – Study Reports Submitted

(Shading indicates lack of previous review)

Document Number	Study Title (Study Report Number)	If reviewed before	
		Reviewer	Date
Pharmacology			
930015740	In Vitro Assessment of Enzyme Inhibitory Activity of BMS-730823, the M1 Metabolite of BMS-562247	RH PH	3/09 3/07
930028738	Antithrombotic And Antihemostatic Effects in Rabbits	DJ*	7/05
930028740	**Apixaban Inhibits Human Clot-Bound Factor Xa Activity in Vitro**		
930028741	**Effects of Apixaban on Tissue-Factor Induced Human Platelet Aggregation In Vitro**		
930028744	Apixaban: Affinity for Proteolytic Enzymes In Vitro	DJ*	7/05
930028745	Apixaban: Mechanism of Inhibition of Factor Xa, Prothrombinase and Development of A Predictive Model for Inhibition of Measured Thrombin Generation	DJ*	7/05
930028747	Effects of BMS-562247 in Standard Coagulation Assays in Human Plasma	DJ*	7/05
930028749	Antithrombotic and Antihemostatic Effects in Dogs	DJ*	7/05
930028752	Effects of Apixaban in Rat Models of Thrombosis and Hemostasis	DJ*	7/05
930028753	The Effects of Factor Xa Inhibitor, Apixaban, on Elevated Thrombotic Biomarkers in Diabetic/Obese Mice		
930037330	Apixaban: Affinity for Factor Xa from Laboratory Animal Species	DJ*	7/05
Safety Pharmacology			
930002567	BMS-562247: Effects on HERG/IKr Currents and Rabbit Purkinje Fiber Action Potentials	DJ	7/05
930036572	Effect of Apixaban (BMS-562247) on Action Potentials in Isolated Rabbit Cardiac Purkinje Fibers (DS08161)	RH	8/09
930036574	Effects of Apixaban (BMS-562247) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (DS08162)	RH	8/09
930015526	BMS-730823 (M1 Metabolite of BMS-562247): Effects on hERG/IKr Potassium Channels and Rabbit Purkinje Fiber Action Potentials (DT05166)	RH	3/09
930010808	Single-Dose Intravenous Cardiovascular Telemetry Study in Dogs (DS04260)	PH RH	09/05 3/10
930002541	Acute Effects of Intravenous Administration On Hemodynamics in Anesthetized Dogs (T01-10-52)	DJ	7/05
930010669	BMS-562247: Exploratory Oral Cardiovascular Telemetry Study in Dogs (T02-1-4)	DJ	7/05
930002531	BMS-562247: Novascreen® Receptor Binding Profile (T01-12-3)	DJ	7/05
Pharmacodynamic Drug Interactions			
930028739	**Arterial Antithrombotic and Bleeding Time Effects of Apixaban in Combination with Antiplatelet Therapy in Rabbits**		
Pharmacokinetics/ADME			
Analytical Assay			
930008990	Method Validation Report for The Determination of BMS-562247 in Rabbit Plasma (K_2EDTA) Using LC-API/MS/MS (ALTCA02A)	PH	3/07

Document Number	Study Title (Study Report Number)	If reviewed before	
		Reviewer	Date
930033949	**Method Validation Report for the Determination of BMS-562247 in Low Sample Volume Rat Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M)**	PH RH	9/05 3/10
930008022	Method Validation Report for The Determination of BMS-562247 in Rat Plasma (K_2EDTA) Using LC-API/MS/MS (AR562B)	PH RH	9/05 3/10
930011627	Method Validation Report for The Determination of BMS-562247 in Dog Plasma (K_2EDTA) Using LC-API/MS/MS (AR562C)	PH RH	9/05 3/10
930008644	Method Validation Report for The Determination of BMS-562247 in Rat Fetal Embryo Extract Using LC-API/MS/MS (AR562D)	PH	3/07
930012207	**Method Validation Report for The Determination of BMS-562247 in Mouse Plasma (K_2EDTA) Using LC-API/MS/MS (AR562F)**		
930015560	**Method Validation Report for The Determination of BMS-562247 in Mouse Fetal Embryo Extract Using LC-API/MS/MS (AR562H)**		
930013967	**Method Validation Report for The Determination of BMS-730823 in Rat Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M3)**		
930014148	**Method Validation Report for The Determination of BMS-730823 in Dog Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M4)**		
930014144	**Method Validation Report for The Determination of BMS-730823 in Mouse Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M5)**		
930013970	**Method Validation Report for The Determination of BMS-730823 in Rabbit Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M6)**		
930013971	**Method Validation Report for The Determination of BMS-730823 in Monkey Plasma (K_2EDTA) Using LC-API/MS/MS (AR562M7)**		
930002436	Quantitative Determination of BMS-562247 in Dog Plasma (K_3EDTA) Using LC/MS/MS (MAP003/562247)	PH RH	9/05 3/10
930002437	Quantitative Determination of BMS-562247 in Rat Plasma (K_2EDTA) Using LC/MS/MS (MAP004/562247)	PH RH	9/05 3/10
930002493	Nonclinical Pharmacokinetics and Metabolism of Factor Xa Inhibitor BMS-56224 (00102)	DJ	7/05
930017758	Assessment of PgP Mediated Transport of BMS-562247 in LLC-PK_1 Cell Monolayers (300797734)	DJ*	7/05
930037388	Evaluation of Apixaban as a Potential Substrate of Uptake Transporters	RH	3/10
930037717	Evaluation of Apixaban in the Caco-2 Permeability Assays Plus/Minus Co-incubation with Naproxen	RH	3/10
930002419	BMS-562247: Absorption, Distribution, Metabolism and Excretion (ADME) Summary (BMS-562247-map-summary)	DJ	7/05
930006278	BMS-562247: Single-Dose Oral Investigative Toxicokinetic Study in Dogs (DN03010)	DJ	7/05
930037784	Bidirectional Transport (Papp) and Inhibition Studies of BMS-562247 on MDCKII and MDCKII-BCRP Monolayers (b) (4) 76-BMS-02-30Sept2008)	RH	3/10
930011880	In Vitro Determination of Serum Protein Binding of BMS-562247 in Mouse	PH	3/07
930037000	Radioactive Peak Profile and Identification in Milk of Rats Following Oral Administration of [^{14}C]Apixaban (BMS-562247)	PH RH	9/05 3/10
930005742	Tissue Distribution of Radioactivity in Male Long-Evans Rats Following Oral Administration of [^{14}C]BMS-562247 and Dosimetry Calculation (DDBS011/562247)	PH RH	9/05 3/10
930009803	BMS-562247: Oral Study of Embryo-Fetal Development in Rats – Toxicokinetics (DN03042)	PH	9/05
930011664	BMS-562247: Oral Study of Embryo-Fetal Development in Rabbits – Toxicokinetics (DN03045)	RH	3/09
930016662	**BMS-562247: Intravenous Study of Embryo-Fetal Development in Rabbits – Toxicokinetics (DN05050)**		
930016586	Ten-Day Oral Range-Finding Study in Pregnant Mice (DN06004)	RH	3/09

Document Number	Study Title (Study Report Number)	If reviewed before	
		Reviewer	Date
930036905	BMS-562247: Quantitative Tissue Distribution of Drug- Related Material Using Whole-Body Autoradiography Following a Single Oral Dose of [^{14}C]BMS-562247 (5 mg/kg) to Sprague Dawley Rats (QPS No. 20N-0807)	RH	3/10
930010961	Disposition of [^{14}C]BMS-562247 after Intravenous Administration to Rats	RH	3/10
930013242	Biotransformation of [^{14}C] BMS-562247 in Aroclor-Induced Rat Liver S9 Fraction	PH	3/07
930014907	Biotransformation of [^{14}C]Apixaban after Intravenous and Oral Administration to Female New Zealand White Rabbits	PH	3/07
930015043	Comparison Of The O-Desmethyl Apixaban Sulfotransferase Activities in Liver S9 of Different Species and Identification of Human Sulfotransferases Mediating Sulfation of O-Desmethyl Apixaban	PH	3/07
930015646	Biotransformation of [^{14}C]Apixaban after Oral Administration to Male CD-1 Mice	PH	3/07
930037129	Identification of Major Human P450 Enzymes Involved in Metabolism of Apixaban (BMS-562247)	DJ RH	7/05 3/10
930006776	Comparative In Vitro Metabolism of [^{14}C]BMS-562247 in Hepatocytes from Mouse, Rat, Dog, Monkey, and Human (DDBS012/562247)	RH	3/10
930007004	Biotransformation of [^{14}C]BMS-562247 in Rats (DDBS016/ 562247)	RH	310
930007005	Biotransformation of [^{14}C]BMS-562247 in Dogs (DDBS017/ 562247)	RH	3/10
930014442	**Mass Balance of Radioactivity after Oral Administration of [^{14}C]BMS-562247 to Male CD-1 Mice (MBA00221)**		
930017359	**Mass Balance of Radioactivity After Intravenous and Oral Administration of [^{14}C]BMS-562247 to Female New Zealand White Rabbits (MBA00222)**		
930037205	**Assessment of Inhibition of Digoxin Efflux in LLC-PK$_1$ Cell Monolayers (300963740)**		
930024178	Evaluation of The Inhibitory Effects of BMS-562247 on the Activity Of Cytochrome P450 Enzymes CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 And CYP3A4 in Human Liver Microsomes	DJ*	7/05
930037853	**Evaluation of Common NSAIDs' Ability to Inhibit P-gp Efflux.**		
930024170	**In Vitro Evaluation Of BMS-562247 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes (b) (4) 063004)**		
930039361	**Effects of Activated Charcoal Administration on Pharmacokinetics of Apixaban (BMS-562247) Following Oral Administration in Male Dogs**		
930006278	Absorption Study in Dogs Using Multiple Particle Sizes of BMS-562247 (DN03010)	DJ	7/05
Toxicology			
Single Dose Toxicology Studies			
930002397	Single-Dose Oral Toxicity Study in Mice (DN02041)	DJ	7/05
930002410	Single-Dose Oral Toxicity Study in Rats (DN02042)	DJ	7/05
930011162	Single-Dose Intravenous Toxicity Study in Mice (DN04095)	PH	9/05
930011032	Single-Dose Intravenous Toxicity Study in Rats (DN04096	PH	9/05
930015741	BMS-730823 (M1 Metabolite of BMS-562247): Single-Dose Oral Toxicokinetics and Tolerability Study in Monkeys (DN05062)	RH	3/09
930016135	Single-Dose Oral Toxicity Study in Dogs (DN06026)	RH	3/09
Repeated Dose Toxicology Studies			
930001603	Two-Week Oral Exploratory Toxicity Study in Rats (DN02004)	DJ	7/05
930009629	Two-Week Oral Exploratory Toxicity Study in Rats (DN03087)	DJ	7/05
930010865	Two-Week Intravenous Toxicity Study in Rats (DN04097)	PH	9/05
930005268	Three-Month Oral Toxicity Study in Rats (DN02043)	DJ	7/05
930012967	**BMS-562247: Six-Month Oral Toxicity Study in Rats (DN03118)**	**PH†**	**12/05**
930006683	Two-Week Oral Exploratory Toxicity Study in Dogs (DN03088)	DJ	7/05

Document Number	Study Title (Study Report Number)	If reviewed before	
		Reviewer	Date
930001671	DPC-Ag0023-00: Two Week Oral Gavage Toxicity And Toxicokinetic Study in Beagle Dogs (T0l-10-9)	DJ	7/05
930010669	Two-Week Intravenous Toxicity Study in Dogs (DN04098)	PH	9/05
930004221	Three-Month Oral Toxicity Study in Dogs (DN02040)	DJ	7/05
	Three-Month Oral Toxicity Study in Dogs, Amendment	PH	3/07
930012966	BMS-562247: One-Year Oral Toxicity Study in Dog (DN03117)	RH	3/09
Genetic Toxicology Studies			
920016149	Exploratory Ames Reverse-Mutation Study in Salmonella (DS02019)	DJ	7/05
930002536	Ames Reverse-Mutation Study in Salmonella and Escherichia Coli (DS02121)	DJ	7/05
930006384	BMS-562247: Cytogenetics Study in Chinese Hamster Ovary Cells (DS03194)	DJ PH	7/05 9/05
930015541	BMS-562247: Cytogenetics Study in Chinese Hamster Ovary Cells (DS05177)	RH	3/09
930002539	Oral Micronucleus Study in Male Rats (DS02117)	DJ	7/05
930015561	One-Month Oral In Vivo/In Vitro Cytogenetics Study In Rat Peripheral Blood Lymphocytes (DS05163)	RH	3/09
Carcinogenicity			
930031442	**BMS-562247: 104-Week Dietary Carcinogenicity Study in Mice (DN05068)**		
930031443	**BMS-562247: 104-Week Dietary Carcinogenicity Study in Rats (DN05069)**		
930031438	Two-Week Dietary and Gavage Exploratory Range Finding Study in Mice (DN04059)	PH	12/05
930031453	A 2-Week Dietary Palatability and Toxicokinetic Study in Rats (DN04087)	PH	12/05
930031440	Three-Month Dietary Dose Range-Finding Toxicity Study in Mice (DN04099)	PH	12/05
930031531	Three-Month Dietary Dose Range-Finding Toxicity Study in Rats (DN04100)	PH	12/05
Reproductive and Developmental Toxicology Studies			
930016567	BMS-562247: Oral Study of Fertility and Early Embryonic Development in Rats (DN05056)	RH	3/09
930004671	BMS-562247: Ten-Day Oral Range-Finding Study in Pregnant Rats (DN03013)	DJ	7/05
930004672	BMS-562247: Thirteen-Day Oral Range-Finding Study in Pregnant Rabbits (DN03014)	DJ	7/05
930009803	BMS-562247: Oral Study of Embryo-Fetal Development in Rats (DN03042)	PH	9/05
930011664	BMS-562247: Oral Study of Embryo-Fetal Development in Rabbits (DN03045)	RH	3/09
930015215	**BMS-562247: Thirteen-Day Intravenous Range-Finding Study in Pregnant Rabbits (DN05006)**		
930016662	**BMS-562247: Intravenous Study of Embryo-Fetal Development in Rabbits (DN05050)**		
930016586	Ten-Day Oral Range-Finding Study in Pregnant Mice Study (DN06004)	RH	3/09
930021450	Apixaban (BMS-562247): Oral Study of Embryo-Fetal Development in Mice (DN06023)	RH	2/09
930036291	Exploratory Oral Range-Finding Peripostnatal Study in Rats (DN07035)	RH	8/09
930036745	Apixaban (BMS-562247): Oral Study of Pre- and Postnatal Development in Rats (DN08001)	RH	3//09
930035423	Oral Tolerability and Toxicokinetic Study in Juvenile Rats (DN08064)	RH	10/09

Document Number	Study Title (Study Report Number)	If reviewed before	
		Reviewer	Date
Other Toxicology Studies			
930010276	BMS-562247: Hemolytic Potential Results (45335)	PH	9/05
930006278	Single-Dose Oral Investigative Toxicokinetic Study in Dogs (DN03010)	DJ	7/05
930015723	BMS-730823 (M1 Metabolite of BMS-562247: One-Week Oral Toxicokinetics Study in Rats (DN05011)	RH	3/09
930015739	BMS-730823 (M1 Metabolite of BMS-562247: One-Week Oral Toxicokinetic Study in Dogs (DN05012)	RH	3/09
930021524	Neutral Red Uptake Phototoxicity Assay of Apixaban (BMS-562247) in Balb/C 3T3 Mouse Fibroblasts (DN06050)	RH	2/09

* Review of study summary only, [T] Partial review
Reviewers: DJ - David Joseph, RH - Ronald Honchel, PH - Patricia Harlow

Appendix 2: Executive CAC Meeting Minutes - November 15, 2005

Executive CAC
Date of Meeting: November 15, 2005

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D., OND IO, Member
Josie Yang, DVM, Ph.D., DAARP, Alternate Member
Albert Defelice, Ph.D., DCRP, Team Leader

Presenting Reviewer and Author of Draft Minutes: Patricia Harlow, Ph.D., DCRP

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 met to consider the adequacy of the protocols for two-year carcinogenicity bioassays in rats and mice. The Committee did not address the sponsor's proposed statistical evaluation for the two-year 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.'

(b) (4)

Drug Name: BMS-562247 (Apixaban)
Sponsor: Bristol-Myers Squibb Company

Background

BMS-562247 is a direct inhibitor of the coagulation Factor Xa. As a result, it decreases the conversion of prothrombin to proteolytically active thrombin thereby decreasing thrombin-mediated activation of both coagulation and platelet function. Addition of BMS-562247 to normal human plasma causes concentration-dependent prolongations in standard coagulation assays.

(b) (4)

Mouse Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed a 104-week study using 60 CD-1 mice/sex/group with two dietary control groups. Based on saturation of the exposure to BMS-562247 in 2-week and 13-week dietary toxicology studies, the sponsor proposed maximum daily doses of 1500 and 3000 mg/kg administered through an *ad lib* diet to males and females, respectively. The low and mid-doses proposed are 150 and 500 mg/kg for both sexes. The maximum proposed doses to male and female mice resulted in total AUC values that are 2.2 and 4.2 times, respectively, the human AUC for a dose of 10 mg BID. Taking into account the higher plasma protein binding in humans, the maximum proposed doses to male and female mice resulted in unbound AUC values that are 8.3 and 16.9 times, respectively, the human AUC.

In a 13-week toxicology study using dietary administration, CD-1 mice were dosed with 0, 1500, 3000, 4500 and 6000 mg/kg. In a 2-week dose range-finding toxicology study, CD-1 mice were dosed with 0, 300, 600 and 1500 mg/kg using dietary administration. No dose-limiting toxicity was observed in either study.

The form of BMS-562247 used in the toxicology studies was not clear. The draft protocol did not specify the form of BMS-562247 that will be used.

The sponsor also proposed separate satellite groups (25 animals/sex/dose group) for monitoring blood concentrations of BMS-562247 at 1, 2, 4, 8 and 24 hours after the start of the dark cycle during week 26.

The protocol indicates that histopathological evaluation of all tissues on the protocol list will be performed for all animals in the main study groups. All macroscopic abnormalities will be evaluated microscopically.

Rat Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed a 104-week study using 60 Sprague Dawley rats/sex/group with two dietary control groups. Based on saturation of the exposure to BMS-562247 in 2- and 13-week dietary toxicology studies, the sponsor proposed daily doses of 0, 50, 200 and 600 mg/kg administered through an *ad lib* diet. The maximum proposed doses to male and female rats resulted in total AUC values that are 5.2 and 8.3 times, respectively, the human AUC for a dose of 10 mg BID. Taking into account the higher plasma protein binding in rats, the maximum proposed doses to male and female rats resulted in unbound AUC values that are 1.6 and 2.5 times, respectively, the human AUC.

In a 13-week dietary toxicology study, Sprague Dawley rats were dosed with 0, 600, 1800, and 2400 mg/kg. Body weight gain in males receiving 1800 and 2400 mg/kg was decreased 9.9 and 8.2%, respectively. In a 2-week dietary palatability and TK study, Sprague Dawley rats were dosed with 200, 600 and 1500 mg/kg.

The form of BMS-562247 used in the toxicology studies was not clear. The draft protocol did not specify the form of BMS-562247 that will be used.

The sponsor also proposed monitoring blood concentrations of BMS-562247 and BMS-730823 (O-demethyl-BMS-562247-O-sulfate, the M1 metabolite) in the main study animals (4 rats/sex/group/timepoint) at 1, 2, 4, 8, and 24 hours after the start of the dark cycle during week 26.

Plasma concentration of the M1 metabolite in humans represents >20% of the parent. This metabolite represented at most 1.3 and 0.6% that of the parent in the plasma of male and female rats, respectively. The maximum proposed doses to male and female rats resulted in total AUC values for BMS-730823 that are 0.2 and 0.1 times, respectively, the human AUC for BMS-730823 with a dose of 10 mg BID.

The protocol indicates that histopathological evaluation of all tissues on the protocol list will be performed for all animals in the main study groups. All macroscopic abnormalities will be evaluated microscopically.

Executive CAC Recommendations and Conclusions:

Mouse:

* The Committee concurred with the sponsor's proposed doses of 0, 150, 500, and 1500 mg/kg/day in diet (*ad lib* feeding) in males and 0, 150, 500, and 3000 mg/kg/day by diet (ad lib feeding) in females based on saturation of absorption with the qualification that the same form of BMS-562247 be used in the cancer bioassay as was used in the 13-week toxicology studies. This recommendation is conditional on submission of a final report, including a quality assurance statement without substantive data changes.

* The proposed TK study is not necessary.

Rat:

* The Committee concurred with the sponsor's proposed doses of 0, 50, 200, and 600 mg/kg/day by diet (*ad lib* feeding) based on saturation of absorption with the qualification that the same form of BMS-562247 be used in the cancer bioassay as was used in the 13-week toxicology studies. This recommendation is conditional on submission of a final report, including a quality assurance statement, without substantive data changes.

* Main study animals should not be bled for TK data. If TK data is needed, it must be collected only from satellite animals.

* The Committee noted that the M1 metabolite is present in human and rat plasma, but not mouse plasma. However, the immediate precursor to the M1 metabolite, M2, was produced in cultures of hepatocytes from mice, rats and humans. Since the M1 metabolite is a phenolic sulfate conjugate, it is more stable thermodynamically than benzylic and aniline sulfate esters and is considered less likely to form a reactive electrophile. Both M1 and M2 are more polar compounds that are expected to be eliminated more rapidly than the parent.

* If the survival of any dose group approaches 20 animals during the study, the sponsor should contact the Division prior to termination of dose groups or stoppage/reduction of dosing. The Committee noted that the sponsor should NOT terminate any dose groups prior to the scheduled study termination or change any dosing without first contacting the Agency.

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

cc:\
/Division File, CDRP
/A. Defelice, Team leader, DCRP
/A. Laniyonu, Team leader, DMIHP
/J. Choudary, Team Leader, DGP
/P. Harlow, Reviewer, DCRP
/M. Pease-Fye, CSO/PM, DCRP
/A. Seifried, OND IO

Appendix 3: Executive CAC Meeting Minutes – November 29, 2011

Executive CAC
Date of Meeting: November 29, 2011

Committee: David Jacobson-Kram, Ph.D., OND-IO, Chair
Abigail Jacobs, Ph.D., OND-IO, Member
Paul Brown, Ph.D., OND-IO, Member
Dan Mellon, Ph.D., DAAAP, Alternate Member
Thomas Papoian, Ph.D., DCRP, Supervisor
Patricia Harlow, Ph.D., DCRP, Reviewer

Presenting Reviewer: Patricia Harlow, Ph.D.
Author of Draft: Patricia Harlow, Ph.D.

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

NDA: 202-155
Drug Name: Apixaban (BMS 562247)
Sponsor: Bristol Myers Squibb Company and Pfizer

Background:
Apixaban is a direct Factor Xa inhibitor. In the Phase 3 trial for prevention of stroke in patients with non-valvular atrial fibrillation, the daily apixaban dose was 10 mg (5 mg BID). Although the cancer bioassays used multiple apixaban lots that differed in particle size distribution and process used for synthesis, the apixaban in all these lots was (b) (4) apixaban.

Rat Carcinogenicity Study:
Sprague Dawley rats (60/sex/group) received daily oral doses of 0, 50, 200, and 600 mg/kg/day of apixaban administered through the diet to males for 104 weeks and to females for 97-100 weeks. The total exposures to apixaban in the high dose males and females were 5.4 and 9.4 fold, respectively, the mean total exposure in patients receiving the recommended human dose (RHD) of 5 mg BID. However, the exposures to unbound apixaban in male and females rats were 1.6 and 3.4 fold the exposure to unbound drug in patients receiving the RHD.

No significant treatment-related effects were observed on mortality, bodyweight gain, and food consumption. However, the mean body weight and body weight gain decreased up to 10% and 15%, respectively, in the high dose male group compared to the control group from Weeks 60 to 104. Some statistically significant non-neoplastic findings, such as increased extramedullary hematopoiesis, increased pigment, and decreased thrombosis are consistent with the pharmacodynamic effect of apixaban as a Factor Xa inhibitor.

The incidences of malignant lymphoma, a common tumor, displayed a tendency to

increase with dosage in both male and female rats. However, in neither sex was either the trend or the pairwise comparison between the concurrent control and the high dose animals found to be statistically significant by CDER criteria.

Mouse Carcinogenicity Study:
CD-1 mice (60/sex/group) received daily oral doses of 0, 10, 20, and 60 mg/kg/day of apixaban administered through the diet for 104 weeks. The total exposures to apixaban in the high dose males and females were 2.4 and 5.4 fold, respectively, the mean total exposure in patients receiving the RHD. However, the exposures to unbound apixaban were 8.8 and 20.1 fold the mean exposure to unbound drug in patients receiving the RHD.

No significant treatment-related effects was observed on mortality, bodyweight gain or food consumption. However, the high dose female group gained approximately 10% less bodyweight than the control group from Week 6 through 76. Although the incidences of convulsions, reported as being similar to the laboratory historical incidence, were not related to apixaban treatment, the high incidence in untreated control mice, particularly in males, was considered unusual in this study. The non-neoplastic findings of extramedullary hematopoiesis in the liver in male mice and hemorrhage in the thymus of female mice are consistent with the pharmacodynamic effect of apixaban as a factor Xa inhibitor.

The incidences of a few tumors increased in the higher dose groups compared to those in the control groups. The incidence of Schwannoma (nerve sheath tumor) was numerically increased in the mandibular salivary gland of high dose males; however, the p value (0.027) for this tumor in the trend test did not attain the significance level of pt < 0.025 required for a rare tumor to be considered positive. The incidence of the combination of hemangiomas and hemangiosarcomas (pt = 0.0487), uterine/cervical glandular polyps alone (pt = 0.0230), and the combination of uterine/cervical glandular polyps and adenocarcinomas (pt = 0.0081) were numerically increased in high-dose females. However, none of these tumors had a p-value that attained the significance level of p < 0.005 required for a common tumor to be considered positive according to CDER statistical criteria. Therefore, no statistically significant neoplastic finding was related to apixaban treatment under the conditions of this study.

Executive CAC Recommendations and Conclusions:

Rat:
The Committee concurred that the study was adequate, noting prior Exec CAC concurrence with the protocol.

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

Mouse:
The Committee concurred that the study was adequate, noting prior Exec CAC concurrence with the protocol.

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

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

cc:\
/Division File, DCRP
/T. Papoian, Supervisor, DCRP
/P. Harlow, Reviewer, DCRP
/A. Blaus, CSO/PM, DCRP
/A.Seifried, OND-IO

Appendix 4: Sponsor's Summary of Tumors in Male Mice – Study DN05068

Table 4: Male Mice: Number of Tumor Bearing Animals / Number of Animals Evaluated, and Peto-Pike Trend Test P-Values.

		DOSE				
ORGAN/TISSUE	NEOPLASM	0	0	150	500	1500
Adrenal Glands	Adenoma, Cortical	0/58	2/60	3/59	0/59	1/59 0.6083
Adrenal Glands	Adenoma, Subcapsular Cell	0/58	2/60	3/59	3/59	2/59 0.2734
Brain	Oligodendroglioma	0/60	0/60	0/60	1/60	0/60 0.4237
Epididymides	Schwannoma	0/60	0/60	0/60	1/60	0/60 0.4257
Gallbladder	Adenoma	0/58	0/56	1/60	0/57	1/56 0.1765
Harderian Glands	Adenocarcinoma	0/60	1/60	0/60	1/60	0/59 0.6106
Harderian Glands	Adenoma	1/60	3/60	2/60	6/60	1/59 0.5341
Kidneys	Neoplasm, Nos	0/60	0/60	0/60	0/60	1/60 0.1484
Kidneys	Papilloma, Transitional Cell	0/60	0/60	0/60	0/60	1/60 0.1683

		DOSE				
ORGAN/TISSUE	NEOPLASM	0	0	150	500	1500
Liver	Fibrosarcoma	0/60	1/60	0/60	0/60	0/60 1.0000
Liver	Adenoma, Hepatocellular	8/60	7/60	12/60	12/60	7/60 0.4341
Liver	Carcinoma, Hepatocellular	3/60	1/60	3/60	1/60	1/60 0.7257
Lung	Adenoma, Bronchiolar Alveolar	10/60	7/60	8/60	8/60	7/60 0.5094
Lung	Carcinoma, Bronchiolar Alveolar	2/60	5/60	8/60	3/60	6/60 0.1823
Multicentric Neoplasm	Leukemia, Granulocytic	1/60	0/60	0/60	0/60	0/60 1.0000
Multicentric Neoplasm	Hemangioma	0/60	1/60	1/60	0/60	2/60 0.1075
Multicentric Neoplasm	Hemangiosarcoma	4/60	3/60	1/60	2/60	3/60 0.4364
Multicentric Neoplasm	Hemangioma or Hemangiosarcoma	4/60	4/60	2/60	2/60	5/60 0.1961
Multicentric Neoplasm	Sarcoma, Histiocytic	1/60	1/60	0/60	1/60	0/60 0.7426

		DOSE				
ORGAN/TISSUE	**NEOPLASM**	**0**	**0**	**150**	**500**	**1500**
Multicentric Neoplasm	Lymphoma	5/60	5/60	0/60	3/60	1/60 0.9113
Multicentric Neoplasm	Mast Cell Tumor	0/60	1/60	0/60	1/60	0/60 0.6105
Parathyroid Glands	Adenoma	0/36	0/36	0/39	1/37	0/35 0.4583
Pituitary Gland	Adenoma, Pars Distalis	0/58	2/58	0/57	1/59	1/57 0.3207
Pituitary Gland	Carcinoma, Pars Intermedia	0/58	0/58	0/57	1/59	0/57 0.3933
Salivary Gland, Mandibular	Schwannoma	0/60	0/60	0/60	0/60	2/60 0.0269
Small Intestine, Duodenum	Sarcoma, Undifferentiated	1/60	0/59	0/60	0/60	0/59 1.0000
Testes	Adenoma, Interstitial Cell	2/60	0/60	1/60	1/60	3/60 0.0525
Testes	Adenoma, Rete Testis	1/60	0/60	0/60	0/60	1/60 0.3097
Thyroid Gland	Carcinoma, C-Cell	2/60	0/59	0/60	0/60	0/59 1.0000
Tongue	Carcinoma, Squamous Cell	0/60	1/60	0/60	0/60	0/60 1.0000
Urinary Bladder	Mesenchymal Tumor	0/60	0/60	0/60	1/60	0/60 0.5676

Appendix 5: Sponsor's Summary of Tumors in Female Mice – Study DN05068

Table 5: Female Mice: Number of Tumor Bearing Animals / Number of Animals Examined, and Peto-Pike Trend Test P-Values

		DOSE				
ORGAN/TISSUE	**NEOPLASM**	**0**	**0**	**150**	**500**	**3000**
Adrenal Glands	Adenoma, Subcapsular Cell	0/59	0/60	1/60	0/60 0.5424	0/59 0.6351
Adrenal Glands	Pheochromocytoma	1/59	0/60	1/60	0/60 0.7885	1/59 0.3187
Gallbladder	Adenoma	0/58	0/59	0/60	0/60 1.0000	1/59 0.1471
Harderian Glands	Adenocarcinoma	1/60	0/60	1/60	0/60 0.7984	0/60 0.8287
Harderian Glands	Adenoma	1/60	0/60	2/60	3/60 0.1302	2/60 0.2121
Kidneys	Carcinoma, Tubular Cell	1/60	0/60	0/60	0/60 1.0000	0/60 1.0000
Liver	Adenoma, Hepatocellular	3/60	1/60	1/60	4/60 0.1366	1/60 0.6269
Liver	Osteosarcoma	0/60	1/60	0/60	0/60 1.0000	0/60 1.0000
Lung	Adenoma, Bronchiolar Alveolar	5/60	7/60	9/60	7/60 0.4831	7/60 0.4898
		DOSE				
ORGAN/TISSUE	**NEOPLASM**	**0**	**0**	**150**	**500**	**3000**
Lung	Carcinoma, Bronchiolar Alveolar	6/60	5/60	3/60	3/60 0.8618	0/60 0.9757
Mammary Gland	Adenocarcinoma	3/60	1/60	1/60	1/60 0.8174	0/60 0.9463
Multicentric Neoplasm	Hemangioma	3/60	3/60	1/60	0/60 0.9922	3/60 0.2814
Multicentric Neoplasm	Hemangiosarcoma	2/60	1/60	2/60	4/60 0.0990	4/60 0.0623
Multicentric Neoplasm	Hemangioma or Hemangiosarcoma	5/60	4/60	3/60	4/60 0.5800	7/60 0.0487
Multicentric Neoplasm	Sarcoma, Histiocytic	3/60	2/60	2/60	1/60 0.8468	0/60 0.9665
Multicentric Neoplasm	Lymphoma	9/60	6/60	10/60	13/60 0.1020	10/60 0.1566
Ovaries	Adenoma, Tubulostromal	2/60	0/60	0/60	0/60 1.0000	0/60 1.0000
Ovaries	Cystadenocarcinoma	0/60	1/60	0/60	0/60 1.0000	0/60 1.0000
Ovaries	Cystadenoma	0/60	0/60	0/60	0/60 1.0000	1/60 0.1456

		DOSE				
ORGAN/TISSUE	**NEOPLASM**	**0**	**0**	**150**	**500**	**3000**
Ovaries	Sex-Cord/Stromal Tumor	1/60	1/60	0/60	2/60 0.2576	1/60 0.3020
Pancreas	Adenoma, Islet Cell	0/60	1/60	0/60	0/60 1.0000	0/60 1.0000
Pituitary Gland	Adenoma, Pars Distalis	3/59	3/60	0/59	4/59 0.2319	1/57 0.6134
Skin, Subcutis	Carcinoma, Basal Cell	1/60	0/60	0/60	0/60 1.0000	0/60 1.0000
Stomach, Glandular	Adenoma	0/60	0/60	1/60	0/60 0.6190	0/60 0.7143
Stomach, Nonglandular	Carcinoma, Squamous Cell	0/60	0/60	1/60	0/60 0.4839	0/60 0.5722
Uterus with Cervix	Adenocarcinoma	0/60	0/60	0/60	1/60 0.2357	1/60 0.0939
Uterus with Cervix	Fibroma	2/60	0/60	0/60	0/60 1.0000	0/60 1.0000
Uterus with Cervix	Fibrous Histiocytoma	0/60	0/60	0/60	0/60 1.0000	1/60 0.2727
Uterus with Cervix	Leiomyoma	1/60	2/60	4/60	0/60 0.7590	3/60 0.2664
				DOSE		
ORGAN/TISSUE	**NEOPLASM**	**0**	**0**	**150**	**500**	**3000**
Uterus with Cervix	Leiomyosarcoma	2/60	2/60	2/60	2/60 0.4791	0/60 0.8887
Uterus with Cervix	Neuroendocrine Tumor	0/60	0/60	1/60	0/60 0.5424	0/60 0.6351
Uterus with Cervix	Endometrial Polyp, Stromal	0/60	1/60	3/60	1/60 0.2968	1/60 0.4309
Uterus with Cervix	Endometrial Polyp, Glandular	2/60	2/60	0/60	1/60 0.8621	5/60 0.0230
Uterus with Cervix	Endometrial Polyp, Glandular or Adenocarcinoma	2/60	2/60	0/60	2/60 0.6039	6/60 0.0081
Uterus with Cervix	Endometrial Polyp (Glandular or Stromal)	2/60	3/60	3/60	2/60 0.6345	6/60 0.0263
Uterus with Cervix	Sarcoma, Undifferentiated	0/60	0/60	1/60	0/60 0.4802	0/60 0.5741
Uterus with Cervix	Sarcoma, Stromal	0/60	2/60	0/60	2/60 0.1988	1/60 0.3108
Uterus with Cervix	Carcinoma, Squamous Cell	0/60	0/60	0/60	0/60 1.0000	1/60 0.2027
Vagina	Carcinoma, Squamous Cell	0/60	0/60	1/60	0/59 0.4907	0/60 0.5880

Appendix 6: Sponsor's Summary of Mouse Historical Control Data - Study DN05068

Historical Control Incidence of Uterine Endometrial Polyps and Adenocarcinoma in Female CD-1 Mice (b) (4) site of BMS-562247 mouse carcinogenicity study)[8]

Study Code	In-life Phase	Incidence (%)	
		Uterine endometrial polyp	Adenocarcinoma
A (C1)	2001-2003	5/70 (7.1)	0/70 (0)
A (C2)	2001-2003	3/70 (4.3)	1/70 (1.4)
B	2001-2003	8/60 (13.3)	0/60 (0)
C	2002-2004	1/50 (2.0)	0/50 (0)
D	2004-2006	4/60 (6.7)	2/60 (3.3)
E	2004-2006	7/60 (11.7)	1/60 (1.7)
F	2004-2006	2/60 (3.3)	1/60 (1.7)
G	2000-2002	3/37 (8.1)	0/37 (0)
H (C1)	2001-2003	6/65 (9.2)	1/65 (1.5)
H (C2)	2001-2003	13/65 (20.0)	1/65 (1.5)

Appendix 7: Sponsor's Summary of Tumors in Male Rats - Study DN05069

Table 4: Male Rats: Number of Tumor Bearing Animals / Number of Animals Examined, and Peto-Pike Trend Test P-Values

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
ADRENAL GLAND	Pheochromocytoma	2/60	7/60	12/60	5/60	4/60
						0.7447
ADRENAL GLAND	Adenoma: cortical cell	1/60	1/60	1/60	0/60	1/60
						0.5149
BRAIN	Oligodendroglioma	1/60	0/60	0/60	0/60	0/60
						1.0000
BRAIN	Reticulosis	1/60	0/60	2/60	0/60	0/60
						0.8499
BRAIN	Meningioma	0/60	0/60	0/60	0/60	1/60
						0.1845
BRAIN	Meningeal granular cell tumor	1/60	0/60	0/60	0/60	0/60
						1.0000
EPIDIDYMIS	Mesothelioma	1/60	0/60	1/60	0/60	0/60
						0.8488
HEART	Schwannoma: endocardial	0/60	1/60	0/60	0/60	0/60
						1.0000
HEART	Schwannoma: intramural	0/60	0/60	0/60	0/60	1/60
						0.1845
KIDNEY	Lipoma	1/60	0/60	1/60	0/60	0/60
						0.8202
LIVER	Adenoma: hepatocellular	2/60	2/60	2/60	1/60	0/60
						0.9439
LIVER	Carcinoma: hepatocellular	2/60	1/60	0/60	1/60	2/60
						0.2279
LIVER	Hepatocellular Adenomas & Carcinomas	4/60	3/60	2/60	2/60	2/60
						0.6910
LIVER	Hemangioma	1/60	0/60	1/60	0/60	0/60
						0.8440

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
MAMMARY GLAND	Adenoma	1/60	0/59	1/59	0/60	1/60
						0.3691
MAMMARY GLAND	Fibroadenoma	0/60	1/59	0/59	0/60	0/60
						1.0000
PANCREAS	Adenoma: islet cell	3/59	3/58	2/59	0/59	3/59
						0.3961
PANCREAS	Carcinoma: islet cell	2/59	2/58	1/59	2/59	2/59
						0.3843
PANCREAS	Islet cell Adenomas & Carcinomas	5/59	5/58	3/59	2/59	5/59
						0.3536
PANCREAS	Adenoma: acinar cell	0/59	0/58	0/59	0/59	1/59
						0.2712
PARATHYROID GLAND	Adenoma	0/52	1/50	0/43	0/53	2/57
						0.0921
PARATHYROID GLAND	Carcinoma	1/52	0/50	0/43	0/53	1/57
						0.3888
PARATHYROID GLAND	Adenomas & Carcinomas	1/52	1/50	0/43	0/53	2/57
						0.1770
PITUITARY GLAND	Adenoma: pars distalis	37/60	37/60	42/60	32/60	32/60
						0.7754

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
PITUITARY GLAND	Carcinoma: pars distalis	0/60	0/60	0/60	1/60	1/60
						0.1073
PITUITARY GLAND	Carcinoma: pars intermedia	1/60	0/60	0/60	0/60	0/60
						1.0000
PITUITARY GLAND	Adenomas & Carcinomas	38/60	37/60	42/60	33/60	33/60
						0.7290
SEMINAL VESICLE	Adenocarcinoma	0/60	1/60	0/60	0/60	0/60
						1.0000
SKIN/SUBCUTIS	Trichoepithelioma	2/60	1/60	1/60	0/60	0/60
						0.9782
SKIN/SUBCUTIS	Liposarcoma	1/60	0/60	0/60	0/60	0/60
						1.0000
SKIN/SUBCUTIS	Keratoacanthoma	2/60	3/60	1/60	0/60	1/60
						0.8154
SKIN/SUBCUTIS	Osteosarcoma: extraskeletal	0/60	1/60	1/60	0/60	0/60
						0.8394
SKIN/SUBCUTIS	Fibrosarcoma	1/60	0/60	1/60	2/60	0/60
						0.6302

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
SKIN/SUBCUTIS	Lipoma	1/60	0/60	1/60	0/60	1/60
						0.3661
SKIN/SUBCUTIS	Schwannoma	0/60	2/60	0/60	0/60	1/60
						0.5313
SKIN/SUBCUTIS	Fibroma	2/60	0/60	2/60	1/60	1/60
						0.5081
SKIN/SUBCUTIS	Adenoma: basal cell	1/60	0/60	0/60	0/60	0/60
						1.0000
SYSTEMIC NEOPLASMS	Lymphoma: malignant	1/60	0/60	1/60	0/60	3/60
						0.0370
SYSTEMIC NEOPLASMS	Hemangiosarcoma	3/60	3/60	4/60	2/60	1/60
						0.8730
SYSTEMIC NEOPLASMS	Histiocytic sarcoma	2/60	1/60	2/60	4/60	4/60
						0.0674
TESTIS	Adenoma: interstitial cell	2/60	0/60	1/60	2/60	3/60
						0.0701
THYROID GLAND	Adenoma: C-cell	9/60	6/58	8/60	3/59	9/60
						0.3614
THYROID GLAND	Carcinoma: C-cell	5/60	3/58	2/60	3/59	1/60
						0.8783

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
THYROID GLAND	C-cell Adenomas & Carcinomas	13/60	8/58	9/60	5/59	10/60
						0.5342
THYROID GLAND	Adenoma: follicular cell	0/60	1/58	3/60	1/59	0/60
						0.7706
THYROID GLAND	Carcinoma: follicular cell	0/60	0/58	2/60	1/59	0/60
						0.5348
ZYMBALS GLAND	Adenocarcinoma	1/59	0/55	2/59	1/54	0/58
						0.7561
ADIPOSE TISSUE[a]	Schwannoma	0/4	1/3	0/3	0/4	0/5
ADIPOSE TISSUE[a]	Hibernoma	0/4	0/3	0/3	1/4	1/5
EARS[a]	Papilloma: squamous cell	0/0	0/0	0/0	0/0	1/1

[a] Non-protocol specified tissue - statistical analysis not reported. Denominators represent the number of animals that received microscopic examination.

Appendix 8: Sponsor's Summary of Tumors in Female Rats - Study DN05069

Table 5: Female Rats: Number of Tumor Bearing Animals / Number of Animals Examined, and Peto-Pike Trend Test P-Values

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
ADRENAL GLAND	Pheochromocytoma	2/60	1/60	3/60	0/60	2/60
						0.4297
ADRENAL GLAND	Adenoma: cortical cell	1/60	2/60	1/60	3/60	0/60
						0.8263
ADRENAL GLAND	Carcinoma: cortical cell	0/60	1/60	0/60	0/60	0/60
						1.0000
ADRENAL GLAND	Pheochromocytoma: complex	0/60	0/60	0/60	0/60	1/60
						0.1892
BRAIN	Reticulosis	1/60	0/60	0/60	0/60	0/60
						1.0000
BRAIN	Astrocytoma	0/60	1/60	0/60	1/60	1/60
						0.2608
BRAIN	Granular cell tumor	0/60	0/60	0/60	0/60	1/60
						0.1892
BRAIN	Meningeal granular cell tumor	0/60	1/60	0/60	0/60	0/60
						1.0000
BRAIN	Granular & Meningeal granular cell tumors	0/60	1/60	0/60	0/60	1/60
						0.3329

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
CECUM	Leiomyoma	0/60	1/60	0/60	0/60	0/60
						1.0000
CERVIX	Granular cell tumor	0/60	3/59	0/60	0/60	1/60
						0.5689
CERVIX	Fibroma	1/60	0/59	0/60	0/60	0/60
						1.0000
CERVIX	Schwannoma	1/60	0/59	1/60	0/60	0/60
						0.8401
CERVIX	Carcinoma: squamous cell	0/60	0/59	0/60	0/60	1/60
						0.1772
CERVIX	Leiomyoma	0/60	1/59	0/60	0/60	0/60
						1.0000
CERVIX	Sarcoma: endometrial stromal	0/60	1/59	0/60	0/60	0/60
						1.0000
JEJUNUM	Adenocarcinoma	0/60	0/60	1/60	0/60	0/60
						0.5972
KIDNEY	Lipoma	1/60	0/60	0/60	0/60	0/60
						1.0000
KIDNEY	Sarcoma: anaplastic	1/60	0/60	0/60	0/60	0/60
						1.0000

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
LIVER	Adenoma: hepatocellular	1/60	2/60	0/60	1/60	0/60
						0.8766
MAMMARY GLAND	Adenoma	6/60	10/60	9/60	7/60	8/60
						0.4750
MAMMARY GLAND	Fibroadenoma	17/60	25/60	24/60	27/60	23/60
						0.4221
MAMMARY GLAND	Adenocarcinoma	9/60	5/60	5/60	11/60	9/60
						0.1391
MAMMARY GLAND	Adenomas & Fibroadenomas	21/60	31/60	25/60	30/60	26/60
						0.4842
MAMMARY GLAND	Adenomas, Fibroadenomas & Adenocarcinomas	26/60	31/60	27/60	32/60	29/60
						0.3474
OVARY	Granulosa cell tumor	2/60	0/60	0/60	0/60	0/60
						1.0000
OVARY	Fibrosarcoma	1/60	0/60	0/60	0/60	0/60
						1.0000
OVARY	Thecoma	0/60	1/60	0/60	0/60	0/60
						1.0000
		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
PANCREAS	Adenoma: islet cell	2/60	1/59	0/60	0/60	0/60
						1.0000
PANCREAS	Carcinoma: islet cell	2/60	2/59	1/60	0/60	1/60
						0.7181
PANCREAS	Schwannoma	0/60	0/59	1/60	0/60	0/60
						0.6303
PITUITARY GLAND	Adenoma: pars distalis	48/60	48/60	42/60	44/60	43/60
						0.4704
PITUITARY GLAND	Carcinoma: pars distalis	5/60	5/60	6/60	3/60	5/60
						0.5276
PITUITARY GLAND	Adenomas & Carcinomas	53/60	53/60	48/60	47/60	48/60
						0.4817
SKIN/SUBCUTIS	Fibrosarcoma	1/60	0/60	0/60	0/60	0/60
						1.0000
SKIN/SUBCUTIS	Lipoma	1/60	0/60	0/60	0/60	0/60
						1.0000
SKIN/SUBCUTIS	Fibroma	0/60	0/60	0/60	0/60	1/60
						0.1772
SKIN/SUBCUTIS	Adenoma: basal cell	0/60	0/60	1/60	0/60	1/60
						0.1844

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
STOMACH	Papilloma: squamous cell	0/60	0/60	1/60	0/60	1/60
						0.1892
SYSTEMIC NEOPLASMS	Lymphoma: malignant	2/60	0/60	1/60	2/60	4/60
						0.0323
SYSTEMIC NEOPLASMS	Hemangiosarcoma	2/60	1/60	0/60	2/60	0/60
						0.7673
SYSTEMIC NEOPLASMS	Histiocytic sarcoma	1/60	0/60	0/60	2/60	0/60
						0.5367
THYMUS	Thymoma	1/60	0/60	0/60	0/60	1/60
						0.3829
THYROID GLAND	Adenoma: C-cell	5/59	2/59	2/59	8/60	6/60
						0.0740
THYROID GLAND	Carcinoma: C-cell	1/59	2/59	4/59	4/60	2/60
						0.4605
THYROID GLAND	C-cell Adenomas & Carcinomas	6/59	4/59	6/59	10/60	8/60
						0.1092
THYROID GLAND	Adenoma: follicular cell	1/59	1/59	2/59	1/60	1/60
						0.5332
THYROID GLAND	Carcinoma: follicular cell	0/59	0/59	2/59	0/60	0/60
						0.6936

		DOSE (mg/kg/day)				
	NEOPLASM	**0**	**0**	**50**	**200**	**600**
TONGUE	Carcinoma: squamous cell	0/60	0/60	1/60	0/60	0/60
						0.5854
UTERUS	Leiomyosarcoma	0/60	0/60	0/60	0/60	1/60
						0.1850
UTERUS	Granular cell tumor	0/60	1/60	0/60	0/60	1/60
						0.3129
UTERUS	Polyp: endometrial stromal	1/60	4/60	2/60	2/60	2/60
						0.5761
UTERUS	Adenoma: endometrial	0/60	0/60	1/60	0/60	0/60
						0.5938
VAGINA	Granular cell tumor	2/60	0/60	0/60	3/60	0/60
						0.6758
COMBINED CERVIX,	Granular cell tumor	2/60	4/60	0/60	3/60	2/60
UTERUS & VAGINA						0.4527
ADIPOSE TISSUE[a]	Schwannoma	1/9	0/7	0/5	0/3	0/7
ADIPOSE TISSUE[a]	Fibrosarcoma	0/9	0/7	0/5	0/3	1/7
ADIPOSE TISSUE[a]	Malignant hibernoma	1/9	0/7	0/5	0/3	0/7
ADIPOSE TISSUE[a]	Mesothelioma	0/9	1/7	0/5	0/3	0/7
EARS[a]	Neural crest tumor		0/1	0/1	1/1	1/1	0/0

[a] Non-protocol specified tissue - statistical analysis not reported. Denominators represent the number of animals that received microscopic examination.

Appendix 9: Sponsor's Summary of Historical Control Data in Rats - Study DN05069

Historical and Concurrent Control Incidences of Malignant Lymphoma in Oral Carcinogenicity Studies in Charles River Sprague Dawley Rats at (b) (4)

(b) (4) Study	In-Life Phase	Incidence (%)			
		Males		Females	
		Control Group 1	Control Group 2	Control Group 1	Control Group 2
Concurrent Controls	2006-2008	1/60 (1.7)	0/60 (0)	2/60 (3.3)	0/60 (0)
B	2001-2003	0/70 (0)	-	0/70 (0)	-
C	2001-2003	1/60 (1.7)	-	1/60 (1.7)	-
H	2004-2006	1/60 (1.7)	-	0/60 (0)	-
J	2004-2006	2/60 (3.3)	-	1/60 (1.7)	-
K	2005-2007	4/60 (6.7)	-	0/60 (0)	-

A dash (-) indicates only 1 control group utilized in this study.

Appendix 10: References

Adams RL, Bird RJ. 2009. Review article: Coagulation cascade and therapeutics update: relevance to nephrology. Part 1: Overview of coagulation, thrombophilias and history of anticoagulants. Nephrology (Carlton).; 14: 462-470.

Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen D, Powers P. 1987. Development of the human coagulation system in the full-term infant. Blood.; 70: 165-172.

Asaoka Y, Sakai H, Sasaki J, Goryo M, Yanai T, Masegi T, Okada K. 2010. Changes in the gene expression and enzyme activity of hepatic cytochrome P450 in juvenile Sprague-Dawley rats. J Vet Med Sci.; 72(4): 471-9.

Bath PM, Butterworth RJ. 1996. Platelet size: measurement, physiology and vascular disease. Blood Coagul Fibrinolysis. 7(2): 157-61.

Borensztajn K, Peppelenbosch MP, Spek CA. 2008. Factor Xa: at the crossroads between coagulation and signaling in physiology and disease. Trends Mol Med; 14: 429-40.

Bouchard BA, Catcher CS, Thrash BR, Adida C, Tracy PB. 1997. Effector cell protease receptor-1, a platelet activation-dependent membrane protein, regulates prothrombinase-catalyzed thrombin generation. J Biol Chem.; 272: 9244 –9251.

Camerer E, Rottingen J-A, Gjernes E, Larsen K, Skartlien AH, Iversen J-G, Prydz H. 1999. Coagulation factors VIIa and Xa induce cell signaling leading to up-regulation of the egr-1 gene. J Biol Chem 274: 32225-32233.

Chu SG, Becker RC, Berger PB, Bhatt DL, Eikelboom JW, Konkle B, Mohler ER, Reilly MP, Berger JS. 2010. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. J Thromb Haemost. 8(1): 148-56.

Connolly AJ, Ishihara H, Kahn ML, Farese RV, Coughlin SR. 1996. Role of the thrombin receptor in development and evidence for a second receptor. Nature; 381: 516–519.

Creasy DM, Jonnassen H. 1999. A modified Davidson's fixative for fixation of small and large animal testes. Vet Pathol 36: 518.

da Silva VA Jr, Vieira AC, Pinto CF, de Paula TA, Palma MB, Lins Amorim MJ, Amorim AA Jr, Manhães-de-Castro R. 2006. Neonatal treatment with naloxone increases the population of Sertoli cells and sperm production in adult rats. Reprod Nutr Dev.; 46(2): 157-66.

De França LR, Hess RA, Cooke PS, Russell LD. 1995. Neonatal hypothyroidism causes delayed Sertoli cell maturation in rats treated with propylthiouracil: evidence that the Sertoli cell controls testis growth. Anat Rec.; 242(1): 57-69.

Dean RB and Dixon WJ. 1951. Anal. Chem. 23: 636.

Dewerchin M, Liang Z, Moons L, Carmeliet P, Castellino FJ, Collen D, Rosen ED 2000. Blood Coagulation Factor X Deficiency Causes Partial Embryonic Lethality and Fatal Neonatal Bleeding in Mice Thromb Haemost; 83: 185–90.

de Zwart L, Scholten M, Monbaliu JG, Annaert PP, Van Houdt JM, Van den Wyngaert I, De Schaepdrijver LM, Bailey GP, Coogan TP, Coussement WC, Mannens GS. 2008. The ontogeny of drug metabolizing enzymes and transporters in the rat. Reprod Toxicol.; 26(3-4): 220-30.

de Zwart LL, Haenen HE, Versantvoort CH, Wolterink G, van Engelen JG, Sips AJ. 2004. Role of biokinetics in risk assessment of drugs and chemicals in children. Regul Toxicol Pharmacol.; 39(3): 282-309.

Duanmu Z, Weckle A, Koukouritaki SB, Hines RN, Falany JL, Falany CN, Kocarek TA, Runge-Morris M. 2006. Developmental expression of aryl, estrogen, and hydroxysteroid sulfotransferases in pre- and postnatal human liver. J Pharmacol Exp Ther.; 316(3): 1310-7.

Fager AM, Wood JP, Bouchard BA, Feng P, Tracy PB. 2010. Properties of procoagulant platelets: defining and characterizing the subpopulation binding a functional prothrombinase. Arterioscler Thromb Vasc Biol. 30(12): 2400-7.

Feistritzer C, Lenta R, Riewald M. 2005. Protease-activated receptors-1 and -2 can mediate endothelial barrier protection: role in factor Xa signaling. J Thromb Haemost.; 3(12): 2798-805.

França RL, Silva Jr VA, Chiarini-Garcia H, Garcia SK, Debeljuk L. Cell proliferation and hormonal changes during postnatal development of the testis in the pig. Biol Reprod 2000, 63: 1629–1636.

Glatt H. 1997. Sulfation and sulfotransferases 4: bioactivation of mutagens via sulfation. FASEB J. 11(5): 314-21.

Hassan HJ, Leonardi A, Chelucci C, Mattia G, Macioce G, Guerriero R, Russo G, Mannucci PM, Peschle C. 1990. Blood coagulation factors in human embryonic-fetal development: preferential expression of the FVII/tissue factor pathway. Blood.; 76(6): 1158-64.

Lee KP, Frame SR, Sykes GP, Valentine R. 1993. Testicular degeneration and spermatid retention in young male rats. Toxicol Pathol.;21(3): 292-302.

Luettgen JM, Knabb RM, He K, Pinto DJ, Rendina AR. 2011. Apixaban inhibition of factor Xa: Microscopic rate constants and inhibition mechanism in purified protein systems and in human plasma. J Enzyme Inhib Med Chem.;26(4): 514-26.

Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. 2001. Proteinase-activated receptors. Pharmacol Rev.; 53: 245-82

Mahmood B, Daood MJ, Hart C, Hansen TW, Watchko JF. 2001. Ontogeny of P-glycoprotein in mouse intestine, liver, and kidney. J Investig Med.; 49(3):250-7.

Mann KG, Brummel K, Butenas S. 2003. What is all that thrombin for? J Thromb Haemost; 1: 1504–14.

McGuire MK, Littleton AW, Schulze KJ, Rasmussen KM. 1995. Pre- and postweaning food restrictions interact to determine reproductive success and milk volume in rats. J Nutr.;125(9): 2400-6.

Nesheim ME, J.B. Taswell, K.G. Mann, 1979.The contribution of bovine Factor V and Factor Va to the activity of prothrombinase, J. Biol. Chem. 254: 10952–10962.

Orth JM. Gunsalus GL, Lamperti AA. Evidence from Sertoli-cell depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 1988, 122: 787–794.

Papapetropoulos A, Piccardoni P, Cirino G, Bucci M, Sorrentino R, Cicala C, Johnson K, Zachariou V, Sessa WC, Altieri DC. 1998. Hypotension and inflammatory cytokine gene expression triggered by factor Xa-nitric oxide signaling. Proc Natl Acad Sci USA, 95: 4738-4742.

Pinto DJ, Orwat MJ, Koch S, Rossi KA, Alexander RS, Smallwood A, Wong PC, Rendina AR, Luettgen JM, Knabb RM, He K, Xin B, Wexler RR, Lam PY. 2007. Discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H -pyrazolo[3,4-c]pyridine-3-carboxamide (apixaban, BMS-562247), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. J Med Chem.;50(22): 5339-56.

Rand MD, J.B. Lock, C. van't Veer, D.P. Gaffney, K.G. Mann, 1996. Blood clotting in minimally altered whole blood, Blood 88: 3432–3445.

Ruf W, Dorfleutner A, Riewald M. 2003. Specificity of coagulation factor signaling. J. Thromb. Haemost. 1: 1495–1503.

Senden NH, Jeunhomme TM, Heemskerk JW, Wagenvoord R, van't Veer C, Hemker HC, Buurman WA. 1998. Factor Xa induces cytokine production and expression of adhesion molecules by human umbilical vein endothelial cells. J Immunol. 161(8): 4318-4324.

Simorangkir DR, Wreford NG, De Kretser DM. 1997. Impaired germ cell development in the testes of immature rats with neonatal hypothyroidism. J Androl.; 18: 186-193.

Sun WY, Witte DP, Degen JL, Colbert MC, Burkart MC, Holmback K, Xiao Q, Bugge TH, Degen SJF. 1998. Prothrombin deficiency results in embryonic and neonatal lethality in mice. Proc Natl Acad Sci USA 95: 7597-7602.

Tai SJ, Herzog RW, Margaritis P, Arruda VR, Chu K, Golden JA, Labosky PA, High KA. 2008. A viable mouse model of factor X deficiency provides evidence for maternal transfer of factor X. J Thromb Haemost.; 6(2): 339-45.

Tanaka KA, Key NS, Levy JH. 2009. Blood coagulation: hemostasis and thrombin regulation. Anesth Analg. 108: 1433-46.

Terry KK, Chatman LA, Foley GL, Kadyszewski E, Fleeman TL, Hurtt ME, Chapin RE. 2005. Effects of feed restriction on fertility in female rats. Birth Defects Res B Dev Reprod Toxicol.; 74(5): 431-41.

Tracy PB, Nesheim ME, Mann KG. 1992. Platelet factor Xa receptor, Methods Enzymol. 215: 329–360.

Traynelis SF, Trejo J. 2007. Protease-activated receptor signaling: new roles and regulatory mechanisms. Curr Opin Hematol.;14(3): 230-5.

Vizioli L, Muscari S, Muscari A. 2009. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. Int J Clin Pract. 63(10): 1509-15.

Watchko JF, Daood MJ, Mahmood B, Vats K, Hart C, Ahdab-Barmada M. 2001. P-glycoprotein and bilirubin disposition. J Perinatol.;21 Suppl 1: S43-7.

Wong PC, Watson CA, Crain EJ. 2008. Arterial antithrombotic and bleeding time effects of apixaban, a direct factor Xa inhibitor, in combination with antiplatelet therapy in rabbits. J Thromb Haemost 2008; 6: 1736-41.

Wong PC, Crain EJ, Watson CA, Jiang X, Hua J, Bostwick JS, Ogletree ML, Schumacher WA, Rehfuss R. 2007. Platelet aggregometry and receptor binding to predict the magnitude of antithrombotic and bleeding time effects of clopidogrel in rabbits. J Cardiovasc Pharmacol; 49: 316-24.

Wong PC, Pinto DJ, Zhang D. 2011. Preclinical discovery of apixaban, a direct and orally bioavailable factor Xa inhibitor. J Thromb Thrombolysis.; 31(4): 478-92.

Wong PC, Watson CA, Crain EJ. 2008. Arterial antithrombotic and bleeding time effects of apixaban, a direct factor Xa inhibitor, in combination with antiplatelet therapy in rabbits. J Thromb Haemost.; 6(10): 1736-41.

Wong PC, Crain EJ, Xin B, Wexler RR, Lam PY, Pinto DJ, Luettgen JM, Knabb RM. 2008. Apixaban, an oral, direct and highly selective factor Xa inhibitor: in vitro, antithrombotic and antihemostatic studies. J Thromb Haemost.; 6(5): 820-9.

Working PK. 1988. Male reproductive toxicology: comparison of the human to animal models. Environ Health Perspect.; 77: 37-44.

Xue J, Wu A, Westfield A, Tuley EA, Lu D, Zhang Q, Shim K, Zheng X, Sadler JE. 1998. Incomplete embryonic lethality and fatal neonatal hemorrhage caused by prothrombin deficiency in mice. Proc Natl Acad Sci USA. 95: 7603-7607.

Yamaguchi Y, Okabe K, Liang J, Matsumura F, Ohshiro H, Ishihara K, Matsuda T, Takeya M, Kuratsu JI, Mori K, Yamada S, Ogawa M. 1999. A synthetic selective inhibitor of factor Xa, DX-9065a, reduces monocyte chemoattractant protein-1 expression after ischemia-reperfusion injury in rat liver. Dig Dis Sci.; 44(12): 2568-76.

Zoetis T, Hurtt ME. 2003. Species comparison of anatomical and functional renal development. Birth Defects Res B Dev Reprod Toxicol.; 68(2): 111-20.

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

/s/

PATRICIA P HARLOW
02/21/2012

THOMAS PAPOIAN
02/21/2012
I concur.

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:	202155
Supporting document/s:	Electronic Document Room (EDR)
Applicant's letter date:	09/28/11, 09/29/10 (Nonclinical, Quality)
CDER stamp date:	09/28/11, 09/19/10 (Nonclinical, Quality)
Product:	Apixaban
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Bristol-Myers Squibb Company and Pfizer
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	Patricia P. Harlow, Ph.D.
Supervisor/Team Leader:	Thomas Papoian, Ph.D., D.A.B.T.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Alison Blaus

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202155 are owned by Bristol-Myers Squibb Company and Pfizer or are data for which Bristol-Myers Squibb Company and Pfizer has obtained a written right of reference. Any information or data necessary for approval of NDA 202155 that Bristol-Myers Squibb Company and Pfizer do not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202155.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

This review focuses solely on the toxicological qualification of the impurities in apixaban, an inhibitor of FXa, proposed for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation under NDA 202155.

1.2 Brief Discussion of Nonclinical Findings

The sponsor identified eight potential impurities in the apixaban drug substance by HPLC. Most of the impurities, except [(b) (4)] and [(b) (4)] were present in lots used for general toxicology studies at levels less than [(b) (4)]% or the specified level. However, the exposure level of each impurity in animals was at least 90-fold higher than the maximum predicted exposure level in humans. Therefore, the eight apixaban impurities are considered qualified in terms of general toxicology.

Since the one lot of apixaban drug substance used for the apixaban Ames assay had very low levels of three impurities, the reviewer requested that the CDER Computational Toxicology Group evaluate all apixaban impurities for genotoxicity. Although the Derek for Windows (DfW) application did not identify structural alerts for any of the apixaban impurities, the FDA Computational Toxicology Group predicted that [(b) (4)] as positive in the Ames assay and [(b) (4)] as positive in the micronucleus assay.

[(b) (4)] was present at adequate levels in the Ames assay of the apixaban drug substance and the reliability of the positive prediction in the Ames assay by Computational Toxicology is considered weak. Although the sponsor synthesized impurity [(b) (4)] and could potentially evaluate this impurity directly in an Ames assay, such an evaluation is not considered necessary.

Although [(b) (4)] was not present at adequate concentrations in the apixaban lot used in the in vivo micronucleus assay, chromosomal aberrations were evaluated in a 1-month repeat dose study using an apixaban lot in which [(b) (4)] was present at [(b) (4)]%. The high dose contained [(b) (4)] at 362-fold the maximum possible human exposure. Therefore, the clastogenic potential of [(b) (4)] was adequately tested and an additional in vivo study for clastogenicity is not necessary.

The sponsor identified six compounds in the apixaban synthesis as genotoxic. The levels of these compounds are controlled [(b) (4)] in the manufacturing process of apixaban. Spiking/purging studies confirmed that each of the genotoxic compounds was adequately purged and the level of each genotoxic compound in the final apixaban drug substance is <10 ppm. Not only is the maximum exposure of each compound, but also is the total exposure of all six compounds to a patient taking the maximum dose of 10 mg of apixaban per day less than the Threshold of Toxicological concern of 1.5 µg per day. Therefore, the levels of the identified genotoxic compounds are being adequately controlled.

1.3 Recommendations

The reviewer recommends that the apixaban impurities identified by HPLC be considered qualified and no additional toxicology or genetic toxicology study be required.
In addition, the identified genotoxic impurities are considered adequately controlled.

2 Drug Information

2.1 Drug

CAS Registry Number: 503612-47-3

Generic Name: Apixaban

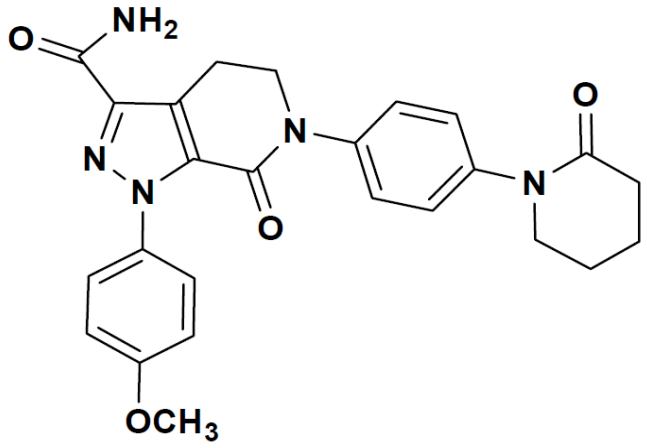
Code Names: BMS-562247, DPC-AG0023

Chemical Name: 1-(4-Methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide

Molecular Formula/Molecular Weight: $C_{25}H_{25}N_5O_4$/459.50

Structure:

Figure 1: Structure of Apixaban



Pharmacologic Class: Apixaban is a Factor Xa inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

(b) (4)

IND 68598, DCRP (11/09/2006)

2.3 Drug Formulation

Apixaban is formulated for oral administration as immediate release, film-coated tablets containing either 2.5 or 5 mg of active compound. The tablets also contain anhydrous lactose, microcrystalline cellulose, croscarmellose sodium, sodium lauryl sulfate, and magnesium stearate. The film coating for the 2.5 mg tablet is (b) (4) which contains hypromellose (b) (4) lactose monohydrate, titanium dioxide, triacetin, and iron oxide yellow. The film coating for the 5 mg tablet is (b) (4), which contains hypromellose (b) (4), lactose monohydrate, titanium dioxide, triacetin, and iron oxide red.

2.4 Comments on Novel Excipients

No novel excipient is used in the manufacture of apixaban tablets.

2.5 Comments on Impurities/Degradants of Concern

Background:

The sponsor identified eight potential impurities by HPLC in the apixaban drug substance. Four impurities ((b) (4) will be specified; four impurities ((b) (4)) will not be specified. The levels of the impurities varied with the process used for manufacturing apixaban (Appendix 1). Since (b) (4)

General toxicological qualification of rivaroxaban impurities

Most of the impurities, except (b) (4) and (b) (4) were present in lots used for general toxicology studies at levels less than (b) (4)% or the specified level (Appendix 1). However, as summarized in Table 1, the exposure level of each impurity in animal toxicology studies was at least 90-fold higher than the maximum predicted exposure level in humans (Appendix 2). Therefore, the eight apixaban impurities are considered qualified in terms of general toxicology.

Table 1: Reviewer's Summary of Apixaban Impurities

Impurity	Proposed criteria (%)	Levels in commercial sized batches by commercial process* (%)	Lot for toxicological qualification[T]			
			Lot number	Impurity level (%)	Toxicology study used	Exposure ratio, animal/ human
Specified						
(b) (4)		(b) (4)	2E55171	(b) (4)	3-month rat, dog	334
(b) (4)			2E55171			196
(b) (4)			5L00821[b]		2-year rat and mouse carcinogenicity	536
(b) (4)			4K83298, 4K86939, 4K89700, 4K85835			392
Not specified (Individual; other)						
(b) (4)		(b) (4)	4K83298, 4K86939, 4K89700, 4K85835		2-year rat and mouse carcinogenicity	612
(b) (4)			5L00821[b]			488
(b) (4)			2E55171		3-month rat, dog	91
(b) (4)			3J69810		6-month rat, 1 year dog	606

(b) (4) chosen for commercial development. [T] Levels from Table 3.2.S.4.4.1.104, Document 930047145. Min mum % in the four lots used during weeks 1-56 in 2-year mouse study. [b] Lot 5L00821 was used weeks 57 to 102 in 2-year mouse study.

Genotoxicity qualification of apixaban impurities

The sponsor did not specifically comment on the potential genotoxicity of the impurities listed above in Table 1. However, the non-genotoxicity of these impurities was implied in the sponsor's discussion of a control strategy for genotoxic impurities (Section 3.2.S.2.6.4, Document 930047145). The sponsor stated that the starting materials, reagents and reaction by-products in the synthetic route were assessed to identify known or potentially genotoxic compounds that could be impurities in the drug substance. The sponsor indicated that an unspecified in silico assessment was used to identify compounds with structural alerts for genotoxic activity. If an Ames assay confirmed the genotoxicity of an identified compound, the sponsor considered the compound genotoxic. The sponsor's list of genotoxic compounds (Table 3) did not include the impurities listed in Table 1.

Since the one lot of apixaban drug substance used for the apixaban Ames assay had very low levels of three impurities ((b) (4) (b) (4) and (b) (4) the reviewer requested that the CDER Computational Toxicology Group evaluate all impurities for apixaban. Although the Derek for Windows (DfW) application did not identify structural alerts for any of the apixaban impurities, the FDA Computational Toxicology Group predicted that (b) (4) and (b) (4) were positive for genotoxicity. The following paragraphs discuss these two positive predictions and the need for additional genotoxicity assays.

(b) (4)

The CDER Computational Toxicology group predicted [redacted] to be positive in the Salmonella Ames assay. However, the Derek for Windows program found no structural alerts for [redacted] and the SciQSAR program gave a negative prediction for [redacted] for Salmonella mutagenesis. Additionally, the Leadscope Model Applier program initially made no call for [redacted] Only the MC4PC program gave a positive prediction for [redacted]

The MC4PC positive prediction is based the [redacted] indicated in red in Figure 1. This structural alert is derived from a statistically strong cluster of training set compounds in which [redacted] Furthermore, [redacted] compounds have [redacted], which is absent in [redacted] Thus, the reliability of the MC4PC basis for the positive prediction for [redacted] for Salmonella mutagenicity is considered weak.

Figure 2: Structural Alert Identified by MC4PC in [redacted]

[redacted]

The structural alert is in red.

In addition, Dr. Naomi Kruhlak re-ran the Leadscope application in a manner to force it to make a prediction. The results indicated a negative prediction for [redacted] with high confidence. The presence of the [redacted] outweigh and mitigate the positive prediction made by Leadscope for the [redacted].

Since the coverage for [redacted] and other apixaban impurities in the current databases is not high, one could argue that the lack of a signal may be due to a lack of relevant training set molecules. However, the FDA Computational Toxicology analysis used four different software applications, three of which predicted [redacted] to be negative for genotoxicity. Only the MC4PC application produced a positive, but questionable, prediction. Therefore, the combined evidence for the positive prediction for [redacted] for Salmonella mutagenicity is considered weak in that it is based solely on the unreliable MC4PC results.

Furthermore, the Ames assay for the apixaban parent (Study DS02121, Document 930002536) used apixaban Lot 2E55171, in which [redacted] was present at [redacted]%. Therefore, [redacted] was present at [redacted] microgram/plate at the highest

concentrations tested (Table 2). These amounts of (b) (4) are similar to the amounts of most of the positive controls, which induced at least a 9-fold increase in the number of revertants/plate. If (b) (4) is a positive genotoxic compound, the reviewer expected that the results of the Ames assay for the parent apixaban also should have been positive. Therefore, an additional evaluation of the genotoxicity of (b) (4) in an Ames assay is not necessary.

Table 2: Reviewer's Summary - Concentrations in the Apixaban Ames Assay

	Maximum concentration of apixaban tested, (BMS 724914), µg/plate		Positive control concentration (Fold induction of revertants)	
Strain	Absence of S-9	Presence of S-9	Absence of S-9	Presence of S-9
TA98	(b) (4)			
TA100				
TA1535				
TA1537				
E.coli WP2 *uvrA*				

Study DS02121, Document 930002536)

(b) (4)

The CDER Computational Toxicology group predicted (b) (4) to be negative in the Ames assay, but positive in the in vivo micronucleus assay. However, the Derek for Windows program found no structural alerts for (b) (4) and the MC4PC program gave a negative prediction for (b) (4) for the in vivo micronucleus assay. Additionally, the Leadscope Model Applier program made no call for (b) (4) The positive prediction for (b) (4) in the micronucleus assay was based solely on the SciQSAR results.

The micronucleus assay for the apixaban drug substance (Study DN02117, Document 930002539) used apixaban Lot 2E55171, in which (b) (4) was present at (b) (4)%. Therefore, (b) (4) was not present at adequate concentrations to assess its genotoxic potential in this assay. However, a 1-month repeat dose study (Study DS05163, Document 930015561) evaluating in vivo/in vitro cytogenetics used Lot 4K83298 in which (b) (4) was present at (b) (4)%. The high dose of 600 mg/kg/day of Lot 4K83298 did not induce an increase in the frequency of lymphocytes with chromosomal aberrations. Each rat in the high dose group received a human equivalent dose of 58 µg/kg/day or 362-fold the maximum possible human exposure to (b) (4) Therefore, the clastogenic potential of (b) (4) was adequately tested in this assay and an additional evaluation of the in vivo genotoxicity of (b) (4) is not necessary.

Furthermore, (b) (4) was present the lots used for the 2 year carcinogenicity studies in rats and mice. During weeks 1 to 56, (b) (4) was present at a minimum of (b) (4)% and during weeks 57 to 102, it was present at (b) (4)%. Given that the high dose in the mouse carcinogenicity study was 1500 mg/kg/day for males and 3000 mg/kg/day for females, the exposure to (b) (4) during 97% of the mouse

carcinogenicity study was at least 450 higher than the maximum possible human exposure to [redacted]

Control of genotoxic impurities in apixaban

The sponsor identified six genotoxic compounds in the apixaban synthesis (Table 3). One compound is [redacted]. In confirmatory Ames assays, the compounds exhibited differences in the mutagenic responses by bacterial strains consistent with the genotoxic class. The sponsor is controlling the levels of these compounds [redacted] in the manufacturing process of apixaban. Studies conducted with spiking levels of at least 4000 ppm confirmed that each of the genotoxic compounds were purged greater than 200-fold during the manufacturing process. The level of each genotoxic compound in the final apixaban drug substance is ≤10 ppm. The maximum exposure of each compound to a patient taking the maximum dose of 10 mg of apixaban per day is [redacted] µg per day, which is less than the Threshold of Toxicological concern of 1.5 µg per day. Furthermore, the maximum level of the sum of all the compounds is [redacted] µg per day or also less than 1.5 µg per day. The reviewer agrees that the identified genotoxic compounds are being adequately controlled.

Table 3: Reviewer's Summary Genotoxic Impurities in Apixaban

Genotoxic class	Compound	Number positive strains in Ames -S9	Number positive strains in Ames +S9	Spiking/purging studies Spiking level, ppm	Spiking/purging studies Level in apixaban, ppm	Maximum exposure level, µg/day*
[redacted]				4000	<10 ppm	[redacted]
				6000	<10 ppm	
				4000	<10 ppm	
				10000	<10 ppm	
				10000	<10 ppm	
				10000	<10 ppm	

-S9 without metabolic activation, +S9 with metabolic activation, * Based on 10 mg apixaban per day.

Conclusions:

1. Although most of the eight impurities, except [redacted] and [redacted] were present at levels less than [redacted]% or the specified level in lots used for general toxicology studies, the exposure level of each impurity in animals was at least 90-fold higher than the maximum predicted exposure level in humans (Appendix 2). Therefore, the eight apixaban impurities are considered qualified in terms of general toxicology.

2. The FDA Computational Toxicology analysis predicted impurity [redacted] to be positive for the Ames assay. Although the sponsor synthesized impurity [redacted] and could potentially evaluate this impurity directly in an Ames assay, such an evaluation is not considered necessary, because: (a) [redacted] was present at adequate levels in the Ames assay of the apixaban drug substance and (b) the reliability of the positive prediction in the Ames assay by Computational Toxicology is weak.

3. The FDA Computational Toxicology analysis predicted impurity (b) (4) to be positive for the micronucleus assay. Although (b) (4) was not present at adequate concentrations in the apixaban in vivo micronucleus assay, chromosomal aberrations were evaluated in a 1-month repeat dose study using an apixaban lot in which (b) (4) was present at (b) (4) %. The high dose contained a (b) (4) human equivalent dose of 58 µg/kg/day or 362-fold the maximum possible human exposure to (b) (4) Therefore, the clastogenic potential of (b) (4) was adequately tested and an additional in vivo study for clastogenicity is not necessary.

4. The levels of six compounds in the apixaban synthesis identified as genotoxic are controlled (b) (4) in the manufacturing process of apixaban. Spiking/purging studies confirmed that each of the genotoxic compounds was adequately purged and the level of each genotoxic compound in the final apixaban drug substance is <10 ppm. Not only is the maximum exposure of each compound, but also is the total exposure of all six compounds to a patient taking the maximum dose of 10 mg of apixaban per day less than the Threshold of Toxicological concern of 1.5 µg per day. Therefore, the levels of the identified genotoxic compounds are being adequately controlled.

Recommendations:

The reviewer recommends that the apixaban impurities identified by HPLC be considered qualified and no additional toxicology or genetic toxicology study be required. In addition, the identified genotoxic impurities are considered adequately controlled.

3 Appendix/Attachments

Appendix 1: Apixaban Impurity Levels in Pre-Clinical Lots

Batch	Used in Toxicology Study Proposed specification, %	Process	Manufacture Year	Apixaban, %	Total Impurities, %	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
	3-mo rat, dog	(b) (4)				(b) (4)						
2E55171	Ames, micronucleus		2002	98.3								(b) (4)
2F52153	2-wk rat, dog, Chr Ab		2002	98.4								
3E68243	EFD rat		2003	98.8								
3J69810	1-yr dog, 6-mo rat		2003	100								
4B81615	EFD rabbit		2004	99								
4K83298	2 yr mouse, rat, 1 mo cytogenetics in rats		2004	99.4								
4K86939	2 yr mouse, rat		2004	99.4								
4K89700	2 yr mouse, rat		2004	98.9								
4K91480			2004	100.1								
4K90125			2004	98.7								
4K85835	2 yr mouse, rat		2004	99.9								
4K85841			2004	99.8								
5B06549	EFD mice		2005	99.6								
5F09130	Mouse DR EFD		2005	99.4								
5L00821	2 yr mouse, rat		2005	99.8								
5L00876			2005	99.6								
6E17751			2005	100.9								
6E17753			2005	100.8								
6E17758			2005	99.7								
6G14181			2006	100.1								
6J12236			2006	100								
6J12238	2 yr mouse, rat		2006	99.6								
6J12241			2006	100.4								
7A28071	In vitro hERG		2006	100.3								
7A29560			2006	100.2								

Data from Table 3.2.S.4.4.1.T02 Apixaban Batch Information and Table 3.2.S.4.4.1.T04 Apixaban HPLC Impurity Profile (Document 930047145), Lots used in the 2-year carcinogenicity studies are in blue text. The values for specific impurities used to calculate animal to human ratios are in bold text on a yellow background.

Appendix 2: Reviewer's Evaluation of Impurity Exposure Levels in Animals and Humans

Impurity	Specification, ≤ %	Maximum % in recent commercial lot	Impurities in selected batches used in toxicology studies, % (% apixaban purity, Manufacture process)						Maximum Exposure, µg/kg/day		Ratio animal to human exposure
			2E55171 (98.3% (b) (4)	3J69810 (98.8%, (b) (4)	4K85835 (99.9% (b) (4)	Minimum 1st yr carc.	5L00821 (99.8%, (b) (4)	6J12238 (99.6% (b) (4)	Human†	Animal‡	
Specified impurities											
(b) (4)											
Non-specified impurities											
(b) (4)											
Lot used in indicated toxicology study (Document number in footnote)			3 month rat[1], dog[2], Ames[3], Micronucleus[4]	6 month rat[6], 1 year dog[7]	2 year rat[8], 2 year mouse[9]						
Maximum animal dose, mg/kg			Rat: 300 Dog: 20	Rat: 600 Dog: 100	Rat: 600 Mouse: 1500						
HED (mg/kg) based on mg/m^2			Rat: 48.6 Dog: 10.8	Rat: 97 Dog: 54	Rat: 97 Mouse: 122						
Genotoxic impurities			Genotoxic Impurities (ppm)								
(b) (4)	*	<10 ppm					<10 ppm				(b) (4)
	*	<10 ppm					<10 ppm				
	*	<10 ppm					<10 ppm				
	*	<10 ppm			(b) (4)		<10 ppm				
	*	<10 ppm					<10 ppm				
	*	<10 ppm					<10 ppm				

† Based on human dose of 5 mg BID or 10 mg/day or 0.166 mg/kg assuming a 60 kg adult and the maximum % of impurity specified ‡ Based on maximum animal dose in study using lot with the value in bold text on a yellow background and the % of impurity in batch used. * Genotoxic impurities are controlled (b) (4) § (b) (4) impurity only expected in lots made with (b) (4) Document numbers: [1] 930005268, [2] 930004221, [3] 930002536, [4] 930002539, 930006384, 930015541), [6] 930012967, 930012966, [8] 930031443, [9] 930031442, In the mouse carcinogenicity study: Lot 4K85835 was used weeks 1-14, Lot 4K83298 was used weeks 15-30, Lot 4K86939 was used weeks 31-40, Lot 4K89700 was used weeks 41-56, Lot 5L00821 was used weeks 57 to 102 and Lot 6J12238 was used weeks 103-105.

Sources: Section 3.2.S Drug substance, Table 3.2.S.4.4.1.T03, Table 3.2.S.4.4.1.T04

Appendix 3: FDA Computational Toxicology Evaluation

To: **Patricia Harlow**
cc: **Thomas Papoian**
From: **CDER/OPS/OTR/DDSR: The CDER Computational Toxicology Group**
Re: **NDA 202155**
Date: **October 20, 2011**

Twenty-two known and potential structures of metabolites and impurities of apixaban were evaluated by CDER/OPS/OTR/DDSR for genetic toxicity against the ICH S2 battery. Four (quantitative) structure-activity relationship [(Q)SAR] computational toxicology software programs were used: Derek for Windows 13 (DfW), Leadscope Model Applier 1.3.3-3 (LMA), MC4PC 2.4.0.7 (MC), and SciQSAR 2.2 (SQ).

The results of the predictions from the software programs were weighted equally and the analysis was optimized for sensitivity (minimizing false negatives) to reach the overall conclusions. The overall genetox predictions for the 22 structures are shown in the first summary table below, followed by predictions for each chemical in detail. All 22 chemical structures are shown in Figure 1.

The predicted toxicological activities are scored as follows in all tables:

+	Positive
Eqv	Equivocal/marginally active
-	Negative
NSA	No structural alerts identified by DfW
NC	No call can be made because the chemical's structural features are not adequately represented in the model (poor coverage)
N/A	No model available for this endpoint with this software

Table 1: Summary Calls for Predicted ICH S2 Battery Genetox Tests

Chem. No.	Chemical Name	*Salm.* Mut.	*E. coli* Mut.	Mouse Lymph.	*In Vitro* Chrom. Abs.	*In Vivo* Micro-nucleus	Overall Prediction
1	Apixaban	-	NC	-	NC	-	-
2	(b) (4)	-	NC	-	NC	-	-
3		+	NC	-	NC	-	+
4		-	NC	-	NC	-	-
5		-	NC	-	NC	-	-
6		-	NC	-	NC	-	-
7		-	NC	-	NC	+	+
8		-	NC	-	NC	-	-
9	M1 (O-desmethyl apixaban sulfate)	-	NC	-	NC	-	-
10	M2 (O-desmethyl apixaban)	-	NC	-	NC	+	+
11	M7 (3-hydroxy apixaban)	-	NC	-	NC	-	-
12	M4 (2-hydroxy apixaban)	-	NC	-	NC	-	-
13	M4 (4-hydroxy apixaban)	-	NC	-	NC	-	-
14	M4 (5-hydroxy apixaban)	-	NC	-	NC	+	+
15	M10 (2-hydroxy O-desmethyl apixaban sulfate-1)	-	NC	-	NC	-	-
16	M10 (3-hydroxy O-desmethyl apixaban sulfate-1)	-	NC	-	NC	-	-
17	M10 (4-hydroxy O-desmethyl apixaban sulfate-1)	-	NC	-	NC	-	-
18	M10 (5-hydroxy O-desmethyl apixaban sulfate-1)	-	NC	-	NC	NC	NC
19	M13 (2-hydroxy O-desmethyl apixaban)	-	NC	+	NC	+	+
20	M13 (3-hydroxy O-desmethyl apixaban)	-	NC	-	NC	+	+
21	M13 (4-hydroxy O-desmethyl apixaban)	-	NC	+	NC	-	-
22	M13 (5-hydroxy O-desmethyl apixaban)	-	NC	+	NC	+	+

Table 2: Genetic Toxicity Predictions in ICH S2 Battery Tests

Chem. No.	Chemical Name	*Salmonella* Mutagenicity				Overall *Salmonella* Call	*E. coli* Mutagenicity				Overall *E. coli* Call
		DfW	LMA	MC	SQ		DfW	LMA	MC	SQ	
1	Apixaban	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
2	(b) (4)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
3		NSA	NC	+	-	+	NSA	NC	NC	N/A	NC
4		NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
5		NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
6		NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
7		NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
8		NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
9	M1 (O-desmethyl apixaban sulfate)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
10	M2 (O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
11	M7 (3-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
12	M4 (2-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
13	M4 (4-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
14	M4 (5-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
15	M10 (2-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
16	M10 (3-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
17	M10 (4-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
18	M10 (5-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
19	M13 (2-hydroxy O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
20	M13 (3-hydroxy O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
21	M13 (4-hydroxy O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC
22	M13 (5-hydroxy O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	N/A	NC

Table 2: Genetic Toxicity Predictions in ICH S2 Battery Tests (continued)

Chem. No.	Chemical Name	Mouse Lymphoma				Overall Mouse Lymph. Call	*In Vitro* Chromosome Aberrations				Overall Chrom. Abs. Call	*In Vivo* Micronucleus				Overall Micro-nucleus Call	Overall Gene-tox Call
		DfW	LMA	MC	SQ		DfW	LMA	MC	SQ		DfW	LMA	MC	SQ		
1	Apixaban	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
2	(b) (4)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
3		NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	+
4		NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
5		NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
6		NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
7		NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	+	+	+
8		NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
9	M1 (O-desmethyl apixaban sulfate)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	Eqv	-	-	-
10	M2 (O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	+	+	+
11	M7 (3-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
12	M4 (2-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
13	M4 (4-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
14	M4 (5-hydroxy apixaban)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	+	+	+
15	M10 (2-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	Eqv	-	-	-
16	M10 (3-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	Eqv	-	-	-
17	M10 (4-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	Eqv	-	-	-
18	M10 (5-hydroxy O-desmethyl apixaban sulfate-1)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	NC	-	NC	NC
19	M13 (2-hydroxy O-desmethyl apixaban)	NSA	NC	-	+	+	NSA	NC	NC	-	NC	NSA	NC	-	+	+	+
20	M13 (3-hydroxy O-desmethyl apixaban)	NSA	NC	-	-	-	NSA	NC	NC	-	NC	NSA	NC	-	+	+	+
21	M13 (4-hydroxy O-desmethyl apixaban)	NSA	NC	-	+	+	NSA	NC	NC	-	NC	NSA	NC	-	-	-	-
22	M13 (5-hydroxy O-desmethyl apixaban)	NSA	NC	-	+	+	NSA	NC	NC	-	NC	NSA	NC	-	+	+	+

In conclusion, one structure (impurity 2) was predicted positive overall for genetic toxicity based upon a positive *Salmonella* mutagenicity prediction by MC4PC. A further six structures were predicted to be positive overall based on positive micronucleus *in vivo* predictions by SciQSAR.

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

/s/

PATRICIA P HARLOW
10/31/2011

THOMAS PAPOIAN
11/01/2011
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202155 **Applicant: Bristol-Myers Squibb Company and Pfizer** **Stamp Date: 09/28/2011**

Drug Name: apixaban **NDA/BLA Type: Commercial**

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		Many of the toxicology studies were conducted with apixaban batches made using the initial (b) (4) rather than the subsequent (b) (4) was chosen as the commercial process. Although the processes result in drug substance lots with different impurity profiles, the impurities are considered qualified toxicologically.
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		Statements are included with individual study reports
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		See review regarding specific apixaban impurities.
11	Has the applicant addressed any abuse potential issues in the submission?		X	The sponsor did not directly address abuse potential in the CTD. However, apixaban has limited distribution to CNS tissues in adult rats. In a 3-month repeated dose study apixaban did not affect the measured parameters of CNS function in dogs. Systemic exposures of unbound drug in dogs at the highest dose 20 mg/kg were 65-times the human exposure.
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_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

No nonclinical review issue has been identified.

Patricia Harlow, Ph.D. — October 27, 2011
Reviewing Pharmacologist — Date

Thomas Papoian, Ph.D. — October 31, 2011
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/

PATRICIA P HARLOW
10/31/2011

THOMAS PAPOIAN
11/01/2011
I concur.