CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

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Product:	Rivaroxaban (Bay 59-7939)
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Janssen Pharmaceuticals Inc
Review Division:	Division of Cardiovascular and Renal Products
Reviewer:	Patricia P. Harlow, Ph.D.
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1 Executive Summary

1.1 Introduction

Rivaroxaban is being developed for the prevention and treatment of multiple thrombosis-mediated conditions. Under the current NDA 202,439, rivaroxaban is proposed for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation. The dosing regimen in the Phase 3 trial was 20 mg of rivaroxaban once a day in patients with normal renal function. Under the related NDA 22,406, rivaroxaban at a dose of 10 mg once a day was approved on July 1, 2011 for the prophylaxis of deep vein thrombosis and pulmonary embolism in patients undergoing hip replacement surgery or knee replacement surgery.

1.2 Brief Discussion of Nonclinical Findings

Rivaroxaban (BAY 59-7939) binds directly to and inhibits the activity of Factor Xa, the coagulation protease that converts prothrombin to thrombin, which in turn converts fibrinogen to fibrin in forming a blood clot. Rivaroxaban inhibition of Factor Xa is independent of other factors, such as antithrombin III, and results in prolongation of coagulation in vitro and anti-thrombotic effects in various animal models of venous and arterial thrombosis in vivo. The major safety concern for rivaroxaban is its low margin of safety for prolongation of bleeding compared to its inhibition of thrombosis.

Rivaroxaban was evaluated in an extensive battery of safety pharmacology studies. In vitro studies showed no effect of rivaroxaban up to 10 μM on the hERG channel or prolongation of action potential. In anesthetized beagle dogs, rivaroxaban had no effect on the cardiovascular function (heart rate, cardiac output, left ventricular pressure, dP/dt, and left ventricular end-diastolic pressure), ECG parameters (PQ, QRS and QT intervals), respiration, acid/base balance, hematocrit and electrolytes. Rivaroxaban did not affect the central nervous system (behavior, body temperature), the convulsive threshold dose of pentylenetetrazol, duration of hexobarbital-induced anesthesia, urine volume, urinary electrolytes, hematological parameters or gastrointestinal motility. However, rivaroxaban significantly delayed the nociceptive response in rats at doses of 10 mg/kg and above

Co-administration of rivaroxaban with heparin, enoxaparin, acetylsalicylic acid or clopidogrel generally produced additive or less than additive effects on thrombosis and bleeding in the rat arteriovenous shunt model, depending on the dosages used. Co-administration of rivaroxaban with acetylsalicylic acid, diclofenac, warfarin, or clopidogrel produced additive or less than additive effects in a rat bleeding model; however, co-administration with naproxen produced additive or more than additive effects. Rivaroxaban is not as potent, on a molar basis, as heparin or enoxaparin at preventing in vitro catheter-induced clotting. The concentration of rivaroxban (12.1 μM) required to prolong clotting in the presence of catheter segments by 8-fold is above the Cmax of rivaroxaban in patients with atrial fibrillation (274 μg/L or 0.6 μM). Studies of bleeding time prolongation in baboons and rats indicated administration of human FVIIa or activated prothrombin complex concentrate could partially reverse the prolongation of bleeding induced by rivaroxaban.

The use of unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) is restricted in patients that develop heparin-induced thrombocytopenia (HIT). These patients with antibodies to a complex of heparin/platelet factor 4 (PF4) can have platelet activation, a hypercoagulable state with decreased platelet counts, and severe thrombotic complications, including death. Sera and platelets in multiple combinations from 89 different patients with established ELISA positive HIT antibodies were used to evaluate rivaroxaban in several types of assays relative to heparin, enoxaprin, fondaparinux and argatroban controls. Experiments included a ^{14}C-serotonin release assay, drug-induced platelet aggregation, flow cytometric analysis of platelet activation, microparticle generation, P-selectin expression, and platelet aggregate formation as well as binding to platelet factor 4 (PF4) and release of PF4 from resting and tissue factor (TF) activated platelets. Rivaroxaban did not show a positive response with any of the HIT patient sera in any assay, whereas heparin and enoxaprin produced the expected positive responses.

The plasma protein binding of rivaroxaban varied greatly among species, with unbound fractions in humans, rats, mice, dogs, and rabbits of 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively. As a result, exposure comparisons between species were made based on the unbound fraction of rivaroxaban. The principal human plasma binding protein was serum albumin.

Whole body autoradiography of Wistar rats at 0.5 hour after administration of a single dose of radioactive rivaroxaban indicated rapid distribution to most tissues with the highest concentrations in the gastrointestinal tract, intermediate levels in the liver, kidneys, bladder and plasma, and lowest concentrations in the brain, spinal cord and testes. A low level of radioactivity was observed in the eyes and pigmented skin of the Long Evans rat 7 days after dosing indicating that radioactive rivaroxaban showed some affinity for melanin-containing tissue in pigmented rats. However, rivaroxaban was not phototoxic in an in vitro assay using 3T3.A31 mouse fibroblasts.

Whole-body autoradiography of pregnant Wistar rats on gestation day 19 indicated distribution of radioactive rivaroxaban through the placental barrier to the fetus. The highest levels of rivaroxaban radioactivity in maternal and fetal blood occurred at 2 hours after dosing when the fetal blood level (0.286 mg-eq/L) was 15% of the maternal blood level (1.86 mg-eq/L). The average exposure in the fetuses based on $AUC_{(0\ 24)}$ reached about 20 % of the exposure in maternal blood.

The in vivo metabolism study in humans showed that unchanged rivaroxaban represented more than 85 % of the AUC of total radioactivity in all four subjects. The principal metabolic pathway of rivaroxaban involves the hydroxylation at the morpholinone moiety leading to the mono-hydroxylated metabolite M-2, which is then further metabolized in subsequent morpholinone ring opening/oxidation steps to form metabolite M-1. Although the plasma levels of the M-1 metabolite varied among the four subjects evaluated, the levels of M1 metabolite were less than 10% of total radioactivity AUC in all four human subjects. The M-1 metabolite was found to be the most prominent metabolite in plasma of humans as well as in the plasma of mice, rats and dogs where the levels of M1 were equal to or greater than the levels of M1 in humans. In vitro studies indicated CYP3A4 and CYP2J2 are the principal CYP enzymes for metabolism of rivaroxaban to metabolite M-2.

Excretion of rivaroxaban in humans is primarily renal (66%). In contrast, rivaroxaban excretion in rats is principally by the biliary/fecal route (66-81%) with only 24-28% excretion into the urine. Excretion of rivaroxaban in dogs is through a combination of routes with 52% of radioactive rivaroxaban excreted in the urine of dogs,

Rivaroxaban did not inhibit biotransformation reactions catalyzed by CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2, and 3A4 or induce the expression of human CYP enzymes CYP1A2, 3A4, 2B6, and 2C19. The most potent inhibitors of rivaroxaban metabolism consist primarily of HIV protease inhibitors and antifungal azoles, particularly ketoconazole and ritonavir.

Chronic toxicology studies of 6 and 12 months duration were conducted in rats and dogs, respectively. In general, compound-related findings, such as prolongation of coagulation parameters and effects from bleeding, were related to the pharmacological activity of rivaroxaban. Although in some studies individual rats and dogs showed increases in ALT, these findings were transient, not associated with liver histopathology and not consistently associated with increases in AST or bilirubin.

Rivaroxaban was not mutagenic in the in vitro Ames test, the in vitro chromosome aberration assay in V79 Chinese hamster lung cells, or the in vivo mouse bone marrow micronucleus test.

In adequate carcinogenicity studies, CD-1 mice and Wistar rats received oral dosages of 10, 20 and 60 mg/kg/day of rivaroxaban for up to 104 weeks. Although the incidences of a few tumors in each species were increased in the higher dose groups compared to the incidences in the control groups, the incidences did not achieve the statistical significance for the tumors to be considered positive, according to current CDER guidance. The Executive Carcinogenicity Assessment Committee concluded that there were no clear drug-related neoplasms in either study.

However, in the rat carcinogenicity study, the incidence of valvular fibrosis in the heart increased with dose in both males and females and was statistically significant in females by the trend test ($p_t = 0.0048$). The incidences of valvular fibrosis in the male (0-4%) and female (0-8%) groups in the rivaroxaban study were at or below the historical incidence range for the control males (12-20%) and females (8-26%) in two previous carcinogenicity studies conducted by the sponsor. The sponsor attributed the differences in incidences to inconsistency of histopathological sampling of heart valves leading to variability in histopathological diagnosis. In all but one rat, the valvular findings were accompanied by chronic cardiomyopathy, a common age-related finding in the rat.

In an acceptable fertility and early embryo development study in rats, the NOAEL for paternal and maternal toxicity was 12.5 mg/kg based on decreased body weight gain and food intake at ≥50 mg/kg. Although the gestation index decreased at 200 mg/kg, the NOAEL for embryo toxicity is considered to be 200 mg/kg, because the number of viable fetuses per litter was not significantly affected at 200 mg/kg.

In an acceptable embryo-fetal development study in rats, the NOAEL for maternal toxicity was 35 mg/kg, because of decreased body weight gain, increased mortality and increased bleeding at 120 mg/kg. However, the NOAEL for placental effect was 10

mg/kg, because the incidence of necrotic placentas increased at ≥35 mg/kg. The NOAEL for fetal toxicity was 35 mg/kg, because fetal body weight and percentage of male fetuses decreased at 120 mg/kg. The percentage of fetuses with malformations for all dose groups was within the historical range and no individual malformation increased significantly with dose. However, ventricular septal defects (VSD) were observed in one fetus in each of the control, mid, and high dose groups, and VSD in conjunction with persistent truncus arteriosus was found in an additional fetus in the high dose group. However, the three fetuses with VSDs in the mid and high dose groups were sired by the same male. In addition, the literature indicates VSDs occur spontaneously in rats. The NOAEL for malformations was 120 mg/kg.

In an acceptable embryo-fetal development study in rabbits, the NOAEL for maternal toxicity was 2.5 mg/kg, because body weight gain decreased, and the incidence of abortions and necrotic placentas increased at ≥10 mg/kg. Since mortality of dams occurred at ≥40 mg/kg, the dose of 160 mg/kg was above a maximum tolerated dose. Each rivaroxaban treated group had one dam having total resorptions and dams displaying evidence of bleeding. Although the percentage of malformed fetuses increased at ≥40 mg/kg, the percentages were within the historical control range. Furthermore, the types and incidences of most malformations were similar to those in the historical control groups and did not display a dose-relationship. The litter incidence of ventricular septal heart defect at 160 mg/kg was slightly higher than the maximum historical control incidence. However, the incidence of this defect involved up to 2 fetuses in 2 litters in any one historical control group and has shown a trend of increased incidence in the most recent years of historical data provided. These malformations also occur regularly in the same strain of rabbit at other laboratories. Therefore, the NOAEL for malformations is 160 mg/kg. In contrast, the NOAEL for fetal toxicity was 2.5 mg/kg, because late resorptions and post-implantation loss increased at ≥10 mg/kg, and the number of live fetuses per litter and fetal body weight decreased at ≥40 mg/kg.

In an acceptable pre-/postnatal development study in rats, the NOAEL for F_0 maternal toxicity was 10 mg/kg, because mortality, abortions, bleeding, and decreased body weight gain were observed at 40 mg/kg. The incidence of stillborn F_1 pups increased at ≥10 mg/kg. The NOAEL for peri-natal fetal toxicity was 10 mg/kg, because survival between delivery and lactation day (LD) 4 decreased at 40 mg/kg. However, treatment of F_0 females with rivaroxaban did not affect postnatal survival after LD 4, sex ratio, body weight at birth, post-natal body weight development, or delay the general physical development of the F_1 offspring. Neurological assessments showed no significant effect of rivaroxaban treatment on F_1 reflexes, sensory functions, learning ability, memory and explorative behavior. Treatment of F_0 dams with rivaroxaban during pregnancy and lactation did not impair the fertility of the F_1 animals or the survival or development of the F_2 embryos through gestation and delivery.

Juvenile Wistar rats received daily oral doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban from postnatal day (PND) 4 through PND 26 in a pilot study and PND 10 through PND 105 in a main study. In the pilot study, body weight gain decreased in mid and high dose male and the high dose female groups. In the main study, body weight gains at study termination for the high dose males and females were greater than those

for the control males and females, although body weight gain decreased in the high dose groups during the second and third week of treatment. Near the end of the main study, the high dose males and females exhibited decreases in mean erythrocyte count and increases in mean MCH, MCHC and reticulocytes compared to the values for the concurrent control group. The increased mean absolute and relative adrenal weights in high dose males and increased mean absolute and relative liver weights in the mid and high dose males and the high dose females showed no histopathological correlates. However, the high dose males exhibited a slightly higher incidence of peri-insular hemorrhage, inflammatory infiltration, and fibrosis in the pancreas and a slightly higher incidence of colloidal alteration in the thyroid. Rivaroxaban administration did not affect the learning and memory of weanling rats. In the functional observational battery, mean body temperature was slightly lower in the high dose males and females compared to the controls. Although no significant effect was observed on either motor or locomotor activity, one low dose female displayed no habituation. The toxicokinetic data in these studies indicated a 6-10 fold higher exposure for rivaroxaban between PND 10 and 15 than on PND 31 or 86.

1.3 Recommendations

1.3.1 Approvability

NDA 202,439 for rivaroxaban is approvable from a nonclinical 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 rivaroxaban or satisfactory safety margins have been demonstrated relative to human therapeutic exposures. However, the label needs to warn women of child-bearing potential of rivaroxaban's embryo/fetal and peri-natal toxicity to offspring and the high risks for bleeding during labor and delivery.

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1.3.2 Additional Non Clinical Recommendations

No additional nonclinical recommendations.

1.3.3 Labeling

Because the goal date for the related NDA 22,406 approval preceded that of the current NDA 202,439, the nonclinical reviewers for both NDAs worked together and with other team members to negotiate with the sponsor on the nonclinical sections of the label indicated below. Because the dose of rivaroxaban differs for the two indications, the exposure ratios for the higher dose of 20 mg for the current NDA 202,439 are indicated in parentheses.

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Pregnancy Category C
There are no adequate or well-controlled studies of XARELTO in pregnant women, and dosing for pregnant women has not been established. Use XARELTO with caution in pregnant patients because of the potential for pregnancy related hemorrhage and/or emergent delivery with an anticoagulant that is not readily reversible. The anticoagulant

effect of XARELTO cannot be reliably monitored with standard laboratory testing. Animal reproduction studies showed no increased risk of structural malformations, but increased post-implantation pregnancy loss occurred in rabbits. XARELTO should be used during pregnancy only if the potential benefit justifies the potential risk to mother and fetus.

Rivaroxaban crosses the placenta in animals. Animal reproduction studies have shown pronounced maternal hemorrhagic complications in rats and an increased incidence of postimplantation pregnancy loss in rabbits. Rivaroxaban increased fetal toxicity (increased resorptions, decreased number of live fetuses, and decreased fetal body weight) when pregnant rabbits were given oral doses of 10 mg/kg rivaroxaban during the period of organogenesis. This dose corresponds to about 11 (4) times the human exposure of unbound drug, based on AUC comparisons at the maximum recommended human dose of 10 (20) mg/day. Fetal body weights decreased when pregnant rats were given oral doses of 120 mg/kg. This dose corresponds to about 40 (14) times the human exposure of unbound drug.

8.2 Labor and Delivery
Safety and effectiveness of rivaroxaban during labor and delivery have not been studied in clinical trials. However, in animal studies maternal bleeding and matemal and fetal death occurred at the rivaroxaban dose of 40 mg/kg (about 17 (6) times maximum human exposure of the unbound drug at the human dose of 10 (20) mg/day).

8.3 Nursing Mothers
It is not known if rivaroxaban is excreted in human milk. Rivaroxaban and/or its metabolites were excreted into the milk of rats. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from rivaroxaban, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

11 DESCRIPTION
Rivaroxaban, a factor Xa inhibitor, is the active ingredient in XARELTO Tablets with the chemical name 5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-1,3-oxazolidin-5-yl }methyl)-2-thiophenecarboxamide.

13 NON-CLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility
Rìvaroxaban was not carcinogenic when administered by oral gavage to mìce or rats for up to 2 years. The systemic exposures (AUCs) of unbound rivaroxaban in male and female mice at the highest dose tested (60 mg/kg/day) were 3- and 5-times (1 and 1.6 times), respectively, the human exposure of unbound drug at the human dose of 10 (20) mg/day. Systemic exposures of unbound drug in male and female rats at the highest dose tested (60 mg/kg/day) were 4- and 10-times (2- and 4-times), respectively, the human exposure.

Rìvaroxaban was not mutagenic in bacteria (Ames-Test) or clastogenic in V79 Chinese hamster lung cells in vitro or in the mouse micronucleus test in vivo.

No impairment of fertility was observed in male or female rats when given up to 200 mg/kg/day of rivaroxaban orally. This dose resulted in exposure levels, based on the unbound AUC, at least 33 (13) times the exposure in humans given 10 (20) mg rivaroxaban daily

2 Drug Information

2.1 Drug

CAS Registry Number: 366789-02-8

Generic Name: Rivaroxaban (Xarelto™)

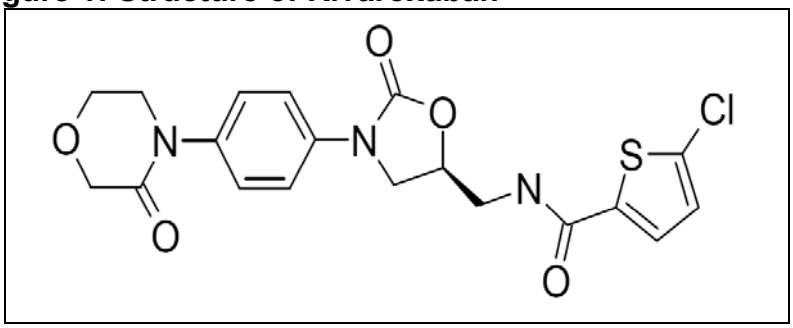
Code Names: JNJ-39039039, BAY 59-7939

Chemical Name: 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide

Molecular Formula/Molecular Weight: $C_{19}H_{18}ClN_3O_5S$/ 435.89 g/mol

Structure or Biochemical Description:

Figure 1: Structure of Rivaroxaban



Pharmacologic Class: Rivaroxaban is a Factor Xa inhibitor.

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA-022406 (Division of Hematology Products (DHP))
IND-064892 (DHP)
IND-075238 (Division of Cardiovascular and Renal Prosucts (DCRP))
IND- (b) (4)
MF-021580 (DRM)
MF-021581 (DRM)
MF-021592 (DRM)

2.3 Drug Formulation

For the proposed indication, rivaroxaban is formulated for oral administration as immediate release, film-coated tablets containing either 15- or 20-mg of active compound. The tablets also contain microcrystalline cellulose (b) (4) NF, croscarmellose sodium NF, hypromellose (b) (4) USP), lactose monohydrate NF, magnesium stearate (b) (4) NF, sodium lauryl sulfate NF, and (b) (4) as excipients. The commercially available film coating for the 15 mg tablet is Opadry Red (b) (4) containing hypromellose (b) (4) USP, polyethylene glycol (b) (4) 3350 NF, ferric oxide red NF, and titanium dioxide USP. The film coating for the 20 mg tablet is Opadry® II Dark Red (b) (4) containing partially hydrolyzed polyvinyl alcohol USP, polyethylene glycol (b) (4) 3350 NF, ferric oxide red NF, titanium dioxide USP, and talc USP.

2.4 Comments on Novel Excipients

All of the excipients, except Opadry® Red (b) (4), are commonly used in oral commercial pharmaceutical dosage forms and are compendial materials. The CMC Review of NDA 202439 by Pei-I Chu on 6/27/2011 indicates that the formulation contains no novel excipients and although the Opadry film coating powders are not compendial, the film coating powders are composed of compendial excipients.

2.5 Comments on Impurities/Degradants of Concern

The qualification of impurities in rivaroxaban was not specifically addressed in the nonclinical review of the related NDA 22406. The following paragraphs summarize the issues identified by the reviewer of the current NDA 202439. For additional information the reader is referred to the impurities review dated 02/17/11, the filing issues letter dated 3/17/11, the sponsor's response (SD 037) dated 4/8/11, and the discipline review letter of 05/04/11.

DMF 21581 indicates the rivaroxaban drug substance has three specified impurities and twelve unspecified impurities. The reviewers of NDA 22406 and 202439 agreed that the rivaroxaban impurities are qualified in terms of general toxicology. However, the nonclinical reviewer of NDA 22406 did not address the genotoxic qualification of these impurities.

The sponsor maintained that none of the potential impurities generated genotoxic alerts using computer applications capable of identifying structural alerts. To confirm the sponsor's statement, the CDER Computational Toxicology Group evaluated all rivaroxaban impurities. Although no structural alerts were identified for the rivaroxaban impurities by the Derek for Windows (DfW) application, some positive predictions for genotoxicity were obtained using other software applications.

Most of the impurities having positive genotoxic predictions were present at levels higher than (b) (4) for unspecified or (b) (4) for specified impurities in at least one of the three rivaroxaban lots used for genetic toxicology studies. However, one impurity, (b) (4) was present at only (b) (4) in one lot and was not detected in the other two lots used for genotoxicity testing.

Both the sponsor's and the FDA's QSAR analyses predicted the [redacted] impurity to be negative in the Ames test with MC4PC and found no structural alerts with Derek for Windows. However the FDA QSAR analysis gave a positive prediction for the in vivo micronucleus test for the [redacted] impurity using MC4PC. This latter prediction was the basis for the overall positive genotoxicity conclusion for the [redacted] impurity.

Despite this positive genetox conclusion, the reviewers acknowledged that the [redacted] impurity was present in the rivaroxaban lots used for carcinogenicity testing at concentrations up to [redacted] and that the Ames QSAR database is larger than the in vivo micronucleus QSAR database. Given the presence of the [redacted] impurity in the lots used for carcinogenicity testing and the lack of a positive finding in the QSAR analysis for the Ames test, the reviewers agreed that the [redacted] impurity in the rivaroxaban drug substance can be consider non-genotoxic and subject to the guidelines outlined in ICH-Q3A(R2). Although the maximum concentration of the [redacted] impurity in rivaroxaban lots used for carcinogenicity testing was only [redacted], the animal exposure to the [redacted] impurity was at least 7.8-fold higher than the human exposure (based on a 60 kg individual) to the impurity if it was present in the 20 mg dose at the maximum of [redacted] Additionally, the animal exposure to the [redacted] impurity in a 13-week rat study was >100-fold higher than the human exposure to the impurity if it was present in the 20 mg dose at the maximum of [redacted]

The sponsor also identified several starting materials or intermediates in the synthesis of rivaroxaban that tested as positive in the Ames test or had genotoxic alerts using computer applications capable of identifying structural alerts. Dr. Chopra reviewed the study report of an Ames test for [redacted](PH-34344). This synthesis intermediate was positive in the Ames test with three of five *Salmonella typhimurium* strains in the presence of metabolic activation. Study reports for the other genotoxic starting materials [redacted] or synthesis intermediate [redacted] of concern were not submitted for review.

The lower limit of detection for these genotoxic materials in the drug substance is [redacted] or less. Since the maximum dose of rivaroxaban for patients with atrial fibrillation is 20 mg/day, a patient will receive a maximum amount of [redacted] of [redacted] µg/day. This level is acceptable, since it is less than the threshold of toxicological concern of 1.5 µg/day indicated in the CDER Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (Dec 2008). Moreover, the sum of [redacted] and the three other genotoxic impurities will be [redacted] or less than [redacted]. The levels of these four materials are considered acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Rivaroxaban is being developed for the prevention and treatment of multiple thrombosis-mediated conditions. Under the current NDA 202439, rivaroxaban is proposed for the prevention of stroke and systemic embolism in patients with non-

valvular atrial fibrillation. The dosing regimen in the Phase 3 trial was 20 mg of rivaroxaban once a day in patients with normal renal function.

2.7 Regulatory Background

IND-64892 for rivaroxaban was submitted to DHP in May 2002. Rivaroxaban (BAY 59-7939) was proposed for the prophylaxis of deep vein thrombosis (DVT), which may lead to pulmonary embolism (PE) in patients undergoing knee or hip replacement surgery.

(b) (4)

In June 2006, the sponsor submitted IND 075238 to DCRP for the proposed indication of prophylaxis of stroke and non-CNS embolism in patients with non-valvular atrial fibrillation. In (b) (4)

The sponsor originally submitted NDA-022406 to DHP for prevention of venous thrombosis on 07/28/08. A complete response letter was issued on 5/27/09. The sponsor re-submitted NDA-022406 on 01/03/11. DHP approved rivaroxaban on 07/01/11 for the prophylaxis of deep vein thrombosis (DVT) which may lead to pulmonary embolism (PE) in patients undergoing knee or hip replacements.

The sponsor submitted NDA 202439 on 01/05/11 for the proposed use of rivaroxaban in preventing stroke and systemic embolism in patients with atrial fibrillation.

3 Studies Submitted

The study reports in GlobalSubmit Review are listed by document number, not by study report number. This review refers to all study reports by their document number.

The sponsor submitted the eight nonclinical study reports listed in Table 1 under NDA 202439. The sponsor referenced all of the nonclinical study reports previously submitted under NDA 22,406. These study reports are listed in Appendix 1. Most of these study reports were reviewed previously and are only briefly summarized in this review. However, a few study reports had not been fully reviewed and are discussed in this review. The study reports for the reproductive toxicology studies were independently reviewed, because of the need to appropriately address the sponsor's proposed label.

3.1 Studies Reviewed

Table 1: List of Nonclinical Study Reports Submitted to NDA 202,439

Document	Study Title
R-8562	The effect of rivaroxaban on catheter-induced clotting
R-8466	Potential of the Factor Xa Inhibitor Rivaroxaban (BAY 59-7939) for the Anticoagulation Management of Patients with Heparin-Induced Thrombocytopenia
PH-36090	BAY 59-7939 (Rivaroxaban): In vitro Studies in MDCKII-BCRP Cells to Evaluate the Substrate Characteristics of BAY 59-7939 for Human BCRP
PH-36243	Carcinogenicity Study in CD-1 Mice (2 Years Administration by Gavage)
PH-36242	Carcinogenicity Study in Wistar Rats (2 Years Administration by Gavage)
PH-36153	Pilot Study in Neonatal Rats (Repeated Administration by Gavage for 23 Days Starting on Postnatal Day 4)
PH36161	Mechanistic Study on Mitochondrial Protein Synthesis after Repeat- Dose (4-Week) Administration to Male Wistar Rats
PH-36347	Repeated Dose Study in Systemic Toxicity in Neonatal Rats (14-Weeks Daily Administration by Gavage Starting on PND 10)

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Table 2: Previous Reviews Referenced

Application	Reviewer	Review Date
IND 64892	S. Chakder	10/15/02
	S. Chakder	04/20/05
	S. Chakder	10/15/05
	S. Chakder	10/19/05
	Y. Chopra	03/02/06
	Y. Chopra	06/30/06
	Y. Chopra	12/06/06
	Y. Chopra	12/13/06
	Y. Chopra	02/27/07
NDA 22406	Y. Chopra	05/12/09
	P. Harlow	06/13/11

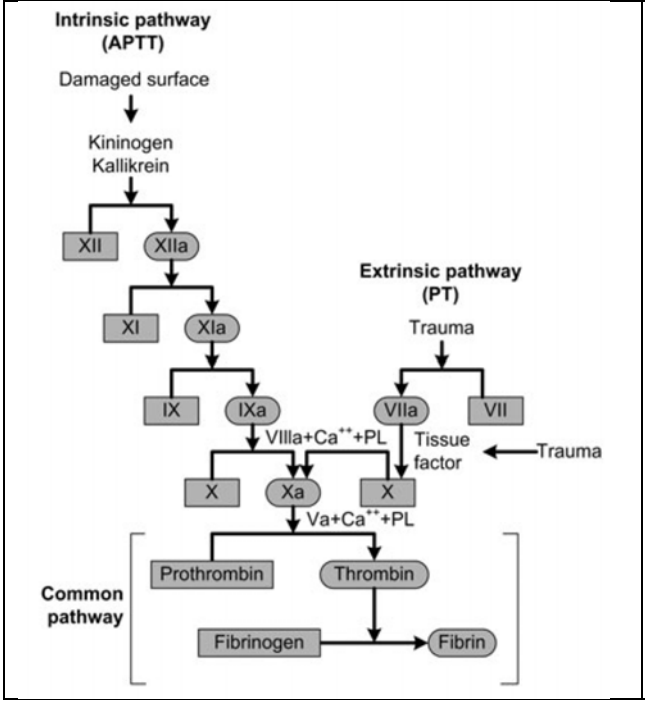
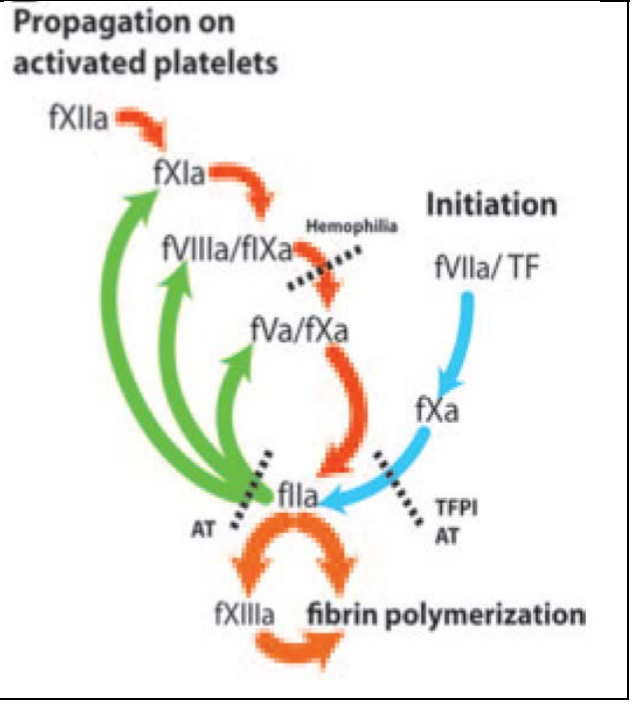
IND 75238	P. Harlow	03/06/07
IND (b) (4)	P. Harlow	03/06/07
NDA 202439	P. Harlow	02/17/11
	M. Jackson	05/11/11
	P. Harlow	06/13/11
	P. Chu	06/27/11

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action:
Rivaroxaban (BAY 59-7939) binds directly to and inhibits the activity of Factor Xa, the human plasma serine protease that converts prothrombin to thrombin. Thrombin (Factor IIa) is the serine protease that converts fibrinogen to fibrin in forming a blood clot. The inhibition of Factor Xa by rivaroxaban 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 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). Soluble FXa activates both PAR-1 and PAR-2 and 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 [Marfarlane et al. 2001]. FXa-dependent signaling can promote cell proliferation, cell migration and fibrosis as well as induce production of pro-inflammatory cytokines. Therefore, inhibition of FXa may have additional consequences beyond direct inhibition of clot formation.

Drug activity related to proposed indication:

In vitro studies

Rivaroxaban binds to purified human FXa with a Ki of 0.4 nM (PH-32009). Lineweaver-Burk analysis of FXa inhibition by rivaroxaban indicates rivaroxaban is a competitive inhibitor of FXa. The X-ray crystal structure of rivaroxaban in a complex with human FXa indicated that the critical active interactions of rivaroxaban are in the S1 and S4 specificity pockets of the active site (Roehrig et al. 2005). Therefore, the mechanism of rivaroxaban inhibition of FXa is independent of anti-thrombin III, unlike that of heparins. Kinetic analysis using purified human FXa indicated the inhibition of purified FXa by rivaroxaban was rapid and reversible (PH-35082). Rivaroxaban also inhibited human FXa activity with a slightly higher IC_{50} when present in the prothrombinase complex on washed human platelets. However, the inhibition of clot-bound FXa required an even higher IC_{50} of 75 nM (Depasse et al. 2005)

Table 3: Reviewer's Summary - PH-32009, PH-35082

Free human FXa enzyme assay	Ki	0.4 ± 0.02 nM
	Half-time	200 sec
	k_{on} k_{off}	$1.7x10^{7}\ M^{-1}\ s^{-1}$ $5\times10^{-3}\ s^{-1}$
	IC_{50}	0.7 nM
Prothrombinase bound FXa, IC_{50}		2.1 nM
Clot-bound FXa, IC_{50}		75 nM (Depasse et al. 2005)

The metabolites of rivaroxaban were evaluated in chromogenic enzyme activity assays using human FXa (PH-35076). The metabolites M-2 and M-7 inhibited FXa with IC_{50}

values of 2.3 and 89 nM, indicating they are 3.8-fold and 148-fold less potent, respectively, than rivaroxaban with an IC_{50} value of 0.6 nM. The metabolites M-1 and M-4 are weak inhibitors with IC_{50} values of 5840 and 52457 nM, respectively. The metabolites M-13, M-15, M-16, M-17 and M-18 are inactive with IC_{50} values greater than 100 µM.

To evaluate the inhibition of FXa from different animal species, endogenous FX in plasma was converted to FXa using Russel's Viper Venom. Although the IC_{50} for FXa in human plasma is 30-fold higher than the IC_{50} for purified FXa based on total concentration, the unbound concentration in human plasma (1.1 nM) is similar to that for purified FXa (0.7 nM). The unbound concentration of the IC_{50} for endogenous FXa in different species was higher than that for endogenous human FXa. These results are consistent with differences in the activation peptide and active site regions demonstrated between rabbit and human FXa (Edwards et al. 2002). Similarly, the concentration of rivaroxaban required to prolong by 2-fold the prothrombin (PT) and activated partial thromboplastin time (aPTT) coagulation assays was higher in the other species tested. However, comparison on the basis of unbound concentration indicates greater differences between human and either rabbit or dog than between human and either rat or mouse.

Table 4: Reviewer's Summary - In vitro Effects in Different Species - PH-32009

		Total concentration of BAY 59-7939, nM				
		Human	Rabbit	Rat	Mouse	Dog
IC_{50}, Purified FXa		0.7				
IC_{50}, Endogenous FXa in plasma		21	21	290	245	114
Concentration required to double	PT	230	120	300	150	230
	aPTT	690	1970	2090	570	1190
Fraction unbound		5.07%	23.4%	1.27%	6.45%	10.4%
		Unbound concentration of BAY 59-7939, nM				
IC_{50}, Endogenous FXa in plasma		1.1	4.9	3.7	15.8	11.8
Concentration required to double	PT	11.7	28	38	9.7	24
	aPTT	35	460	26.5	36.8	124

Rivaroxaban at 200 µM did not affect platelet aggregation in human platelet rich plasma induced by ADP (0.3 or 0.5 µg/mL), collagen (1 or 3 µg/mL), a thromboxane mimetic (U 46619 at 1 µg/mL), or the thrombin receptor activating peptide-6 (TRAP-6, 30 or 5 µg/mL). Platelet aggregation induced by γ- thrombin (3 or 5 µg/mL) was inhibited by rivaroxaban with an IC_{50} = 81 µM. However, in studies of thrombin generation induced by low concentrations of tissue factor, rivaroxaban at 10 and 20 nM prolonged by 2-fold the initiation phase of thrombin generation (lag time) in platelet-rich plasma and in whole blood, respectively (Gerotziafas et al. 2007). Similarly, Wong and Jiang (2010) found platelet aggregation induced by a low concentration of tissue factor in human platelet-rich plasma was inhibited by rivaroxaban with an IC_{50} of 8 nM.

Specificity for FXa

In vitro assays with purified enzymes indicated that rivaroxaban has high selectivity for FXa compared to related serine proteases (Table 5). Although rivaroxaban inhibited plasma kallikrein with an IC_{50} of 45 µM, selectivity for FXa remained >10,000 fold.

Table 5: Reviewer's Summary – Rivaroxaban Specificity

Document	Protease	Highest concentration	Selectivity for Fxa
PH-32009	Thrombin	69 µM	>10,000
	Factor XI	69 µM	
	Plasmin	69 µM	
	Urokinase	69 µM	
	Trypsin	69 µM (25%)	
	Activated Protein C	69 µM	
PH-33906	Tissue Plasminogen Activator (t-PA)	69 µM	>10,000
PH-33916	α-Chymotrypsin	69 µM	>10,000
PH-33918	Human Plasma Kallikrein	69 µM (60%) (IC_{50} = 45 µM)	>10,000
PH-34952	α and ß FXIIa	30 µM	>10,000

After oral or intravenous administration to animals, rivaroxaban showed *in vivo* anti-thrombotic activity in various arterial and venous thrombosis models in rats, mice, and rabbits as summarized in Table 6. The doses of rivaroxaban required to inhibit thrombus formation were lower in venous models than in arterial models.

Table 6: Reviewer's Summary – Pharmacology Studies

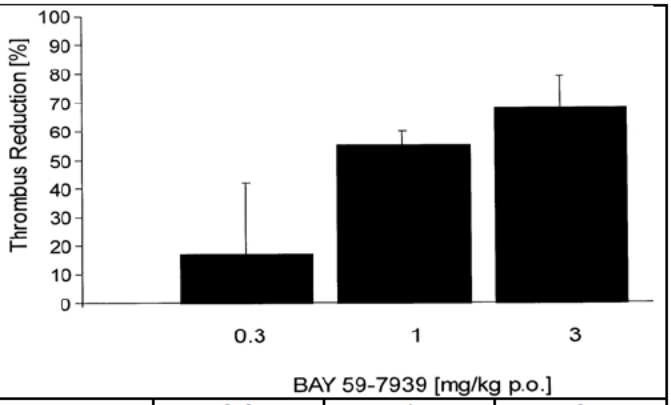
Document	Model	Species	Route	Doses, mg/kg	Result	Comment
PH-31612	Arterio-venous shunt	Rat	Oral	1, 2, 3, 6, 10	At ED_{50} = 6 mg/kg, PT increase 3.6 fold, FXa inhibited by 78%	Thrombus wt. at 1 mg/kg > control
PH-32791		Rabbit	Oral	0.3, 1, 3	See Figure 3 below	
PH-31614		Rabbit	IV	0.1, 0.3, 1, 3	58% inhibition of thrombus wt. at 1 mg/kg	PT increase 3.5 fold at 1 mg/kg
PH-31613	Mechanical injury-induced thrombosis	Rat	Oral	1, 3, 10	Carotid artery ED_{50} 10 mg/kg; Jugular vein ED_{50} 2 mg/kg	
PH-32794	$FeCl_3$-induced thrombosis	Rat	Oral, IV	1, 3 p.o. 3 i.v.	43% and 69% inhibition of thrombus wt. at 3 mg/kg p.o. and 3 mg/kg i.v	
PH-34125		Mouse	IV	0.3, 1, 3	44% inhibition of thrombus wt at 1 mg/kg	
R-8473 R-8473A	Electrolytic-ally injured carotid artery thrombosis	Rat	IV	0.3, 1, 3	At 1 mg/kg, mean time to occlusion > 30 min, control 13 min. and 80% arteries non-occluded	PT increase 3 fold, TAT inhibited by 75%

Document	Model	Species	Route	Doses, mg/kg	Result	Comment
PH-32793	Venous stasis	Rat	IV	0.03, 0.1, 0.3, 1	At 0.3 mg/kg, 86% inhibition of thrombus wt. at 0.3 mg/kg	3.2 fold PT increase, 65% inhibition FXa at 0.3 mg/kg
PH-33917	Thromboem-bolic death	Mouse	IV	0.03, 0.1, 0.3, 1.0	At 0.3 mg/kg, survival rate was 47% and FXa inhibited 45%	
PH-35083	Tissue factor-induced hypercoagulability	Rat	IV	0.0009, 0.0027, 0.009, 0.027, 0.090, 0.270, 0.900	At 0.27 mg/kg, TAT was inhibited 85%, PT increase 2 fold, and FXa activity inhibited by 40%	Mean rivaroxaban concentration was 481 µg/L at 0.27 mg/kg
PH-31659	Tail bleeding time (BT)	Rat	Oral	3, 6, 10 mg.kg	At 3 mg/kg BT not change and PT increased 2-fold	At 6 mg/kg 2-fold increase BT, PT increase 4.4-fold
PH-32792	Ear bleeding time	Rabbit	Oral, IV	0.3, 1, 3	75 min after dosing, 1 mg/kg increase BT 2-fold, but no change at 0.3 mg/kg	

PT = prothrombin time

As an example of the efficacy of rivaroxaban demonstrated in the pharmacology studies, Figure 3 summarizes the results of the rabbit arterio-venous shunt model (PH-32791). Rivaroxaban at 1 mg/kg inhibited FXa by 91%, reduced thrombus formation by 55%, and prolonged PT 1.3 fold. The unbound plasma concentration of rivaroxaban for individual animals ranged from 6.6 to 16 µg/L, which is comparable to the unbound concentration of rivaroxaban (14 µg/L) at the Cmax (274 µg/L) in patients with atrial fibrillation. These results suggest that rivaroxaban at appropriate concentrations would likely be anti-thrombotic in humans. In a separate study (PH-32792) in rabbits, rivaoxaban at 1 mg/kg did not increase bleeding time at 105 minutes after dose administration, but did induce a 2-fold increase in bleeding time at 75 minutes after dose administration. Therefore, the margin of safety concerning bleeding is narrow.

Figure 3: Reviewer's Summary – Rabbit AV Shunt Model

Inhibition of clot formation in rabbit arteriovenous shunt model (PH-32791) (Thrombus induced in shunt with nylon string 90-105 min. after oral administration of rivaroxaban) * p< 0.05					
Rivaroxaban dose, mg/kg			0.3	1	3
Reduction in thrombus weight, %			17	55*	68*
Prolongation of aPTT ex vivo, fold			1.1	1.3*	1.7*
Prolongation of PT ex vivo, fold			1.1	1.3*	2.1*
Inhibition Fxa, %			59*	91*	100*
Total rivaroxaban plasma conc, µg/L			9-21	28-69	55-465
Unbound rivaroxaban conc., µg/L			2.1-4.9	6.6-16	13-109
Rabbit ear bleeding, fold increase at indicated time after oral dose (PH-32792)	75 min.		1.1	2.0	2.1*
	90 min		1.4	1.7	1.6
	105 min		1.1	1.1	1.3*

Study title: The Effect of Rivaroxaban on Catheter-Induced Clotting

Document no.: R-8562
Study no.: EDMS-ERI-14389810
Study report location: NDA 202439 EDR, Module 4
Conducting laboratory and location: (b) (4)
Date of study initiation: April 2, 2009
GLP compliance: Not indicated
QA statement: Not present
Drug, lot #: a) BAY 59-7939 (rivaroxaban), lot not indicated
Note: purity and lot numbers were not provided
b) fondaparinux,
c) enoxaparin,
d) heparin
e) tick anticoagulant protein (TAP)

Coronary catheters (Mach 1, 6F, 2 mm) were cut into 1.5 cm segments, which were placed next to the outer walls of wells of a 96-well plate. Citrated plasma was prepared from blood obtained from healthy donors. Aliquots of plasma and varying concentrations of rivaroxaban, fondaparinux, enoxaparin, heparin or TAP were added to wells of a 96-well plate that either did or did not contain a catheter segment. After incubation for 15 min, clotting was initiated by addition of $CaCl_2$ and turbidity was monitored at 405 nm for 5 hours.

The presence of coronary catheter segments decreased the mean clotting time from 992 seconds to 508 seconds. The concentrations of rivaroxaban and fondaparinux

required to achieve a clotting time of 4000 sec were similar. However, the concentrations of enoxaparin (5.6 µM), TAP (4.7 µM), and heparin (0.4 µM) required to achieve a clotting time of 4000 sec in the presence of catheter segments were lower than that of rivaroxaban (12.1 µM). This rivaroxaban concentration is above the Cmax of rivaroxaban in patients with atrial fibrillation (274 µg/L or 0.6 µM). Therefore, rivaroxaban is not as potent as heparin or enoxaparin at preventing catheter-induced clotting in vitro.

4.2 Secondary Pharmacology

In vitro binding of rivaroxaban to a panel of physiologically important receptors (R-6463) and enzymes (R-8462) was tested at a single concentration of 6.9 µM. Binding of reference compounds to the receptor panel was minimally inhibited by rivaroxaban with the greatest inhibition being 34% to the β2-adrenergic receptor. In the panel of in vitro enzyme assays, rivaroxaban significantly inhibited only FXa and thrombin activity. However, in different in vitro assays (Table 5), rivaroxaban did not inhibit thrombin at 69 µM. Data submitted for a (b) (4) of rivaroxaban indicated that this compound had no effect on 5-HT_{1A} and 5-HT_3 receptors or the serotonin transporter.

Table 7: Reviewer's Summary – Relevant Results from R-8462, R-8463

Enzyme/receptor (IC_{50} of reference compound)	Species	Ligand/Substrate	Conc. µM	% inhibition*
Rivaroxaban, BAY 59-7939 (BR-4093†)				
Enzyme				
Factor Xa (1 µM)	Human	N-α-Z-D-Arg-Gly-Arg-pNA	6.9	99*
Factor Iia, thrombin (0.11 nM)	Human	Z-Gly-Pro-Arg-AMC	6.9	88*
Factor VIIa (1.85 µM)	Human	N-CH3-SO2-D-Phe-Gly-Arg-4-nitranilide	6.9	33
CYP450, 2C9 (583 nM)	Human	3-Cyano-7-ethoxycoumarin	6.9	27
MEK1 (1.7 nM)	Rabbit	Myelin basic protein	6.9	24
Protein Ser/Thr kinase PKBα/Akt1 (0.22 nM)	Human	Crosstide	6.9	22
Monoamine oxidase A (3.1 nm)	Rat brain	[^{3}H]-serotonin	6.9	9
Receptor				
Adrenergic β1 (0.94 nM	Human	[^{125}I] Cyanopindolol	6.9	21
Adrenergic β2 (0.31 nM)	Human	[^{3}H] CGP-12177	6.9	34
Monoamine transporter (4.5 nM)	Rabbit	[^{3}H] Dihydrotetrabenazine	6.9	22
Neuropeptide Y2 (0.14 nM)	Human	[^{125}I] Peptide YY	6.9	21
Serotonin, 5-HT_1, nonselective (3 nM)	Rat brain	[^{3}H]-serotonin	6.9	-10
Serotonin, 5-HT_2, nonselective (16.8 nM)	Rat brain	[^{3}H]-ketanserin	6.9	14
(b) (4)				
Serotonin transporter (10 nM)	Human	[^{3}H]-paroxetine	10	4
Serotonin, 5-HT_{1A} (2 nM)	Human	[^{3}H] 8-OH-DPAT	10	3
Serotonin, 5-HT_3 (16.8 nM)	Human	[^{3}H] GR-65630	10	-5
Significant inhibition or stimulation considered >50%, †Molecular weight assumed to be 300, therefore, reviewer adjusted concentration,‡ (b) (4) of rivaroxaban, molecular weight of (b) (4) Data from R 8463, R 8462 and SD 59 (05/20/11)				

Rivaroxaban has structural similarity to the oxazolidinone derivative linezolid, which has antibacterial activity. For each of three Gram positive bacterial strains, the minimal

inhibitory concentration (MIC) of rivaroxaban and its metabolites M-1, M-2, and M15 was greater than >128 µg/mL (PH-35331). In contrast, the MIC of linezolid ranged from 0.5 to 4 µg/mL. Furthermore, co-incubation of rivaroxaban and linezolid at various concentrations did not affect the antibacterial activity of linezolid (PH-35332). These studies indicate that rivaroxaban does not have antibacterial activity.

4.3 Safety Pharmacology

Rivaroxaban was evaluated in a battery of safety pharmacology studies, which were reviewed previously by Drs. Chakder and Chopra under IND 64,892 and NDA 22,406.

In vitro studies showed no effect of rivaroxaban at concentrations up to 10 µM on the hERG channel or prolongation of action potential. In anesthetized beagle dogs, rivaroxaban had no effect on the cardiovascular function (heart rate, cardiac output, left ventricular pressure, dP/dt, and left ventricular end-diastolic pressure), ECG parameters (PQ, QRS and QT intervals), respiration, acid/base balance, hematocrit and electrolytes. In rats, rivaroxaban did not affect the central nervous system (behavior, body temperature), the convulsive threshold dose of pentylenetetrazol, duration of hexobarbital-induced anesthesia, urine volume, urinary electrolytes, hematological parameters or gastrointestinal motility. However, rivaroxaban significantly delayed the nociceptive response in rats at doses of 10 mg/kg and above.

Although unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) are generally the current standards of care for patients requiring anticoagulation, their use is restricted in patients that develop heparin-induced thrombocytopenia (HIT). These patients have antibodies to a complex of heparin/platelet factor 4 (PF4) that can result in platelet activation, a hypercoagulable state with decreased platelet counts, and severe thrombotic complications, including death (Kelton 2002). The following study evaluated whether rivaroxaban interacts with HIT antibodies and induces platelet activation.

Study title: Potential of the Factor Xa Inhibitor Rivaroxaban (BAY 59-7939) for the Anticoagulation Management of Patients with Heparin-Induced Thrombocytopenia (HIT)

Document no.:	R-8466
Study no.:	EDMS-ERI-14285442
Study report location:	NDA 202439 EDR, Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not indicated; report date December 2006
GLP compliance:	Indicated
QA statement:	Not present
Drug, lot #: Note: purity was not provided	f) BAY 59-7939 (rivaroxaban), Lot BX018AW g) Unfractionated sodium heparin, lot # 096026. h) Enoxaparin, Sanofi-Aventis, lot # 89445. i) Fondaparinux, Sanofi-Synthelabo, lot # 0010000003. j) Hypersulfated form of pentasaccharide from heparin, a gift from (b) (4) k) Argatroban, Texas Biotechnology Corp, lot # 3601. l) Melagatran, AstraZeneca, lot # 84004

Sera and platelets in multiple combinations from 89 different patients with established ELISA positive HIT antibodies were used to evaluate various concentrations of rivaroxaban in several types of platelet assays relative to control compounds. Three platelet function assays included a ^{14}C-serotonin release assay, drug-induced platelet aggregation using a light-transmission aggregometer, and flow cytometric analysis of platelet activation that evaluated platelet microparticle generation, P-selectin expression on platelets, and platelet aggregate formation. Additional studies examined binding to platelet factor 4 (PF4) as well as the release of PF4 from resting and tissue factor (TF) activated platelets in whole blood and platelet-rich plasma (PRP). Experiments were performed immediately after blood collection.

The results from these studies were presented only as summary figures and are summarized in Table 8. Rivaroxaban did not show a positive response with any of the HIT patient sera in any of the assays. In contrast, heparin and enoxaprin produced the expected positive responses and argatroban produced expected negative responses. Although fondaparinux produced negative responses in most of the assays, it was positive with 1 of 18 sera positive for HIT antibodies in the platelet aggregation assay using an aggregometer. Rivaroxaban did not interact with PF4 based on the lack of an effect in FXa inhibition. Not only did rivaroxaban not induce PF4 release from resting platelets, but it also reduced the release of PF4 from TF activated platelets by 20-30%.

Table 8: Reviewer's Summary – R8466 Study Results

	Result, drug concentration range tested, number of HIT sera/platelets)				
Assay (Figure # in Document R-8466)	**Rivaroxaban**	**Heparin**	**Enoxaparin**	**Fondaparinux**	**Argatroban**
^{14}C-serotonin release study 1 (Figure 3, 5)	Negative 0.1-10 µg/mL, 51	Positive^ 0.1-100 U/ mL, 8	Positive^ 0.1-100 U/ mL, 8		
^{14}C-serotonin release study 2 (Figure 4)	Negative 0.1-10 µg/mL, 28)			Negative 0.1-100 µg/mL, 10	Negative 0.1-100 µg/mL, 10
Platelet aggregation – aggregometer study 1 (Figure 6)	Negative 0.02- 20 µg/mL, 12	Positive^ 0.1-100 U/mL 12			
Platelet aggregation – aggregometer study 2 (Figure 7)	Negative 100% *, 18	Positive^ 100% *, 18	Positive^ 13/18 *, 18	Positive 1/18 *, 18	
Flow cytometry – microparticle formation (Figure 8, 9, 10, 11)	Negative 0.02- 20 µg/mL, 21 + 5	Positive^ 0.1-100 U/mL 17 + 4	Positive^ 0.1-100 U/mL 4	Negative 0.1-100 µg/mL, 5	Negative 0.1-100 µg/mL, 5
Flow cytometry – platelet aggregation (Figure 8, 9, 10, 11)	Negative 0.02- 20 µg/mL, 21 + 5	Positive†^ 0.1-100 U/mL 17 +		Negative 0.1-100 µg/mL, 5	Negative 0.1-100 µg/mL, 5
Flow cytometry – % P-selectin positive platelets (Figure 8, 9, 10, 11)	Negative 0.02- 20 µg/mL, 21 + 5	Positive^ 0.1-100 U/mL 17 + 4	Positive^ 0.1-100 U/mL 4	Negative§ 0.1-100 µg/mL, 5	Negative 0.1-100 µg/mL, 5
Flow cytometry – P-selectin MFI‡ (Figure 8, 9, 10, 11)	Negative 0.02- 20 µg/mL, 21 + 5	Positive^ 0.1-100 U/mL 17		Negative 0.1-100 µg/mL, 5	Negative 0.1-100 µg/mL, 5
PF4 release – resting platelets in whole blood (Figure 12)	Negative 1 µg/mL, 5	Positive 5 µg/mL, 5	Positive 5 µg/mL, 5	Negative 1 µg/mL, 5	
PF4 release – resting platelets in PRP (Figure 13)	Negative 1 µg/mL, 5	Positive 5 µg/mL, 5	Positive 5 µg/mL, 5		Negative 1 µg/mL, 5
PF4 release – TF activated platelets in whole blood (Figure 14)	Negative 1 µg/mL, 5 PF4 release inhibited	Positive 5 µg/mL, 5	Positive 5 µg/mL, 5	Negative 1 µg/mL, 5	
PF4 release – TF activated platelets in PRP (Figure 15)	Negative 1 µg/mL, 5 PF4 release inhibited	Positive 5 µg/mL, 5	Positive 5 µg/mL, 5		Negative 1 µg/mL, 5 PF4 release inhibited
PF4 binding – drug activity measured (Figure 16)	Negative 1 µg/mL, 3	Positive 5 µg/mL, 3	Positive 5 µg/mL, 3	Negative 1 µg/mL, 3	Negative 1 µg/mL, 3

^ Heparin and enoxaprin were positive at the lower concentrations used, * No concentrations were provided for these experiments, § Although fondaparinux was considered negative for induction of P selectin positive platelets, the percentage of P selectin positive platelets was non statistically significantly higher at 1 µg/mL than at 0.1, 10 or 100 µg/mL and the negative saline control was not shown, † Platelet aggregation was statistically decreased by heparin using flow cytometry and considered consistent with the increase in platelet microparticle formation, ‡MFI = mean fluorescence intensity

The sponsor argues that rivaroxaban showed anti-thrombotic activity without prolongation of bleeding time in rats and rabbits. However, the safety margin for bleeding is minimal in the rabbit as shown in Figure 3. In the rat arteriovenous shunt

model (PH-31612), the rivaroxaban dose of 3 mg/kg produced a 41% decrease in thrombus weight. In a separate rat study (PH-31659), the dose of 3 mg/kg did not increase in tail bleeding time, although PT was prolonged 2-fold. However, at only a 2-fold higher dose (6 mg/kg), the bleeding time increased 2 fold. Thus, the safety margin for bleeding is minimal in both the rat and the rabbit.

Table 9: Reviewer's Summary of Antithrombotic Activity and Bleeding in Rats

Document	PH-31659		PH-31612		
Dose, mg/kg	Bleeding time, fold	Fold PT prolongation	% reduction thrombus weight	Fold PT prolongation	% inhibition FXa activity†,
1	-	-	-14 (7)	1.7 (0.2)*	38 (5)
2	-	-	16 (7)*	1.8 (0.1)*	46 (3)
3	1.0	2.0*	41 (7)*	2.4 (0.2)*	61 (2)
6	2.0*	4.4*	51 (6)*	3.7 (0.4)*	78 (2)
10	2.7*	3.6*	73 (2)*	5.1 (0.1)*	89 (2)

P< 0.05; † No statistical evaluation

4.4 Pharmacodynamic drug interactions:

Pharmacodynamic drug interactions were evaluated either in the rat arteriovenous shunt model or in the rat tail bleeding model. In the rat arteriovenous shunt model, coadministration of rivaroxaban with either heparin or enoxaparin produced additive or less than additive effects on reduction of thrombosis formation depending on the dosages used. Coadministration of rivaroxaban with either acetylsalicylic acid or clopidogrel or the combination of both also produced additive effects in the dosage ranges tested. In general, the inhibition of thrombus formation was less than fully additive. However, the increases in bleeding time varied depending on the combination and the dosages used. For example, in study PH-34970 combinations of ASA and rivaroxaban did not affect bleeding time, but the inhibition of thrombus formation was less than additive. Although combinations with clopidogrel also increased the reduction in thrombus formation, more than additive effects were observed for increases in bleeding time, indicating potential for a narrowing of the therapeutic margin with combination anti-platelet therapy.

Table 10: Reviewer's Summary of Pharmacodynamic Interactions

Document	Study Description	Model	Results
PH-32386	Interaction with enoxaparin and heparin	Rat arterio-venous shunt model, oral dose	Interaction with heparin was additive in decreasing thrombus formation. Interaction with enoxaparin was not fully additive and depended on the dosages used
PH-34950	Interaction with acetylsalicylic acid (ASA)	Rat arterio-venous shunt with BT, i.v. dose	Interaction with ASA was less than fully additive in decreasing thrombus formation and BT only slightly increased
R-8474	Interaction Between BAY 59-7939 and 3 Antiphlogistic Drugs (ASS, Diclofenac and Naproxen)	Bleeding time (BT) in the anesthetized rat (oral dose)	BAY 59-7939 significantly prolonged BT at 3 and 10 mg/kg, but not 1 mg/kg. Prolonged BT with ASS (30, 100 mg/kg) or naproxen (20, 80 mglkg), but not with diclofenac (1, 3 and 10 mg/kg). No clear additive effects on BT when BAY 59-7939 combined with ASS (10, 30 mg/kg) or diclofenac (1, 3 mg/kg). However, some additive effects on BT when BAY 59-7939 combined with naproxen (5, 20 mg/kg).
PH-32735	Interaction between BAY 59-7939 and acetylsalicylic acid (ASA)	BT in the anesthetized rat (oral dose)	1, 3, 10 mg/kg BAY 59-7939 or 10, 30, 100 mg/kg ASA dose-dependently prolonged BT. Co-administration produced additive or more than additive effects depending on dosages
PH-32913	Interaction Between BAY 59-7939 and Naproxen	BT in the anesthetized rat (oral dose)	1, 3, 10 mg/kg BAY 59-7939 or 5, 20, 80 mg/kg naproxen dose-dependently prolonged BT. Co-administration produced additive or more than additive effects depending on dosages
PH-32914	Interaction Between BAY 59-7939 and Diclofenac	BT in the anesthetized Rat (oral dose)	1, 3, 10 mg/kg BAY 59-7939 or 1, 3, 10 mg/kg diclofenac dose-dependently prolonged BT. Co-administration produced additive or less than additive effects depending on dosages
PH-32948	Potential interaction with warfarin	BT in the anesthetized rat (oral dose)	1, 3, 10 mg/kg BAY 59-7939 or 0.1, 0.3, 0.5 mg/kg warfarin dose-dependently prolonged BT. Co-administration produced additive or less than additive effects depending on dosages
PH-34951	Interaction with clopidogrel	Rat arterio-venous shunt model & BT, i.v. dose	BAY 59-7939 interaction with clopidgrel was less than fully additive in decreasing thrombus formation. Depending on the dosages used, increase in BT was either slightly changed or more than additive.
PH-32946	Interaction with clopidogrel	BT in the Anesthetized rat (oral dose)	BT prolonged by both BAY 59-7939 and clopidgrel. Co-administration increased BT either in additive or less than additive manner.
PH-34970	Interaction with ASA & clopidogrel	Rat arterio-venous shunt model & BT, i.v.	Various combinations of rivaroxaban, clopidogrel and ASA generally produced less than fully additive effects on inhibition of thrombus formation. BT increased less for rivaroxaban /ASA combinations than for combinations with clopidogrel

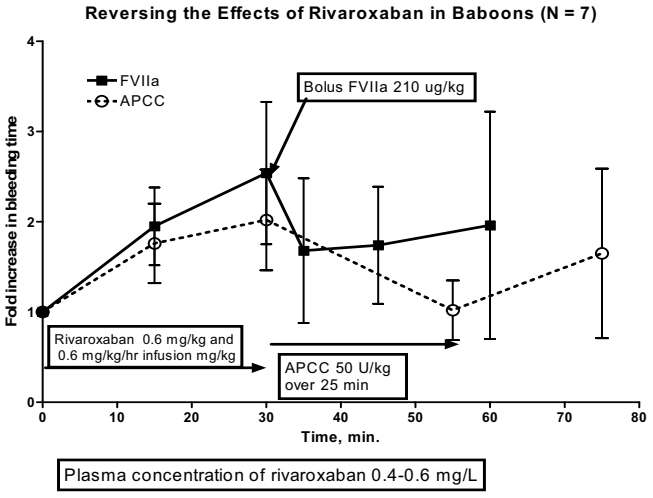
BT = bleeding time

Study title: Hemostatic effects of activated factor VII and activated prothrombin complex concentrate in rivaroxaban-anticoagulated primates

Document no.:	R-8472
Study no.:	GDD-GTR-TCH-ACR
Study report location:	NDA 22406 EDR, Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not indicated; report date March 2008
GLP compliance:	Not indicated
QA statement:	Not present
Drug:	a) BAY 59-7939 (rivaroxaban)
Note: Lot numbers and purity were not provided	b) Human FVIIa (NovoSeven, NovoNordisk) c) activated prothrombin complex concentrate (APCC, FEIBA VH, Baxter Healthcare)

This study, briefly reviewed by Dr. Chopra, indicated that administration of FVIIa and FEIBA VH reversed the template bleeding time (BT) in baboons treated with rivaroxaban. However, the current reviewer noted that overall 0.2 mg/kg FVIIa or 50 U/Kg APCC only partially reversed the 2-2.5 fold increase in BT induced by rivaroxaban as indicated in Table 11. Five minutes after FVIIa administration, the mean BT decreased to 1.68 fold of baseline compared to 2.54 fold of baseline at the end of rivaroxaban administration. At the end of APCC administration, the mean BT decreased to 1.02 fold of baseline compared to 2.02 fold of baseline at the end of rivaroxaban administration. Although four animals at the end of APCC treatment and one animal treated with FVIIa had BTs less than baseline, some individual animals, particularly those treated with FVIIa, showed little reversal of BT. In addition, the reversal of BT prolongation induced by rivaroxaban was not sustained in some animals after treatment termination.

Table 11: Reviewer's Summary – Bleeding Times (BT) in R-8472



Summary of individual animals	
FVIIa - Maximum % fold BT decrease (#)	# with BT increase 30 min post FVIIa bolus (value)
100% (3)	1 (4.5 fold of baseline BT)
59-70% (2)	1 (2 fold of baseline BT)
23-42% (2)	2 (2, 2.3 fold of baseline BT)
APCC- Maximum % fold BT decrease	# with BT increase 20 min post APCC infusion
100% (5)	3 (1.1, 1.5, 3.4 fold of baseline BT)
63% (1)	1 (2.5 fold of baseline BT)
42% (1)	

Study title: Recombinant factor VIIa (NovoSeven) reduced the bleeding time prolongation induced by rivaroxaban (BAY 59-7939) in anaesthetized rats

Document no.:	PH-34870
Study no.:	GDD LGO PRR Pharmacology
Study report location:	NDA 22406 EDR, Module 4
Conducting laboratory and location:	Pharma R&D Discovery Research, Bayer HealthCare AG, Wuppertal, Germany
Date of study initiation:	Not indicated; report date March 2007
GLP compliance:	Not indicated
QA statement:	Not present
Drug:	a. BAY 59-7939 (rivaroxaban)
Note: Lot numbers and purity were not provided	b. Human FVIIa, recombinant (NovoSeven, NovoNordisk)

The ability FVIIa to reverse prolongation of bleeding time induced by rivaroxaban was tested in the mesenteric artery bleeding model in Wistar rats. Bleeding times were obtained from three vessel incisions before treatment (baseline) and one after intravenous administration of vehicle, rivaroxaban alone or rivaroxaban with FVIIa (Novo Nordisk) at two concentrations. In one experiment, rivaroxaban and FVIIa were added simultaneously and the cut was made 5 minutes later. In the other experiment, the cut was made 5 minutes after rivaroxaban administration and FVIIa was administered 1 minute later. Table 12 shows that FVIIa partially reversed the prolonged bleeding time in rats treated with rivaroxaban after delayed administration. However, the highest dosage of FVIIa (400 µg/kg) only reduced by 50% the prolonged BT induced by rivaroxaban.

Table 12: Sponsor's Summary - Bleeding Times in PH-34870

Rivaroxaban and FVIIa simultaneous administration

	Number of animals	Change in bleeding time (x-fold vs.baseline)
Vehicle	11	0.99 ± 0.02
Rivaroxaban 2 mg/kg	10	3.57 ± 0.87
Rivaroxaban 2 mg/kg + Novoseven 100 µg/kg	10	2.42 ± 0.39
Rivaroxaban 2 mg/kg + Novoseven 400 µg/kg	10	1.85 ± 0.35*

* $P < 0.05$ vs. rivaroxaban 2 mg/kg

Delayed administration of FVIIa

	Number of animals	Change in bleeding time (x-fold vs.baseline)
Vehicle	11	0.99 ± 0.02
Rivaroxaban 2 mg/kg	9	3.49 ± 0.65
Rivaroxaban 2 mg/kg + Novoseven 100 µg/kg	10	2.45 ± 0.25
Rivaroxaban 2 mg/kg + Novoseven 400 µg/kg	9	1.71 ± 0.15**

** $P < 0.01$ vs. rivaroxaban 2 mg/kg

Study title: FEIBA (Factor Eight Bypassing Activity) reduced the bleeding time prolongation induced by rivaroxaban (BAY 59-7939) in anaesthetized rats

Document no.:	PH-34871
Study no.:	GDD LGO PRR Pharmacology
Study report location:	NDA 22406 EDR, Module 4
Conducting laboratory and location:	Pharma R&D Discovery Research, Bayer HealthCare AG, Wuppertal, Germany
Date of study initiation:	Not indicated; report date April 2007
GLP compliance:	Not indicated
QA statement:	Not present
Drug:	c. BAY 59-7939 (rivaroxaban)
Note: Lot numbers and purity were not provided	d. Factor VIII-Inhibitor Bypassing Activity (FEIBA)(Baxter BioScience)

The ability of FEIBA to reverse prolongation of bleeding time induced by rivaroxaban was tested in the mesenteric artery bleeding model in Wistar rats. In each rat bleeding times were obtained from three vessel incisions before treatment (baseline) and one after intravenous administration of vehicle, rivaroxaban alone or rivaroxaban with FEIBA at two concentrations. In one experiment, rivaroxaban and FEIBA were added simultaneously and the cut was made 5 minutes later. In the other experiment, the cut was made 5 minutes after rivaroxaban administration and FEIBA was administered 1 minute later. Table 13 shows that FEIBA partially reversed the prolonged bleeding time in rats treated with rivaroxaban after delayed administration. However, the highest dosage of FEIBA (100 U/kg) only reduced BT by approximately 50%.

Table 13: Sponsor's Summary - Bleeding Times - PH34871

Simultaneous application of rivaroxaban and FEIBA

	Number of animals	Change in bleeding time (x-fold vs.baseline)
Vehicle	10	0.99 ± 0.02
Rivaroxaban 2 mg/kg	10	2.82 ± 0.31
Rivaroxaban 2 mg/kg + FEIBA 50 U/kg	10	1.80 ± 0.12**
Rivaroxaban 2 mg/kg + FEIBA 100 U/kg	12	1.61 ± 0.14**

** $P < 0.01$ vs. rivaroxaban 2 mg/kg

Application of FEIBA 1 minute after rivaroxaban

	Number of animals	Change in bleeding time (x-fold vs.baseline)
Vehicle	10	0.99 ± 0.02
Rivaroxaban 2 mg/kg	10	2.98 ± 0.36
Rivaroxaban 2 mg/kg + FEIBA 50 U/kg	10	1.73 ± 0.20*
Rivaroxaban 2 mg/kg + FEIBA 100 U/kg	10	1.43 ± 0.07***

* $P < 0.05$, *** $P < 0.001$ vs. rivaroxaban 2 mg/kg

Study title: Prothrombin complex concentrate reduced the bleeding time prolongation induced by rivaroxaban (BAY 59-7939) in anaesthetized rats

Document no.:	PH-35374
Study no.:	Not indicated
Study report location:	NDA 22406 EDR, Module 4
Conducting laboratory and location:	Pharma R&D Discovery Research, Bayer HealthCare AG, Wuppertal, Germany
Date of study initiation:	Not indicated; report date May 2008
GLP compliance:	Not indicated
QA statement:	Not present
Drug:	a. BAY 59-7939 (rivaroxaban)
Note: Lot numbers and purity were not provided	b. prothrombin complex concentrate (PPC), (Beriplex®, CSL Behring)

The ability of PPC to reverse prolongation of bleeding time induced by rivaroxaban was tested in the mesenteric artery bleeding model in Wistar rats. In each rat bleeding times were obtained from three vessel incisions before treatment (baseline) and one after intravenous administration of vehicle, rivaroxaban alone or rivaroxaban with PPC at two concentrations. Rivaroxaban pretreatment time was not specified. PPC was administered 1 minute after the cut was made. Table 14 shows that PPC at 50 U/kg partially reversed the prolonged bleeding time by 72% in rats treated with rivaroxaban; however PPC at 25 U/kg was ineffective.

Table 14: Sponsor's Summary - Bleeding Times - PH35374

Treatment	Bleeding time baseline (seconds)	Bleeding time post-treatment (seconds)	Bleeding time relative to baseline (x-fold)
Rivaroxaban 2 mg/kg vehicle	162±10	861±213$^{\$\$\ 1}$	5.4±1.4
Rivaroxaban 2 mg/kg Beriplex® 50 U/kg	165± 6	242± 56*$^{\ \dagger}$	1.5 ± 0.4
Rivaroxaban 2 mg/kg Beriplex® 25 U/kg	196±11	991±184$^{\$\$\ 2}$	5.3± 1.1

Values are presented as mean ± SEM from 7 animals per group (rivaroxaban plus vehicle) and from 10 animals per group (rivaroxaban plus Beriplex® 50 or 25 U/kg)

* $p < 0.05$ vs rivaroxaban 2 mg/kg plus vehicle

† $p < 0.05$ vs rivaroxaban plus Beriplex® 25 U/kg

$^{\$\$}$ $p < 0.01$ vs baseline

[1] One animal with bleeding time >1800 seconds

[2] Two animals with bleeding time >1800 seconds

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Studies describing the absorption, distribution, metabolism and excretion of rivaroxaban were previously reviewed by Drs. Chakder and Chopra. The sections below briefly summarize this information.

Methods of Analysis

Plasma concentrations of rivaroxaban were determined by validated HPLC/MS/MS methods using an internal standard.

Absorption

Absolute bioavailability of rivaroxaban was 60 and 60 - 86% in rats and dogs, respectively. After administration of single IV and oral doses of up to 10 mg/kg of rivaroxaban drug substance, Cmax and AUC values increased dose proportionately in rats and dogs. However, since the oral bioavailability of micronized rivaroxaban was low, the sponsor initially utilized a 10% co-precipitate of rivaroxaban in polyethylene glycol 6000 (10% active drug substance; 90% PEG-6000) for most of the nonclinical studies submitted. A direct comparison of the absorption of micronized rivaroxaban and the 10% co-precipitate was not made in a single study. However, the exposures obtained in two 13-week rat toxicology studies (PH-33051, PH-34379) are summarized in Table 15. Higher exposure levels obtained with the 10% co-precipitate in PH-33051 increased with dose, but were less than dose-proportional. In contrast, exposure to the micronized rivaroxaban showed saturation, particularly after repeated dosing.

Table 15: Reviewer's Summary - Exposure in PH-33051 and PH-34379

			Dose, mg/kg					
PH-33051 – Wistar rat			**Male**			**Female**		
BAY 59-7939 co-precipitate, Batch: J20020703, 9.6 % API, 90 % PEG 6000	Parameter	Unit	12.5	50	200	12.5	50	200
	Day 1							
	$AUC_{(0-24)}$	mg*hr/L	13.4	42.2	117	14.7	63.0	152
	Cmax	mg/L	2.03	5.94	12.7	2.73	8.89	15.6
	Day 85							
	$AUC_{(0-24)}$	mg*hr/L	5.87	76.7	185	15.0	118	237
	Cmax	mg/L	2.4	12.5	21	4.88	21.5	26.0
PH-34379 – Wistar rat			**Male**			**Female**		
BAY 59-7939 micronized, Batch: BX01UNC, purity: 99.3%	Parameter	Unit	60	300	1500	60	300	1500
	Day 1							
	$AUC_{(0-24)}$	mg*hr/L	23.7	44.5	44.6	43.3	63.8	90.2
	Cmax	mg/L	2.94	4.30	5.09	4.1	5.49	7.6
	Day 80							
	$AUC_{(0-24)}$	mg*hr/L	26.1	25.6	31.2	54.6	50.3	57.7
	Cmax	mg/L	3.8	3.21	4.0	6.99	8.52	6.87

Distribution

The volume of distribution, Vss, in single dose pharmacokinetic studies was 0.3 and 0.4 L/kg for the rat and dog, respectively, indicating distribution to the extracellular space. Plasma to blood concentration ratios were 1.53, 1.14, 1.4 for, rat, dog and man, respectively, indicating that rivaroxaban is primarily located in plasma.

The plasma protein binding of rivaroxaban varied greatly among species with the highest in rats (98.73%) and the lowest in rabbits (76.6%) (Table 16). As a result, exposure comparisons between species were made based on the unbound fraction of rivaroxaban. The principal human plasma binding protein was serum albumin with lesser contributions from α1-acidic glycoprotein, α-globulins, β-globulins, γ-globulins and LDL.

Table 16: Reviewer's Summary of Protein Binding - PH-32966; PH-33395

Species/Strain	Rivaroxaban Concentration, mg/L	Fraction Bound %	Fraction unbound, %
Mouse / CD-1	0.1-3.0 11-1140	93.55 90.29	**6.45*** 9.71
Rat / Wistar Rat	0.1 – 3.0 9.7-34 101-113	98.73 98.01 96.84	**1.27*** 1.99 3.16
Rabbit / CHBB	0.1-3.2 10.6-107	76.6 73.1	**23.4*** 26.9
Dog / Beagle	0.1-2.8 10-103	89.6 85.0	**10.4*** 15.0
Monkey / Rhesus	0.1-3.0 11-112	81.7 77.2	18.3 22.8
Pig/Mini LEWE	0.1-1.4	92.87	7.13
Human	0.1-3.1 10.6-340	94.9 92.0	**5.07*** 7.96
Human albumin	1.15	80.4	19.6
Human α1-AGP	1.13	32.3	67.7
Human α-globulin	1.1	14.2	85.8
Human β-globulin	1.1	41.9	58.1
Human γ-globulin	1.4	5.9	94.1
LDL	1.1	18.0	82.0

* Asterisks mark the values for the fraction unbound in calculations of safety margins.

The binding of rivaroxaban to human plasma was not displaced by the presence of therapeutic or super-therapeutic concentrations of other common drugs, including warfarin, clofibrate, ibuprofen, propranolol, nifedipine, phenytoin, and digitoxin (PH-33395). However, three of the drugs tested increased the unbound fraction of rivaroxaban. Gemfibrocil at super-therapeutic concentrations (500 mg/L) increased the unbound fraction of rivaroxaban marginally to 9.8 %, Glibenclamide at super-therapeutic concentrations (100 and 500 mg/L) increased the free fraction of rivaroxaban to 9.5 % and 25.9%, respectively. High therapeutic concentrations of salicylic acid (200 mg/L) increased the unbound fraction of rivaroxaban to 11.6 % and super-therapeutic concentrations of 1000 mg/L increased the unbound fraction of rivaroxaban to 29.0 %. Therapeutic concentrations of other highly protein bound drugs are not expected to greatly affect the unbound fraction of rivaroxaban.

Whole body autoradiography (PH-32339) of Wistar rats at 0.5 hour after administration of a single dose of radioactive rivaroxaban indicated rapid distribution to most tissues with the highest levels of radioactivity in the gastrointestinal tract, and intermediate levels in the liver, kidneys, bladder and plasma. The lowest concentrations were in the brain, spinal cord and testes. At 24 hours after administration of radioactive rivaroxaban, the levels of radioactivity in most tissues were close to the limit of detection indicating rapid elimination. However, 7 days after administration of radioactive rivaroxaban, low residual levels of rivaroxaban radioactivity were still detected in the gastrointestinal tract, liver, and kidneys. The tissue distribution of radioactivity after repeated dosing (PH-34647) was similar to that after a single dose. However, the level of residual radioactivity at 168 hours after repeated administration was higher than after a single dose. The accumulation ratios in the liver and kidneys were 7 and 11-fold, respectively. Quantitative examination of tissues (PH-33719, Table 17) indicated similar concentrations and distribution of radioactivity in the tissues of the pigmented Long Evans rat and the albino Wistar rats. However, a low level of radioactivity was observed in the eyes and pigmented skin of the Long Evans rat indicating that radioactive rivaroxaban showed some affinity for melanin-containing tissue in pigmented rats.

Table 17: Reviewer's Summary - Distribution in Long-Evans and Wistar Rats

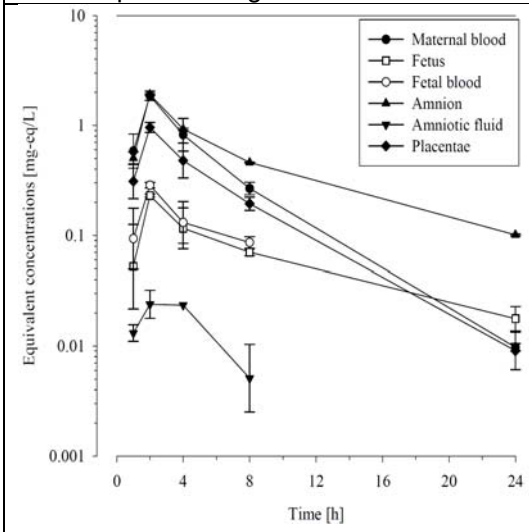
	Equivalent concentration CEQ (mg-eq/L) at time post single dose				
	Long-Evans		Wistar		
	Single dose		Single dose		14 doses
Tissue	24 hr	168 hr	24 hr	168 hr	168 hr
Blood	0.00447	<0.00277	0.00281	<0.00280	<0.0139
Body excl. GIT	0.0577	0.00269	0.0121	0.00212	
Carcass	0.0729	0.00099	0.00310	<0.000963	0.00537
Eyes	0.0505	0.0109	<0.00276	<0.00262	<0.00874
Kidneys	0.0229	0.0127	0.00378	0.0177	0.201
Liver	0.143	0.0377	0.167	0.0403	0.292
Pigmented skin	0.0454	0.00175			
Plasma carotis	0.00518	<0.00139	0.00606	<0.00143	0.0506
Skeletal muscle	0.00185	<0.000465	0.00606	<0.00144	0.00375
Skin	0.0209	0.00196	0.00747	0.00203	0.0191
PH 33719, PH 34647					

Whole-body autoradiography of pregnant Wistar rats on gestation day 19 indicated distribution of radioactive rivaroxaban through the placental barrier to the fetus (PH-34872). The highest levels of rivaroxaban radioactivity in maternal and fetal blood occurred at 2 hours after dosing when the fetal blood level (0.286 mg-eq/L) was 15% of the maternal blood level (1.86 mg-eq/L). The average exposure in the fetuses based on $AUC_{(0\ 24)}$ reached about 20 % of the exposure in maternal blood. Maximum concentrations in most maternal and fetal tissues also occurred around 2 hours post-dose. The highest maternal tissue levels of radioactivity in the liver and kidneys slightly exceeded the maternal blood concentration. Most of the fetal tissue levels were less than the fetal blood levels with the liver having the highest concentration of rivaroxaban radioactivity. The fetal tissue levels were 10 to 88% of the corresponding maternal tissue level with the skeletal muscle having the highest fetal:maternal tissue ratio. At the Tmax of 2 hours, a total of 17 % of the dose was located in maternal tissues and 0.44 %

in fetal tissues. Rivaroxaban radioactivity was rapidly eliminated from all fetal and maternal tissues with 0.27 % of the dose remaining in the dam and 0.033 % of the dose remaining in the fetuses at 24 hours post-dose.

Table 18: Maternal and Fetal Rivaroxaban Radioactivity Levels - PH34872

Sponsor's Figure – PH-34872



nc = not calculated

Reviewer's Summary of Tissue Concentrations

Mean equivalent concentrations [mg-eq/L]

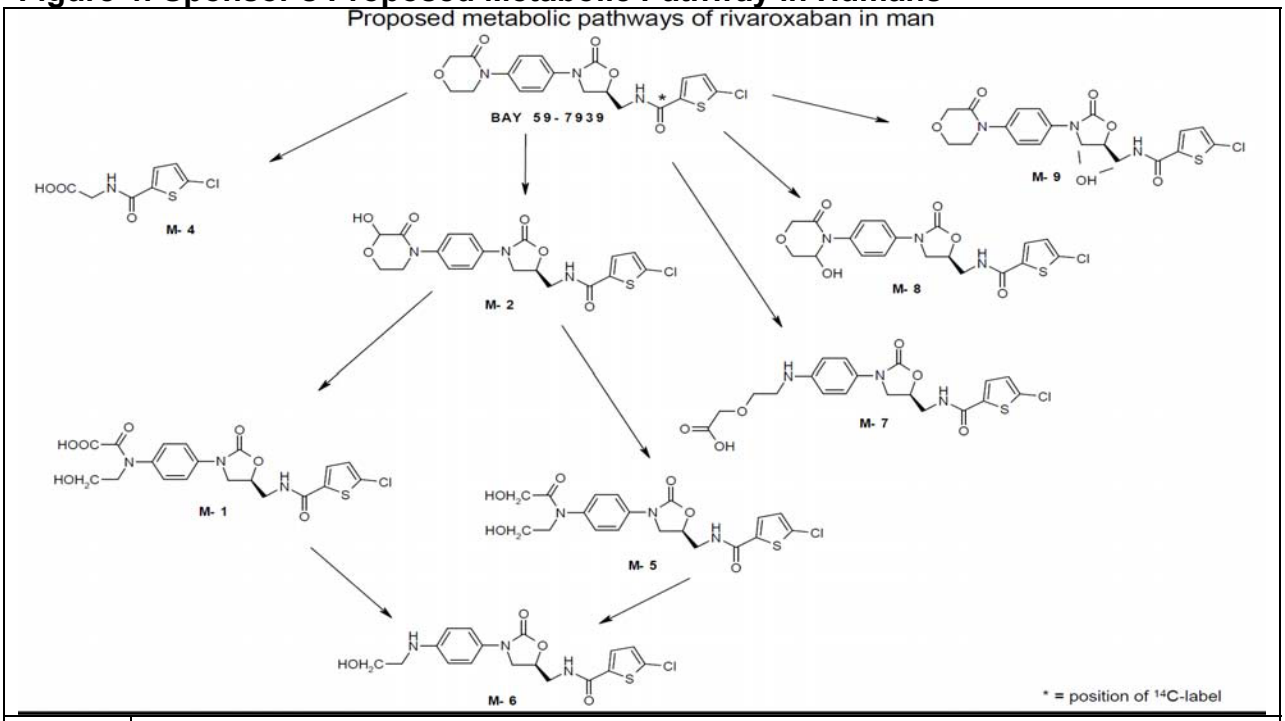
Time post dose	Maternal			Fetal		
	2 hr	4 hr	8 hr	2 hr	4 hr	8 hr
Adipose, brown	0.499	0.260	0.118	0.195	0.088	0.086
Amnion	1.91	0.926	0.459			
Amniotic fluid	0.024	0.024	0.005			
Blood	1.86	0.823	0.267	0.286	0.131	0.086
Brain	0.039	0.022	0.005	0.041	0.022	0.009
Kidney	2.14	1.08	0.551	nc	0.137	0.078
Liver	2.81	1.26	0.561	0.316	0.155	0.118
Lungs	1.67	0.636	0.226	0.185	0.092	0.068
Myocard	0.935	0.419	0.208	0.169	0.091	0.058
Placentae	0.957	0.479	0.194			
Skeletal muscle	0.268	0.131	0.062	0.237	0.094	0.074
Skin	0.474	0.204	0.076	0.274	0.087	0.086
Fetus (average)				0.230	0.116	0.070

2.6.4.5 Metabolism

In vivo metabolism

Evaluation of metabolites in animals and man indicated that metabolism of rivaroxaban by cleavage and hydroxylation was generally similar in all species evaluated. As summarized in the sponsor's proposed metabolic pathway for rivaroxaban in humans (Figure 4), the principal metabolic pathway is the hydroxylation at the morpholinone moiety leading to the monohydroxylated metabolite M-2, which is then further metabolized in a subsequent morpholinone ring opening/oxidation steps to form metabolite M-1. Although other metabolic pathways, such as amide hydrolysis at the morpholinone ring leading to M-7, exist for rivaroxaban, M-1 was identified as the most prominent metabolite in plasma of humans as well as in the plasma of mice, rats and dogs. However, some differences in metabolism of rivaroxaban exist between individual species, such as the absence of minor metabolites (M-5 in mice and M-7 in rats).

Figure 4: Sponsor's Proposed Metabolic Pathway in Humans



	Compound in proposed metabolic pathway								
Human	Parent	M-1	M-2	M-4	M-5	M-6	M-7	M-8	M-9
Rat	+	+	+	+	+	+		+	+
Mouse	+	+	+	+		+	+	+	+
Dog	+	+	+	+	+	+	+	+	+

+ indicates the presence of the individual metabolite in the proposed pathway for the species.

The in vivo metabolism study in humans (PH-33230) showed that unchanged rivaroxaban represented more than 85 % of the AUC of total radioactivity in all four volunteers (Table 19). The levels of the most prominent metabolite M1 in human plasma varied among the four subjects evaluated. The level of M1 metabolite level was as high as 18% of radioactivity at one timepoint in the plasma of one of the four subjects. However, based on total radioactivity AUC, the levels of M1 metabolite were less than 10% in all four subjects. In vivo metabolism studies in mice, rats and dogs, also indicated that unchanged rivaroxaban was the major compound in the plasma of these species. The levels of the M1 metabolite in rats and dogs were similar to that in humans and the levels of the M1 metabolite in mice were greater than that in humans. Therefore, the M1 metabolite can be considered adequately evaluated in the general toxicology studies.

Table 19: Reviewer's Summary - Rivaroxaban Metabolites in Plasma

Species (times post dose)	Metabolite as % of radioactivity in plasma at individual timepoint (% $AUC_{(0-t)}$ of total radioactivity)					
	Parent	M-1	M-2	M-5	Others	Document
Human (0.25 – 12 hr)	82-100 (88.8)	0-6.0 (4)	ND	0-2.5	M-4, M-7, M-8/M-9	PH-33230
Subject 001	92-100 (90.9)	0-1.7 (0.4)	ND	0-2.5	M-4, M-7, M-8/M-9	
Subject 002	90.5-100 (93.6)	0-9.5 (3.2)	ND	ND	ND	
Subject 003	93.7-100 (84.8)	0-3.0 (1.2)	ND	0-2.8	M-4, M-7, M-8/M-9	
Subject 004	82-100 (86.0)	0-18 (7.2)	ND	ND	M-4, M-7, M-8/M-9	
Mouse (0.25 – 8 hr)	36-63 (43)	11-24 (15)	6.9-11.6		M-4, M-6, M-7, M-8/M-9	PH-33897
Rat (1-8hr)	69-88 (83.3)	5.3-11.1 (6.3)	1.3-5.4		M8/M9	PH-31969
Dog (0.25 – 10 hr)	31-88 (70.9)	0.5-11.1 (5.2)	0-4.2		M-6, M7	PH-33092

ND Not determined

In vitro metabolism

In vitro studies (PH-34783) examined the metabolism of [^{14}C]-rivaroxaban during incubation with liver microsomes from Wistar rat, beagle dog, cynomolgus monkey, CD-1 mouse, NMRI mouse, Himalaya rabbit, and human (pool) and with hepatocyte sandwich cultures of Wistar rat, Beagle dog, and human. As summarized in Table 20, the major metabolite in the microsome incubations was the M-2 metabolite with lesser amounts of the M-1, M-3, M-7, and M-8/M-9 metabolites. In the incubations with hepatocytes, the major metabolite was M-1 with lesser amounts of M-2, M-7, and M-8/M-9. The former finding is important because the M-1 and M-2 metabolites are expected to have been formed in the presence of metabolic activation by rat liver microsomes in the bacterial reverse mutation assays. Therefore, the genotoxicity of M-1 and M-2 metabolites were evaluated at least at the higher concentrations of rivaroxaban used in those assays (see Table 29)

Table 20: Reviewer's Summary of In Vitro Metabolism in Various Species

PH-34783	% of radioactivity						
Species	Parent	M-1	M-2	M-3	M-7/M-10	M-8/M-9	Others
Liver microsomes							
Mouse	34.7-46	1.1-1.5	22.8-25.7	2.2-2.7	6.2-10.5	12.6	M-11, M-12, M-13
Rat	M: 66.1 F: 86.9	1.8 1.1	21.9 8.9	0.8 0	-	3.5 1.1	M-10, M-11, M-12, M-13
Dog	63.8	0.8	20.3	2.7	1.3	3.3	M-11, M-12, M-13
Human	69.1	3.2	15.4	0.7	1.5	6.0	M-12
Hepatocytes							
Rat	62.5-64.2	12.1-13.0	1.4	-	0.0	1.4-1.5	M-4, M-12
Dog	35.6-44.5	28.1-28.3	3.6-4.1	-	2.5-3.3	3.0-4.2	M-4, M-5, M-6, M-11
Human	47.2-56.3	16.6-17.4	0.7-1.6	-	3.1-5.2	3.8-4.1	M-4, M-5, M-6, M-11

Three different methods were applied to identify the principal P450 enzyme(s) involved in the biotransformation of rivaroxaban (PH-32627). The first method using incubation of [^{14}C]-rivaroxaban with a panel of 18 recombinant CYP isoforms indicated that CYP2J2 was the most effective isoform catalyzing formation of M-2 followed by CYP3A4 and to an even lesser extent CYP3A5 and CYP2D6. In contrast, formation of metabolite M-9 was mediated almost exclusively by CYP3A4. Kinetic analyses using recombinant CYP2J2 and CYP3A4 indicated Km-values of 7.8 µM and 66.4 µM, respectively for M-2 formation. The next method of incubation using [^{14}C]-rivaroxaban with human liver microsomes in the presence or absence of CYP isoform-selective inhibitors indicated that turnover of rivaroxaban was decreased in the presence of CYP3A4 inhibitors (azamulin, ketoconazole, troleandomycin) as well as in the presence of a CYP2J2 inhibitor HET0016. Co-incubations with CYP3A4 inhibitors decreased formation of M-9, whereas co-incubations with the CYP3A4-selective inhibitor azamulin and the CYP2J2 inhibitor HET0016 decreased formation of M-2. Correlation analyses indicated CYP3A4 is the principal CYP enzyme for M-9 formation, whereas CYP3A4 and CYP2J2 are the principal CYP enzymes for M-2 formation.

2.6.4.6 Excretion

The plasma half-life of the parent compound was 1.46 and 1.0 hours in rats and dogs, respectively, indicating rapid elimination from both species. The plasma clearance in rats (0.4 L/(kg.h)) and dogs (0.3 L/(kg.h)) is similar to the GRF (0.31 and 0.37 L/(kg.h), respectively) reported for these species (Davies and Morris 1993).

In rats, radioactive rivaroxaban was excreted principally by the biliary/fecal route (66-81%) with only 24-28% excretion into the urine after either oral or IV administration. However, following oral and IV administration a higher percentage (51 and 52%, respectively) of radioactive rivaroxaban was excreted in the urine of dogs and a lower percentage (40 and 43%, respectively) of the radioactivity was excreted in the feces. In contrast, excretion of rivaroxaban in humans is primarily renal (66%) with a lower amount excreted in the feces (28%).

Administration of radioactive rivaroxaban to lactating rats between Day 8 to 10 post partum indicated that 2.1% of the dose was excreted into milk within 32 hours after dosing, assuming that continuous milk production represents 20% of the maternal body weight per day. The concentration of radioactive rivaroxaban in both milk and maternal plasma was highest at 1 hour after dosing and declined to low levels at 24 hours after dosing. However, the ratio of milk to plasma rivaroxaban concentration was higher at 4 and 8 hours after dosing (2.68 and 4.72, respectively) compared to that observed at 1 hour after dosing (1.57).

2.6.4.7 Pharmacokinetic drug interactions

Two studies (PH-31634 and PH-34858) examined the ability of rivaroxaban to inhibit specific human cytochrome P-450 isoforms. Rivaroxaban at concentrations >50 µM did not inhibit biotransformation reactions catalyzed by CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2, and 3A4.

The potential of rivaroxaban to induce expression of human CYP1A2, CYP3A4, CYP2B6 and 2C19 was investigated using cultured human hepatocytes from four 4 different donors (PH-33718). Although CYP1A2 and 3A4 were induced in all cultures by the prototypic inducers omeprazole and rifampicin, respectively, the expression of human CYP enzymes CYP1A2, 3A4, 2B6, and 2C19 was not affected by rivaroxaban.

The potential for drug-drug interactions between rivaroxaban and 82 drugs from various compound classes was evaluated based on the inhibition of the formation of rivaroxaban metabolites M-2 and M-9 in co-incubations with pooled human liver microsomes (PH-34973). The most potent inhibitors of rivaroxaban metabolism listed in Table 21 consist primarily of HIV protease inhibitors and antifungal azoles. The most potent inhibitors of both M2 and M9 formation are ketoconazole and ritonavir.

Table 21: Reviewer's Summary of Inhibitor of Rivaroxaban Metabolism

Inhibitor	M-2, IC_{50} [µM]	M-9, IC_{50} [µM]
Atazanavir (reyataz)	2.4	1.2
Clotrimazole	10	0.25
Cyclosporin	9.7	3.6
Indinavir sulfate	4.3	1.7
Itraconazole	5.6	4.0
Ketoconazole	0.28	0.28
Miconazole	3.6	2.2
Ritonavir	0.54	0.42
Saquinavir (invirase)	11	10
Sildenafil	34	12
Vardenafil	34	3.0

Table 22 summarizes a series of in vitro studies investigating the transport of rivaroxaban and potential interaction effects on rivaroxaban clearance. Rivaroxaban was found to be highly permeable in the Caco-2 cell assay. Although rivaroxaban did not inhibit P-glycoprotein (P-gp), it is a weak to moderate substrate of P-gp. Three known P-gp inhibitors (verapamil, cyclosporine A and ivermectin) at concentrations higher than the clinical relevant concentrations inhibited the efflux of rivaroxaban in L-MDR1 cells and may decrease the renal clearance of rivaroxaban. Rivaroxaban is a substrate of the breast cancer resistance protein (mouse Bcrp, human BCRP). Although rivaroxaban only slightly inhibited Bcrp mediated transport of topotecan, ABZSO, and prazosin transport, rivaroxaban transport by Bcrp was inhibited by ritonavir and ketoconazole.

Table 22: Reviewer's Summary of In Vitro Transport Studies

Document	Study title	Result
PH-34936	Investigation of cell permeability in Caco-2 cells with regard to BCS classification	Relative to 22 reference compounds in a Caco-2 cell assay, BAY 59-7939 was found to be highly permeable according to the FDA guidelines for biowaiver classification system (BCS).
PH-34937	BAY 59-7939 (Rivaroxaban): Determination of the inhibitory potency towards human P-gp	In a MDR1 flux assay, BAY 59-7939 up to 10 µm showed no inhibitory potential towards the human P-gp multidrug resistance protein.

Document	Study title	Result
PH-34985	BAY 59-7939 (Rivaroxaban): Plasma and Brain Concentrations of [^{14}C]BAY 59-7939 Radioactivity and of Unchanged BAY 59-7939 in Male P-gp Double Knock-out mdr1a/1b Mice and Wild-type Mice after Single Intravenous and Oral Administration of [^{14}C]BAY 59-7939 or BAY 59-7939	Following intravenous or oral administration of BAY 59-7939, the brain to plasma concentration ratios in mdr1a/1b (-/-, -/-) mice were slightly higher (1.9 - 2.3–fold and 1.6 – 3.2-fold, respectively) compared to wild-type mice. BAY 59-7939 is classified as a weak P-gp substrate and is not likely to penetrate the blood-brain barrier in man in large amounts.
PH-34986	BAY 59-7939 (Rivaroxaban): In vitro studies in L–MDR1 cells to evaluate the P-gp substrate characteristics	The effect of several drugs on the bidirectional transport of BAY 59-7939 across L-MDR1 cells indicated BAY 59-7939 is a moderate substrate for the human P-gp efflux pump with a low affinity and high velocity. P-gp is most likely involved in the active renal secretion of BAY 59-7939
PH-34987	BAY 59-7939 (Rivaroxaban): In Vitro Studies in MDCKII-Bcrp Cells to evaluate the Bcrp-Substrate Characteristics of BAY 59-7939	Using Madin-Darbin canine kidney cells over-expressing the multidrug transport protein mouse breast cancer resistance protein (BCRP) (MDCKII-Bcrp), BAY 59-7939 was found to be a substrate for Bcrp and its transport was inhibited by ritonavir and ketoconazole
PH-35219	BAY 59-7939 (Rivaroxaban): Determination of the inhibitory potency towards Breast Cancer Resistance Protein	In co-incubation studies with MDCKII-Bcrp cells, increasing concentrations of BAY 59-7939 slightly inhibited Bcrp mediated topotecan, ABZSO, and prazosin transport.
PH-35258	BAY 59-7939 (Rivaroxaban): In vitro Studies to evaluate the inhibitory potential of various drugs on the efflux of BAY 59-7939 across L-MDR1 Cells	Three known P-gp inhibitors (verapamil, cyclosporine A and ivermectin) inhibited the efflux ratio of BAY 59-7939 in L-MDR1 cells in a concentration dependent manner and may decrease renal clearance of BAY 59-7939.
PH-35272	Rivaroxaban (BAY 59-7939): In vitro Studies in MDCKII-Bcrp Cells to evaluate the influence of various drugs on Bcrp mediated Rivaroxaban transport	The efflux of BAY 59-7939 across MDCKII cells overexpressing human BCRP was not reduced in the presence of CYP3A4 inhibitors (atazanavir, clarithromycin, clotrimazole, cyclosporine A, erythromycin, indinavir, itraconazole, miconazole, saquinavir, and verapamil)
PH-35323	BAY 59-7939 (Rivaroxaban): In vitro Studies to evaluate the inhibitory potential of Quinidine on the efflux of BAY 59-7939 across L-MDR1 Cells	The P-gp mediated efflux of BAY 59-7939 across L-MDR1 cells was reduced by quinidine in a concentration-dependent manner, although complete blockage of the efflux was not observed even at 100 µM.
PH-36090	BAY 59-7939 (Rivaroxaban): In vitro Studies in MDCKII-BCRP Cells to Evaluate the Substrate Characteristics of BAY 59-7939 for Human BCRP	The efflux of BAY 59-7939 across MDCKII cells overexpressing human BCRP was reduced in the presence of Ko143, a known BCRP inhibitor.

5.2 Toxicokinetics

Toxicokinetics are discussed with the appropriate toxicity study.

6 General Toxicology

6.1 Single-Dose Toxicity

Drs. Chakder and Chopra previously reviewed acute toxicology studies in mice and rats.

The maximum non-lethal oral single dose of the BAY 59-7939 co-precipitate with PEG 6000 in mice and rats was greater than 500 mg/kg. The maximum single oral dose of the BAY 59-7939 co-precipitate with PEG 6000 administered to dogs was 150 mg/kg in a 4-week subactute toxicology study (PH-31848).

No deaths of male or female rats were observed after oral administration of 500 mg/kg rivaroxaban or intravenous administration of 0.66 mg/kg. No deaths of male or female mice were observed after oral administration of 500 mg/kg rivaroxaban or intravenous administration of 25 mg/kg. However, following intravenous dosing at 25 mg/kg, the mice showed treatment-related clinical signs including decreased motility and labored breathing.

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. Chakder and Chopra have been filed and can be found in DARRTS. Table 23 summarizes the pivotal toxicology studies in rats and dogs submitted to IND 64892 and NDA 22406. Although only one of these studies used the micronized formulation of the drug substance proposed for marketing, the exposures of rivaroxaban achieved using the PEG 6000 melt coprecipitate were approximately 5-fold higher than that using the micronized drug substance.

Compound-related findings (effects on coagulation parameters, spontaneous bleeding in dogs with secondary anemia) were related to the pharmacological activity of rivaroxaban. Although transient increases in ALT and sometimes bilirubin and AST were observed in individual rats and dogs, these increases were not associated with any adverse histopathology findings in the liver.

Table 23: Reviewer's Summary of Pivotal Toxicology Studies

Species	Duration, weeks	Doses, mg/kg	Test article	Maximum AUC, mg*hr/L	Comments	Document
Rat, Wistar	4	0, 12.5, 50, 200	PEG 6000 Melt coprecipitate	M: 156 F: 227	Dose related effects on red cell & coagulation parameters. ALT slightly increased. NOAEL 50 mg/kg	PH-32303 PH-32333
	13	0, 12.5, 50, 200	PEG 6000 Melt coprecipitate	M: 185 F: 237	Dose related effects on red cell & coagulation parameters. Transient ALT & bilirubin increases. NOAEL 200 mg/kg	PH-33051 PH-33051A
	13	0, 60, 300, 1500	Micronized drug substance	M: 31.2 F: 57.7	Drug related effects on red cell & coagulation parameters. Transient ALT, AST & bilirubin increases NOAEL 1500 mg/kg	PH-34379

Species	Duration, weeks	Doses, mg/kg	Test article	Maximum AUC, mg*hr/L	Comments	Document
	26	0, 12.5, 50, 200	PEG 6000 Melt coprecipitate	M: 137 F: 280	See summary in Table 24	PH-33611
Dog Beagle	4	0, 15, 50, 150	PEG 6000 Melt coprecipitate	M +F: 37.1	Dose related effects coagulation times. Hemorrhage & spleen Extra-medullary hematopoiesis.	PH-31848 PH-32348
	13	0, 15, 50, 150	PEG 6000 Melt coprecipitate	M+F: 37.5	Dose related effects on red cell & coagulation parameters. Bleeding and hemorrhage NOAEL 50 mg/kg	PH-33056
	52	0, 5, 15, 50	PEG 6000 Melt coprecipitate	M+F: 22.0	See summary in Table 24	PH-34235

Table 24: Reviewer's Summary of Chronic Toxicology Studies

Parameter	Rat	Dog
Strain	Wistar (Hsd Cpb:WU)	Beagle
Study duration	6 months	12 months
Document code	PH-33611	PH-34235
Study code	T0073127	T4073149
GLP/QA	Indicated	Indicated
Drug batch, purity	BAY 59-7939, Batch 030618, 9.4% (Co-precipitate with PEG 6000)	BAY 59-7939, Batch 031212-100, 9.3% (Co-precipitate with PEG 6000)
Formulation	Solutol/water (20/80; v/v)	Suspension in water
Doses	0, 12.5, 50, 200 mg/kg	0, 5, 15, 50 mg/kg
Route	Oral gavage	Oral gavage
Number/sex/group	Main: 20	4/sex
Mortality	M: 1 at 200 mg/kg, F: 1 each at 0, 12.5, 200 mg/kg and 2 at 50 mg/kg Three deaths attributed to blood sampling, causes of others not determined	F: 1 at 50 mg/kg due to abdominal bleeding
Adverse clinical signs	No treatment related effect	Emesis and hematoma in legs and eyes of males at 50 mg/kg. Increased incidence of red, discolored feces at all doses
Body weight	BW gain decreased 12 and 15% in males at 50 and 200 mg/kg	Decreased BW and BW gain in 15 and 50 mg/kg in F
Food intake	No treatment related effect	No treatment related effect
Water intake	Increased 10-17% and 12-15% in M and F at 50 and 200 mg/kg	Not monitored
Ophthalmoscopy	No treatment related effect	No treatment related effect
Electrocardiography	Not monitored	Heart rate and QT interval not significantly different
Hematology	No clear treatment related effect	Decreased Hct and increased reticulocytes in two F at 50 mg/kg
Coagulation – (Note: time of blood collection relative to dosing was not indicated)	Increase in thromboplastin time (HQuick) at 50, 200 mg/kg in M and all treated groups in F;	Increase in PT and aPTT at 15 and 50 mg/kg

Clinical chemistry	Increased ALT (35%) on D88 in M at 50 and 200 mg/kg. Increased bilirubin (14-29%) on D178 in M and F at 200 mg/kg. Decreased serum K (10-15%) in M and F at 200 mg/kg on D88 and D178, but not plasma K					Increased ALT in some individual animals				
Urinalysis	Increased urine volume at 200 mg/kg in M (55-140%) and F (67-140%), presence of crystals in sediment at 50, 200 mg/kg in M and F					Urinary triple phosphate crystals observed in individuals at 15, 50 mg/kg in M and at 15 mg/kg in F				
Macroscopic pathology	Increased lesions and adhesion in epididymides of M at 200 mg/kg					Discoloration of duodenal lymph nodes in some treated males at all doses				
Organ weights	Slight increase in relative kidney wt in M at 200 mg/kg and F at 50, 200 mg/kg (NSS).					No treatment related effect				
Microscopic pathology	Increased incidence mucosal cysts in stomach, sperm granuloma in epididymides, lacrimal gland atrophy in M at 200 mg/kg. Increase incidence of ovarian follicular cyst, hemorrhage in multiple tissues and lacrimal gland atrophy in F at 200 mg/kg					Lymph node hemosiderin in treated males, peribronchial inflammation in both treated M and F				
Toxicokinetics AUC (mg*hr/L)	Dose, mg/kg	Male		Female		Dose, mg/kg	Male		Female	
		D1	D176	D1	D176		D170	D352	D170	D352
	12.5	10.4	18.0	20.9	32.4	5	6.6	8.1	4.6	5.0
	50	33.2	75.6	79.0	114	15	12.6	13.0	20.6	9.4
	200	93.5	137	171	280	50	27.3	32.1	34.9	14.4
NOAEL	12.5 mg/kg based on decreased BW gain, increased water intake and presence of urinary crystals at 50, 200					5 mg/kg based on decreased BW at 15 and 50 mg/kg in F and increased urinary crystals in M at 15 and 50 mg/kg				

Based on the NOAELs in the chronic toxicology studies, safety margins were calculated below based on total and unbound exposures. The 3 to 5-fold safety margin from the chronic dog study is higher than the 1.4 to 2.4 safety margin in the chronic rat study.

Table 25: 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
General toxicology						
6 month - rat	M	12.5	18	0.23	5.5	1.4
	F	12.5	32	0.4	9.7	2.4
12 month - dog	M	5	8.1	0.84	2.5	5.0
	F	5	5.0	0.52	1.5	3

† Unbound fractions in humans, rats, mice, dogs, and rabbits are 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively.
‡ Comparison to human exposure at 20 mg/day corresponding to 0.33 mg/kg in a 60 kg patient or 3.3 mg*hr/L. Unbound human exposure was 0.167 mg*hr/L.

Rivaroxaban is structurally similarity to linezolid, an antibiotic compound that affects mitochondrial activity (De Vriese et al. 2006). The sponsor submitted the following mechanistic toxicology study comparing linezolid and rivaroxaban with NDA 202439.

Study title: Mechanistic Study on Mitochondrial Protein Synthesis after Repeat-Dose (4-Week) Administration to Male Wistar Rats

Study no.:	T6080116 (PH-36161)
Study report location:	NDA 202439 EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG Wuppertal, Germany
Date of study initiation:	March 11, 2009
GLP compliance:	No
QA statement:	Not present
Drug, lot #, and % purity:	a. BAY 59-7939 micronized, Lot BXA1AJV, Purity 100% b. Linezolid, unknown lot and purity

Key Study Findings

Consistent with the study of De Vriese et al. (2006), linezolid treatment in the current rat study resulted in decreased protein level and enzyme activity of mitochondrially encoded Complex IV MT-COI in heart and liver, but not the protein level and associated activity of the nuclear encoded mitochondrial Complex II or protein levels of nuclear encoded VDAC1. Although BAY 59-7939 shows structural similarity to linezolid, it did not significantly affect the protein or enzyme activity levels of either Complex IV MT-COI or complex II subunit SDHB in the hearts and livers of most of the BAY 59-7939 treated rats. However, slightly lower levels of MT-COI protein, MT-COI enzyme activity and complex II subunit SDHB enzyme activity were found in the livers of two rats compared to the levels in control animals.

Methods

Doses:	0, 60 mg/kg of BAY 59-7939 0, 250 mg/kg of Linezolid
Frequency of dosing:	Daily (28 days for BAY 59-7939, 21 days for Linezolid)
Route of administration:	Oral by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	BAY 59-7939 in Ethanol/Solutol HS15/tap water (1/4/5, v/v/v) and Linezolid in 0.5% aqueous Tylose MH 300
Species/Strain:	Rat, Wistar (Hsd Cpb.WU)
Number/Sex/Group:	10 males/group
Age:	Not indicated
Weight:	221-246 gm
Satellite groups:	None
Unique study design:	Liver and heart were harvested for Western blot analysis of mitochondrial proteins and determination of activities of mitochondrial respiratory chain complexes (II and IV) after repeated dosing with BAY 59-7939 or Linezolid, a positive control compound.
Deviation from study protocol:	Not indicated

Observations and Results

Mortality

On regular workdays, the animals were examined visually at least twice daily for mortality, morbidity and reaction to treatment. On weekends and during the pretest period, the animals were examined once a day.

One animal treated with linezolid died on Day 22 just before necropsy. No treatment-related mortality occurred in animals treated with BAY 59-7939.

Clinical Signs

In addition to a more detailed weekly physical examination, general observations during treatment were made at least once daily on regular work days.

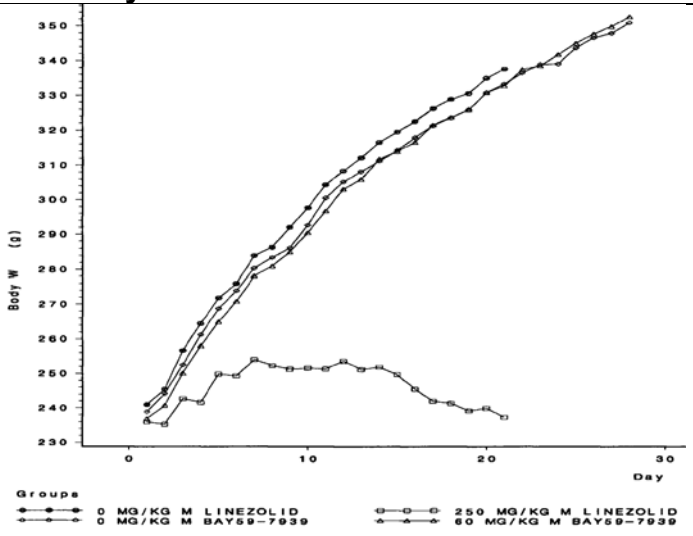
One animal male treated with BAY 59-7939 had a bloody muzzle on Day 9. However, multiple animals treated with linezolid had clinical findings such as piloerection (5), squatting position (4), hair loss (2), reduced motility (2) or pallor (1).

Body Weights

The animals were weighed daily until necropsy.

As shown in Table 26, body weight was not affected by treatment with BAY 59-7939; however, mean total body weight was reduced 30% by treatment with linezolid.

Table 26: PH 36161: Sponsor's Body Weight Graphs, Reviewer's Tabular Summary



	Mean body weight (BW), gm			
Day	Linezolid		BAY 59-7939	
mg/kg	0	250	0	60
1	241	236*	239	237
7	284	254*	280	278
14	317	252*	311	312
21	338	237*	333	333
28			351	353
% control BW Day 21 Day 28		70		 100 100
BW, Day 1-21 Mean Gain Range	97 79 121	1 -60 69	95 64 110	96 50 118
BW, Day 1-28 Mean Gain Range			112 76 129	116 58 144
* p < 0.01				

Food and Water Intake

Food and water intake were determined weekly. Data was provided per group and not by cage (2-3 animals).

As shown in Table 27, food and water intakes were not affected by treatment with BAY 59-7939. However, mean food intake per animal was reduced 38% and water intake per kg of body weight was increased 33% by treatment with linezolid.

Table 27: Sponsor's Summaries of Food and Water Intake – Study

Mean Food Intake					
Dose	Days	g/animal		g/kg body weight	
mg/kg		total	per day	total	per day
males		Linezolid			
0	1-22	559	26 6	1783	84 9
250	1-22	344	16 4	1393	66 3
males		BAY 59-7939			
0	1-29	699	25 0	2192	78 3
60	1-29	717	25 6	2248	80 3

Mean Water Intake					
Dose	Days	g/animal		g/kg body weight	
mg/kg		total	per day	total	per day
males		Linezolid			
0	1-22	604	28 8	1933	92 1
250	1-22	635	30 3	2570	122 4
males		BAY 59-7939			
0	1-29	955	34 1	3000	107 2
60	1-29	940	33 6	2949	105 3

Special Evaluations

Isolation of mitochondria and mitochondrial proteins

Pieces of liver and heart were frozen in liquid nitrogen and stored at -80°C. After the mitochondria were isolated using differential centrifugation in sucrose-containing buffers, the mitochondrial proteins were solubilized. Protein was determined according to Bradford et al. (1976) using bovine serum albumin as a standard.

Western Blot analysis of mitochondrial proteins

Mitochondrial protein samples (20 µg) were run on 12% SDS polyacrylamide gels and blotted onto PVDF membranes with an Invitrogen iBlot System. The blots were probed with mouse monoclonal antibodies against three mitochondrial proteins. The Voltage-dependent anion channel 1 (VDAC I), a loading control, and the succinate dehydrogenase complex subunit B iron sulfur (SDHB) are encoded by the nuclear genome. The complex IV subunit cytochrome c oxidase (MTCO1) is encoded by the mitochondrial genome. The protein bands for these three proteins were visualized on the blots by first binding the appropriate goat-antimouse IgG antibody coupled to alkaline phosphatase, washing, and then incubating the blots with a chemiluminescent substrate for alkaline phosphatase followed by detection of the chemiluminescence by exposure to X-ray films.

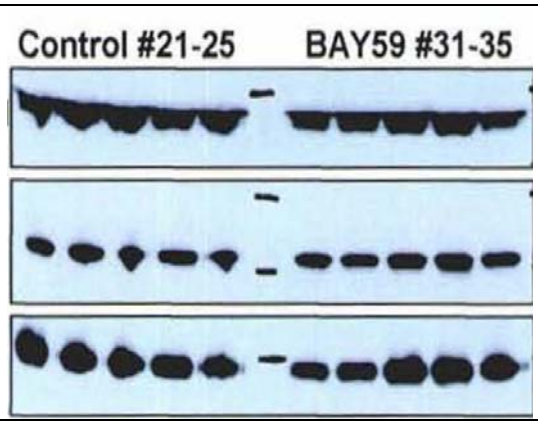
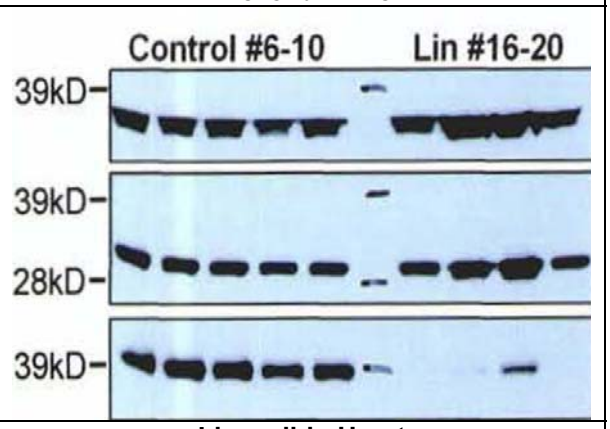
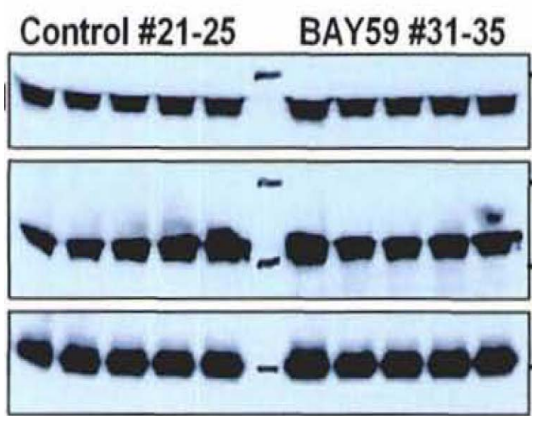
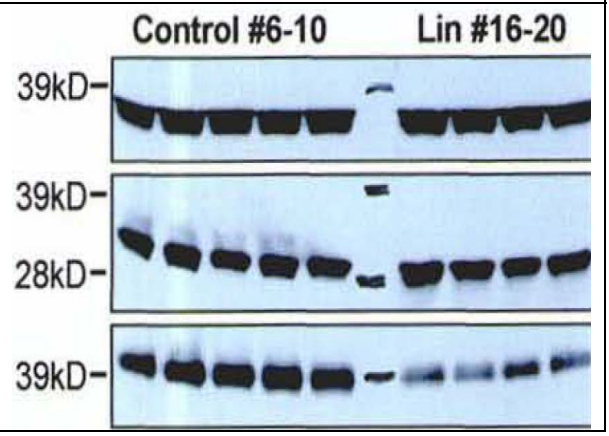
Enzyme activities of Complex II (Succinate dehydrogenase-coenzyme Q reductase) and Complex IV (Cytochrome C Oxidase)

Spectrophotometric assays were used to measure the activity of Complexes I and IV. The indirect reduction of 2, 6-dichloroindophenol by decylubiquinol generated from decylubiquinone in the presence of intact Complex II and succinate was used to determine Complex II activity, as described by Ziegler and Rieske (1967). The amount of mitochondrial protein corresponded to 40 µg/mL and 15 µg/mL for liver and for heart, respectively. The oxidation of reduced cytochrome C by intact Complex I was used to determine Complex IV cytochrome C oxidase activity as described by Sherratt et al. (1988). The amount of mitochondrial protein corresponded to 4 µg/mL for liver and 1 µg/mL for heart.

Western Blot Results

The Western blots below (Figure 5) show that the levels of the nuclear encoded complex II subunit SDHB and the mitochondrial outer membrane channel subunit VDACI were not affected either by linezolid or BAY 59-7939 treatment in either the liver or heart. However, the levels of the complex IV subunit MT-COI protein encoded by the mitochondrial genome were decreased in the hearts and almost completely eliminated in the livers of linezolid treated animals relative to levels in control animals. In contrast, the levels of the MT-COI protein in the hearts and livers of BAY 59-7939 treated animals did not appear to be reduced, except for slightly lower levels of MT-COI protein in the livers of animals 31 and 32, compared to the levels in control animals.

Figure 5: Sponsor's Figures – Selected Western Blots - PH-36161

Protein	BAY 59-7939 - Liver	Linezolid - Liver
VDAC SDHB MT-CO1		
	BAY 59-7939 – Heart	**Linezolid - Heart**
VDAC SDHB MT-CO1		

Linezolid did not affect Complex II activity compared to the vehicle control, but significantly decreased Complex IV activity by 68% and 46% in liver and heart mitochondrial fractions, respectively (Table 28). Similarly, the ratio of Complex IV activity to Complex II activity was decreased by 67% and 43% in mitochondrial fractions from male rat liver and heart from linezolid-treated animals, respectively, In contrast, BAY 59-7939 did not significantly affect the mean Complex II, Complex IV activities or the ratio of Complex IV to Complex II activity in mitochondrial fractions from rat livers and hearts compared to mean activities in the vehicle controls. However, animals 31 and 32 had individual values for Complex IV and Complex II activities in their livers, but not the heart, that are below the minimum value of the concurrent control group.

Table 28: Reviewer's Summary of Mitochondrial Enzyme Activites - PH-36161

		Liver (ΔE/min/mg)			Heart (ΔE/min/mg)		
		Complex IV	Complex II	Ratio Cn:CII	Complex IV	Complex II	Ratio Cn:CII
Control	Mean	12.98	3.66	3.69	38.13	11.93	3.22
	SD	1.94	0.97	0.75	10.08	2.61	0.63
	Minimum	10.60	2.80	2.37	23.0	8.80	2.02
	Maximum	16.08	5.18	2.37	53.0	16.13	4.13
Linezolid	Mean	**4.18***	3.47	**1.21***	**20.74***	11.22	**1.85***
	SD	2.10	1.58	0.31	3.75	1.90	0.19
	Minimum	1.88	1.91	0.64	17.67	9.16	1.46
	Maximum	7.92	6.53	1.75	26.33	13.93	2.13
Control	Mean	9.72	2.48	4.06	49.73	8.72	6.04
	SD	1.13	0.52	0.84	10.98	3.50	1.37
	Minimum	8.0	1.78	2.42	37.33	6.11	3.99
	Maximum	12.17	3.30	5.42	72.0	18.07	7.91
BAY 59-7939	Mean	10.27	2.60	4.25	48.77	9.02	5.70
	SD	2.18	0.85	1.18	7.27	2.87	1.48
	Minimum	5.42	1.03	3.22	37.67	5.22	3.98
	Maximum	12.17	3.58	7.07	61.0	13.73	9.0
Individual animals	31	**7.25**	**1.03**	7.07	61.0	12.82	4.83
	32	**5.42**	**1.19**	4.55	40.33	7.16	5.64

* p< 0.05

Linezolid, an oxazolidinone antibiotic, is known to inhibit bacterial protein synthesis by preventing the formation of the 70S ribosome initiation complex. De Vriese et al. (2006) previously demonstrated decreased activity of mitochondrial complexes I and IV in the liver, muscle, and kidney samples from linezolid-treated patients compared with the activities in samples from a control population. Additionally, decreased activity of mitochondrial complexes I and IV in the liver and muscle from linezolid-treated rats correlated with a decrease in individual subunits from complexes I and IV on Western blots. Similarly, in the present rat study, linezolid treatment resulted in decreased protein levels and enzyme activities of mitochondrially encoded Complex IV MT-COI in heart and liver, but not the protein level and associated activity of the nuclear encoded mitochondrial Complex II or protein levels of nuclear encoded VDAC1. Although BAY 59-7939 shows structural similarity to linezolid, it did not significantly affect the protein or enzyme activity levels of either Complex IV MT-COI or complex II subunit SDHB in the hearts and livers of most of the BAY 59-7939 treated rats. However, slightly lower levels of MT-COI protein, MT-COI enzyme activity and complex II subunit SDHB enzyme activity were observed in the livers of two animals (31 and 32), compared to the levels in control animals.

Dosing Solution Analysis

Homogeneity and stability of BAY 59-7939 and linezolid in the vehicles was evaluated prior to start of the study. Both test articles were shown to be homogeneously distributed and stable beyond the period of use in the formulations in the concentration range to be used. Analyses during the study verified that the contents of BAY 59-7939 and linezolid formulations were 105% and 102% of the target concentrations and that both were homogeneously distributed in the vehicles.

7 Genetic Toxicology

Rivaroxaban was not mutagenic in the Ames test, the chromosome aberrations assay in V79 Chinese hamster lung cells, or the mouse bone marrow micronucleus test.

Study reports for genetic toxicology studies for BAY 59-7939 were previously submitted to IND 64892 and NDA 22-406 and reviewed as indicated in Table 29.

Table 29: Genetic Toxicology Study Reports for BAY 59-7939

Document number	Study number	Study	Lot BAY 59-7939	Reviewed under	
				IND 64892	NDA 22406
PH 31770	T 1070545	Ames - Salmonella	010621	S. Chakder	Y. Chopra
PH 33561	T 9073243	Ames – Salmonella*	BX01JU3	S. Chakder	
PH 34016	T 8074764	Ames – Salmonella	BX01SFS		
PH 31537	T 2070546	Chromosomal Aberration	010621	S. Chakder	Y. Chopra
PH 34198	T 0073244	Chromosomal Aberration*	BX01SFS		Y. Chopra
PH 31536	T 3070547	Mouse Micronucleus	010621	S. Chakder	Y. Chopra
PH 33256	T 6072638	TK in mice after two IP doses	010621	S. Chakder	
* Reviews lacked data tables					

The table below (Table 30) summarizes the previously reviewed studies conducted with micronized rivaroxaban (BAY 59-7939, Lot 010621).

Table 30: Reviewer's Summary – Genetic Toxicology Studies Using Micronized Rivaroxaban

	In vitro		***In vivo***	
Assay	Ames	Chromosomal Aberration	Micronucleus	
Document code	PH 31770	PH 31537	PH 31536	PH-33256
Study code	T 1070545	T 2070546	T 3070547	T6072638
Drug lot/purity	BAY 59 7939 (micronized rivaroxaban); Batch No. 010621, purity 98.8%.			
Conducting lab and location	Bayer AG, PH PD Toxicology, Wuppertal, Germany			
Study dates	7/12/01 12/6/01	8/7/01 10/11/01	8/22/01 9/25/01	3/26/03 4/3/03
GLP/QA	Yes	Yes	Yes	Yes
Vehicle	DMSO	DMSO	0.5% aqueous Cremophor El	1% aqueous Cremophor El
Metabolic activation	Yes, induced rat liver S9 prep 3/27/01	Yes, induced rat liver S9 prep 3/27/01	Not applicable in vivo	
Doses	Maximum 5000 µg/plate	Maximum: 120 µg/mL 4 hr treatment 90 µg/mL 18 hr treatment	Two IP doses of 0, 35, 70, 140 mg/kg/day (10 mL/kg) based on pilot test using 100, 400 and 1000 mg/kg	Two IP doses of 140 mg/kg/day (10 mL/kg) No controls.
Strain/cell type	*Salmonella typhimurium* TA 1535, TA 1537,TA 98 TA 100, and TA 102	V79 Chinese hamster lung cells (22 chromosomes confirmed)	NMRI mice (HsdNVin: NMRI)	NMRI mice (HsdNVin: NMRI)
Appropriate replication	Triplicate plates each dose in two assays	Duplicate cultures each dose	5 males/group	3 males/time point
Toxicity or exposure	Precipitation in both assays ≥1581 µg/plate Slight toxicity TA102 ±S9; both assays, TA100 +S9 plate incorporation only	Mitotic index decreased with dose. Preliminary study indicated precipitation ≥100 µg/mL	Deaths in males and females at 400 and 1000 mg/kg in pilot. Clinical signs in main study included apathy, spasms, and difficulty in breathing	No death or clinical signs. Plasma samples collected at 0.25, 0.5, 1, 2, 4, 7 and 24 h after the second dose for exposure only.

	In vitro		*In vivo*	
Assay	Ames	Chromosomal Aberration	Micronucleus	
Document code	PH 31770	PH 31537	PH 31536	PH-33256
Appropriate controls	Without S9: yes; with S9: no, since only used 2 AA as positive control. However, same S9 lot used for PH31537	Yes, mitomycin C and cyclophosphamide as positive controls	Yes, cyclophosphamide as positive control	TK only indicates Cmax was 4.0 mg/L and $AUC_{(0-24)}$ was 25.7 mg*hr/L. No evaluation of micronuclei, no formulation analysis
Study Comments	Used both plate incorporation & pre incubation. TA100 background slightly high, but positive controls acceptable	4 hr treatment ±S9 and harvest at 18 and 30 hr, 18 treatment S9 Coded slide evaluation	IP administration is different than oral clinical route. Vehicle is different in PH 31536 and PH 33256	
			Formulation 98 102 % of nominal Coded slide evaluation	Unbound exposure of 0.326 mg*hr/mL is 2 fold the unbound human AUC of 0.167 mg*hr/L for 20 mg/day
Study result	**Negative**	**Negative**	**Negative**	**Exposure only**

The report PH 34016 had not been previously reviewed. In addition, the reviews for reports PH 33561 and PH 34198 lacked data tables. These three genetic toxicology reports, reviewed below, used two lots of BAY 59-7939 that were spiked with most, but not all, identified impurities. In particular, the [redacted] (b) (4) impurity was either not present or present at [redacted] (b) (4) in these lots. A micronucleus assay was not conducted with these spiked lots of BAY 59-7939.

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

Study title: BAY 59-7939 *Salmonella* Microsome Test - Plate Incorporation and Preincubation Method

Document no.:	PH-34016 (Study T 8074764)
Study report location:	EDR
Conducting laboratory and location:	Bayer HealthCare AG, PH-PD Toxicology, Wuppertal, Germany
Date of study initiation:	01/05/2005 (Experimental start 03/04/2005)
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7039, Batch BX01SFS, purity 88.4% According to DMF 21581, batch BX01SFS also contained the following impurities: [redacted] (b) (4)

Key Study Findings

In two valid assays, BAY 59-7039 Batch BX01SFS, containing most of the identified impurities, did not induce excess reverse mutations in five recommended bacterial strains (*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA102) either in the absence or presence of metabolic activation at ≥1581 µg/plate, doses at which precipitation occurred.

Methods

Strains:	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102
Concentrations in definitive study:	16, 50, 158, 500, 1581, and 5000 µg/plate
Basis of concentration selection:	A prior study with BAY 59-7039 indicated that the maximum concentration (5 mg/ plate) recommended by the OECD guideline could be used.
Negative control:	DMSO
Positive control:	Without metabolic activation: TA98: 4-Nitro-1,2-phenylene diamine (4-NPDA, 0.5 µg/plate) TA100: Nitrofurantoin (0.2 µg/plate) TA1535: Na azide (10 µg/plate) TA1537: 4-NPDA (10 µg/plate) TA102: Mitomycin C (0.2 µg/plate) and Cumene hydroperoxide (50 µg/plate) With metabolic activation – all strains: 2-Aminoanthracene (3 µg/plate)
Formulation/Vehicle:	DMSO
Formulation analysis and stability	The lowest and highest formulation concentrations were assayed for BAY 59-7039 at 0 and 24 hours after preparation. The BAY 59-7039 content was 90-110% of the nominal concentration.
Incubation & sampling time:	Plate incorporation: Test article (0.1 mL/plate) in the absence and presence of rat liver S9 mix was added to cells and top agar and immediately poured onto plates. Preincubation: Test article (0.1 mL/plate) in the absence and presence of rat liver S9 mix was added to cells and pre-incubated at 37°C for 20 minutes prior to addition of top agar and pouring onto plates All plates were incubated at 37°C for 48 hours prior to counting revertants using an Artek automatic counter.

Study Validity

The assays were considered valid because all strains exhibited the number of spontaneous revertants per plate within or very close to the historical background control ranges. In addition, the positive controls induced a significant increase in the number of revertants. However, the only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. The report indicated the activity of the S9 preparation of each batch of S9 was characterized with appropriate indirect mutagens prior to first use. However, these results were not provided in the submitted report.

Results

Although BAY 59-7039 precipitated at ≥1581 µg/plate, all doses could be evaluated. Toxicity did not limit the concentration of test article evaluated.

BAY 59-7039 Batch BX01SFS did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) compared to the vehicle control either in the absence or presence of metabolic activation at any dose (Table 31). All revertant counts for test article samples were within or very close to the historical negative control range. A repeat experiment using the preincubation method confirmed the results using the plate incorporation method.

Table 31: Reviewer's Summary – Ames Assays - Study PH-34016

		Mean of 3 replicates									
		Plate incorporation					Pre-incubation				
Group		TA 1535	TA 100	TA 1537	TA 98	TA 102	TA 1535	TA 100	TA 1537	TA 98	TA 102
Without S9											
BAY 59-7939	0	18	159	6	21	236	15	150	6	27	217
	16	16	164	5	26	235	18	159	8	28	198
	50	20	159	5	25	231	14	150	7	24	202
	158	26	172	6	24	231	16	159	6	30	182
	500	15	178	5	25	225	14	165	5	29	206
	1581	21	176	5	22	231	16	156	8	35	180
	5000	14	176	4	17	192	16	145	6	31	170
Na azide		683					776				
NF			344					398			
4-NPDA				75	133				90	131	
MMC						581					
Cumene											435
With S9											
BAY 59-7939	0	10	228	8	38	335	12	184	8	47	197
	16	11	204	8	35	269	13	206	7	43	199
	50	9	200	6	39	282	12	187	9	41	177
	158	8	195	6	41	256	12	207	6	49	180
	500	11	227	8	41	289	11	217	7	44	176
	1581	9	216	9	36	296	9	199	8	44	153
	5000	6	205	6	43	290	10	196	6	36	113
2-AA		211	1594	231	1306	940	167	1447	404	1145	481

The historical control data for 2003 and 2004 are summarized in Table 32.

Table 32: Reviewer's Summary - Prior Historical Control Data – Study PH-34016

Group	Plate incorporation*					Pre-incubation*				
	TA 1535	TA 100	TA 1537	TA 98	TA 102	TA 1535	TA 100	TA 1537	TA 98	TA 102
Without S9										
Negative (DMSO)										
2003	14	144	7	24	227	15	137	8	20	236
	(3)	(13)	(1)	(5)	(28)	(3)	(18)	(1)	(5)	(24)
2004	16	135	7	26	199	15	134	8	24	209
	(4)	(15)	(1)	(9)	(14)	(3)	(10)	(1)	(6)	(19)
Positive										
2003	679	358	91	157	530	664	463	120	164	477
	(90)	(42)	(12)	(18)	(63)	(88)	(37)	(18)	(17)	(44)
2004	651	339	91	153	541	609	460	111	157	470
	(121)	(28)	(18)	(16)	(59)	(107)	(56)	(18)	(18)	(34)
With S9										
Negative (DMSO)										
2003	11	169	9	34	294	11	159	9	34	297
	(2)	(22)	(2)	(6)	(32)	(2)	(24)	(2)	(5)	(28)
2004	11	153	10	40	255	12	156	10	36	251
	(2)	(23)	(2)	(9)	(24)	(2)	(24)	(2)	(7)	(25)
Positive										
2003	170	1526	286	1247	721	187	1527	345	1255	613
	(28)	(143)	(78)	(138)	(114)	(28)	(159)	(60)	(144)	(94)
2004	150	1436	259	1164	573	163	1515	293	1145	512
	(29)	(116)	(57)	(112)	(79)	(14)	(134)	(25)	(120)	(56)

* Median (Semi Q range)

Study title: BAY 59-7939 *Salmonella* Microsome Test - Plate Incorporation and Preincubation Method

Document no.:	PH-33561 (Study T 9073243)
Study report location:	EDR
Conducting laboratory and location:	Bayer HealthCare AG, PH-PD Toxicology, Wuppertal, Germany
Date of study initiation:	03/04/04 (Experimental 08/13/04)
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7039, Batch BX01JU3, purity 94.9% According to DMF 21581, batch BX01JU3 also contained the following impurities: (b) (4) (b) (4) .

Key Study Findings

In two valid assays, BAY 59-7039 Batch BX01JU3 containing most of the identified impurities did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537, and TA102) either in the absence or presence of metabolic activation at ≥1581 µg/plate, doses at which precipitation occurred..

Methods

Strains:	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102
Concentrations in definitive study:	16, 50, 158, 500, 1581, and 5000 µg/plate
Basis of concentration selection:	A prior study with BAY 59-7039 indicated that the maximum concentration (5 mg/ plate) recommended by the OECD guideline could be used.
Negative control:	DMSO
Positive control:	Without metabolic activation: TA98: 4-Nitro-1,2-phenylene diamine (4-NPDA, 0.5 µg/plate) TA100: Nitrofurantoin (0.2 µg/plate) TA1535: Na azide (10 µg/plate) TA1537: 4-NPDA (10 µg/plate) TA102: Mitomycin C (0.2 µg/plate) and Cumene hydroperoxide (50 µg/plate) With metabolic activation – all strains: 2-Aminoanthracene (3 µg/plate)
Formulation/Vehicle:	DMSO
Formulation analysis and stability	The lowest and highest formulation concentrations were assayed for BAY 59-7039 at 0 and 24 hours after preparation. The BAY 59-7039 content was 90-110% of the nominal concentration.
Incubation & sampling time:	Plate incorporation: Test article (0.1 mL/plate) in the absence and presence of rat liver S9 mix was added to cells and top agar and immediately poured onto plates. Preincubation: Test article (0.1 mL/plate) in the absence and presence of rat liver S9 mix was added to cells and pre-incubated at 37°C for 20 minutes prior to addition of top agar and pouring onto plates All plates were incubated at 37°C for 48 hours prior to counting revertants using an Artek automatic counter.

Study Validity

The assays were considered valid because all strains exhibited the number of spontaneous revertants per plate within or very close to the historical background control ranges. In addition, the positive controls induced a significant increase in the number of revertants. However, the only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. The report indicated the activity of the S9 preparation of each batch of S9 was characterized with appropriate indirect mutagens prior to first use. However, these results were not provided in the submitted report.

Results

Although BAY 59-7039 precipitated at ≥1581 µg/plate, all doses could be evaluated. Toxicity did not limit the concentration of test article tested.

BAY 59-7039 Batch BX01JU3 did not induce excess reverse mutations in five recommended bacterial strains (*S. typhimurium* TA98, TA100, TA1535, TA1537 or TA102) compared to the vehicle control either in the absence or presence of metabolic activation at any dose (Table 33). All revertant counts for test article samples were within or very close to the historical negative control range. A repeat experiment using the preincubation method confirmed the results using the plate incorporation method.

Table 33: Reviewer's Summary – Ames Assays – Study PH-33561

		Mean of 3 replicates									
		Plate incorporation					Pre-incubation				
Group		TA 1535	TA 100	TA 1537	TA 98	TA 102	TA 1535	TA 100	TA 1537	TA 98	TA 102
Without S9											
BAY 59-7939	0	10	94	6	15	216	20	142	7	18	207
	16	11	104	7	15	202	21	148	6	18	197
	50	10	95	5	16	216	22	137	5	15	216
	158	14	82	4	13	202	19	125	6	16	202
	500	10	96	6	21	207	16	135	6	18	207
	1581	9	89	5	17	193	24	125	4	18	193
	5000	10	100	5	14	192	12	136	4	13	192
Na azide		656					685				
NF			251					478			
4-NPDA				72	114				72	114	
MMC						476					
Cumene											476
With S9											
BAY 59-7939	0	9	115	11	30	234	10	137	8	30	248
	16	9	119	10	26	226	13	136	7	31	230
	50	9	116	9	31	239	10	144	7	26	221
	158	8	109	9	25	227	11	132	9	33	230
	500	9	109	10	31	231	11	142	8	35	235
	1581	12	103	9	33	201	10	131	8	29	227
	5000	10	90	6	28	207	9	140	5	22	170
2-AA		182	1238	364	1061	523	167	1384	379	994	454

Table 34: Reviewer's Summary Prior Historical Control Data – Study PH-33561

	Plate incorporation*					Pre-incubation*				
Group	TA 1535	TA 100	TA 1537	TA 98	TA 102	TA 1535	TA 100	TA 1537	TA 98	TA 102
Without S9										
Negative (DMSO0										
2002	14	136	7	31	216	12	145	8	29	255
	(5)	(15)	(2)	(9)	(35)	(3)	(13)	(2)	(6)	(32)
2003	14	144	7	24	227	15	137	8	20	236
	(3)	(13)	(1)	(5)	(28)	(3)	(18)	(1)	(5)	(24)
Positive										
2002	735	323	88	156	556	740	447	114	165	480
	(80)	(30)	(11)	(24)	(92)	(95)	(31)	(14)	(17)	(35)
2003	679	358	91	157	530	664	463	120	164	477
	(90)	(42)	(12)	(18)	(63)	(88)	(37)	(18)	(17)	(44)
With S9										
Negative (DMSO)										
2002	12	151	8	39	270	11	155	9	38	314
	(3)	(25)	(2)	(12)	(24)	(2)	(16)	(1)	(9)	(38)
2003	11	169	9	34	294	11	159	9	34	297
	(2)	(22)	(2)	(6)	(32)	(2)	(24)	(2)	(5)	(28)
Positive										
2002	158	1506	256	1283	554	170	1550	316	1234	509
	(30)	(126)	(60)	(139)	(64)	(22)	(122)	(57)	(149)	(34)
2003	170	1526	286	1247	721	187	1527	345	1255	613
	(28)	(143)	(78)	(138)	(114)	(28)	(159)	(60)	(144)	(94)

* Median (Semi Q range)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: BAY 59-7939 In Vitro Chromosomal Aberration Test with Chinese Hamster V79 Cells

Document no.:	PH34198 (Study T 0073244)
Study report location:	EDR
Conducting laboratory and location:	Bayer AG, PH-PD Toxicology, Wuppertal, Germany
Date of study initiation:	03/18/05
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7039, Batch BX01SFS, purity 88.4% According to DMF 21581, batch BX01SFS also contained the following impurities: (b) (4) (b) (4) .

Key Study Findings

In acceptable in vitro assays, BAY 59-7039 Batch BX01JU3, containing most of the identified impurities, did not induce excess chromosomal aberrations in V79 Chinese hamster lung cells.

Methods

Cell line:	Chinese hamster V79 cells having a modal number of 22 chromosomes were karyotyped on 02/21/2005.
Concentrations in definitive study:	4 hr treatment – S9, 18 hr harvest: 0, 30, 60, 120 µg/mL 4 hr treatment + S9, 18 hr harvest: 0, 30, 90, 180 µg/mL 18 hr treatment – S9, 18 hr harvest: 0, 15, 30, 60 µg/mL 4 hr treatment – S9, 30 hr harvest: 0, 120 µg/mL 4 hr treatment + S9, 30 hr harvest: 0, 180 µg/mL
Basis of concentration selection:	The concentrations were based on the results of two previously conducted cytogenetic studies [a pilot study, Report 303 14P and a GLP-study T 2070546, Report 3 1537E]. BAY 59-7939 precipitated in the medium at ≥90 µg/mL in the presence and absence of metabolic activation.
Negative control:	DMSO

Positive control:	Mitomycin C was used at final concentrations of 0.1 and 0.03 µg/mL for the 4 and 18 hr treatments, respectively. Cyclophosphamide was used at a final concentration of 2 µg/mL in the presence of metabolic activation.
Formulation/vehicle:	DMSO
Incubation & sampling times:	Two treatment schedules were used to treat duplicate cultures of $1x10^6$ cells at each concentration of BAY 59-7039 along with negative and positive controls. The first schedule used a 4 hour treatment in the absence or presence of metabolic activation followed by washing with phosphate buffered saline, addition of fresh media and continued culture until harvest at 18 or 30 hours. The second schedule used a 18 hour treatment with harvest at 18 hour. Two hours prior to harvest, colcemid (40 µg/mL) was added to arrest dividing cells in metaphase. Cells were collected by centrifugation and resuspendedin 0.4% KCl for hypotonic treatment. After fixation in ethanol/glacial acetic acid, the cells were dropped onto slides, and stained with 3% Giemsa stain. At least two coded slides were prepared per culture.
Slide evaluation:	Cytotoxicity and mitotic index were determined from 1000 cells per culture. At least, 200 cells per concentration (100 cells per replicate culture) were evaluated for chromatid and chromosome aberrations (gaps, breaks, fragments, deletions, exchanges and multiple aberrations). The number of polyploid cells was recorded for an unknown number of cells.
Assessment criteria:	An assay was acceptable, if the negative controls were in the laboratory historical range and the positive controls induced a relevant increase in chromosome aberrations consistent with the laboratory historical range. A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate. A test was considered negative, if there was no such increase at any time interval or if there were statistical significant values, which were within the range of historical negative controls.

Study Validity

The study was considered valid, since all negative controls were within the historical range. Although the positive controls for the 4 hour treatment were lower than the historical positive control range in 2004, the values for both mitomycin C and cyclophosphamide were significantly elevated for all treatments.

Results

The results are summarized in the table below. In the absence of metabolic activation, no concentration of BAY 59-7939 induced statistically significant increases in the percentage of metaphases with aberrations. In the presence of metabolic activation, a

statistically significant increase in the percentage of metaphases with aberrations was observed for the cultures treated for 4 hours with 90 µg/mL and harvested at 18 hours. However, the mean and individual culture values are within the historical negative control range. Additionally, no increase in the percentage of metaphases with aberrations was observed for the cultures treated for 4 hours with 180 µg/mL and harvested at either 18 hours or 30 hours. Therefore, BAY 59-7939 did not induce excess chromosomal aberrations in V79 Chinese hamster lung cells in vitro.

Table 35: Reviewer's Modification of Sponsor's Tables – Study PH34198

4 hour treatment without metabolic activation – harvest at 18 hours

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)			% mitoses	% survival
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges		
DMSO 0	18	37	100	0	0	1	0	0	0	2	0	0	0	0	0	2.0	2.0	0.0	100	100
		4	100	0	0	0	0	0	0	0	0	1	0	0	0	1.0	1.0	1.0		
			200	0	0	1	0	0	0	2	0	1	0	0	0	1.5	1.5	0.5		
BAY 59-7939 30	18	15	100	0	0	0	1	0	0	0	0	0	0	0	0	0.0	1.0	0.0	123	98.8
		8	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0		
			200	0	0	0	1	0	0	0	0	0	0	0	0	0.0	0.5	0.0		
BAY 59-7939 60	18	22	100	0	0	1	0	0	0	0	0	0	0	0	0	1.0	1.0	0.0	108	82.1
		35	100	0	0	0	0	0	1	0	0	4	0	0	0	4.0	4.0	3.0		
			200	0	0	1	0	0	1	0	0	4	0	0	0	2.5	2.5	1.5		
BAY 59-7939 120	18	17	100	0	0	0	0	0	0	0	0	3	0	0	0	2.0	2.0	2.0	79.7	81.5
		18	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0		
			200	0	0	0	0	0	0	0	0	3	0	0	0	1.0	1.0	1.0		
Mitomycin C 0.1	18	6	100	1	1	4	1	0	3	4	0	7	0	0	0	20.0	18.0	7.0	98.7	110
		44	100	3	0	11	1	0	2	0	0	11	0	0	0	21.0	19.0	9.0		
			200	4	1	15	2	0	5	4	0	18	0	0	0	20.5**	18.5**	8.0**		

4 hour treatment with metabolic activation – harvest at 18 hours

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)			% mitoses	% survival
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges		
DMSO 0	18	26	100	0	0	0	0	0	0	0	0	3	0	0	0	2.0	2.0	2.0	100	100
		33	100	0	1	0	0	0	0	0	0	2	0	0	0	2.0	1.0	1.0		
			200	0	1	0	0	0	0	0	0	5	0	0	0	2.0	1.5	1.5		
BAY 59-7939 30	18	20	100	0	0	0	0	0	1	2	0	0	0	0	0	3.0	3.0	0.0	94.7	93.8
		41	100	0	0	1	1	0	0	1	0	1	0	0	1	4.0	4.0	1.0		
			200	0	0	1	1	0	1	3	0	1	0	0	1	3.5	3.5	0.5		
BAY 59-7939 90	18	39	100	0	0	1	0	0	1	0	0	3	0	0	0	4.0	4.0	3.0	115	80.3
		31	100	2	0	1	0	0	0	0	0	9	0	0	1	6.0	6.0	5.0		
			200	2	0	2	0	0	1	0	0	12	0	0	1	5.0	5.0*	4.0		
BAY 59-7939 180	18	14	100	0	0	0	0	0	0	0	0	3	0	0	0	2.0	2.0	2.0	112	86
		9	100	0	0	0	0	0	0	0	0	3	0	0	0	3.0	3.0	3.0		
			200	0	0	0	0	0	0	0	0	6	0	0	0	2.5	2.5	2.5		
Cyclophosphamide 2	18	3	100	1	0	8	1	0	12	1	0	10	0	0	0	25.0	25.0	10.0	66.4	86.5
		2	100	3	0	11	2	0	0	0	0	4	2	0	0	22.0	21.0	6.0		
			200	4	0	19	3	0	12	1	0	14	2	0	0	23.5**	23.0**	8.0**		

4 hour treatment with & without metabolic activation – harvest at 30 hours

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatid Type			Chromosome Type			other				Metaphases with Aberrations (%)			% mitoses	% survival
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges		
without metabolic activation																				
DMSO 0	30	28	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	100	100
		25	100	1	0	0	0	0	0	0	0	0	0	0	0	1.0	0.0	0.0		
			200	1	0	0	0	0	0	0	0	0	0	0	0	0.5	0.0	0.0		
BAY 59-7939 120	30	7	100	1	0	1	0	0	0	0	0	1	0	0	0	3.0	2.0	1.0	48.6	60.3
		5	100	1	0	1	0	0	0	0	0	1	0	0	0	3.0	2.0	1.0		
			200	2	0	2	0	0	0	0	0	2	0	0	0	3.0	2.0	1.0		
with metabolic activation																				
DMSO 0	30	19	100	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	100	100
		21	100	0	0	0	0	0	0	0	0	3	0	0	0	3.0	3.0	3.0		
			200	0	0	0	0	0	0	0	0	3	0	0	0	1.5	1.5	1.5		
BAY 59-7939 180	30	32	100	0	0	1	0	0	0	0	0	2	0	0	1	3.0	3.0	2.0	120	73.4
		13	100	0	0	0	0	0	0	0	0	0	2	0	0	2.0	2.0	2.0		
			200	0	0	1	0	0	0	0	0	2	2	0	1	2.5	2.5	2.0		

18 hour treatment without metabolic activation – harvest at 18 hours

Experimental Group and Concentration in µg/ml	Harvest Time in Hours	Culture Number	Cells scored	Gaps		Classes of Aberrations: Chromatic Type			Chromosome Type			other				Metaphases with Aberrations (%)			% mitoses	% survival
				g	ig	b	f	d	ib	if	id	ex	maE	ma	cd	incl. gaps	excl.gaps	exchanges		
DMSO 0	18	55	100	1	0	0	0	0	1	1	0	6	0	0	0	6.0	5.0	3.0	100	100
		51	100	1	1	0	0	0	0	0	0	2	0	0	0	4.0	2.0	2.0		
			200	2	1	0	0	0	1	1	0	8	0	0	0	5.0	3.5	2.5		
BAY 59-7939 15	18	49	100	1	0	1	1	0	0	0	0	7	0	0	1	8.0	7.0	5.0	118	86.3
		57	100	0	0	2	0	0	1	0	0	0	0	0	0	3.0	3.0	0.0		
			200	1	0	3	1	0	1	0	0	7	0	0	1	5.5	5.0	2.5		
BAY 59-7939 30	18	47	100	0	0	0	0	0	0	1	0	3	0	0	1	2.0	2.0	2.0	76.5	79.5
		50	100	0	0	0	0	0	0	0	0	2	0	0	1	2.0	2.0	2.0		
			200	0	0	0	0	0	0	1	0	5	0	0	2	2.0	2.0	2.0		
BAY 59-7939 60	18	46	100	1	0	0	0	0	1	0	0	2	0	0	1	4.0	3.0	2.0	32,7	51.6
		56	100	0	0	1	0	0	0	0	0	1	0	0	0	2.0	2.0	1.0		
			200	1	0	1	0	0	1	0	0	3	0	0	1	3.0	2.5	1.5		
Mitomycin C 0.03	18	52	100	0	0	13	6	0	6	1	0	4	0	0	0	26.0	26.0	4.0	79.6	75.8
		53	100	0	0	10	5	0	4	0	0	11	0	0	0	23.0	23.0	9.0		
			200	0	0	23	11	0	10	1	0	15	0	0	0	24.5**	24.5**	6.5*		

Table 36: Reviewer's Modification of Sponsor's 2004 Historical Control Data

Treatment Time, hr.	S9 mix	Solvent or Substance	Number of Studies	Harvest Time in Hours	Metaphases with Aberrations including Gaps (in %)			Metaphases with Aberrations excluding Gaps (in %)			Metaphases with Exchanges (in %)		
					Minimum	Median	Maximum	Minimum	Median	Maximum	Minimum	Median	Maximum
4	-	DMSO	16	18	0.5	2.3	7.5	0.5	2.3	6.5	0.0	0.0	1.0
			16	30	0.5	1.8	4.0	0.5	1.5	4.0	0.0	0.0	0.5
		Mitomycin C	19	18	29.5	42.5	64.0	29.5	38.5	61.5	9.5	19.5	36.0
4	+	DMSO	17	18	0.0	2.5	6.0	0.0	2.5	6.0	0.0	0.5	2.5
			16	30	0.5	2.5	8.0	0.5	2.5	8.0	0.0	0.3	1.5
		Cyclophosphamide	20	18	37.0	51.0	61.5	31.0	49.8	59.0	5.5	21.8	36.0
18	-	DMSO	15	18	0.5	3.0	7.5	0.0	2.5	5.5	0.0	0.0	1.0
		Mitomycin C	19	18	20.5	29.5	48.5	20.5	29.5	44.5	4.5	9.0	16.0

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

Document PH 31536 for the in vivo micronucleus study was reviewed by Drs. Chaker and Chopra under IND 64892 and NDA 22-406, respectively. A brief summary is in

above. Rivaroxaban did not induce the formation of excess micronuclei in mice treated with two intraperitoneal doses of 140 mg/kg/day of rivaroxaban. A subsequent toxicokinetic study, PH-33256, indicated that the total exposure (AUC) was 25.7 mg*hr/L, which corresponds to an unbound exposure of 0.326 mg*hr/L or 2-fold the human exposure of unbound drug (0.167 mg*hr/L) following a rivaroxaban dose of 20 mg/day.

8 Carcinogenicity

Two year carcinogenicity studies were conducted in CD-1 mice and Wistar rats using two lots of micronized rivaroxaban. The complete reviews of these study reports were

placed in DARRTS on 6/13/11 as a separate document. The findings are briefly summarized below.

Study title: Carcinogenicity Study in CD-1 Mice

Document no.:	PH-36243
Study no.:	T3076596 (AT05917)
Study report location:	EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED Toxicology, Wuppertal Germany
Date of study initiation:	October 17, 2006
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7939 (rivaroxaban) a) Lot BXO23BS, purity 100% b) Lot BXA18UX, purity > 99.7%
Vehicle:	Solutol HS15®/ethanol/tap water (40/10/50, v/v/v)
CAC concurrence - protocol:	On August 1, 2006, the Executive CAC did not concur with the sponsor's proposed doses of (b) (4) mg/kg/day and instead recommended doses of 0, 10, 20, and 60 mg/kg/day by oral gavage, based on saturation of absorption. The Executive CAC meeting minutes are in Appendix 2.
CAC concurrence – study results:	On April 15, 2011, the Executive CAC 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 mice received oral doses of BAY 59-7939 (rivaroxaban) for up to 104 weeks. At dosages of 10, 20 and 60 mg/kg/day the mean $AUC_{(0\ 24h)}$ during Week 52 was 980, 1240, and 2520 ng.hr/mL in males, respectively, and 1590, 2370, and 3090 ng.hr/mL in females, respectively.

Summary of Non-neoplastic Findings

Consistent with the anti-coagulant pharmacodynamic action of BAY 59-7939, the mean thromboplastin time at 1 hour after dosing was significantly prolonged in all treated groups of males and females on all sampling days; however, not all members of each treated group had values above the normal range. Some of the non-neoplastic microscopic findings, such as increased pigment deposits, were also related to the pharmacodynamic action of BAY 59-7939. The combined incidence of necrosis in the liver slightly increased in the high dose males. In addition, the incidence of biliary cysts in the liver and dilation/atrophy in the preputial gland increased in mid and high dose male groups.

Adequacy of Carcinogenicity Study

The mouse carcinogenicity study used the doses (0, 10, 20, and 60 mg/kg/d) that were recommended by the Exec CAC. The study length was acceptable since the male and female mice were treated for up to 104 weeks. No statistically significant difference in mortality was observed between control and treated groups for either sex.

Appropriateness of Test Model

The Crl: CD-1™ (ICR) BR mouse strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data have been established. The proposed metabolic pathway of BAY 59-7939 in mice and man is similar involving structural cleavage and hydroxylation, although a minor metabolite, M-5, is not formed in mice.

Summary of Tumor Findings

Consistent with the increase in liver nodules macroscopically, hepatocellular tumors (adenoma and carcinoma) increased with BAY 59-7939 dosage in the males, but not in the females. If the hepatocellular tumors are combined, the statistical evaluations by the sponsor and the FDA statistician indicated p values in the trend test (p_t) of 0.0076 and 0.036, respectively. Since hepatocellular tumors are common tumors in mice, no p value for hepatocellular tumors attains the significance level ($p < 0.005$) necessary for the tumors to be considered positive, according to current CDER guidance. Furthermore, the incidence of either basophilic foci or total foci of alterations in the liver was similar across control and treated groups. The incidences of hepatocellular tumors for the male treated groups in the current study are within historical ranges.

The incidences of a few other tumors were increased in the higher dose groups compared to the incidences in the control groups. The tumors with overall incidences greater than 1% in the (b) (4) listing (2003) for spontaneous tumors in CD-1 mice include histocytic sarcoma in the high dose females (incidence of 1.6%, sponsor's $p_t = 0.176$, FDA $p_t = 0.174$), malignant lymphoma in the high dose males ((incidence of 4.5%, sponsor's $p_t = 0.136$, FDA $p_t = 0.043$) and in mid- and high dose females (incidence of 9.9%, sponsor's $p_t = 0.071$, FDA $p_t = 0.164$). The tumors with overall incidences less than 1% in the (b) (4) listing (2003) for spontaneous tumors in CD-1 mice include ovarian cystadenoma in the high dose females (incidence of 0.74%, sponsor's $p_t = 0.032$, FDA $p_t = 0.055$), testicular Leydig cell tumor in mid and high dose males (incidence of 0.85%, sponsor's $p_t = 0.070$, FDA $p_t = 0.155$) and uterine hemangiosarcoma in the high dose females (incidence of 0.47%, sponsor's $p_t = 0.086$, FDA $p_t = 0.058$). In RITA historical control database, the mean incidences of testicular Leydig cell tumor and ovarian cystadenoma are 3.2% and 1.7%, respectively. However, none of the p values obtained for these tumors attain the significance level of $p < 0.025$ required for even a rare tumor to be considered positive. In addition, the incidences are within historical ranges.

Evaluation of Tumor Findings

The FDA nonclinical and statistical reviewers concur with the sponsor that no significant evidence of neoplasia related to BAY 59-7939 treatment was observed in CD-1 mice.

Study title: Carcinogenicity Study in Wistar Rats (2 Years Administration by Gavage)

Document no.:	PH-36242
Study no.:	T8076429 (AT05916)
Study report location:	EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED Toxicology, Wuppertal Germany
Date of study initiation:	September 6, 2006
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7939 (rivaroxaban, micronized) a) Lot BXO23BS, purity 100% b) Lot BXA18UX, purity > 99.7%
Vehicle:	Solutol HS15®/ethanol/tap water (40/10/50, v/v/v)
CAC concurrence - protocol:	On August 1, 2006, the Executive CAC did not concur with the sponsor's proposed doses of (b) (4) mg/kg/day and instead recommended doses of 0, 10, 20, and 60 mg/kg/day by oral gavage, based on saturation of absorption. The Executive CAC meeting minutes are in Appendix 2.
CAC concurrence – study results:	On April 15, 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

Wistar rats received oral doses of BAY 59-7939 for up to 104 weeks. At dosages of 10, 20, and 60 mg/kg/day, the mean $AUC_{(0\ 24h)}$ was 13.4, 15.4, and 20.3 mg.hr/L in males, respectively, and 34.7, 47.5, and 48.2 mg.hr/L in females, respectively.

Summary of Non-neoplastic Findings

Consistent with the pharmacodynamic action of BAY 59-7939, the mean values for thromboplastin time for all treated groups at 1 hour after dosing on all sampling days were significantly greater than those for the control groups. Likewise, the incidence of increased pigment deposition increased in some organs and across all organs in the high dose groups. The incidence of valvular fibrosis in the heart increased with dose in both males and females and the incidence was statistically significant in females using a trend test ($p = 0.0048$).

Adequacy of Carcinogenicity Study

The rat carcinogenicity study used the doses (0, 10, 20 and 60 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.

Appropriateness of Test Model

The Wistar strain is an appropriate model because this rat strain is known to be responsive to known carcinogens and historical control data are available. The most predominant form of BAY 59-7939 in both rat and human plasma was unchanged compound. The proposed metabolic pathway of BAY 59-7939 in rats and man is similar involving structural cleavage and hydroxylation, although a minor metabolite, M-7, is not formed in rats.

Summary of Tumor Findings

Squamous cell carcinoma was present in the clitoral gland of two high dose females. Statistical evaluations by the sponsor and the FDA statistician indicated p values in the trend test (pt) of 0.030 and 0.070, respectively. Neither p value attain the significance in the trend test ($p_t < 0.025$) required for this finding of a rare tumor to be considered positive, according to current CDER guidance. Additionally, squamous cell papilloma was present in both a control female and a high dose female. Although the incidence of squamous cell carcinoma was statistically significant (p = 0.03), squamous cell papilloma was present in both a control female and a high dose female. Therefore, statistical significance for squamous cell carcinoma plus papilloma is lacking.

Adrenal cortical adenomas were present only in treated animals with the incidence significantly higher in the mid and high dose females (p = 0.012, trend test). No adrenal adenocarcinoma was found in any group. Since adrenal cortical adenoma is a common tumor, the p value for females did not attain the significance in the trend test (p< 0.005) required for this common tumor to be considered positive.

Evaluation of Tumor Findings

The nonclinical and statistical reviewers concur with the sponsor that no significant evidence of neoplasia related to BAY 59-7939 treatment was observed in Wistar rats.

Overall evaluation of carcinogenicity

The nonclinical and statistical reviewers concurred with the sponsor that no significant evidence of neoplasia related to rivaroxaban treatment was observed either in Wistar rats or CD-1 mice. The Executive Carcinogenicity Assessment Committee also concluded that there were no clear drug-related neoplasms in either study.

The safety margins for the highest dosage of rivaroxaban (60 mg/kg/day) used in the carcinogenicity studies were calculated based on AUC values for exposures to total and unbound rivaroxaban. Because the protein binding of rivaroxaban differs significantly among species, the safety margins based on exposures to unbound drug are considered more relevant for comparisons between humans and different animal species.

The recommended daily dosage of rivaroxaban for patients (a) undergoing hip and knee surgery or (b) with atrial fibrillation is 10 mg and 20 mg, respectively. Consequently, the safety margins reported in the labels for NDA 22406 and NDA 202439 will differ as indicated in Table 37 and Table 38.

Table 37: Safety Margins for Human Dose of 20 mg Rivaroxaban Daily

Study/ Species		Sex	NOAEL (mg/kg) M/F	Exposure at NOAEL		Safety Margin‡	
				Total $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Unbound† $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Based on Total AUC at NOAEL	Based on Unbound AUC
Carcinogenicity – 2 year							
Rat	Tumors	M	60	20.3	0.257	6.2	1.5
		F		48.2	0.612	14.6	3.7
Mouse	Tumors	M	60	2.52	0.162	0.76	1.0
		F		4.24	0.273	1.28	1.6

† Unbound fractions in humans, rats, mice, dogs, and rabbits are 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively.
‡ Comparison to human exposure at 20 mg/day corresponding to 0.33 mg/kg in a 60 kg patient or 3.3 mg*hr/L. Human exposure to unbound drug was 0.167 mg*hr/L.

Table 38: Safety Margins for Human Dose of 10 mg Rivaroxaban Daily

Study/ Species		Sex	NOAEL (mg/kg) M/F	Exposure at NOAEL		Safety Margin‡	
				Total $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Unbound† $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Based on Total AUC at NOAEL	Based on Unbound AUC
Carcinogenicity – 2 year							
Rat	Tumors	M	60	20.3	0.257	17.4	4.3
		F		48.2	0.612	41.2	10.3
Mouse	Tumors	M	60	2.52	0.162	2.2	2.7
		F		4.24	0.273	3.6	4.6

† Unbound fractions in humans, rats, mice, dogs, and rabbits are 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively.
‡ Comparison to human exposure at 10 mg/day corresponding to 0.16 mg/kg in a 60 kg patient or 1.17 mg*hr/L. Human exposure to unbound drug was 0.0593 mg*hr/L.

Unexpected Non Neoplastic Lesion in the Rat Carcinogenicity Study

The incidence of valvular fibrosis in the heart increased with dose in both male and female rats and was statistically significant in females by the trend test (p_t = 0.0048). In contrast, the relatively high incidence of cardiomyopathy in rats was similar across male groups and only slightly increased with dose in female groups.

Table 39: Reviewer's Summary - Non-neoplastic Lesions - Document PH-36242

Rat Carcinogenicity Study		BAY 59-7939 Dose level (mg/kg/day)							
Non-Neoplastic Findings		Male				Female			
	All main study animals	0	10	20	60	0	10	20	60
Organ/Tissue	Finding #/group	50	50	50	50	50	50	50	50
Heart	Examined	50	50	50	49	50	50	50	50
Cardiomyopathy	#	39	39	41	41	27	28	30	33
	%	78	78	82	82	54	56	60	66
Valvular fibrosis (p_t = 0.0048,	#	0	1	1	2	0	0	2	**4***
p_e = 0.0587)	%	0	2	2	4	0	0	4	**8**
	Severity	-	3	2	2	-	-	2	**2.5**

* $p<0.05$, p_t = p value for the trend test, p_e = p value for Exact Fisher pairwise test

Valvular fibrosis was not observed in the 6-month rat (PH-33611), 12-month dog (PH-34235) or 2-year mouse studies (PH-36243) using rivaroxaban. Valvular fibrosis was not listed in the Charles River 2011 Compilation of Neoplastic and Non-neoplastic Lesions in Wistar Rats; however, valvular endocardiosis was observed in 1/1217 male and 1/1217 female fetuses. In the discipline review letter of 5/4/11, the sponsor was asked to provide historical control data for valvular fibrosis and an additional description (specific valves and structures involved) of the valvular lesion.

The sponsor provided the data below (Table 40) for the untreated control groups from two rat carcinogenicity studies that used the same Wistar strain obtained from the same animal source as used in the rivaroxaban 2-year carcinogenicity study. The study dates and identification of the study pathologist were not provided. Because one study (T9072749) had two control groups, the incidence of valvular fibrosis and cardiomyopathy in these studies is presented as percentages. The incidence of valvular fibrosis varied 1.7-fold in males and 3.2-fold in females between these two control studies. In contrast, the incidences of valvular fibrosis in all male (0-4%) and female (0-8%) groups in the rivaroxaban study were at or below the control range in males (12-20%) and females (8-26%). The sponsor attributed the differences in incidences to inconsistency of histopathological sampling of heart valves leading to variability in histopathological diagnosis. The carcinogenicity reports indicated that the laboratory conducting the studies (Bayer Schering Pharma AG) is a member of the RITA group (Morawietz et al. 1992). Therefore, only one section of the heart was probably examined based on the published RITA guidelines (Morawietz et al. 2004). In addition, the reported incidence of valvular fibrosis may vary with the pathologist making the diagnosis.

Table 40: Reviewer's Summary – Sponsor's Historical Data from SD 59

		Males		Females	
	Study	AT043333	T9072749*	AT043333	T9072749*
Heart	# examined	50	100	50	100
Cardiomyopathy	%	60	79	52	33
Valvular fibrosis	%	20	12	26	8
* Two control groups combined					

Between the two control studies, the incidence of cardiomyopathy varied less (1.3 fold in males and 1.6 fold in females) than that for valvular fibrosis. The ranges for cardiomyopathy (60-79% in males and 33-52% in females) are within the ranges (12-100% in males and 6-93% in females) reported in the Charles River 2011 Compilation of Neoplastic and Non-neoplastic Lesions in Wistar Rats. In the rivaroxaban rat carcinogenicity study, the incidence of cardiomyopathy was 78 to 82% in males and 54 to 66% in females (Table 39). The incidences of cardiomyopathy in the rivaroxaban study were at or slightly above the high end of the laboratory historical control ranges. The sponsor maintains that valvular fibrosis is an integral part of the chronic cardiomyopathy common in aging rats.

The sponsor described valvular fibrosis, also referred to as endocardial myxomatous change (EMC), as

> "a fibro-myxomatous septal or nodular thickening of the valves, predominantly in the subendocardial region. In cases not affecting the whole valve, the alteration

> was located towards the free edge of the valve. Occasionally, a segmental thickening with a broad base along the valve surface was noticed. Slight to moderate cellular infiltration was seen consisting predominantly of fibroblasts with an elongated nucleus and an indistinct cell border. Additional mononuclear cell infiltration was seen; however, to a lower degree. The basophilic slightly edematous appearance of the lesions corresponds to the myxomatous nature of the finding."

The sponsor stated that the valvular fibrosis was "almost exclusively seen on the left atrioventricular valve" (mitral valve). In contrast to this preference for the mitral valve, only single cases additionally involved the right atrioventricular (tricuspid) valves, the aortic or the pulmonary valves, but at a lower severity than for the mitral valves. Elangbam et al. (2002) was cited as showing a high incidence of valvular fibrosis in the mitral valve in aged Sprague-Dawley rats. However, Elangbam et al. (2002) found an incidence of 68% for the mitral valve, 57% for the aortic valve, 33% for the pulmonary, and 30% for the tricuspid valve at the end of their 2-year rat study in which 85% of the animals had valvular findings. Elangbam et al. (2002) concluded that EMC has a strong microscopic resemblance to the valvular disease in humans induced by ergotamine, methysergide, and fenfluramine-phentermine. In a review of cardiac valvular pathology, Donnelly (2008) stated that all of these drugs have been reported to be associated with mitral and/or tricuspid regurgitation with histologic valve lesions similar to carcinoid syndrome. In contrast, three month administration of serotonin to 10 Sprague-Dawley rats was shown to induce pulmonary and aortic insufficiency in 3 and 1 rats, respectively (Gustaffsson et al. 2005).

The sponsor also noted that in all but one rat, the valvular findings were accompanied by pronounced chronic cardiomyopathy, a common age-related finding in the rat. Lewisand Maron (1992) reported that Sprague-Dawley rats display an age-related increase in severity and incidence of both cardiomyopathy and cardiac valvular lesions with the latter present at 28 and 17% in males and females, respectively. Elangbam et al. (2002) found the incidence of cardiomyopathy was 82% in male and 39% in female 2-year old Sprague Dawley rats. EMC was associated with 90% and 84% of the incidences of cardiomyopathy in males and female rats, respectively.

Because drug-induced valvular heart disease in humans has been associated with increased serotonin levels, activation of the 5-HT_{2B} receptor, and increased serotonin transporter binding (Bhattacharyya et al. 2009), the sponsor was asked to provide additional information about the methods, the species, and the isoforms of the receptors used for the serotonin receptors and transporters evaluated in study report R-8463 (Effects of Rivaroxaban in Radioligand Binding Assays). In addition, an evaluation was requested of the binding of rivaroxaban and its major metabolites to the human and rat 5-HT_{2B} receptors relative to the binding of appropriate agonist and antagonist positive controls for these receptors.

The sponsor stated that Wistar rat brain was the source of non-selective 5-HT_1 and 5-HT_2 serotonin receptors used in the radioligand binding assays of rivaroxaban. As indicated in Table 7, rivaroxaban was considered inactive in these assays, because the effects were less than 50%. The sponsor also pointed to the lack of an effect of rivaroxaban on rat brain monoamine oxidase A (MAO_A), which metabolizes serotonin.

Although binding of rivaroxaban to serotonin transporters was not evaluated, the sponsor showed that [redacted] (b) (4) of rivaroxaban, was inactive in binding to a human recombinant 5-HT transporter and implied that rivaroxaban should also be inactive.

The sponsor provided additional information regarding the relationship of rivaroxaban to effects of serotonin. The sponsor stated that at 5 µM rivaroxaban and other closely related oxazolidinone structures were considered inactive in a cell-based assay specific for the human 5-HT_{2B} receptor subtype compared to a positive antagonist control compound. In addition, rivaroxaban in the presence of heparin-induced thrombocytopenia antibodies did not induce the in vitro activation of platelets and release of serotonin in a ^{14}C-serotonin release assay (R-8466, Table 8).

In summary, the sponsor reasonably concluded that valvular fibrosis in the 2-year carcinogenicity study in rats is not the result of treatment with rivaroxaban, but is due to common physiological changes in aging rats. In addition, the sponsor search of the safety database for adverse events related to cardiac valves, but excluded congenital valvular disease, in completed clinical trials involving rivaroxaban use for at least 90 days indicated a similar incidence in rivaroxaban treated groups and in the active comparator groups (SD 89, 7/20/11).

9 Reproductive and Developmental Toxicology

Study reports for the reproductive and developmental studies (Fertility and Early Embryo Development [FEED], Embryo-Fetal Development [EFD], and Pre-/Postnatal Development [PPND]) were previously reviewed under IND 64892 and NDA 22406. The full reviews of these studies by Drs. Chakder and Chopra can be found in DARRTS.

Table 41: Overview of Reproductive and Developmental Studies

Document number	Study number	Study	Lot BAY 59-7939	Reviewed under	
				IND 64892	NDA 22406
PH 33273	T 2062789	Rat FEED	J20020528	S. Chakder	Y. Chopra
PH 33582	T3063590	Rat EFD	J20020528	S. Chakder	Y. Chopra
PH 33380	T0062930	Rabbit EFD	J20020430	S. Chakder	Y. Chopra
PH 33368	T0062930	TK in rabbit EFD	J20020430	S. Chakder	Y. Chopra
PH 34608	T9062957	Rat PPND	030723-100		Y. Chopra

The following table (Table 42) summarizes the four reproductive and developmental toxicology studies. All these studies were conducted using the BAY 59-7939 co-precipitate drug substance.

Table 42: Brief Summaries of Reproductive and Developmental Toxicology Studies for Rivaroxaban

Study	FEED - Rat	EFD - Rat	EFD - Rabbit	PPND - Rat
Species	Rat	Rat	Rabbit	Rat
Document no.	PH 33273	PH 33582	PH 33380/PH 33368	PH 34608
Study code	T 2062789	T3063590	T0062930	T9062957
Drug lot/purity	J20020528, 9.5%	J20020528, 9.5%	J20020430, 9.5%	030723 100, 9.2%
Drug comment	All lots were of BAY 59 7939 Coprecipitate (active ingredient 9.5%)			
Conducting lab and location	Bayer AG, PH PD Toxicology, Wuppertal, Germany			
Study dates	8/19/02 to 4/7/04	7/3/02 11/12/04	6/12/02 8/5/04	1/13/04 9/26/06
GLP/QA	Yes	Yes	Yes	Yes
Vehicle	20% Solutol HS 15/80% water with PEG 6000	Water with PEG 6000	Water with PEG 6000	20% Solutol HS 15/80% water with PEG 6000
Strain	Wistar rats (Hsd Cpb:WU)	Wistar rats (Hsd Cpb:WU)	Rabbits (CHBB:HM strain)	Wistar rats (Hsd Cpb:WU)
Number/group	24/sex/group	22 female/group	18 20 female/group	25 female/group
Doses	0, 12.5, 50, and 200 mg/kg	0, 10, 35, and 120 mg/kg	0, 2.5, 10, 40, 160 mg/kg	0, 2.5, 10, 40 mg/kg
Route	oral	oral	oral	oral
Treatment duration Males(M) and female (F)	M: daily 4 wks prior to mating through GD 7 F: daily 2 wks prior to mating through GD 7	F: daily from GD 6 through GD17	F: GD 6 through GD 20	F_0: GD 6 to PND 21
Cesarean section day	GD 14 16	GD 20	GD 29	PND 21
Study acceptability	Litter numbers/group and high dose adequate	Litter numbers/group and high dose adequate	Litter numbers/group and high dose adequate	Litter numbers/group and high dose adequate
Comments: Parental	Mortality attributed to dosing errors M: 1 each in control, 50 & 200 mg/kg groups; F: 2 at 200 mg/kg Salivation: increased in M & F at ≥50 & ≥12.5 mg/kg BW gain: decreased at ≥50 mg/kg in M and F. Food intake decreased in M & F at 200 mg/kg during Wk 1. One F at 200 mg/kg had no viable fetuses.	Mortality: 1 at 120 mg/kg due to BW loss, lack of food intake and bleeding. BW gain & food intake decreased at 120 mg/kg Red vaginal discharge in 5/19 at 120 mg/kg, # female with necrotic placentas increased at 35 and 120 mg/kg	Mortality: 2 each at 40 and 160 mg/kg. Abortions at ≥10 mg/kg; Total resorptions at ≥2.5 mg/kg Decreased food intake and BW gain at ≥10 mg/kg Placental alterations ≥10 mg/kg 160 mg/kg above a MTD	Mortality F_0: 7 F dying or killed at 40 mg/kg Bleeding and impaired delivery at 40 mg/kg Decreased body weight and food intake at 40 mg/kg Decreased rearing index and F1 pup viability index (PND 4) at 40 mg/kg
Comments: Embryo/fetal/ offspring	Although mean post implantation loss/litter and % post implantation loss increased at 50 & 200 mg/kg, values were within historical control range and number of viable fetuses was not affected. However, number of litters with any post implantation loss and with loss of >1 fetus post implantation increased at 50 and 200 mg/kg.	Although not statistically significant, % of males decreased at 120 mg/kg and fetal weight decreased at 120 mg/kg. No increased Incidence of malformations, but the incidence of incomplete ossification of some bones increased and the incidence of incomplete ossification of other bones decreased	Post implantation loss increased at ≥10 mg/kg Number of live fetuses decreased at ≥40 mg/kg % of males decreased at 40 mg/kg and fetal weight decreased at ≥40 mg/kg. Delayed ossification at 40 and 160 mg/kg and increased % of malformed fetuses, but % within historical range and no specific defect	Increased pup loss and decreased pup survival through LD 4 at 40 mg/kg Increased # pups at 40 mg/kg with no milk spot, milk in stomach or intestines Decreased pup weight at 40 mg/kg. No effect on postnatal development after PND 4 and reproductive ability of F1 pups,
Key findings:	BAY 59 7939 had no significant effect on mating, fertility and implantation up to 200 mg/kg.	Although no effect on the incidence of fetal malformations up to 120 mg/kg, decreased fetal body weight and % males at 120 mg/kg was associated with maternal toxicity.	Although BAY 59 7939 had no effect on the incidence of fetal malformations up to 160 mg/kg, fetal toxicity was present ≥40 mg/kg in association with maternal toxicity.	BAY 59 7939 up to 40 mg/kg had no effect on development of F1 pups after LD 4; however, prior to LD4 pups of dams at 40 mg/kg had decreased survival along with high mortality of their dams.
Parental NOAEL	12.5 mg/kg	35 mg/kg (placenta 10 mg/kg)	2.5 mg/kg	10 mg/kg
Offspring NOAEL	Embryo toxicity: 50 mg/kg Fertility: 200 mg/kg	Malformation: 120 mg/kg Toxicity: 35 mg/kg	Malformation: 160 mg/kg Toxicity: 2.5 mg/kg	Peri natal <LD4: 10 mg/kg Post natal >LD4: 40 mg/kg

In the table below, exposure comparisons are made between animals and humans based on the NOAEL dose and the dose at which toxicity was observed in the reproductive toxicology study.

Table 43: Exposure Comparisons for Reproductive Toxicology Studies

Study	FEED	EFD	EFD	PPND
Species	Rat	Rat	Rabbit	Rat
Document no.	PH 33273	PH 33582	PH 33380/PH 33368	PH 34608
Comparisons based on NOAEL dose				
Comparison NOAEL dose/ human, mg/m^2	Parental = 12.5 mg/kg: 2.0/0.33 = 6.1 Embryo = 50 mg/kg: 8.1/0.33 = 24.5 Fertility = 200 mg/kg: 32.1/0.33 = 97	Placental = 10 mg/kg: 1.62/0.33 = 4.9 Maternal & fetal toxicity = 35 mg/kg: 5.7/0.33 = 17 Malformation = 120 mg/kg: 19.4/0,33 = 59	Maternal & fetal toxicity 2.5 mg/kg: 0.81/0.33 = 2.5 Malformation: 160 mg/kg: 51.8/0.33 = 157	Maternal & peri natal toxicity = 10 mg/kg: 1.62/0.33 = 4.9 Postnatal toxicity = 40 mg/kg: 6.5/0.33 = 19.6
Total exposure (AUC) at NOAEL to human AUC	Parental = 12.5 mg/kg: 17.7 22.3/3.3 = 5.4 6.8 Embryo = 50 mg/kg: 83.3 99.5/3.3 = 25.2 30 Fertility = 200 mg/kg: (156 to 227)/3.3 = 47 69	Placental = 10 mg/kg: 18.9/3.3 = 5.7 Maternal & fetal = 35 mg/kg: 77.7/3.3 = 23.5 Malformation = 120 mg/kg: 188/ 3.3 = 57	Placental = 10 mg/kg: 18.9/3.3 = 5.7 Maternal & fetal toxicity = 2.5 mg/kg: 0.74/3.3 = 0.22 Malformation = 160 mg/kg: 23.9/3.3 = 7.2	Maternal & peri natal = 10 mg/kg: 18.9/3.3 = 5.7 Postnatal toxicity = 40 mg/kg: >77.7/3.3 = 23.5
Unbound exposure at NOAEL to human AUC	Parental = 12.5 mg/kg: (0.225 to 0.283)/0.167 = 1.3 1.7 Embryo = 50 mg/kg: (1.06 to 1.26)/0.167 = 6.3 7.5 Offspring = 200 mg/kg: (2.10 to 2.88)/0.167 = 12.5 17.2	Placental = 10 mg/kg: 0.24/0.167 = 1.4 Maternal & fetal = 35 mg/kg: 0.99/0.167 = 5.9 Malformation = 120 mg/kg: 2.39/0.167 = 14.3	Maternal & fetal toxicity 2.5 mg/kg: 0.173/0.167 = 1.0 160 mg/kg: 5.59/0.167 = 33.5	Maternal & peri natal = 10 mg/kg: 0.240/0.167 = 1.4 Postnatal toxicity = 40 mg/kg: >0.99/0.167 = >5.9
Comparisons based toxic dose				
Toxic dose/ human, mg/m^2	Parental = 50 mg/kg: 8.1/0.33 = 24.5 Offspring =200 mg/kg: 32.1/0.33 = 97	Maternal (placental) & Fetal toxicity = 120 mg/kg: 19.4/0.33 = 595.7/0.33 = 17 Malformation = >120 mg/kg: 19.4/0.33 = 59	Maternal & fetal toxicity 10 mg/kg: 3.24/.33 = 9.8 Malformation >160 mg/kg: 51.8/0.33 = 157	Maternal & peri natal toxicity = 40 mg/kg: 6.5/0.33 = 19.6 Postnatal toxicity > 40 mg/kg: 6.5/0.33 = 19.6
Total exposure (AUC) at toxic dose to human AUC	Parental = 50 mg/kg: 83 100/3.3 = 25 30 Offspring = 200 mg/kg: 156 227/3.3 = 47 69	Maternal (placental) & Fetal toxicity = 120 mg/kg: 188/ 3.3 = 57 Malformation > 120 mg/kg: >188/ 3.3 = 57	Maternal & fetal toxicity = 10 mg/kg: 2.78/3.3 = 0.84 Malformation > 160 mg/kg: 23.9/3.3 = 7.2	Maternal & peri natal = 40 mg/kg: >77.7/3.3 = >23.5 Postnatal toxicity > 40 mg/kg: >77.7/3.3 = >23.5
Unbound exposure at toxic dose to human AUC	Parental = 50 mg/kg: 1.05 1.27/0.167 = 6.3 7.6 Offspring = 200 mg/kg: 2.10 2.88/0.167 = 12.5 17.2	Maternal (placental) & fetal toxicity = 120 mg/kg: 2.39/0.167 = 14.3 Malformation > 120 mg/kg: 2.39/0.167 = 14.3	Maternal & fetal toxicity = 10 mg/kg: 0.65/0.167 = 3.9 Malformation > 160 mg/kg: 5.59/0.167 = 33.5	Maternal & peri natal = 40 mg/kg: >0.99/0.167 = >5.9 Postnatal toxicity > 40 mg/kg: >0.99/0.167 >5.9
Comment	No TK in study. Use AUC from 4 week rat study (PH 32333)	TK in study	TK in study	No TK in study. Use AUC from rat EFD study (PH 33582)
Unbound fraction in humans, rats, and rabbits is 5.07%, 1.27%, and 23.4%, respectively. Human exposure at 20 mg/day corresponding to 0.33 mg/kg in a 60 kg patient was 3.3 mg*hr/L. Unbound human exposure is 0.167.				

In the following sections, the reviewer summarizes for each reproductive and developmental study those parameters that affect the determination of the reviewer's NOAELs above.

9.1 Fertility and Early Embryonic Development

In an acceptable fertility and early embryo development study in rats, the NOAEL for paternal and maternal toxicity was 12.5 mg/kg, because body weight gain and food intake decreased at ≥50 mg/kg. Although the gestation index decreased at 200 mg/kg, the NOAEL for embryo toxicity can be considered 200 mg/kg, because the number of viable fetuses per litter was not significantly affected at 200 mg/kg.

Table 44: Reviewer's Summary Rat FEED - PH 33273

Rat FEED	0	12.5	50	200	Historical^
Body weight gain: M, D1 22 F, GD1 14	55.6 60.5	54.5 64.1	50.2 56.1	46.7 55.2	
Females mated	23	24	24	23	(11 studies)
Time to mate, d	2.0	1.8	1.9 (3.8[1])	2.7	2.9 (2.0-4.3)
Females re-mated	0	0	3	0	
Females inseminated	22	24	24	23	
Mating Index	95.7	100	100	100	99.6 (96-100)
Females with implantations	19	20	21	21	(17-23)
Fertility Index	86.4	83.3	87.5	91.3	83.1 (70.8-87.5)
Females with viable embryos	19	20	21	19[†]	
Gestation Index	100	100	100	**90.5**	99.1 (94.7-100)
Females with no live fetus	0	0	0	1	0.01 (0-1) (2/205 litters)
Cesarean section parameters (range)					
Females evaluated	19	20	21	20	
Corpora lutea/litter	13.9 (4-19)	14.3 (11-17)	14.2 (11-19)	13.8 (10-17)	13.5 (12.2-14.4)
Implantation sites/litter	11.5 (1-18)	13.0 (7-16)	12.4 (5-17)	11.9 (2-16)	11.24 (8.3-13.2)
Pre-implantation loss, #/litter	2.5 (0-7)	1.3 (0-4)	1.8 (0-5)	2.0 (0-12)	2.24 (1-4.9)
% Pre-implantation loss	18.0 (0-83.3)	9.1 (0-30)	12.7 (0-50)	14.5 (0-84.6)	16.6 (8.1-34.1)
Post-implantation loss (PoIL), #/litter	0.2 (0-1)	0.4 (0-1)	0.7 (0-4)	0.8 (0-4)	0.96 (0.6-1.3)
# litter with PoIL	4	8	**11**	**11**	
# litter with PoIL ≥2	0	0	2	3	
% Post-implantation loss	1.7 (0-10)	3.0 (0-10)	**5.6** (0-18.2)	**6.7** (0-36.4 [100[‡]])	8.5 (4.5-10.9)
Live fetuses/litter	11.3 (1-17)	12.6 (7-16)	11.7 (6-17)	11.1 (0-16)	10.32 (7.6-12.6)
$AUC_{(0-24)}$[1], mg*hr/L (M, F)		17.7, 22.3	83.3, 99.5	156, 227	

^ 11 studies from 1995 to 2001, [†] Excludes moribund Female180 with implantation sites that was euthanized. [‡] Female 195 had 85% pre implantation loss and 100% post implantation loss of the two implantations. [1] From PH 32333

9.2 Embryonic Fetal Development

Rat

In an acceptable embryo-fetal development study in rats, the NOAEL for maternal toxicity was 35 mg/kg, because of decreased body weight gain, increased mortality and increased bleeding at 120 mg/kg. However, the NOAEL for placental effect was 10 mg/kg, because the incidence of necrotic placentas increased at ≥35 mg/kg. The NOAEL for fetal toxicity was 35 mg/kg, because fetal body weight and percentage of male fetuses decreased at 120 mg/kg. The percentage of fetuses with malformations for all dose groups was within the historical range. Although no individual malformation

increased significantly with dose, ventricular septal defects (VSD) were observed in one fetus in each of the control, mid, and high dose groups, and VSD in conjunction with persistent truncus arteriosus was found in an additional fetus in the high dose group. However, the three fetuses in the mid and high dose groups were sired by the same male. VSDs also occur spontaneously in rats (Solomon et al. 1997; Burdan et al. 2006) and humans (Hoffman and Kaplan 2002). Since at least the smaller VSDs close postnatally in rats (Solomon et al. 1997; Fleeman et al. 2004) and humans (Miyake 2004), some investigators consider VSDs as developmental delays rather than malformations (Solomon et al. 1997; Fleeman et al. 2004). Regarding variations, ossification of some fetal bones was delayed and the ossification of other bones was accelerated at 120 mg/kg. The NOAEL for malformations was 120 mg/kg.

Table 45: Reviewer's Summary Rat EFD - Document PH-33582

Rat EFD	0	10	35	120	Historical[†]
Body weight gain: F, GD 0-20	107.3	105.8	102.7	93.9	
Females mated	22	22	22	22	
Females with implantations	19	22	20	20	
Females evaluated	19	22	20	19 °	
Females with red vaginal discharge	0	1	0	**5**	0
Females with necrotic placenta	5 (26%)	6 (27%)	**12* (55%)**	**13* (68%)**	0-11.8%
Females with viable fetuses	19	22	20	19	
Corpora lutea/litter	12.5 (11[1']-16)	13.5 (9-17)	13.1 (10-16)	13.5 (12 [5^]-16)	13.0-15.9
Implantation sites/litter	11.6 (8[1']-15)	12.3 (6[2]-15)	11.6 (2-15)	12.7 (9 [1^]-15)	11.8-13.8
Pre-implantation loss, #/litter	0.9 (0-3)	1.2 (0-8[2])	1.6 (0-9[a])	0.8 (0-4)	0.8-2.7
Pre-implantation loss, ≥3	0	4	2	1	
Early resorption/litter	0	0	0	0	
Late resorption/litter	0.4	0.9	0.7	0.6	0.4-1.5
Total resorption/litter	0.4	0.9	0.7	0.6	
Post-implantation loss (PoIL), #/litter	0.4 (0-2)	1.0 (0-5[2])	0.7 (0-2)	0.6 (0-2[9°])	0.5-1.6
# litter with PoIL	6	13	12	10	
# litter with PoIL ≥2	2	4	2	4	
Dead fetuses/litter	0	0.1[3]	0	0	0-0.1
Live fetuses/litter	11.2 (8[1']-15)	11.3 (5-14)	10.9 (4[2]-15)	12.1 (10[1^]-16)	10.9-12.9
% males	56.6 (33-77[100'])	48.7 (36-80)	50.1 (33-73)	**43.2** (21[0^]-62)	46.9-56.3
# litters % male <33%	0	0	0	3	
Fetal weight, gm	3.59 (3.18-3.89)	3.57 (2.76-3.92)	3.70 (3.37-4.22)	**3.48** (2.6-4.27)	3.54-3.77
% malformed fetuses	1.4	2.8	1.8	2.6	0.0-6.9
# litter with malformations	3	6	4	5	0-7
% litters with malformations	15.8	27.3	20.0	26.3	0-35
Ventricular septal defect, with & without truncus arterious, # (%)	1 (1/101 = 0.99%)	0	1 (1/104 = 0.96%	2 (2/110) = 1.9%	
$AUC_{(0-24)}$, mg*hr/L		18.9	77.7	188	

[†] 18 studies from 1998-2000, [1] Female 1057 had only 1 corpora lutea, 1 implantation, and 1 live male fetus [2] Female 1067 had 14 corpora lutea, 6 implantation sites, and 5 live fetuses, [3] Female 1116 had 2 dead fetuses and 13 live fetuses, [a] Females 1069 and 1127 had 8 and 9 preimplantation losses and 2 and 4 live fetuses, respectively, ^ Female 1088 had 5 corpora lutea, 1 implantation and 1 live female fetus, ° Female 1068, who had 9 implantations and 1 late resorption, was excluded from mean calculation because she was euthanized on GD 16 due to red vaginal discharge, blood weight loss, absence of food intake and hypoactivity.

Rabbit

In an acceptable embryo-fetal development study in rabbits, the NOAEL for maternal toxicity was 2.5 mg/kg, because body weight gain decreased and the incidence of abortions and necrotic placentas increased at ≥10 mg/kg. Since mortality of dams occurred at ≥40 mg/kg, the dose of 160 mg/kg was above a maximum tolerated dose. Each treated group had one dam having total resorptions and dams displaying evidence of bleeding. Although the percentage of malformed fetuses increased at ≥40 mg/kg, the percentages were within the historical control range. Furthermore, the types and incidences of most malformations were similar to those in the historical control groups and did not display a dose-relationship. However, the litter incidence of ventricular septal heart defect at 160 mg/kg was slightly higher than the maximum historical control incidence. The incidence of this defect involved up to 2 fetuses in 2 litters in any one historical control group and has shown a trend of increased backround incidence in the most recent years of data provided. These malformations also occur regularly in the same strain of rabbit at other laboratories (Viertell and Trieb 2003). Therefore, the NOAEL for malformations is 160 mg/kg. In contrast, the NOAEL for fetal toxicity was 2.5 mg/kg, because late resorptions and post-implantation loss increased at ≥10 mg/kg and the number of live fetuses per litter and fetal body weight decreased at ≥40 mg/kg.

Table 46: Reviewer's Summary Rabbit EFD - Document PH-33380

Rabbit EFD	Dose, mg/kg					Historical† mean range
	0	2.5	10	40	160	
Body weight gain, GD 6-20	47	102	29	10	12	
Females mated	20	20	20	20	12	
Female deaths	0	0	0	2[a]	2[d]	
Females with implantations	20	20	20	18	9[o]	
Females aborted	0	0	1[2] (5%)	2[b] (11%)	2[e] (22%)	0-10%
Females with total resorption (% implanted)	0 (0)	1[1] (5%)	1[3] (5%)	1[c] (5.6%)	1^ (11%)	0-5%
Females with red discharge	0	2 (10%)	2 (10%)	11 (61%)	8 (89%)	0-5%
Females with live fetuses	20	19	18	15	6	
Gestation rate	100	95	90	83.3	66.7	90-100%
Females with necrotic placenta (% with live fetus)	1 (5)	1 (5.3)	7 (38.9)	10* (66.7)	3 (50)	0-4.5%
Females with coarse grained placenta (%)	0 (0)	0 (0)	3 (16.7)	4 (26.7)	3* (50)	0-11.5%
Corpora lutea/litter (range)	8.0 (5-11)	8.3 (4-13)	7.4 (5-10)	7.9 (6-10)	8.7 (7-11)	7.6-8.8
Implantations/litter (range)	6.7 (0-10)	7.3 (0-13)	6.9 (5-9)	7.3 (2-10)	7.8 (5-11)	6.8-8.1
Pre-implantation loss, #/litter	1.4 (0-7)	1.0 (0-6)	0.6 (0-5)	0.9 (0-6)	0.8 (0-3)	0.2-1.6
Early resorption/litter	0	0	0	0.2 (0-3)	0	0
Late resorption/litter {§} (range)	0.4 (0-3)	0.3 {0.6} (0-1[8[1]])	0.7 {1.2} (0-4[9[3]])	1.3 {1.2} (0-4)	2.8 {3.4} (0-8)	0.1-0.9
Post-implantation loss (PoIL), #/litter {§} (range)	0.4 (0-3)	0.3 {0.6} (0-1 [8[1]])	0.7 {1.2} (0-5 [9[3]])	1.3 {1.4} (0-4 [7, 8[b]])	2.8 {3.4*} (0-8 [9, 10[e]])	0.1-0.9
# litter with PoIL (% implanted) {§, (% implanted)}	7 (35)	5 (25) 6 (32)	6 (30) 7 (39)	10 (55) 14 (78)	4 (44) 8 (88)	
Dead fetuses/litter	0	0	0	0	0	0
Live fetuses/litter (range)	6.2 (1-9)	7.0 (0-13)	6.2 (0-9)	6.0 (0-9)	5.0 (0-9)	6.1-7.5
% of implantations/litter	93.5	95.9	89.5	82.8	67.5*	87.4-98.1
% males	52.7	55.6	53.5	44.1	50.9	46-60

Rabbit EFD	Dose, mg/kg					Historical† mean range
	0	2.5	10	40	160	
Fetal weight, gm (range)	40.5 (31.7-41.5)	38.6 (28.6-45.3)	39.0 (31.5-43.5)	**35.4*** (27.1-40.8)	**32.6*** (26.0-38.6)	32.4-39.0
% malformed fetuses	3.2	3.0	5.4	12.2	13.3	0-13.4%
# litter with malformations	3	4	5	8	2	
% litters with malformations	15.0	21.1	27.8	53.3	33.3	0-47%
Ventricular septal heart defect with/without additional heart defects, # fetuses, # litters, (% implanted litters)	0, 0 (0)	1, 1 (5.3)	1, 1 (5.6)	1, 1 (6.7)	2, 1 (16.7)	0-12.5%
$AUC_{(0-24)}$, mg*hr/L		0.74	2.78	13.1	23.9	

† 17 studies from 1998 2001, * p < 0.05, º Female 5428 excluded on GD before dosing initiated. ¹ Female 5492 had 8 late resorptions and no viable fetuses, ² Female 5454 aborted and was excluded from calculations ³ Female 5455 had 9 late resorptions and no viable fetuses [a] Females 5403 and 5410 were euthanized because of abortions and were excluded from calculations [b] Females 5383 and 5414, who died, had 7 and 8 post implantation losses, respectively and were excluded from calculations, [c] Female 5426 had 6 and 3 pre and post implantation losses, respectively, and no viable fetuses, [d] Females 5377 and 5401, who died, had 9 and 7 post implantation losses, respectively and were excluded from calculations, [e] Females 5379 and 5399 were euthanized because of abortions and were excluded from calculations ^ Female 5390 had 3 and 7 pre and post implantation losses, respectively, and no viable fetuses. {§} Calculation including litters with total resorption.

9.3 Prenatal and Postnatal Development

In an acceptable pre-/postnatal development study in rats, the NOAEL for maternal toxicity was 10 mg/kg, because mortality, abortions, bleeding, and decreased body weight gain was observed at 40 mg/kg. The incidence of stillborn F1 pups increased at ≥10 mg/kg. Although the number of stillborn pups increased and the number of live pups on LD 0 and LD 4 decreased at both 10 and 40 mg/kg, the NOAEL was considered to be 10 mg/kg based on the higher percentage of pup survival at 10 mg/kg (95.5%) compared to that at 40 mg/kg (75.8%). The higher incidence of the clinical observations of milk spot absence and hypoactivity correlated with offspring necropsy findings on LD 0 through LD 2 of empty stomach and/or empty intestines, particularly in three litters (#54, #55 and #100). Despite the peri-natal effects, treatment of F0 females with BAY 59-7939 did not affect postnatal survival after LD 4, sex ratio, body weight at birth, post-natal body weight development, or delay general physical development of the F1 offspring. Neurological assessments showed no significant effect of BAY 59-7939 treatment on F1 reflexes, sensory functions, learning ability, memory and explorative behavior. Treatment of F0 dams with BAY 59-7939 during pregnancy and lactation did not impair the fertility of the F1 animals or the survival or development of the F2 embryos through gestation and delivery.

Table 47: Reviewer's Summary - Pre-/Postnatal Development – PH 34608

Rat PPND	Dose, mg/kg				Historical controls[†] Min/Max[‡]
Parameter	0	2.5	10	40	
F0 females mated	25	25	25	25	
Number with implantations	19	21	23	24	
Implantations/litter	13.06	12.29	12.61	12.23	9.9-13.8
Fertility index, %	76	84	92	96	65-92
F0 females excluded	1	0	0	2	
Prenatal loss/litter	0.67	0.52	1.22	1.05	0.4-1.75
F0 Dams dying prior to or at delivery (F0 #, GD died)	1 (#71d19)	0	0	**2** (#84d20, #95d23)	1 (12 studies)
Dams delivering pups	18	21	23	22	
Dams with viable pups	18	21	23	22	
Dams with only dead pups	0	0	0	0	
Dams with stillborn pups	0	1	**4**	**3**	0-6
# stillborn pups	0	1	**11***	3	0-13
Pups delivered /litter	12.39	11.76	11.39	11.18	
Gestation index, %	94.7	100	100	91.7	82-100
Gestation duration, days	22.6	22.4	22.5	22.6	22.3-22.8
F0 Dams with bleeding delivery to PND4	0	0	0	**8** (#51, 54, 55, 61, 84, 93, 95, 100)	1 (8 studies)
F0 Dams dying LD 0-4	0	0	0	**3** (# 2d3, #54d1, #100d0)	0 (8 studies)
F0 Dams killed LD 0-4 – all pups died (F0#, LD)	0	0	0	**2** (#51d0, #55d2)	
Dams rearing pups to LD 4	18	21	23	17	
Rearing index, %	100	100	100	**77.3**	90.5-100
Dams with live born pups, but no pups alive on LD 4	0	0	0	**5**	
Pups dead, missing, killed or cannibalized on LD 0 LD 1-4 LD 0-4 LD 5-21 LD 1-21	5 5 10 0 10	0 2 2 3 5	4 6 10 3 13	**28*** **34*** **62*** 2 **64***	0-23 (after LD0 to end of rearing
% pup survival LD 1-4	96.0	98.9	95.5	**75.8***	94.3-100
Live pups/litter – LD 0	12.4	11.7	10.9	11.0	8.9-12.5
Live pups/litter – LD 4	11.8	11.6	10.5	10.6	8.8-12.4
% male – LD 0	51.4	48.3	47.9	55.2	
F1 Pups with No milk spot, #pups/# litters, (F0 #, day of death) Hypoactivity, #pups/# litters, (F0 #, day of death)	1/1 0/0	2/2 0/0	1/1 (#22d9) 3/1 (#22d9)	**36*/4** (#27d6, 54d0, #55d0-1, #100d0) **13*/4** (#27d6, #54d1, #55d1, #61d4)	0-3 0-6
F1 Birth Weight on LD 1: Offspring Mean/litter Range litter means	 6.39 5.7-7.4	 6.39 5.5-7.7	 6.41 5.1-7.6	 6.10 5.1-7.0	5.8-6.5
F1 Birth Weight on LD 4: Offspring Mean/litter Range litter means	 10.52 9.0-13.7	 10.55 7.7-13.7	 10.71 9.0-13.6	 10.26 8.7-13.2	8.6-10.7
F1 Necropsy, # pups Stomach empty(F0 #) Intestines empty (F0 #)	 0 0	 0 0	 0 1	 **16*** (#54, #55) **17*** (#54, #55, #100)	 0 5 0 1

* $p < 0.05$

10 Special Toxicology Studies

Local tolerance

Rivaroxaban was administered by the paravascular and intraarterial routes at 0.33 mg/animal, and by i.v. infusion at 4.95 mg/animal/day for one or 6 days to male and female dogs (1/sex/group) (PH-33414). No hemolysis was observed in any animal. Macroscopic and histopathological examinations of the tissues from the injection sites did not show any differences in local tolerance following paravascular, intraarterial or intravenous administration of the drug, when compared with saline controls.

Phototoxicity

The potential phototoxicity of rivaroxaban was assessed in an in vitro model using mouse fibroblasts (3T3.A31). Rivaroxaban at concentrations up to 250 µg/mL had a photo irradiation factor (PIF) of 1, indicating that rivaroxaban was not phototoxic in this assay. In contrast, the positive control (75 µg/ml chlorpromazine) was photoxic with a PIF of 13.

Juvenile Toxicology

Study title: Pilot Study in Neonatal Rats (Repeated Administration by Gavage for 23 Days Starting on Postnatal Day 4)

Document no.:	PH-36153
Study no.:	T8080929 (AT05792)
Study report location:	EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG, GDD-GED Toxicology, Wuppertal, Germany
Date of study initiation:	9/9/09
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BAY 59-7939 (micronized), Batch BXA18UX, 99.8%

Key Study Findings

Daily doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban in 0.5% aqueous Tylose were administered by oral gavage to 12 Wistar rats/sex/group from postnatal day (PND) 4 through PND 26. No mortality occurred. Body weight gain decreased in mid and high dose male and the high dose female groups. Alanine aminotransferase values were slightly higher in the treated male and female groups and alkaline phosphatase values were slightly higher in the treated female groups. However, a dose relationship was not evident for either parameter. Although the mean absolute kidney and liver weights decreased with dose in the males, the mean relative kidney and liver weights did not change with dose indicating a lack of a treatment related effect. Exposure (AUC) was generally similar in males and females on all sampling days and increased with dose. However, the increase in exposure was less than dose-proportional. Exposure on Day 11 (PND 15) was higher than the exposure on the other sampling days.

Methods

Doses:	0, 6, 20 and 60 mg/kg
Frequency of dosing:	Daily from PND 4 through PND 26
Rationale for dose selection	Doses were based on prior toxicity studies in rats that indicated saturation of absorption above 60 mg/kg.
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	0.5% aqueous Tylose
Species/Strain:	Rat (Wistar, Hsd Cpb:WU)
Number/Sex/Group:	12/sex/group, except control groups had 5/sex
Age:	From PND 4 through PND 26
Weight:	PND 4, M: 9.8-13.7 gm; F: 9.8-12.9 gm
Satellite groups:	For TK, 5-6/sex/group
Unique study design:	Because dosing was to begin on PND 4, pregnant dams (GD 15/16) were obtained and allowed to deliver. On PND 1-3, half of the litters were reduced to 6 male pups and half were reduced to 6 female pups. The litters were assigned randomly to treatment groups. One male and one female litter (5 pups per litter) were used as controls. See dam assignments below.

	Dam number							
	0		6		20		60	
Group	M	F	M	F	M	F	M	F
1-4	1	8	2, 3	9, 10	4, 5	11, 12	6, 7	13, 14
Groups 1-4, dosed once, were used for TK on Days 1/2 (PND 4/5)								
5-8	15	22	16, 17	23, 24	18, 19	25, 26	20, 21	27, 28
Groups 5-8, dosed 11 times, were used for TK on Days 11/12 (PND 14/15)								
9-12	29	36	30, 31	37, 38	32, 33	39, 40	34, 35	41, 42
Main groups 9-12, dosed 23 times, were used for TK on Days 21/22 (PND 21/22)								

Deviation from study protocol:	Protocol deviations were not indicated.

Observations and Results

Mortality

The animals were examined visually for mortality and morbidity twice daily, except on weekends and holidays when they were examined once daily.

No deaths occurred during the study.

Clinical Signs

Clinical observations were made daily once before dose administration and then after dose administration. Detailed clinical observations were made weekly in Groups 9-12 during treatment.

No treatment–related clinical sign was observed.

Body Weights

The animals in all groups were weighed daily up to scheduled necropsy and immediately before necropsy. However, only body weight data for the main study animals (Groups 9-10) were included in the study report.

The mid and high dose male groups exhibited statistically significant 10% and 11%, respectively, decreases in total cumulative body weight gain. These decreases were relatively constant over the course of the study. The high dose female group exhibited a non-significant decrease of 5% in total cumulative body weight gain, whereas the low and mid dose female groups exhibited increased body weight gain. The decrease in the high dose females was maximal on Days 2 (17%), 3 (12%), 16 (10%), and 17 (11%).

Table 48: Sponsor's Summaries of Body Weight – Document PH-36153

Body weight, gm

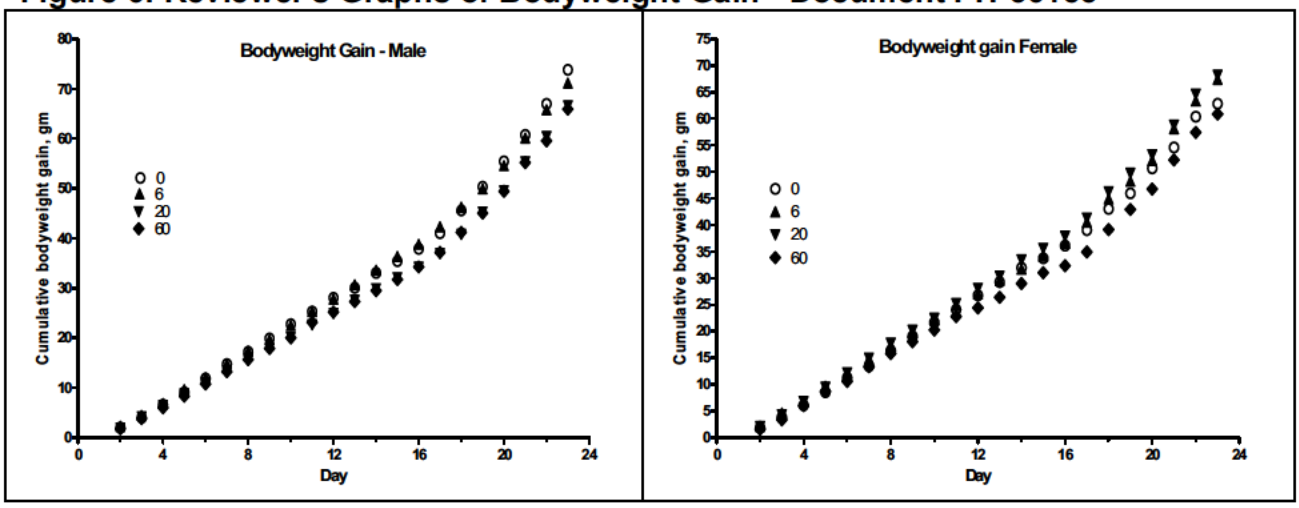
Sex	m	m	m	m	f	f	f	f
Dose (mg/kg)	0	6	20	60	0	6	20	60
Day								
1	12.62	12.42	11.58	11.15 ++	10.48	11.63 ++	11.47 ++	10.80
8	29 82	29 78	27.38	26.79 +	26.80	28.98	29.22 +	26.61
15	48.02	48.78	43.67 +	42.87 ++	44.20	45.77	47.09	41.83
22	79 60	78.21	71.99 +	70.69 ++	70.88	74.95	79.08 +	68.22

Body weight gain, gm

Sex	m	m	m	m	f	f	f	f
Dose (mg/kg)	0	6	20	60	0	6	20	60
Day					Relative			
1 - 8	2 46	2.48	2.26	2.23	2.33	2.48	2 54	2.26
8 - 15	2.60	2.71	2.33 ++	2.30 ++	2.49	2.40	2.55	2 17
15 - 22	4.51	4.20	4.05	3.97 +	3.81	4.17	4.14	3.77
				Cumulative				
1 - 22	66 98	65.79	60.41 +	59.54 +	60.40	63.33	64.61	57.42

Day 1 = PND 4 Day 8 = PND 11 Day 15 = PND 18 Day 22 = PND 25

Figure 6: Reviewer's Graphs of Bodyweight Gain - Document PH-36153



Clinical Chemistry

Blood samples for clinical chemistry were collected from all animals in Groups 9-12 on Day 22/23. The following parameters were measured: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, glutamate dehydrogenase, and gamma glutamyl transferase.

The mean values for alanine aminotransferase were slightly higher in the treated male and female groups; however, a dose relationship was not evident. The mean values for alkaline phosphatase were slightly higher in the treated female groups; however, a dose relationship was not evident and the finding was not observed in males.

Dose mg/kg	ALAT (GPT) U/l	ASAT (GOT) U/l	APh U/l	GLDH U/l	GGT U/l	
m	Day 22 = PND 25					
0	62.64	119.12	583 2	6.88	0.90	*
6	76.68 +	107.02	481.3	7 77	3.55	
20	75 36 +	100.48	479 8	6.61	1.27	
60	74.81 +	105.70	493.9	7.69	1.64	
f	Day 23 = PND 26					
0	62.96	107.92	401.6	7.16	0.32	
6	67.86	105.28	492.2 +	7.06	0.50	
20	76.93 +	108.85	543.0 ++	6.97	0.38	
60	70.32	101.46	436.4	7.18	0.46	

* This mean GGT for low dose males includes a clear outlier value of 28. The mean without this outlier is 1.3

Gross Pathology

The main study animals in Groups 9-12 were sacrificed for scheduled necropsy during week 14. The animals were subjected to systematic examination and the abnormalities, adrenals, brain, femur, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, thymus, uterus/cervix and vagina were fixed in 10% formalin. The eyes, testes, the left and two thirds of the right kidney were fixed in Davidson's solution.

No gross finding was related to treatment.

Organ Weights

The following organs from Groups 9-12 were weighed before fixation: brain, heart, kidneys, liver, and spleen.

The mean absolute kidney and liver weights decreased with dose in the males, but not females. However, the mean relative kidney and liver weights did not change with dose indicating a lack of a treatment related effect.

Toxicokinetics

Blood samples were obtained and plasma was prepared from three satellite treated animals/sex/group at 0.5, 2, 7, and 24 hours after dosing on Days 1 (PND 4/5, Groups 2-4), 11 (PND 14/15, Groups 6-8), and 18 (PND 21/22, Groups 10-12) of treatment. Blood samples were obtained from control animals (Groups, 1, 5, and 9) at 2 hours after dosing on days 1, 11, and 18. After addition of an internal standard and protein precipitation with acetonitrile, analysis of BAY 59-7939 was performed using a validated LC-MS/MS assay with a lower limit of quantification of 0.002 mg/L. The concentration of BAY 59-7939 in control animals was below the limit of quantification on Days 11 and 18, but ranged from 0.0107 to 0.0658 mg/L on Day 1.

Exposure was generally similar in males and females on all sampling days. Exposure increased with dose; however, the increase was less than dose-proportional. Exposure on Day 11 (PND 15) was higher than the exposure on the other sampling days.

Reviewer's Summary TK Parameters - Document PH-36153

		Male			Female		
Dose (mg/kg)		6	20	60	6	20	60
Day 1 – PND 4							
$AUC_{(0-24)}$	mg*hr/L	10.5	20.6	40.3	5.3	22.5	38.8
$AUC_{(0-24)\ norm}$	mg*hr/L	1.75	1.03	0.672	0.883	1.13	0.647
C_{max}	mg/L	2.51	3.31	6.19	0.95	3.87	6.37
t_{max}	hr	2.0	2.0	2.0	2.0	2.0	2.0
Day 11 – PND 15							
$AUC_{(0-24)}$	mg*hr/L	16.1	28.2	48.9	17.8	38.1	50.9
$AUC_{(0-24)\ norm}$	mg*hr/L	2.68	1.41	0.815	2.97	1.90	0.849
C_{max}	mg/L	2.41	3.01	6.71	2.70	4.05	5.71
t_{max}	hr	2.0	2.0	2.0	2.0	2.0	2.0
Day 18 – PND 22							
$AUC_{(0-24)}$	mg*hr/L	8.57	24.7	25.9	8.89	12.3	33.9
$AUC_{(0-24)\ norm}$	mg*hr/L	1.43	1.23	0.431	1.48	0.616	0.566
C_{max}	mg/L	1.57	2.05	3.39	1.22	1.98	3.78
t_{max}	hr	0.5	2.0	0.5	2.0	2.0	0.5

Study title: Repeated Dose Study in Systemic Toxicity in Neonatal Rats (14-Weeks Daily Administration by Gavage Starting on PND 10)

Document no.:	PH-36347
Study no.:	T1081417 (AT06105)
Study report location:	EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG, GDD-GED Toxicology, Wuppertal, Germany
Date of study initiation:	12/10/09
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BAY 59-7939 (micronized), Batch BXA18UX, 99.8%

Key Study Findings

Daily doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban in 0.5% aqueous Tylose were administered by oral gavage to 12 Wistar rats/sex/group from postnatal day (PND) 10 through PND 105. The death of one low dose female of an unknown cause was not attributed to treatment. Although the body weight gains at study termination for the high dose males and females were greater than those for the control males and females, body weight gain decreased in the high dose groups during the second and third week of treatment. Near the study end, the high dose males and females exhibited decreases in mean erythrocyte count and increases in mean MCH, MCHC and reticulocytes compared to the values for the concurrent control group. The high dose females also exhibited an increase in mean thrombocyte count. Females exhibited dose-related statistically significant increases in calcium and treated male groups exhibited increases in calcium that were not dose-related. The high dose males had increased mean absolute and relative adrenal weights. The mid and high dose males and the high dose females had increased mean absolute and relative liver weights. However, neither the adrenal gland nor the liver showed histopathological correlates. The high dose males exhibited a slightly higher incidence of peri-insular hemorrhage, inflammatory infiltration, and fibrosis in the pancreas and a slightly higher incidence of colloidal alteration in the thyroid.

BAY 59-7939 administration did not affect the learning and memory of weanling rats. In a functional observational battery, mean body temperature was slightly lower in the high dose males and females compared to the controls. No significant effect was observed on either motor or locomotor activity; however, one low dose female displayed no habituation with her highest motor activity value in the last monitoring interval.

The toxicokinetic data in this study confirm the higher exposure in rats less than 31 days old observed in the pilot study. Maximal exposures between PND 10 and 15 were 6-10 fold higher than exposures on PND 31 or 86.

Methods

Doses:	0, 6, 20 and 60 mg/kg
Frequency of dosing:	Daily from PND 10 through PND 105 (14 weeks)
Rationale for dose selection	Doses were based on a non-GLP dose range finding study (Document PH-36153) from PND 4 to 27. Males treated with 20 and 60 mg/kg and females treated with 60 mg/kg exhibited decreases in body weight gain.
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	0.5% aqueous Tylose
Species/Strain:	Rat (Wistar, Hsd Cpb:WU)
Number/Sex/Group:	12/sex/group
Age:	From PND 10 until week 14
Weight:	PND 10, M: 22.8-30 gm; F: 21.8-29.1 gm
Satellite groups:	For TK, 5-6/sex/group
Unique study design:	Because dosing began on PND 10, pregnant dams (GD 15/16) were obtained and allowed to deliver. On PND 1-9, half of the litters were reduced to 6 male pups and half were reduced to 6 female pups. The litters were assigned randomly to treatment groups. Group 5 had one male and one female litter (5 pups per litter) that were used as TK controls. See dam assignments below.

	Dam number							
	0		6		20		60	
Group	M	F	M	F	M	F	M	F
1-4	1, 2	9, 10	3, 4	11, 12	5, 6	13, 14	7, 8	15, 16
Main groups 1-4 were dosed for at least 95 days and used for TK on Days								
5-8	17	24	18, 19	25, 26	20, 21	27, 28	22, 23	29, 30
Groups 5-8 were dosed 1 time and were used for TK on Days 21/22 (PND 30/31) and Days 76/77 (PND 86/87).								

Deviation from study protocol:	Protocol deviations were not indicated.

Observations and Results

Mortality

The animals were examined visually for mortality and morbidity twice daily, except on weekends and holidays when they were examined once daily.

One low dose female rat died of an unknown cause.

Clinical Signs

Clinical observations were made daily once before dose administration and then 30 minutes after dose administration. Detailed clinical observations were made once weekly in all groups during treatment.

No treatment–related clinical sign was observed.

Body Weights

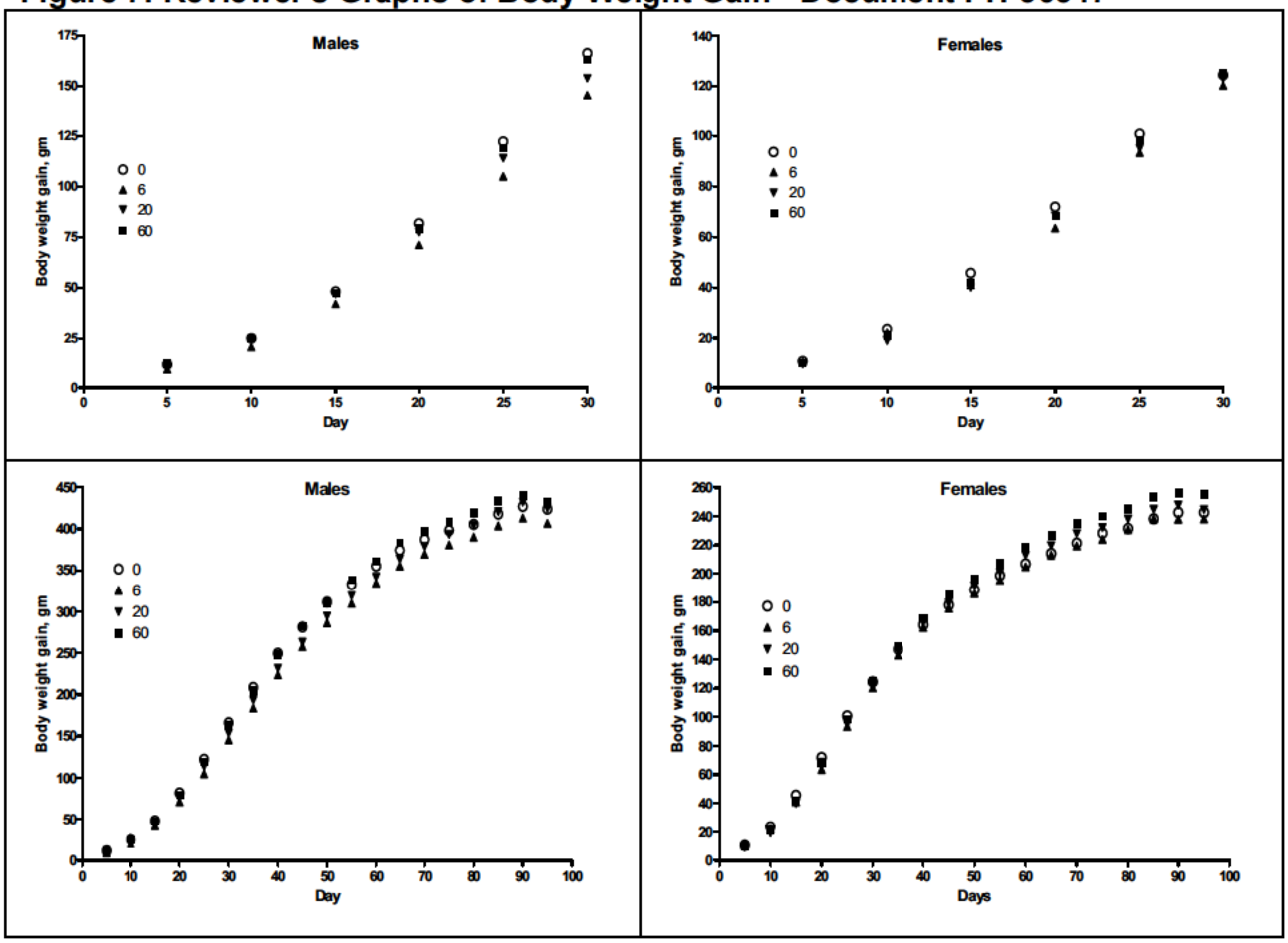
The animals in all groups were weighed daily up to scheduled necropsy and immediately before necropsy. However, only body weight data for the main study animals was included in the study report.

At study termination the body weight gains for the high dose males and females were greater than those for the control males and females. Although this indicated a lack of an overall toxic effect, the reviewer notes that during the second and third week of treatment the mean body weight gains were less in the high dose males and females (-4% and -8.3%, respectively) than those in the control groups. Body weight gains were even lower in the low and mid dose groups, particularly in the males, as illustrated in the figures below.

Table 49: Sponsor's Table of Body Weights – Document PH-36347

Sex	Males						Females					
Dose mg/kg Days	0	6		20		60	0	6		20		60
1	28	25	**	26	**	25 **	26	29	*	25		26
8	48	41	**	46		45	44	46		40	**	43
15	76	67	**	73		72	72	70		65	**	68
22	125	108	**	116	*	118	109	103		100	*	104
29	184	163	**	171	*	178	145	144		145		146
36	243	217	**	225	*	237	172	173		174		178
43	299	269	**	279	*	295	202	199		200		207
50	340	312	**	320		336	215	215		216		222
57	375	348	*	357		377	229	229		231		240
64	399	376		385		403	237	240		245		250
71	420	399		409		425	251	249		254		263
78	427	410		424		436	255	256		260		267
85	446	429		447		459	264	267		270		280
92	453	437		453		464	262	260		266		272
Gain D1-D92, gm	425	411		428		439	236	232		241		246

Figure 7: Reviewer's Graphs of Body Weight Gain - Document PH-36347



Ophthalmoscopy

On Day 83, the ophthalmological evaluation of all main group animals included evaluation of the pupillary reflex of both eyes and inspection of the anterior regions of the eye followed by examination using an indirect ophthalmoscope and a photo-slit lamp after pupil dilation.

No treatment–related ophthalmological finding was observed.

Hematology

Blood samples for hematology were collected from 10 non-fasting animals per group on Day 93. The following parameters were measured: differential blood count (lymphocytes, eosinophils, monocytes, neutrophils, and atypical lymphocytes), erythrocyte count, erythrocyte morphology, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), hemoglobin concentration, hematocrit, leucocyte count, reticulocyte count, thrombocyte count, and thromboplastin time (Hepato-Quick). The time of blood sampling relative to dose administration was not indicated.

The high dose males exhibited statistically significant decreases in mean erythrocyte count and increases in mean MCH, MCHC and reticulocytes compared to the values for the concurrent control group. The high dose females exhibited non-statistically significant changes in these parameters, as well as an increase in mean thrombocyte count. All female treated groups exhibited statistically significant increases in leucocyte and lymphocyte counts that were attributed to the low values for the control females, since the mean control values were below the mean historical control values (7.24 X 10^9/L and 6.37 X 10^9/L, respectively). However, all individual values were within historical control ranges. The changes in erythrocyte parameters are consistent with the pharmacodynamic effect of BAY 59-7939. However, no effect was observed on mean thromboplastin times. This lack of an effect is most likely attributable to blood collection immediately prior to dosing when rivaroxaban concentrations were low.

Table 50: Sponsor's Summary - Mean Hematology Values – Document PH-36347

Dose mg/kg	ERY T/l	HB g/l	HCT l/l	MCH pg	MCHC g/l Ery	MCV fl	RETI %	THRO G/l	HQUICK Sec.	LEUCO G/l	LYM G/l
m	Day 93									Day 93	
0	8.723	145.7	0.4764	16.73	306.2	54.63	1.75	1101.5	45.14	10.046	8.647
6	8.506	144.0	0.4699	16.91	306.3	55.25	1.94	1070.5	45.47	9.609	7.996
20	8.761	145.3	0.4737	16.61	307.0	54.09	1.91	1128.2	47.42	10.217	8.759
60	8.419 *	144.9	0.4612	17.24 *	314.2 **	54.78	2.12 **	1110.4	45.37	9.811	8.184
f	Day 91									Day 91	
0	8.335	144.5	0.4585	17.35	315.2	55.03	2.25	1063.3	35.72	6.803	5.573
6	8.243	143.7	0.4580	17.45	314.0	55.60	1.90	1017.6	38.42	8.787 *	7.152 *
20	8.486	147.5	0.4725	17.39	312.2	55.70	1.97	1024.7	37.47	9.663 **	8.089 **
60	8.142	142.6	0.4481	17.53	318.3	55.07	2.21	1165.4	36.95	8.955 **	7.389 **
Historical range - ±3 SD											
M	7.92, 10.34	137, 169	0.424, 0.526	14.9, 18.8	303, 343	45.7, 58.5	7, 32	795, 1603	28.8, 50.4	4.45, 16.46	3.53, 14.89
F	7.52, 9.63	133, 166	0.409, 0.505	15.7, 19.2	306, 350	47.2, 59.4	2, 32	805, 1588	25.0, 41.8	1.32, 13.17	0.85, 11.89

Clinical Chemistry

Blood samples for clinical chemistry were collected from 10 non-fasting males and females per group on Days 29/30 (for plasma enzyme activities only) and from 10 fasting animals per group on Days 93/91. The following parameters were measured: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, glutamate dehydrogenase, gamma glutamyl transferase, lactate dehydrogenase, creatine kinase, albumin, total bilirubin, cholesterol, creatinine, total protein, triglycerides, urea, glucose, chloride, calcium, inorganic phosphate, potassium, and sodium.

The sponsor's summary tables below summarize the mean chemistry values. The changes in plasma enzyme values were not considered toxicologically significant. The toxicological relevance of other parameters with statistical significance was dismissed, because the changes were considered too small, clear dose dependences were lacking or the change occurred only in one sex. In addition, most of the mean and individual values were within the laboratory's historical control ranges for rats 12 to 25 weeks of age.

However, three parameters deserved comment. First, all mean potassium values and almost all individual potassium values were above the historical control range (3SD: 3.9-5.7 and 3.5-5.5 mmol/L for males and females). Second, all mean and individual phosphate values were above the historical control range (3SD: 1.4-2.47 and 0.67-2.25 mmol/L for males and females). Together these results indicate some hemolysis of the samples used for electrolyte analyses.

Third, females exhibited a dose-related increase in calcium that was statistically significant in the mid- and high dose groups. Although all treated male groups also exhibited an increase in calcium, the increases were not dose-related. The mean values for the control groups were close to the maximum of the historical control range and the maximum individual values of control males and females were 2.76 and 2.73 mmol/L, respectively. The mean values for all treated groups were above the maximum of the historical control range. The number of males and females in the low, mid and high dose groups having calcium values above the concurrent maximum values were 2, 5, 1 and 3, 6, 7, respectively. In a systematic study, Lippi et al. (2006) showed that hemolysis did not affect calcium measurements using a colorimetric method with o-cresolphthalein complexone. However, calcium measurements in this study were made using flame photometry and the details of the method were not provided.

Table 51: Sponsor's Summary - Mean Chemistry Values – Document PH-36347

Dose mg/kg		ALAT U/l		ASAT U/l		APh U/l		GLDH U/l	Gamma-GT U/l	LDH U/l		CK U/l
m		Day 29										
0		93.28		88.22		605.4		7.15	0.12			
6		92.55		90.25		531.8		6.78	0.00			
20		71.82	**	82.64		545.1		6.62	0.03			
60		86.75		81.80		564.3		5.93	0.08			
m		Day 93										
0		42.96		80.71		91.4		16.11	0.22	77.6		184.5
6		41.19		82.85		87.6		16.63	0.07	68.6		170.2
20		39.28		74.66		83.7		12.31	0.34	57.6		157.8
60		39.83		73.56		89.3		14.89	0.22	53.1		173.3
f		Day 30										
0		80.50		72.60		483.4		5.45	0.59			
6		76.12		77.64		457.8		5.18	0.57			
20		75.35		70.88		446.7	*	4.77	0.65			
60		66.99	**	67.65		402.8	**	4.99	0.56			
f		Day 91										
0		31.36		78.22		78.7		15.35	0.23	89.0		143.5
6		32.62		82.42		75.5		15.11	0.10	75.7		149.2
20		31.27		86.53		74.2		10.90	0.18	105.2		170.3
60		27.86		71.00		63.1	**	12.29	0.17	54.8	*	98.3
Historical range												
±3 SD	M	12.5, 59.3		31.9, 92.6		38, 111		Up to 26.4	Up to 5	Up to 250		Up to 277
	F	7.1, 59.5		32.9, 102.3		18, 78		Up to 23.1	Up to 4	Up to 359		Up to 537

Dose mg/kg		Na mmol/l		K mmol/l		Cl mmol/l		Ca mmol/l		P mmol/l		Protein g/l		Albumin g/l	
m		Day 93													
0		147.1		6.73		97.3		2.600		3.374		62.21		34.25	
6		146.6		6.36		99.9	*	2.723	*	3.230		63.47		34.33	
20		146.4		6.68		97.0		2.776	**	3.216		62.96		34.07	
60		146.8		6.67		97.3		2.689		3.055	**	61.62		33.03	*
f		Day 91													
0		145.0		6.66		100.8		2.617		2.826		63.18		36.48	
6		144.5		6.61		102.0		2.682		3.015		64.81		36.67	
20		144.2		6.25		102.2		2.738	**	3.109		64.41		36.12	
60		143.4	**	6.38		100.2		2.759	**	3.059		64.48		36.08	
Historical range															
±3	M	139, 146		3.9, 5.7		94, 106		2.26, 2.63		1.40, 2.47		57.9, 74.1		29.1, 39.2	
SD	F	138, 146		3.5, 5.5		96, 109		2.20, 2.62		0.67, 2.25		56.6, 75.0		30.9, 41.8	

Dose mg/kg		GLUCOSE mmol/l		CHOL mmol/l		TRIGL mmol/l		CREA µmol/l		UREA mmol/l		Bili-t µmol/l	
m		Day 93											
0		4.077		1.607		0.645		58.8		5.445		0.33	
6		4.315		1.457		0.526		60.8		6.364	**	0.30	
20		4.334		1.463		0.648		53.9	*	5.325		0.51	
60		4.384	*	1.659		0.875		53.8	*	5.525		0.32	
f		Day 91											
0		4.395		1.576		0.409		64.0		6.224		0.47	
6		4.550		1.581		0.376		61.6		6.220		0.61	
20		4.626		1550		0.355		60.8		6.167		0.55	
60		4.693		1.536		0.412		55.9	**	5.392		0.65	
±3	M	2.93, 5.34		0.58, 3.31		0.29, 2.04		40, 63		2.94, 7.97		0.5, 2.5	
SD	F	2.74, 5.40		0.62, 3.04		0.26, 1.03		41, 66		2.83, 8.49		0.7, 2.7	

Urinalysis

The following parameters were determined: volume, density, protein, urea, creatinine, blood, pH, bilirubin, glucose, ketone bodies, urobilinogen, and sediment microscopy. Urine was collected for 16 hr from fasted animals from Day 93 in males and Day 91 in females.

No change in urinalysis parameters was related to treatment.

Gross Pathology

The surviving main study animals were sacrificed for scheduled necropsy during week 14. 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 15 were fixed in 10% neutral buffered formalin, except for the eyes and testes which were fixed in Davidson's solution and the optic nerve and kidneys which were fixed in Davidson's solution followed by neutral buffered formalin. The urinary bladder and lungs were initially inflated with 10% neutral buffered formalin prior to fixation by immersion.

No gross finding was related to treatment.

Table 52: Reviewer's List of Collected Tissues - PH-36347

Abnormal tissues	Larynx	Seminal vesicles with coagulating glands
Adrenals	Liver	Skeletal muscle - thigh
Aorta	Lungs	Skin (mammary area)
Brain (cerebrum, cerebellum, brain stem)	Lymph nodes (mandibular – mesenteric, popliteal)	Spinal cord (cervical, thoracic, lumbar)
Cecum	Mesentary	Spleen
Colon	Nasal cavity/nasopharynx	Sternum with bone marrow
Duodenum	Optic nerves	Stomach
Epididymides	Ovaries with oviduct	Testes
Esophagus	Pancreas	Thymus
Eyes and eyelids	Peyers patches	Thyroid with parathyroids
Extraorbital lacrimal glands	Pharynx	Tongue
Femur with joint	Pituitary	Trachea
Harderian glands	Preputial gland	Ureters
Head with skull cap	Prostate	Urethra
Heart	Rectum	Urinary bladder
Ileum	Salivary glands (submandibular, sublingual and parotid)	Uterus with cervix
Jejunum	Sciatic nerve	Vagina
Kidneys		Zymbal's glands

Organ Weights

The following organs were weighed before fixation: adrenals, brain, heart, kidneys, liver, spleen, thymus, testes epididymides and uterus.

The mean absolute and relative adrenal weights increased 17-18% for the high dose males compared to those for the control males. The mean absolute and relative liver weights increased up to 11% in the mid and high dose males and females. Neither the adrenal nor the liver findings correlated with any histopathological finding.

Histopathology

Adequate Battery

Tissue samples from all main study animals were dehydrated, embedded in Paraplast, sectioned, and stained with hematoxylin and eosin. All tissues listed in Table 52 and gross abnormalities identified at macroscopic examination from all animals in the control and high dose groups sacrificed at the end of the scheduled treatment period and from the female that died during the study were examined by histology. The pancreas and thyroid gland were examined from all animals in all groups. Cryosections from the liver were stained with Oil Red O.

Peer Review

Peer review included examination of the pancreas, prostate and thyroid glands of all groups and all slides of two animals per sex from the high dose group.

Histological Findings

In the four treatment groups, the incidences in the pancreas of peri-insular hemorrhage (0/0/0/3), inflammatory infiltration (0/0/0/3), and fibrosis (1/1/0/4) were slightly higher in the high dose males.

In the thyroid gland, colloidal alteration was observed in the mid and high dose males (0/0/1/5) and the severity of follicular cell hypertrophy (3/1/2/5) in the same high dose males increased slightly compared to the severity of follicular cell hypertrophy in the control group.

Special Evaluations

Learning and Memory Test
During Weeks 6 and 7, the first ten animals in each dose group were evaluated at an unspecified time after dosing in a water-M-maze test. In the first phase, each animal was placed in the water six times each one hour apart. If the animal found the way out, the score was considered positive. However, swim speeds and the times to exit were not reported. A week later, memory was tested by repeating the first phase one time. The exit was then moved to test the reversal learning ability and each animal was again placed in the water six times each one hour apart.

The sponsor concluded that BAY 59-7939 administration did not affect the learning and memory of weanling rats. However, the reviewer noted that fewer treated males scored positive than control males in trials 1 and 2 of Phase 1, although thereafter the number of males scoring positive was not significantly different between control and treated groups.

Table 53: Reviewer's Summary - Learning and Memory – Document PH-36347

	Number of animals scoring positive												
	Phase 1 trials						Phase 2 trials						
Dose	1	2	3	4	5	6	M	1	2	3	4	5	6
Males													
0	6	6	8	9	8	8	9	0	3	7	9	10	10
6	4	4	6	9	9	8	9	0	2	5	9	10	9
20	3	2	9	8	7	7	8	0	2	8	7	8	9
60	3	2	9	8	10	8	8	0	2	9	8	10	10
Females													
0	4	7	8	9	7	9	7	0	3	7	7	9	9
6	3	10	10	9	9	10	10	4	4	7	9	10	9
20	2	5	9	10	10	10	10	1	2	6	8	9	10
60	5	4	7	9	9	9	8	0	6	8	8	8	8

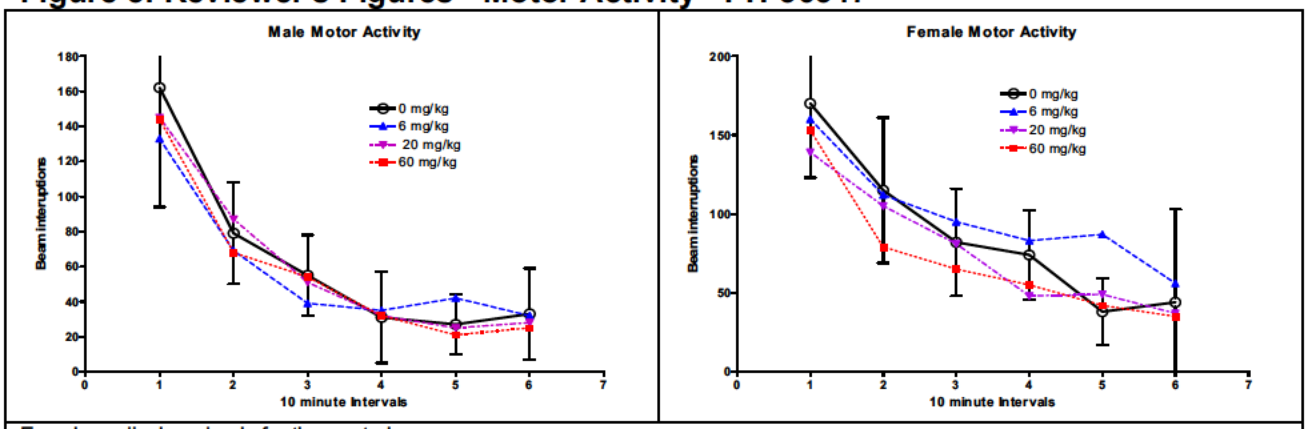
Functional Observational Battery
Functional observations of the first ten animals/sex/dose group were performed unblinded once on Days 82/83 and 84/85, respectively. The observations were performed at an unspecified time after dosing. The functional observational battery (Moser 1989) included home cage observations, handling observations, open field observations, neuromuscular and reflex/ physiological observations.

The mean landing foot splay was significantly decreased 17% in the mid dose males, but not the high dose males. The mean landing foot splay was decreased from 17% to 23% in all females treated groups with statistical significance attained in the low and high dose groups; however the decrease was not dose-dependent. Although not statistically significant the mean body temperature was lower in the high dose males and females (37.7º, 38.4ºC) compared to the controls (38.0º, 38.7ºC).

Motor Activity Assessment
Motor activity was evaluated for 60 minutes using an automated activity measuring device in a figure-eight maze (Reiter 1983). Locomotor activity was measured by eliminating consecutive counts for a given beam. The report indicated that the laboratory had previously conducted studies with rats treated with reference compounds that increase (triadimefon) and decrease (chlorpromazine) motor activity, when compared with untreated rats, to establish the sensitivity and reliability of these procedures. Each animal was evaluated individually at an unspecified time after dose administration on Days 89 and 90.

No significant effect was observed on either motor or locomotor activity. The total motor activity for each 10 minute period for males and females in the figures below (Figure 8) indicate that all groups showed habituation. However, three low dose females had motor activity values in interval 5 that were greater than the values in interval 4. One of these females displayed no habituation with her highest motor activity value in interval 5.

Figure 8: Reviewer's Figures - Motor Activity - PH-36347



Error bars displayed only for the control groups.

Toxicokinetics

Blood samples were obtained and plasma was prepared from three satellite treated animals/sex/group at 0.5, 2, 7, and 24 hours after dosing on Days 1 (PND 10/11), 21 (PND 30/31), and 76 (PND 86/87) of treatment. Blood samples were obtained from three satellite control animals/sex/group at 2 hours after dosing. After addition of an internal standard and protein precipitation with acetonitrile, analysis of BAY 59-7939 was performed using a validated LC-MS/MS assay with a lower limit of quantification of 0.002 mg/L. The concentration of BAY 59-7939 in control animals was below the limit of quantification on Days 21 and 76, but ranged from 0.0023 to 0.0118 mg/L on Day 1.

On Day 1 exposure to rivaroxaban was similar in males and females, whereas on Days 21 and 76 exposure was slightly lower in males compared to females. The sponsor attributed the later finding to higher cytochrome P450 activity in male livers (Mungford 1998). Although exposure increased with dose, the increase was less than dose-proportional. The higher exposure on Day 1 (PND 10) was attributed to the solubilizing

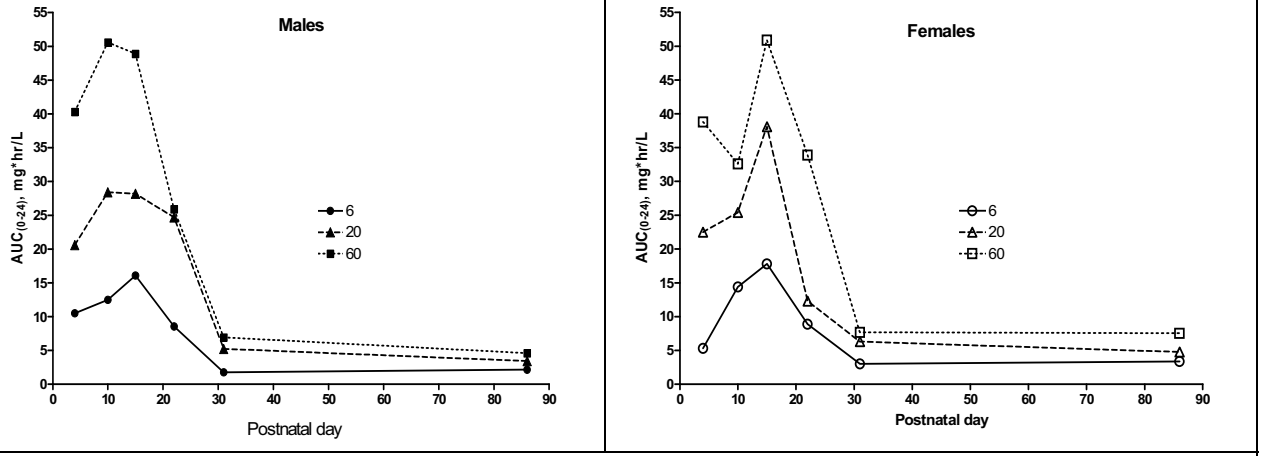
effect of milk and resulting increased absorption. However, the higher plasma concentrations may also be due to differences in cytochrome P450 profiles in younger animals (Strolin Benedetti et al. 2007, Ginsberg et al. 2004; De Zwart et al. 2004; De Zwart et al. 2008) or immaturity of the kidney (Zoetis and Hurtt 2003).

Table 54: Reviewer's Summary TK Parameters - Studies PH-36347

		Male			Female		
Dose (mg/kg)		6	20	60	6	20	60
Day 1 – PND 10							
$AUC_{(0-24)}$	mg*hr/L	12.5	28.4	50.6	14.4	25.4	32.6
$AUC_{(0-24)\ norm}$	mg*hr/L	2.09	1.42	0.84	2.40	1.27	0.54
C_{max}	mg/L	2.75	4.44	5.18	1.97	3.31	4.59
t_{max}	hr	2.0	2.0	2.0	2.0	2.0	2.0
Day 21 – PND 31							
$AUC_{(0-24)}$	mg*hr/L	1.77*	5.23	6.93	3.02	6.30	7.66
$AUC_{(0-24)\ norm}$	mg*hr/L	0.294*	0.261	0.115	0.504	0.315	0.128
C_{max}	mg/L	0.359	0.496	0.736	0.564	0.857	0.920
t_{max}	hr	0.5	2.0	2.0	2.0	2.0	2.0
Day 76 – PND 86							
$AUC_{(0-24)}$	mg*hr/L	2.16	3.42	4.60	3.36	4.76	7.53
$AUC_{(0-24)\ norm}$	mg*hr/L	0.360	0.171	0.077	0.561	0.238	0.125
C_{max}	mg/L	0.259	0.50	0.419	0.510	0.695	0.975
t_{max}	hr	2.0	2.0	2.0	2.0	2.0	2.0
*$AUC_{(0\ 7)}$							

The data in this study confirm the results found in the pilot study of higher exposure in rats less than 31 days old. Figure 9 below shows that exposures were maximal between PND 10 and 15 in these two juvenile rats studies using micronized rivaroxaban (Lot BXA18UX) and 0.5% tylose as the vehicle.

Figure 9: Reviewer's Graphs – Juvenile Rat Studies PH-36347 and PH-36153



Data on PND 10, 31 and 86 from PH 36347. Data on PND 4, 15, 22 from PH 36153

However, the above data appear to be inconsistent with the exposure observed on Day 1 of the rat carcinogenicity study and a 13-week rat toxicology study in which micronized rivaroxaban was also used. At the dosage of 60 mg/kg/day of rivaroxaban, the mean $AUC_{(0\ 24h)}$ was 3-8 higher in males and females on Day 1 of the

carcinogenicity study or 13-week repeat dose study when the rats were 42-49 days old than in the juvenile studies on postnatal days 31 and 86, which bracket the age of 42-49 days (Table 55). The use of Solutol HS 15 in the carcinogenicity and 13-week studies may account for the higher exposure. A 2-fold higher exposure was observed for cyclosporin A in a Solutol HS 15 based formulation compared to a microsuspension of cyclosporin (Gonzalez et al. 2002).

Table 55: Reviewer's Comparison - Juvenile and Other Toxicology Studies

Study #		**PH-34379**	**PH-36242**	**PH-36153**	**PH-36347**	
Study type		13-week repeat dose	Carcinogenicity	Juvenile pilot	14-week Juvenile	
Rivaroxaban lot		BX01UNC	BX023BS and BXA18UX	BXA18UX	BXA18UX	
Drug formulation		Micronized	Micronized	Micronized	Micronized	
Vehicle		Solutol HS15®/ ethanol/ tap water (40/10/50, v/v/v)	Solutol HS15®/ ethanol/ tap water (40/10/50, v/v/v)	0.5% aqueous Tylose	0.5% aqueous Tylose	
Doses, mg/kg		0, 60, 300, 1500	0, 10, 20, 60	0, 6, 20, 60	0, 6, 20, 60	
TK on postnatal day		~49 (Study day 1)	42-49 (Study day 1)	22	31	86
$AUC_{(0\text{-}24h)}$ (mg.hr/L) for 60 mg/kg dose	Males Females	23.7 43.3	27.5 36.0	25.9 33.9	6.93 7.66	4.6 7.53

Dosing Solution Analysis

Prior to the start of the study, test article formulations at concentrations of 1 and 30 mg/mL were shown to be homogenous and stable for 8 days at room temperature. During the study test article formulations were prepared as needed based on the 8 day stability. Analysis of the dose formulations on three days throughout the study showed the formulations were homogenous and the measured concentrations ranged from 99% to 106% of nominal.

11 Integrated Summary and Safety Evaluation

Because of its central role in blood coagulation, the coagulation serine protease, Factor Xa, is a major target for inhibition by therapeutic drugs for use in thromboembolic diseases. Rivaroxaban (BAY 59-7939), an orally active non-peptide inhibitor of FXa, is approvable for the proposed indication from a nonclinical perspective because rivaroxaban 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) effect. These effects included hemorrhage, extramedullary hematopoiesis, pigment deposition and secondary effects on red cell parameters. However, a few issues deserve discussion.

(b) (4)

(b) (4)

Although the two materials have a different profile of impurities, the toxicology studies conducted with the co-precipitate are acceptable in support of the NDA for the micronized material. Importantly, the two year carcinogenicity studies in mice and rats were conducted with the micronized rivaroxaban. These studies, critical for the qualification of the impurities, particularly the (b) (4) in the micronized material, generally indicated the toxic effects were due to the pharmacodynamic effect of rivaroxaban. However, an increase in incidence of vavular fibrosis was unexpected in the rat carcinogencity study. Sponsor concluded reasonably that valvular fibrosis in the 2-year carcinogenicity study in rats was not the result of treatment with rivaroxaban, but was associated with pronounced chronic cardiomyopathy, a common physiological change in aging rats. The low safety margins (<4-fold) in the carcinogenicity studies are a result of saturation of absorption of the micronized rivaroxaban.

Table 56 Summary of Animal to Human Exposure Ratios

			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
General toxicology (10% co-precipitate in polyethylene glycol 6000)						
6 month - rat	M	12.5	18	0.23	5.5	1.4
	F	12.5	32	0.4	9.7	2.4
12 month - dog	M	5	8.1	0.84	2.5	5.0
	F	5	5.0	0.52	1.5	3
Reproductive and Developmental Toxicology (10% co-precipitate in polyethylene glycol 6000)						
FEED – Rat Fertilization to implant	Paternal Maternal Embryo toxicity Fertility	12.5 12.5 50 200	17.7-22.3 17.7-22.3 83-100 156-227	0.23-0.28 0.23-0.28 1.06-1.26 2.1-2.88	5.4-6.8 5.4-6.8 25-30 47-69	1.3-1.7 1.3-1.7 6.3-7.5 12.5-17.2
EFD – Rat Implantation to GD 17	Maternal Placental effect Fetal - toxicity Malformations	35 10 35 120	77.7 18.9 77.7 188	0.99 0.24 0.99 2.39	23.5 5.7 23.5 57	5.9 1.4 5.9 14.3
EFD – Rabbit Implantation to GD 20	Maternal Placental effect Fetal - toxicity Malformations	2.5 2.5 2.5 160	0.74 0.74 0.74 23.9	0.173 0.173 0.173 5.59	0.22 0.22 0.22 7.2	1.0 1.0 1.0 33.5
Pre/Post-natal – Rat Implantation to weaning	F0 (maternal) F1 (perinatal) F1 (postnatal) F1 fertility/ F2	10 10 >40 >40	18.9 18.9 >77.7 >77.7	0.24 0.24 >1.0 >1.0	5.7 5.7 >24 >24	1.4 1.4 >5.9 >5.9
Carcinogenicity – 2 year (micronized rivaroxaban)						
Rat	Tumors	60	20.3/48.2	0.257/0.612	6.2/14.6	1.5/3.7
Mouse	Tumors	60	2.52/3.09	0.162/0.199	0.76/0.94	1.0/1.2

† Unbound fractions in humans, rats, mice, dogs, and rabbits are 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively.
‡Human exposure at 20 mg/day corresponding to 0.33 mg/kg in a 60 kg patient was 3.3 mg*hr/L. Human exposure to unbound drug was 0.167 mg*hr/L.

Administration of rivaroxaban resulted in significant embryo/fetal/offspring toxicity in the developmental toxicity studies in rats and rabbits. The NOAELs for embryo/fetal toxicity in the FEED study and the EFD study in rats were 12.5 mg/kg and 35 mg/kg, respectively. These NOAELs corresponded to safety margins for embryo/fetal/offspring toxicity of 6.3 and 5.9 fold, respectively, based on unbound exposure comparisons to the steady state unbound AUC of about 0.167 mg*hr/L for the 20 mg daily dose in patients with atrial fibrillation. The NOAEL for embryo/fetal toxicity in the EFD study in rabbits was 2.5 mg/kg and no safety margin was achieved based on unbound exposure. Likewise, in the rat PPND study, the NOAEL for peri-natal fetal toxicity was 10 mg/kg which corresponded to essentially no safety margin (1.4 fold) based on unbound exposure.

However, comparisons based solely on total exposure do not fully describe the drug treatment of the animals in the reproductive toxicology studies. Table 57 compares the plasma concentrations of rivaroxaban in the EFD studies relative to the mean Cmax concentrations in patients with atrial fibrillation. In the rat EFD study, the plasma concentrations of rivaroxaban at the toxic dose of 35 mg/kg in the dams were 4 to 6-fold the mean human Cmax plasma concentrations during the first 7 hours after dosing. In the rabbit EFD study, the plasma concentrations of rivaroxaban at the toxic dose of 10 mg/kg in the dams were 2.6-4.4 fold the mean human Cmax plasma concentrations for 8 hours after dosing. Thus, the animals in the rat and rabbit reproductive toxicology studies were subjected to supra therapeutic doses of rivaroxaban for at least 29% and 33%, respectively, of each day following dosing. Some of the adverse effects seen in the reproductive toxicity studies in rats and rabbits with rivaroxaban, especially loss occurring during parturition, may be the result of supra-pharmacodynamic effect of the drug (occult or overt bleeding). However, the animal studies do not clearly reveal the extent to which normal pharmacodynamic (i.e., therapeutic) as well as supra pharmacodynamic activity contribute to the fetal and embryonic loss.

Table 57: Reviewer's Comparison of Plasma Concentrations in the EFD Studies

	Rat EFD = toxic at 35 mg/kg Maternal TK on GD 17			**Rabbit EFD = toxic at 10 mg/kg, Maternal TK on GD 20**		
Time post dose, hrs	Plasma Conc, mg/L	Unbound Conc, mg/L	Unbound Relative To human	Plasma Conc, mg/L	Unbound Conc, mg/L	Unbound Relative To human
1	4.91	0.062	4.4	0.255	0.062	4.4
2				0.250	0.061	4.4
3	6.02	0.076	5.4			
4				0.208	0.050	3.6
7	6.32	0.080	5.7			
8				0.150	0.036	2.6
24	0.375	0.005	0.35	0.036	0.009	0.6
Cmax of rivaroxaban in SPAF patients = 0.274 mg/L Mod 2.7.2 Summary of Clinical Pharmacology Studies, Figure 2 77; Protein binding 5.07%, Unbound Cmax = 0.014 mg/L						

In the rat EFD study, rivaroxaban treatment at 35 and 120 mg/kg resulted in 4% and 12.5% decreases, respectively, in absolute body weight gain compared to values in the control group. Fleeman et al. (2005) demonstrated that feed restriction of Sprague-

Dawley rats to 50% of ad lib values resulted in 21% decrease in absolute maternal body weight and 7% decrease in fetal body weight, but had no effect on the number of viable fetuses, the number of resorptions, incidence of malformations or delayed ossification. Therefore, the 3.1% decrease in fetal body weight in Wistar rats treated with 120 mg/kg rivaroxaban may be at least partially attributable to maternal toxicity, unless the two rat strains differ considerably in their response to decreased food consumption.

Cappon et al. (2005) demonstrated that feed restriction of rabbits to 50% of ad lib values resulted in 64% decrease in absolute maternal body weight gain and a 3% decrease in fetal body weight, but no change in post-implantation loss or the number of viable fetuses. Likewise, 63% restriction of food resulted in a 10% decrease in fetal body weight and one abortion (1/15), but no change in post-implantation loss or number of viable fetuses. In the rabbit EFD study, rivaroxaban treatment at 10 mg/kg resulted in a 38% decrease in absolute body weight gain associated with one abortion (1/20), a 3.8% decrease in fetal body weight, and no decrease in number of viable fetuses, but a 1.8-fold increase in post-implantation loss. Rivaroxaban treatment at 40 mg/kg resulted in a 78% decrease in absolute matertnal body weight gain associated with two abortions, a 12.5% decrease in fetal body weight and a decrease in number of viable fetuses, and a 3-fold increase in post-implantation loss. Although maternal toxicity likely contributed to the fetal toxicity at 40 mg/kg, maternal toxicity less likely contributed to the fetal toxicity at 10 mg/kg. Furthermore, the increase in post-implantation loss at either 10 or 40 mg/kg is not likely attributable to just maternal toxicity.

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 FII die either mid-gestation (9.5 days post coitum) or immediately after birth (Sun et al. 1998; Xue 1998). Likewise, approximately 50% of PAR1-deficient mouse embryos die at midgestation days (9-10 post coitum) (Connolly et al. 1996).

The sponsor maintains that only a limited amount of [^{14}C]-rivaroxaban is transferred through the placenta to the fetus after oral administration of rivaroxaban to the dam based on whole body autoradiography in pregnant albino rats on gestation day 19. However, the average exposure in the fetuses based on $AUC_{(0\ 24)}$ reached about 20 % of the exposure in maternal blood. In addition, the level of radioactivity in the fetus skeletal muscle was similar to that in maternal skeletal muscle. Therefore, the level of rivaroxaban crossing the placenta into the fetus is not insignificant.

In the human fetus at 19-23 weeks of gestation, levels of procoagulant factors (FX and FII (prothrombin)) are only 10-25% of their corresponding adult values (Andrew and Paes 1990; Andrew et al. 1987, Hassan et al. 1990). At the end of gestation, the levels of FX and FII in the neonate at birth are about 37-40% and 44-48%, respectively, of the adult human level (Andrew et al. 1987, Hassan et al. 1990). Similar to the human neonate, the rabbit neonate has plasma FII levels in that are 50% of the adult mean (Hathaway et al. 1964; Karpatkin et al. 1991). On gestation day 25 and 29, the rabbit

fetus has 10% and 49% of adult FII protein levels, respectively, even though the fetus has >60 and 99% of adult FII mRNA levels (Karpatkin et al. 2000).

The critical levels of FX and prothrombin protein earlier in development may be even lower than that indicated above. Tai et al. 2007 generated transgenic mice that had FX activity levels of 1–3% of wild-type and could rescue the lethality of FX knockout mice. Similarly, Sun et al. (2002) generated a strain of thrombin deficient transgenic mice that was able to rescue both the embryonic and the neonatal lethality of FII deficiency. The adults of this transgenic line had thrombin activity that was 5-10% of wild-type adult levels. Assuming that the levels of prothrombin and FX in the rat fetus are similar to that in the rabbit (10-20%% of adult levels), the level prothrombin and FX needed to rescue the embryonic and the neonatal lethality may be only 0.5-2% of adult levels. Therefore, a low level of placental transfer of rivaroxaban to the fetus may still be sufficient to inhibit the low level fetal FXa in the fetus and induce an adverse effect in the embryo/fetus.

Rivaroxaban appears not to be an overt teratogen, but it is an embryo/fetal toxicant, especially at suprapharmacodynamic dosages that cause bleeding. However, the use of rivaoxaban in treating thromboembolic disorders may be acceptable, despite the potential risks to the offspring as long as the label clearly indicates the risk.

In the rat PPND study, peri-natal maternal toxicity was associated with bleeding and death of dams at 40 mg/kg. In the peri-natal period, survival of the pups to LD 4 was also greatly decreased at 40 mg/kg. Although the number of stillborn pups increased and the number of live pups on LD 0 and LD 4 decreased at both 10 and 40 mg/kg, the NOAEL was considered 10 mg/kg based on the higher percentage of pup survival at 10 mg/kg (95.5%) compared to that at 40 mg/kg (75.8%). The decreased survival of the pups may be related to the peri-natal bleeding observed in some dams treated at 40 mg/kg. Some bleeding during partuition may be expected; however, the presence of rivaroxaban likely enhanced bleeding and resulted in both maternal and offspring deaths.

During human labor and delivery, the need to reverse bleeding is sometimes necessary. However, in several studies in rats and baboons, low increases in bleeding time (2 to 5-fold) induced by rivaroxaban were only partially reversed by use of prothrombin complex concentrate, FEIBA, human FVIIa, or activated prothrombin concentrate. Therefore, 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 rivaroxaban covered the period of PND 4-26 and PND 10-105, respectively, corresponding to the human age ranges of neonate to toddler and infant to adolescent, respectively. Although these studies did not identify any specific toxicity not observed in toxicology studies with adult animals, two points deserve mention. First, exposure to rivaroxaban was 6-10 fold higher in the younger rats, particularly between PND 10 and 15, than exposure in rats ≥31 days old. 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, DeZwart et al. 2004). Second, in the juvenile studies blood collection for monitoring coagulation parameters apparently took place prior to dose administration,

because no effect of rivaroxaban dosing was observed on the coagulation test (thromboplastin time). Therefore, the effect of rivaroxaban on coagulation, particularly in the younger animals, was not evaluated. Given that the levels of FX in the neonate at birth are about 37-40% of the adult human level (Andrew et al. 1987, Hassan et al. 1990), any pediatric studies in humans will need to be carefully designed to determine the appropriate levels of rivaroxaban for a therapeutic effect in neonates and infants undergoing rapid changes in their hemostatic, renal and metabolic systems.

12 Appendix/Attachments

Appendix 1: Nonclinical Study Reports Submitted to NDA 22, 406

	Report No.	Study topic
Pharmacology		
	PH-32009	In vitro Pharm
	PH-35082	kinetic study binding to Fxa
	PH-35297	in vitro dog & mouse FXa - PT, aPTT & FXaactivity
	PH-35076	BAY metabolite on Fxa
	PH-34952	Effect on FXII
	PH-33906	Effect on t-PA
	PH-33916	effect on chymotrypsin
	PH-33918	Effect on kallikrein
	R-8462	Effect on enzyme assays
	R-8463	Radioligand binding assays
	PH-31659	Rat Bleeding time
	PH-31611	Rabbit Ear bleeding time
	PH-32792	Bleeding time rabbit
	PH-32793	Venous stasis rat
	PH-31612	AV shunt in rat
	PH-31613	arterial & Venous thrombosis in Rat
	PH-32794	Ferric chloride arterial thrombosis rat
	R-8473	Electrolytically injured rat artery
	R-8473A	Electrolytically injured rat artery
	PH-35083	TF induced coagulation rat
	PH-31614	AV shunt in rabbit
	PH-32791	AV shunt rabbit
	PH-33917	Protection embolism in mice
	PH-34125	Ferric chloride arterial thrombosis mouse
Safety Pharmacology		
	PH-31410	Single administration - glucose fasted and fed rat
	PH-31411	Single administration - renal function, pharm, lipid rat
	PH-31412	Single administration - CNS rat
	PH-31413	Single administration - CNS2 rat
	PH-31414	Single administration - GI motility rat
	PH-31503	Contractility isolate guinea pig ileum
	PH-31616	CVS, ECG, respiration in dog single dose
	PH-33320	Action potential rabbit purkinje fiber
	R-8312	HERG in vitro
Drug Interaction		
	PH-32386	Interaction Enoxaparin/heparin AV shunt in rat
	PH-32735	Interaction ASA bleeding time rat
	PH-32913	Interaction naproxen bleeding time rat
	PH-32914	Interaction diclofenac bleeding time rat
	PH-32946	Interaction clopidogrel bleeding time rat
	PH-32948	Interaction clopidogrel bleeding time rat
	PH-34950	Interation ASA AV shunt and bleeding
	PH-34951	Interaction clopidogrel AV shunt and bleeding
	PH-34970	Interaction clopidogrel+ASA AV shunt and bleeding
	PH-34870	NovoSeven interaction rat

	PH-34871	FEIBA interaction rat
	R-8472	Effect Factor VII and prothrombin in monkey in vivo
	R-8474	Interaction ASA, diclofenac, naproxen bleeding
	PH-35374	Prothrombin concentrate interaction rat
ADME/PK		
	PH-30779	Synthesis 14C BAY 59-7939
	PH-34971	Methods validation TK
	PH-31990	PK Dog IV and Oral
	PH-32007	PK Rat IV and Oral
	PH-33681	TK post single SC in rat
	PH-34594	TK rat single dose
	PH-32333	TK in 4-week tox in rat
	PH-32348	TK 4-week tox in dog
	PH-34045	PK in rat simul & delayed charcoal
	PH-34588	PK mouse single dose
	PH-33250	PK dog labeled BAY
	PH-35039	PK dog single dose - different tablet form
	PH-32966	Studies in Plasma Stability binding etc
	PH-33395	Plasma binding Labeled BAY
	PH-32339	WB autoradio rat single dose IV and oral
	PH-33719	Dist/excre albino & pigmented rat
	PH-34647	Dist/excre albino rat repeat doses
	PH-34872	Dist/excre pregnant rat single doses
	PH-33434	Milk secretion
	PH-34783	in vitro metabolite microsomes & hepatocytes
	PH-31969	Metabolite profile in Rat
	PH-33092	metabolite profile in dog
	PH-33230	metabolite profile in human
	PH-33897	metabolite profile in mouse
	PH-34610	metabolites from human and rat
	PH-34935	Metabolism o (b) (4) in vitro and in vivo
	PH-32627	Human CYP in vitro metabolism BAY
	PH-34973	Interaction of drugs Metabolism BAY in vitro
	PH-31634	Inhibition Human CYP isoform
	PH-34858	Inhbition of human CYP
	PH-33718	CYP induction human hepatocyte
	PH-34937	inhibition PgP
	PH-34986	In vitro MDR1 cells - PgP
	PH-34985	Study in WT & PgP KO mice IV and oral
	PH-35258	in vitro inhibition of BAY efflux by drugs MDR1 cells
	PH-35323	in vitro inhibition of BAY efflux by quinidine MDR1 cells
	PH-34936	Caco 2 permeability
	PH-35219	inhbition breast cancer resistance protein
	PH-34987	In vitro MDCKII Bcrp cells
	PH-35272	in vitro effect on BAY transport by drugs MDCKII-Bcrp cells
Toxicology		
	PH-31843	Acute Tox - mouse & rat
	PH-33496	Acute IV rat
	PH-32076	A/E in Rat oral and IV

	PH-34534	2 week tox rat IV
	PH-33780	2 week tox rat diet PEG coppt
	PH-33623	2 week tox mice diet PEG coppt
	PH-33599	2 week tox rat - micronized in diet
	PH-33609	2 week tox mouse - micronized in diet
	PH-34646	2 week IV tox rat nanosuspension
	PH-34189	2 week IV tox rat
	PH-34088	2-week tox rat diet coppt
	PH-32303	4-week tox rat
	PH-34480	4-week tox rat gavage
	PH-34944	4-week tox rat - id urine crystals
	PH-34379	13-week tox rat gavage
	PH-34553	13-week tox rat diet
	PH-33051	3-month Tox rat
	PH-33051A	3-month Tox rat
	PH-33611	6 month tox rat
	PH-34107	2-week tox mouse diet coppt
	PH-33755	4-week tox mouse
	PH-33902	13-week tox mouse
	PH-34138	13-week tox mouse diet
	PH-34378	13-week tox mouse gavage
	PH-31848	4-week Tox dog
	PH-33056	13-week dog tox
	PH-34235	52-week tox dog
Genetic toxicology		
	PH-31770	Ames - plate & preincubation
	PH-34016	Ames - plate & preincubation T 8074764
	PH-33561	Ames - plate & preincubation T 9073243
	PH-31537	Chrom Ab CH V79 cell
	PH-34198	Chrom Aberr CH V79 cells T0073244
	PH-31536	Micronucleus mouse
	PH-33256	TK mouse IP administration
Reproductive and Developmental Toxicology		
	PH-33273	Fertility & EED rat
	PH-33368	TK EFD rabbit
	PH-33380	EFD Rabbit
	PH-33582	EFD rat
	PH-34608	PPND rat
Other Toxicology		
	PH-34442	Local tolerability Dog
	PH-33414	Local tolerability Dog
	PH-33880	in vitro phototoxicity
	PH-35331	Antibacterial MI concentration
	PH-35332	Interaction BAY & Linezolid
	PH-34996	Melt coppt for tox studies
	PH-34876	4-week tox of AML rat gavage
	PH-34344	AML - Ames
	PH-35342	In vitro mitochondrial tox testing

Appendix 2

Executive CAC
Date of Meeting: August 1, 2006

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abigail C. Jacobs, Ph.D., OND IO, Member
William Taylor, Ph.D., DSPTP, Alternate Member
Laniyonu, Adebayo A, Ph.D., DMIHP, Supervisory Pharmacologist
Yash M. Chopra, M.D., Ph.D., DMIHP, Presenting Reviewer

Author of Draft: Yash M. Chopra

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

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

IND #64,892
Drug Name: BAY 59-7939
Sponsor/Applicant: Bayer Health Pharmaceuticals Corporation

Introduction:
BAY 59-7939 is an orally active competitive Factor Xa inhibitor. It produced an inhibition of human Factor Xa activity, prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT) in a dose dependent manner. The sponsor is developing the drug for the prophylaxis and treatment of deep venous thrombosis (DVT).

(b) (4)

Mouse Carcinogenicity Study and Dose Selection:
The sponsor sent in a protocol for a 2-year oral mouse carcinogenicity study with micronized BAY 59-7939 at doses of (b) (4) mg/kg/day in ethanol/solutolHS15/tapwater (10/40/50 v/v/v) (60/sex/group) of CD-1 mice. Four additional groups of animals (20/sex/group) are

included for toxicokinetic determination and hematology evaluation. Morbidity and mortality of animals would be evaluated daily. Clinical signs would be recorded once before treatment and at a weekly interval up to the time of necropsies. Hematology parameters would be assessed from the blood samples from non-fasted animals after 12, 18 and at 24 months of treatment from all animals of the satellite groups. Histopathology of all the animals of each of the toxicokinetic study groups will be conducted.

In a 13-week oral gavage study with micronized BAY 59-7939, a dose related increase in the plasma concentration was observed at up to 60 mg/kg/day. However, the sponsor proposed (b) (4) mg/kg/day as the high dose in the 2-year study.

Rat Carcinogenicity Study and Dose Selection:

Protocol #T8076429: Carcinogenicity Study in Wistar Rats (2-Year administration by gavage)
The sponsor sent in a protocol for the 2-year oral rat carcinogenicity study with micronized BAY 59-7939 at the doses of (b) (4) mg/kg/day in ethanol/solutolHS15/tapwater (10/40/50 v/v/v) (50/sex/group) of Wistar rats. Four additional groups of animals (20/sex/group) are included for the toxicokinetic determination, hematology, and clinical chemistry evaluation. Morbidity and mortality of animals would be evaluated daily. Clinical signs would be recorded once before treatment and at weekly intervals up to the time of necropsies. Hematology and clinical chemistry parameters would be assessed for all animals (non-fasted) of the satellite groups after 6, 12,18 and at 24 months of treatment. Blood samples (0.2 ml/animal) for toxicokinetic evaluation would be collected after 1 year of treatment and near the end of the 2 year treatment period. Histopathology of all the animals of each of the study groups will be conducted.

In the 13-week oral gavage study with micronized BAY 59-7939, a dose related increase in the plasma concentration was observed at up to 60 mg/kg/day. However, the sponsor proposed (b) (4) mg/kg/day as the high dose for the 2-year study.

Executive CAC Recommendations and Conclusions:

Mice:

- The Committee did not concur with the sponsor's proposed doses of (b) (4) mg/kg/day and instead recommended doses of 0, 10, 20, and 60 mg/kg/day by oral gavage, based on saturation of absorption.

Rats:

- The Committee did not concur with the sponsor's proposed doses of (b) (4) mg/kg/day and recommended doses of 0, 10, 20, and 60 mg/kg/day BAY 59-7939 by oral gavage, based on saturation of absorption.

Any increase in tumors in an organ for a tumor type should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al., JNCI 76:283, 1986)

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

cc:\
/Division File, DMIHP
/Adebayo Laniyonu, Supervisory Pharmacologist, DMIHP
/Yash M. Chopra, Reviewer, DMIHP
/Diane Leaman, PM, DMIHP
/Adele Seifried, OND IO.

Appendix 3

Executive CAC
Date of Meeting: April 19, 2011

Committee: David Jacobson-Kram, Ph.D., OND-IO, Chair
Abigail Jacobs, Ph.D., OND -IO, Member
Paul Brown, Ph.D., OND-IO, Member
Karen Davis Bruno, Ph.D., DMEP, Alternate Member
Thomas Papoian, Ph.D., DCRP, Supervisor
Patricia Harlow, Ph.D., DCRP, Reviewer

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

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

NDAs: 202-439, 22-406
Drug Name: Rivaroxaban (BAY 59-7939)
Sponsor: Ortho McNeil Janssen Pharmaceuticals Inc

Background:
Rivaroxaban is a direct Factor Xa inhibitor. In the Phase 3 trial for prevention of stroke in patients with non-valvular atrial fibrillation, the maximum daily dose was 20 mg.

Rat Carcinogenicity Study:
In a 104-week study using 50 Wistar rats/sex/group, daily doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban in ethanol/solutol HS/tap water (10/40/50 v/v) were administered by oral gavage. The exposures in the high dose males and females were 6.2 and 14.6 fold, respectively, the mean human exposure in subjects receiving 20 mg.

No significant treatment-related effect was observed on mortality, bodyweight gain, and food consumption. Although only slight effects were observed on red cell parameters on Days 184, 366, 548, and 716 , the mean values for thromboplastin time for all treated groups on all sampling days were significantly greater (up to 1.9 and 2.5-fold in males and females, respectively) than those for the control groups. Likewise, the incidence of pigment deposition increased in some organs and across all organs in the high dose groups consistent with the pharmacodynamic action of BAY 59-7939. However, the incidence of valvular fibrosis in the heart increased with dose in both males and females with the incidence in females statistically significant (p 0.0048).

The incidences of a few tumors, including squamous cell carcinoma of the clitoral glands, adrenal cortical adenoma, adrenal pheochromocytoma, mammary fibroadenoma, histocytic sarcoma, and skin fibroma, were numerically increased in the higher dose groups compared to those in the control groups. However, the incidences were within historical ranges, and the attained p values do not reach the thresholds to classify these tumors as drug-related according to the CDER statistical criteria.

Mouse Carcinogenicity Study:

In a 104-week study using 60 CD-1 mice/sex/group, daily doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban in ethanol/solutol HS/tap water (10/40/50 v/v) were administered by oral gavage. The exposures in the high dose males and females were 0.8 and 0.9 fold, respectively, the mean human exposure in subjects receiving 20 mg.

No significant treatment-related effect was observed on mortality, bodyweight gain or food consumption. At study end, slight decreases in hemoglobin concentration and hematocrit, slightly prolonged thromboplastin times, and increased incidences of microscopic pigment deposits were consistent with the pharmacodynamic action of rivaroxaban.

Consistent with the increase in liver nodules macroscopically, hepatocellular tumors (adenoma and carcinoma) increased with rivaroxaban dosage in the males, but not in the females. However, the incidences of hepatocellular tumors were within historical ranges, and the attained p values do not reach the thresholds to classify these tumors as drug-related.. Similarly, the incidences of a few other tumors, including histiocytic sarcoma, malignant lymphoma, ovarian cystadenoma, uterine hemangiosarcoma, and testicular Leydig cell tumors, were numerically increased in the higher dose groups compared to those in the control groups. However, the incidences were within historical ranges, and the attained p values do not reach the thresholds to classify these tumors as drug-related.

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, Team leader, DCRP
/P. Harlow, Reviewer, DCRP
/A. Blaus, CSO/PM, DCRP
/A.Seifried, OND-IO

Appendix 4: 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

Andrew M and Paes B. 1990. Development of the hemostatic system in the neonate and young infant. Am J Pediatr Hematol Oncol.; 12: 95-104.

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.

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.

Bradford M, 1976. A rapid and sensitive method for the quantitation of protein utilising the princple of protein-dye binding. Anal Biochem; 72: 248-254

Bhattacharyya S, Schapira AH, Mikhailidis DP, Davar J. 2009. Drug-induced fibrotic valvular heart disease. Lancet. ; 374(9689): 577-85.

Burdan F, Szumilo J, Dudka J, Korobowicz A, Klepacz R. 2006. Congenital ventricular septal defects and prenatal exposure to cyclooxygenase inhibitors. Braz J Med Biol Res. ; 39(7): 925-34.

Cappon GD, Fleeman TL, Chapin RE, Hurtt ME. 2005. Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit Birth Defects Research (Part B); 74: 424–430.

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.

Davies B, Morris T. 1993. Physiological parameters in laboratory animals and humans. Pharm Res.; 10(7): 1093-5.

Depasse F, Busson J, Mnich J, Le Flem L, Gerotziafas GT, Samama MM. 2005. Effect of BAY 59-7939 — a novel, oral, direct factor Xa inhibitor — on clot-bound factor Xa activity in vitro. J. Thromb. Haemost. Abstr. 3 (Suppl. 1), P1104.

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 Vriese AS., Coster RV, Smet J, Seneca S, Lovering A, Van Haute LL, Vanopdenbosch LJ, Martin JJ, Groote CC, Vandecasteele S, Boelaert JR. 2006. Linezolid-induced inhibition of mitochondrial protein synthesis. Clin Infect Dis, 42(8): 1111-7

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.

Donnelly K. 2008. Cardiac Valvular Pathology: Comparative pathology and animal models of acquired cardiac valvular diseases. Toxicologic Pathology.;36: 204-17.

Edwards ST, Betz A, James HL, Thompson E, Yonkovich SJ, Sinha U. 2002. Differences between human and rabbit coagulation factor X-implications for in vivo models of thrombosis. Thromb Res.;106(1): 71-9.

Elangbam C, Colman K, Lightfoot R, Tyler RD, Wall HG. 2002. Endocardial Myxomatous Change in Harlan Sprague-Dawley Rats (Hsd:S–D) and CD-1 Mice: Its Microscopic Resemblance to Drug-Induced Valvulopathy in Humans. Toxicologic Pathology.; 30: 483-91.

Ginsberg G, Hattis D, Miller R, Sonawane B. 2004. Pediatric pharmacokinetic data: implications for environmental risk assessment for children. Pediatrics; 113 (4): 973-83

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.

Fleeman TL, Cappon GD, Chapin RE, Hurtt ME. 2005. The effects of feed restriction during organogenesis on embryo-fetal development in the rat. Birth Defects Res B Dev Reprod Toxicol. ;74: 442-9.

Fleeman TL, Cappon GD, Hurtt ME. 2004. Postnatal closure of membraneous ventricular septal defects in Sprague-Dawley rat pups after maternal exposure with trimethadione. Birth Defects Research (Part B); 71: 185-190.

Gerotziafas GT, Elalamy I, Depasse F, Perzborn E, Samama MM. 2007. In vitro inhibition of thrombin generation, after tissue factor pathway activation, by the oral, direct factor Xa inhibitor rivaroxaban. J Thromb Haemost.; 5(4): 886-8.

Gonzalez RCB, J. Huwyler, I. Walter, R. Mountfield, and B. Bittner. 2002. Improved oral bioavailability of cyclosporin A in male Wistar rats comparison of a Solutol HS 15 containing selfdispersing formulation and a microsuspension. Intl. J. Pharm.; 245: 43–151.

Gustafsson BI, Tømmerås K, Nordrum I, Loennechen JP, Brunsvik A, Solligård E, Fossmark R, Bakke I, Syversen U, Waldum H. 2005. Long-term serotonin administration induces heart valve disease in rats. Circulation.; 111(12): 1517-22.

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.

Hathaway WE, Hathaway HS, Belbusen LP. 1964. Coagulation factors in newborn animals. J Lab Clin Med.; 63: 784-90.

Hoffman JI and Kaplan S. 2002. The incidence of congenital heart disease. J Am Coll Cardiol.; 39: 1890–1900.

Karpatkin M, Blei F, Hurlet A, Greco A, Tang Z. 1991. Prothrombin expression in the adult and fetal rabbit liver. Pediatr Res.; 30: 266-269.

Karpatkin M, Lee M, Cohen L, McKinnell J, Nardi M. 2000. Synthesis of coagulation proteins in the fetus and neonate. J Pediatr Hematol Oncol.; 22: 276-80.

Kelton JG. 2002. Heparin-induced thrombocytopenia: an overview. Blood Rev.;16(1): 77-80

Lewis JF and Maron BJ. 1992. Cardiovascular consequences of the aging process. Cardiovasc Clin.; 22(2): 25-34

Lippi G, Salvagno GL, Montagnana M, Brocco G, Guidi GC. 2006. Influence of hemolysis on routine clinical chemistry testing. Clin Chem Lab Med.; 44(3): 311-6.

Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. 2001. Proteinase-activated receptors. Pharmacol Rev.; 53: 245-82

Mann KG, Brummel K, Butenas S. 2003. What is all that thrombin for? J Thromb Haemost; 1: 1504–14.

Miyake T, Shinohara T, Nakamura Y, Fukuda T, Tasato H, Toyohara K, Tanihira Y. 2004. Spontaneous closure of ventricular septal defects followed up from <3 months of age. Pediatr Int; 46: 135-140.

Morawietz G, Rittinghausen S, Mohr U. 1992. RITA--Registry of Industrial Toxicology Animal-data--progress of the working group. Exp Toxicol Pathol.; 44(6): 301-9.

Morawietz G, Ruehl-Fehlert C, Kittel B, Bube A, Keane K, Halm S, Heuser A, Hellmann J. 2004. Revised guides for organ sampling and trimming in rats and mice – Part 3. A joint publication of the RITA and NACAD groups. Exp Toxic Pathol.; 55: 433–449

Moser VC 1989. Screening Approaches to Neurotoxicity. A Functional Observational Battery. J. Amer. Coll. Toxicol.; 8(1): 85-93.

Mugford CA, Kedderis GL 1998. Sex-dependent Metabolism of Xenobiotics. Drug Metabolism Reviews, 30: 441-498.

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

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.

Reiter LW. 1983. Chemical Exposure and Animal Activity: Utility of the Figure 8 Maze, in: Developments in the Science and Practice of Toxicology, edited by A.W. Hayes, R.C. Schnell and T.S. Miya, New York, Elsevier Press, 1983, pp. 73-84

Roehrig S, Straub A, Pohlmann J, Lampe T, Pernerstorfer J, Schlemmer KH, Reinemer P, Perzborn E. 2005. Discovery of the novel antithrombotic agent 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide (BAY 59-7939): an oral, direct factor Xa inhibitor. J Med Chem.; 48(19): 5900-8.

Ruf W, Dorfleutner A, Riewald M. 2003. Specificity of coagulation factor signaling. J. Thromb. Haemost. 1: 1495–1503

Sherratt HS, Watmough NJ, Johnson MA, Turnbull DM. 1988 Methods for study of normal and abnormal skeletal muscle mitochondria. Meth Biochem Anal, 33: 243-335

Solomon HM, Wier PJ, Fish CJ, Hart TK, Johnson CM, Posobiec LM, Gowan CC, Maleeff BE, Kerns WD. 1997. Spontaneous and induced alterations in the cardiac membranous ventricular septum of foetal, weanling, and adult rats. Teratology 55: 185–194.

Strolin Benedetti M, Whomsley R, Baltes EL. 2007. Drug metabolism in the pediatric population and the elderly. Drug Discov Today; 12: 599-610.

Sun WY, Coleman MJ, Witte DP, Degen SJ. 2002. Rescue of prothrombin-deficiency by transgene expression in mice. Thromb Haemost. 88: 984-991.

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.

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.

Viertell B and Trieb G. 2003. The Himalayan rabbit (Oryctolagus cuniculus L.): spontaneous incidences of endpoints from prenatal developmental toxicity studies. Lab Animals (2003) 37: 19–36

Wong PC and Jiang X. 2010. Apixaban, a direct factor Xa inhibitor, inhibits tissue-factor induced human platelet aggregation in vitro: comparison with direct inhibitors of factor VIIa, XIa and thrombin. Thromb Haemost.;104(2): 302-10.

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.

Ziegler D, Rieske JS. 1967. Preparation and properties of Succinate Dehydrogenase-CoenzymeQ Reductase (Complex II). Methods Enzymol, 10: 231-235

Zoetis T, Hurtt ME. 2003. Species comparison of anatomical and functional renal development. Birth Defects Res B Dev Reprod Toxicol.; 68(2): 111-20.

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/s/

PATRICIA P HARLOW
08/01/2011

THOMAS PAPOIAN
08/01/2011
I concur.

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

NDA REVIEW AND EVALUATION OF CARCINOGENICITY DATA

Application numbers:	22406, 202439
Supporting documents:	In Electronic Document Room (EDR)
Applicant's letter dates:	22406: resubmission on 12/30/2010 202439: original submission 01/04/11
CDER stamp dates:	22406: resubmission on 01/03/11 202439: original submission on 01/05/11
Product:	Rivaroxaban (Bay 59-7939)
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Ortho McNeil Janssen Pharmaceuticals Inc [Bayer Schering Pharma AG (Bayer) and Johnson & Johnson Pharmaceutical Research and Development, L.L.C. (J&JPRD)]
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.
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Rivaroxaban is a direct factor Xa inhibitor being developed for the prevention and treatment of multiple thrombosis-mediated conditions, including short-term prophylaxis of deep vein thrombosis in patients undergoing knee or hip replacement surgery under NDA 22406 and for the longer-term prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation under NDA 202439.

According to the ICH Guideline S1A (1996), “Carcinogenicity studies should be performed for any pharmaceutical whose expected clinical use is continuous for at least 6 months. For the longer-term indication under NDA 202439, the study reports of the carcinogenicity studies were submitted and reviewed. However, this document was written separately from the remainder of the nonclinical review for NDA 202439 to support similar incorporation of the carcinogenicity results into the label for the shorter-term indication under NDA 22406.

1.2 Brief Discussion of Nonclinical Findings

Two year carcinogenicity studies were conducted in CD-1 mice and Wistar rats.

In an adequate 104-week study using 60 CD-1 mice/sex/group, daily doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban in ethanol/solutol HS/tap water (10/40/50% v/v) were administered by oral gavage. The systemic exposures (AUCs) of unbound rivaroxaban in male and female mice at the highest dose tested (60 mg/kg/day) were 1- and 1.6 times, respectively, the human exposure of unbound drug at the human dose of 20 mg per day, and 3- and 5-times, respectively, the human exposure of unbound drug at the human dose of 10 mg daily.

No significant treatment-related effects were observed in mice on mortality, bodyweight gain or food consumption. At study end, slight decreases in hemoglobin concentration and hematocrit, slightly prolonged thromboplastin times, and increased incidences of microscopic pigment deposits were consistent with the pharmacodynamic action of rivaroxaban as a factor Xa inhibitor. Consistent with the increase in liver nodules macroscopically, hepatocellular tumors (adenoma and carcinoma) increased with rivaroxaban dosage in the male, but not in the female mice. However, the incidences of hepatocellular tumors were within historical ranges, and the attained p values do not reach the thresholds to classify these tumors as drug-related.. Similarly, the incidences of a few other tumors, including histiocytic sarcoma, malignant lymphoma, ovarian cystadenoma, uterine hemangiosarcoma, and testicular Leydig cell tumors, were numerically increased in the higher dose groups compared to those in the control groups. However, the incidences were within historical ranges, and the attained p values do not reach the thresholds to classify these tumors as drug-related.

In an adequate 104-week study using 50 Wistar rats/sex/group, daily doses of 0, 10, 20, and 60 mg/kg/day of rivaroxaban in ethanol/solutol HS/tap water (10/40/50% v/v) were administered by oral gavage. The systemic exposures (AUCs) of unbound rivaroxaban

in male and female rats at the highest dose tested (60 mg/kg/day) were 1.5- and 3.7 times, respectively, the human exposure of unbound drug at the human dose of 20 mg per day, and 4- and 10-times, respectively, the human exposure of unbound drug at the human dose of 10 mg daily.

No significant treatment-related effect was observed in rats on mortality, bodyweight gain, and food consumption. Although only slight effects were observed on red cell parameters on Days 184, 366, 548, and 716 , the mean values for thromboplastin time for all treated groups on all sampling days were significantly greater (up to 1.9 and 2.5-fold in males and females, respectively) than those for the control groups. Likewise, the incidence of pigment deposition increased in some organs and across all organs in the high dose groups consistent with the pharmacodynamic action of BAY 59-7939. However, the incidence of valvular fibrosis in the heart increased with dose in both male and female rats with the incidence in females statistically significant ($p = 0.0048$) in a trend test, but not in a pair-wise test ($p = 0.0587$).

The incidences of a few tumors in rats, including squamous cell carcinoma of the clitoral glands, adrenal cortical adenoma, adrenal pheochromocytoma, mammary fibroadenoma, histocytic sarcoma, and skin fibroma, were numerically increased in the higher dose groups compared to those in the control groups. However, these incidences were within historical ranges, and the attained p values do not reach the thresholds to classify these tumors as drug-related according to the CDER statistical criteria.

The nonclinical and statistical reviewers concurred with the sponsor that no significant evidence of neoplasia related to BAY 59-7939 treatment was observed either in Wistar rats or CD-1 mice. The Executive Carcinogenicity Assessment Committee also concluded that there were no clear drug-related neoplasms in either study.

1.3 Recommendations

1.3.1 Approvability

The results of the carcinogenicity studies support approvability of rivaroxaban.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical recommendation is necessary.

1.3.3 Labeling

For Section 13.1 of the label for NDA 22406 and NDA 202439; however, the exposure ratios below are for NDA 22406.

Rivaroxaban was not carcinogenic when administered by oral gavage to mice or rats for up to 2 years. The systemic exposures (AUCs) of unbound rivaroxaban in male and female mice at the highest dose tested (60 mg/kg/day) were 3- and 5-times, respectively, the human exposure of unbound drug at the human dose of 10 mg per

day. Systemic exposures of unbound drug in male and female rats at the highest dose tested (60 mg/kg/day) were 4- and 10-times, respectively, the human exposure.

2 Drug Information

2.1 Drug

CAS Registry Number: 366789-02-8

Generic Name: Rivaroxaban (Xarelto™)

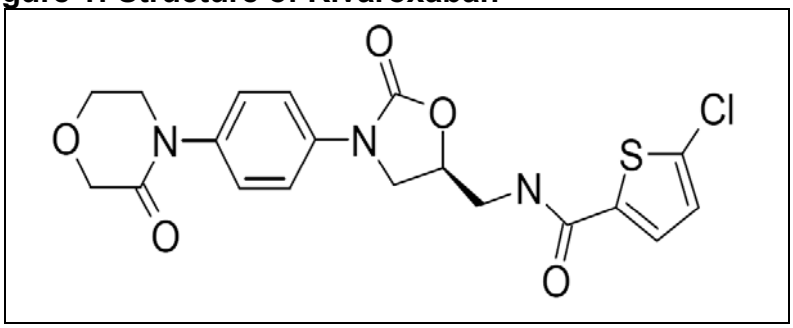
Code Names: JNJ-39039039, BAY 59-7939

Chemical Name: 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide

Molecular Formula/Molecular Weight: $C_{19}H_{18}ClN_3O_5S$/ 435.89 g/mol

Structure or Biochemical Description:

Figure 1: Structure of Rivaroxaban



Pharmacologic Class: Rivaroxaban is a direct Factor Xa inhibitor.

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA-022406 (DHP)
IND-064892 (DHP)
IND-075238 (DCRP)
IND- (b) (4)
MF-021580 (DRM)
MF-021581 (DRM)
MF-021592 (DRM)

2.3 Drug Formulation

Rivaroxaban is formulated for oral administration as immediate release, film-coated tablets containing either 15- or 20-mg of active compound. The tablets also contain microcrystalline cellulose (b) (4) NF, croscarmellose sodium NF, hypromellose (b) (4) USP), lactose monohydrate NF, magnesium stearate (b) (4) NF, sodium lauryl sulfate NF, (b) (4) as excipients. The commercially available film coating for the 15 mg tablet is Opadry Red (b) (4) containing hypromellose (b) (4) USP, polyethylene glycol (b) (4) 3350 NF, ferric oxide red NF, and titanium dioxide USP. The film coating for the 20 mg tablet is Opadry® II Dark Red (b) (4) containing partially hydrolyzed polyvinyl alcohol USP, polyethylene glycol (b) (4) 3350 NF, ferric oxide red NF, titanium dioxide USP, and talc USP.

2.4 Comments on Novel Excipients

All of the excipients are commonly used in oral commercial pharmaceutical dosage forms. The CMC Review of NDA 22406 indicates that the formulation excipients are conventional.

2.5 Comments on Impurities/Degradants of Concern

Impurities and degradants will be discussed in the nonclinical review of NDA 202439.

2.6 Proposed Clinical Population and Dosing Regimen

Rivaroxaban is being developed for the prevention and treatment of multiple thrombosis-mediated conditions. Under NDA 22406, rivaroxaban at 10 mg daily is proposed for the prophylaxis of deep vein thrombosis (DVT) in patients undergoing knee or hip replacement surgery. Under NDA 202439, rivaroxaban at 20 mg daily is proposed for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.

2.7 Regulatory Background

The carcinogenicity protocols were submitted and reviewed under IND 64,892. In April 2005, the Exec CAC reviewed rat and mouse carcinogenicity protocols that proposed dietary administration of rivaroxaban (b) (4) . The Exec CAC did not concur with the proposed protocols and made recommendations to the sponsor. In January 2006, the Exec CAC reviewed additional data submitted by the sponsor and concurred with the sponsor's originally proposed dietary doses. However, a subsequent addendum to the January 2006 meeting minutes indicates the Exec CAC rescinded this concurrence, because the sponsor failed to submit the stability and pharmacological activity data (b) (4) as requested by DMIHP in a teleconference on February 6, 2006. The carcinogenicity protocols and the Exec CAC's action were discussed with the sponsor in a teleconference on February 23, 2006. Subsequently, in a teleconference on June 1, 2006, the sponsor

revealed that [redacted] Consequently, the sponsor proposed to use micronized rivaroxaban [redacted] as the drug product for development. On August 1, 2006, the Exec CAC evaluated carcinogenicity protocols that used micronized rivaroxaban. For both the rat and mouse protocols, the Exec CAC did not concur with the sponsor's proposed doses of [redacted] mg/kg/day and instead recommended doses of 0, 10, 20, and 60 mg/kg/day by oral gavage, based on saturation of absorption in 13-week gavage studies.

3 Studies Submitted

The following study reports for the carcinogenicity studies were submitted under NDA 202439 and are reviewed in Section 4 below

Document Number	Study Number	Study Title
PH-36243	T3076596	BAY 59-7939: Carcinogenicity Study in CD-1 Mice (2 Years Administration by Gavage)
PH-36242	T8076429	BAY 59-7939: Carcinogenicity Study in Wistar Rats (2 Years Administration by Gavage)

4 Carcinogenicity

Two year carcinogenicity studies were conducted in CD-1 mice and Wistar rats. The nonclinical reviews of the study reports are below. The complete statistical review by Dr. Matthew Jackson dated May 11, 2011 is in DARRTS.

Study title: BAY 59-7939: Carcinogenicity Study in CD-1 Mice (2 Years Administration by Gavage)

Document no.:	PH-36243
Study no.:	T3076596 (AT05917)
Study report location:	EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED Toxicology, Wuppertal Germany
Date of study initiation:	October 17, 2006
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7939 (rivaroxaban) a) Lot BXO23BS, purity 100% b) Lot BXA18UX, purity > 99.7%
CAC concurrence - protocol:	On August 1, 2006, the Executive CAC did not concur with the sponsor's proposed doses of (b) (4) and instead recommended doses of 0, 10, 20, and 60 mg/kg/day by oral gavage, based on saturation of absorption. The Executive CAC meeting minutes are in Appendix 1.
CAC concurrence – study results:	On April 15, 2011, the Executive CAC concurred that the study was adequate and there were no clear drug-related neoplasms. The Executive CAC meeting minutes are in Appendix 2.

Key Study Findings

Introduction

CD-1 mice received oral doses of BAY 59-7939 (rivaroxaban) for up to 104 weeks. At dosages of 10, 20 and 60 mg/kg/day the mean $AUC_{(0\ 24h)}$ during Week 52 was 980, 1540, and 2520 ng.hr/mL in males and 1710, 3290, and 4240 ng.hr/mL in females, respectively.

Summary of Non-neoplastic Findings

Consistent with the anti-coagulant pharmacodynamic action of BAY 59-7939, the mean thromboplastin time at 1 hour after dosing was significantly prolonged in all treated groups of males and females on all sampling days; however, not all members of each treated group had values above the normal range. Some of the non-neoplastic microscopic findings, such as increased pigment deposits, were also related to the pharmacodynamic action of BAY 59-7939. The combined incidence of necrosis in the liver slightly increased in the high dose males. In addition, the incidence of biliary cysts in the liver and dilation/atrophy in the preputial gland increased in mid and high dose male groups.

Adequacy of Carcinogenicity Study

The mouse carcinogenicity study used the doses (0, 10, 20, and 60 mg/kg/day) 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. No statistically significant difference in mortality was observed between control and treated groups for either sex.

Appropriateness of Test Model
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. The proposed metabolic pathway of BAY 59-7939 in mice and man is similar involving structural cleavage and hydroxylation, although a minor metabolite, M-5, is not formed in mice.

Summary of Tumor Findings
Consistent with the increase in liver nodules macroscopically, hepatocellular tumors (adenoma and carcinoma) increased with BAY 59-7939 dosage in the males, but not in the females. If the hepatocellular tumors are combined, the statistical evaluations by the sponsor and the FDA statistician indicated p values in the trend test (p_t) of 0.0076 and 0.036, respectively. Since hepatocellular tumors are common tumors in mice, no p value for hepatocellular tumors attains the significance level ($p < 0.005$) necessary for the tumors to be considered positive, according to current CDER guidance. Furthermore, the incidence of either basophilic foci or total foci of alterations in the liver was similar across control and treated groups. The incidences of hepatocellular tumors for the male treated groups in the current study are within historical ranges.

The incidences of a few other tumors were increased in the higher dose groups compared to those in the control groups. The tumors with overall incidences greater than 1% in the (b) (4) listing (2003) for spontaneous tumors in CD-1 mice include histocytic sarcoma in the high dose females (incidence of 1.6%, sponsor's p_t = 0.176, FDA p_t = 0.174), malignant lymphoma in the high dose males ((incidence of 4.5%, sponsor's p_t = 0.136, FDA p_t = 0.043) and in mid- and high dose females (incidence of 9.9%, sponsor's p_t = 0.071, FDA p_t = 0.164). The tumors with overall incidences less than 1% in the (b) (4) listing (2003) for spontaneous tumors in CD-1 mice include ovarian cystadenoma in the high dose females (incidence of 0.74%, sponsor's p_t = 0.032, FDA p_t = 0.055), testicular Leydig cell tumor in mid and high dose males ((incidence of 0.85%, sponsor's p_t = 0.070, FDA p_t = 0.155) and uterine hemangiosarcoma in the high dose females ((incidence of 0.47%, sponsor's p_t = 0.086, FDA p_t = 0.058). In RITA historical control database, the mean incidences of testicular Leydig cell tumor and ovarian cystadenoma are 3.2% and 1.7%, respectively. However, no p value for these tumors attained the significance level of $p < 0.025$ required for even a rare tumor to be considered positive. In addition, the incidences are within historical ranges.

Evaluation of Tumor Findings
The FDA nonclinical and statistical reviewers concur with the sponsor that no significant evidence of neoplasia related to BAY 59-7939 treatment was observed in CD-1 mice.

Methods

Doses:	0, 10, 20, and 60 mg/kg/day
Frequency of dosing:	Daily for up to 729 days
Dose volume:	10 mL/kg
Route of administration:	Orally by gavage
Formulation/Vehicle:	Ethanol/Sotutol HS 15/Tap Water (10/40/50 v/v/v)
Basis of dose selection:	A 13-week dose range finding study in the same strain of mice indicated that absorption of BAY 59-7939 saturated at 60 mg/kg
Species/Strain:	Mice (Mus musculus)/ Crl:CD-1(ICR) BR (b) (4)
Number/Sex/Group:	Main: 60 animals/sex/dose Satellite: 20 animals/sex/dose
Age:	6-7 weeks on first day of treatment
Animal housing:	Individual cages
Paradigm for dietary restriction:	None; food was administered ad libitum
Dual control employed:	None
Interim sacrifice:	None
Satellite groups:	Yes, for clinical laboratory and toxicokinetic measurements
Deviation from study protocol:	Not indicated

Observations and Results

Mortality

The animals were examined visually for mortality and morbidity twice daily, except on weekends and holidays when they were examined once daily.

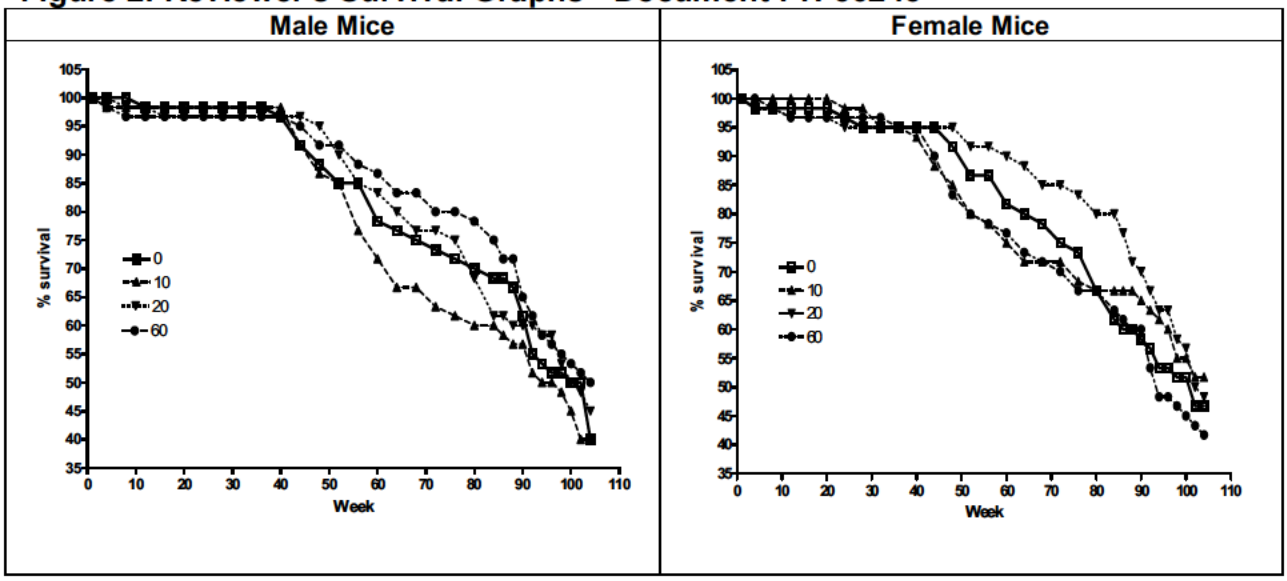
Although no statistically significant difference in mortality was observed (Table 1), the high dose males showed a slight decrease in mortality compared to the control males and the high dose females showed a slight increase in mortality compared to control females at the end of the study.

Table 1: Reviewer's Table Summarizing Mortality in Mice - Document PH-36243

	Males				Females			
Dose, mg/kg	**0**	**10**	**20**	**60**	**0**	**10**	**20**	**60**
Main animals								
Total number/group	60	60	60	60	60	60	60	60
Intercurrent deaths	37	36	34	31	32	29	31	36
% mortality	61.6	60	56.6	51.6	53.3	48.3	51.6	60
Satellite animals								
Total number/group	20	20	20	20	20	20	20	20
Intercurrent deaths	15	11	11	9	12	13	11	13
% mortality	75	55	55	45	60	65	55	65

The sponsor did not provide Kaplan-Meier survival graphs. The reviewer's graphs (Figure 2) below indicate survival was greater in the high dose male group compared to control male group beginning at Week 50. The survival in the mid dose female group was greater than that in the control female group during Weeks 50 to 104.

Figure 2: Reviewer's Survival Graphs - Document PH-36243



The pathologist noted two factors, malignant lymphoma and amyloidosis, that contributed to mortality of some decedents. The percentage of mid and high dose female decedents (30-47%) with moderate to severe amyloidosis in the duodenum, liver, kidneys and spleen was lower than the percentage of control female decedents (57-60%) with amyloidosis. However, the percentage of male decedents with amyloidosis generally was similar across dose groups, except in a few organs, such as the heart (control: 29%, high dose: 11%) and stomach (control 32%, high dose: 7%). The percentage of mid and high dose female decedents (17-23%) with malignant lymphoma was higher than the percentage of control female decedents (6.7%) with malignant lymphoma. Likewise, the percentage of high dose male decedents (21%) with malignant lymphoma was higher than the percentage of control male decedents (2.9%) with malignant lymphoma.

Clinical Examinations

Detailed clinical examinations were made once before the start of treatment and once weekly in all groups during treatment.

The most frequent clinical signs included increased urine excretion, piloerection, pallor, and increased girth (Table 2). The incidence of animals with increased girth was greater in the low, mid and high dose male groups and the mid and high dose female groups compared to the incidence in the control groups. However, the finding of increased girth did not correlate with the numbers of animals with palpable masses. Importantly, the incidence of signs associated with bleeding (vaginal, discolored feces, and blood in bedding) was low and did not show a dosage relationship.

Table 2: Reviewer's Summary of Clinical Findings - Document PH-36243

	Cumulative number of animals in main groups (in satellite groups)							
	Male mice				Female mice			
Finding/Dose (mg/kg)	0	10	20	60	0	10	20	60
Number of animals	60 (20)	60 (20)	60 (20)	60 (20)	60 (20)	60 (20)	60 (20)	60 (20)
Increased urine excretion	25 (14)	29 (9)	31 (8)	25 (12)	25 (11)	27 (10)	26 (9)	19 (11)
Piloerection	28 (15)	21 (15)	32 (10)	22 (13)	23 (8)	20 (8)	22 (3)	19 (10)
Pallor	23 (14)	27 (13)	24 (6)	23 (6)	20 (9)	24 (12)	22 (8)	18 (10)
Increased girth	7 (8)	19 (2)	17 (1)	16 (3)	12 (7)	12 (5)	16 (7)	17 (8)
Palpable masses	2 (1)	5 (0)	6 (0)	3 (1)	4 (2)	0 (0)	2 (0)	1 (0)

Body Weights

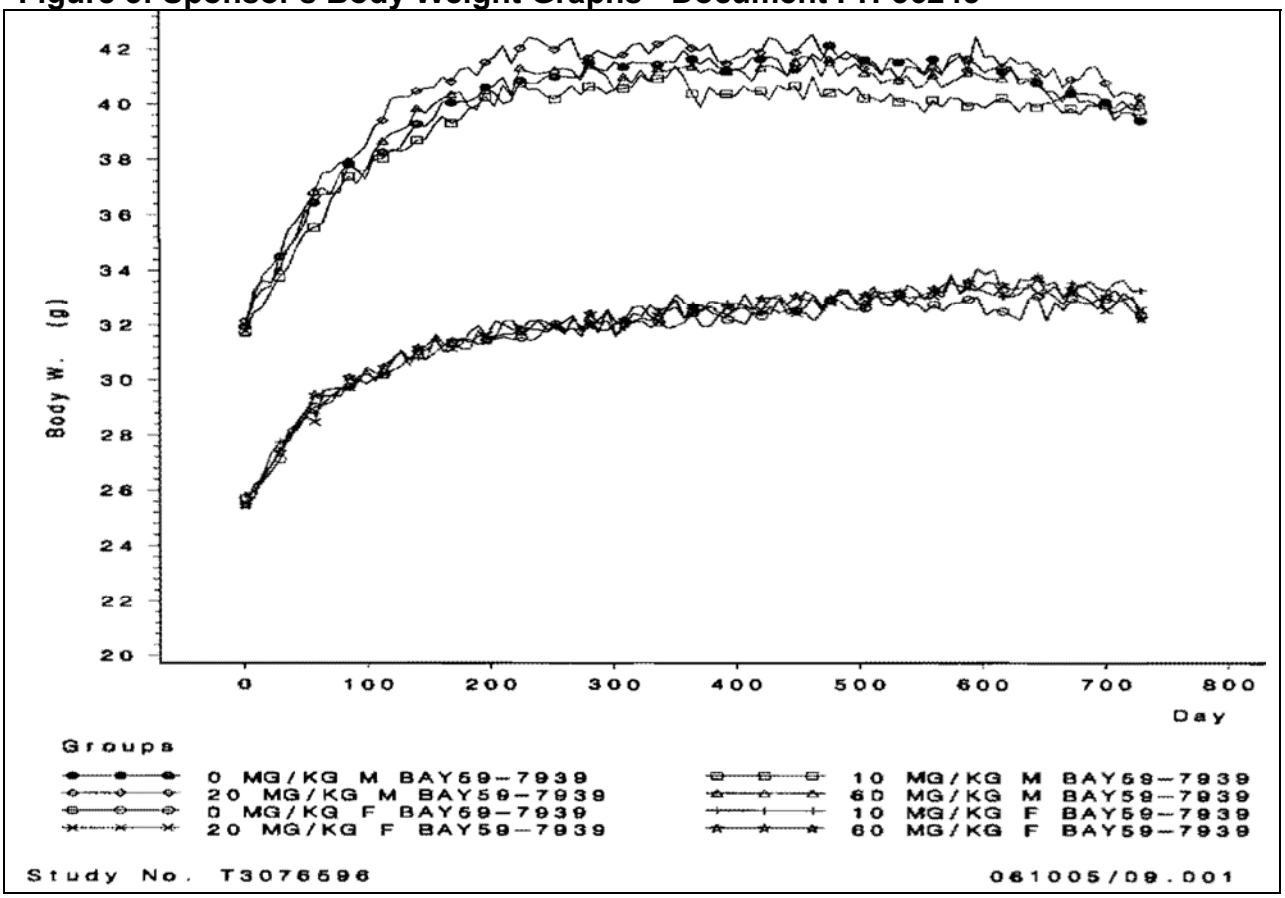
The animals in all groups were weighed on Day 1 of treatment and weekly up to scheduled necropsy and immediately before necropsy.

Body weight and body weight gain in the main study groups were not significantly affected by treatment with BAY 59-7939 (Table 3, Figure 3).

Table 3: Reviewer's Summary of Body Weights - Document PH-36243

Main Study	Dose (mg/kg)							
	Male mice				Female mice			
Day Dose (mg/kg)	0	10	20	60	0	10	20	60
Mean body weight (gm)								
1	31.9	31.7	32.1	31.7	25.7	25.8	25.5	25.5
92	37.6	37.1	38.1	37.6	30.0	29.9	30.1	29.9
183	40.1	39.8	41.3	39.9	31.1	31.7	31.3	31.4
274	41.0	40.3	41.6	40.8	31.8	31.9	31.7	31.6
365	41.6	40.4	42.0	41.4	32.4	32.6	32.5	32.7
456	41.3	40.1	42.2	41.7	32.4	32.6	33.2	32.9
547	41.4	40.0	41.3	40.6	32.7	33.0	33.2	33.0
631	40.7	39.7	41.4	40.9	32.1	33.6	33.1	33.3
722	39.6	40.0	40.4	39.8	32.5	33.2	32.9	33.0
729	39.4	39.7	40.2	40.0	32.3	33.2	32.5	32.2
Body weight gain (gm)								
Day 1 – Day 183	8.2	8.1	9.2	8.2	5.4	5.9	5.8	5.9
Day 1 – Day 365	9.7	8.7	9.9	9.7	6.7	6.8	7.0	7.2
Day 1 – Day 729	7.5	8.0	8.1	8.3	6.6	7.4	7.0	6.7

Figure 3: Sponsor's Body Weight Graphs - Document PH-36243



Food and Water Consumption

Food and water consumption were determined weekly for individual main group animals.

The sponsor's summary tables below (Table 4) indicate that no treatment effect was observed on group mean food or water intake relative to the control group.

Table 4: Sponsor's Summaries of Food and Water Intake - Document PH-36243

	Male mice					Female mice				
	Group means					Group means				
Dose mg/kg	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day
Food intake										
0	728	4084	5.61	102692	141.06	728	3524	4.84	112076	153.95
10	728	3946	5.42	100668	138.28	728	3574	4.91	112556	154.61
20	728	4040	5.55	99998	137.36	728	3545	4.87	111668	153.39
60	728	3997	5.49	100588	138.17	728	3560	4.89	112214	154.14

Water intake									
0	728	5176	7.11	130443	179.18	728	5016	6.89	158952	218.34
10	728	4950	6.80	126301	173.49	728	4929	6.77	154867	212.73
20	728	4929	6.77	122348	168.06	728	4834	6.64	152356	209.28
60	728	4827	6.63	121896	167.44	728	4834	6.64	152640	209.67
Reviewer's modification of sponsor's tables										

Hematology

Blood samples for hematology were collected from 10 non-fasting satellite animals per group during weeks 50, 77, and 103. The following parameters were measured: hematocrit, hemoglobin concentration, erythrocyte count, erythrocyte morphology, reticulocyte count, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume, platelet count, total white cell count, and differential white cell count, including lymphocytes, eosinophils, monocytes, segmented neutrophils, banded neutrophils, and atypical lymphocytes. Additional blood samples taken for measurement of thromboplastin time (Hepato Quick) were collected from 10 satellite animals per group during weeks 51, 78, and 104 approximately 1 hour after drug administration. Samples for blood smears were collected satellite groups during weeks 49, 76, and 101 as well as from all animals killed in moribund condition and the surviving main study animals in the control and high dose groups near the end of the study.

The sponsor concluded that no treatment-related effects on red blood cell parameters were observed in either males or females at any dose of BAY 59-7939 after 49 and 76 weeks of treatment. Evaluation of hematology parameters at these timepoints is complicated by some control animals having values outside of the reference range (Table 5). For males on Day 347 the aberrant values were close to the reference range. For males on Day 535 and females on Day 347 the aberrant control value could be rejected on the basis of a statistical Q test (Dean and Dixon, 1951). For females on Day 536, two control females had values below the reference range and the concurrent control range for some parameters was narrower than the reference range. On Days 716 and 717 only the concurrent control values were available for comparison. Some values for individual animals in the treated groups were below the concurrent control range and some values were above the concurrent control range. However, the erythrocyte count, hemoglobin concentration, and hematocrit values were significantly lower for the high dose females and a dose relationship was evident. Mean values for these parameters for the high dose females on Days 347 and 536 were also lower, but were not statistically significant. The high dose males had non-statistically significant increases in these parameters on Days 535 and 716.

Although statistically significant increases were observed on Day 346 for MCH in the low dose males and for MCHC in the low and mid dose males, these increases were not considered toxicologically significant because of a lack of a dose response relationship with the high dose group. Increased leucocyte counts on Day 716 in the mid and high dose females were attributable to the very high values for single females (617 and 632) in each group. Platelet and differential blood counts showed no significant treatment-related effects.

Table 5: Reviewer's Summary - Hematology Results - Document PH-36243

			Mean (# outside reference range) [# outside concurrent control range]					
	Day	Dose Mg/kg	ERY 10e12/L	HB Gm/L	HCT L/L	MCH pg	MCHC Gm/L ERY	RETI %
Male mice	346	0	8.52	131 (1)	0.410 (1)	15.4 (2)	319 (1)	31
		Min., Max	7.32,10.08	**96**,153	0.339, 0.459	**13.3**, 16.4	**288**, 332	24, 35
		10	8.20	134	0.403	***16.4**** (2)[4]	***332**[5]	32 [4]
		20	8.58 [1]	139	0.414	16.2 (3)[3]	***335**(3)[6]	27
		60	8.51 [2]	137 (1)	0.416 (1) [1]	16.2 (3)[3]	330 (1)[3]	28
		2SD	7.03	107	0.352	13.9	294	6
		2SD+	10.52	163	0.492	16.6	343	58
	535	0	7.55 (1){8.1}	113 (1){122}	0.393 (1){0.373}	14.9 (2)	283 (1) {293}	68 (1) {28}
		Min., Max	**2.61**, 9.63	**33**, 129	**0.180**, 0.438	**13.0,** 16.0	**190**, 307	21, **426**
		10	7.56 (1)	113 (2)[1]	0.390 (2)[2]	14.9 (1)	289	33 (4)[1]
		20	8.17 (2)	121 (1)[5]	0.419 (1)[4]	14.8 (3)[2]	288	35 (2)[1]
		60	8.49	127 [3]	0.434 (1)[5]	14.9	292	29 (2)[1]
		2SD	6.56	102	0.343	14.2	266	21
		2SD+	9.80	150	0.490	16.7	341	35
	716§	0	7.72	115	0.381	14.7	298	49
		Min., Max	7.02, 8.76	105, 129	0.351, 0.435	14.1, 16.1	294, 308	26, 73
		10	7.64 [5]	115 [7]	0.373 [6]	14.9 [3]	303 [9]	55 [7]
		20	8.13 [5]	125 [4]	0.401 [3]	15.4 [3]	308 [6]	43 [3]
		60	8.78 [5]	128 [5]	0.421 [5]	14.6 [1]	305 [4]	46 [4]
Female mice	347	0	8.54 (1){8.82}	132 (2){138}	0.415 (1){431}	15.4 (1){15.7}	318 (1)	28
		Min., Max	**6.0**, 9.27	**75**, 147	**0.264**, 0.441	**12.7,** 16.4	**290,** 336	**15**, 41
		10	8.65 (1)[3]	138 (2)[3]	0.427 (2)[3]	16.0 (1)[2]	324	29 (2)[2]
		20	8.42 (2)[1]	133 (1)	0.413 (2)[2]	15.8 (1)[3]	320	27
		60	8.23 (4)[1]	128 (2)[2]	0.390 (4)[2]	15.4 (2)[2]	324 (2)[2]	33 (2)[2]
		2SD	8.21	129	0.404	14.5	294	17
		2SD+	9.74	152	0.493	16.8	334	41
	536	0	7.98 (2)	119 (2)	0.411 (2)	15.0	290	45 (3)
		Min., Max	**5.19**, 9.84	**75**, 141	**0.267**, 0.486	14.4, 15.4	284, 300	17, **99**
		10	7.92	121 (1)	0.417 (1)	15.4 (1)[5]	291 (2)[3]	30
		20	7.75 (1)[1]	119 (2)	0.413 (3)	15.3 (1)[7]	286 (2)[3]	59 (1)[1]
		60	7.53 (2)	111 (2)	0.385 (5)	14.8 (2)[3]	288 (2)[3]	44 (2)
		2SD	6.99	112	0.376	14.4	281	12
		2SD+	9.99	153	0.508	16.9	320	58
	717§	0	8.70	128	0.428	14.7	298	35
		Min., Max	7.38, 10.02	102, 141	0.345, 0.471	13.9, 15.5	286, 313	12, 58
		10	7.38 [3]	113 [2]	0.380 [2]	15.3 [3]	295 [1]	53 [3]
		20	7.06 [5]	104 [3]	0.354 [2]	14.5 [5]	283 [4]	87 [4]
		60	***5.82**** [5]	***86**[4]	***0.296**** [4]	14.6 [2]	286 [3]	74 [4]

* $p < 0.05$, HD = high dose, ERY = erythrocytes, HB = hemoglobin concentration, HCT = hematocrit,
MCV = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration,
RETI = % reticulocytes, 2SD+ = 2 standard deviations above the mean, 2SD = 2 standard deviations below the mean
Min. = minimum, Max. = maximum, § Only concurrent control range available

Although the mean thromboplastin time was significantly prolonged in all treated groups of males and females on all sampling days (Table 6), examination of individual values indicates that not all members of each treated group had values above the normal range. Despite collection of blood samples at 1 hour after dose administration or close to the T_{max}, the maximum individual value on each sampling day was at most 1.8-fold the mean of the respective control group. Furthermore, the maximum individual value did not always occur in the highest dose group.

Table 6: Reviewer's Summary Thromboplastin Times - Document PH-36243

		Dose	HQUICK, sec			Reference range		Number	
	Day	Mg/kg	Mean	Min.	Max.	3SD-	3SD+	≤ 3SD+[†]	>3SD+[‡]
Male mice	345	0	18.0	16.3	20.6	13.3	22.7	10	0
		10	21.9**	19.5	25.0			7	3
		20	22.0**	19.7	27.2			8	2
		60	22.2**	18.3	16.7			6	4
	535	0	19.9	16.9	22.0	15.0	24.7	10	0
		10	24.0**	20.7	26.3			7	3
		20	24.1**	20.6	31.4			7	3
		60	23.1**	21.9	24.5			10	0
	716	0	20.0	16.7	23.5	16.7	23.5	5[§]	0
		10	23.2**	21.1	25.3			5[§]	4
		20	27.0**	23.4	35.6			1[§]	8
		60	24.4**	21.7	28.2			4	6
Female mice	347	0	19.5	16.2	21.4	15.1	23.8	10	0
		10	24.5**	20.4	33.6			5	5
		20	24.0**	18.1	28.5			3	7
		60	25.6**	21.9	29.6			3	7
	536	0	19.0	16.3	20.5	15.1	22.9	10	0
		10	23.2**	21.0	25.6			4	6
		20	23.6**	20.2	27.0			2	8
		60	24.2**	19.3	28.3			4	6
	717	0	19.8	17.6	21.1	17.6	21.1	8[§]	0
		10	25.6**	19.7	28.9			1[§]	6
		20	24.6**	22.2	27.0			0[§]	9
		60	24.9**	16.2	32.7			1[§]	6

[†] Number of values <3 standard deviations above mean, [‡] Number of values ≥ 3 standard deviations above mean, [§] Less than 10 animals/groups

Gross Pathology

The surviving satellite animals were sacrificed for scheduled necropsy during week 105. The surviving main study animals were sacrificed for scheduled necropsy during weeks 105-107. 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 7 were fixed in 10% neutral buffered formalin. The urinary bladder and lungs were initially inflated with 10% neutral buffered formalin prior to fixation by immersion.

Table 7: Reviewer's List of Organs Collected - Document PH-36243

Abnormal tissues	Kidneys	Seminal vesicles with coagulating glands
Adrenal glands	Larynx	Skeletal muscle (thigh)+
Aorta	Liver[#]	Skin (mammary area)
Brain (cerebrum, cerebellum, brain stem)	Lungs	Spinal cord (cervical, thoracic, lumbar)
Cecum	Lymph nodes (mandibular, mesenteric)	Spleen
Clitoral gland	Nasal cavity/nasopharynx	Sternum with bone marrow
Colon	Optic nerves	Stomach
Duodenum	Ovaries	Testes
Epididymides	Oviduct	Thymus
Esophagus	Pancreas	Thyroid with parathyroids
Eyes and eyelids	Peyers patches	Tongue
Extraorbital lacrimal glands	Pharynx	Trachea
Femur with joint	Pituitary	Ureters
Gall bladder[#]	Preputial gland	Urethra
Harderian glands	Prostate	Urinary bladder
Head with skull cap	Rectum	Uterus with cervix
Heart	Salivary glands (submandibular, sublingual and parotid)	Vagina
Ileum	Sciatic nerve	Zymbal's glands
Jejunum		

[#] Satellite animals were also examined by histopathology.

The principal gross lesions were nodules, cysts, dilations and discolorations (Table 8). Although nodules were found in many tissues, the highest incidence of nodules was in the liver, lung, ovaries, and uterus. The incidence of nodules was increased in the livers of mid and high dose males, the uteri of high dose females and the ovaries of mid and high dose females. Lower incidences of nodules were found in mesenteric lymph node, skin, spleen, heart, testes, thymus, body cavity and Harderian gland. The incidence of nodules was increased in spleen of high dose males, hearts of high dose males, skin of treated male groups, and mesenteric lymph node of mid and high dose male and females. The incidence of nodules in other tissues (adrenal glands, gallbladder, kidney, pancreas, pituitary, preputial glands, seminal vesicles, skull cap, sternum, and stomach) was limited to a single incidence per group. Higher incidences of cysts in the liver were observed in mid and high dose males compared to the incidence in the control group. Higher incidences of lung discoloration were observed in mid and high dose females compared to that in the control group. Higher incidence of gallbladder dilations were observed in the high dose females compared to that in the control group. Necropsy findings in the satellite animals confirm the increased cysts in the high dose male livers, increased nodules in the mid and high dose male livers, and increased nodules in the mid and high dose female ovaries.

Table 8: Reviewer's Summary of Necropsy Findings - Document PH-36243

		Male mice				Female mice			
	Dose, mg/kg	0	10	20	60	0	10	20	60
Number of animals	Main study (decedents)	60 (37)	60 (36)	60 (34)	60 (31)	60 (32)	60 (29)	60 (31)	60 (36)
Finding	Tissue								
Cyst	Liver	0	0	3 (2)	3	0	1	1	1
	Ovaries	-	-	-	-	18 (7)	24 (9)	18 (7)	18 (10)
Dilations	Gallbladder	2 (2)	0	2 (2)	0	6 (4)	5 (1)	6 (3)	**12 (8)**
	Uterus	-	-	-	-	0	0	2 (1)	2 (1)
Discoloration	Lung	4 (4)	4 (4)	6 (6)	7 (6)	6	6	**12**	**12**
Nodules	Liver	4 (4)	8 (4)	**12 (7)**	**12 (6)**	3 (2)	1 (0)	3 (3)	1 (1)
	Lung	6 (3)	10 (4)	5 (2)	8 (4)	5 (1)	3	**6 (3)**	**6 (1)**
	Ovaries	-	-	-	-	2 (2)	2 (2)	**5 (3)**	**4 (2)**
	Uterus	-	-	-	-	4 (3)	3	5 (3)	**9 (7)**
	Testes	0	0	3 (1)	1	-	-	-	-
	Spleen	0	1 (1)	0	**3** (1)	0	0	0	0
	Thymus	1 (1)	2 (2)	0	2 (2)	2 (2)	3 (1)	1 (1)	1 (1)
	Lymph node mesenteric	0	1 (1)	**3 (1)**	**2 (2)**	1	1 (1)	**2 (1)**	**2**
	Skin	0	**2 (2)**	**2 (2)**	**2 (2)**	1	0	1 (1)	1
	Body cavity	1 (1)	0	0	0	2 (2)	0	3 (1)	0
	Harderian gland	2	0	1	1 (1)	2	0	0	0
	Heart	0	0	1 (1)	**2** (2)	0	0	0	0
Satellite animals	Number	23	24	26	29	28	31	29	24
Cyst	Liver	0	0	**1**	**3**	0	1	1	1
	Ovaries	-	-	-	-	11	18	11	8
Dilations	Gallbladder	0	0	0	0	2	4	3	4
Discoloration	Lung	0	0	0	0	0	2	2	0
Nodules	Lung	3	6	3	4	4	3	3	5
	Liver	0	4	**5**	**6**	1	1	0	0
	Ovaries	-	-	-	-	0	0	**2**	**2**
	Uterus	-	-	-	-	2	3	3	0

Organ Weights

The following organs were weighed before fixation: adrenals, brain, kidneys, liver, spleen, and testes.

Sponsor concluded that no treatment-related effect was observed on organ weights (Table 9). However, the reviewer notes a slight increase in absolute and relative liver weights in the high dose male group. This is best illustrated by a comparison of the median liver weights. Some correlation of liver weight with the presence of nodules and cysts exists in that 13 of the 19 males for whom liver weights were available had liver weights above the median of the group.

Table 9: Sponsor's Summaries of Organ Weights - Document PH-36243

Dose mg/kg	Body W. G	Brain mg	Adrenals mg	Liver mg	Spleen mg	Kidneys mg	Testes mg	Median Liver Weight
Mean Absolute Organ Weights								
m	Terminal Sacrifice							
0	39	497	8	2331	106	780	212	2297
10	40	512	8	2480	120	783	203	2403
20	40	501	7	2303	137	730	217	2224
60	40	507	7	2579	137	771	215	2431
f	Terminal Sacrifice							
0	33	508	10	1962	146	509		1847
10	33	496	9	1901	120	477		1759
20	33	488 +	8	1920	122	487		1795
60	33	497	9	1904	145	486		1872
Mean Relative Organ Weights								
Dose mg/kg	Body W. G	Brain mg/100g	Adrenals mg/100g	Liver mg/100g	Spleen mg/100g	Kidneys mg/100g	Testes mg/100g	
m	Terminal Sacrifice							
0	39	1273	20	5939	273	1996	547	5724
10	40	1291	19	6252	299	1967	513	5894
20	40	1264	18	5690	349	1812	547	5583
60	40	1288	17	6524	343	1947	544	6331
f	Terminal Sacrifice							
0	33	1571	30	6001	439	1561		5684
10	33	1505	26	5765	360	1439		5301
20	33	1497	25	5803	369	1480		5551
60	33	1537	27	5861	452	1494		5692

Histopathology

Tissue samples from all main study animals were dehydrated, embedded in Paraplast, sectioned, and stained with hematoxylin and eosin. All tissues listed in Table 7 and gross abnormalities identified at macroscopic examination from all animals sacrificed at the end of the scheduled treatment period and from all animals killed or dying during the study were examined by histology. In addition, the liver and gallbladder were examined microscopically from animals in all satellite groups.

Peer Review

Peer review included examination of the liver, pituitary glands and mesenteric lymph nodes as well as all tumors and pre-neoplastic lesions of all groups. In addition, approximately 25% of frequent lesions and all slides of six animals per sex from the high dose group were also examined.

Neoplastic Lesions

The incidences of the most notable tumors in the mouse carcinogenicity study are summarized in Table 10 below. The sponsor's listing of tumor incidences is in Appendix 3. The statistical evaluations of the sponsor and the FDA statistician are in Appendix 4 and 5, respectively. Historical control data provided by the sponsor are in Appendix 9.

Consistent with the increase in liver nodules macroscopically, hepatocellular tumors (adenoma and carcinoma) increased with BAY 59-7939 dosage in the males, but not in the females. The sponsor's evaluation of hepatocellular adenoma and hepatocellular carcinoma indicated p values in the trend test of 0.052 and 0.046, respectively. The FDA statistician's evaluation of hepatocellular adenoma and hepatocellular carcinoma indicated p values in the trend test of 0.128 and 0.143, respectively. If the hepatocellular tumors are combined, the statistical evaluations by the sponsor and the FDA statistician indicated p values in the trend test (p_t) of 0.0076 and 0.036, respectively. Since hepatocellular tumors are common tumors in mice, no p value attains the significance level ($p < 0.005$) necessary for the tumors to be considered positive, according to current CDER guidance. Furthermore, the incidence of either basophilic foci or total foci of alterations in the liver was similar across control and treated groups. The incidences of hepatocellular tumors for the male treated groups in the current study are within historical ranges.

The incidences of a few other tumors were increased in the higher dose groups compared to those in the control groups. The tumors with overall incidences greater than 1% in the [redacted] (b) (4) listing (2003) for spontaneous tumors in CD-1 mice include histocytic sarcoma in the high dose females (incidence of 1.6%, sponsor's p_t = 0.176, FDA p_t = 0.174), malignant lymphoma in the high dose males (incidence of 4.5%, sponsor's p_t = 0.136) and in mid- and high dose females (incidence of 9.9%, sponsor's p_t = 0.071). The tumors with overall incidences less than 1% in the [redacted] (b) (4) listing (2003) for spontaneous tumors in CD-1 mice include ovarian cystadenoma in the high dose females (incidence of 0.74%, sponsor's p_t = 0.032, FDA p_t = 0.055), testicular Leydig cell tumor in mid and high dose males (incidence of 0.85%, sponsor's p_t = 0.070) and uterine hemangiosarcoma in the high dose females (incidence of 0.47%, sponsor's p_t = 0.086, FDA p_t = 0.058). In RITA historical control database, the mean incidences of testicular Leydig cell tumor and ovarian cystadenoma are 3.2% and 1.7%, respectively. However, no p value for these tumors attained the significance level of $p < 0.025$ required for even a rare tumor to be considered positive. In addition, the incidences are within historical ranges.

Table 10: Reviewer's Summary of Neoplastic Findings – Document PH-36243

Mouse Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
Neoplastic Findings†		Male				Female			
All main study animals		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	60	60	60	60	60	60	60	60
Liver	#	58	60	58	57	58	60	59	58
Hepatocellular Adenoma B	#	0	3	**4**	**4**	0	0	0	0
(RITA range: Male, 0 21.7%) (p_t = 0.053)	%	0	5.0	6.9	7.0	0	0	0	0
[redacted] (b) (4) maximum: Male, 28%)	p_e				0.057				
Hepatocellular Adenocarcinoma M	#	2	4	**7**	**7**	1	1	0	0
(RITA range: Male, 4 22%) (p_t = 0.046)	%	3.5	6.7	12.1	12.3	1.7	1.7	0	0
([redacted] (b) (4) maximum: Male, 16%)	p_e				0.077				
Hepatocellular Adenoma + Adenocarcinoma	#	2	7	**11**	**11**	1	1	0	0
(RITA range: 8.0 36.1%) (p_t = 0.0076)	%	3.5	11.7	19.0	19.3	1.7	1.7	0	0
	p_e								
Systemic tumors	#	58	60	58	57	58	60	59	58
Histocytic sarcoma M (F: p_t = 0.176)	#	2	0	0	2	3	3	4	**5**
[redacted] (b) (4) maximum: Male: 8.0, Female: 18.3%)	%	3.5	0	0	3.5	5.2	5.0	6.8	8.6
Lymphoma M (M: p_t = 0.111; F: p_t = 0.059)	#	3	3	3	**7**	4	4	**11**	**7**
(RITA range: Male: 0 17.6%, Female: 4 43.3%)	%	5.2	5.0	5.2	12.3	6.9	6.7	18.6	12.1
[redacted] (b) (4) maximum: Male: 21.7%, Female: 50%)	p_e				0.14			0.05	0.26

Mouse Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
Neoplastic Findings†		Male				Female			
All main study animals		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	60	60	60	60	60	60	60	60
Ovaries	#	0	0	0	0	54	54	54	63
Cystadenoma B (p_t = 0.033)	#					0	0	1	**2**
(RITA range: 0 5.0%) (CR maximum: 7.3%)	%					0	0	1.8	3.5
	p_e								0.248
Luteoma B	#					0	0	2	1
(b) (4) maximum: 4.0%)	%					0	0	3.5	1.8
Granulosa cell B	#					0	0	2	0
((b) (4) maximum: 2.9%)	%					0	0	3.5	0
Granulosa cell M	#					0	0	1	0
(b) (4) maximum: 1.7%)	%					0	0	1.7	0
Granulosa cell combined	#					0	0	3	0
	%					0	0	5.2	0
Testes	#	57	60	58	57	0	0	0	0
Granulosa cell B	#	0	0	1	0				
	%	0	0	1.7	0				
Leydig cell B (p_t = 0.07)	#	0	2	**3**	**3**				
(RITA range: Male, 0 10%)	%	0	3.3	5.2	5.3				
Vascular system									
Hemangiosarcoma									
Liver	#	1/58	2/60	0	2/57	0	0	1/59	0
	%	1.7	3.3	0	3.5	0	0	1.7	0
Spleen	#	1/58	0	0	0	1/58	0	1/59	0
	%	1.7	0	0	0	1.7	0	1.7	0
Skin	#	0	0	0	0	1/58	0	0	0
	%	0	0	0	0	1.7	0	0	0
Uterus (p_e = 0.248, Trend p_t = 0.086)	#					0	0	1/58	**2**/58
(b) (4) maximum: 4.1%)	%					0	0	1.7	3.5
Body cavity	#	0	0	0	0	0	0	1/4	0
	%	0	0	0	0	0	0	N<10	0
Combined hemangiosarcomas	#	2	2	0	2	2	0	4	2
	%	3.4	3.3	0	3.5	3.4	0	>6.8	3.5
Hemangioma									
Spleen	#	0	0	1/58	0	0	0	0	0
	%	0	0	1.7	0	0	0	0	0
Skin	#	0	0	0	1/57	0	0	0	0
	%	0	0	0	1.8	0	0	0	0
Uterus	#					2/58	0	1/58	0
(b) (4) maximum: 4.6%)	%					3.5	0	1.7	0
Spinal Cord	#	0	1/60	0	0	0	0	0	0
	%	0	1.7	0	0	0	0	0	0
Combined hemangiomas	#	0	1	1	1	2	0	1	0
	%	0	1.7	1.7	1.8	3.5	0	1.7	0
Total hemangiosarcomas + hemangiomas	#	2	3	1	3	4	0	5	2
	%	3.4	5.0	1.7	5.3	7.0	0	>8.5	3.5

† All p values are from the sponsor's study report for T3076596. p_e = Exact p value, p_t = Trend p value, RITA(Registry of International Toxicology Animal) Data, 2009, (b) (4) March 2005

Non Neoplastic Lesions

The study pathologist considered the significant increases in biliary cysts in the liver of males and dilation/atrophy in the preputial gland to be background variation (Table 11). The pathologist noted non-statistically significant increases in ovarian hemorrhages often associated with large cyst formation in the treated females groups and a statistically significant increase in ovarian pigment deposits. Likewise, extramedullary hematopoiesis in the spleen and liver increased in incidence and severity in the treated male groups and reached statistical significance for the mid-dose group. The pathologist considered the observations of hemorrhage, pigment deposits and hematopoiesis to be related to the pharmacodynamic effect of BAY 59-7939.

Although the reviewer agrees that these observations could be related to the anti-coagulant effects of BAY 59-7939, the reviewer notes that the incidence of hemorrhage in other organs did not always increase with dose. In the urinary bladder, the incidence of hemorrhage decreased with dose. Furthermore, the incidence of hemorrhage across all organs did not increase with dose. However, the incidence of pigment deposits increased in male liver, female ovaries, and female lungs. Also, the incidence of pigment deposits across all organs did increase in the mid and high dose males and the high dose females.

Table 11: Reviewer's Summary Non-neoplastic Lesions – Document PH-36243

Mouse Carcinogenicity Study			BAY 59 7939 Dose level (mg/kg/day)							
	All animals		Male				Female			
			0	10	20	60	0	10	20	60
Organ/Tissue	Finding	#/group	60	60	60	60	60	60	60	60
Liver		#	58	60	58	57	58	60	59	58
	Amyloidosis	#	25	23	19	25	22	28	14	19
		%	43.1	38.3	32.8	43.9	37.9	46.7	23.7	32.8
	Basophilic foci	#	2	2	2	4	0	0	0	1
		%	3.4	3.4	3.4	6.8	0	0	0	1.7
	Clear cell foci	#	3	1	1	1	1	0	0	0
		%	5.2	1.7	1.7	1.7	1.7	0	0	0
	Eosinophilic foci	#	0	0	4	1	0	0	0	1
		%	0	0	6.8	1.7	0	0	0	1.7
	Total foci	#	5	3	7	6	1	0	0	2
		%	8.6	5.0	12.1	10.5	1.7	0	0	3.4
	Biliary cysts	#	0	0	**3**	**3**	0	1	2	2
		%	0	0	5.2	5.2	0	1.7	3.4	3.4
					p = 0.012					
	Increased hemopoiesis	#	0	1	**5**	3	7	3	4	4
		%	0	1.7	8.6	5.2	12.0	5.2	6.8	6.8
					p = 0.022					
	Increased pigment deposit	#	2	1	3	4	0	2	0	1
		%	3.5	1.7	5.2	7.0	0	3.3	0	1.7
	Focal necrosis	#	10	9	13	12	11	10	8	10
		%	17.2	15.0	22.4	21.1	19.0	16.7	13.6	17.2
	Diffuse necrosis	#	0	1	0	0	0	0	0	0
		%	0	1.7	0	0	0	0	3.4	0
	Single cell necrosis/degeneration	#	5	5	2	8	1	3	0	1
		%	8.6	8.3	3.5	14.0	1.7	5.0	0	1.7
	Total necrosis	#	15	15	15	20	12	13	8	11
		%	25.8	25.0	25.8	35.0	20.6	21.6	13.6	19.0
Gall bladder		#	58	60	58	57	58	60	59	58
	Amyloidosis	#	1	1	0	1	3	4	0	1
		%	1.8	1.8	0	1.9	5.4	6.8	0	1.9
	Dilation	#	2	1	3	0	5	5	6	10
		%	3.5	1.8	5.5	0	8.9	9.5	10.3	18.5
Urinary bladder			57	60	57	57	58	59	57	57
	Hemorrhage		4	1	0	1	0	0	0	0
			7.0	1.7	3.5	3.5	0	0	0	0
Ovaries		#					57	60	57	57
	Amyloidosis	#					11	17	5	9
		%					19.3	*28.3*	8.8	15.9
	Hemorrhage	#					4	10	10	8
		%					7.0	16.7	17.5	14.0
	Pigment deposits	#					0	0	**1**	**3**
		%					0	0	1.8	5.3
		p							p = 0.012	
Testes		#	57	60	58	57				
	Hemorrhage	#	0	1	1	0				
		%	0	1.7	1.7	10				
	Leydig cell hyperplasia, diffuse	#	17	17	21	19				
		%	29.9	28.3	36.7	33.0				
	Leydig cell hyperplasia, focal	#	1	0	1	1				
		%	1.8	0	1.7	1.8				

Mouse Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
All animals		Male				Female			
		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	60	60	60	60	60	60	60	60
Total Leydig cell hyperplasia	#	18	17	22	20				
	%	31.6	28.3	37.9	35.0				
Epididymides	#	58	60	58	57				
Hemorrhage	#	0	1	0	1				
	%	0	1.7	0	1.8				
Oligospermia	#	2	6	5	7				
	%	3.5	10.0	8.6	12.3				
Prostate	#	58	60	58	57				
Hemorrhage	#	1	0	0	0				
	%	1.8	0	0	0				
Lymphoid infiltrates	#	3	6	4	6				
	%	5.3	10.2	6.9	10.5				
Preputial gland	#	58	60	58	57				
Dilation/atrophy	#	43	43	**47**	**50**				
	%	74.1	71.7	81.0	87.7				
	p			p = 0.019					
Lungs	#	58	60	58	57	57	60	59	58
Alveolar hemorrhage	#	5	8	6	7	5	3	3	8
	%	8.6	13.3	10.3	12.3	8.8	5.0	5.1	
Pigment deposits	#	1	1	1	0	1	0	0	3
	%	1.7	1.7	1.7	0	1.8	0	0	5.2
Urinary bladder	#	57	60	57	57	58	59	57	57
Hemorrhage	#	4	1	2	2	0	0	0	0
	%	7.0	1.7	3.5	3.5	0	0	0	0
Stomach	#	57	60	58	55	57	60	56	58
Amyloidosis	#	11	13	5	2	9	15	5	7
	%	29.3	21.7	8.6	3.6	15.8	25.0	8.9	12.1
Hyperplasia, squamous cell	#	3	1	5	5	6	4	2	0
forestomach	%	5.3	1.7	8.6	9.1	10.5	6.7	3.6	0
Hyperplasia, fundic mucosal	#	11	18	20	14	8	12	10	12
	%	19.3	30.0	34.5	25.5	14.0	20.0	17.9	20.7
Pancreas	#	57	60	58	55	57	60	56	58
Amyloidosis	#	1	1	0	0	2	1	2	0
	%	1.9	1.7	0	0	3.5	1.7	3.5	0
Heart	#	58	60	58	57	58	60	59	58
Amyloidosis	#	10	12	5	3	6	7	4	6
	%	17.2	20.0	8.6	5.3	10.3	11.7	6.8	10.3
Cardiomyopathy	#	6	7	8	8	5	6	3	4
	%	10.3	11.7	13.8	14.0	8.6	10.0	5.1	6.9
Spleen	#	58	60	58	57	58	60	59	58
Amyloidosis	#	27	28	24	28	25	27	20	22
	%	46.6	46.7	41.4	49.1	43.1	45.0	33.9	37.9
Pigment deposits	#	0	2	2	1	0	1	1	1
	%	0	3.3	3.5	1.8	0	1.7	1.7	1.7
Increased hemopoiesis	#	6	13	**14***	10	10	11	14	9
	%	10.3	21.7	24.1	17.5	17.2	18.3	23.7	15.5
				P = 0.042					
All organs Hemorrhage/hematoma	#	10	9	9	6	13	13	14	11
	%	17.6	15.1	15.6	10.7	22.7	21.7	24.4	19.1
Pigment deposits	#	3	4	7	8	3	4	4	8
	%	5.2	6.7	12.1	14.1	5.2	6.7	6.9	13.9

Toxicokinetics

Blood samples were obtained and plasma was prepared from three satellite animals/sex/group at 0.5, 1, 2, 4, 7, and 24 hours after dosing on Days 1 and 361 of treatment. On Day 710, blood samples were obtained from three satellite animals/sex/group at 0.5 hour after dosing. After addition of an internal standard and protein precipitation with acetonitrile, analysis of BAY 59-7939 was performed using a validated LC-MS/MS assay with a lower limit of quantification of 0.002 mg/L. The

concentration of BAY 59-7939 in control animals was below the limit of quantification on all three sampling days.

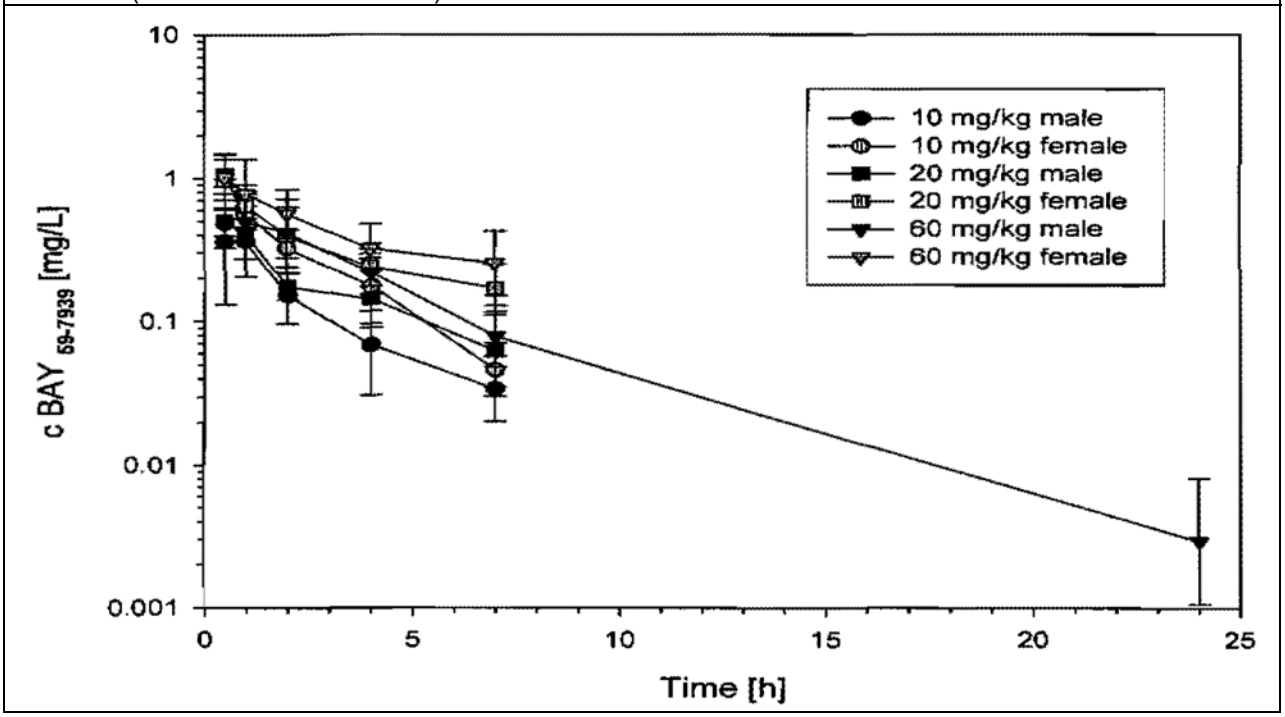
The t_{max} was 0.5 - 1 hour, indicating rapid absorption of BAY 59-7939. Exposure to BAY 59-7939 on Day 1 was only slightly lower in males than in females; however, exposure during Week 52 was lower in males by a factor of 0.5 to 0.8 (Table 12). Although exposure increased with dose in both males and females, the increase was less than dose-proportional. Exposure decreased with repeated dosing for 52 weeks by a factor of 0.3 to 0.5 in males and 0.65 to 0.7 in females. Plasma concentrations at 0.5 hour after dosing during Week 102 were not significantly different from plasma concentrations at 0.5 hour after dosing during Week 52. Since the human exposure at the highest recommended daily dose of 20 mg/day was 3.3 mg*hr/L, the exposure multiples for male and female mice were 0.76 and 1.28 times, respectively, the human exposure based on total AUC values. These multiples of the human exposure for male and female mice increase to 1.0 and 1.6 based on unbound AUC values.

Table 12: Reviewer's Summary of Toxicokinetics in Mice - Document PH-36243

Pharmacokinetic Parameters – Day 1 versus Week 52							
		Male			Female		
Dose (mg/kg)		10	20	60	10	20	60
Day 1							
$AUC_{(0-24)}$	mg*hr/L	2.47	4.95	4.79	2.61	NC	6.15
C_{max}	mg/L	0.806	1.25	1.38	1.13	1.28	2.33
t_{max}	hr	0.5	0.5	1.0	0.5	0.5	0.5
Week 52							
$AUC_{(0-24)}$	mg*hr/L	0.98	1.54	2.52	1.71	3.29	4.24
C_{max}	mg/L	0.363	0.503	1.09	0.568	0.963	1.02
t_{max}	hr	1.0	0.5	0.5	1.0	0.5	0.5
Plasma concentrations (mg/L) at 0.5 hour after drug administration							
		Male			Female		
Dose (mg/kg)		10	20	60	10	20	60
Day 1	Mean (SD)	0.826 (0.222)	1.26 (0.129)	1.29 (0.122)	1.16 (0.298)	1.34 (0.457)	2.35 (0.318)
Week 52	Mean SD	0.469 (0.313)	0.536 (0.237)	1.11 (0.241)	0.495 (0.110)	1.03 (0.495)	1.07 (0.404)
Week 102	Mean SD	0.321 (0.119)	0.446 (0.031)	0.809 (0.164)	0.739 (0.220)	0.706 (0.271)	0.956 (0.316)

Figure 4: Sponsor's Concentration-Time Profiles in Mice - Document PH-36243

Plasma concentrations of BAY 59-7939 versus time after oral administration to male and female mice in Week 52 (Geometric mean of n = 3)



Dosing Solution Analysis

Prior to the start of the study, test article formulations at concentrations above 6 mg/mL and below 1 mg/mL were shown to be homogenous and stable for 15 days at room temperature. During the study test article formulations were prepared as needed based on the 15 day stability. Analysis of the dose formulations on eleven days throughout the study showed the formulations were homogenous and the measured concentrations ranged from 84% to 114% of nominal (Table 13).

Table 13: Reviewer's Summary of Formulation Analysis - Document PH-36243

	Nominal concentration, mg/mL		
	1	2	6
Number	11	11	11
Mean recovery, %	101.2	103.5	101.1
SD	3.7	4.6	6.0
Maximum	107	114	107
Minimum	93	97	84

Study title: BAY 59-7939: Carcinogenicity Study in Wistar Rats (2 Years Administration by Gavage)

Document no.:	PH-36242
Study no.:	T8076429 (AT05916)
Study report location:	EDR, Module 4
Conducting laboratory and location:	Bayer Schering Pharma AG GDD-GED Toxicology, Wuppertal Germany
Date of study initiation:	September 6, 2006
GLP compliance:	Indicated
QA statement:	Present
Drug, lot #, and % purity:	BAY 59-7939 (rivaroxaban) a) Lot BXO23BS, purity 100% b) Lot BXA18UX, purity > 99.7%
CAC concurrence - protocol:	On August 1, 2006, the Executive CAC did not concur with the sponsor's proposed doses of (b) (4) mg/kg/day and instead recommended doses of 0, 10, 20, and 60 mg/kg/day by oral gavage, based on saturation of absorption. The Executive CAC meeting minutes are in Appendix 1
CAC concurrence – study results:	On April 15, 2011, the Executive CAC discussed the study results and concurred that the study was adequate and there were no clear drug-related neoplasms. The Executive CAC meeting minutes are in Appendix 2.

Key Study Findings

Introduction

Wistar rats received oral doses of BAY 59-7939 for up to 104 weeks. At dosages of 10, 20, and 60 mg/kg/day, the mean $AUC_{(0\ 24h)}$ was 13.4, 15.4, and 20.3 mg.hr/L in males and 34.7, 47.5, and 48.2 mg.hr/L in females, respectively, during week 54 of treatment.

Summary of Non-neoplastic Findings

Consistent with the pharmacodynamic action of BAY 59-7939, the mean values for thromboplastin time for all treated groups at 1 hour after dosing on all sampling days were significantly greater than those for the control groups. Likewise, the incidence of increased pigment deposition increased in some organs and across all organs in the high dose groups. The incidence of valvular fibrosis in the heart increased with dose in both males and females and the incidence was statistically significant in females ($p = 0.0048$) by the trend test.

Adequacy of Carcinogenicity Study

The rat carcinogenicity study used the doses (0, 10, 20 and 60 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.

Appropriateness of Test Model
The Wistar strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data are available. The most predominant form of BAY 59-7939 in both rat and human plasma was unchanged compound. The proposed metabolic pathway of BAY 59-7939 in rats and man is similar involving structural cleavage and hydroxylation, although a minor metabolite, M-7, is not formed in rats.

Summary of Tumor Findings
Squamous cell carcinoma was present in the clitoral gland of two high dose females. Statistical evaluations by the sponsor and the FDA statistician indicated p values in the trend test (pt) of 0.030 and 0.070, respectively. Neither p value attain the significance in the trend test ($p_t < 0.025$) required for this finding of a rare tumor to be considered positive, according to current CDER guidance. Additionally, squamous cell papilloma was present in both a control female and a high dose female. Although the incidence of squamous cell carcinoma was statistically significant (p = 0.03), squamous cell papilloma was present in both a control female and a high dose female. Therefore, statistical significance for squamous cell carcinoma plus papilloma is lacking.

Adrenal cortical adenomas were present only in treated animals with the incidence significantly higher in the mid and high dose females (p = 0.012, trend test). No adrenal adenocarcinoma was found in any group. Since adrenal cortical adenoma is a common tumor, the p value for females did not attain the significance in the trend test (p< 0.005) required for this common tumor to be considered positive.

Evaluation of Tumor Findings
The nonclinical and statistical reviewers concur with the sponsor that no significant evidence of neoplasia related to BAY 59-7939 treatment was observed in Wistar rats.

Methods

Doses:	0, 10, 20, and 60 mg/kg/day
Frequency of dosing:	Daily for up to 732 days
Dose volume:	10 mL/kg
Route of administration:	Orally by gavage
Formulation/Vehicle:	Ethanol/Sotutol HS 15/Tap Water (10/40/50 v/v/v)
Basis of dose selection:	A 13-week dose range finding study in the same strain of rats indicated that absorption of BAY 59-7939 saturated at 60 mg/kg
Species/Strain:	Rat (Rattus norvegicus)/(Hsd Cpb:WU, Wistar) [redacted] (b) (4)
Number/Sex/Group:	50
Age:	6-7 weeks at study initiation
Animal housing:	2-3 rats/cage
Paradigm for dietary restriction:	None; food was administered ad libitum
Dual control employed:	None

Interim sacrifice: None
Satellite groups: Yes, for clinical laboratory and toxicokinetic measurements
Deviation from study protocol: Not indicated

Observations and Results

Mortality

The animals were examined visually for mortality and morbidity twice daily, except on weekends and holidays when they were examined once daily.

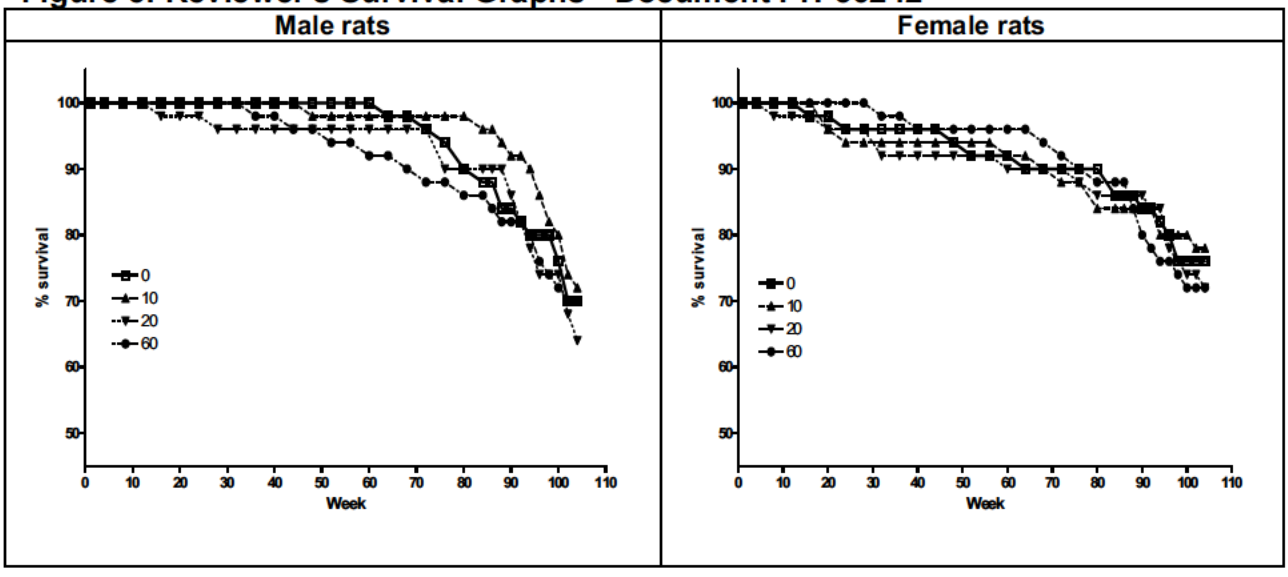
Mortality was not related to treatment (Table 14).

Table 14: Reviewer's Summary of Rat Mortality – Document PH-36242

	Males				Females			
Dose, mg/kg	**0**	**10**	**20**	**60**	**0**	**10**	**20**	**60**
Main animals								
Total number/group	50	50	50	50	50	50	50	50
Intercurrent deaths	15	14	19	16	12	11	14	14
% mortality	30	28	38	32	24	22	28	28
Satellite animals								
Total number/group	20	20	20	20	20	20	20	20
Total intercurrent deaths	5	12	5	9	4	10	7	6
% total mortality	25	60	25	45	20	50	35	30
Deaths during blood sampling	0	3	1	0	1	1	0	1

The sponsor did not provide Kaplan-Meier survival graphs. The reviewer's graphs (Figure 5) below indicate survival was reduced in the high dose male group compared to control male group during Weeks 40 to 80. The survival in the female groups was similar across all dose groups.

Figure 5: Reviewer's Survival Graphs - Document PH-36242



The pathologist noted that chronic progressive nephropathy contributed to mortality of the male decedents and gross lesions in the uterus or mammary gland contributed to the mortality of female decedents.

Clinical Signs
Detailed clinical examinations were made once before the start of treatment and once weekly in all groups during treatment.

The most frequent clinical signs included piloerection, hair loss, bloody eye, and palpable masses (Table 15). Although the incidence of main study animals with palpable masses was slightly higher in the mid and high dose male groups than the incidence in the control group, the incidence of satellite animals with palpable masses was slightly lower in the mid and high dose male groups than the incidence in the control group. Importantly, bleeding (general or vaginal) did not increase with dose.

Table 15: Reviewer's Summary of Notable Clinical Signs – Document PH-36242

	Cumulative number of animals in main groups (in satellite groups)							
	Male rats				Female rats			
Finding/Dose (mg/kg)	0	10	20	60	0	10	20	60
Number of animals	50 (20)	50 (20)	50 (20)	50 (20)	50 (20)	50 (20)	50 (20)	50 (20)
Piloerection	22 (5)	18 (4)	15 (2)	15 (6)	11 (1)	7 (2)	14 (3)	11 (3)
Hair loss	17 (4)	17 (5)	11 (7)	18 (7)	10 (11)	15 (9)	17 (10)	18 (7)
Bloody eye	7 (2)	5 (4)	7 (5)	5 (7)	8 (5)	7 (5)	5 (1)	7 (8)
Palpable masses	6 (5)	2 (5)	9 (2)	10 (2)	14 (4)	16 (9)	15 (8)	16 (8)

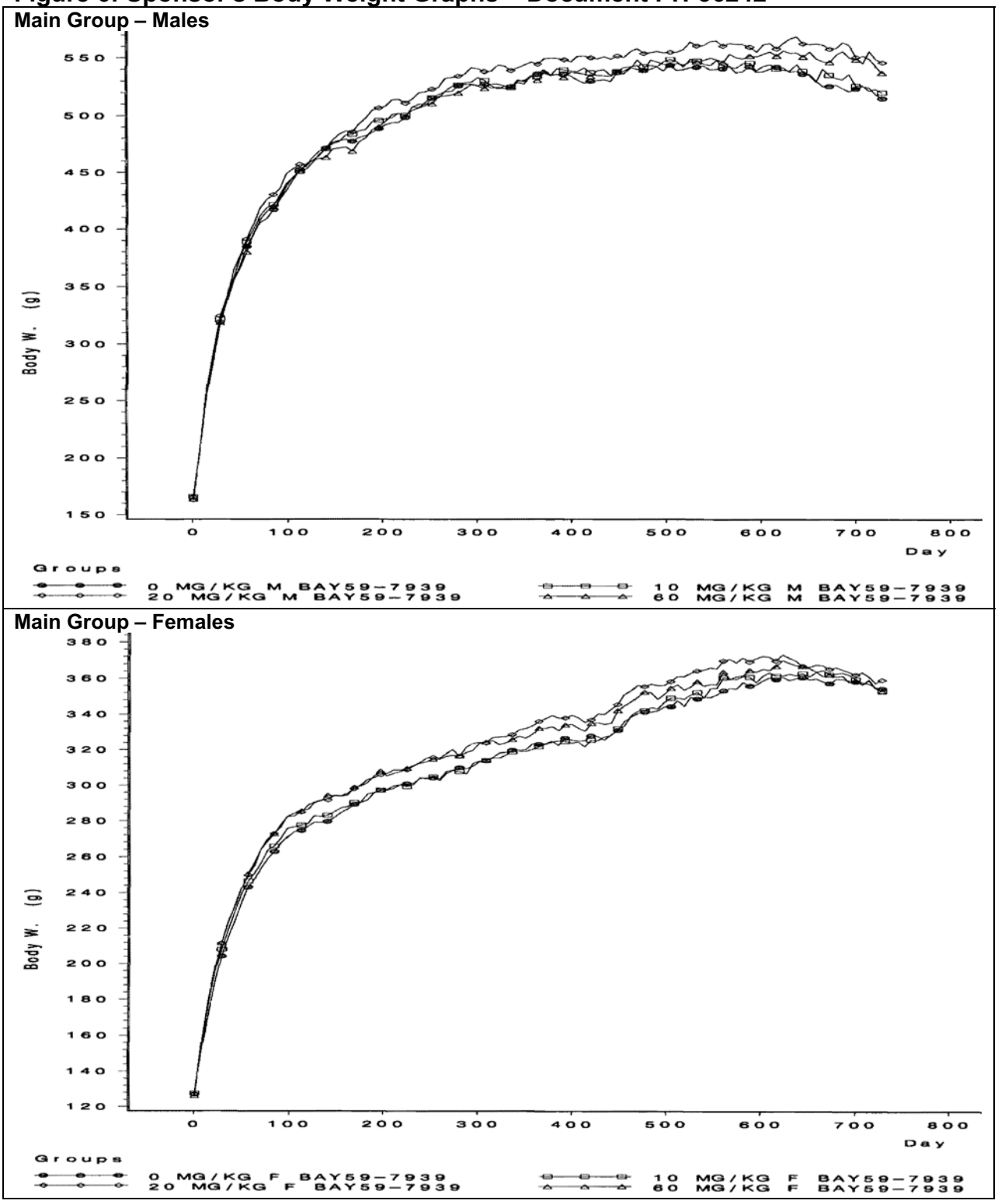
Body Weights
The animals in all groups were weighed on Day 1 of treatment and weekly up to scheduled necropsy and immediately before necropsy.

Body weight and body weight gain in the main study groups were not significantly affected by treatment with BAY 59-7939 (Table 16, Figure 6).

Table 16: Reviewer's Summary of Body Weights – Document PH-36242

Main Study	Dose (mg/kg)							
	Male rats				Female rats			
Day Dose (mg/kg)	0	10	20	60	0	10	20	60
Mean body weight (gm)								
1	165	165	163	164	127	127	127	126
92	426	427	435	430	268	270	276	278
183	482	487	497	480	292	295	303	301
274	521	525	533	518	308	308	320	317
365	536	533	544	534	323	322	336	332
456	537	535	550	541	336	333	351	344
547	539	543	564	548	349	354	365	356
631	538	543	568*	554	359	362	371	368
722	518	522	548	542	354	354	357	355
729	514	519	545	536	354	353	359	352
Body weight gain (gm)								
Day 1 – Day 183	317	322	334	316	165	168	176	175
Day 1 – Day 365	371	368	381	370	196	195	209	206
Day 1 – Day 729	349	354	382	372	227	226	232	226

Figure 6: Sponsor's Body Weight Graphs – Document PH-36242



Food and Water Consumption
Food and water consumption were determined weekly for individual main group animals.

The sponsor's summary tables (Table 17) indicate no treatment effect was observed for group mean food or water intake relative to the control group.

Table 17 : Sponsor's Summaries of Food and Water Intake – Document PH-36242

	Males					Females				
	Group means					Group means				
Dose mg/kg	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day
Food intake										
0	1-730	15732	21.6	32706	44.9	1-730	11733	16.1	38574	52.9
10	1-730	15612	21.4	32281	44.3	1-730	11605	15.9	37901	52.0
20	1-730	16052	22.0	32380	44.4	1-730	11922	16.4	37950	52.1
60	1-730	15528	21.3	31963	43.9	1-730	11847	16.3	37964	52.1
Water intake										
0	1-730	22936	31.5	47329	64.9	1-730	18674	25.6	60612	83.1
10	1-730	22907	31.4	47042	64.5	1-730	18819	25.8	60745	83.3
20	1-730	23941	32.8	48016	65.9	1-730	19259	26.4	60486	83.0
60	1-730	22927	31.5	47056	64.5	1-730	19407	26.6	61692	84.6

Reviewer's modification of sponsor's tables

Ophthalmology
The eyes of all main study animals were examined before treatment initiation. Only the eyes of control and high dose animals were examined during weeks 53/54, 79, and 103-105. After testing papillary reflex, the eyes were dilated and examined using an indirect ophthalmoscope and a photo slit-lamp.

No treatment related findings were observed for the ophthalmoscopic parameters.

Hematology
Blood samples for hematology were collected from 10 fasting satellite animals per group during weeks 27/28, 53/54, 79/80, and 103/104. The following parameters were measured: hematocrit, hemoglobin concentration, erythrocyte count, erythrocyte morphology, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, platelet count, total white cell count, and differential white cell count, including lymphocytes, eosinophils, monocytes, neutrophils, basophils, and atypical lymphocytes. Additional blood samples taken for measurement of thromboplastin time (Hepato-Quick, Roche) were collected from 10 satellite animals per group during weeks 51, 78, and 104 approximately 1 hour after drug administration. Samples for blood smears were collected satellite groups during weeks 54, 80, and 103 as well as from all animals killed in moribund condition and the surviving main study animals in the control and high dose groups near the end of the study.

Most of the statistically significant changes in means for red cell parameters in treated groups were less than 6% of the control means (Table 18) and individual values were within or close to the reference range. For example, statistically significant decreases in

erythrocytes, hemoglobin and hematocrit in the mid-dose group on Day 717 were attributed to Male 448, whose values (8.07, 140, and 0.418, respectively) are within the reference ranges for these parameters. Although none of the group means for hematology parameters for the high dose females on Day 716 were significantly different from the control means, the reviewer notes that the values for all hematology parameters for Female 544 are considerably outside the reference range. This female had the clinical sign of pallor from Day 715 to 729. At necropsy at study termination, she had pale liver and red nodules in her uterus. These findings and changes in hematology parameters are consistent with bleeding, a pharmacodynamic effect of rivaroxaban.

Table 18: Reviewer's Summary of Hematology Parameters – Document PH-36242

	Day	Dose Mg/kg	ERY 10e12/L	HB Gm/L	HCT L/L	MCV fL	MCH pg	MCHC Gm/L ERY	RETI %	Platelet 10e9/L
Males	185	0	9.33	157	0.466	50.0	16.8	337	13	1204
		10	9.01	156	0.452	50.2	17.4	**347***	13	1145
		20	8.93	155	0.452	50.7	17.3	342	14	1220
		60	8.96	155	0.451	50.4	17.3	**343***	14	1127
	367	0	8.88	154	0.452	51.0	17.4	341	12	1220
		10	8.92	156	0.455	51.1	17.5	343	13	1191
		20	8.81	154	0.452	51.3	17.5	341	13	1238
		60	9.00	155	0.453	50.3	17.2	342	13	1201
	550	0	9.12	156	0.494	54.3	17.2	316	14	1438
		10	8.68	153	0.478	55.1	17.6	320	16	1369
		20	8.69	151	0.472	54.4	17.4	320	14	1412
		60	8.94	153	0.477	53.4	17.1	320	14	1360
	717	0	9.01	157	0.469	52.2	17.5	336	14	1195
		10	8.75	156	0.461	52.8	17.9	339	14	1245
		20	**8.48***	**150***	**0.442***	52.2	17.6	338	13	1250
		60	8.65	153	0.452	52.2	17.7	339	14	1264
Reference range for Day 717		2SD+	10.09	171	0.517	56.8	18.4	346	26	1810
		2SD-	6.98	122	0.374	46.8	15.5	310	10	858
Female	184	0	8.74	156	0.47	53.8	17.8	332	15	1209
		10	8.57	154	0.46	53.7	18.0	335	16	1166
		20	8.51	154	0.462	54.3	18.1	334	17	1171
		60	8.49	152	**0.452***	53.3	18.0	**337***	16	1121
	366	0	8.03	152	0.442	55.0	19.0	345	18	1128
		10	7.91	149	0.434	54.9	18.8	343	17	1107
		20	7.86	150	0.432	55.0	19.1	347	18	1152
		60	8.04	150	0.433	54.0	18.7	347	16	1112
	548	0	8.16	151	0.475	58.2	18.6	319	19	1187
		10	8.05	149	0.457	56.9	18.5	**326***	16	1203
		20	8.03	153	0.464	57.9	19.0	**329***	17	1261
		60	7.96	149	**0.453***	57.0	18.7	**328***	16	1243
	716	0	7.68	148	0.436	57.1	19.4	340	24	1123
		10	7.70	148	0.428	55.6	19.2	346	17	1114
		20	7.77	150	0.427	55.1	19.3	**351***	18	1176
		60	7.24	139	0.407	57.0	19.3	340	45	1200

Reference range for Day 717	2SD+	9.16	163	0.493	59.4	19.7	350	29	1419
	2SD-	7.43	138	0.412	49.9	16.8	318	7	792
717	F544	**3.83**	**78**	**0.257**	**67.3**	**20.5**	**304**	**284**	**1644**
Remaining HD females	Max.	8.82	154	0.451	58.8	19.9	351	24	1267
	Min.	7.1	138	0.404	51.2	18.6	338	13	1056

* $p < 0.05$, HD = high dose, ERY = erythrocytes, HB = hemoglobin concentration, HCT = hematocrit, MCV = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration, RETI = % reticulocytes, 2SD+ = 2 standard deviations above the mean, 2SD = 2 standard deviations below the mean

Since blood samples for measurement of coagulation times were collected 1 hour after dosing, the values for thromboplastin time were expected to indicate prolonged coagulation times. The mean values for thromboplastin time for all treated groups on all sampling days were significantly greater (1.5 to 1.9-fold in males, 1.8 to 2.5-fold in females) than those for the control groups (Table 19). Individual values of all treated animals, except one (low dose female 507 on Day 723), were greater than three standard deviations above the reference mean. Dose dependence is more evident in the females compared to the males. However, the reviewer notes that the mean value and all individual values for control males on Day 192 were greater than three standard deviations above the reference mean. Individual values for other control males and females were also greater than three standard deviations above the reference mean. These included control males on Day 375, control males on Day 724, control females on Day 191 and one control female on Day 723.

Table 19: Reviewer's Summary of Thromboplastin Times – Document PH-36242

		Dose	HQUICK, sec			Reference range		Number	
	Day	Mg/kg	Mean	Min.	Max.	3SD-	3SD+	< 3SD+[†]	>3SD+[‡]
Males	192	0	44.9	**42.8**	**49.1**	26.8	41.5	0	10
		10	**71.9***	53.5	86.4			0	10
		20	**71.3***	56.9	86.6			0	10
		60	**82.4***	56.7	105.8			0	10
	375	0	39.3	35.8	**43.7**	24.3	39.2	7	3
		10	**61.8***	46.2	69.9			0	10
		20	**63.1***	49	84.6			0	10
		60	**74.7***	52	109.7			0	10
	556	0	38.7	33.4	42.6	18.8	47.2	10	0
		10	**68.3***	51.9	88.1			0	8[§]
		20	**66.3***	49.9	88.9			0	10
		60	**73.5***	57.2	95.6			0	10
	724	0	39.1	33.6	**43.9**	22.5	39.6	5	5
		10	**63.7***	49.7	88.8			0	10
		20	**67.5***	50.8	81.6			0	10
		60	**68.7***	61.6	95.5			0	10

	Day	Dose Mg/kg	HQUICK, sec Mean	Min.	Max.	Reference range 3SD-	3SD+	Number < 3SD+[†]	>3SD+[‡]
Females	191	0	37.9	34.3	**41.1**	25.2	40.6	8	2
		10	**70.4***	56.6	86.2			0	10
		20	**72.2***	57.8	87.2			0	10
		60	**86.5***	72.9	103.4			0	10
	373	0	34.1	30.2	36.3	23.3	36.4	10	0
		10	**61.8***	52.6	81.1			0	10
		20	**72.3***	59.9	81.3			0	10
		60	**85.6***	69.6	95.8			0	10
	555	0	36.8	33.3	38.9	21.1	42.4	10	0
		10	**70.8***	55.9	83.9			0	10
		20	**77.5***	70	85.6			0	10
		60	**88.3***	72.6	101.5			0	10
	723	0	36.1	33.1	**38.3**	23.4	37.6	9	1
		10	**66.2***	**36.9**	81.9			1	9
		20	**70.1***	57.3	85.6			0	10
		60	**79.4***	59.9	104			0	10

[†] Number of values <3 standard deviations above mean, [‡] Number of values > 3 standard deviations above mean, [§] Only 8 animals

Clinical Chemistry

Blood samples for clinical chemistry were collected from 10 fasting satellite animals per group during weeks 27, 53, 79, and 103. The following parameters were measured: alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase, glucose, total bilirubin, total cholesterol, triglycerides, creatinine, urea, total protein, sodium, potassium, chloride, calcium, and inorganic phosphate.

The findings are summarized in the sponsor's tables below (Table 20, Table 21). No significant changes were observed in plasma enzyme activities, including ALT or AST. Glucose, urea, total protein, and sodium are parameters for which a treated group exhibited a statistically significant difference from the concurrent control group. For these parameters, no dose or time-dependence was observed and values for individual animals were within the sponsor's reference ranges (± 2 SD) or within the range of concurrent control values.

However, other parameters (chloride, phosphate, and potassium) with a statistically significant difference from the concurrent control group had individual values outside the reference range. On Day 717, the high dose male group exhibited statistically significant higher chloride concentration than the control males Although five of ten high dose males had individual values above the reference range (94-102), only one had a value (106) that was more than 7% above the mean of the reference range. Furthermore, this increase was not observed in the high dose female group.

The high dose male group on Days 367 and 717 also showed a statistically significant decrease in phosphate. However, only one high dose male had a phosphate value (1.12) below the reference range (1.19 – 1.93) and all individual values for females were within the reference range.

Statistically significant decreases in potassium were observed in the high dose males on Day 550 and mid- and high dose females on Day 548. Table 22 summarizes these results with respect to the time-matched reference range and individual animal values. On Day 550, not only were the values for six high dose males less than two standard deviations below the reference mean, but also the mean potassium value for the high dose male group was less than two standard deviations below the reference mean. Interestingly, the values for six low dose males were also less than two standard deviations below the reference mean, but all values for control males were within the reference range. In contrast, the values for individual mid- and high dose females on Day 548 were within the reference range, but the values for five control females were above two standard deviations above the reference mean. Additionally, although no statistically significant difference was observed among the male groups on Day 717, one, three and two individual values in the control, mid, and high dose groups, respectively, were less than two standard deviations below the reference mean. Furthermore, values for two control males (401 and 405) of 9.2 and 7.1 mmole/L, respectively, were greater than three standard deviations above the reference mean. No explanation was provided for these excessive values for control animals, although hemolysis of these control samples is a potential explanation.

Table 20: Sponsor's Summary of Clinical Chemistry - A – Document PH-36242

Dose mg/kg	GLUCOSE mmol/l	CHOL mmol/l	TRIGL mmol/l	CREA mcmol/l	UREA mmol/l	BILIt mcmol/l	PROT g/l	ALBUMIN g/l
m	**Day 185**							
0	3.18	2.21	0.82	57	6.29	2.1	71.8	35.7
10	3.01	2.30	0.98	54	5.23 ++	1.9	69.3	35.9
20	3.08	2.19	1.00	55	5.77	1.8	69.4	35.9
60	3.19	2.21	0.88	55	5.57	1.9	69.9	36.6
m	**Day 367**							
0	3.48	2.87	1.34	56	6.03	1.7	70.9	32.8
10	3.36	2.84	1.50	54	5.57	1.8	69.0	33.1
20	3.53	2.78	1.38	55	6.19	1.5	70.2	33.2
60	3.57	3.09	1.54	54	5.89	1.7	72.0	34.1
m	**Day 550**							
0	3.85	3.71	2.13	59	7.61	1.9	73.3	32.7
10	3.66	3.39	1.77	57	6.73	1.7	69.7 +	32.9
20	3.66	3.35	1.57	56	6.74	1.7	70.3	33.3
60	3.96	3.83	1.88	59	7.59	2.0	72.8	34.1
m	**Day 717**							
0	3.44	3.96	1.89	60	7.24	2.1	70.5	33.1
10	3.59	3.58	2.01	55	6.14	1.9	69.1	33.3
20	3.46	3.71	2.24	59	7.11	1.8	69.4	32.6
60	3.53	4.26	2.40	58	6.56	2.0	72.2	33.7
f	**Day 184**							
0	3.54	2.08	1.11	56	6.53	2.4	72.5	40.9
10	3.30	1.75	0.86	56	6.56	2.3	70.8	40.5
20	3.27 +	2.11	0.93	56	7.06	2.2	72.4	41.6
60	3.33	1.66	0.94	61	7.61	2.0	70.1	39.6
f	**Day 366**							
0	3.31	2.01	1.51	57	6.46	1.8	73.2	38.3
10	3.13	1.86	1.33	57	6.69	1.6	70.5	37.3
20	3.25	1.97	1.50	56	6.94	1.7	71.5	38.0
60	3.37	1.71	1.55	56	6.63	1.9	71.3	37.4
f	**Day 548**							
0	3.96	2.28	1.52	54	6.27	2.2	75.1	37.7
10	3.64	2.18	1.28	53	6.16	1.9	75.3	39.1
20	3.63 +	2.22	1.49	52	6.86	2.2	74.2	39.2
60	3.80	2.22	1.55	55	6.80	2.3	75.1	39.2
f	**Day 716**							
0	4.07	2.55	2.16	57	6.30	1.7	77.8	38.2
10	3.69	2.81	2.23	55	6.31	2.2	76.4	38.6
20	3.80	2.69	2.78	55	6.31	1.8	75.3	37.5
60	3.93	2.71	3.41	56	6.31	1.8	77.0	38.0

Table 21: Sponsor's Summary of Clinical Chemistry - Part B - Document PH-36242

Dose mg/kg	ASAT (GOT) U/l	ALAT (GPT) U/l	APh U/l	GLDH U/l	GGT U/l
m	Day 185				
0	66.4	58.6	67	8.8	1
10	64.8	61.1	63	8.8	1
20	64.0	67.1	60	8.6	1
60	62.0	51.1	63	8.5	1
m	Day 367				
0	72.3	62.4	62	15.4	0
10	74.5	72.2	62	14.6	1
20	59.9	59.1	57	11.8	0
60	58.9	58.2	57	12.7	1
m	Day 550				
0	80.1	53.5	60	14.4	3
10	65.7	46.2	57	17.5	2
20	57.7	48.1	58	12.4	3
60	60.0	53.1	59	10.1	3
m	Day 717				
0	75.7	58.7	67	8.9	3
10	67.7	62.8	62	12.4	3
20	55.2 +	61.3	61	7.4	4
60	59.3	67.5	61	7.4	4
f	Day 184				
0	62.1	47.8	34	7.2	0
10	66.4	46.3	33	4.0	0
20	64.6	44.0	32	6.0	0
60	68.6	49.1	35	14.2	1
f	Day 366				
0	75.6	55.5	33	15.1	1
10	66.0	48.6	24	9.4	1
20	62.0	51.4	27	9.5	0
60	75.2	56.3	31	15.4	1
f	Day 548				
0	81.8	47.0	31	19.8	0
10	83.4	49.0	29	15.5	0
20	82.6	44.1	27	12.7	0
60	78.8	43.2	25	15.9	0
f	Day 716				
0	80.5	47.3	33	20.5	0
10	72.4	50.6	29	11.7	0
20	60.8	50.0	31	8.2	0
60	67.3	49.5	27	18.2	0

Dose mg/kg	Na mmol/l	K mmol/l	Cl mmol/l	Ca mmol/l	P mmol/l
m	Day 185				
0	144	5.0	97	2.45	1.70
10	143	4.9	98	2.42	1.64
20	143	4.9	98	2.42	1.73
60	143	4.7	97	2.46	1.77
m	Day 367				
0	144	4.8	97	2.44	1.69
10	143	5.0	96	2.43	1.57
20	144	4.9	97	2.43	1.53
60	143	4.9	98	2.47	1.52 +
m	Day 550				
0	141	5.0	99	2.44	1.44
10	141	4.6	98	2.41	1.50
20	140	4.8	99	2.43	1.49
60	140	4.4 ++	99	2.47	1.47
m	Day 717				
0	144	5.6	99	2.54	1.59
10	142	4.9	97	2.51	1.47
20	143	4.5	99	2.55	1.47
60	142 ++	4.6	102 ++	2.57	1.33 ++
f	Day 184				
0	142	4.4	100	2.52	1.43
10	142	4.2	100	2.45	1.28
20	142	4.2	100	2.49	1.35
60	141 +	4.2	100	2.45	1.30
f	Day 366				
0	142	4.4	97	2.49	1.30
10	141	4.3	97	2.48	1.26
20	141 ++	4.5	96	2.54	1.41
60	141 ++	4.4	98	2.49	1.26
f	Day 548				
0	139	4.8	98	2.43	1.12
10	139	4.4	99	2.44	1.00
20	139	4.3 ++	99	2.48	1.19
60	139	4.2 ++	97	2.50	1.16
f	Day 716				
0	140	4.4	97	2.53	1.12
10	140	4.4	98	2.53	1.14
20	139 +	4.3	96	2.57	1.20
60	140	4.3	98	2.59	1.19

\+ significantly different at $p \leq 0.05$

++ significantly different at $p \leq 0.01$

Table 22: Reviewer's Summary of Serum Potassium Values - Document PH-36242

		Dose	K, mmole/L			Reference range		Number	
	Day	Mg/kg	Mean	Min.	Max.	2SD-	2SD+	< 2SD-[†]	>2SD+[‡]
Males	185	0	5.0	4.4	**5.7**	4.3	5.6	0	1
		10	4.9	4.6	5.2			0	0
		20	4.9	4.4	5.5			0	0
		60	4.7	4.3	5.2			0	0
	367	0	4.8	4.6	5.0	4.4	5.5	0	0
		10	5.0	4.5	**5.6**			0	1
		20	4.9	**4.3**	5.4			2	0
		60	4.9	4.4	5.5			0	0
	550	0	5.0	4.6	5.7	4.5	5.8	0	0
		10	4.6	**3.8**	5.8			**6**	0
		20	4.8	4.4	5.4			0	0
		60	**4.4***	**3.8**	5.0			**6**	0
	717	0	5.6 [5.0]	**4.1**	**9.2** [5.5]	4.3	5.9	1	2 [§]
		10	4.9	4.5	5.6			0	0
		20	4.5	**4.0**	5.0			**3**	0
		60	4.6	**4.0**	4.9			**2**	0
Females	184	0	4.4	3.9	5.0	3.7	5.1	0	0
		10	4.2	3.7	4.6			0	0
		20	4.2	3.9	4.6			0	0
		60	4.2	**3.5**	4.6			1	0
	366	0	4.4	4.0	4.8	3.5	5.0	0	0
		10	4.3	3.8	4.7			0	0
		20	4.5	4.0	4.7			0	0
		60	4.4	3.8	4.7			0	0
	548	0	4.8	4.4	**5.2**	3.5	4.9	0	5
		10	4.4	3.8	**6.2**			0	1
		20	**4.3***	3.8	4.6			0	0
		60	**4.3***	3.8	4.6			0	0
	716	0	4.4	3.9	**5.3**	3.4	5.0	0	1
		10	4.4	4.1	4.8			0	0
		20	4.3	3.9	4.9			0	0
		60	4.3	4.1	4.8			0	0

[†] Number of values < 2 standard deviations below mean, [‡] Number of values >2 standard deviations above mean, [§] Control males 401 and 405 had values of 9.2 and 7.1, respectively. [ ] Values omitting males 401 and 405.

Urinalysis

Urine samples were collected for a period of 16 hours from 10 fasting satellite animals per group during weeks 26, 52, 78, and 102. The following parameters were measured: volume, density, pH, blood, bilirubin, protein, glucose, ketone bodies, and urobilinogen. The urine sediment was examined microscopically for epithelial cells, leucocytes, erythrocytes, bacteria, amorphous salts, triple phosphate crystals, and other abnormal components.

Although the low and high dose males on Day 180 showed a statistically significant decrease in urine density compared to the density for the control group, urinalysis

parameters were considered to be unaffected by treatment, because all individual values were within the reference range.

Gross Pathology

The surviving satellite animals were sacrificed for scheduled necropsy during week 105. The surviving main study animals were sacrificed for scheduled necropsy during weeks 105-107. 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 23 were fixed in 10% neutral buffered formalin. The urinary bladder and lungs were initially inflated with 10% neutral buffered formalin prior to fixation by immersion.

Table 23: Reviewer's Summary of Tissues Collected - Document PH-36242

Abnormal tissues	Kidneys	Seminal vesicles with coagulating glands
Adrenals	Larynx	Skeletal muscle - thigh
Aorta	Liver	Skin (mammary area)
Brain (cerebrum, cerebellum, brain stem)	Lungs	Spinal cord (cervical, thoracic, lumbar)
Cecum	Lymph nodes (mandibular – mesenteric, popliteal)	Spleen
Clitoral gland	Nasal cavity/nasopharynx	Sternum with bone marrow
Colon	Optic nerves	Stomach
Duodenum	Ovaries with oviduct	Testes
Epididymides	Pancreas	Thymus
Esophagus	Peyers patches	Thyroid with parathyroids
Eyes and eyelids	Pharynx	Tongue
Extraorbital lacrimal glands	Pituitary	Trachea
Femur with joint	Preputial gland	Ureters
Harderian glands	Prostate	Urethra
Head with skull cap	Rectum	Urinary bladder
Heart	Salivary glands (submandibular, sublingual and parotid)	Uterus with cervix
Ileum	Sciatic nerve	Vagina
Jejunum		Zymbal's glands

The pathology report commented on kidney discoloration, nodules in the pituitary and preputial glands, and cysts in the ovary. A decreased incidence of kidney discoloration was found in the mid and high dose males. However, the reviewer notes that discoloration of the adrenal glands was present in a few treated animals from all dose groups, but not in the control group (Table 24). In contrast, discoloration in the liver was similar across groups and discoloration in the lungs was slightly decreased in the mid and high dose females. Although lung discoloration occurred primarily in decedents, discoloration in the other tissues did not.

The pathologist noted the absence of nodules of the pituitary glands of high dose females and the increased incidence of nodules in the preputial glands of high dose males. The reviewer also noted the decreased incidence of nodules in uteri of the treated female groups. In contrast, the incidence of nodules in the skin was increased in the high dose females and the mid and high dose males. However, the incidence of nodules in other tissues was generally evenly distributed across groups.

The pathologist noted a decreased incidence of ovarian cysts in the high dose females. The reviewer noted the incidence of cysts in other tissues, exemplified by the liver and kidney, was generally similar across groups.

Table 24: Reviewer's Summary of Gross Pathology - Document PH-36242

		Male				Female			
	Dose, mg/kg	0	10	20	60	0	10	20	60
Finding	Number (decedents)	50 (5)	50 (12)	50 (5)	50 (9)	50 (4)	50 (10)	50 (7)	50 (6)
Discoloration	Kidney	9 (3)	8 (4)	**3 (1)**	**2 (0)**	2 (1)	2 (1)	3 (2)	2 (2)
	Adrenal glands	0	2 (0)	1 (0)	2 (1)	0	1 (0)	2 (0)	1 (0)
	Lungs	9 (8)	7 (6)	10 (8)	8 (8)	6 (5)	5 (5)	3 (3)	3 (3)
	Liver	7 (3)	9 (1)	6 (0)	6 (2)	6 (2)	12 (3)	3 (2)	10 (3)
Nodules	Preputial glands	1 (0)	0	0	**4 (1)**	-	-	-	-
	Pituitary	0	0	0	1 (0)	6 (1)	5 (1)	7 (3)	**0**
	Lung	0	0	1 (1)	3 (2)	0	1 (0)	0	1 (1)
	Liver	2 (1)	1 (0)	0	1 (0)	2 (1)	0	0	2 (0)
	Pancreas	0	2 (0)	0	1 (0)	0	0	0	0
	Kidney	1 (1)	1 (0)	0	0	0	1 (0)	1 (0)	2 (2)
	Uterus	-	-	-	-	16 (4)	**9 (4)**	**10 (2)**	**11 (3)**
	Adrenal glands	3 (0)	4 (2)	5 (2)	2 (0)	1 (0)	2 (1)	3 (1)	2 (1)
	Skin	3 (1)	0	**6 (2)**	**6 (3)**	6 (1)	9 (3)	5 (2)	**11 (5)**
Cyst	Ovaries	-	-	-	-	6 (0)	6 (2)	7 (0)	**2 (1)**
	Liver	5 (0)	3 (0)	1 (1)	3 (0)	4 (1)	10 (1)	5 (1)	6 (2)
	Kidney	7(2)	4(2)	6(0)	5(2)	1(0)	0	0	0
Enlarged	Liver	1 (0)	5 (3)	4 (2)	1 (0)	2 (1)	1 (0)	0	0
Decreased size	Testes	1 (0)	1 (0)	4 (0)	2 (0)	-	-	-	-

Organ Weights

The following organs were weighed before fixation: adrenals, brain, kidneys, liver, spleen, and testes. The methods indicate that the weights of organs exhibiting a severe pathological alteration (e.g. nodule, tumor, cyst) were excluded from the calculation of the mean value, if the individual weight was at least three times higher than the median value of the respective group. The rationale for these criteria was not provided.

Most of these exclusions were for male adrenal weights, which had a 32 to 85 fold intra-group variation consistent with the high overall incidence of pheochromocytoma in male adrenal glands. Intra-group variation of female adrenal weights was less (6 to 28 fold) and fewer values were excluded.

The testes weight (12006 mg) for low dose male 53 was excluded, since the testes had nodules and Leydig cell tumor. However, testes weight (10110 mg) for control male 23 was not excluded, although those testes also had Leydig cell tumor. The low testes weight for mid-dose male 103 (812 mg) was included and was attributable to severe (Grade 5) atrophy of the testes and other sexual organs. Likewise, the low testes weight for mid-dose male 111 (1752 mg) was included and was attributable to severe (Grade 5) atrophy of the testes.

No absolute kidney weights were excluded. Although no significant difference in mean absolute kidney weights was observed for either males or females, the high dose males showed a statistically significant decrease in mean relative kidney weight (Table 25). The mean relative kidney weight in the low and mid-dose males was also decreased, but was not statistically significant. The decreases in mean relative kidney weight in male treated groups correlated best with combined incidence of hyperplasia in the kidney.

Although mean absolute and relative spleen weights for female treated groups were lower than those in the control group, the differences were not statistically significant and a clear dose relationship was not evident (Table 26). The spleen absolute weight for control female 244 (17028 mg, 26 times the group median of 652 mg) was excluded even though severe pathology was not noted, probably because this value was a clear outlier. However, the spleen absolute weight for control female 207 (3582 mg, 5.5 times the group median of 652 mg) was included even though this organ was noted as being enlarged at necropsy, because the enlargement was consistent with markedly (grade 4) increased hematopoiesis found microscopically, not the presence of a nodule, tumor, or cyst. Similarly, the spleen absolute weight for high dose female 382 (2411 mg, 3.6 times median of 670 mg) was included, even though this spleen was noted as being enlarged at necropsy, because the enlargement was consistent with moderate (grade 3) increased hematopoiesis found microscopically. If values for females 207 and 382 are omitted, the decrease in mean female spleen weights is no longer evident.

In contrast, statistically significant decreases in absolute spleen weight were found for the low and mid-dose males (15%) and decreases in relative spleen weight were found for all treated male groups (16%, 20% and 14%). However, the sponsor discounted these changes because they were considered small and lacking in a dose relationship. The spleen of high dose male 165 (2549 mg, 2.8 times group median of 912 mg) was included even though nodules (0.8 cm diameter) were found at necropsy and lymphoma grade 3 was found microscopically. Likewise, the spleen of high dose male 175 (1807 mg, 2 times group median of 912 mg) was included even though nodules were found at necropsy and a grade 4 histocytic sarcomatous infiltrate was found microscopically. Omission of the values for these two high dose males results in a dose-dependent decrease spleen absolute and relative weights in the males. However, this decrease did not correlate with a specific histopathological finding.

Table 25: Sponsor's Summaries of Organ Weights - Document PH-36242

Absolute organ weights

Dose mg/kg	Body W. G	Brain mg	Adrenals mg	Liver mg	Spleen mg	Kidneys mg	Testes mg
m							
0	512	2189	73	19851	1095	3856	3744
10	517	2180	77	18757	928 ++	3667	3558
20	546	2182	86	20183	926 ++	3856	3414
60	546	2168	70	19824	991	3725	3672
f							
0	352	1998	69	13981	772	2708	
10	353	2042	73	13627	692	2706	
20	355	2003	73	13425	665	2711	
60	354	2008	74	13046	723	2662	

Relative organ weights

Dose	Body W.	Brain	Adrenals	Liver	Spleen	Kidneys	Testes
mg/kg	G	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
m							
0	512	431	15	3882	215	759	731
10	517	426	15	3634	180 ++	717	695
20	546	407	16	3734	171 ++	727	630 ++
60	546	401 +	13	3649	185 ++	690 +	676
f							
0	352	571	20	3982	221	772	
10	353	582	21	3857	197	770	
20	355	570	20	3798	188	767	
60	354	573	21	3690	205	754	

+ significantly different at $p \leq 0.05$ ++ significantly different at $p \leq 0.01$

Table 26: Reviewer's Summary of Spleen Weights - Document PH-36242

	Dose	Spleen Absolute Weight, mg				Spleen Relative, mg/100 gm			
	mg/kg	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Males	0	1095	202	727	1446	215	43	153	319
	10	928*	164	549	1423	180*	29	127	236
	20	926*	156	477	1222	171*	30	109	236
	60	991	336	676	2549	185*	72	113	522
	60, omit M165 & M175	917*	121	676	1160	169*	28	113	212
Females	0	772	499	520	3582	221	152	143	1093
	10	692	120	490	981	197	38	144	312
	20	665	113	456	1003	188	30	137	235
	60	723	317	482	2411	205	90	138	691
	0, omit F207	694	162	520	1267	197	42	138	294
	60, omit F382	675	113	482	1046	191	30	138	296

Histopathology

Tissue samples from all main study animals were dehydrated, embedded in Paraplast, sectioned, and stained with hematoxylin and eosin. All tissues listed in Table 23 and gross abnormalities identified at macroscopic examination from all main animals sacrificed at the end of the scheduled treatment period and from all main animals killed or dying during the study were examined by histology. Tissues of the satellite groups were not examined microscopically.

Peer Review

The peer review included examination of all tumors and pre-neoplastic/hyperplastic lesions of all groups. In addition, approximately 25% of frequent lesions and all slides of 5 animals per sex from the high dose group were also examined.

Neoplastic lesions

The incidences of the most notable tumors in the rat carcinogenicity study are summarized in Table 27 below. The sponsor's listing of tumor incidences is in Appendix

6. The statistical evaluations of the sponsor and the FDA statistician are in Appendix 7 and 8, respectively. Historical control data provided by the sponsor are in Appendix 9.

Squamous cell carcinoma was present in the clitoral gland of two high dose females. Statistical evaluations by the sponsor and the FDA statistician indicated p values in the trend test (p_t) of 0.030 and 0.070, respectively. Neither p value attains the significance in the trend test ($p_t < 0.025$) required for this finding of a rare tumor to be considered positive, according to current CDER guidance. Additionally, squamous cell papilloma was present in both a control female and a high dose female. Therefore, statistical significance for squamous cell carcinoma plus papilloma is also lacking. Furthermore, squamous cell hyperplasia in the clitorial gland was present in females of the control, low and mid-dose groups, but not the high dose group further confirming the lack of a treatment relationship.

Adrenal cortical adenomas were present only in treated animals with the incidence significantly higher in the mid and high dose females (sponsor's pt = 0.0126). However, no adrenal adenocarcinoma was found in any group. The FDA statistician's evaluation indicated a p value for the trend test of 0.041. Based on a mean background incidence of 3.5% in the RITA database, adrenal cortical adenoma is a common tumor. Therefore, neither p value for females attained the significance in the trend test ($p < 0.005$) required for this finding to be considered positive, according to current CDER guidance. In addition, cortical adrenal hyperplasia (zona fasciculata and zona glomerulosa) did not show a dose relationship.

The incidence of benign and malignant pheochromocytoma in the adrenal medulla was higher in males than in females. The incidence in males was similar across all groups. However, the incidence in females slightly increased in the treated groups, but was without statistical significance. In addition, focal medullary hyperplasia was similar across control and treated groups in both males and females.

The incidence of adenoma and adenocarcinoma in the mammary gland of females did not show a positive dose relationship. However, the incidence of fibroadenoma increased in the high dose females. The sponsor's statistical analysis for fibroadenoma indicated a p value of 0.0526 for the trend test. The FDA statistician's analysis indicated a p value of 0.031 for the pairwise test and 0.062 for the trend test. Based on a mean background incidence of 14% in the RITA database, mammary fibroadenoma in female rats is a common tumor. Therefore, the p value for fibroadenoma in females did not attain the significance in the trend test ($p< 0.005$) required for this finding of a common tumor to be considered positive. Even if fibroadenomas and fibromas are combined, the p value would not attain the required significance level. Furthermore, hyperplasia in the mammary gland did not show a positive dose relationship.

The sponsor's evaluation indicated that histiocytic sarcoma (pt = 0.0268) and skin fibroma ($p_t = 0.0294$) were statistically significant in males. The FDA statistician's evaluation indicated p values in the trend test of 0.056 and 0.18 for histiocytic sarcoma and skin fibroma, respectively. Based on the Charles River (2011) listing of spontaneous tumors in Wistar rats, the overall incidences of histiocytic sarcoma and skin fibroma are 0.74% and 0.41%, respectively. Neither p value attains the

significance in the trend test ($p_t < 0.025$) required for these findings of rare tumors to be considered positive, according to current CDER guidance.

In contrast to the mouse carcinogenicity study in which hepatocellular tumors in males increased with dose, only two hepatocellular tumors were observed in rats, one in a low dose male and another in a high dose female. Leydig cell tumors in rats decreased with dose in contrast to an increase in Leydig cell tumors in mice.

Table 27: Reviewer's Summary – Neoplastic Lesions - Document PH-36242

Rat Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
Neoplastic Findings		Male				Female			
All main study animals		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	50	50	50	50	50	50	50	50
Liver	#	50	50	50	49	50	50	50	50
Hepatocellular adenoma B	#	0	1	0	0	0	0	0	1
(CR max.: M 17.5%, F 9.2%)	%	0	2	0	0	0	0	0	2
Pancreas	#	49	50	50	49	50	50	50	50
Acinar adenoma B	#	0	0	2	1	0	1	0	1
(CR max.: M 1.8%)	%	0	0	4	2	0	2	0	2
Acinar adenocarcinoma M	#	0	1	0	0	0	0	0	0
(CR max.: M 1.5%)	%	0	2	0	0	0	0	0	0
Combined acinar adenoma/carcinoma	#	0	1	2	1	0	1	0	1
	%	0	2	4	2	0	2	0	2
Islet cell adenoma	#	0	1	1	1	0	0	1	0
(CR max.: M 7.0%, F 2.0%)	%	0	2	2	2	0	0	2	0
Testes		50	50	50	49	0	0	0	0
Leydig cell tumor B	#	6	6	3	3				
(CR max.: M 6.7%)	%	12	12	6	6				
Clitorial glands	#					43	40	42	45
Squamous cell carcinoma M (p = 0.03)	#					0	0	0	**2***
	%					0	0	0	4.4
Squamous cell papilloma B	#					1	0	0	1
	%					2.3	0	0	2.2
Squamous cell carcinoma + papilloma	#					1	0	0	3
	%					2.3	0	0	6.6
Basal cell tumor B	#					0	0	1	0
	%					0	0	2.4	0
Basal cell carcinoma M	#					1	0	2	0
	%					2.3	0	2.4	0
Adenoma B	#					0	0	1	0
	%					0	0	1.2	0
Adenocarcinoma M	#					0	1	0	0
	%					0	2.5	0	0
Pituitary gland	#	50	49	48	49	50	50	50	50
Adenoma pars distalis B	#	3	7	6	5	9	7	13	3
(CR max.: M 37.3%, F 75%)	%	6	14.2	12.5	10.2	18	14	26	6
Adenocarcinoma pars distalis M	#	0	0	0	0	2	0	0	0
(CR max.: M 6.7%, F 5.4%)	%	0	0	0	0	4	0	0	0
Combined Adenoma + Adenocarcinoma	#	3	7	6	5	11	7	13	3
	%	6	14.2	12.5	10.2	22	14	26	6
Adrenal gland		50	49	50	49	50	50	50	50
Cortical Adenoma B (p_t = 0.012)	#	0	2	2	1	0	1	**4***	**4***
RITA range: Male: 0 5.9%, Female: 0 8%	%	0	4.1	4	2	0	2	8	8
(CR max.: M 6.7%, F 3.0%)									
Pheochromocytoma B	#	20	22	12	18	1	3	4	3
	%	40	44.8	24	36.7	2	6	8	6
Pheochromocytoma M	#	1	4	4	2	0	0	1	0
	%	2	8.2	8	4.1	0	0	2	0
Combined Pheochromocytoma	#	21	26	16	20	1	3	5	3
	%	42	53.0	32	40.8	2	6	10	6
Hemolymphoreticular System	#	50	50	50	50	50	50	50	50
Sarcoma histiocytic (p_t = 0.026)	#	0	0	0	**2***	1	0	0	0
(CR max.: M 2.0%, F 1.3%)	%	0	0	0	4	2	0	0	0
Malignant fibrous histiocytoma M	#	0	0	0	1	0	0	0	0
	%	0	0	0	2	0	0	0	0
Mammary gland	#	50	50	50	49	50	50	50	50

Rat Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
Neoplastic Findings		Male				Female			
All main study animals		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	50	50	50	50	50	50	50	50
Adenoma B	#	0	0	0	0	1	2	0	0
(CR max.: F 8.0%)	%	0	0	0	0	2	4	0	0
Adenocarcinoma M	#	0	0	0	0	3	1	1	1
(CR max.: F 12.0%)	%	0	0	0	0	6	2	2	2
Adenoma + Adenocarcinoma	#	0	0	0	0	4	3	1	1
	%	0	0	0	0	8	6	2	2
Fibroadenoma B (p_t= 0.052)	#	0	0	0	0	4	7	4	**11***
(RITA range: 5 28%, CR max.: F 32%)	%	0	0	0	0	8	14	8	**22**
Fibroma B	#	0	0	0	0	0	0	0	1
	%	0	0	0	0	0	0	0	2
Fibroadenoma + Fibroma	#	0	0	0	0	4	7	4	**12***
	%	0	0	0	0	8	14	8	**24**
Skin	#	50	50	50	49	50	50	50	50
Papilloma B	#	1	0	0	1	0	0	0	0
(CR max.: M 2.0%)	%	2	0	0	2	0	0	0	0
Basal cell carcinoma, basosquamous M	#	1	0	0	0	0	0	0	0
(CR max.: M 1.3%)	%	2	0	0	0	0	0	0	0
Fibroma B (p = 0.029)	#	0	0	**2***	**2***	0	0	0	0
(CR max.: M 2.0%)	%	0	0	4	4	0	0	0	0
Fibrosarcoma M	#	0	0	0	1	0	0	0	0
(CR max.: M 0.67%)	%	0	0	0	2	0	0	0	0
Keratoacanthoma B	#	1	0	1	1	0	0	0	0
(CR max.: M 10%)	%	2	0	2	2	0	0	0	0
Liposarcoma M	#	1	0	1	0	0	0	0	0
	%	2	0	2	0	0	0	0	0
Squamous cell carcinoma M	#	0	0	1	0	0	0	0	0
(CR max.: M 2.7%, F 2.0%)	%	0	0	2	0	0	0	0	0
Fibroma + Fibrosarcoma	#	0	0	2	3	0	0	0	0
	%	0	0	4	6	0	0	0	0

RITA: Registry of Industrial Toxicology Animal Data, CR: Charles River (March 2011)

Non Neoplastic lesions

In the two year studies, the percentage of rats (54-84%) with chronic cardiomyopathy was higher than the percentage of mice with (5-14%) cardiomyopathy. Although the incidence of cardiomyopathy in rats was similar across male groups, a slight non-statistically significant increase in the incidence of cardiomyopathy was observed in female groups with dose (Table 28). However, the incidence of valvular fibrosis in the heart increased with dose in both males and females and was statistically significant in females by a trend test (p_t = 0.0048), but not by a pairwise test (p = 0.0587).

Although the incidence of hemorrhage did not increase significantly with dose in any organ or across all organs, the incidence of pigment deposition increased with dose in some organs. The increased pigment deposition representing the remains of previous micro-hemorrhages was attributed to the pharmacological action of BAY 59-7939. In the pancreas, peri-vascular and/or peri-insular pigment deposition was increased of high dose rats with statistical significance attained in the high dose females. In the adrenal gland, pigment deposits increased in the high dose males, the mid- and high dose females without statistical significance. In the high dose males, the incidence of pigment deposition also increased in the mesenteric lymph nodes. In the high dose females, the incidence of pigment deposition also increased with statistical significance in the popliteal lymph nodes and the uterus. Across all organs the incidence of pigment deposition increased in the high dose males and females.

Table 28: Reviewer's Summary - Non-neoplastic Lesions - Document PH-36242

Rat Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
Non Neoplastic Findings		Male				Female			
All main study animals		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	50	50	50	50	50	50	50	50
Liver	#	50	50	50	49	50	50	50	50
Hyperplasia bile duct diffuse	#	39	39	40	38	20	20	16	22
Hyperplasia bile duct focal/multifocal	#	4	0	2	1	5	2	3	3
Foci Basophilic (NOS)	#	4	2	0	0	2	0	1	2
Foci Basophilic Tigroid	#	5	8	7	9	10	5	9	9
Foci Eosinophilic	#	1	1	0	0	0	0	0	1
Foci Clear cell	#	33	39	34	37	10	7	13	13
Congestion/hemorrhage	#	5	6	12	7	1	3	3	3
Hemorrhage	#	0	1	0	0	0	0	0	0
Pigment deposits	#	5	1	1	1	0	0	0	2
Necrosis focal/multi focal	#	3	3	2	3	3	2	2	5
Necrosis single cell	#	1	0	0	0	0	0	0	0
Heart		50	50	50	49	50	50	50	50
Cardiomyopathy		39	39	41	41	27	28	30	33
Valvular fibrosis (p = 0.0048)		0	1	1	2	0	0	2	**4***
Pancreas	#	49	50	50	49	50	50	50	50
Hyperplasia focal acinar	#	1	3	1	0	0	1	0	0
Hyperplasia focal ductular	#	1	1	1	1	0	0	0	0
Metaplasia focal hepatocytic	#	0	0	0	0	0	0	0	1
Hemorrhage	#	0	1	0	0	0	0	0	0
Pigment deposits perivascular (p=0.021)	#	21	21	20	25	7	10	13	**15***
Pigment deposits periinsular (p = 0.031)	#	12	10	8	15	3	1	5	**7***
Kidneys	#	50	50	50	49	50	50	50	50
Chronic progressive nephropathy	#	45	48	49	45	41	37	40	33
Hemorrhage/hematoma	#	1	1	0	0	0	0	0	0
Testes	#	50	50	50	49	0	0	0	0
Leydig cell hyperplasia diffuse	#	2	0	0	0				
Leydig cell hyperplasia focal	#	8	7	4	5				
Clitorial glands	#	0	0	0	0	43	40	42	45
Hyperplasia focal squamous cell	#					1	0	1	0
Hyperplasia diffuse squamous cell	#					1	1	1	0
Hyperplasia focal acinar cell	#					0	1	0	1
Hyperplasia diffuse reactive (p = 0.016)	#					0	1	0	**4***
Hyperplasia focal reactive	#					1	1	0	0
Pituitary gland	#	50	49	48	49	50	50	50	50
Hyperplasia pars distalis diffuse	#	1	0	0	0	2	2	0	0
Hyperplasia pars distalis focal	#	19	16	11	13	8	6	5	4
Hyperplasia pars intermedia diffuse	#	2	1	1	0	0	0	0	0
Pigment deposits	#	0	2	1	2	6	5	4	5
Adrenal gland	#	50	49	50	49	50	50	50	50
Hyperplasia focal medullary	#	31	34	34	36	17	21	12	15
Hyperplasia focal zona fasciculata	#	11	12	7	10	3	9	4	5
Hyperplasia focal zona glomerulosa	#	0	1	0	0	0	1	0	0
Hyperplasia cortical (combined fas + glom)	#	11	13	7	10	3	10	4	5
RITA: Male: 4.1 73, Female: 8.3 49	%	22	26.5	14	20	6	20	8	10
Hemorrhage/congestion	#	1	1	3	2	0	3	0	0
Pigment deposits/ zona reticularis	#	18	14	13	**26**	25	28	32	**32**
				p = 0.058				p = 0.055	
Mammary gland	#	50	50	50	49	50	50	50	50
Hyperplasia diffuse	#	0	0	0	0	6	8	6	5
Hyperplasia focal	#	0	0	0	0	4	**12***	7	2
Hyperplasia focal with atypia	#	0	0	0	0	1	3	0	1
Hyperplasia ductular	#	0	0	0	0	0	1	0	0
Pigment deposits (p = 0.032)	#	14	19	16	14	1	0	1	**4***
Skin	#	50	50	50	49	50	50	50	50
Hyperplasia focal	#	0	0	0	0	0	0	2	0
Hyperplasia squamous cell	#	0	1	0	0	0	0	0	0
Hemorrhage	#	0	0	0	0	0	1	0	0
Skeletal Muscle	#	50	50	50	49	50	50	50	50
Myodegeneration (p = 0.016)	#	0	2	**6***	4	1	1	1	1
Hemorrhage/hematoma in other tissues									
Spinal cord	#	0/50	0/50	1/50	1/49	0/50	0/50	0/50	0/50

Rat Carcinogenicity Study		BAY 59 7939 Dose level (mg/kg/day)							
Non Neoplastic Findings		Male				Female			
All main study animals		0	10	20	60	0	10	20	60
Organ/Tissue Finding	#/group	50	50	50	50	50	50	50	50
Nose	#	0/50	0/50	0/50	2/49	0/50	1/50	0/50	0/50
Larynx	#	0/49	0/50	0/49	2/48	0/49	0/50	0/50	0/50
Trachea	#	0/50	0/50	0/49	0/48	0/49	0/50	1/50	0/50
Lungs aveolar	#	7/50	6/50	3/50	4/49	1/50	0/50	0/50	0/50
Urinary bladder	#	0/50	0/49	0/49	0/49	1/50	0/50	0/50	0/50
Spleen hematoma	#	0/50	0/50	0/50	0/49	0/50	0/50	0/50	1/50
Lymph node mesenteric	#	0/49	0/50	1/49	0/49	0/50	0/50	0/50	0/50
Lymph node mandibular	#	3/50	1/50	5/49	3/49	1/49	2/50	1/50	0/49
Total hemorrhage in other tissues		10	7	10	12	3	3	2	1
Total hemorrhage from liver, etc. above		2	4	3	2	0	4	0	0
Total hemorrhage		12	11	13	14	3	7	2	1
Increased pigment deposits in other tissues									
Lung	#	0/50	0/50	1/50	1/49	0/50	0/50	1/50	0/50
Ovaries	#					0/50	0/50	0/50	1/50
Uterus (p = 0.011)	#					1/50	3/50	4/50	**7*/50**
Spleen	#	23/50	19/50	28/50	20/49	21/50	21/50	21/50	26/50
Thymus	#	2/44	2/47	1/49	3/46	1/46	1/48	0/48	1/49
Lymph nodes other	#	0/1	0/1	3/4	0/2	2/4		0/1	
Lymph node mesenteric (p = 0.054)	#	5/49	7/50	7/49	**11/49**	4/50	5/50	3/50	3/50
Lymph node popliteal (p = 0.015)	#	6/48	7/44	8/48	4/46	9/50	8/48	12/50	**17*/48**
Lymph node mandibular	#	21/50	13/50	15/49	19/49	21/49	20/50	19/50	25/49
Lacrimal glands	#	2/49	2/50	7/50	3/48	0/50	0/50	0/50	0/50
Eyes	#	0/49	1/50	0/50	0/48	0/50	0/50	0/50	0/50
Total pigment deposits in other tissues		59	51	70	61	59	58	60	80
Total pigment deposits from liver, etc. above		70	67	59	83	42	44	55	65
Total increased pigment deposits		129	118	129	144	101	102	115	145
Other statistically significant findings									
Hyperkeratosis extremities (p = 0.042)	#	0/0	0/0	0/1	**3*/3**	0/4	0/3	0/4	0/4
Lungs Inflam infiltrate (p = 0.012)	#	1/50	2/50	1/50	2/49	0/50	1/50	3/50	**4*/50**
Ovary hyperplasia sex cord stromal (p = 0.034)	#					10/50	5/50	14/50	**15*/50**
Thymus hyperplasia cord & tubule	#	7/44	5/47	5/49	2/46	12/46	**24*/48**	13/48	14/48
Lacrimal glands inflame. infiltrate (p = 0.013)	#	3/49	0/50	0/50	1/50	0/50	1/50	0/50	**4*/50**
Sternum myelofibrosis (p = 0.04)	#	1/50	1/50	3/50	**3*/49**	0/50	0/50	0/50	1/50

* p<0.05

Toxicokinetics

Blood samples were obtained and plasma was prepared from three satellite animals/sex/group at 0.5, 1, 2, 4, 7, and 24 hours after dosing on Days 1 and 381 of treatment. Blood samples were obtained from control animals at 1, 7 hours after dosing. On Day 726, blood samples were obtained from three satellite animals/sex/group at 1.0 hour after dosing. After addition of an internal standard and protein precipitation with acetonitrile, analysis of BAY 59-7939 was performed using a validated LC-MS/MS assay with a lower limit of quantification of 0.002 mg/L. The concentration of BAY 59-7939 in control animals was below the limit of quantification on all three sampling days.

The t_{max} was 1-2 hours on Day 1 and 1 hour on Day 381. Exposure to BAY 59-7939 on all sampling days was lower in males than in females with exposure on Day 381 lower in males by a factor of 0.32 to 0.42 (Table 29, Figure 7). Although exposure increased with dose, the increase was less than dose-proportional. Exposure increased with repeated dosing for 52 weeks by a factor of 1.3 to 1.7 in females. In contrast, exposure in males was essentially unchanged at the two lower dosages and decreased by a factor of 0.74 at the highest dosage. Plasma concentrations at 1.0 hour after dosing on Day 726 were not significantly different from plasma concentrations at 1.0 hour after dosing on Day 381. Since the mean human exposure at the highest recommended daily dose of 20 mg/day was 3.3 mg*hr/L, the exposure multiples for male and female rats on

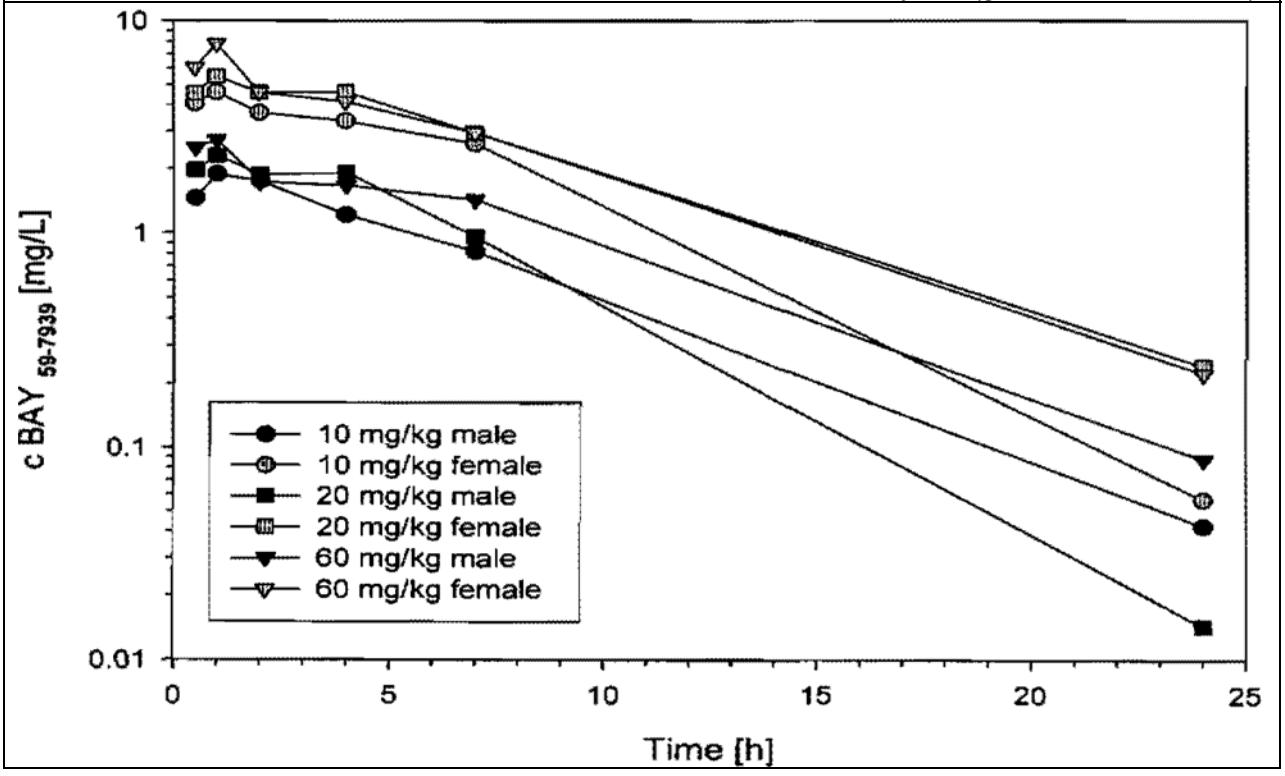
Day 381 were 6.2 and 14.6, respectively, of the human exposure based on total AUC values. These multiples of the human exposure for male and female rats decrease to 1.5 and 3.7, when correction is made for percent protein binding (i.e., amount of unbound drug).

Table 29: Reviewer's Summary of Toxicokinetic Results – Document PH-36242

Toxicokinetic Parameters – Day 1 versus Day 381							
		Male			Female		
Dose (mg/kg)		10	20	60	10	20	60
Day 1							
$AUC_{(0-24)}$	mg*hr/L	11.5	16.6	27.5	20.0	27.8	36.0
C_{max}	mg/L	1.98	2.39	2.92	2.63	3.26	4.09
t_{max}	hr	1.0	2.0	2.0	2.0	2.0	2.0
Day 381							
$AUC_{(0-24)}$	mg*hr/L	13.4	15.4	20.3	34.7	47.5	48.2
C_{max}	mg/L	1.89	2.31	2.73	4.61	5.48	7.81
t_{max}	hr	1.0	1.0	1.0	1.0	1.0	1.0
Plasma concentrations (mg/L) at 1.0 hour after drug administration							
		Male			Female		
Dose (mg/kg)		10	20	60	10	20	60
Day 1	Mean (SD)	1.98 (1.28)	1.91 (1.14)	2.33 (1.08)	2.50 (1.19)	2.65 (1.26)	3.82 (1.30)
Day 381	Mean SD	1.89 (1.19)	2.31 (1.22)	2.73 (1.08)	4.61 (1.16)	5.48 (1.55)	7.81 (1.03)
Day 726	Mean SD	2.31 (1.21)	2.61 (1.46)	2.99 (1.21)	4.05 (1.33)	5.78 (1.15)	5.70 (1.02)

Figure 7: Sponsor's Plasma Concentration Profiles – Document PH-36242

Plasma BAY 59-7939 concentrations after oral administration to rats on Day 381 (geometric mean, n = 3)



Dosing Solution Analysis
Prior to the start of the study, test article formulations at concentrations above 6 mg/mL and below 1 mg/mL were shown to be homogenous and stable for 15 days at room temperature. During the study test article formulations were prepared as needed based on the 15 day stability. Analysis of the dose formulations on eleven days throughout the study showed the formulations were homogenous and the measured concentrations ranged from 90% to 116% of nominal (Table 30).

Table 30: Reviewer's Summary of Formulation Analyses – Document PH-36242

	Nominal concentration, mg/mL		
	1	2	6
Number	11	11	11
Mean recovery, %	103.0	103.7	105.5
SD	4.6	3.8	3.5
Maximum	115	115	116
Minimum	90	95	102

5 Integrated Summary and Safety Evaluation

The nonclinical and statistical reviewers concurred with the sponsor that no significant evidence of neoplasia related to rivaroxaban treatment was observed either in Wistar rats or CD-1 mice. The Executive Carcinogenicity Assessment Committee also concluded that there were no clear drug-related neoplasms in either study.

The safety margins for the highest dosage of rivaroxaban (60 mg/kg/day) used in the carcinogenicity studies were calculated based on AUC values for exposures to total and unbound rivaroxaban. Because the protein binding of rivaroxaban differs significantly among species, the safety margins based on exposures to unbound drug are considered more relevant for comparisons between humans and different animal species.

The recommended daily dosage of rivaroxaban for patients: (a) undergoing hip and knee surgery and (b) with atrial fibrillation is 10 mg and 20 mg, respectively. Consequently, the safety margins reported in the labels for NDA 22406 and NDA 202439 will differ as indicated in Table 31 and Table 32.

Table 31: Safety Margins for Human Dose of 20 mg Rivaroxaban Daily

Study/ Species		Sex	NOAEL (mg/kg) M/F	Exposure at NOAEL		Safety Margin‡	
				Total $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Unbound† $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Based on Total AUC at NOAEL	Based on Unbound AUC
Carcinogenicity – 2 year							
Rat	Tumors	M	60	20.3	0.257	6.2	1.5
		F		48.2	0.612	14.6	3.7
Mouse	Tumors	M	60	2.52	0.162	0.76	1.0
		F		4.24	0.273	1.28	1.6

† Unbound fractions in humans, rats, mice, dogs, and rabbits are 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively.
‡ Comparison to human exposure at 20 mg/day corresponding to 0.33 mg/kg in a 60 kg patient or 3.3 mg*hr/L. Human exposure to unbound drug was 0.167 mg*hr/L.

Table 32: Safety Margins for Human Dose of 10 mg Rivaroxaban Daily

Study/ Species		Sex	NOAEL (mg/kg) M/F	Exposure at NOAEL		Safety Margin‡	
				Total $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Unbound† $AUC_{(0\text{-}24\ hr)}$ (mg*hr/L)	Based on Total AUC at NOAEL	Based on Unbound AUC
Carcinogenicity – 2 year							
Rat	Tumors	M	60	20.3	0.257	17.4	4.3
		F		48.2	0.612	41.2	10.3
Mouse	Tumors	M	60	2.52	0.162	2.2	2.7
		F		4.24	0.273	3.6	4.6

† Unbound fractions in humans, rats, mice, dogs, and rabbits are 5.07%, 1.27%, 6.45%, 10.4%, and 23.4%, respectively.
‡ Comparison to human exposure at 10 mg/day corresponding to 0.16 mg/kg in a 60 kg patient or 1.17 mg*hr/L. Human exposure to unbound drug was 0.0593 mg*hr/L.

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/s/

PATRICIA P HARLOW
06/10/2011

THOMAS PAPOIAN
06/13/2011
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:	NDA 202439, DMF-02581
Supporting document/s:	EDR
Applicant's letter date:	01/04/11, 07/23/08
CDER stamp date:	01/05/11, 07/24/08
Product:	Rivaroxaban (Bay 59-7939)
Indication:	Prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation.
Applicant:	Ortho McNeil Janssen Pharmaceuticals Inc [Bayer Schering Pharma AG (Bayer) andJohnson & Johnson Pharmaceutical Research and Development, L.L.C. (J&JPRD)]
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

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1 Executive Summary

1.1 Introduction

This document focuses on the toxicological qualification of the impurities in rivaroxaban, an inhibitor of FXa, proposed for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation under NDA 202439 as well as the prevention and treatment of multiple thrombosis-mediated conditions under NDA 22406.

1.2 Brief Discussion of Nonclinical Findings

DMF 21581 indicates the rivaroxaban drug substance has three specified impurities and twelve unspecified impurities. The reviewer agrees with the nonclinical reviewer of NDA 22406 that the rivaroxaban impurities are qualified in terms of general toxicology. However, the nonclinical reviewer of NDA 22406 did not address the genotoxic qualification of these impurities.

Most of the impurities were present at levels higher than (b) (4) for unspecified or (b) (4) for specified impurities in at least one of the three rivaroxaban lots used for genetic toxicology studies. However, one impurity, (b) (4) was present at only (b) (4) in one lot and was not detected in the other two lots.

The sponsor maintains that none of the potential impurities generated genotoxic alerts using computer applications capable of identifying structural alerts. To confirm the sponsor's statement, the CDER Computational Toxicology Group evaluated all rivaroxaban impurities. Although no structural alerts were identified for the rivaroxaban impurities by the Derek for Windows (DfW) application, some positive predictions for genotoxicity were obtained using other software applications.

Of the five most critical genotoxic alerts, the reviewer's concerns regarding four impurities are mitigated by the presence of these impurities at levels higher than (b) (4) for unspecified or (b) (4) for specified impurities in at least one of the three rivaroxaban lots used for the Ames and/or chromosomal aberration assays. However, the concern regarding the structural alert for (b) (4) can not be mitigated, because this impurity was present at only (b) (4) in one lot and was not detected in the other two lots used in the genetic toxicology studies.

1.3 Recommendations

1. The sponsor needs to justify why the impurity (b) (4) should be specified at (b) (4) when the maximum percentage in lots used for toxicology studies was (b) (4) and was either not detected or present at (b) (4) in the lots used for the genotoxicity assays. Alternatively, the sponsor should lower the specifcation limit for (b) (4) to (b) (4).

2. If the sponsor is unable to lower the specification limit for (b) (4) then the sponsor needs to conduct an Ames assay and a in vitro chromosomal aberration assay either with (b) (4) itself or with the percentage of (b) (4) in the drug substance being at least equal to or greater than 3-fold the specification of

(b) (4). If these assays are negative, then no additional studies are necessary. If either of these assays is positive, then an in vivo micronucleus assay should be conducted.

2 Drug Information

2.1 Drug

CAS Registry Number: 366789-02-8

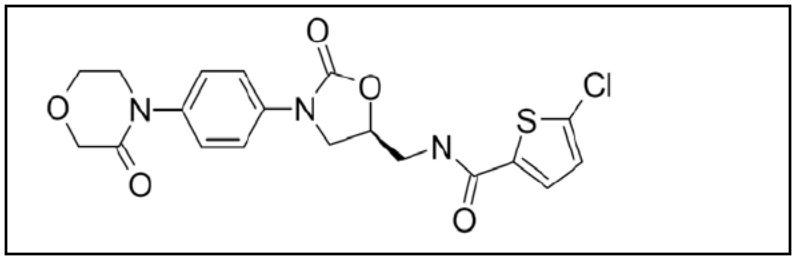
Generic Name: Rivaroxaban (Xarelto™)

Code Names: JNJ-39039039, BAY 59-7939

Chemical Name: 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide

Molecular Formula/Molecular Weight: $C_{19}H_{18}ClN_3O_5S$/ 435.89 g/mol

Structure or Biochemical Description:



Pharmacologic Class: Rivaroxaban is a direct Factor Xa inhibitor.

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA-022406 (DHP)
IND-064892 (DHP), IND-075238 (DCRP), IND-(b) (4)
MF-021580 (DRM), MF-021581 (DRM), MF-021592 (DRM)

2.3 Drug Formulation

Rivaroxaban is formulated for oral administration as immediate release, film-coated tablets containing either 15- or 20-mg of active compound. The tablets also contain microcrystalline cellulose (b) (4) NF, croscarmellose sodium NF, hypromellose (b) (4) USP), lactose monohydrate NF, magnesium stearate (b) (4) NF, sodium lauryl sulfate NF (b) (4) as excipients. The commercially available film coating for the 15 mg tablet is Opadry Red (b) (4) containing hypromellose (b) (4) USP, polyethylene glycol (b) (4) 3350 NF, ferric oxide red NF, and titanium dioxide USP. The film coating for the 20 mg tablet is Opadry® II Dark Red (b) (4) containing partially hydrolyzed polyvinyl alcohol USP, polyethylene glycol (b) (4) 3350 NF, ferric oxide red NF, titanium dioxide USP, and talc USP.

2.4 Comments on Novel Excipients

All of the excipients are commonly used in oral commercial pharmaceutical dosage forms and are compendial materials.

2.5 Comments on Impurities/Degradants of Concern

1. Background:
   a. The nonclinical review of N22406 did not address impurities in the rivaroxaban drug substance, although the review of DMF 21581 for rivaroxaban indicates that the previous nonclinical reviewer considered all impurities qualified.
   b. The review of DMF 21581 indicates only three impurities (b) (4) are specified at the threshold of qualification (b) (4). The remaining impurities are not specified, since the sponsor maintains their levels are below (b) (4)

2. General toxicological qualification of rivaroxaban impurities
   a. Most of the impurities were present at levels higher than (b) (4) in at least one lot used for a general toxicology study (Appendix 1). The exceptions are (b) (4)
   b. However, a comparison for selected lots (Appendix 2) of the exposure of the impurities in animals to the maximum possible exposure in humans indicates the ratio of animal exposure to maximum possible human exposure is above 100. Therefore, the reviewer agrees with the previous nonclinical reviewer that the rivaroxaban impurities are qualified in terms of general toxicology.

3. Genetox qualification of impurities
   a. Only three lots (010621, BX01JU3, and BX01SFS) were used for genetic toxicology assays.
   b. Most of the impurities were present in at least one lot used for genetic toxicology studies (Appendix 3) at levels higher than (b) (4) for unspecified or (b) (4) for specified impurities. However, (b) (4) was present at only (b) (4) in Lot BX01SFS and was not detected in the other two lots.
   c. In the DMF 21581 Summary (p 44), the sponsor maintains that none of the potential impurities generated genotoxic alerts using specific computer applications capable of identifying alerting structural elements.

d. To confirm the sponsor's statement, the CDER Computational Toxicology Group was requested to evaluate all impurities for rivaroxaban. These CompTox results are provided in Appendix 4.

e. Although no structural alerts were identified for the rivaroxaban impurities by the Derek for Windows (DfW) application, some positive predictions for genotoxicity were obtained when using other QSAR software applications and models in the standard battery. The following discussion focuses on the five positive predictions summarized in Table 1 and excludes the other positive predictions, including those obtained for the mouse lymphoma assay.

   i. One impurity, (b) (4) was positive for an alert for the Ames assay (Salmonella) in one (SciQSAR 2.2) of the four programs used. This impurity was present at 3-4 fold higher levels in two lots used for Ames assays, although it was not detected in the lots used for carcinogenicity studies. In these Ames assays where the maximum amount of rivaroxaban was 5000 µg per plate, the amount of (b) (4) would have been (b) (4) per plate, an amount equivalent to or greater than the amount of the positive control compounds. Since both Ames studies produced negative results, the reviewer does not believe a separate Ames assay for (b) (4) is necessary.

   ii. One impurity, (b) (4) was positive for an alert for the in vitro chromosomal aberration (CA) assay in one (SciQSAR 2.2) of the four programs used. This impurity was present at 4 fold higher levels in one lot used for a chromosomal aberration assay, but was not detected in the lots used for carcinogenicity studies. In this CA assay where the maximum concentration of rivaroxaban was 180 µg/mL, the concentration of (b) (4) would have been (b) (4), a concentration in the range of the positive control compounds (b) (4). Since this chromosomal aberration study produced negative results, the reviewer does not believe a separate chromosomal aberration assay for (b) (4) is necessary.

   iii. One of the three impurities positive for an alert for the in vivo micronucleus assay in one (MC4PC 2.3.0.36) of the four programs is the (b) (4) This impurity was present at a 2-3 fold higher percentage in the lot used for the in vivo micronucleus assay than the percentage in the specification, but was not detected in the lots used for carcinogenicity studies. Although the micronucleus assay produced negative results, the reviewer notes that the maximum dose of rivaroxaban was 140 mg/kg IP and the dose of (b) (4) would have been (b) (4) a dose less than that of the positive control (b) (4) However, the (b) (4) was present at a 5-6 fold higher percentage in the lots used for the Ames and chromosomal

aberration assays than the percentage in the specification and these assays produced negative results. Therefore, the reviewer does not believe a separate in vivo micronucleus assay for (b) (4) is necessary.

iv. Another impurity positive for an alert for the in vivo micronucleus assay in one (MC4PC 2.3.0.36) of the four programs is (b) (4) This specified impurity was not detected in the lot used for the in vivo micronucleus assay, but was present at (b) (4) of the proposed specification in the lots used for carcinogenicity studies as well as present at 3-4 fold higher percentages in the lots used for the Ames and chromosomal aberration assays than the percentage in the specification. Since the Ames and CA assays were negative, the reviewer does not believe that a separate in vivo micronucleus assay for (b) (4) is necessary.

v. The last impurity positive for an alert for the in vivo micronucleus assay in one (MC4PC 2.3.0.36) of the four programs is (b) (4) This impurity was not detected in the lot used for the in vivo micronucleus assay. Although (b) (4) was present at (b) (4) of the proposed specification (b) (4)) in the lots used for carcinogenicity studies, this impurity was either not detected or present at (b) (4) in the lots used for the Ames and chromosomal aberration assays. Additionally, the levels of (b) (4) in the later clinical batches ranged from (b) (4) If present at the maximum specification level, the amount of (b) (4) in the human dose (20 mg) would be (b) (4) per day or (b) (4) the Threshold of Toxicologic Concern for genotoxicity of (b) (4) per day. Therefore, the reviewer concludes that the genotoxicity of (b) (4) has not been adequately evaluated.

f. Recommendations:

i. The sponsor needs to justify why (b) (4) should be specified at (b) (4) when the maximum percentage in lots used for toxicology studies was (b) (4) and was either not detected or present at (b) (4) in the lots used for the genotoxicity assays (Ames, chromosomal aberration, and micronucleus). Alternatively, the sponsor should lower the specifcation limit for (b) (4) to (b) (4)

ii. If the sponsor is unable to lower the specification limit for (b) (4) then the sponsor needs to conduct an Ames assay and an in vitro chromosomal aberration assay either with (b) (4) itself or with the percentage of (b) (4) in the drug substance at least equal to or greater than 3-fold the specification of (b) (4) If these assays are negative, then no

additional studies are necessary. If either of these assays is positive, then an in vivo micronucleus assay should be conducted.

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/s/

PATRICIA P HARLOW
02/17/2011

THOMAS PAPOIAN
02/17/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202439 **Applicant: Ortho-McNeil-Janssen-Pharmaceuticals/OMJPI (Johnson & Johnson / Bayer)** **Stamp Date: 1/5/2011**

Drug Name: rivaroxaban **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		Although most of the toxicology studies were conducted using the coprecipitate formulation of the rivaroxaban, the mouse and rat carcinogenicity studies and the 13-week studies used to determine dosing in the carcinogenicity studies were conducted with the micronized drug substance, which will be the marketed formulation.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Statements are included with individual study reports

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
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		The nonclinical review of NDA 22406 did not discuss impurities. The sponsor maintains that only three of 17 possible impurities need to be specified and no impurity has a structural alert. However, it is not clear that these impurities were submitted for FDA CompTox evaluation.
11	Has the applicant addressed any abuse potential issues in the submission?		X	The sponsor did not directly address abuse potential. However, rivaroxaban has limited distribution to the brain. Single doses produced no central nervous system effect in behavior studies; however, it did slightly delay nociceptive reactions to heat indicating a weak analgesic effect.
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.

The reviewer will submit the specified and unspecified impurities for FDA CompTox evaluation and assumes that these evaluations will confirm the sponsor's statement that no structural alert was found for any of the impurities.

Patricia Harlow, Ph.D. — February 3, 2011
Reviewing Pharmacologist — Date

Albert DeFelice, Ph.D. — February 4, 2011
Team Leader/Supervisor — Date

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

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/s/

PATRICIA P HARLOW
02/04/2011

ALBERT F DEFELICE
02/04/2011