

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**022406Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

Xarelto (rivaroxaban)

**Date:** June 30, 2011

**To:** File for NDA #22-406

**From:** John K. Leighton, PhD, DABT

Associate Director for Pharmacology/Toxicology  
Office of Oncology Drug Products

The pharmacology/toxicology review of Dr Chopra was examined previously and no additional pharmacology/toxicology studies were deemed necessary to support the proposed indication, and no additional nonclinical studies are needed to support the current approval. A labeling review was previously deferred.

The pharmacology/toxicology information in the labeling is acceptable and supported by the studies submitted for review. The pharmacologic classification “factor Xa inhibitor” was chosen as it is pharmacologically valid and the terminology is consistent with other drugs in the class. Currently, there is no need or scientific justification to distinguish in pharmacologic classification, whether an inhibitor is “direct” or “indirect” in relation to factor Xa inhibitors, regardless of the pharmacologic mechanism of action. This approach is consistent with the FDA guidance: Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information.

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/s/  
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JOHN K LEIGHTON  
06/30/2011

## MEMORANDUM

Xarelto (rivaroxaban)

**Date:** May 12, 2009

**To:** File for NDA #22-406

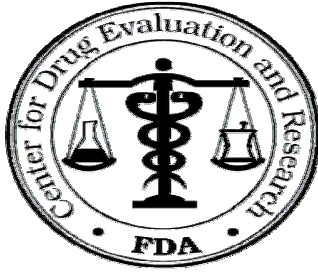
**From:** John K. Leighton, PhD, DABT  
Associate Director for Pharmacology  
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review provided by Drs. Chopra and Laniyonu. I concur with their conclusions that Xarelto may be approved. No additional pharmacology or toxicology studies are necessary for the proposed indication. A review of the labeling will be deferred.

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this page is the manifestation of the electronic signature.**  
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/s/

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John Leighton  
5/12/2009 06:20:25 AM  
PHARMACOLOGIST



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

**PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

**NDA NUMBER:** 022-406

**SERIAL NUMBER:** 000

**DATE RECEIVED BY CENTER:**

**PRODUCT:** BAY 59-7939 (JNJ-39039039; Xarelto®/Rivaroxaban Micronized) Tablets

**INTENDED CLINICAL USE:** For the Prevention of Venous Thromboembolism (VTE) and pulmonary embolism

**SPONSOR:** Johnson & Johnson Pharmaceutical  
Research & Development L.L.C., Raritan, NJ.

**CONTACT PERSON:** Shelly Chandler, Johnson & Johnson  
Pharmaceutical; Phone (908) 704-4579

**DOCUMENTS REVIEWED:** Electronic submission (eCTD)

**REVIEW DIVISION:** Division of Medical Imaging and Hematology  
Drug Products (DMIHP)

**PHARM/TOX REVIEWER:** Yash M. Chopra, M.D.; Ph.D.

**PHARM/TOX SUPERVISOR:** Adebayo Lanijonu, Ph.D.

**DIVISION DIRECTOR:** Rafel D. Rieves, M.D.

**PROJECT MANAGER:** Diane Leaman, D. Pharm.

## **Executive Summary**

### **I. Recommendations**

#### **A. Recommendation on approvability**

NDA 22-406 is recommended for approval from P/T perspective.

#### **B. Recommendation for nonclinical studies** **None**

#### **C. Recommendations on labeling**

A separate labeling review will be written.

### **II. Summary of nonclinical findings**

#### **A. Brief overview of nonclinical findings**

##### **Pharmacologic activity**

BAY 59-7939 (Xarleto) a selective and competitive oral Factor Xa and thrombin generation inhibitor ( $K_i$  of  $0.5 \pm 0.02$  nM;  $IC_{50}$  of 2.1 nM) acts by preventing the activation of prothrombin to thrombin. Reduction of thrombus formation was demonstrated in arteriovenous shunt model in rats/rabbits and, injury induced venous thrombus formation in rats. BAY 59-7939 did not show effects on the cardiovascular function, ECG, respiration, acid/base balance, hematocrit and electrolytes in anesthetized beagle dogs up to oral dose of 30 mg/kg. It showed no effects on pentylenetetrazol convulsions or hexobarbital-induced anesthesia in rodents. It did not exert effect on gastrointestinal motility, urine excretion or on the respiratory system.

##### **Absorption, Distribution, Metabolism and Excretion (ADME):**

Orally administered BAY 59-7939 was absorbed and showed an absolute oral bioavailability of about 60% in both rats and dogs. In rats, the  $T_{1/2}$  of the drug was 1.2 to 2.3 hours after oral and 0.9 hr after IV dose. The  $T_{1/2}$  of tagged compound was prolonged by 18.8 and 42.1 hrs by the IV and oral routes, respectively suggesting the tagging of the radioactivity with metabolite/s. In dogs, intravenously administered compound achieved dose related concentration. An IV dose of 0.3 and 3 mg/kg attained AUC of 1.0 and 6.03 mg.h/ml with similar half life of 0.95 hours. The protein binding was more than 90% and is variable among species. The labeled [ $^{14}C$ ]BAY 59-7939 was heterogeneously distributed in different organs and tissues without any specific tissues affinity. The compound is oxidized to morpholino moiety (M1) by CYP3A4 and M-1 was its major metabolite. In rat/human hepatocyte in vitro preparations, M-1 was

formed and was the major plasma metabolite and was excreted in the urine, bile, and feces of rats. Fecal route was the major route of excretion in rats. After an oral dose, 66.9% of the radioactivity was excreted in feces and 24.7% in the urine by day 7. About 0.2% of the administered radioactivity remained as unaccounted in the rat.

#### **Single dose Toxicity Study:**

Acute single oral dose of 500 mg/kg in mice and rats did not produce any effect in the animals and lethal/sublethal doses were not estimated. A single intravenous 25 mg/kg dose in dogs produced CNS related effect of reduced activity, abdominal positioning, labored breathing, narrowed palpebral and piloerection.

#### **Repeat dose Toxicity Studies:**

a. Mouse: In a 4-week toxicity study in rats at the oral gavage doses of 0, 12.5, 50 and 200 mg/kg/day (6/sex/group) there were no treatment related effects seen in the animals. The target organs of toxicity and the highest tolerated dose were not identified. Similar plasma concentrations in male and female mice were seen.

BAY 59-7939 at oral gavage doses of 50, 100 and 200 mg/kg/day (PEG-6000 co-precipitate) for 13 weeks produced linear non-dose proportional plasma concentrations. There were higher incidences of fibrosis of the heart, mononuclear cell infiltration in kidneys, hyperplastic spindle cells in adrenal and increased cellularity of marginal zones of the spleen in males and, high incidences of Kupffer cell foci in the liver and mononuclear cell infiltration in the kidneys in females of high dose group. The liver, adrenal, kidneys and spleen were the target organs of toxicity in both sexes. A 100 mg/kg/day dose was the MTD in the study. The plasma exposure was 20 and 29.5 times the exposure produced by the clinical dose in humans. In another 13-week toxicity study, CD-1 mice were fed 1250, 2500 and 5000 ppm of BAY 59-7939 in dietary admixture (10% PEG-6000 coprecipitate). The mean drug administered in diet was 237, 476 and 1007 mg/kg/day in males, and 237, 476 and 1007 mg/kg/day in females. A dose dependent increase in coagulation time and, increased liver enzymes activities and incidences of focal renal tubular hypertrophy in males, and focal necrosis of liver in females were noted. The kidney and liver were the target organs of toxicity in males and females and 5000 ppm in diet appears to be the MTD. The exposure levels (AUC<sub>0-24h</sub>) at study MTD were 31.3 and 43.0 mg.h/L in males and females, i.e., about 7 and 10 times the human exposures of the clinical dose of 60 mg/day (30 mg b.i.d).

b. Rat:

BAY 59-7939 at intravenous doses from 0.0657 to 0.657 mg/kg/day for 14 days in rats produced a dose proportional plasma concentration. Foamy macrophages in lung parenchyma, swelling/vacuolation of proximal convoluted



renal tubules and, extramedullary hematopoiesis of spleen was reported in high dose group and high dose of 0.1971 mg/kg/day (1.183 mg/m<sup>2</sup>) was a 'maximal tolerable dose'. Target organs of toxicity were lungs, kidneys and spleen.

In 4-week oral gavage toxicity study conducted at doses of 0, 12.5, 50 and 200 mg/kg/day, a 12% decrease in body weight of high dose males and, treatment-related increase in several liver enzyme activities in treated animals were noted. There were insignificant decrease in CD45<sub>total</sub> cells, slight increase in IgA levels females and IgG levels in males. High incidences of bilateral retinal atrophy, focal inflammation of the pancreas unilateral diffuse dilatation of the testes were suggestive of eyes, pancreas and testes as the target organs of toxicity. A dose between 50 and 200 mg/kg/day was the highest tolerable dose and will provide the plasma exposure of 68 to 162 multiples of human plasma concentration.

Sponsor submitted three 13-week oral gavage toxicity studies and in the first 3-month study with 4 weeks of recovery, oral doses of 12.5, 50 and 200 mg/kg/day produced treatment-related increase of ALT and, decrease of GLDH and LDH enzymes significantly in high dose treatment group. These changes were not of clinical importance. The increased incidences of pigment deposition in the pancreas, mesenteric lymph node hemorrhage and focal retinal atrophy in the eye were seen in only males. Females of high dose group showed higher incidences of epicarditis, lung congestion and thymic hemorrhage. The high dose of 200 mg/kg/day was considered as an MTD and drug induced adverse effects were not completely resolved in the recovery period. In the 13-week dietary study, BAY 59-7939 was administered at the doses of 75 to 300 mg/kg/kg in diet containing 0.5% of BAY 59-7939. A non-dose proportional plasma concentration and minor change of hyper pigmentation in periductal pancreatic islets was seen in males of the high dose group. The target organs of toxicity were not identified. CAC-Ex committee concluded that the proposed MFD in diet was not identified in the study and the maximum amount of the active compound was 0.5% and not 5% as required by ACH Guidelines 51C.

The third 13-week oral gavage toxicity study in Wistar rat conducted with the micronized form at 0, 60, 300 and 1500 mg/kg/day BAY 59-7939 doses showed that there was dose-dependent increase in the coagulation time in rats and no treatment related effects in any of the treated group animals. The plasma concentrations were erratic and non-dose related. The target organs of toxicity and MTD were not identified in the study.

Six-month chronic study was performed at oral doses of 12.5 to 200 mg/kg/day and treatment related increase in the plasma concentration of animals was seen within 60 minutes of the dosing. No other relevant data could be obtained as the histopathology report of the study animals was not included.

**c. Dogs:**

BAY 59-7939 at 5 to 50 mg/kg/day for 4 weeks produced a treatment but non-dose proportional peak plasma concentrations in 1.5 to 3 hr. Alveolar macrophages and hemosiderin deposits in lymph node were produced in greater intensity in animals of 50 mg/kg/day group. The NOEL was 5 mg/kg/day and the highest tolerable dose was 15 mg/kg/day in the study and the target organs of toxicity were lungs and lymph nodes.

In 4-week oral toxicity study, vomits containing white colored fluid/foam and discolored (green/bright) feces were seen in 150 mg/kg/day group. On study week 2, a dose related increase of 141.7%, 46.2% and 63.6% in males and 100%, 120% and 290% in females in reticulocytes at 15, 50 and 150 mg/kg/day groups. Treatment related subcutaneous hemorrhage was present in 1, 3, 1 and 3 males and, 0, 3, 3 and 3 females of the treated groups. The hemorrhage and hematomas were observed at the venous puncture sites for blood pressure measurement sites. Extramedullary hematopoiesis in spleen of treated males and females of the study indicated that the target organ of toxicity was the spleen. The highest tolerated dose was 50 mg/kg/day.

13-Week subchronic oral gavage toxicity study in dogs was conducted at 15 to 150 mg/kg doses. White fluid/foam vomiting, discolored (green/bright) feces and subcutaneous hemorrhage in tissues and, extramedullary hematopoiesis in the spleen were reported in high dose group animals. A dose of 150 mg/kg/day was identified as the highest tolerated dose. In 52-week toxicity study in dogs, oral gavage doses of 0, 5, 25 and 50 mg/kg/day produced a treatment but non-dose proportional increase in plasma concentrations from 1.75 to 2.25 hr of administration in males and, 1 to 3 hr in females. The study did not include histopathology evaluation. The target organs of toxicity and NOAEL could not be identified and the study was not full and complete.

### **Carcinogenicity Studies:**

The 104-week mouse and rat carcinogenicity studies were not required for the short term indication.

### **Reproduction Toxicology:**

In segment I. Fertility and reproductive performance study, BAY-59-7939 from the oral doses of 12.5 to 200 mg/kg/day in male and female rats during the fertility and reproductive performance period produced a reduction in number of dams (90.5%) with viable fetuses and a slight increase in post implantation loss and a dose related reduction in ovarian weight by 8.8% in the dams of the high dose group. It was embryo- and feto- toxic NOAEL was 50 mg/kg/day. Based on body surface area ( $\text{mg}/\text{mm}^3$ ), it provides 41 times greater plasma exposure than the proposed clinical dose exposure.

BAY 59-7939 from 10 to 120 mg/kg/day doses during organogenesis period of day 6 to 17 postcoitum produced dose related increase in rat plasma concentrations and was not

teratogenic in rats. Based on body surface exposure ( $\text{mg}/\text{mm}^3$ ), the highest dose provides 97 times greater exposure than the proposed clinical dose. BAY 59-7939 in pregnant rabbits during organogenesis produced a linear increase in plasma within 0.5 to 2 h of administration and was not teratogenic. Systemic maternal NOEL and intrauterine development safe dose was 2.5 mg/kg/day in the study and provides 4 times greater exposure in the study animals.

In prenatal and post-natal toxicity study in pregnant rats, BAY 59-7939 Coprecipitate 10 % 100 at oral gavage doses of 2.5 to 40 mg/kg/day in pregnant female produced generalized tissue bleeding and still births. The dose related increase incidences of empty stomach, no detectable milk spots in stomach and intestines indicating delayed/abnormal sucking reflex in the pups born to 10 and 40 mg/kg/day treated dams was reported. The other postnatal common adverse effects in pups were hypoactivity, pale skin, cold to touch surface. The NOAEL for dams (FO) and, pre- and postnatal development of the F1 generation was 10 mg/kg/day and based on body surface area it was 8 times the exposures of the proposed human dose.

**Mutagenicity:**

BAY 59-7939 was not mutagenic in Ames test, chromosome aberrations assay in V79 Chinese hamster lung cells and the mouse bone marrow micronucleus induction test by the intraperitoneal route.

**B. Nonclinical safety issues relevant to clinical use**

None.

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 22-406

**Review number:** 001

**Sequence number/date/type of submission:** 000/July 22, 2008/Original

**Information to sponsor:** Yes ( ) No (X)

**Manufacturer for drug substance:** Johnson & Johnson Pharmaceutical Research & Development L.L.C., Raritan, NJ.

**Reviewer name:** Yash M. Chopra, M.D.; Ph.D.

**Division name:** Division of Medical Imaging & Hematology Drug Products

**HFD #:** 160

**Review completion date:** May 11, 2009

**Drug:**

Trade name: Xarelto® Tablets

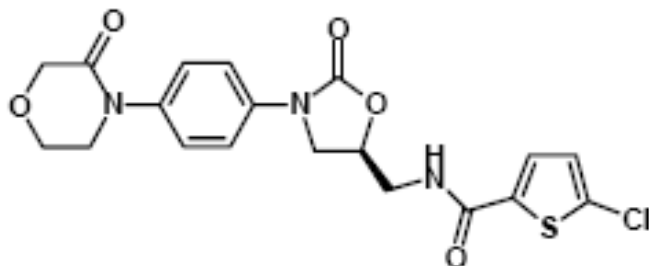
Generic/Code name: Rivaroxaban micronized/BAY 59-7939/JNJ-9039039

Chemical name: 5-Chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl) phenyl]-1, 3-oxazolidin-5-yl) methyl}-2-thiophenecarboxamide. BAY 59-7939 is a pure S-enantiomer.

CAS registry number: 496775-62-3.

Molecular formula/molecular weight: C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S/435.89.

**Structure:**



**Drug Class:** Factor Xa Inhibitor/Antithrombotic Agent

**Indication:** For the Prevention of Venous Thromboembolism (VTE) and pulmonary embolism.

**Clinical formulation (and components):** Each film-coated tablet contains (b) (4) BAY 59-7939. In addition to the active ingredient, each tablet contains the following inactive ingredients: microcrystalline cellulose, croscarmellose, lactose monohydrate, hypromellose, sodium lauryl sulfate, purified water, magnesium stearate, (b) (4) and titanium oxide.

**Relevant INDs/NDAs/DMFs:** IND 64,892

**Intended clinical population:** For the prevention of recurrent venous thromboembolic events (VTE) and pulmonary embolism in patients undergoing hip replacement surgery or knee replacement surgery.

**Route of administration:** Oral

**Recommended Dose:** The recommended dose of XARELTO™ is 10 mg (0.2 mg/kg) taken orally once daily taken at least 6 to 10 hours after surgery once hemostasis has been established.

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

**Studies reviewed within this submission:**

## PRECLINICAL STUDIES AND TESTING LABORATORIES

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5. Metabolism & Excretion of [ <sup>14</sup> C]BAY 59-7939 in Wistar rat Plasma, Urine, and Bile	PH 3461600/1 3001444	-	27

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Rats		precipitate 10% 100; Batch no. 040526-100	
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3. Bacterial Reverse Mutation Assay of Anilino-morpholinone (a by product of BAY 59-7939 co-precipitate)	T 1076143/PH-34344	BXR387U (Sample BGQ 0210-85 rein)	105
4. In Vitro Chromosomal Aberration Test with Chinese Hamster V79 Cells	Study No.: T 0073244/ AT02611/PH34198	Batch No. BX01SFS	106
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Study title: Developmental Toxicity Study in Rats after Oral Administration	#T3063590/PH-33582)	# J20020528; Coprecipitate 10%, 101	119
Study title: BAY 59-7939 Developmental Toxicity Study in Rabbits after Oral Administration	# TO062930/PH-33380/AT01303)	#J20020430	124
Study for Effects on Pre- and Postnatal Development in Rats Including Maternal Function after	T9062957/PH34608	#030723-100 BAY 59-7939 Coprecipitate 10 % 100	128

Oral Administration			
<b>SPECIAL TOXICOLOGY:</b>			
BAY 59-7939 Nanosuspension 2% (w/v) (IFT 163 Nanosuspension 2% (w/v) Subacute Toxicity Study in Wistar Rats (2 Weeks Administration by Intravenous Administration)	#T7076284/PH34646	Nanosuspension 2% (w/v)/[IFT 163 Nanosuspension 2% (w/v)]; batch # - BXOIGFL	136
Local Tolerability Study in Beagle Dogs After Paravasal, Intravenous and Intra-arterial Injections.	#. T4073400/ AT01346/PH3	#BXOINB2	141
3. In vitro 3T3 NRU (neutral red uptake) phototoxicity assay	#T9075016/AT02074/ PH-33880	#BXOINB2	144



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## 2.6.2 PHARMACOLOGY & TOXICOLOGY REVIEW

### 2.6.2.1 BRIEF SUMMARY

**Disclaimer:** Some of the Tabular and Graphical information from the sponsor's submissions are incorporated in the review.

**Introduction and drug history:** BAY 59-7939 a new molecular entity, is an oral bioavailable selective and competitive inhibitor of Factor Xa and will be used for the treatment and prevention of arterial and venous thromboembolism. Activation of Factor Xa plays a central role in the cascade of blood coagulation. Factor Xa directly converts prothrombin (Factor II) to thrombin (Factor IIA) and the catalytically active thrombin then activates platelets. The hydrolysis of fibrinogen to fibrin leads to clot formation and thromboembolism. The inhibition of Factor Xa by BAY 59-7939 blocks the generation of thrombin and prevents the formation of venous thromboembolism.

### PHARMACOLOGY:

#### Mechanism of Action:

BAY 59-7939 acts by preventing the activation of prothrombin to thrombin via the inhibition of activated factor X (FXa).

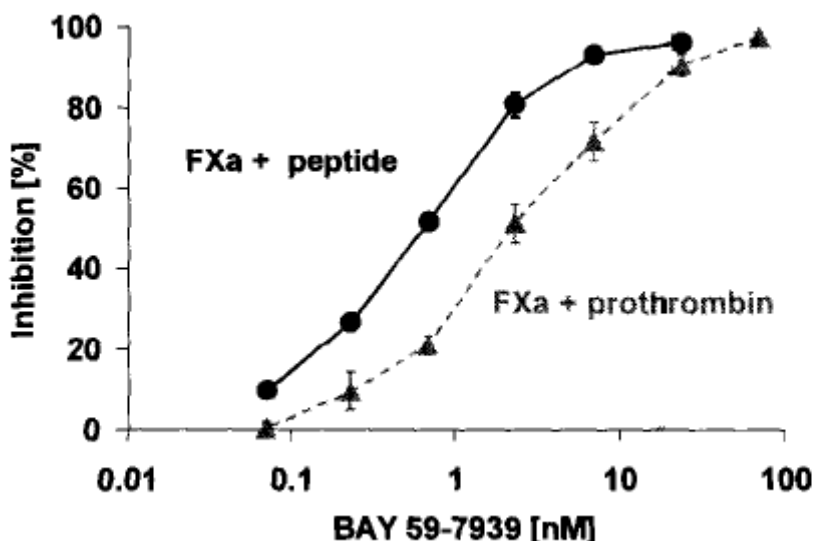
#### Drug Activity Related to Proposed Indication (Primary Pharmacology)

#### *In Vitro* Studies

##### Inhibitory Effects of BAY 59-7939 in Enzyme Assays:

The inhibitory effects of BAY 59-7939 on free Factor Xa and other serine proteases were determined using chromogenic substrates. BAY 59-7939 caused concentration-dependent inhibition of human FXa with a  $K_i$  of  $0.4 \pm 0.02$  nM. The Lineweaver-Burk analysis suggested it to be a competitive inhibitor of FXa. It did not cause inhibition of other serine proteases, such as thrombin, Factor XI, plasmin, urokinase or activated protein C at concentrations up to 69  $\mu$ M. The only serine protease affected by BAY 59-7939 was trypsin which was inhibited by 25% at 69  $\mu$ M. BAY 59-3979 exhibited a ~10,000-fold lower affinity for trypsin as compared with FXa.

The inhibitory effect of BAY 59-7939 on FXa was also shown by measuring the inhibition of prothrombin-bound FXa in a reconstituted prothrombinase complex and measuring the generation of thrombin. BAY 59-7939 inhibited thrombin generation with an  $IC_{50}$  value of  $2.1 \pm 0.4$  nM. The inhibitory effect of BAY 59-7939 on unbound and prothrombinase-bound human FXa is shown below taken from sponsor's submission.



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The inhibition of endogenous factor Xa activity in plasma was measured by converting FX to FXa by Russel's Viper Venom. The IC<sub>50</sub>s for the BAY 59-7939 inhibition of the human and rabbit FXa activity were similar (21±1 and 21±2 nM, respectively); in the rat plasma, a 15-fold higher concentration was needed to inhibit endogenous FXa (IC<sub>50</sub> = 290±18 nM).

#### Activity of compound BR-4276 (BAY 59-7939) in Enzyme Assay (PT# 1027604).

The effect of BAY 59-7939 on various *in vitro* enzyme biochemical assays was determined. Significant results are displayed in the following table.

PRIMARY TESTS							
CAT. #	PRIMARY BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC <sub>50</sub> *	K <sub>i</sub>	n <sub>H</sub>
113600	Peptidase, Factor Xa	hum	10 µM	99			
165000	Peptidase, Thrombin	hum	10 µM	88			

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\* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.

‡ Denotes item meeting criteria for significance

† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)

#### Anticoagulant Activity:

##### Platelet aggregation:

The effect of orally administered BAY 59-7939 was studied on platelet aggregation. Bay 59-7939 did not affect the human rich plasma platelet aggregation induced by collagen, U46619, ADP or thrombin receptor activated peptide (TRAP-6), at

concentrations up to 200  $\mu$ M. There was some inhibition of gamma thrombin-mediated platelet aggregation by BAY 59-7939 with an  $IC_{50}$  of 81  $\mu$ M.

**Radioligand Binding assays of compound BR-4093 (BAY 59-7939):** (PT# 1009442).

The available data of the binding of BAY 59-7939 with various receptors was consolidated.  $IC_{50}$  values were determined by a non-linear, least squares regression analysis using Data Analysis Toolbox™ (b) (4). The historical values for the  $K_d$  of the ligand (obtained experimentally (b) (4)) were used. BAY 59-7939 was found not to produce a significance binding with the selected substrates. The binding data is presented in the following table.

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<b>EXPERIMENTAL RESULTS - BIOCHEMICAL ASSAYS</b>	

CAT. #	TARGET	BATCH* SPP. n=	CONC.	70% INHIBITION					$IC_{50}$	$K_i$	$n_H$	R
				-100	-50	0	50	100				
268000	Progesterone	23932	2	10 $\mu$ M	0							
268700	Purinergic $P_{2X}$	23926	2	10 $\mu$ M	-15							
271000	Serotonin 5-HT <sub>1</sub> , Non-Selective	23778	2	10 $\mu$ M	-10							
271600	Serotonin 5-HT <sub>2</sub>	23780	2	10 $\mu$ M	14							
278300	Sigma, Non-Selective	23766	2	10 $\mu$ M	8							
279500	Sodium Channel, Site 2	23767	2	10 $\mu$ M	-12							
255510	Tachykinin NK <sub>1</sub>	23758	2	10 $\mu$ M	-5							
285000	Testosterone	23918	2	10 $\mu$ M	15							

**In vivo studies:**

**Antithrombotic Activity in an Arteriovenous Shunt Model in Rats:**

The antithrombotic activity of BAY 59-7939 was investigated in an arterio-venous shunt rat model in which the right common carotid artery and the left jugular vein were connected to affect shunt. The extracorporeal circulation was opened for 15 minutes, and the formed thrombus was weighed. BAY 59-7939 was administered orally 90 min before damage. Enoxaparin, a reference standard, was administered subcutaneously 60 min before damage.

Treatment with oral doses of BAY 59-7939 produced a dose-dependent decrease in thrombus weight with an  $ED_{50}$  value of 5.0 mg/kg (SC). The inhibition of thrombus formation was 41%, 51% and 73% by 3.0, 6.0 and 10 mg/kg BAY 59-7939 doses, respectively. At oral doses of 3-6 mg/kg in rats, the FXa activity and the concentration of TAT (antithrombin III/thrombin complex) were both reduced by 60-80% and the PT was prolonged by 2.4-3.7 fold; in contrast, the aPTT was prolonged by <1.5-fold. Thus Bay 59-7939 produced prolongation of PT and

APTT, and anti-FXa activity and TAT in the arterio-venous shunt model in rats as shown in the table.

Dose (mg/kg)	Fold PT Prolongation Mean $\pm$ S.E.	Fold aPTT Prolongation Mean $\pm$ S.E.	Anti FXa Inhibition (%) Mean $\pm$ S.E.	TAT Inhibition (%) Mean $\pm$ S.E.
1.0	1.71 $\pm$ 0.16**	N.D.	38 $\pm$ 5***	26 $\pm$ 9**
2.0	1.80 $\pm$ 0.09***	1.12 $\pm$ 0.05*	46 $\pm$ 3**	51 $\pm$ 10**
3.0	2.38 $\pm$ 0.20***	N.D.	61 $\pm$ 2***	75 $\pm$ 14*
6.0	3.66 $\pm$ 0.36***	1.40 $\pm$ 0.02***	78 $\pm$ 2***	78 $\pm$ 4*
10.0	5.06 $\pm$ 0.14***	1.77 $\pm$ 0.07***	89 $\pm$ 2	105 $\pm$ 4

N.D., not determined; \*,  $p < 0.05$ ; \*\*,  $p < 0.02$ ; \*\*\*,  $p < 0.001$

Enoxaparin also affected these parameters and ED<sub>50</sub> was 21 mg/kg for TAT inhibition in the test.

### **Effect on Thrombus Formation in Injury-Induced Arterial and Venous Thrombosis in Rats:**

The antithrombotic activity of BAY 59-7939 was determined in rats with mechanically damaged carotid artery and the jugular vein. Thrombus development was induced by chilling the vessels, and after 4 hours the thrombi were removed and weighed. BAY 59-7939 was administered orally 90 minutes before damage, whereas enoxaparin (reference standard) was administered 15 minutes before damage of the blood vessels.

On the venous site, BAY 59-7939 caused a dose-dependent reduction of the thrombus mass with an ED<sub>50</sub> value of 2.0 mg/kg. In the arterial site, the thrombus formation was more resistant to inhibition by BAY 59-7939 and at 10 mg/kg, the thrombus weight was reduced by 51  $\pm$  8%. Enoxaparin had ED<sub>50</sub> values were 1 and 3 mg/kg for the venous and arterial thrombus, respectively.

### **Prothrombin complex concentrate reduced the rivaroxaban (BAY 59-7939) induced bleeding time prolongation in anaesthetized rats (Report #PH-35374/#2008-05-13)**

In rat mesenteric artery bleeding model, the effect of BAY 59-7939 on prothrombin complex concentrate (PPC) formation and the prolongation of the bleeding time was evaluated in anesthetized rats. Bleeding was induced by a pretreatment with intravenous rivaroxaban (2 mg/kg, 1 ml/kg). The catheter was rinsed with 0.2 ml solvent. PPC (50 U/kg in 2 ml/kg or 25 U/kg in 2 ml/kg; n=10 per dose group) or vehicle (0.9 % NaCl, n=7) were injected one minute after induction of bleeding with high doses of rivaroxaban and Beriplex® (50 U/kg) was given 1 min after the induction of bleeding. Rivaroxaban (2 mg/kg i.v.) significantly prolonged the mesenteric bleeding time relative to baseline by 5.4 $\pm$ 1.4-fold (162 $\pm$ 10 versus 861 $\pm$ 213 seconds). Administration of 50 U/kg Beriplex®, given one minutes after induction of bleeding, nearly completely normalized the bleeding time to 242 $\pm$ 56 seconds (1.5 $\pm$ 0.4-fold,  $p > 0.05$  compared to baseline). The low dose of 25 U/kg Beriplex® was ineffective. Thus the data suggest that PPC may have the potential as a possible antidote to the direct FXa inhibitor rivaroxaban.

**Hemostatic effects of activated factor VII and activated prothrombin complex concentrate in rivaroxaban-anticoagulated primates (Study No.: R-8472/Product Report 2008-03-10)**

The study was done in animals with impaired hemostasis induced by a 0.6 mg/kg bolus dose of intravenous rivaroxaban followed by continuous infusion at a rate of 0.6 mg/kg/h for 1 hour (lowest proposed clinical dose). To the anticoagulated animals, either an intravenous bolus dose of 210 ug/kg Recombinant human FVIIa or APCC (Feiba VH) infusion dose of 50 U/kg over 25min (2u/kg/min) was administered to reverse hemostasis impairment by rivaroxaban. In another set of experiments, primary hemostasis was assessed as template bleeding time (BT). Rivaroxaban prolonged BT to about 202 to 254% (95% CI  $\pm$ 30%;  $P < 0.019$ ). FVIIa (n=7) shortened the BT to 168% at a dose of 210  $\mu$ g/kg that was lower than the safe (not prothrombotic) dose of FVIIa. Feiba VH (N=7) reversed the bleeding time to the baseline level at the end of Feiba infusion. FVIIa and FEIBA VH administration during antithrombotic treatment with rivaroxaban supported hemostasis. The study did not determine if either FVIIa or FEIBA VH increased the rate of thrombus propagation in rivaroxaban-treated animals.

**Effects of JNJ-39039039 (Rivaroxaban) on Thrombotic Occlusion in Electrolytically Injured Rat Carotid Artery (DD07446) JNJ-39039039 (Rivaroxaban)**

The antithrombotic activity of JNJ-39039039 (rivaroxaban) was evaluated in a model of arterial thrombosis in anesthetized rats with thrombotic occlusion (induced by electrolytic injury of a carotid artery). No significant difference in the time to occlusion between vehicle control and the lowest dose of 0.3 mg/kg JNJ-39039039 was seen. However, single intravenous infusion doses of 1 or 3 mg/kg JNJ-39039039 produced marked antithrombotic activity (median occlusion time  $> 30$  min). JNJ-39039039 at 1 and 3 mg/kg produced greater increase in median time to occlusion than a single dose of enoxaparin infused at a dose of 10 mg/kg (median time to occlusion = 21.6 min). BAY 59-7939 showed a dose-dependent increase in activated clotting time (ACT), prothrombin time (PT) and Russell's viper venom time (RVVT), and substantially decreased the formation of TAT complexes, but did not substantially affect aPTT. The study supported the use of BAY 59-7939 in arterial thrombotic disorders such as ACS.

**Antithrombotic Activity in an Arteriovenous Shunt Model in Rabbits:**

The antithrombotic activity of BAY 59-7939 was evaluated in an arteriovenous shunt model in rabbits in which the right common carotid artery and the left jugular vein were connected by a polyethylene catheter containing a rough thrombogenic nylon thread. The extracorporeal circulation was opened 10-20 minutes after IV administration of BAY 59-7939 or enoxaparin. The extracorporeal circulation was opened for 15 minutes and after which the thrombi were removed and weighed. Intravenous administration of BAY 59-7939 caused

a dose-dependent reduction of thrombus mass in the rabbit model with an ED<sub>50</sub> of 0.6 mg/kg. At the doses of 0.3, 1.0 and 3.0 mg/kg, thrombus formation was inhibited by 40 ± 10%, 58 ± 5% and 83 ± 7%, respectively. BAY 59-7939 also caused a dose dependent prolongation of PT (1.1 to 1.3 times the control value) and aPTT (1.3 to 8.6 times the control value) in the rabbit. Enoxaparin caused a dose-dependent reduction of thrombus mass with an ED<sub>50</sub> value of 1.8 mg/kg (based on body surface area: HED= 0.58 mg/kg and was about 3 times the intended clinical dose).

#### **Effect of BAY 59-7939 on Rat Tail and Rabbit Ear Bleeding Time:**

The effect of BAY 59-7939 on the rat tail bleeding time was examined 90 minutes after oral administration of the drug. Subcutaneous enoxaparin was administered 60 minutes before measurement of bleeding time. The bleeding time was determined as the time from cutting the tip of the tail to the time of cessation of blood flow for longer than 30 seconds. The effect of the drug on the rabbit ear bleeding time was examined after IV dosing. BAY 59-7939 caused a dose-dependent increase in the bleeding time at or greater than 3.0 mg/kg dose, which was identified as the antithrombotic dose in the rat arteriovenous shunt model. At this dose there was 41% reduction in thrombosis in the rat model. There was a 2-fold increase in the bleeding time at 6.0 mg/kg of BAY 59-7939, and at 10 mg/kg dose, a 2.7-fold increase in the bleeding time was observed. With subcutaneous enoxaparin, a 2-fold increase in the bleeding time was observed at 10 mg/kg (the lowest antithrombotic dose in the rat arterio-venous shunt model). Prolongation of bleeding time was greater with enoxaparin as compared with BAY 59-7939. The effects of oral doses of BAY 59-7939 and SC doses of enoxaparin on the bleeding time in rat tails are summarized in the Table below.

<b>BAY 59-7939</b>		<b>Enoxaparin</b>	
<b>Group</b>	<b>Bleeding time (sec) mean ± S.E</b>	<b>Group</b>	<b>Bleeding time (sec) mean ± S.E</b>
Control	179 ± 13	Control	168 ± 5.0
3.0 mg/kg	172 ± 11	3.0 mg/kg	170 ± 5.0
Control	185 ± 5	Control	187 ± 5.0
6.0 mg/kg	381 ± 42*	10 mg/kg	365 ± 37*
Control	171 ± 7	Control	258 ± 13
10 mg/kg	466 ± 29*	30 mg/kg	592 ± 8.0*

In the rabbit ear model, BAY 59-7939 caused a dose-dependent prolongation of bleeding time from 1.0 mg/kg. There was a 4-fold increase in the bleeding time at 1.0 mg/kg and 4.7-fold increase was at 3.0 mg/kg. Enoxaparin, at IV doses of 3.0 and 10 mg/kg, caused 1.5 and 2.7-fold increases, respectively, in the rabbit ear bleeding time.

#### **Effect on Drug Metabolism by Different Cytochrome P450 Isoforms:**

The ability of BAY 59-7939 to inhibit different human cytochrome P450 (CYP) isoforms (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) was studied after incubation with standard substrates in the absence or presence of the drug. The substrates used for different CYP isoforms were: 7-

ethoxycoumarin (CYP1A2), coumarin (CYP2A6), taxol (CYP2C8), tolbutamide (CYP2C9), mephenytoin (CYP2C19), dextromethorphan (CYP2D6), chlorzoxazone (CYP2E1) and testosterone (CYP3A4).

BAY 59-7939 (100  $\mu$ M) did not inhibit the formation of the metabolites by the CYP isoforms except the CYP2C9 induced metabolites. The tolbutamide metabolite formation which is formed by CYP2C9 isoform was reduced by 34% in the presence of 100  $\mu$ M BAY 59-7939, as compared with the control. This indicated CYP2C9 could have a role in the metabolism of BAY 59-7939.

### **In vitro Studies in L-MDR1 Cells to Evaluate the P-gp-substrate Characteristics**

In L-MDR1 cells preparation, Ketoconazole and amiodarone showed a significant inhibition on the efflux ratio of BAY 59-7939 with IC<sub>50</sub> values of 8.98 $\pm$ 3.44  $\mu$ M and 14.1  $\mu$ M, respectively. Oral doses of 400 mg Ketoconazole and 400 mg Amiodarone o.d. produced peak plasma concentrations (C<sub>max</sub>) of 17.5 and 2.2 to 3.5  $\mu$ M, respectively in human. The C<sub>max</sub> after repeated oral administration in combination with 10 mg BAY 59-7939 was significantly high. Sponsor proposed the observed decrease of the renal clearance for BAY 59-7939 in patients receiving Ketoconazole was due to the inhibition of P-gp. The partial renal clearance in these patients after co-administration of Ketoconazole suggested for the involvement of a second transport protein. The co-administration of Amiodarone with BAY 59-7939 resulted in insignificant effect. Ritonavir inhibited the efflux ratio of BAY 59-7939 in L-MDR1 cells in a concentration dependent manner with an IC<sub>50</sub> of 27.9 $\pm$ 10.3  $\mu$ M.

In conclusion, the *in vitro* study with L-MDR1 cells, BAY 59-7939 was a moderate P-gp substrate.

### **Pharmacology summary:**

BAY 59-7939 (Xarleto) a selective and competitive oral inhibitor of Factor Xa, produced concentration dependent inhibition (human FXa activity with a K<sub>i</sub> of 0.5  $\pm$  0.02 nM) with no inhibitory activity on serine proteases such as thrombin, Factor X1, plasmin, urokinase, or activated protein C. It inhibited thrombin generation with an IC<sub>50</sub> of 2.1 nM and induced a prolongation of PT and aPTT in human plasma. Increased bleeding times in rats and rabbits was observed in a concentration dependent manner. A two-fold increase in bleeding time) was observed at 26  $\mu$ M. It showed no effect on collagen, ADP or thrombin receptor-activated peptide (TRAP-6) induced platelet aggregation. However, an inhibition of thrombus formation in an arteriovenous shunt model in rats and rabbits and, injury induced venous thrombus formation in rats was seen. Thus, BAY 59-7939 has the potential of being useful in the prevention of arterial and venous thrombosis through inhibition of FXa.



## **SAFETY PHARMACOLOGY:**

### **Effects on Cardiovascular and Respiratory Systems of anesthetized Dogs after Single Intraduodenal Administration:**

The influence of BAY 59-7939 on the cardiovascular and respiratory function was examined in anesthetized, artificially respired beagle dogs after single intraduodenal doses of 3, 10 and 30 mg/kg of the drug. The effects of the drug on heart rate, cardiac output, cardiac left ventricular pressure, rise of left ventricular pressure (dP/dt) rate, left ventricular end-diastolic pressure were determined. Three leads standard limb EKGs were recorded to measure PQ-interval, QT-interval and QRS-interval.

Respiration rate and respiration pressures (peak inspiratory and expiratory pressures) were measured with a pressure transducer. Arterial pH, partial O<sub>2</sub> and CO<sub>2</sub> pressure, plasma sodium- and potassium concentrations, and hematocrit were measured using an automatic blood gas/electrolyte analyzer.

BAY 59-7939, at intraduodenal doses up to 30 mg/kg, had no effects on cardiovascular function, EKG, respiration, acid/base balance, hematocrit and electrolytes in beagle dogs. After intraduodenal administration of BAY 59-7939 to dogs, the plasma exposure levels increased with increasing dose. The T<sub>max</sub> was 1 hr at the low dose and 2-4 hr at the high dose and, the maximum plasma concentrations (C<sub>max</sub>) at high dose was up to 8.5 µg/L.

### **Effect on the Central Nervous System (CNS) of Rats:**

Effects of a Single Oral Dose of BAY 59-7939 on the behavior and physiological state: The effects of single oral gavage dose of 3, 10 or 30 mg/kg BAY 59-7939 on the behavioral and physiological state, open-field behavior and body temperature were examined in rats. The animals were observed at 30 minute intervals for changes in body temperature, and at 30, 60 and 120 minutes after dosing for changes in behavior and physiological state. Single oral dose of BAY 59-7939 had no effect on the normal behavior, behavior in the open field and the body temperature of the animals. One (of six) animal receiving the low dose of 3 mg/kg dose showed tremor that was considered not related to treatment with the drug.

Effects on pentylenetetrazole convulsive threshold, nociceptive responsiveness to heat and hexobarbital induced anesthesia in rats: The effects of BAY 59-7939 on pentylenetetrazole convulsive threshold, nociceptive responsiveness to heat (hot plate test) and hexobarbital-induced anesthesia were examined in male rats after oral administration of 3, 10 and 30 mg/kg doses. The convulsive threshold and nociceptive responsiveness in rats were examined 30 minutes after the dosing, and hexobarbital-induced anesthesia was evaluated 45 minutes post-administration of BAY 59-7939. No significant effect on the convulsive threshold dose of pentylenetetrazol or the duration of hexobarbital-induced anesthesia was seen at oral doses up to 30 mg/kg. However, dose-dependent delay (control, 25.6±7.5 sec,

low dose  $26.4 \pm 7.3$  sec, mid dose  $34.9 \pm 6.0$  sec, high dose  $32.4 \pm 10.4$  sec) at 10 and 30 mg/kg doses was observed in nociceptive reactions to heat indicating a weak analgesic effect of the drug.

**Effects on Renal Function, Hematology, Blood Glucose and Lipid Metabolism in Rats:**

BAY 59-7939, at oral doses up to 30 mg/kg produced no effect on urine volume or urinary potassium and chloride excretion. A slight insignificant increase in the sodium excretion was observed at all doses compared with the vehicle treated animals ( $0.10 \pm 0.06$ ,  $0.14 \pm 0.13$ ,  $0.19 \pm 0.06$  and  $0.16 \pm 0.07$  mmol/kg/2 hours in the control, low, mid and high doses, respectively).

BAY 59-7939 had no effect on the RBC, WBC or platelet counts, hemoglobin and hematocrit values. The coagulation parameters, such as thrombin time and thromboplastin time were dose-dependently prolonged by BAY 59-7939. Fasted or fed blood glucose concentrations and cholesterol or triglycerides levels were not affected by BAY 59-7939.

**Effects on the Gastrointestinal Tract:**

Male rats were treated with oral doses of 3, 10 and 30 mg/kg of the drug and the control animals received the vehicle. A 20% suspension of barium sulfate in 0.5% aqueous tylose was administered by oral gavage 30 minutes after BAY 59-7939 or vehicle administration to observe GI motility. Animals were then sacrificed 30 min after barium sulfate administration and the length of the intestinal segment covered with barium sulfate was measured. Oral administration of 30 mg/kg BAY 59-7939 had no effect on the intestinal barium sulfate transit time in rats, and was considered to have no effect on the gastrointestinal motility.

**Effects on the Contractility of the Isolated Guinea Pig Ileum:** The effects of BAY 59-7939 ( $10^{-7}$  and  $10^{-6}$  g/ml) on the guinea pig ileum baseline tone or on the contractions induced by acetylcholine, serotonin, histamine and barium chloride were examined in vitro. BAY 59-7939 addition in the bath 2 minutes before the contractile substances, showed no contractile or relaxant effect on the isolated guinea pig ileal segments at concentrations up to  $10^{-6}$  g/ml. It produced insignificant effect on the acetylcholine, histamine, serotonin or barium chloride induced contractions of the ileum.

Thus, BAY 59-7939 had no effect on the *in vivo* motility of the gastrointestinal tract of rats or the *in vitro* contractions of the isolated guinea pig ileum.

**Safety pharmacology summary:**

BAY 59-7939 had no effect on the cardiovascular function, EKG, respiration, acid/base balance, hematocrit and electrolytes in anesthetized beagle dogs at intraduodenal doses up to 30 mg/kg. It had no central nervous system (CNS) effects in male rats up to the oral doses of 30 mg/kg. BAY 59-7939 did not alter

the convulsive threshold dose of pentylenetetrazol or duration of hexobarbital-induced anesthesia in rats but showed mild analgesic effect as it produced a delay in nociceptive response. Oral administration of BAY 59-7939 to rats did not cause changes in urine volume, urinary electrolytes or hematological parameters. It showed no effect on the gastrointestinal motility in rats.

#### **2.6.2.5 Interaction Studies:**

##### **In Vitro Interaction of rivaroxaban and linezolid:**

Rivaroxaban is structurally similar to the oxazolidinone derivative linezolid, which has ability to bind to the bacterial ribosome and to inhibit bacterial protein synthesis. Linezolid showed good and specific activity against Gram positive bacteria. Possible interactions of rivaroxaban against three Gram positive reference strains were studied. At concentrations from 0.006 to 0.2 ug/mL and 0.25 to 8 ug/mL, respectively, of rivaroxaban and linezolid, this combination showed no effect on the antibacterial activity of linezolid. It showed that rivaroxaban does not exert antagonistic or agonistic activity with regard to the antibacterial activity of linezolid.

##### **Interaction between BAY 59-7939 and 3 Antiphlogistic Drugs (ASA, Diclofenac and Naproxen) on Bleeding Time in the Anesthetized Rat (P.O. Administration) Study No.: T 6063070**

BAY 59-7939 at oral doses of 3 and 10 mg/kg produced a significant prolongation of bleeding time (+279% and +213%, respectively;  $p < 0.01$  each), and a high tendency towards an increase was observed (+91%,  $p = 0.054$ ) at 1 mg/kg BAY 59-7939 dose. Combinations of BAY 59-7939 (1, 3 and 10 mg/kg p.o.) with ASA (10 mg/kg p.o.) or BAY 59-7939 (1 and 3 mg/kg p.o.) with ASA (30 mg/kg p.o.) did not significantly differ the bleeding time of BAY 59-7939 alone-treated groups.

BAY 59-7939 (1, 3 and 10 mg/kg p.o.) with diclofenac (1 mg/kg p.o.) or BAY 59-7939 (1 and 3 mg/kg p.o.) with diclofenac (3 mg/kg p.o.) had no significant effects on bleeding time as compared with the BAY 59-7939-treated groups. But combination of BAY 59-7939 (1 mg/kg p.o.) with naproxen (5 mg/kg p.o.) or BAY 59-7939 (1 mg/kg p.o.) with naproxen (20 mg/kg p.o.) showed significant increase in bleeding time as compared with the BAY 59-7939 (+151% and +142%,  $p < 0.001$  and  $p < 0.01$ , respectively). Combinations of BAY 59-7939 (3 and 10 mg/kg p.o.) with naproxen (5 mg/kg p.o.) or BAY 59-7939 (3 mg/kg p.o.) with naproxen (20 mg/kg p.o.) had no statistically significant effects on bleeding time as compared with the BAY 59-7939-treated groups. These results suggest that BAY 59-7939 (3 and 10 mg/kg p.o.) prolonged bleeding time in the anesthetized rat. ASA (30 and 100 mg/kg p.o.) or naproxen (20 and 80 mg/kg p.o.) treatments prolonged the bleeding time and diclofenac (1, 3 and 10 mg/kg p.o.) did not produce an effect on the bleeding time. In contrast, BAY 59-7939

when combined with naproxen (5 and 20 mg/kg p.o.) showed additive effects on bleeding time.

BAY 59-7939 (3 and 10 mg/kg p.o.) produced a significant prolongation of bleeding time in the anesthetized rat. A prolongation of bleeding time was reported in groups of animals treated with ASA (30 and 100 mg/kg p.o.) or naproxen (20 and 80 mg/kg p.o.), but not with diclofenac (1, 3 and 10 mg/kg p.o.). No clear additive effects on bleeding time were observed in groups treated with BAY 59-7939 and ASA (10 and 30 mg/kg p.o.) or diclofenac (1 and 3 mg/kg p.o.). Some additive effects on bleeding time were reported when BAY 59-7939 was combined with naproxen.

## **PHARMACOKINETICS/TOXICOKINETICS**

Pharmacokinetics of BAY 59-7939 was investigated in Wistar rats and beagle dogs after oral and IV administration of unlabeled BAY 59-7939 or [<sup>14</sup>C]BAY 59-7939.

### **Absorption/PK:**

#### **RATS:**

##### **1. PK of a single IV or PO dose of unlabeled BAY 59-7939 to rats: (Study #PH-32007)**

Plasma pharmacokinetics of BAY 59-7939 in male rats (3 animals/group) was investigated following single IV doses of 1.0 and 3.0 mg/kg and single oral doses of 1.0, 3.0 and 10 mg/kg of unlabeled BAY 59-7939 in PEG 400 diluted in distilled water. The plasma concentrations of the drug were determined by high pressure liquid chromatography and mass spectrometric analysis. The pharmacokinetic parameters were calculated from the mean plasma concentrations by non-compartmental analysis. After oral administration of single doses of BAY 59-7939 to male rats, the drug was rapidly absorbed. The AUC and C<sub>max</sub> values increased with increasing doses and the t<sub>1/2</sub> ranged from 1.2 hours, at the high dose to 2.3 hours, at the low dose. The absolute bioavailability was approximately 60% (65.5% and 59.2% at 1.0 and 3.0 mg/kg, respectively). After 1.0 and 3.0 mg/kg single IV doses, the maximum plasma concentrations and the exposure levels increased with increasing doses. After IV dosing, the t<sub>1/2</sub> was less than 1.0 hour (0.83 and 0.94 hour, at 1.0 and 3.0 mg/kg doses, respectively). PK parameters after oral and IV administration of single doses of unlabeled BAY 59-7939 to male rats are shown in the sponsor's Table below.

Pharmacokinetic parameters calculated from the geometric means of the individual plasma concentrations after intravenous and oral administration as a solution to fasted male Wistar rats (n = 3 per time point).

Route		intravenous		oral		
Dose [mg/kg]		1	3	1	3	10
AUC	[mg·h/l]	2.27	8.51	1.49	5.04	14.7
AUC <sub>norm</sub>	[kg·h/l]	2.27	2.84	1.49	1.68	1.47
AUC(t <sub>0</sub> -∞)	[%]	1.88	0.0996	2.88	0.914	1.24
C <sub>max</sub>	[mg/l]	4.52	11.5	0.926	3.11	6.01
C <sub>max, norm</sub>	[kg/l]	4.52	3.85	0.926	1.04	0.601
t <sub>1/2</sub>	[h]	0.830	0.935	2.29	1.41	1.19
points*	terminal	3.00	3.00	3.00	3.00	4.00
Interval*	[h]	1-4	4-8	4-8	4-8	2-8
V <sub>ss</sub>	[l/kg]	0.323	0.277	n.c.	n.c.	n.c.
CL	[l/(h·kg)]	0.440	0.352	n.c.	n.c.	n.c.
f	[%]	n.c.	n.c.	58.2**	65.6**	57.4**

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n.c. = not calculated

\* = used for regression to determine t<sub>1/2</sub>

\*\* = calculated with the mean AUC<sub>norm</sub> (2.56 kg·h/L) after 1 and 3 mg/kg i.v.

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The pharmacokinetics of IV bolus and oral dose of 3 mg/kg [<sup>14</sup>C]BAY 59-7939 was studied in male Wistar rats (PH-32007). Additionally, the studies were conducted in bile duct-cannulated rats following intraduodenal and IV administration of the radiolabeled compound. Absorption of the radioactivity from GI tract was 66.8% after oral administration. The t<sub>1/2</sub> for the parent compound was 1.46 hours and for the total radioactivity 42.1 hours. The pharmacokinetic parameters after oral and IV administration a 3 mg/kg dose to male Wistar rats are summarized in the sponsor's Table:

**Table 1: Pharmacokinetics of radioactivity in male Wistar rats after single administration of [<sup>14</sup>C]BAY 59-7939. Pharmacokinetic parameters calculated from geometric means of 3 male animals per time point.**

Dose	[mg/kg]	3	3
Route		i.v.	p.o.
AUC	[mg·eq·h/L]	8.78	4.20
AUC <sub>norm</sub>	[kg·h/L]	2.82	1.35
CEQ <sub>max</sub>	[mg·eq/L]	n.c.	2.04
CEQ <sub>max, norm</sub>	[kg/L]	n.c.	0.657
T <sub>max</sub>	[h]	n.c.	0.25
t <sub>1/2app</sub>	[h]	1.81	1.46
Interval*	[h]	4 – 8	4 – 8
t <sub>1/2</sub>	[h]	18.8	42.1
Interval*	[h]	8 – 48	24 – 72

\* = used for regression to determine terminal t<sub>1/2</sub>

n.c. = not calculated

## DOGS:

### 2. PK of a single IV or PO dose unlabeled BAY 59-7939 to fasted female

**dogs:** (Study #PH-33250/31990/I 6001555)

In fasted female dogs, PK after a single IV administration (0.25 hr infusion) of 0.3 mg/kg and single oral dose of 0.3 and 3.0 mg/kg of unlabeled BAY 59-7939 (batch#507018) was determined. Samples of blood were withdrawn at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 24, 30 and 48 hours after administration and the plasma drug concentrations determined by high pressure liquid chromatography (HPLC) with mass spectrometric (MS/MS) detection. The pharmacokinetic parameters were calculated from the mean plasma drug concentrations by non-compartmental analysis.

A single IV bolus dose of <sup>14</sup>BAY 59-7939 to female dogs has a  $t_{1/2}$  of about 1.0 hour and the plasma concentrations were about 5% of  $C_{max}$  within 4 hr. An oral dose of 1 mg/kg achieved a peak ( $C_{max}$ ) of unchanged compound of 1.28 mg/L in plasma in  $T_{max}$  of 0.57 h after dosing. The total plasma clearance of unchanged compound was 0.31 L/kg·h. The volume of distribution at steady state ( $V_{ss}$ ) was to 0.4 L/kg and unchanged BAY 59-7939 occurred with half-lives of about 1 h. The  $t_{1/2}$  of radioactivity elimination was prolonged to 3-8 h, similar to oral administration. The  $t_{1/2}$  of radioactivity amounted to 1.1 h and the  $t_{1/2}$  of unchanged compound was 0.79 h in the time interval 3-6 h after administration. The volume of distribution at the steady state was 0.4 l/kg. After oral dose of 0.3 and 3.0 mg/kg, the  $C_{max}$  and AUC values increased in non-dose proportional manner, and the  $t_{1/2}$  was about 1.0 hour. The absolute bioavailability in dogs was 60%. The pharmacokinetic parameters in female dogs after IV and oral dosing are summarized in the sponsor's Table below.

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Route Dose [mg/kg]		Intravenous		Oral			
		0.3		0.3		3	
		Mean geom.	S.D. geom.	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.
AUC	[mg·h/L]	1.00	1.35	0.605	2.04	6.03	1.39
AUC <sub>norm</sub>	[kg·h/L]	3.34	1.35	2.02	2.04	2.01	1.39
C <sub>max</sub>	[mg/L]	0.762	1.26	0.254	1.35	2.72	1.23
C <sub>max, norm</sub>	[kg/L]	2.54	1.26	0.848	1.35	0.906	1.23
t <sub>max</sub>	[h]	n.c.	n.c.	0.454	1.74	0.500	2.00
t <sub>1/2</sub>	[h]	0.952	1.27	0.876	1.12	0.924	1.09
Interval*	[h]	3 - 6		2 - 8		3 - 8	
V <sub>ss</sub>	[L/kg]	0.402	1.33	n.c.	n.c.	n.c.	n.c.
CL	[L/(h·kg)]	0.300	1.35	n.c.	n.c.	n.c.	n.c.
F	[%]			60.4		60.2	

n.c. = not calculated

\* = used for regression to determine  $t_{1/2}$

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**Dog:** (Study #PH33250) In female Beagle dogs, the PK of radioactivity and of unchanged substance were investigated after a single intravenous short-term infusion ( $T = 0.25$  h), and after oral administration by gavage at different doses in a cross-over fashion. The estimated PK of radioactivity in rats and dogs is shown in the following sponsor's table:

Table 3-1: Pharmacokinetics of radioactivity after single administration of [<sup>14</sup>C]rivaroxaban (PH-32076, Version 3; PH-33250)

Species		Rat	Rat	Dog	Dog
Dose	[mg/kg]	3	3	1	1
Route		i.v.	p.o.	i.v.	p.o.
AUC	[mg-eq-h/L]	8.78	4.20	5.55	5.10
AUC <sub>0-∞</sub>	[kg-h/L]	2.82	1.35	5.55	5.10
CEQ <sub>max</sub>	[mg-eq/L]	NC	2.04	2.80	1.67
CEQ <sub>max, nom</sub>	[kg/L]	NC	0.657	2.80	1.67
t <sub>max</sub>	[h]	NC	0.25	0.25	0.572
t <sub>1/2app</sub>	[h]	1.81	1.46	1.39	1.14
Interval <sup>a</sup>	[h]	4 – 8	4 – 8	3 – 8	3 – 6
t <sub>1/2</sub>	[h]	18.8	42.1	111	128
Interval <sup>a</sup>	[h]	8 – 48	24 – 72	48 – 168	48 – 168

a = used for regression to determine half-life

NC = not calculated

Source: M4.2.2.2.1, PH-32076, Version 3; M4.2.2.2.3, PH-33250

The absorption of radioactivity in rats was 67 % and 92 % in dogs after oral administration of rivaroxaban.

### Tissue Distribution:

#### 3. Qualitative tissue distribution of the radioactivity after IV (1 mg/kg) or oral (3 mg/kg) doses of [<sup>14</sup>C]BAY 59-7939 in Wistar rats and in oral (3 mg/kg) doses of [<sup>14</sup>C]BAY 59-793 in Pigmented Rats (Study No.: I 8001449/#32339)

The qualitative tissue distribution patterns of the radioactivity in Wistar rats after IV (1 mg/kg) or oral (3 mg/kg) doses of [<sup>14</sup>C]BAY 59-7939 was determined and, PK of a single 3 mg/kg oral dose in pigmented Long Evans rat was also determined. The rats were sacrificed at selected times up to seven days after administration.

The qualitative distribution patterns of the radioactivity were similar in male and female rats after oral and IV administration. No significant penetration of the radioactivity across the blood brain barrier was observed in rats. As compared with the Wistar rats, slightly higher radioactivities were observed in some melanin containing tissues (eye wall, Harderian gland) of the pigmented rat. Two to 8 hours following oral administration of a radiolabeled dose to male Wistar rats, the highest concentrations were detected in the GI contents and contents of the bile ducts and urinary bladder followed by liver, kidney, skin, intestinal mucosa, coagulation gland, blood, heart, lung, skeletal muscles, testes, seminal vesicles, salivary and lachrymal glands, lymphatic system, pancreas, thyroid, adrenals and adipose tissues. Twenty-four hours after an oral dose, the highest radioactivity concentrations were observed in the intestinal contents and moderate concentrations were found in the liver and contents of the urinary bladder. After 7 days, moderate to low concentrations were detectable in the liver, kidneys, skin, hair follicles and the GI contents.

#### 4. In Vitro Protein Binding and Distribution into Blood Cells: (study #PH-32966)

Binding of BAY 59-7939 to plasma proteins was determined by ultrafiltration method after incubating [<sup>14</sup>C]BAY 59-7939 with plasma from humans, rats, mice, dogs and rabbits. Plasma from human, Wistar rats, dogs, rabbits and mice were

incubated with 1.08, 0.84, 0.91, 1.12 and 1.01 mg/l concentrations of radiolabeled BAY-59-7939 and the bound and the unbound fractions were separated.

Trial no.	Batch-no.	Radiochem. purity [%]	Chemical purity [%]	Specific radio-activity [MBq/mg]	Date of control
54, 56	PLS 0451-1-03A	99.88	98.48	2.69	Jan. 19 and 26, 2001
65	PLS 0451-12A	99.5	99.5	2.66	Feb. 03, 2003

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[<sup>14</sup>C]BAY 59-7939 was bound extensively to plasma proteins and the extent of binding varied among different species. The fraction of unbound BAY 59-7939 ( $f_u$ ) was lowest in rats (0.86%) and highest in rabbits (18.2%). In humans, the  $f_u$  was 2.8% at a BAY 59-7939 concentration of approximately 1 mg/l. Thus, BAY 59-7939 was 98.9%, 99.1%, 93.4%, 71.8% and 96.1% bound to plasma proteins from human, rats, dogs, rabbits and mice, respectively. The main binding was in human plasma proteins and; acidic  $\alpha_1$ -glycoprotein played only a minor role in the binding of the compound in human plasma.

### **Metabolism:**

#### **In vivo:**

#### **5. Metabolism & Excretion of [<sup>14</sup>C]BAY 59-7939 in Wistar rat Plasma, Urine, and Bile after Oral, Intraduodenal and Intravenous Dose: (Study #PH-3461600)**

The metabolite profile of [<sup>14</sup>C]BAY 59-7939 was determined in the Wistar rat plasma, urine, and bile after oral, intraduodenal and intravenous administration. Radiolabeled [<sup>14</sup>C]-BAY 59-7939 was administered in bile duct-cannulated male Wistar rats by the intraduodenal and IV routes at a dose of 3 mg/kg. In addition, plasma and urine samples were obtained after oral and IV administration of a 3 mg/kg dose. Samples were collected at 1, 2, 4 and 8 hours after administration of the drug. Samples of plasma and excreta were analyzed by HPLC and NMR spectroscopy and LCMS analysis were used to determine the structures of the metabolites.

At all the time points, the unchanged drug was the main component in the plasma. The unchanged drug represented 88% of the total radioactivity at 1 hour and 78% at 8 hours after administration. M-1 was the main metabolite and represented 8% of the radioactivity at 8 hours. Two other metabolites M-2 and M-3 were the minor metabolites and accounted for less than 3% of the radioactivity at 1 or 8 hours. The metabolite profiles in the plasma samples 8 hours after oral administration of a 3 mg/kg dose is shown in the sponsor's Table below.



**Table 4: [<sup>14</sup>C]BAY 59-7939**

Metabolite profiles (8 hours) in different plasma samples after oral administration of 3 mg/kg body weight to male Wistar rats (study no. I 4001472/01).

animal no.		(b) (4)	arithmetic mean
data file			
work up recovery[%]			84.3
retention time [min]	identification		
8.3	unknown		2.7
11.5	unknown		0.0
13.0	unknown		0.3
15.1	M-1		7.7
18.0	unknown		0.0
18.3	unknown		0.7
19.1	M-2		3.3
19.4	M-3		3.0
20.4	unknown		4.1
21.5	drug		78.1
22.5	unknown		0.3
compounds balanced			100.0
compounds identified			92.0
total number of compounds			

About 24-28% of the administered dose was excreted in the urine in 48 hours after IV or oral administration. Unchanged drug accounted for 22-31% of the total radioactivity in the urine and 3 major metabolites were identified (M-1, M-3 and an unidentified metabolite). The main metabolite, M-1 accounted for 33-39% of the radioactivity (about 9% of the dose), and M-3 accounted for 16-17% of the radioactivity (about 4% of the dose). The unidentified metabolite accounted for 13-14% of the radioactivity (3-4% of the dose) and M-1 represented less than 1% of the dose.

In the 24-hour urine of the bile duct cannulated (BDC) rats (Study #PH34616600/I3001444), the unchanged drug accounted for 3% of the dose that was similar to that observed in intact animals. The major metabolites were M-1, M-3 and an unknown metabolite. M-2 was a minor metabolite representing <1% of the dose. The metabolite profiles in the 0-48 hour urine fractions after oral administration of a 3 mg/kg dose in rats is shown in sponsor's Table below.

**Table 5: [<sup>14</sup>C]BAY 59-7939**

Metabolite profiles in the 0-48 h urine fractions after oral administration of 3 mg/kg body weight (animal no. 797, study no. I 3001444/04).

animal no.		797	(b) (4)
data file			
excretion (% of dose)		total calculated	
		21.99	
		0-48 h urine % of dose	
retention time [min]	identifica- tion		
3.4	unknown	0.41	
8.0	unknown	3.11	
9.1	unknown	0.13	
12.5	unknown	0.22	
13.1	unknown	0.05	
13.5	unknown	0.14	
15.1	M-1	7.73	
17.1	unknown	0.00	
17.6	unknown	0.00	
18.0	unknown	0.16	
19.4	M-2	0.60	
20.2	M-3	4.01	
21.1	unknown	0.13	
22.2	drug	5.29	
compounds balanced		21.98	
compounds identified		17.63	
total number of compounds			

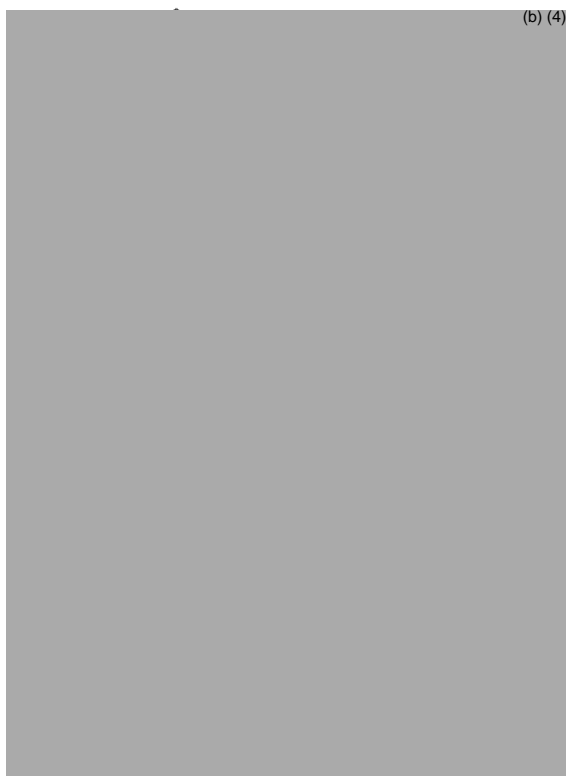
After intraduodenal administration, 32-39% of the administered dose was excreted in bile in 24 hours. Only a small fraction of the unchanged drug was detected in the bile (<1% of the dose). The major metabolite, M-1 accounted for 87-91% of the radioactivity, and M-2 and M-3 accounted for <1% of the dose. The metabolite profiles in 0-24 bile fractions after intraduodenal administration of a 3 mg/kg dose to bile duct-cannulated rat is shown in the sponsor's Table below.

**Table 14:** [<sup>14</sup>C]BAY 59-7939  
Metabolite profiles in the 0 - 24 h bile fractions after intraduodenal administration of  
3 mg/kg body weight  
(BDC rat, animal no. 792, study no. I 3001444/03).

animal no.		792	
data file		(b) (4)	
excretion (% of dose)		total calculated	
		33.82	
		0-24 h bile % of dose	
retention time [min]	identifica- tion		
1.6	unknown	0.37	
4.6	unknown	0.00	
5.0	unknown	0.04	
12.8	unknown	1.32	
14.7	M-1	30.50	
16.5	unknown	0.05	
18.1	unknown	0.07	
19.5	M-2	0.42	
20.0	M-3	0.44	
22.0	drug	0.62	
compounds balanced		33.82	
compounds identified		31.97	
total number of compounds			

In bile-duct cannulated rats, the labeled compound administered intraduodenally was excreted by 40-49% in the feces as the unchanged drug. The major metabolites were M-1 and M-3 and accounted for 11-19% and 21-31% of the radioactivity, respectively. An unknown metabolite accounted for 9-16% of the radioactivity. The sponsor's proposed metabolic pathways of BAY 59-7939 in rats are shown in the Figure below.

Figure 1: Proposed metabolic pathways observed *in vitro* and *in vivo* in rats.



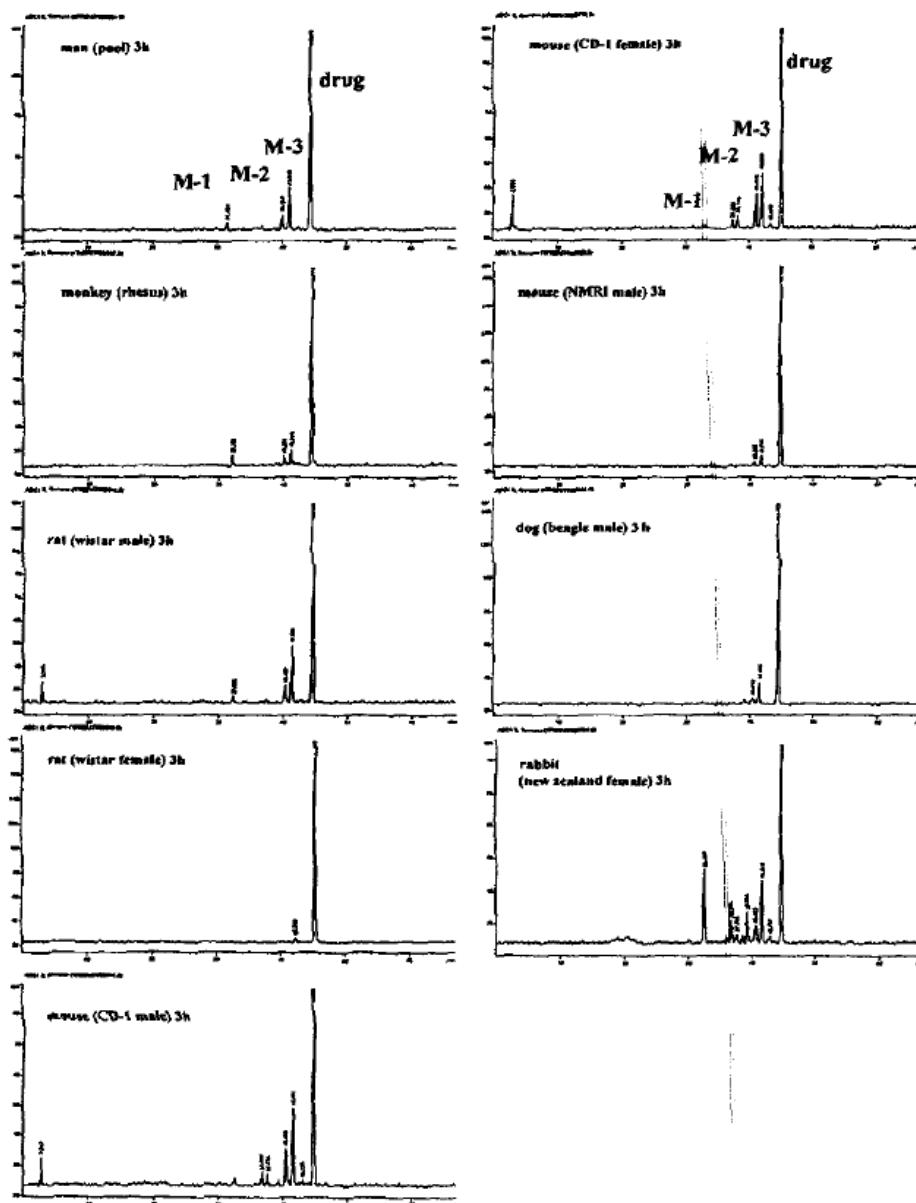
**In Vitro:**

**6. In Vitro Metabolism & Excretion of BAY-59-7939 in Liver Microsomes of Rhesus Monkey, Beagle Dog, New Zealand Rabbit, Wistar Rat, NMRI mouse and Man: (Study #PH34783)**

In vitro metabolism of BAY-59-7939 was examined in liver microsomes of Rhesus monkey, Beagle dog, New Zealand rabbit, Wistar rat, NMRI mouse and humans. The incubations were stopped at 60 and 180 minutes and the samples were analyzed by HPLC-<sup>14</sup>C-MS/MS analysis.

Hydroxylation of the morpholino moiety leading to metabolites M-2 and M-3 was the major phase-I metabolic pathway. Oxidative opening of the morpholino ring leading to M-1, a minor biotransformation pathway was detected in most species. With respect to the phase-1 metabolism, Rhesus monkeys and Wistar rats were the closest to humans. Beagle dogs and NMRI mice also had metabolic patterns, similar to humans; however, in CD-1 mice and rabbits, several additional metabolites were detected. The radioactive peaks for the different metabolites after incubating radiolabeled BAY 59-7939 with the liver microsomes from different species are shown in the sponsor's Figure below.

**Figure 1:** [ $^{14}\text{C}$ ]BAY 59-7939  
Radio-chromatograms from incubations with liver microsomes of different species.



The metabolism of BAY 59-7939 was also investigated after incubating [ $^3\text{H}$ ]BAY 59-7939 with cultured rat and human hepatocytes. The metabolite profiles were examined after 24 and 48 hours incubation. The turnover of the drug after 48 hours of incubation was 60% in human and 82% in rat hepatocytes. Qualitatively, the metabolic profiles in rat and human hepatocytes were similar as M-1 was the major metabolite in both human and rat hepatocytes and accounted for 29-49% and 65% of the radioactivity, respectively. M-3 and M-6 (formed by amide hydrolysis of M-1) were identified as minor metabolites in both species.

## Excretion:

### 7. Excretion of Orally or Intravenously administered [<sup>3</sup>H]BAY 59-7939 in Rats and Dogs:

Rat: (Study #PH32076)

Orally or intravenously administered [<sup>3</sup>H]BAY 59-7939 in rats was mainly excreted by the fecal route. After an IV dose of 3 mg/kg [<sup>3</sup>H]BAY 59-7939, about 65.5% of the radioactivity was excreted in the feces and 28.1% in the urine in 7 days. After oral dosing, 66.9% of radioactivity was excreted in the feces and 24.7% in the urine in 7 days. About 37-55% of the IV dose of 3 mg/kg of [<sup>3</sup>H]BAY 59-7939 was recovered in bile fractions of bile duct cannulated rats within 24 hr of its administration. However, approximately 32-39% of the intraduodenal dose was excreted in the bile showing an incomplete absorption of the drug from the duodenum.

**Table 4:** Comparison of radioactivity balances in per cent of the administered dose after single administration of [<sup>14</sup>C]BAY 59-7939 to male Wistar rats.

Statistics: Arithmetic means (Mean arithm.) and coefficients of variation (C.V.)

Dose [mg/kg] Route Duration [h]	3 i.v. 168		3 p.o. 168		3 i.d. (BDC) 24		3 i.v. (BDC) 24	
	Mean arithm.	C.V. [%]	Mean arithm.	C.V. [%]	Mean arithm.	C.V. [%]	Mean arithm.	C.V. [%]
Exp. Air	n.a.	n.a.	0.0248	13.6	n.a.	n.a.	n.a.	n.a.
Urine	28.1	5.68	24.7	4.91	17.1	27.0	30.3	22.9
Bile	n.a.	n.a.	n.a.	n.a.	34.7	10.2	48.4	14.8
Feces	65.5	5.09	66.9	3.80	46.4	6.95	12.9	24.5
Body excl. GIT*	0.224	9.45	0.147	26.8	0.421	13.9	0.755	38.2
GIT*	0.0220	12.3	0.0292	40.3	0.183	81.8	0.337	158
Total Body	0.246	7.93	0.176	28.7	0.605	32.4	1.09	74.9
Balance	93.9	3.22	91.8	3.45	98.8	2.64	92.7	4.31

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\* GIT = gastrointestinal tract  
n.a. = not applicable  
BDC = bile duct-cannulated rat

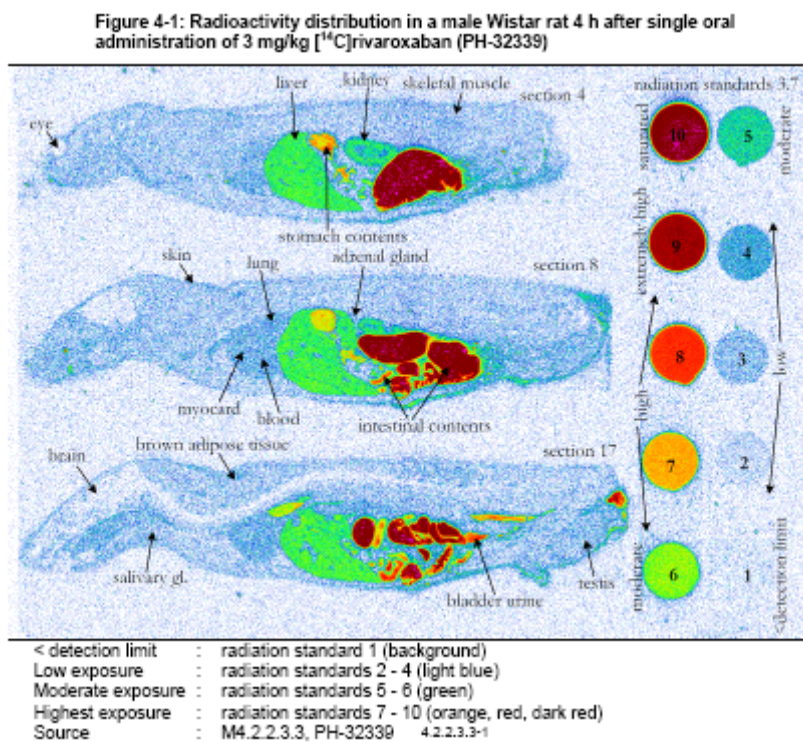
In male Wistar rats, pharmacokinetics of rivaroxaban and of [<sup>14</sup>C]rivaroxaban-related radioactivity (unchanged compound and radiolabeled metabolites) in a formulation consisting of 20% ethanol, 60% PEG 400 and 20% demineralized water were investigated after single intravenous bolus injection and after oral (i.d. administration) doses of [<sup>14</sup>C]rivaroxaban or non-labeled rivaroxaban. [<sup>14</sup>C]BAY 59-7939-radioactivity was excreted via the biliary/fecal route as well as via the renal route after intravenous and oral administration to Wistar rats. About 65.5 % of the radioactivity was found in feces and 28.1 % were excreted via urine until Day 7 after intravenous administration in intact rats.

In intact rats, the orally administered compound was excreted in 66.9 % and 24.7 % in feces and urine and >95 % of the dose was excreted within the first day of the administration. In bile duct-cannulated rats, all radioactive doses were excreted within 24 h after intravenous and intraduodenal administration. About 17.1 % and 34.7% of the intraduodenal dose was excreted in urine and bile. About 46.4 % of the administered radioactivity was recovered in feces. The

presence of radioactivity in feces suggested incomplete absorption of the compound and extrabiliary radioactivity excretion.

In the bile duct-cannulated rats, the intravenously administered tagged radioactivity was excreted by 48.4 % and 12.9 % in bile and feces, respectively, suggesting extrabiliary radioactivity excretion as well.

In rats (study#32339), the bioavailability of the compound was 67.1 % after intraduodenal administration of [ $^{14}$ C]rivaroxaban to BDC rats and about 31, 17.3 and 0.426% of the radioactivity was excreted in bile, urine and gastrointestinal tract. In addition, 14.3% was excreted as extra biliary excretion. The administered radioactivity was distributed widely in rat liver, kidneys and skeletal muscle, intestines and, other tissues as shown in the following figure.



## 8. Rats Following Single Dose Administration (Placental Transfer) (Study #PH48872)

In pregnant Wistar rats, 3 mg/kg orally administered [ $^{14}$ C]Rivaroxaban (in a 60 % PEG 400 and 40 % demineralized water) on the 19<sup>th</sup> day of gestation showed a moderate amount of radioactivity in the maternal and fetal organs and tissues and the distribution was similar in pregnant and non-pregnant rats. The highest concentrations were in liver and kidneys followed by high concentrations in the amnion. In fetal tissues, the radioactivity was homogeneous with low radioactivity in brain and the exposure (as maximum concentrations or AUC) was 20% which exceeded the maternal blood exposure (exception of brain). In fetal organs and tissues, the concentrations were lower than maternal organs. The radioactivity concentration in mammary glands and blood was similar and the

radioactivity was excreted into milk. The concentration was 0.27 % of the dose in dam milk and 0.033 % in the fetuses.

**Table 4-8: Pharmacokinetic parameters of radioactivity in organs and tissues of pregnant Wistar rats after single oral administration of 3 mg/kg [<sup>14</sup>C]rivaroxaban (PH-34872)**

	CEQ <sub>max</sub>	CEQ <sub>max</sub> ratio	t <sub>max</sub>	AUC(0-24)	AUC	t <sub>1/2</sub>	Regression range
	(mg-eq/L)		(h)	(mg-eq-h/L)	(mg-eq-h/L)	(h)	(h)
Organ/tissue	Mean geom.	Organ/ blood	Mean geom.	Mean geom.	Mean geom.	Mean geom.	
Adipose tissue (brown)	0.499	0.269	2.00	2.43	2.45	3.27	4 – 24
Adipose tissue (white)	0.0829	0.0447	1.26	0.533	0.540	4.39	2 – 8
Adrenal glands	1.40	0.754	2.00	6.75	6.83	3.66	4 – 24
Aminion	1.92	1.03	2.00	10.6	11.6	6.53	4 – 24
Amniotic fluid	0.0263	0.0142	2.80	0.148	0.151	2.68	2 – 8
<b>Blood (myocard)</b>	<b>1.85</b>	<b>1.00</b>	<b>2.00</b>	<b>7.40</b>	<b>7.44</b>	<b>3.18</b>	<b>4 – 24</b>
Brain	0.0389	0.0210	2.00	0.159	0.160	2.07	2 – 8
Kidneys	2.15	1.16	2.00	12.2	12.5	4.31	4 – 24
Liver	2.82	1.52	2.00	13.3	13.6	4.19	4 – 24
Lungs	1.68	0.904	2.00	6.31	6.35	3.22	4 – 24
Mammary glands	0.853	0.461	2.00	6.47	7.06	6.53	4 – 24
Myocard	0.936	0.505	2.00	4.56	4.66	4.25	4 – 24
Ovaries	0.921	0.497	2.00	3.54	3.56	3.08	4 – 24
Pancreas	0.813	0.439	2.00	3.68	3.72	3.46	4 – 24
Placentae	0.960	0.518	2.00	4.49	4.54	3.52	4 – 24
Skeletal muscles	0.269	0.145	2.00	1.35	1.37	3.90	4 – 24
Skin	0.473	0.255	2.00	2.09	2.18	4.35	4 – 24
Spleen	0.510	0.275	2.00	2.47	2.46	3.25	2 – 8
Thymus	0.476	0.257	2.00	1.88	1.87	2.55	2 – 8
Thyroid	0.873	0.471	2.00	3.70	3.74	3.62	4 – 24
Uterus	1.07	0.575	2.00	5.27	5.39	4.33	4 – 24
Fetal adipose tissue (brown)	0.195	0.105	2.00	1.41	1.51	6.14	2 – 8
Fetal adrenal glands	0.134	0.0721	4.00	1.03	1.08	3.87	4 – 8
Fetal blood	0.286	0.154	2.00	1.54	1.54	3.75	2 – 8
Fetal brain	0.0415	0.0224	2.00	0.207	0.208	2.89	2 – 8
Fetal kidneys	0.141	0.0761	3.02	1.29	1.35	4.89	4 – 24
Fetal liver	0.317	0.171	2.00	1.92	1.96	4.17	4 – 24
Fetal lungs	0.186	0.100	2.00	1.15	1.19	4.64	4 – 24
Fetal myocard	0.169	0.0912	2.00	1.03	1.04	4.19	2 – 8
Fetal skeletal muscles	0.237	0.128	2.00	1.27	1.31	4.74	4 – 24
Fetal skin	0.273	0.147	2.00	1.43	1.50	5.36	4 – 24
Fetus (average)	0.231	0.125	2.00	1.51	1.71	7.59	4 – 24

Source: M4.2.2.3.6, PH-34872 4.2.2.3.6-1

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The parent compound concentration in plasma was below the lower limit of quantification in the rat and dog. Radioactivity was eliminated in 72 h in rat plasma and in 168 h in dog plasma. The half-lives were up to 42 h in rats and up to 128 h in dogs.

## Other ADME Studies:

### 9. Identification of Human CYP Isoforms Involved in the *In Vitro* Metabolism of BAY 59-7939.

**Methods:** This *in vitro* CYP reaction phenotyping study was conducted to evaluate the CYP isoforms contributing to the oxidative metabolic pathways of BAY 59-7939. BAY-59-7939 was incubated with human liver microsomes in the absence and presence of CYP isoform-selective inhibitors, and recombinant CYP isoforms with correlation analyses performed.

**Results:** Isoforms CYP2J2 was most effective in catalyzing the formation of M-2 followed by CYP3A4. CYP3A5 as well as CYP2D6 were also capable of forming M-2. The kinetic parameters for M-2 formation performed with recombinant CYP2J2 as well as with recombinant CYP3A4 revealed K<sub>m</sub>-values



of 7.8  $\mu\text{M}$  and 66.4  $\mu\text{M}$ , respectively. A higher affinity of BAY 59-7939 to CYP2J2 was seen in comparison to CYP3A4. The CYP3A4 inhibitors (azamulin, ketoconazole, troleandomycin) and the CYP2J2 inhibitor (HET0016) decreased the turnover of BAY 59-7939 in human liver microsome. M-9 formation was reduced when incubated in the presence of CYP3A4 inhibitors and M-2 formation was inhibited by 50% in the presence of CYP2J2 inhibitor HET0016. Ebastine was extensively metabolized by hydroxylation via the mediation of CYP2J2. CYP3A4 activities of single donors highly correlated with M-9 formation rates. Overall, the involvement of CYP2J2 was determined in M-2 formation from BAY 59-7939 and CYP3A4 was involved in M-9 formation. The data of formation of the metabolites is shown in the following table (table 7 of sponsor Report #PH-32627).

**Table 7:** BAY 59-7939: Inhibition of M-2 and M-9 formation from BAY 59-7939 (1  $\mu\text{M}$ ) in human liver microsomes by CYP isoform-selective inhibitors.

RMA 1482G	Inhibitor	c [inhibitor] [ $\mu\text{M}$ ]	Mean M-2 [ng/mL]	M-2 % of control	Mean M-9 [area ratio]	M-9 % of control
1 + 2	Control*	-	13.9	1.	0.002262	1.
3 + 4	1-Amino-1H-benzotriazole*	1000	0.4	2.7	0.000032	1.4
5 + 6	Furaphylline*	20	13.3	95.7	0.002239	99.0
7 + 8	Control	-	20.7	1.	0.003134	1.
9 + 10	Azamulin	10	9.1	43.9	0.000230	7.3
11 + 12	Azamulin	1	12.3	59.4	0.000722	23.0
13 + 14	Ketoconazole	10	5.3	25.8	0.000227	7.2
15 + 16	Ketoconazole	1	10.1	48.8	0.000599	19.1
17 + 18	Methylpyrazole	200	15.0	72.6	0.003512	112.1
19 + 20	Quinidine	5	23.0	111.1	0.003685	117.6
21 + 22	Benzylphenobarbital	5	20.6	99.5	0.004157	132.6
23 + 24	Sulfaphenazole	10	19.9	96.2	0.003028	96.6
25 + 26	Quercetin	50	8.1	39.0	0.002291	73.1
27 + 28	Quercetin	10	15.2	73.5	0.002759	88.0
33 + 34	Astemizole	50	12.1	58.5	0.002427	77.5
35 + 36	Astemizole	10	15.6	75.1	0.002863	91.4
37 + 38	HET0016	10	9.7	46.7	0.003150	100.5

\* = preincubation 20 min

### PK/TK summary:

The absolute oral bioavailability of unlabeled BAY 59-7939 or [ $^{14}\text{C}$ ]-BAY 59-7939 was 60% in both rats and dogs and  $T_{1/2}$  of the unchanged drug was 1.2 to 2.3 hours after oral and 0.9 hr after IV dosing. The  $T_{1/2}$  for the total radioactivity was 18.8 and 42.1 hrs by the IV and oral routes, respectively. The  $T_{1/2}$  was 0.95 hour after a 0.3 mg/kg IV dose and the plasma concentration at steady state (AUC) was 1.0 mg.h/ml. BAY 59-7939 was 97.1%, 99.1%, 93.4%, 71.8% and 96.1% bound to plasma proteins from human, rats, dogs, rabbits and mice, respectively. The radioactivity was distributed in different organs and tissues without any preferred affinity for any specific organ. The morpholino moiety is the main target of oxidative metabolism and catalyzed by CYP3A4 and metabolites M-2 and M-3 are formed by further oxidation. M-1 was detected as the major metabolite in rat urine, bile and feces. After oral dosing, 66.9% of radioactivity was excreted in feces and 24.7% in the urine in 7 days. After 7 days of dosing, only about 0.2% of the administered radioactivity remained in the rat.

Figure 3-1: Proposed metabolites of [<sup>14</sup>C]rivaroxaban from *in vitro* and *in vivo* studies (main metabolic pathway is indicated by larger arrowheads)



## 2.6.6 TOXICOLOGY

### 2.6.6.1 Acute Toxicology:

**Study Title:** Acute Toxicity Studies in the Mouse and Rat after Oral Administration and in the Mouse after Intravenous Administration.

**Key study findings:** The acute toxicity in rats and mice was performed at the maximum feasible oral dose of 500 mg/kg or intravenous dose of 25 mg/kg. None of the animals died at either of these doses and the minimal lethal doses could not be determined. The treatment related clinical signs in male and female mice treated with IV dose of 25 mg/kg were decreased motility, abdominal

position, labored breathing, narrowed palpebral fissure and piloerection. Because of solubility/ suspendibility problems, higher doses were not evaluated.

**Study no:** T 1070941, T 0070940 and T 5070936.

**Conducting Laboratory:** Department of Toxicology/Pharma, Institute of Toxicology, BAYER AG, 42096 Wuppertal, Germany.

**Dates of study initiation & completion:** June 22, 2001 & March 05, 2002.

**GLP compliance:** Yes

**QA Report Yes (X) No ( )**

**Drug, Lot #, radiolabel (if applicable), and % purity:** BAY 59-7939, batch No. 010507.

**Formulation/vehicle:** For oral administration, BAY 59-7939 was suspended in Solutol HS15 (a surface active agent)/water (20g/80g). The maximum feasible dose by the oral route was 500 mg/kg (25 ml/kg). For IV administration, the drug was dissolved in polyethylene glycol. The maximum feasible dose by the IV route was 25 mg/kg (in 5 ml/kg) because of its limited solubility.

**Methods:**

**Dosing:**

Species/strain: Hsd WIN:NMRI mice and Hsd Cpb:WU rats

#/sex/group or time point: 5 animals/sex/group

Age: not provided

Weight Range: Mice 22g - 25 g, males; 17g – 23g, females

Rats: 214g – 228g, males; 178g – 192g, females

Doses administered: 500 mg/kg by the oral route and 25 mg/kg (5 ml/kg) by the IV route.

Route, form, volume, and infusion rate: The drug was administered by oral gavage (25 ml/kg), and by a single intravenous injection into a tail vein (5 ml/kg) for approximately 30 seconds.

**Observations and times:**

Clinical signs: The animals were observed several times on the day of administration, and at least once daily for the 14-day observation period.

Body weights: Once a week.

Gross pathology: At the end of the 14-day observation period, the animals were sacrificed and examined macroscopically. The animals that died during the observation period were examined as soon as possible.

**Results:**

**Mortality:**

Rats: There were no deaths of male or female rats receiving an oral dose of 500 mg/kg BAY 59-7939

Mice: There were no deaths of male or female animals receiving an oral dose of 500 mg/kg or an IV dose of 25 mg/kg.

**Clinical signs:**

Rats: No treatment related clinical signs were observed in male and female rats treated at an oral maximum feasible dose of 500 mg/kg BAY 59-7939.

Mice: No treatment related clinical signs were observed in male and female mice treated with a single oral dose of 500 mg/kg.

Mice treated at IV dose of 25 mg/kg BAY-59-7939 had decreased motility, abdominal position, labored breathing, narrowed palpebral fissure and piloerection. The clinical signs were observed within 1 hour of dosing and lasted for up to 3 days in the males, and for up to 5 days in the females.

**Body weights:** No changes in the body weights were observed in any group.

**Gross pathology:** No treatment related changes were observed in any group.

In summary, no animal died in the acute oral toxicity studies conducted at the maximum feasible dose of 500 mg/kg. The minimal lethal dose was not identified. Treatment related clinical signs of decreased motility, abdominal position, labored breathing, narrowed palpebral fissure and piloerection were observed only in male and female mice treated at the maximum feasible oral dose in rats and mice.

**2.6.6.2 Subacute/Subchronic/Chronic Toxicity Studies:**

**MOUSE:**

**1. Subacute Oral Toxicity Study in CD-1 Mice (4 Weeks Administration by Gavage) Study No: T7072792**

**Key study findings:** The 4-week oral gavage treatment with BAY 59-7939 in CD-1 mice at the doses of 0, 12.5, 50 and 200 mg/kg/day (as PEG-6000 co-precipitate in solutol/water. 20/80 v/v) produced a dose proportional plasma increase in drug concentration. No treatment related changes in the body weight gain, hematology or enzyme contents were observed in any of the treatment groups. The histopathology of the tissues was not included in the study and the MTD was not identified.

**Study no:** T8074494/Study ID BHC (PH-PD-TA CV)

**Conducting laboratory and location:** PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation & completion:** June 13, 2003 & February 14, 2005

**GLP compliance:** Yes

**QA report:** yes ( ) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939, PEG 6000 co-precipitate 10% 100; Batch no. J20020703.

**Formulation/vehicle:** The drug was dissolved in solutol/water (20/80 v/v), and administered by oral gavage.

**Methods:**

**Dosing:**

**Species/strain:** Crl:CD-1 (ICR)BR mice

**#/sex/group or time point (main study):** 10 animals/sex/group

**Satellite groups used for toxicokinetics or recovery:** 18 animals/sex/group

**Age:** 6-7 weeks old

**Weight:** 25.2-34.2 g for males, 23.0-27.8 g for females.

**Doses in administered units:** BAY 59-7939 was administered by oral gavage at dosing schedule as shown in sponsor's table 5-5 and scanned below:

**Table 5-2: Dosing Schedule**

Group No.	Dose (mg/kg)	Sex	Number of Animals	Animal Number
<b>Main Groups</b>				
1	0	male	10	1-10
2	12.5	male	10	11-20
3	50	male	10	21-30
4	200	male	10	31-40
5	0	female	10	41-50
6	12.5	female	10	51-60
7	50	female	10	61-70
8	200	female	10	71-80
<b>Satellite Groups</b>				
9	0	male	3	81-83
10	12.5	male	18	84-101
11	50	male	18	102-119
12	200	male	18	120-137
13	0	female	3	138-140
14	12.5	female	18	141-158
15	50	female	18	159-176
16	200	female	18	177-194

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**Route, form, volume, and infusion rate:** The drug as (PEG-6000 co-precipitate) was formulated with the vehicle solutol/water (20/80), and administered by oral gavage (13 ml/kg). Control animals received the same amount of PEG as the high dose group.

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and abnormal clinical signs.

**Body weights:** Body weights were measured prior to initiation of dosing, and then weekly thereafter.

**Food consumption:** Food and water consumptions were measured once a week, and the daily food intake per animal was calculated.

**Hematology:** Blood samples for hematological and clinical chemistry parameters estimation were collected from 5/sex/group on Days 1 and 27.

**Gross pathology:** All animals were subjected to complete necropsy examinations at scheduled sacrifice. Animals that died or sacrificed moribund, were also subjected to necropsy examination.

**Organs weighed:** The weights of the following organs were recorded: brain, heart, kidney, liver, spleen, ovary, testes.

**Histopathology:** Histopathological examinations were not performed.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from the satellite animals (3 animals/time point) on Days 1 and 27 at 0.5, 1, 2, 4, 7 and 24 hours after administration.

## **Results:**

**Mortality:** Two mice (1/sex) of 200 mg/kg/day group died during the blood collection and gavage error.

**Clinical signs:** No treatment-related clinical signs were observed in any group.

**Body weights:** The mean body weights of the control male and female animals on day 1 of dosing were 32.3 and 24.0 g, respectively. There was no statistically significant reduction in the body weight gain seen in the treatment groups.

**Food and water consumption:** The mean food and water consumptions were not changed in any of the treatment groups compared to control male and female mice.

**Hematology:** No change in the hematology parameters were seen in the study animals. Clotting time (HQuick) was not changed in either males or females receiving the drug (pharmacological effect).

**Clinical chemistry:** No treatment-related changes in the clinical chemistry parameters were observed in any group.

**Organ weights:** There was a reduction of 12% in the absolute weight of ovaries of 200 mg/kg/day group. The relative liver weight was insignificantly increased in males of 50 and 200 mg/kg/day groups and a 9% increase of liver weight was observed in females of 200 mg/kg/day group.

**Gross pathology:** Discolorations of the spleen were observed in 1 male of 10 of 200 mg/kg group and this was not treatment related.

**Histopathology:** The histopathology exam of the tissues of the animals was not done in the study.

**Toxicokinetics:** Plasma peak exposure levels (AUC) of BAY 59-7939 was determined in TK group (3/sex/group) and the Tmax was seen in 0.5 hr. The data indicated a dose proportional increase with no evidence of accumulation of the compound. The toxicokinetic parameters for BAY 59-7939 in male and female mice at steady state (Day 27) are summarized in the Table below.

**Table 6-10: Toxicokinetic Investigations**

Dose	[mg/kg]	12.5	50	200
AUC(0-7)	[mg·h/L]	2.92	13.9	32.0
AUC(0-7)norm	[kg·h/L]	0.234	0.279	0.160
AUC(0-24)	[mg·h/L]	2.98	14.6	34.0
AUC(0-24)norm	[kg·h/L]	0.239	0.292	0.170
C <sub>max</sub>	[mg/L]	2.03	9.99	14.7
C <sub>max, norm</sub>	[kg/L]	0.163	0.200	0.0734
t <sub>max</sub>	[h]	0.500	0.500	0.500
RA1	[%]	109	90.8	122
RA3	[%]	94.1	90.4	59.4

$$RA1 = C_{\text{max, Day 27}} / C_{\text{max, Day 1}}$$

$$RA3 = AUC(0-24)_{\text{Day 27}} / AUC(0-24)_{\text{Day 1}}$$

### Summary of individual study findings:

In the 4-week oral gavage toxicity study in CD-1 mice, the drug was administered at 0, 12.5, 50 and 200 mg/kg/day doses. A dose proportional increase in plasma concentration was seen in study animals and no treatment related changes in the body weight gains, hematology or enzyme contents in any of the animals of treatment groups. The histopathology of the tissues was not included in the study and the MTD was not identified in this study.

## 2. Study title: Thirteen (13)-Week Oral (gavage) Toxicity study of BAY 59-7939 in Mice:

**Key study findings:** In the 13-week oral gavage toxicity study with BAY 59-7939 in CD-1 mice, the drug was administered at 50, 100 and 200 mg/kg/day doses. The target organs of toxicity were the heart, kidneys, adrenal and spleen in males, and the liver and kidneys in females. 100 mg/kg/day was well tolerated and provided 20 and 29.5 times the plasma exposure of the proposed human dose in male and female animals, respectively.

**Study no:** T8074494

**Conducting laboratory and location:** PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation:** Jun 09, 2004

**GLP compliance:** Yes

**QA report:** yes ( ) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939, PEG 6000 co-precipitate 10% 100; Batch no. 031212-100.

**Formulation/vehicle:** The drug was dissolved in solutol/water (20/80 v/v), and administered by oral gavage.

**Methods:**

**Dosing:**

**Species/strain:** Crl:CD-1 (ICR)BR mice

**#/sex/group or time point (main study):** 10 animals/sex/group

**Satellite groups used for toxicokinetics or recovery:** 18 animals/sex/group were used for toxicokinetic studies.

**Age:** 6-7 weeks old

**Weight:** 25.2-34.2 g for males, 23.0-27.8 g for females.

**Doses in administered units:** BAY 59-7939 was administered by oral gavage at 0, 50, 100 and 200 mg/kg/day (13 ml/kg) doses for 94 days. Control group received the vehicle (solutol/water, 20/80 v/v).

**Route, form, volume, and infusion rate:** The drug as (PEG-6000 co-precipitate) was formulated with the vehicle solutol/water (20/80), and administered by oral gavage (13 ml/kg). Control animals received the same amount of PEG as the high dose group.

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and abnormal clinical signs.

**Body weights:** Body weights were measured prior to initiation of dosing, and weekly thereafter.

**Food consumption:** Food and water consumptions were measured once a week,

**Hematology:** Blood samples for hematological examinations were collected on Days 79 and 80 for males and females, respectively.

**Clinical chemistry:** Blood samples for clinical chemistry examinations were collected on Days 87 and 86 for males and females, respectively.

**Gross pathology:** All animals were subjected to complete necropsy examinations at scheduled sacrifice. Animals that died or sacrificed moribund, were also subjected to necropsy examination.

**Organs weighed:** The weights of the following organs were recorded: brain, adrenal gland, heart, kidney, liver, spleen, ovary, testes, epididymides and uterus.

**Histopathology:** Histopathological examinations of the following organs from the controls and the high dose groups were conducted.

Adrenal gland, epididymides, esophagus, eyes, femur, heart, kidneys, liver and gall bladder, lungs, mesenteric lymph node, submandibular lymph node, optic nerve, ovaries, oviducts, pancreas, salivary glands, skeletal muscle, spleen, sternum, testis, thymus, thyroid glands, trachea, urinary bladder, uterus and vagina. Histopathological examinations were also conducted of the macroscopic abnormalities, spleen and eyes (with optic nerves) from the low and mid dose animals.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from the satellite animals (3 animals/time point) on Days 1 and 86 at 0.5, 1, 2, 4, 7 and 24 hours after administration.

**Other:** At necropsy, liver specimens from 5 animals/sex/dose group were collected for determination of the activity of the compound on the following enzyme activities: 7-ethoxycoumarin deethylase (CYP1A1, 2B1, 2D1, 2E1), 7-Ethoxyresorufin deethylase (CYP 1A1), Aldrin Epoxidase (CYP 2B1, 3A1, 3A4, 2C11), Epoxide hydrolase, UDP-Glucuronyl transferase and Glutathione S-transferase.

**Results:**



**Mortality:** There was no mortality in any group.

**Clinical signs:** No treatment-related clinical signs were observed in any group.

**Body weights:** The mean body weights of the control male and female animals on Day 1 of dosing was 30.8 g and 24.2 g, respectively. Body weight gains of the treatment group males were slightly higher than that of controls (not dose-dependent). Females receiving the mid dose had lower body weight gains. Percent changes in body weight gains of male and female animals on Day 92 are shown in the Table below.

Parameter	Males				Females			
	Control	50 mg/kg	100 mg/kg	200 mg/kg	Control	50 mg/kg	100 mg/kg	200 mg/kg
Body Wt. (g) Day 1	30.8	30.2	29.8	30.7	24.2	24.8	24.4	24.1
Body Wt. (g) Day 92	35.5	36.3	34.9	36.4	29.8	30.5	28.4	29.9
Body Wt. gain (g)	4.7	6.1	5.1	5.7	5.6	5.7	4.0	5.8
Body Wt. gain (% control)	100%	129.8%	108.5%	121.3%	100%	101.8%	71.4 %	103.6%

**Food consumption:** The mean food consumptions of the control male and female mice were 5.82 and 5.72 g/animal/day, respectively. No changes in food consumption were observed in any group.

**Hematology:** Males receiving the high dose had slightly decreased thrombocyte levels (10%). Clotting time (HQuick) was significantly increased in both males and females receiving the drug (pharmacological effect). The effects of BAY 59-7939 on the HQuick values (sec) in male and female mice are shown in the Table below

	Control	50 mg/kg	100 mg/kg	200 mg/kg
<b>Males</b>	19.1	41.0	44.4	46.3
<b>Females</b>	19.9	43.1	43.8	60.3

**Clinical chemistry:** No treatment-related changes in the clinical chemistry parameters were observed in any group. Determinations of the enzyme activities of liver homogenates showed decreased activities of 7-Ethoxyresorufin deethylase (65%) and Aldrin Epoxidase (about 70%) at 50 mg/kg in males. In females, a dose dependent decrease in the levels of Ethoxyresorufin deethylase (18.3%, 22.9% and 37.5% at low, mid and high doses, respectively) and Aldrin Epoxidase (17.9%, 18.9% and 29.2% at low, mid and high doses, respectively) were observed.

**Organ weights:** A slight increase in the liver weights (relative to body wt.) was observed in mid (11% in males, 4% in females) and high dose (7% in males, 9% in females) male and female mice. The relative spleen weights of the mid dose male (8.4%) and female (17.7%) animals were slightly higher than the respective controls.

**Gross pathology:** Discolorations of the spleen were observed in males at 100 (1 of 10) and 200 (2 of 10) mg/kg/day doses.

**Histopathology:** Males of the high dose (200 mg/kg) treatment group had higher incidences of lymphoid cellularity of the marginal zones of the spleen (6 of 10 animals), higher incidences of fibrosis of the heart (control, 0 of 10; high dose, 2 of 10), mononuclear cell infiltration in the kidneys (control, 0 of 10; high dose, 4 of 10) and, hyperplastic spindle cells in the adrenal (control 1 of 10; high dose, 3

of 10). Females of the high dose group had higher incidences of Kupffer cell foci in the liver (control, 1 of 10; high dose, 3 of 10) and mononuclear cell infiltration in the kidneys (control, 1 of 10; high dose, 4 of 10) mononuclear cell infiltration in the kidneys, hyperplastic spindle cells in the adrenal gland and fibrosis of the heart. Histopathological changes observed in male and female animals are summarized in the Table below.

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Organ/Finding	Control	50 mg/kg	100 mg/kg	200 mg/kg
<b>MALES</b>				
Heart -fibrosis	0/10	--	--	2/10
Kidney -mononuclear cell infiltration	0/10	--	--	4/10
Adrenal -hyperplastic spindle cells	1/10	--	--	3/10
Spleen -increased cellularity of marginal zones	1/10	0/10	1/10	6/10
<b>FEMALES</b>				
Liver -Kupffer cell foci	1/10	--	--	3/10
Kidney -mononuclear cell infiltration	1/10	--	--	4/10

**Toxicokinetics:** Plasma exposure levels (AUC) of BAY 59-7939 in male and female mice increased with increasing doses at 50 and 100 mg/kg/day doses, and no further increase in the exposure levels were observed at the 200 mg/kg/day dose. The plasma exposure levels (AUC<sub>0-24h</sub>) at low, mid high doses in male and female mice were 9.4, 20.4 and 22.8 mg.h/L, and 11.4, 30.3 and 28.1 mg.h/L, respectively and exposure levels in both sexes were similar. The toxicokinetic parameters for BAY 59-7939 in male and female mice at steady state (Day 86) are summarized in the Table below.

Parameter	50 mg/kg		100 mg/kg		200 mg/kg	
	Male	Female	Male	Female	Male	Female
AUC <sub>0-24h</sub> (mg.h/L)	9.4	11.4	20.4	30.3	22.8	28.1
C <sub>max</sub> (mg/L)	6.78	7.43	10.5	10.2	11.0	11.4
T <sub>max</sub> (h)	0.50	0.50	0.50	0.50	0.50	0.50

#### Summary of individual study findings:

In the 13-week oral gavage toxicity study in CD-1 mice, BAY 59-7939 administered at 0, 50, 100 and 200 mg/kg/day doses for 94 days caused linear non-dose proportional increase in plasma concentrations. The histopathological abnormalities of higher incidences of fibrosis of the heart, mononuclear cell infiltration in kidneys, hyperplastic spindle cells in adrenal and increased cellularity of marginal zones of the spleen were reported in males of high dose group. Higher incidences of Kupffer cell foci in the liver and mononuclear cell infiltration in the kidneys were in females of this group. These findings indicated the liver and kidneys being the target organs of toxicity in both sexes and, adrenal and spleen were additional targets in males of the study. A dose of 100 mg/kg/day was identified as the MTD and the males and females in this group had 20 and 29.5 times the human plasma exposure of the proposed clinical dose.

### 3. Study title: **Thirteen (13)-Week Oral (Dietary) Toxicity study of BAY 59-7939 in Mice:**

**Key study findings:** In the 13-week oral dietary toxicity study in CD-1 mice, BAY 59-7939 at 0 (diet only), 0 (diet plus PEG), 1250, 2500 and 5000 ppm concentrations in dietary admixture caused dose dependent increase in coagulation time (HQUICK) and, increase in liver enzymes in males and females. Increased incidences of focal tubular hypertrophy of the kidney in males and focal necrosis in liver in females indicated kidney and liver as the target organs of toxicity in males and females. Based on these findings, the high dose appears to be the MTD. The plasma exposure levels (AUC<sub>0-24h</sub>) at low, mid and high doses in male and female mice were 14.4, 21.3 and 31.3 mg.h/L in males and, 20.1, 31.8 and 43.0 mg.h/L in females, respectively. The plasma exposure levels in male and female mice at the high dose were about 1.2 and 1.7 times the human exposures at the proposed clinical dose of 10 mg/day.

**Study no:** T1073975

**Conducting laboratory and location:** PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation:** July 19, 2004

**GLP compliance:** Yes

**QA report:** yes ( ) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939 Co-precipitate 10% 100; Batch no. 040526-100.

**Formulation/vehicle:** The drug was administered as PEG-6000 co-precipitate in dietary admixtures. The animals of the control and treatment groups received the same amount of PEG-6000.

**Methods:**

**Dosing:**

**Species/strain:** Crl:CD-1 (ICR)BR mice

**#/sex/group or time point (main study):** 10 animals/sex/group

**Satellite groups used for toxicokinetics or recovery:** 18 animals/sex/group

**Age:** 6 weeks old

**Weight:** 26.3-32.7 g for males, 22.6-28.1 g for females.

**Doses in administered units:** BAY 59-7939 was administered at doses of 0 (pure diet), 0 (PEG control), 1250, 2500 and 5000 ppm in diet for 93 days. Diet mixtures containing the test article were for *ad libitum* consumption and based on daily food intake from day 1 to day 92, the mean drug intake was 237, 476 and 1007 mg/kg in males, and 323, 710 and 1304 mg/kg in females. Homogeneity and stability of the drug in the feed mixture were checked at regular intervals.

**Route, form, volume, and infusion rate:** The drug as (PEG-6000 co-precipitate) was administered as a dietary admixture.

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and abnormal clinical signs.

**Body weights:** Body weights were measured prior to initiation of dosing, and weekly thereafter.

**Food consumption:** Food and water consumptions were measured once a week, and the daily food intake per animal was calculated.

**Hematology:** Blood samples for hematological examinations were collected on Days 79 and 78 for males and females, respectively.

**Clinical chemistry:** Blood samples for clinical chemistry examinations were collected on Days 87 and 86 for males and females, respectively.

**Gross pathology:** All animals were subjected to complete necropsy examinations at scheduled sacrifice. Animals that died or sacrificed moribund, were also subjected to necropsy examinations.

**Organs weighed:** The weights of the following organs were recorded: brain, adrenal gland, heart, kidney, liver, spleen, ovary, testes, epididymides and uterus.

**Histopathology:** Histopathological examinations of the following organs from the two controls and the high dose groups were conducted.

Adrenal gland, aorta, epididymides, esophagus, eyes, extraorbital lacrimal glands, femur, heart, kidneys, larynx, liver and gall bladder, lungs, mesenteric lymph node, submandibular lymph node, optic nerve, ovaries, oviducts, pancreas, salivary glands, skeletal muscle, spleen, sternum, testes, thymus, thyroid glands, trachea, urinary bladder, uterus and vagina. Histopathological examinations were also conducted of the macroscopic abnormalities and the liver (with gallbladder) from the low and mid dose animals.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from the satellite group animals (3 animals/time point) on Days 4, 30 and 91/92. On Days 4 and 30, blood samples were collected at 8 a.m. and 4 p.m., and on days 91/92, blood samples were collected at every 4 hours. Plasma concentrations of BAY 59-7939 were determined by a LC-MS/MS method.

**Other:** At necropsy, liver specimens from 5 animals/sex/dose group were collected for determination of the following enzyme activities: UDP-Glucuronyl transferase, 7-Ethoxycoumarin deethylase, 7-Ethoxyresorufin deethylase, Aldrin Epoxidase, Epoxide hydrolase and Glutathione S-transferase.

## **Results:**

**Mortality:** There was no treatment related mortality in any group. One female from the low dose group died during blood collection, and was not related to treatment with the drug.

**Clinical signs:** No treatment-related clinical signs were observed in any group.

**Body weights:** The mean body weights of the control male and female animals on Day 1 of dosing were 29.6 g and 25.5 g, respectively. Treatment with BAY 59-7939 for 13 weeks had no treatment related effects on the body weight gains of male mice. In female mice, an increase in the body weight gains was observed in animals receiving BAY 59-7939. No differences in body weight gains were observed between animals receiving PEG and pure diet as controls. Percent

changes in body weight gains of male and female animals on Day 92 are shown in the Table below.

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Parameter	Males				Females			
	Control	237	476	1007	Control	323	710	1304
	(PEG)	mg/kg	mg/kg	mg/kg	(PEG)	mg/kg	mg/kg	mg/kg
Body Wt. (g) Day 1	29.6	29.3	28.9	29.2	25.5	26.0	25.5	25.3
Body Wt. (g) Day 92	39.7	38.7	38.8	40.5	28.8	30.4	31.2	30.8
Body Wt. gain (g)	10.1	9.4	9.9	11.3	3.3	4.4	5.7	5.5
Body Wt. gain (% control)	100%	93.1%	98.0%	111.9%	100%	133.3%	172.7%	166.7%

**Food consumption:** The mean food consumptions of the control male and female mice were 6.09 and 6.45 g/animal/day, respectively. Treatment group males (8.2% to 20.2%) and females (6% to 28.2%) had increased food intakes during the study period (Days 0-92), but was not dose-dependent. Water intake was also slightly higher in the treatment group males (up to 26%) and females (up to 10%).

**Hematology:** Males receiving the high dose (1007 mg/kg/day) had slightly increased mean corpuscular volume (MCV, 8.9%) and mean corpuscular hemoglobin (MCH, 6.4%) values. Coagulation time (HQUICK) was significantly and dose dependently increased in both males (47.5% to 66.9%) and females (32.4% to 61.3%) receiving the drug. This effect is related to the pharmacological actions of the drug. No differences in the hematological parameters were observed between PEG and pure diet control groups. The effects of BAY 59-7939 on hematological parameters of male and female mice are summarized in the Table below.

Table 6-5: Hematology

	ERY	HB	HCT	MCV	MCH	MCHC	RETI	THRO	HQUICK
Dose ppm	10E12/l	g/l	l/l	fl	pg	g/l ERY	o/oo	10E9/l	sec
males	Day 79 (HQUICK Day 73)								
0 + PEG 6000	8.32	130	0.440	53.0	15.6	294	42	1370	18.1
1250	8.56	137	0.467	54.6	16.0	293	44	1350	26.7 ++
2500	8.18	131	0.451	55.2	16.0	290	56 +	1398	28.2 ++
5000	7.66	126	0.433	57.7 +	16.6 ++	289	101	1289	30.2 ++
females	Day 78 (HQUICK Day 72)								
0 + PEG 6000	8.59	143	0.484	56.5	16.7	295	50	1956	21.3
1250	8.49	141	0.476	56.2	16.6	297	49	1359	28.3 ++
2500	8.78	144	0.491	56.0	16.4	294	52	1330	35.6 ++
5000	8.31	137	0.468	56.4	16.5	292	50	1342	40.5 ++

The statistical analysis was based on a comparison of treatment groups vs. the PEG 6000 control

**Clinical chemistry:** ALT and bilirubin concentrations were affected only slightly in treatment group animals as shown below in the table. No differences in the clinical chemistry parameters were observed between the two control groups.

MALES (Day 87)		Control (PEG)	Low Dose	Mid Dose	High Dose
ALT (U/L)		57.2	26.5	28.1	24.2
Cholesterol (mmol/L)		3.34	3.37	3.47	3.26
Bilirubin-total (μmol/L)		1.4	1.7	1.6	1.5
FEMALES (Day 86)		Control (PEG)	Low Dose	Mid Dose	High Dose
ALT (U/L)		31.0	24.1	24.4	22.4
Cholesterol (mmol/L)		2.63	2.78	2.40	3.13
Bilirubin-total (μmol/L)		1.7	2.0	2.3	2.4

Determinations of the enzyme activities of liver homogenates showed increased levels of Epoxide hydrolase (EH), UDP-glucuronyl transferase (GLU-T) and Glutathione S-transferase (GS-T) levels in treatment group males and females. The levels of these enzymes in different groups of mice are shown in the Table below.

**Table 6-9: Determinations in Liver Tissue**

Dose ppm	ECOD nmol/g*min	EROD nmol/g*min	ALD nmol/g*min	EH nmol/g*min	GS-T nmol/g*min	GLU-T nmol/g*min
<b>males</b>						
0 + PEG 6000	8.8	1.10	30.4	708	350	244
0	12.2	1.22	32.3	868	ns 363	352
1250	10.7	1.04	32.4	789	+	404
2500	18.3	++ 1.12	46.2	++ 946	++ 516	++ 498
5000	11.5	0.88	32.5	904	++ 449	++ 328
<b>females</b>						
0 + PEG 6000	24.9	1.13	38.4	472	124	341
0	24.5	1.40	36.3	486	121	371
1250	26.8	1.22	40.3	586	+	171
2500	29.1	1.36	41.5	576	++ 191	++ 476
5000	25.4	1.26	39.4	629	++ 220	++ 436

The statistical evaluation of the liver enzyme activities was carried out by Student t-test (MS-Excel 97): pure diet control and treated groups against PEG 6000 control.

**Organ weights:** No treatment-related changes in organ weights were observed in any group.

**Gross pathology:** Brown discoloration of the liver was observed in males (3 of 10) receiving the high dose of BAY 59-7939. Discoloration of the liver was not observed in any other group.

**Histopathology:** Males receiving the high dose had higher incidences of focal tubular hypertrophy of the kidney. One mid dose and one high dose female had focal necrosis of the liver. Histopathological changes observed in male and female animals are summarized in the Table below

Organ/Finding	Control (Pure Diet)	Control (PEG 6000)	BAY 59-7939 (1250 ppm)	BAY 59-7939 (2500 ppm)	BAY 59-7939 (5000 ppm)
<b>MALES</b>					
Kidney					
-focal tubular hypertrophy	0/10	1/10	--	--	3/10
<b>FEMALES</b>					
Liver					
-focal necrosis	0/10	0/10	0/10	1/10	1/10

### Toxicokinetics:

On day 91/92, a treatment related and non-dose proportional increase in plasma concentrations in about 4 to 8 hr of the drug administration. The TK data of the study is shown below in the table.

**Table 6-10: Summary on exposure in mice at steady state (day 91/92) after administration of BAY 59-7939 in the diet**

Dose	[ppm]	Males			Females		
		1250	2500	5000	1250	2500	5000
AUC <sub>(0-24)</sub>	[mg·h/L]	14.4	21.3	31.3	20.1	31.8	43.0
C <sub>max</sub>	[mg/L]	1.39	1.89	1.93	1.77	1.82	2.81
C(24)/C <sub>max</sub>	[%]	41.4	61.7	83.4	56.0	89.7	80.1
t <sub>max</sub>	[h]	8	4	4	4	8	4

### Summary of individual study findings:

In the 13-week oral dietary toxicity study in CD-1 mice, BAY 59-7939 at 0 (diet only), 0 (diet plus PEG), 1250, 2500 and 5000 ppm concentrations in dietary admixture caused dose dependent increase in coagulation time and, increase in

liver enzymes in males and females. Increased incidences of focal tubular hypertrophy of the kidney in males and focal necrosis in liver of 1 female indicated kidney and liver as the target organs of toxicity in males and females and the high dose was identified as an MTD. The plasma exposure levels (AUC<sub>0-24h</sub>) at low, mid and high doses in male and female mice were 14.4, 21.3 and 31.3 mg.h/L in males and, 20.1, 31.8 and 43.0 mg.h/L in females, respectively. The plasma exposure levels in male and female mice at the high dose were about 1.2 and 1.7 times the human exposures at the proposed clinical dose of 10 mg/day.

#### **4. BAY 59-7939: Subchronic Oral Toxicity Study in CD-1 Mice (13 Weeks Administration by Gavage) Study No.: T6075725**

##### **Key study findings:**

BAY 59-7939 at oral gavage doses of 0, 60, 300 and 1500 mg/kg body weight for a period of at least 93 days produced no treatment-related effects and none of the animals died. The body weight, food and water consumptions in treated animals were comparable to those of the control animals. Coagulation time was prolonged from 60 mg/kg or greater dose in both sexes and no histopathological effects were observed in animals. The absorption was inconsistent but treatment related and plasma concentration in male mice was slightly lower than females. Under the conditions described the no-observed-adverse-effect level (NOAEL) for males and females was >1500 mg/kg/day.

**Study no:** AT02903/T6075725/PH-34378

**Conducting laboratory and location:** BHC PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation & completion:** January 28, 2005 and March 23, 2006

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot # and % purity:** BAY 59-7939 micronized, Batch #BX01NB2; 99.3%.

**Formulation/vehicle:** The drug was administered in ethanol/Solutol HS15/demineralized water (1/4/5) in a volume of 10 ml/kg. The animals of the control and treatment groups received the same amount of vehicle consisted of ethanol/Solutol HS15/tap water.

##### **Methods:**

**Species/strain:** CD-1 mice

**#/sex/group or time point (main study):** 10 animals/sex/group

**Satellite groups used for toxicokinetics:** 18/sex/treatment group and 9/sex in control group.

**Age:** 7 weeks old

**Weight:** 34.9 to 35.2 g for males, 21.1 to 30.0 g for females.

**Doses in administered units:** BAY 59-7939 was administered in animals at the doses shown in the table below in a volume of 10 ml/kg:

**Table 5-2: Dosing Schedule**

Group		Dose (mg /kg b.w.)	Sex	Number of Animals	Animal number
1	main group	0	male	10	1-10
2	"	60	male	10	11-20
3	"	300	male	10	21-30
4	"	1500	male	10	31-40
5	"	0	female	10	41-50
6	"	60	female	10	51-60
7	"	300	female	10	61-70
8	"	1500	female	10	71-80
9	satellite group	0	male	9	81-89
10	"	60	male	18	90-107
11	"	300	male	18	108-125
12	"	1500	male	18	126-143
13	"	0	female	9	144-152
14	"	60	female	18	153-170
15	"	300	female	18	171-188
16	"	1500	female	18	189-206

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**Route, form, volume, and infusion rate:** Oral

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and morbidity and once daily in cage for abnormal clinical signs.

**Body weights:** Body weights were measured prior to initiation of dosing, weekly thereafter and immediately before necropsy.

**Food consumption and water intake:** Food and water consumptions were measured once a day and from the weekly data, the daily food and water intake per animal was computed.

**Hematology:** Blood samples for hematological examinations were collected at week 12 for Hepato Quick (HQUICK) and week 13 for hematology data for main groups males/females.

**Clinical chemistry:** Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, albumin, total protein, cholesterol, creatinine, urea, bilirubin-total, bilirubin-direct were estimated in week 12 of males/females of main study groups.

**Gross pathology:** Animals that died or sacrificed moribund, were also subjected to necropsy examinations. The surviving animals were also subjected to complete necropsy on day 92/93 and examined for pathological changes.

**Organs weighed:** The weights of the organs recorded were: brain, heart, liver, spleen, kidneys, testes, ovaries, and uterus. At the end of the study, liver samples



were collected from animals of main study groups for the determination of CYP450 (P450), triglycerides, n-demethylase (N-DEM), O-demethylase (O-DEM), cytochrome P450-dependent monooxygenase (ECOD – isozymes CYP1A1, 2B1, 2D1, 2E1), EROD isozyme 1A1, ALD (for isozyme 2B1, 3A1, 3A4, 2C11), epoxide hydrolase, conjugated GS-T and GLU-T, and carnitine acetyltransferase (CAT).

**Histopathology:** Histopathological examinations of the following organs from the control and high dose treatment groups of the main study groups were conducted. Adrenal gland, aorta, epididymides, esophagus, eyes, extra orbital lacrimal glands, femur, heart, head, kidneys, larynx, liver and gall bladder, lungs, mesenteric lymph node, mandibular and popliteal lymph nodes, optic nerve, ovaries, oviducts, pancreas, salivary glands, skeletal muscle, spleen, sternum, testes, thymus, thyroid glands, trachea, urinary bladder, uterus and vagina. The liver, kidneys, lungs, pancreas, eyes and optic nerves of the animals of all animals of the study were examined.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from retro-orbital venous plexus from the satellite group animals. Frequency and Dates of Toxicokinetic Investigation: i) day 1 – blood samples collected from 3/sex/group at 0.5, 1, 2, 4, 7 and 24 hours after administration and, ii) on day 85 at 1, 7 and 24 hours after administration of the compound.

## **Results:**

**Mortality:** Three animals of study groups died and were; 2 from the 150 mg/kg/day group and 1 from the 300 mg/kg/day group). These deaths were related to the withdrawal of blood and not related to treatment.

**Clinical signs:** No treatment related effects were noted in study treatment group animals.

**Body weights:** Treatment with BAY 59-7939 for 13 weeks produced no treatment related significant changes in the body weight of male or female mice compared to control group animals.

**Food consumption:** No treatment related change in the food consumption was seen.

**Hematology:** Coagulation time (HQUICK) was significantly and dose-dependently increased (up to 45.3% in males and 62.6% in females) in both treated males and females on day 78/79. The other changes observed were insignificant and not of any relevance.

**Table 6-5: Hematology –  
HQuick - Main Groups**

HQUICK		
Dose mg/kg	sec	
m	Day 78/ 79	
60	18.1	
300	25.1	++
1500	24.5	++
200	26.3	++
f	Day 78/ 79	
0	19.8	
60	28.0	++
300	32.5	++
1500	32.2	++

Legends: ++ significantly different at  $p \leq 0.01$

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**Clinical chemistry:** On day 31, serum AST, ALT and GLDH were increased in study males of 1500 mg/kg/day treatment group but these were not significant. There were no other significant/meaningful effects on clinical chemistry parameters observed in this study. Treatment with the compound for 89 days did not affected T3, T4 and TSH in males or females of the study.

**Organ weights:** The mean absolute and relative weight of liver was insignificantly increased in males and females of the high dose group. The absolute liver weights were 2058, 2063, 2126 and 2150 mg in males and, 1499, 1552, 1557 and 1668 g in females of 0, 60, 300 and 1500 mg/kg/day groups, respectively.

**Gross pathology:** No treatment related pathological changes were seen in any of the animals of the study groups.

**Histopathology:** No treatment related histopathological changes were seen in the males and females included in the treatment groups

#### **Liver Enzymes:**

BAY59-7939 administration for 13 weeks in mice, produced significant decrease in activities of ECOD, EROD, ALD and GLU-T in 1500 mg/kg/day group up to about 70% of the control activities in male mice, whereas in female mice activities were not significantly affected.

**Toxicokinetics:** Plasma concentrations of BAY 59-7939 in week 13 were lower than on week 1 for the high dose group animals. The peak plasma concentrations were seen within 0.5 to 1 hr in males and 1 to 2 hr in females after the administration of the compound but it was neither dose related nor dose proportional. The toxicokinetic parameters for BAY 59-7939 in male and female mice on day 85 are shown in sponsor's summary Table below.

**Table 6-10: Summary on Exposure in Mice at Steady State (Day 85)**

Sex		Male			Female		
Dose	[mg/kg]	60*	300	1500	60*	300	1500*
AUC(0-24)	[mg·h/L]	3.44	5.00	6.11	5.11	7.00	9.41
AUC(0-24) <sub>norm</sub>	[kg·h/L]	0.0573	0.0167	0.00407	0.0852	0.0233	0.00627
C <sub>max</sub>	[mg/L]	1.06	1.28	1.73	1.86	2.21	2.53
C <sub>max, norm</sub>	[kg/L]	0.0177	0.00428	0.00115	0.0311	0.00737	0.00169
C(24)/C <sub>max</sub>	[%]	0.342	1.59	2.03	0.296	0.656	2.34
t <sub>max</sub>	[h]	0.500	0.500	0.500	0.500	0.500	0.500
R <sub>A1</sub>	[%]	75.7	53.9	76.1	74.6	76.3	96.8
R <sub>A3</sub>	[%]	63.4	60.5	62.7	70.0	76.9	67.6

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R<sub>A1</sub> = ratio of C<sub>max</sub> from Week 13 to Day 1

R<sub>A3</sub> = ratio of AUC(0-24) from Week 13 to Day 1

\* = (n=2)

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### Summary of individual study findings:

In the 13-week oral gavage toxicity study in CD-1 mice, the micronized form of BAY 59-7939 up to 1500 mg/kg/day (in ethanol/Solutol/tap water) produced a non-dose proportional increase of plasma concentrations in mice. The coagulation times in males and females were changed and no other treatment related changes were seen. The target organs of toxicity and MTD were not identified.

### RAT:

#### 5. Subacute 2-Week Intravenous Toxicity study in Wistar rats (Study #T2073138/PH-34189)

**Key study findings:** Intravenous doses of 0.0657, 0.1971 and 0.657 mg/kg/day (dose multiples of 1, 3 and 10) /kg/day were administered daily for 14 consecutive days. A dose related foamy macrophages in lung parenchyma (enlarged cytoplasm with eosinophilic granules), swelling/vacuolation of proximal convoluted renal tubules and extramedullary hematopoiesis of spleen was seen. A dose of 0.1971 mg/kg/day (1.183 mg/m<sup>2</sup>) was considered as a 'maximal tolerable dose' and lungs, kidneys and spleen were identified as the target organs of toxicity in this study.

Testing Laboratory: Bayer HealthCare AG, PH-PD Toxicology International, Wuppertal (Germany)

Dates of Start and Completion of Study: September 26, 2003 and November 17, 2005

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted.

Species and Strain: Wistar rats (Hsd Cpb:WU; SPF bred) approximately 7 to 8 weeks old with mean body weight of 160 to 168 g (males) and 111 to 137 g (females)

Batch #: 0850001

Methods: Eighty rats (40/sex) were randomly divided into 4 main study groups (10/sex/group) and intravenously administered the doses of 0, 0.0657, 0.1971 and 0.6570 mg/kg/day BAY 59-7939 in hydroxypropyl  $\beta$ -cyclodextrin (volume = 1, 3 or 10 ml/kg/day, respectively). Additionally, 4 groups of non-fasted animals (8/sex/group) were included in the toxicokinetic part of the study. The blood samples from these animals were drawn on day 1 and 10 at 0.5, 1, 2, 4, 7 and 24 post dose period. The animals were observed prior to dosing and at 1 hr after the dose for mortality and morbidity and detailed changes in clinical changes were noted daily. The body weights, food and water consumption were recorded daily prior to treatment during the study and at the termination of the study. Hematological (including blood coagulation) and blood chemistry parameters were determined on the samples collected from the non-fasted treatment groups animals at the termination of the study (day 15). Liver samples from 5/group animals were tested for enzyme activity. A necropsy on each of the animals was performed at termination; the examination of each of the organs, external surfaces of the body, orifices and cavities of each animal was done. The tissues/organs separated, cleaned and preserved for histopathological examination were: adrenals\*, aorta, brain\*, cecum, colon, duodenum, injection site, jejunum, esophagus, heart\*, ileum, kidneys\*, large intestines, liver\*, lungs, mammary glands, pancreas, pituitary, spleen\*, prostate, rectum, stomach, seminal vesicles, eye/optic nerve, femur, gall bladder, heart, ileum, spinal cord, testes\*/ovaries\* with epididymides\*, rectum, thymus\*, thyroid, urinary bladder, mesenteric lymph nodes and vagina/uterus. The organs marked with asterisk (\*) were weighed.

Results:

a. Observed Effects: The sponsor stated that there were no treatment related effects in the study animals but the table/material were not submitted. Because of the thick viscosity of the solution, all of animals were not injected. None, 0, 1, 4 males and 2, 2, 2, 6 females of 0, 1, 3 and 10 ml/kg/day treatment groups, respectively, were not injected on 2 or more days during the study. No treatment related clinical signs were noted in the animals. The rectal temperature of the study animals was not affected and the initial temperature in male and female animals of control group was 38.56 and 38.21°C, respectively.

b. Mortality: None of the animals of the study group died

c. Body Weight/Food Consumption/Water Consumption Changes: The body weight increase in the rats belonging to treatment groups was not different from the control group animals. The initial and final mean body weights were 160 and 217 g, respectively, in control males and 124 and 161 g, respectively, in control females. On day 15, the mean body weights of males and females included in 10 ml/kg/day group were 210 and 163 g, respectively, which were not different from

the animals of control group. The daily water and food intake was not affected during the study.

d. Hematology/Coagulation/Bone Marrow Changes: There were no treatment related changes in the hematology parameters in the study animals during treatment period. Coagulation parameter and bone marrow changes were not observed during the study.

e. Blood Chemistry/Urinalysis Changes: On day 14, no dose or treatment related significant changes ( $p < 0.01$ ) in hematology parameters were observed. Serum calcium concentrations were increased in all treatment groups (male and female) of the study, but these were not clinically significant (within  $2 \pm$  SD values or in the normal range). The activity of liver O-DEM was increased in a treatment related manner in males and females but was not of a clinical importance. The monooxygenase [ECOD CYP 1A1, 2B1, 2D1 and 2E1) EROD (1A1), ALD 2B1, 3A1, 3A4 and 2C11)] epoxide hydrolase (EH) and conjugation enzymes (GSH, GLU-T) activities were measured. The enzymes activity in liver tissues of males and females were decreased slightly in a non-dose proportional but treatment related manner as shown below in the table 2-3 and 2-4 for males and females (vol 1.3, pp 180).

**Table 2-3 Enzyme activities in the liver of rats (m) after intravenous infusion of BAY 59-7939 for 2 weeks (mean values)**

Dose [ml/kg]	ECOD	EROD	ALD	EH	GS-T	GLU-T
0	14.2	1.11	334.5	562	89	1612
1	8.0 ++	0.47 ++	235.0 ++	437 -	91 -	1079 ++
3	11.4 +	0.82 -	280.7 -	511 -	112 ++	1395 -
10	10.3 ++	0.73 +	230.7 ++	476 -	81 -	1093 ++

**Table 2-4 Enzyme activities in the liver of rats (f) after intravenous infusion of BAY 59-7939 for 2 weeks (mean values)**

Dose [ml/kg]	ECOD	EROD	ALD	EH	GS-T	GLU-T
0	5.6	0.53	34.6	359	97	746
1	3.1 ++	0.29 +	20.2 ++	229 +	81 +	394 ++
3	3.9 +	0.39 -	25.2 +	290 -	109 -	514 -
10	3.6 ++	0.37 +	25.7 +	250 +	104 -	408 +

ECOD = 7-Ethoxycoumarin deethylase [nmol/g\*min]  
 EROD = 7-Ethoxyresorufin deethylase [nmol/g\*min]  
 ALD = Aldrin epoxidase [nmol/g\*min]  
 EH = Epoxide hydrolase [nmol/g\*min]  
 GS-T = Glutathione-S-transferase [μmol/g\*min]  
 GLU-T = UDP-Glucuronyltransferase [nmol/g\*min]

Student t-Test:  
 $p > 0.050$ : -  
 $p \leq 0.050$ : +  
 $p \leq 0.010$ : ++

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The decrease in liver enzymes was of no toxicological relevance. TSH, T3 and T4 levels in male and female animals were not affected during the study. No changes of clinical importance in the urinalysis were seen in study animals.

f. Toxicokinetics & Exposure: On study day 1 and 14, a dose proportional increase in drug plasma concentrations were seen after 30 min of the administration of the compound in both males and female. The half-life of the compound was not estimated. The plasma concentrations of the compound were similar in male and female animals on day 1 and 14 indicating that the compound did not accumulate. The toxicokinetic of the compound in both male and females are shown in the following tables (sponsor's table 6-12, Amendment #28, vol 3 of 4, pp 45).

TABLE  
Toxicokinetic Parameters of BAY 59-7939 in Wistar Rats

Group #	Dose in ml/kg/day (mg/kg)	Tmax (hr)	Cmax (mg/ml)	AUC <sub>(0-24hr)</sub> ng.hr/ml)
Day 1:	1 (0.0657)	0.5	0.031	0.0506 (0.044-AUC <sub>0-nhr</sub> )*
	3 (0.1971)	0.5	0.176	0.275 (0.270-AUC <sub>0-nhr</sub> )*
	10 (0.657)	0.5	0.541	0.830 (0.825-AUC <sub>0-nhr</sub> )*
Day 14:	1 (0.0657)	0.5	0.059	0.0835 (0.0815-AUC <sub>0-n8hr</sub> )*
	3 (0.1971)	0.5	0.194	0.276 (0.269-AUC <sub>0-n8hr</sub> )*
	10 (0.657)	0.5	0.683	0.981 (0.977-AUC <sub>0-n8hr</sub> )*
Day 1	1 (0.0657)	0.5	0.0972	0.156 (0.149-AUC <sub>0-nhr</sub> )*
	3 (0.1971)	0.5	0.260	0.396 (0.396-AUC <sub>0-nhr</sub> )*
	10 (0.657)	0.5	0.930	1.423 (1.42-AUC <sub>0-nhr</sub> )*
Day 14	1 (0.0657)	0.25	0.0888	0.125 (0.121-AUC <sub>0-nhr</sub> )*
	3 (0.1971)	0.5	0.238	0.354 (0.350-AUC <sub>0-nhr</sub> )*
	10 (0.657)	0.25	0.827	1.22 (1.21-AUC <sub>0-8hr</sub> )*

(\*) = AUC<sub>0-8hr</sub>

i. Organ Weight Changes: There was no treatment related effect on the organ weights other than the absolute liver weight of females in the high dose treatment group was increase by 10.9% compared to control group females.

j. Gross Pathology Findings: Bilateral pale discoloration was noted in males and females of 0.1971 and 0.6570 mg/kg/day BAY 59-7939 treatment groups and about 20% (2 of 10) of females of high dose treatment group had swollen spleen. The injection sites in the treated animals showed similar reaction to those of control group.

k. Histopathological Changes: Foamy macrophages in lung parenchyma were present in dose related manner in 0, 0, 6 and 10 males and, 0, 1, 4 and 9 females in the 0, 0.0657, 0.1971 and 0.6570 mg/kg/day groups, respectively. The macrophages were enlarged and contained eosinophilic granules, however the sponsor argued that this was not treatment related as similar changes were not noted in the previous rat studies. In the animals of the high dose treatment group, cytoplasmic enlargement was reported (see the table below). Swelling/vacuolation of proximal convoluted renal tubules was present in 0, 4, 10 and 10 males and 0, 6, 10 and 10 females in the 0, 0.0657, 0.1971 and 0.6570 mg/kg/day groups, respectively. This finding was dose related, and the animals in low dose group had grade 1 and the high dose treatment group showed greater swelling (see details in table below). Extramedullary hematopoiesis of spleen was seen in 1, 2, 1 and 5 males and, 0, 4, 2 and 4 females in the 0, 0.0657, 0.1971 and 0.6570 mg/kg/day groups, respectively. The incidences and severity were higher in high dose group males and females as shown below in the table:

**TABLE**  
Microscopic Incidences in 14-Day Intravenous Toxicity Study in Rats  
(10 Animals/sex/Group)

Organs		BAY 59-7939 Used (mg/kg/day)			
		0	2	3	4
Lungs (Enlarged Foamy Macrophages)	Male	0	0	6 (grade1)	10 (3-gr. 1, 2-gr 2 and 2-gr. 3)
	Female	0	1 (gr. 1)	4 (gr.1)	9 (5-gr 1, 2-gr. 2, 2-gr 3)
Kidney – Prox. convoluted tubules (Swelling/Vacuolation)	Male	0	4 (gr 1)	10 (8-gr 1; 2 gr 2)	10 (10-gr 3)
	Female	0	6 (5-gr 1; 1-gr 3)	10 (1-gr. 1; 9-gr 2)	10 (10-gr 3)
Epididymides Inflamm. Infiltr.	Male	2 (1-gr 1; 1-gr2)	0	1(1-gr 1)	5 (4-gr 1; 1-gr 3)
Uterus Dilatation	Female	1(1-gr. 1)	0	1 (1-gr 2)	3(gr 1)
Mesent. Lymph Node	Male	3(3-gr 1)	3(3-gr 1)	2(2-gr 1)	5(5 gr. 1)
	Female	1(1-gr 1)	4(4-gr 1)	2(2-gr 1)	4(4-gr 1)

‘gr’ = grade/intensity of changes (1=slight, 2=mild, 3=moderate)

In summary, intravenous doses of 0.0657, 0.1971 and 0.657 mg/kg/day BAY 59-7939 produced a dose proportional increase in plasma drug concentration and dose related findings of foamy macrophages in lung parenchyma (enlarged cytoplasm with eosinophilic granules), swelling/vacuolation of proximal convoluted renal tubules and extramedullary hematopoiesis of spleen. A dose of 0.1971 mg/kg/day (1.183 mg/m<sup>2</sup>) was considered as a ‘maximal tolerable dose’ and lungs, kidneys and spleen were identified as the target organs of toxicity in this study.

**6. Study Title: Four-Week Oral Toxicity Study with BAY 59-7939 in Rats:**  
Study no: T 7070622

**Key Findings:** In the 4-week oral gavage toxicity study, 4 groups of rats (10 sex/group) were given 0, 12.5, 50 and 200 mg/kg/day of the drug. A 12% decrease in body weight gain in high dose males at the end of the dosing period and, treatment related increases in several liver enzyme activities in all treated groups animals were noted. The insignificant decrease in CD45<sub>total</sub>, and slight increase in IgA levels in females and IgG levels in males were not of clinical significant. The plasma exposure of animals treated with high dose was 162 times the exposure achieved in man treated with a proposed clinical dose. A slight decrease in the body weight and treatment related increase in several liver enzyme activities in males was not associated with histopathological changes in the liver. The target organs of toxicity were not identified and 200 mg/kg/day dose was the maximum tolerated dose.

**Conducting Laboratory:** Department of Toxicology/Pharma, Institute of Toxicology, BAYER AG, 42096 Wuppertal, Germany.

**Dates of Study Initiation and Completion:** May 18, 2001 and April 23, 2002.

**GLP Compliance:** yes

**QA Report:** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939, Batch no. 507 047

**Formulation/vehicle:** The test substance was suspended in a mixture of Solutol HS 15/water (20g/80g). The formulation of the compound was stable for 2 hours at the room temperature.

**Methods:**

**Dosing Information:**

**Species/strain:** Hsd Cpb.Wu rats

**#/sex/group or time point:** 10 animals/sex/group

**Satellite groups used for toxicokinetics or recovery:** For toxicokinetic analysis 6 animals/sex/group in the control and 8 animals/sex/group in each treatment group received the vehicle or the drug.

**Age:** 5 – 6 weeks

**Weight:** 123 g – 162 g males, 112 g – 149 g females.

**Doses Used:** 0, 12.5, 50 and 200 mg/kg

**Route, form, volume and infusion rate:** The drug was administered by oral gavage and the dosing volume was 10 ml/kg.

**Observations and times:**

**Clinical signs** – twice daily

**Body weights** – prior to initiation of dosing and once a day during the dosing period.

**Food and water consumption** – once a week

**Hematology-** at the end of the dosing period (week 5); recovery groups, at the end of the recovery period.

**Clinical Chemistry-** At the end of the dosing period (week 4).

**Urinalysis-** On days 3/4 and 23/24 of the dosing period.



**Gross pathology** – At the end of the dosing period, the animals were sacrificed and necropsies performed.

**Organs weighed**- the weights of the following organs were measured: brain, heart, liver, spleen, kidneys, adrenals, testes, epididymides and thymus.

**Histopathology**- Histopathological examinations of the following organs of the control and the high dose group animals were conducted.

Adrenal glands, aorta, brain, epididymides, esophagus, eyes, eyelids, exorbital lacrimal glands, femur, harderian glands, nasal cavity, heart, duodenum, jejunum, ileum, cecum, colon, rectum, kidneys, larynx, liver, lungs, lymph nodes (mandibular and mesenteric), optic nerves, ovaries, oviducts, pancreas, pharynx, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid glands, tongue, trachea, ureters, urethra, urinary bladder, uterus, vagina, Zymbal's glands and organs and tissues with macroscopic findings.

**Toxicokinetics**- Blood samples for toxicokinetic analysis were collected on dosing days 1 and 28 from 4 animals/sex at 0.5, 1, 2, 4, 7 and 24 hours after dosing.

**Liver Enzymes**: The activities of the cytochrome P-450 dependent monooxygenases [ethoxycoumarin deethylase (ECOD), ethoxyresorufin deethylase (EROD), aldrin epoxidase (ALD)], epoxide hydrolase (EH) and the conjugation enzymes [glutathione transferase (GS-T), UDP-glucuronyl transferase (GLUT)] of the liver tissues of 5 animals/sex/dose group were determined at the end of the dosing period.

**Immunotoxicity**: To examine the potential immunotoxicity of BAY 59-7939 in rats, the following parameters were examined: cell counts in the spleen, Flow cytometry analyses (FACScan) to determine the subpopulations of spleen cells and the determination of the antibody (IgG, IgM and IgA) titers in the sera using the Sandwich ELISA method.

## **Results:**

**Clinical signs**- No treatment related clinical signs were observed in any group.

**Body weights**- The mean body weights of the control males and females at the beginning of dosing (Day 1) were  $139.0 \pm 4.7$  and  $122.0 \pm 7.63$  g, respectively, and at the end of the dosing were  $287.0 \pm 18.6$  and  $189.0 \pm 11.4$  g, respectively. A slight decrease in the body weight was observed in the high dose males during most part of the dosing period (7.4%, 8.0%, 7.5% and 7.3% on days 7, 14, 21 and 28, respectively). At the end of the dosing period, a 12% suppression of the body weight gain was observed in the high dose males as compared with the controls.

**Food consumption**- The mean food consumptions of the control males and females in Week-1 of dosing were  $20.5 \pm 1.34$  and  $17.2 \pm 1.4$  g/animal/day, respectively. The water consumption was increased in all treatment group males and females (during week 2, 11.9%, 32.9% and 20.2% increases in males and 8.9%, 15.9% and 28.4% increases in females at low, mid and high doses, respectively).

**Ophthalmoscopy**- No treatment-related ophthalmologic changes were observed in any group.

**Hematology-** Males receiving the mid and the high dose had slight increases in the reticulocyte levels (19% and 14% at mid and high doses, respectively). No other changes were observed in any other group.

**Clinical Chemistry-** The high dose males had higher bilirubin levels (27.3%); these values were within the historical control values in this strain of rat.

**Urinalysis-** No treatment-related changes were observed in any group.

**Gross pathology-** No treatment-related gross pathological changes were observed in any group at the end of the treatment or recovery periods.

**Organ weights-** Males and females receiving the high dose had lower heart weights as compared with the controls (relative, 15.6% in males, 11.5% in females). High dose females had slightly lower thymus weights (13.8%, relative). However, no histopathological changes in the heart or thymus were observed in the high dose animals.

**Histopathology-** Histopathological examinations of all tissues were conducted only of the control and high dose animals (except kidney and liver). High dose males had higher incidences of bilateral retinal atrophy (control 4/10, high dose 9/10), focal inflammation of the pancreas (control 1/10, high dose 4/10) and unilateral diffuse tubular dilatation of the testes (control 0/10, high dose 4/10). The severity of incidences of bilateral retinal atrophy in males was higher in the high dose group as compared with that in controls (minimal- control 4/9, high dose 5/9; slight- control 0/9, high dose 4/9).

**Toxicokinetics:** Orally administered BAY 59-7939 was rapidly absorbed in rats with the  $T_{max}$  ranging from 0.5 to 1.0 hour and  $C_{max}$  and AUC values on Days 1 and 28 increased with the dose. The plasma drug exposure levels in females were higher than the males (the AUC values at the low dose on Day 1 in males and females were 12.6 and 22.8 mg.h/ml, respectively). The TK parameters of the male and female rats on Day 1 and Day 28 of dosing are summarized in the following Table.

	Male			Female		
	12.5 mg/kg	50 mg/kg	200 mg/kg	12.5 mg/kg	50 mg/kg	200 mg/kg
<b>Day -1</b>						
AUC <sub>0-24</sub> (µg.h/L)	12631	45037	133996	22779	69780	166116
C <sub>max</sub> (µg/L)	4409	6982	17089	7492	16881	30564
T <sub>max</sub> (h)	0.50	0.50	0.50	1.00	0.50	0.50
<b>Day- 28</b>						
AUC <sub>0-24</sub> (µg.h/L)	17735	83341	155755	22343	99469	227123
C <sub>max</sub> (µg/L)	6107	16588	26002	6008	24644	41955
T <sub>max</sub> (h)	0.50	0.50	0.50	0.50	1.00	0.50

**Liver Enzymes:** The male rats treated with oral doses of BAY 59-7939 for 4 weeks had dose-dependent statistical significant increases in EH (5.9%, 65.4% and 100.9% at low, mid and high doses, respectively) and GLU-T (55.2% and 94.9% at mid and high doses, respectively) levels compare to controls. GS-T level was also slightly increased in the high dose males (26.0%). The EH (36.3%, 54.9% and 77.2% higher at low, mid and high doses, respectively) and GLU-T (22.3%, 36.5% and 43.5% higher at low, mid and high doses, respectively) levels in females were also higher than controls. The high dose females had statistical significant higher ECOD (37%) and EROD (26.5%) levels and the 12.5 mg/kg

females had slightly higher GS-T levels (19.3%). The effects of treatment with BAY 59-7939 on the liver enzyme activities of the male and female rats are summarized in the sponsor tables which are scanned below.

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**Table 3**

**Enzyme activities in the liver of rats (m) after administration of BAY 59-7939 for 4 weeks**

Dose [mg/kg]	ECOD	EROD	ALD	EH	GS-T	GLU-T
0	9.1	0.47	155.8	442	96	706
12.5	9.6	0.27	159.7	468	101	715
	-	-	-	-	-	-
50	10.5	0.39	166.4	731	100	1096
	-	-	-	*	-	**
200	10.7	0.44	161.8	888	121	1376
	-	-	-	*	**	**

**Table 4**

**Enzyme activities in the liver of rats (f) after administration of BAY 59-7939 for 4 weeks**

Dose [mg/kg]	ECOD	EROD	ALD	EH	GS-T	GLU-T
0	4.6	0.34	21.8	215	83	400
12.5	5.1	0.46	25.8	293	99	489
	-	-	-	-	**	-
50	5.4	0.38	27.5	333	86	546
	-	-	-	-	-	-
200	6.3	0.43	26.6	381	95	574
	-	-	-	-	-	-

ECOD	= 7-Ethoxycoumarin deethylase	[nmol/g*min]	Student t-Test
EROD	= 7-Ethoxyresorufin deethylase	[nmol/g*min]	
ALD	= Aldrin epoxidase	[nmol/g*min]	
EH	= Epoxide hydrolase	[nmol/g*min]	
GS-T	= Glutathione-S-transferase	[μmol/g*min]	
GLU-T	= UDP-Glucuronyltransferase	[nmol/g*min]	
			p > 0.050 -
			p <= 0.050 *
			p <= 0.010 **

**Immunotoxicity:** Treatment with 12.5 to 200 mg/kg BAY 59-7939 for 28 days had no effect on the spleen cell counts or the size of the cells in the male and female rats. The CD4<sub>total</sub> (16.9%, 22.1% and 26.5% lower at low, mid and high doses, respectively) and CD45<sub>total</sub> (21.3%, 23.5% and 27.1% lower at low, mid

and high doses, respectively) levels of the treated females were lower than those of controls. The IgA levels in the treated females (64.8%, 26.1% and 23.9% increases at low, mid and high doses, respectively) and the IgG levels of the mid (106.4%) and high (108.7%) dose males were higher than those of controls.

In 4-week oral gavage toxicity study, 4 groups of animals were treated with 0, 12.5, 50 and 200 mg/kg/day doses and a slight decrease in the body weight and treatment related statistically significant increase in several liver enzyme activities not associated with histopathological changes in either sex and of no clinical significance was observed. The CD4<sub>total</sub> levels were lower, IgA and IgG levels in the treated females were only slightly higher than those of controls. The animals of high dose treatment group were exposed to 162 times the plasma exposure achieved in man with clinical dose. The target organs of toxicity were not identified and 200 mg/kg/day dose was the maximum tolerated dose.

**7. Study title: Three (3)-Month Oral (Gavage) Toxicity study of BAY 59-7939 in Rats with a 4-Week Recovery Period (Study no: T7072116)**

**Key study findings:** In the 13-week oral gavage toxicity study in rats, BAY 59-7939 administered at oral doses of 12.5, 50 and 200 mg/kg/day produced a decrease in mean body weight gains in males and, increase in clotting time in both males and females. Increased incidences of pigment deposition in the pancreas, mesenteric lymph node hemorrhage, focal retinal atrophy, thymic hemorrhage and mesenteric lymph node hemorrhage were not completely resolved during the recovery period. The increase in the plaque cell counts and slight increase IgG only in males of all treatment groups was not consistent as not found in females. The highest tolerable dose was 200 mg/kg/day and based on the body surface area, the exposure was 162 multiples of the clinical dose.

**Study no:** T7072116

**Conducting laboratory and location:** PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation:** October 28, 2002

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939, PEG 6000 co-precipitate 10% 100; Batch no. J20020703.

**Formulation/vehicle:** The drug was dissolved in Solutol/water (20/80 v/v). The formulations were prepared fresh once weekly. Control animals were administered the vehicle (Solutol/water). The drug was stable in the formulation during the 7-day storage period (95% to 112% of nominal concentrations).

**Methods:**

**Dosing:**

**Species/strain:** Wistar rats (Hsd Cpb:WU).

**#/sex/group or time point (main study):** 10 animals/sex/group

**Satellite groups used for toxicokinetics or recovery:** 12 animals/sex/group were

used for toxicokinetic studies. Ten (10) animals/sex/group in the control and the high dose groups were left untreated for a 4-week recovery period at the end of the 3-month dosing period.

**Age:** 4 weeks old.

**Weight:** 95 -138 g for males, 93-112 g for females.

**Doses in administered units:** BAY 59-7939 was administered at oral doses of 0, 12.5, 50 and 200 mg/kg/day for 97/98 days. The dosing volume was 10 ml/kg.

**Route, form, volume, and infusion rate:** The drug (formulated in Solutol/water) was administered by oral gavage at a dosing volume of 10 ml/kg.

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and abnormal clinical signs. In addition, detailed clinical examinations were performed weekly.

**Body weights:** Body weights were measured prior to initiation of dosing, and then weekly thereafter.

**Food consumption:** Food and water consumptions were measured once a week, and the daily food intake per animal was calculated.

**Ophthalmoscopy:** All animals were subjected to ophthalmologic examinations before the start of the study, and at the end of the treatment or recovery period.

**Hematology:** Blood samples for hematological examinations were collected on Days 31/32 and 94/95.

**Clinical chemistry:** Blood samples for clinical chemistry examinations were collected on Days 31/32 and 94/95.

**Urinalysis:** Urine samples from all groups of animals were collected on Days 29/30 and 85/86 of the dosing period and at the end of the recovery period.

**Gross pathology:** All animals were subjected to complete necropsy examinations at scheduled sacrifice time. Animals that died or were sacrificed moribund, were also subjected to necropsy examinations.

**Organs weighed:** The weights of the following organs were recorded: brain, adrenal gland, heart, kidney, liver, spleen, thymus, kidneys, testes, epididymides, ovary, and uterus.

**Histopathology:** Histopathological examinations of the following organs from the two controls and the high dose groups were conducted. Adrenal glands, aorta, brain, epididymides, esophagus, eyes, exorbital lacrimal glands, femur, Harderian glands, heart, intestine (Peyer's patches, duodenum, jejunum, ileum, cecum, colon, rectum), kidneys, larynx, liver and gall bladder, lungs, mesenteric lymph nodes, submandibular lymph nodes, optic nerves, ovaries, oviducts, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testis, thymus, thyroid glands, tongue, trachea, ureters, urinary bladder, uterus and vagina.

Histopathological examinations were also conducted for abnormalities of the eyes, kidneys, liver, lungs, pancreas and the sternum with bone marrow from the low and mid dose animals.

**Immunotoxicity:** Ten/sex animals of control, 12.5, 50 and 200 mg/kg/day were dissected and spleen dissected and prepared for the Plaque forming cell assay.

The cells were separated and suspensions made and tested for their induced cells and antibody titres in spleens of all treatment groups for IgA by ELISA.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from the retro-orbital venous plexus of the satellite group animals (3 animals/time point) on Day 1 and Day 85 at 0.5, 1, 2, 4, 7 and 24 hours after administration.

## Results:

**Mortality:** Sixteen rats of the study were found dead or were killed in moribund condition. The mortalities among males and females of the main groups of animals are shown in the Table below.

Males				Females			
0 mg/kg	12.5 mg/kg	50 mg/kg	200 mg/kg	0 mg/kg	12.5 mg/kg	50 mg/kg	200 mg/kg
0/10	1/10	0/10	2/10 (#32,39)	1/10 (#44)	2/10 (# 51, 50)	1/10 (#62)	3/10 (#73,74,77)

In addition, 3 control and 1 high dose males, and 1 control and 1 high dose female of the recovery groups were found dead or killed moribund. The mortalities were unrelated to treatment with the drug.

**Clinical signs:** No treatment-related clinical signs were observed in any group.

**Body weights:** On day 1, mean body weights of the control male and female animals were 117 g and 105 g, respectively. The body weight gains of the treated males were lower than that of controls (5% to 10%; not dose-dependent). Females receiving the high dose had increased body weight gains (119% of control), as compared with the controls. The body weights and the percent changes in body weight gains of male and female animals on Day 92 are shown in the Table below.

Parameter	Males				Females			
	Control	12.5 mg/kg	50 mg/kg	200 mg/kg	Control	12.5 mg/kg	50 mg/kg	200 mg/kg
Body Wt. (g) Day 1	117	120	117	118	105	103	103	101
Body Wt. (g) Day 92	446	423	413	429	236	233	242	257
Body Wt. gain (g)	329	303	296	311	131	130	139	156
Body Wt. gain (% control)	100%	92.1%	90%	94.5%	100%	99.2%	106.1%	119.1%

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**Food consumption:** The mean food consumptions of the control male and female rats were 25.3 and 16.7 g/animal/day, respectively. No treatment-related changes in food consumption were observed in male and female rats receiving BAY 59-7939.

**Ophthalmoscopy:** No treatment related ophthalmologic changes were observed in any group at the end of the dosing or the recovery period.

**Hematology:** Clotting time (HQuick) was significantly increased in both males and females receiving BAY 59-7939, and this effect was found to be reversible following 4 weeks of recovery period. The effects of BAY 59-7939 on the HQuick values (sec) in male and female mice are shown in the Table below

	Control	12.5 mg/kg	50 mg/kg	200 mg/kg
<b>Day 31/32</b>				
<b>Males</b>	33.4	36.6	38.6	73.8
<b>Females</b>	31.9	34.9	38.7	78.9
<b>Day 94/95</b>				
<b>Males</b>	31.3	34.8	38.1	68.4
<b>Females</b>	30.5	35.5	41.1	76.6

**Clinical Chemistry:** Clinical chemistry determinations were conducted on days 31/32 and 94/95 of the dosing period (for main study groups) and on Day 122 (recovery groups). Clinical chemistry changes were insignificant in male and female rats on Days 31/32 and 94/95 and are shown in the Table below.

Clinical chemistry changes in male and female rats on Days 31/32 and 94/95 are shown in the Table below.

Parameter	MALES				FEMALES			
	Control	12.5 mg/kg	50 mg/kg	200 mg/kg	Control	12.5 mg/kg	50 mg/kg	200 mg/kg
<b>Days 31/32</b>								
ALT (U/L)	43.7	42.5	56.6**	71.9**	37.3	36.0	49.9**	63.6**
GLDH (U/L)	2.7	4.3	5.1	4.7	5.1	1.7	3.2	3.2
LDH (U/L)	112	96	114	98	159	130	121	112*
Cholesterol (mmol/L)	1.41	1.56	1.54	1.83*	1.48	1.46	1.61	1.68
Triglyceride (mmol/L)	0.58	0.64	0.74	0.82**	0.58	0.65	0.62	0.56
Urea (mmol/L)	4.19	4.89	5.00	5.82**	4.36	4.30	4.21	5.43*
Bilirubin (μmol/L)	1.3	1.3	1.5	1.7**	1.2	1.2	1.3	1.6**
<b>Days 94/95</b>								
ALT (U/L)	42.3	47.1	46.5	46.3	43.0	39.4	51.2	47.5
GLDH (U/L)	6.9	5.1	6.1	5.6	15.8	5.6	5.5	4.4
LDH (U/L)	123	150	74**	76**	122	81**	73**	70**
Cholesterol (mmol/L)	2.03	1.95	1.92	1.86	1.82	1.91	2.00	1.90
Triglyceride (mmol/L)	1.12	0.98	1.08	0.85	0.83	0.91	0.68	0.55
Urea (mmol/L)	5.24	5.21	4.83	5.46	5.35	5.63	6.11	5.78
Bilirubin (μmol/L)	1.9	1.8	1.7	2.2	1.8	1.7	2.0	1.9

\*, p<0.05; \*\*, p<0.01

**Urinalysis:** There was a dose-related increase in urine volume in male and female animals at both times of the sampling period (up to 41% and 185% on Days 29/30 and up to 41% and 67% on Days 85/86 in males and females, respectively). No increases in the urine volume were observed in the high dose recovery animals at the end of the recovery period.

**Organ weights:** No treatment-related changes in organ weights were observed in any of the groups.

**Gross pathology:** No treatment-related gross pathological changes were observed in any group.

**Histopathology:** Males treated with the high dose had higher incidences of pigment deposits in the pancreas, mesenteric lymph node hemorrhage and focal retinal atrophy. Females receiving the high dose had higher incidences of congestion and pigmentation of lungs, uterine dilatation, pituitary cyst, thymic hemorrhage and mesenteric lymph node hemorrhage. No differences in histopathological changes were observed between the control and the high dose recovery groups at the end of the recovery period. Histopathological changes observed in male and female animals are summarized in the Table below.

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Organ/Finding	Control	12.5 mg/kg	50 mg/kg	200 mg/kg
<b>MALES</b>				
Pancreas -pigment	6/10	0/1	0/1	9/10
Mesenteric lymph nodes -hemorrhage	0/10	0/1	0/1	5/9
Eyes -retinal atrophy, focal	1/10	0/10	1/10	5/9
<b>FEMALES</b>				
Heart -epicarditis	0/10	1/2	1/1	3/10
Lungs -congestion -pigment	0/10 0/10	1/10 1/10	1/10 2/10	3/10 2/10
Pancreas -pigment	0/10	0/2	0/1	2/10
Uterus -dilatation	2/10	1/2	0/1	5/10
Pituitary -cyst	0/10	0/2	0/1	2/10
Thymus -hemorrhage -pigment	0/10 1/10	0/2 0/2	0/1 0/1	5/10 4/10
Mesenteric lymph node -hemorrhage	0/10	0/2	0/1	3/10

**Toxicokinetics:** Plasma exposure levels (AUC) of BAY 59-7939 in male and female rats increased with increasing doses. Plasma exposure levels in female rats were slightly higher than that of male rats at both times of sampling and at all the doses examined. Peak plasma concentrations of BAY 59-7939 on Day 85 were higher than those on Day 1, suggesting that the steady state plasma concentrations were not reached on Day 1. Toxicokinetic parameters for BAY 59-7939 in male and female rats on Day 1 and Day 85 are summarized in the Table below.

Table 5-30: Summary of Pharmacokinetic Parameters

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		Day 1					
Gender Dose (mg/kg)		12.5	Male 50	200	12.5	Female 50	200
AUC(0-24)	[mg·h/L]	13.4	42.2	117	14.7	63.0	152
AUC(0-24) <sub>norm</sub>	[kg·h/L]	1.07	0.844	0.583	1.17	1.26	0.758
C <sub>max</sub>	[mg/L]	2.03	5.94	12.7	2.73	8.89	15.6
C <sub>max, norm</sub>	[kg/L]	0.162	0.119	0.0637	0.219	0.178	0.0780
C(24)/C <sub>max</sub>	[%]	5.77	1.79	15.6	0.385	4.55	14.6
t <sub>max</sub>	[h]	1.00	2.00	1.00	1.00	1.00	0.500
		Day 85					
Gender Dose (mg/kg)		12.5	Male 50*	200	12.5	Female 50	200
AUC(0-24)	[mg·h/L]	5.87	78.7	185	15.0	118	237
AUC(0-24) <sub>norm</sub>	[kg·h/L]	0.469	1.57	0.924	1.20	2.37	1.18
C <sub>max</sub>	[mg/L]	2.40	12.5	21.0	4.88	21.5	26.0
C <sub>max, norm</sub>	[kg/L]	0.192	0.250	0.105	0.390	0.430	0.130
C(24)/C <sub>max</sub>	[%]	0.178	0.907	6.05	0.331	1.95	17.0
t <sub>max</sub>	[h]	0.500	0.500	0.500	1.00	0.500	0.500
R <sub>A1</sub>	[%]	119	210	165	179	242	167
R <sub>A3</sub>	[%]	43.7	187	158	102	188	156

$$R_{A1} = C_{max, Day 85} / C_{max, Day 1}; R_{A3} = AUC(0-24)_{Day 85} / AUC(0-24)_{Day 1}$$

\* n=2/3

**Immunotoxicity:**

a. Cell counts (spleen)

No increase in the cell counts were seen at up to 200 mg/kg/day.

b. Plaque Formina Cells Assay (PFCA):



There was an increase in the Plaque cell counts at 200 mg/kg/day group in both males and females. A slight increase IgG in males of all treatment groups and no consistent effect in females was of not significance. The IgA and IgM antibodies were increased in the males and females of high dose group.

**Table 3**

**Antibody titer in the sera**  
(means and standard deviations)

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(animals/group = cf. Table I)

	Group No.		Means (µg/ml) male	+/- SD	Means (µg/ml) female	+/- SD
<b>IgA</b>	1/5	(Vehicle)	59.0	12.4	106.2	46.2
	2/6	(12.5 mg/kg)	85.3↑	30.2	129.7	28.5
	3/7	(50 mg/kg)	85.6↑	27.9	90.7	38.6
	4/8	(200 mg/kg)	122.8↑	24.5	105.1	41.9
<b>IgM</b>	1/5	(Vehicle)	653.8	133.3	868.7	155.7
	2/6	(12.5 mg/kg)	791.2	146.5	861.9	240.1
	3/7	(50 mg/kg)	647.2	188.9	727.5	397.4
	4/8	(200 mg/kg)	818.4↑	86.9	1051.4↑	270.8
<b>IgG</b>	1/5	(Vehicle)	3774.0	1149.1	3293.8	1687.5
	2/6	(12.5 mg/kg)	5848.3↑	3789.7	4428.6	1534.5
	3/7	(50 mg/kg)	4540.5↑	3351.3	3786.3	1707.6
	4/8	(200 mg/kg)	4875.5↑	1159.4	3016.4	1746.1

↑ = slight increase

↑ = statistically significant increase

**Others:** Drug metabolizing enzyme activities in liver homogenates from male and female rats were determined at the end of the dosing period. High dose males had increased levels of epoxide hydroxylase, glutathione S-transferase and UDP-glucuronyltransferase levels, and high dose females had higher levels of 7-ethoxyresorufin deethylase and UDPglucuronyl transferase levels.

**Summary of the study findings:**

In the 13-week oral gavage toxicity study in rats, BAY 59-7939 was administered at oral doses of 12.5, 50 and 200 mg/kg/day produced a decrease in mean body weight gains in males and, increase in clotting time in both males and females. Increased incidences of pigment deposition in the pancreas, mesenteric lymph node hemorrhage, focal retinal atrophy, thymic hemorrhage and mesenteric lymph node hemorrhage were not completely resolved during the recovery period. The plaque cell counts increase and a slight increase IgG only in males of all treatment groups were not consistent as not found in females. The highest

tolerable dose was 200 mg/kg/day (provides 162 multiples exposure of the clinical dose).

**8. 13-Week Subchronic Oral Dietary Toxicity Study in Rats with 10% co precipitate.** (Study # T 8074601/PH-34553)

In the study, BAY 59-7939 containing 0.5% of the active compound (not 5% as required under ICH guidelines 51C) in diet was administered for 13 weeks at the doses of 75 to 300 mg/kg/kg and a non-dose proportional plasma concentration was seen in rats. A minor change of hyper pigmentation in periductal pancreatic Islets was noted in males, there was no effect in study females. Sponsor should have employed higher doses of the compound.

Testing Laboratory: Bayer HealthCare AG, PH-PD Toxicology International, Wuppertal (Germany)

Dates of Start and Completion of Study: June 24, 2004 and August 1, 2006

GLP & QAU Requirements: A statement of compliance with German GLP regulations and OECD principles of GLP 1997 was submitted.

Species and Strain: Wistar rats (Hsd Cpb:WU; SPF bred) approximately 7 to 8 weeks old with mean body weight of 166 to 169 g (males) and 140 to 142 g (females)

Batch #: 031212-100

Methods: One hundred rats (50/sex) were randomly divided into 5 main study groups (10/sex/group) and administered 0, 0, 75, 150 and 300 mg/kg/day BAY 59-7939 co precipitate 10% 100 in diet (diet mixed compound prepared weekly). The sponsor calculated effective dose as the ratio of the product of the test compound concentration (ppm) and actual diet intake to the actual body weight (g). Additionally, 5 groups of sixty rats (6/sex/group) were included in the toxicokinetic part of the study. No appropriate basis of dose selection was provided by the sponsor. The highest dose of 300 mg/kg/day was considered to produce 50000 ppm of the formulation in the diet. On day 91, the intake of the compound calculated from the weekly means was 77.33, 157.46 and 315.55 mg/kg/day in males and, 78.31, 158.97 and 319.98 mg/kg/day in females. The blood samples from 3/sex/group were drawn from TK study groups on day 3 (1 sample at 8AM), week 5 (1 sample at 8AM) and on week 11 6 samples at 8 and 12 PM, 4 AM, 8AM, 12 AM and 4 PM. The animals were observed once a day for mortality and morbidity and detailed clinical changes were noted daily. The body weights, food and water consumption were recorded prior to treatment and weekly during the study and immediately prior to the necropsy. The hematological and blood chemistry parameters and, urinalysis were determined on the samples collected from retro-orbital venous plexus of the non-fasted

anesthetized animals of the treatment groups. The liver samples from the animals of treatment groups were taken at termination for determining the effect of the treatment on aminopyrine-N-demethylase, p-nitroanisole-O-demethylase, cytochrome P450 content, triglyceride content in all animals and, liver 7-ethoxycoumarin de-ethylase (ECOD), 7-ethoxyresorofin de-ethylase (EROD), aldrin epoxidase (ALD), epoxide hydrolase (EH), glutathione-S-transferase (GSH), UDP-glucuronyltransferase (UDP-GT) were determined. TSH (week 12 to 25), TBC (week 8 to 11), T3 and T4 hormones were estimated. Necropsy on each of the animals was performed at termination and examination of the organs, external surfaces of the body, orifices and cavities of each animal was done. The tissues/organs separated and cleaned were: adrenals\*, aorta, brain\*, cecum, colon, duodenum, injection site, jejunum, esophagus, heart\*, ileum, kidneys\*, large intestines, liver\*, lungs, mammary glands, pancreas, pituitary, spleen\*, prostate, rectum, stomach, seminal vesicles, eye/optic nerve, femur, gall bladder, ileum, spinal cord, testes\*/ovaries\* with epididymides\*, rectum, thymus\*, thyroid, urinary bladder, mesenteric lymph nodes and vagina/uterus\* and preserved for histopathological examination. The organs marked with asterisk (\*) were weighed.

#### Results:

- a. Observed Effects: The sponsor stated that there were no treatment related effects in the study animals.
- b. Mortality: None of the study animals of treatment groups died
- c. Body Weight/Food Consumption/Water Consumption Changes: The reduction in the body weight gain was 21.4, 38.5 and 36.5% in males and, 27.4, 32.1 and 33.1% in females belonging to 75, 300 and 300 mg/kg/day treatment groups, respectively. The daily water and food intake was not affected during the study.
- d. Hematology/Coagulation/Bone Marrow Changes: The PT and APTT were significantly ( $p < 0.002$ ) increased in a dose related manner in animals at doses of 75 mg/kg/day or more. The table 6-7 data has been scanned below (pp 33 of e-CTD Revised Final Report, 1st revision of study #T8074601).

**Table 6-7: Hematology - coagulation**

Hematology - coagulation		
dose mg/kg	THRU 10E9/l	HQUICK sec
males (day 91)		
0	1140	28.8
0 (PEG)	1091	29.2
0 (PEG)	1091	29.2
75	1067	42.5 ++
150	937 ++	50.4 ++
300	981	62.6 ++
females (day 92)		
0	1040	26.2
0 (PEG)	1038	25.8
0 (PEG)	1038	25.8
75	1034	36.1 ++
150	1018	41.6 ++
300	1038	51.5 ++

For reference values see Annex

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No other changes in the hematological parameters of statistical/clinical importance were seen in animals included in 75, 150 or 300 mg/kg/day treatment groups.

e. Blood Chemistry/Urinalysis Changes: On day 91, an increase in serum urea (males of 75 mg/kg/day or more treatment groups and females of 300 mg/kg/day group), a decrease in glucose in females of 300 mg/kg/day group was also seen. The other slight changes like a decrease in creatinine, protein, and albumin was noted in 75 mg/kg/day or more treatment groups in females only. Total bilirubin was increased in treatment related manner. Thyroid hormone profile (TSH, T3 and T4 amounts) were not affected in treated animals.

The activity of ECOD and EROD and GS-T enzymes was increased slightly in both males and females in a non-dose related manner. The phase II enzymes GS-T was not affected in males and GLU-T activity was decreased marginally in females. Liver O-DEM was increased in a treatment related manner in study males and females but was of no clinical importance.

**Table 6-10: Liver tissue**

Liver tissue							
dose mg/kg	P450 nmol/l	ECOD nmol/g*min	EROD nmol/g*min	ALD nmol/g*min	EH nmol/g*min	GS-T μmol/g*min	GLU-T nmol/g*min
males (day 93)							
0	40.7	7.9	0.52	221.4	736	142	1165
0 (PEG)	39.9	6.2	0.47	169.9	602	131	859 +
0 (PEG)	39.9	6.2	0.47	169.9	602	131	859
75	47.8 +	11.9 +	0.83 +	220.3	872 +	170 ++	1301 +
150	44.6	11.3 ++	0.62	206.6	946 +	178 ++	1252 +
300	46.8	10.9 ++	0.54	201.9	1143 ++	123	1450 ++
females (day 95)							
0	41.6	6.0	0.72	33.9	312	94	860
0 (PEG)	39.6	6.3	0.50 +	34.2	350	107	962
0 (PEG)	39.6	6.3	0.50	34.2	350	107	962
75	40.4	7.3 ++	0.73 +	39.3 +	490 ++	120	1100
150	41.1	7.4	0.84 +	36.1	428 +	113	914
300	42.5	7.8 +	0.77 +	38.0	474	86 +	1068

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**Table 6-9: Clinical chemistry**

Clinical chemistry						
dose mg/kg	ASAT U/l	ALAT U/l	Aph U/l	GLDH U/l	LDH U/l	CK U/l
males (day 91)						
0	56.8	49.1	126	7.0	61	136
0 (PEG)	54.5	51.6	144 ++	5.6	40 +	99 +
0 (PEG)	54.5	51.6	144	5.6	40	99
75	56.2	54.7	145	7.5	39	84
150	49.3	50.7	156	5.5	38	124
300	58.3	54.3	148	6.4	33	118
females (day 92)						
0	62.5	51.6	119	17.1	49	111
0 (PEG)	63.1	51.4	104	13.4	47	92
0 (PEG)	63.1	51.4	104	13.4	47	92
75	61.7	51.5	108	8.2	38	112
150	62.9	46.2	94	7.2	35	107
300	58.6	49.4	112	4.9	29	73

For reference values see Annex

Clinical chemistry							
dose mg/kg	GLUCOSE mmol/l	CHOL mmol/l	CREA μmol/l	UREA mmol/l	BILI-t μmol/l	PROT g/l	ALBUMIN g/l
males (day 91)							
0	5.96	2.16	59	6.17	1.2	71.8	36.2
0 (PEG)	5.61	2.27	59	6.26	1.3	70.2	36.0
0 (PEG)	5.61	2.27	59	6.26	1.3	70.2	36.0
75	5.09	2.08	61	8.63 ++	1.8 +	69.8	36.9
150	5.35	2.11	59	8.77 ++	1.8 +	68.6	36.3
300	5.39	2.21	60	7.88 +	1.8 +	68.8	37.1
females (day 92)							
0	5.29	1.89	65	7.49	1.3	72.5	39.5
0 (PEG)	5.28	2.06	64	6.04 ++	1.3	74.1	39.2
0 (PEG)	5.28	2.06	64	6.04	1.3	74.1	39.2
75	5.00	1.87	60 ++	6.96	1.8 ++	67.5 ++	36.3 ++
150	5.14	1.87	59 ++	6.89	1.6 +	68.4 ++	37.5
300	4.84 +	2.01	57 ++	7.32 +	2.1 ++	66.9 ++	36.4 ++

For reference values see Annex

Clinical chemistry – thyroid parameters			
dose mg/kg	T3 nmol/l	T4 nmol/l	TSH μg/IU/l
males (day 91)			
0	1.94	68	8.95
0 (PEG)	2.03	72	8.14
0 (PEG)	2.03	72	8.14
75	2.04	71	8.49
150	2.00	65	8.28
300	2.21	66	9.37
females (day 92)			
0	1.82	32	6.04
0 (PEG)	1.86	47	6.89
0 (PEG)	1.86	47	6.89
75	1.67	43	6.57
150	1.71	47	6.21
300	1.95	56	6.99

For reference values see Annex

f. Toxicokinetics & Exposure: On study day 72/73, a linear and dose proportional increase in the plasma concentrations were seen in both males and females as shown in the following table (Sponsor's Table 6-11, vol. 1.1, pp 16). The concentration of the compound was similar in both sexes. The half-lives of the compound were not estimated by the sponsor. The plasma concentration of the females was generally higher than males.

**Table 6-11: Summary on exposure in rats at steady state (day 72/73) after administration of BAY 59-7939 in the diet**

Dose	[ppm]	Males			Females		
		75	150	300	75	150	300
AUC <sub>(0-24)</sub>	[mg·h/L]	21.0	57.3	79.8	28.4	44.9	107
C <sub>max</sub>	[mg/L]	1.15	3.51	4.26	1.48	2.31	5.84
t <sub>max</sub>		4:00 am	4:00 am	8:00 am	4:00 am	12:00 pm	8:00 am
Trough/peak ratio*	[%]	28	40	55	46	50	41

\* ratio between highest and lowest concentration measured

The corresponding human exposure was taken from the 30 mg bid dose of the 7 day repeat-dose study with an AUC of 5.46 mg·h/L

i. Organ Weight Changes: The absolute weights of liver and heart of males and females included in the 3 treatment groups were significantly ( $p < 0.05$ ) decreased as shown in the following table (sponsor's table 6-12, vol 1.2, pp 39).

**Table 6-12: Absolute organ weights**

Absolute organ weights						
dose	Body W	Brain	Adrenals	Heart	Liver	Spleen
mg/kg	G	mg	mg	mg	mg	mg
males						
0	441	2028	52	1513	15637	770
0 (PEG)	424	2022	51	1252 ++	14179 +	672 +
0 (PEG)	424	2022	51	1252	14179	672
75	361 ++	1952	47	1163	11302 ++	601
150	327 ++	1905	45	1077 +	10863 ++	565 +
300	329 ++	1932	51	1067 +	10399 ++	569 +
females						
0	249	1365	75	1018	8747	521
0 (PEG)	254	1872	70	988	8152	528
0 (PEG)	254	1872	70	988	8152	528
75	221 ++	1850	65	789 ++	6960 ++	480
150	222 ++	1831	60 ++	816 ++	7041 ++	481
300	216 ++	1842	60 ++	786 ++	6491 ++	489

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The relative organ to body weight of brain and testes was increased in treatment related manner in males and females of all treatment groups.

dose mg/kg	Absolute organ weights				
	Kidneys mg	Testes mg	Epididym. mg	Ovaries mg	Uterus mg
males					
0	2650	3372	1664		
0 (PEG)	2607	3378	1560		
0 (PEG)	2607	3378	1560		
75	2195 ++	3315	1416		
150	2221 ++	3222	1294 ++		
300	2167 ++	3144	1301 ++		
females					
0	1590			153	989
0 (PEG)	1580			150	813
0 (PEG)	1580			150	813
75	1402 +			131	891
150	1476			136	799
300	1387 +			123 +	605

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j. Gross Pathology Findings: On necropsy, no treatment-related changes were reported.

k. Histopathological Changes: The incidences and/or severity score of iron positive pigment deposits around pancreatic islets and ducts or within the intralobular connective tissue were 1, 4, 4 and 9 out of 10 males/group and, 0, 1, 4 and 9 out of 10 females/group for the 0, 75, 150 and 300 mg/kg/day treatment groups, respectively. The incidences of focal fibrosis and/or inflammation of the islet of Langerhans and pancreatic ducts were seen in 2, 1, 4 and 8 males and, 1, 0, 0, 0 females out of 10/sex of 0, 0, 75, 150 and 300 mg/kg/day treatment groups, respectively. There was reduced glycogen in the males and females of mid and high dose treatment groups as shown in the following table:

TABLE  
Microscopic Incidences in 3-Month Oral Toxicity Study in Rats (10 Animals/sex/Group)

Organs		BAY 59-7939 Used (mg/kg/day)			
		0	75	150	300
Pancreas (Islet/periductal pigment)	Male	1 ((gr - 1))	4 (gr - 1)	4 (Gr 1 - 3, Gr 2- 1)	9 (Gr 1 - 2, Gr 2- 7)
	Female	0	1 (gr. 1)	4 (gr.1)	9 (5-gr 1, 2-gr. 2, 2-gr 3)
Pancr. Intralobular pigment	Male	0	0	3 (1-gr 1; 2 gr 2)	1 (10-gr 1)
	Female	0	0	0	0
Pancre. Focal Fibrosis Inflamm. Infiltr.	Male	2 (2-gr)	1 (1 - gr -1)	4(4-gr 2)	8 (4-gr 1; 3-gr 2, 1-gr 3)
	Female	1 (Gr 1)	0	0	0
Focal Acinar Degeneration/Atrophy	Male	1(1-gr. 1)	0	4 (4-gr 2)	2 (1 gr 1; 1-Gr 2)
	Female	0	0	0	0
Liver Reduced Glycogen	Male	0	0	1 (gr 2- 1)	2(2 gr. 1)
	Female	0	0	2(2-gr 1)	1(1-gr 1)

'gr' = grade/intensity of changes (1=slight, 2=mild, 3=moderate)

In summary, orally administered BAY 59-7939 from 75 to 300 mg/kg/kg in diet for 13 weeks produced a non-dose proportional plasma concentration in rats and hyperpigmentation in periductal pancreatic Islets was noted in males and, focal



fibrosis and/or inflammation of the islet of Langerhans and pancreatic ducts were seen in males of 150 and 300 mg/kg/day treatment groups and not in females. There was reduced plasma glycogen in the males and females of mid and high dose treatment groups. The target organ of toxicity was pancreas.

**9. Study title: Thirteen (13)-Week Oral Gavage Toxicity study of BAY 59-7939 in Rats**

**Key study findings:**

The 13-week oral gavage toxicity study in Wistar rats was conducted at the doses of 0, 60, 300 and 1500 mg/kg/day micronized BAY 59-7939 for 91/92 (M/F) days. The satellite group animals were treated for 81 days. A dose-dependent increase in the coagulation time in males and females rats was observed and no treatment related changes were seen in animals. The plasma concentration of the compound were non-dose or treatment related. The target organs of toxicity and

**Study no:** T4075697

**Conducting laboratory and location:** BHC PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation & completion:** June 16, 2005 and March 23, 2006

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot # and % purity:** BAY 59-7939 micronized, Batch #BX01UNC; 99.3%.

**Formulation/vehicle:** The drug was administered in ethanol/Solutol HS15/demineralized water (1/4/5) in a volume of 10 ml/kg. The animals of the control and treatment groups received the same amount of vehicle consisted of ethanol/Solutol HS15/tap water.

**Methods:**

**Species/strain:** Wistar Hsd Cpb:WU (b) (4) rats

**#/sex/group or time point (main study):** 10 animals/sex/group

**Satellite groups used for toxicokinetics:** 6/sex/treatment group and 3/sex in control group.

**Age:** 7 weeks old

**Weight:** 144 to 150 g for males, 123 to 128 g for females.

**Doses in administered units:** BAY 59-7939 was administered at dose levels of 0 (ethanol/Solutol/tap water - 10/40/50), 60, 300 and 1500 mg/kg/day for 91/92 (M/F) days. The satellite group animals treated for 81 days. Homogeneity and stability of the drug in the vehicle were stable at least for 15 days.

**Route, form, volume, and infusion rate:** The drug in ethanol/Solutol/tap water (10/40/50) vehicle was administered at 10 ml/kg volume.

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and morbidity and once daily in cage for abnormal clinical signs.

**Body weights:** Body weights were measured prior to initiation of dosing and then weekly thereafter and immediately before necropsy.

**Food consumption and water intake:** Food and water consumptions were measured once a day and from the weekly data, the daily food and water intake per animal was computed.

**Hematology:** Blood samples were collected weekly/every treatment group on day 30 for females and day 31 for males and on day 88/89 for males/females. Hepato Quick (HQUICK) and differential hematology data were collected on day 30/31 for main groups males/females. Urinalysis was done on days 24 and 86 for females and on day 25 and 87 for males.

**Clinical chemistry:** Blood samples for clinical chemistry examinations were collected on day 91/92 of males/females of main study groups. Urinalysis was done on urine samples collected on day 24 and 86.

**Gross pathology:** Animals that died or sacrificed moribund, were also subjected to necropsy examinations. The surviving animals were also subjected to complete necropsy on day 92/93 and examined for pathological changes.

**Organs weighed:** The weights of the organs were recorded for: adrenals, brain, heart, kidney, liver, spleen, ovary/testes, epididymides, prostate, seminal vesicles with coagulation glands, thymus and uterus. At the end of the study, liver samples were collected from animals of main study groups for the determination of CYP450 (P450), triglycerides, n-demethylase (N-DEM), O-demethylase (O-DEM), cytochrome P450-dependent monooxygenase (ECOD – isozymes CYP1A1, 2B1, 2D1, 2E1), EROD isozyme 1A1, ALD for isozyme 2B1, 3A1, 3A4, 2C11, epoxide hydrolase, conjugated GS-T and GLU-T, and carnitine acetyltransferase (CAT).

**Histopathology:** Histopathological examinations of the following organs from the control and high dose treatment groups of the main study groups were conducted. The liver, kidneys, lungs, pancreas, eyes and optic nerves of the animals of all animals of the study were examined. Adrenal gland, aorta, epididymides, esophagus, eyes, extra orbital lacrimal glands, femur, heart, head, kidneys, larynx, liver and gall bladder, lungs, mesenteric lymph node, mandibular

and popliteal lymph nodes, optic nerve, ovaries, oviducts, pancreas, salivary glands, skeletal muscle, spleen, sternum, testes, thymus, thyroid glands, trachea, urinary bladder, uterus and vagina from control and high dose groups only.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from retro-orbital venous plexus from the satellite group animals (3 animals/time point) on Day 1 at 1, 2, 4, 7 and 24 hr post dose in animals of each of drug treatment groups and, at 1, 7 and 24 hr post dose in control group animals.

## **Results:**

**Mortality:** During the study, 3 males (#39, 40 and 97) of 1500 mg/kg/day treatment group belonging to main study died. Two of these died on study day 41 and the 3<sup>rd</sup> animal died on day 72. An additional male died on day 47 and was of 1500 mg/kg/day treatment group of satellite part of the study. The animal #39 showed dilation of esophagus and thickening of stomach area of pylorus and animal #40 showed blood on muzzle, reduced activity and breathing sounds and a weight loss with ruptured esophagus. Sponsor attributed the deaths to thick viscous nature of the compound.

**Clinical signs:** One male #32 of 1500 mg/kg/day treatment group showed tremors from week 1 to 13 and the observations were not considered as treatment related by sponsor.

**Body weights:** Treatment with BAY 59-7939 for 13 weeks produced no treatment related significant changes in the body weight of male or female rats. The food and water intakes in study animals were not affected during the study.

**Hematology:** Coagulation time (HQUICK) was significantly and dose-dependently increased (up to 23.2% in males and 31.3% in females) in both treated males and females on day 31 and 30, respectively. The coagulation parameters were similarly affected on day 89, i.e., by 16.1 and 35.9% in male and female animals, respectively. The other changes observed were insignificant and not of any relevance.

**Clinical chemistry:** On day 31, serum AST, ALT and GLDH were increased in study males of 1500 mg/kg/day treatment group but were not of clinical significant. The treatment with the compound for 89 days did not affect T3, T4 and TSH in males or females of the study.

The enzyme activities of liver homogenates showed no significant changes and the levels of CYP450, triglyceride, mono-oxygenases (ECOD and EROD), N-demethylase (N-DEM), and O-demethylase (N-DEM) were similar to those of control group. Isozymes CAT was increased in males only but of only statistically significance. No changes were observed in urinalysis of the study animals on day 25/24 or 87/86 (M/F) of the study.

**Organ weights:** The mean absolute and relative (to body weight) organ weights of the study animals were not affected.

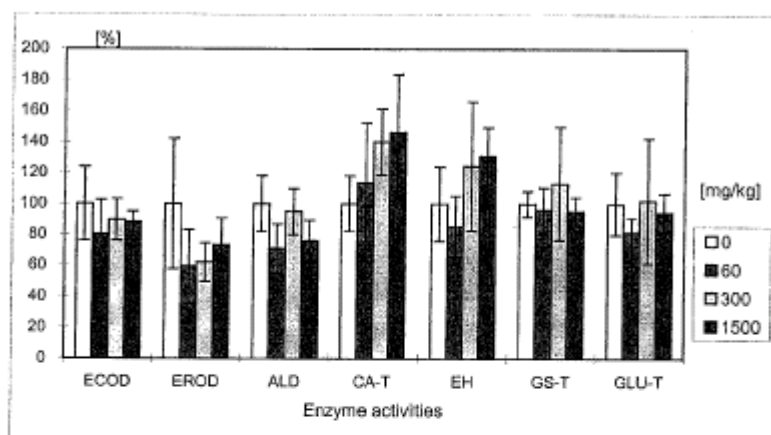
**Gross pathology:** No treatment related pathological changes were seen in any of the animals of 300, 600 and 1500 mg/kg/day treatment groups.

**Histopathology:** No treatment related histopathological changes were seen in the males and females included in the treatment groups

### Liver Enzymes:

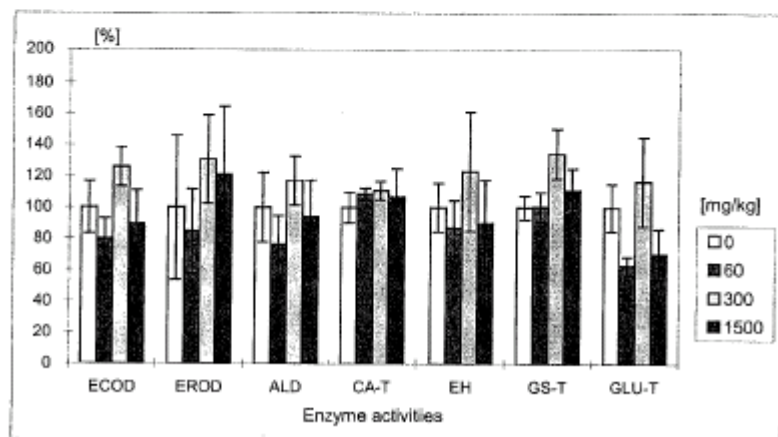
BAY59-7939 administration for 13 weeks in rats, produced a dose dependent slight but statistical significant increase in CA-T and, decrease in ALD and GLU-T in males of 1500 mg/kg/day treatment group. A decrease ( $p < 0.05$ ) in GLU-T enzymes in females of 1500 mg/kg/day treatment group was also seen. This and the other changes in enzymes levels are shown in the following tables.

Figure 2-1: Enzyme activities in the liver of rats (m) after administration of BAY59-7939 for 13 weeks



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Figure 2-2: Enzyme activities in the liver of rats (f) after administration of BAY59-7939 for 13 weeks



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**Table 2-1 Enzyme activities in the liver of rats (m) after administration of BAY59-7939 for 13 weeks**

Animal	Dose [mg/kg]	ECOD	EROD	ALD	CA-T	EH	GS-T	GLU-T
1	0	8.8	0.52	227.5	66	723	125	1127
2		7.9	0.57	190.6	103	399	133	677
3		13.2	1.29	272.9	96	677	140	1164
4		10.5	0.85	236.6	103	614	153	1125
5		7.7	0.58	171.1	110	469	128	939
M		9.6	0.76	219.7	96	576	136	1006
SD ±		2.3	0.32	40.0	17	138	11	204
11	60	7.8	0.20	180.6	88	499	153	890
12		5.4	0.34	135.9	74	416	113	736
13		6.8	0.59	111.2	103	484	120	856
14		7.4	0.65	153.0	110	677	118	709
15		11.2	0.49	198.9	169	383	150	917
M		-	-	+	-	-	-	-
SD ±		7.7	0.45	155.9	109	492	131	822
		2.1	0.18	34.9	37	114	19	93
21	300	8.8	0.41	229.0	118	1121	218	1733
22		7.1	0.45	190.6	125	645	150	716
23		7.6	0.43	238.4	132	730	170	821
24		10.1	0.44	159.7	169	500	150	904
25		9.6	0.64	224.8	125	590	80	963
M		-	-	-	+	-	-	-
SD ±		8.6	0.47	206.5	134	717	154	1028
		1.3	0.10	32.7	20	240	49	405
31	1500	8.5	0.42	173.2	118	855	143	905
32		9.1	0.47	157.8	110	586	130	1114
33		7.5	0.53	152.2	125	794	128	770
34		9.1	0.76	139.8	199	721	110	982
35		8.1	0.61	213.9	147	818	135	983
M		-	-	+	+	-	-	-
SD ±		8.5	0.56	167.4	140	755	129	951
		0.7	0.13	28.6	36	106	12	126

Deviations between manually calculated and computer-determined figures can thus arise due to rounding

ECOD = 7-Ethoxycoumarin deethylase [nmol/g\*min]  
 EROD = 7-Ethoxyresorufin deethylase [nmol/g\*min]  
 ALD = Aldrin epoxidase [nmol/g\*min]  
 CA-T = Carnitine acetyl transferase [nmol/g\*min]  
 EH = Epoxide hydrolase [nmol/g\*min]  
 GS-T = Glutathione-S-transferase [μmol/g\*min]  
 GLU-T = UDP-Glucuronyltransferase [nmol/g\*min]

Student t-Test:

p > 0.050: -  
 p <= 0.050: +  
 p <= 0.010: \*\*

**Table 2-2 Enzyme activities in the liver of rats (f) after administration of BAY59-7939 for 13 weeks**

Animal	Dose [mg/kg]	ECOD	EROD	ALD	CA-T	EH	GS-T	GLU-T
41	0	4.6	0.75	45.0	221	322	75	773
42		3.8	0.48	34.7	287	364	80	808
43		4.8	0.62	38.6	250	323	90	680
44		3.1	0.27	25.0	250	265	75	557
45		3.9	0.25	29.8	272	247	83	626
M		4.0	0.47	34.8	256	304	81	689
SD ±		0.7	0.22	7.7	25	47	6	103
51	60	3.0	0.46	30.6	272	283	88	434
52		2.5	0.19	15.3	272	201	70	379
53		3.2	0.46	27.1	272	226	78	419
54		3.7	0.52	30.6	294	278	83	477
55		3.7	0.38	28.5	279	337	88	462
M		3.2	0.40	26.4	276	265	81	434
SD ±		0.5	0.13	6.4	10	53	7	38
61	300	4.3	0.45	34.4	272	250	88	533
62		5.5	0.66	39.8	309	285	105	803
63		5.4	0.73	49.4	287	512	118	791
64		4.8	0.51	39.5	279	347	118	807
65		5.3	0.75	39.8	272	476	115	1088
M		5.1	0.62	40.6	284	374	109	804
SD ±		0.5	0.13	5.5	15	116	13	196
71	1500	2.6	0.35	21.4	191	155	85	408
72		3.4	0.43	32.7	301	241	80	382
73		3.1	0.51	28.4	287	299	98	422
74		4.2	0.81	42.0	287	385	105	591
75		4.7	0.77	38.3	301	288	80	614
M		3.6	0.57	32.6	274	274	90	483
SD ±		0.9	0.21	8.1	47	84	11	110

Deviations between manually calculated and computer-determined figures can thus arise due to rounding

ECOD	= 7-Ethoxycoumarin deethylase	[nmol/g*min]
EROD	= 7-Ethoxyresorufin deethylase	[nmol/g*min]
ALD	= Aldrin epoxidase	[nmol/g*min]
CA-T	= Carnitine acetyl transferase	[nmol/g*min]
EH	= Epoxide hydrolase	[nmol/g*min]
GS-T	= Glutathione-S-transferase	[μmol/g*min]
GLU-T	= UDP-Glucuronyltransferase	[nmol/g*min]

Student t-Test:

p > 0.050: -  
p <= 0.050: +  
p <= 0.010: ++

**Toxicokinetics:** Plasma concentrations of BAY 59-7939 were determined on Days 1 and 86 at 0.5, 1, 2, 4, 7 and 24 hr post dose in animals of each of drug treatment groups and, at 1, 7 and 24 hr post dose in control group animals. The peak plasma concentrations were seen within 0.5 to 1 hr in males and, 1 to 2 hr in females of the administration of the compound but it was neither dose related nor dose proportional. The TK parameters for BAY 59-7939 in male and female rats at steady state (week 13) are shown below in summary Table of sponsor.

**Table 1:** Short Summary: Mean pharmacokinetic parameters of BAY 59-7939 after oral administration to male and female rats [BAY 59-7939/T 4075697] on Day 80 calculated from the geometric mean concentrations of n = 3.

Sex		Male			Female		
Dose	[mg/kg]	60	300	1500*	60	300	1500
AUC(0-24)	[mg·h/L]	26.1	25.6	31.2	54.6	50.3	57.7
AUC(0-24) <sub>norm</sub>	[kg·h/L]	0.435	0.0853	0.0208	0.909	0.168	0.0385
C <sub>max</sub>	[mg/L]	3.80	3.21	4.00	6.99	8.52	6.87
C <sub>max, norm</sub>	[kg/L]	0.0634	0.0107	0.00267	0.116	0.0284	0.00458
C(24)/C <sub>max</sub>	[%]	2.74	3.36	3.62	4.53	4.06	10.4
t <sub>max</sub>	[h]	1.00	0.500	1.00	1.00	1.00	2.00
R <sub>A1</sub>	[%]	130	74.8	78.5	170	155	90.3
R <sub>A3</sub>	[%]	110	57.5	69.9	126	78.9	64.0

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R<sub>A1</sub> = ratio of C<sub>max</sub> from Day 80 to Day 1

R<sub>A3</sub> = ratio of AUC(0-24) from Day 80 to Day 1

\* = animal No. 97 died, only (n=2) available at 0.5 h, 2 h and 7 h

### Summary of study findings:

In the 13-week oral gavage toxicity study in Wistar rats, the micronized BAY 59-7939 from 60 to 1500 mg/kg/day (in ethanol/Solutol/tap water) produced a dose-dependent increase in the coagulation time in males and females rats. No treatment related histopathological changes were seen. The plasma concentrations produced were non-dose or treatment related and the concentration were similar at all the selected doses of the study. The target organs of toxicity and MTD were not identified.

### 10. Study Title: BAY 59-7939: 6-Month Chronic Toxicity Study in Rats

#### Key study findings:

Bay 59-7939 when administered at an oral dose from 12.5 to 200 mg/kg/day for 6 months in rats produced treatment related increase in the plasma drug concentration in animals within 60 minutes of the administration. The histopathology changes were not examined for all the treated study animals. The target organs of toxicity and NOAEL could not be identified and the study was incomplete.

**Study no:** T0073127/PH-33611/TO0731 27

**Conducting laboratory and location:** BHC PH-R&D Toxicology International, Bayer HealthCare AG, 42096 Wuppertal, Germany.

**Date of study initiation & completion:** July 28, 2003 and December 2, 2004

**GLP compliance:** Yes

**QA report:** yes (X) no ( )

**Drug, lot # and % purity:** BAY 59-7939 co-precipitate 10% 100, Batch #030618; 9.5%.

**Formulation/vehicle:** The drug was administered in Solutol HS15/demineralized water (20/80 v/v) in a volume of 10 ml/kg. The animals of the control and treatment groups received the same amount of vehicle consisted of Solutol/tap water.

**Methods:**

**Species/strain:** Wistar Hsd Cpb:WU (b) (4) rats with mean weight of 167 (range:149 -194 g) males and 129 (range 109 -153 g) females.  
**#/sex/group or time point (main study):** 20 animals/sex/group

**Satellite groups used for toxicokinetics:** 6/sex/treatment group and 3/sex in control group.

**Age:** 7 weeks old

**Weight:** 144 to 150 g for males, 123 to 128 g for females.

**Doses in administered units:** BAY 59-7939 was administered at dose levels of 0 (Solutol/tap water - 20/80, v/v), 12.5, 50 and 200 mg/kg/day for 183 days. Homogeneity and stability of the drug in the vehicle were stable at least for 15 days.

Table 5-2: Dosing Schedule				
Group No.	Dose mg/kg	Sex	Number of Animals	Animal Number
1	0	m	20	1-20
2	12.5	m	20	21-40
3	50	m	20	41-60
4	200	m	20	61-80
5	0	f	20	81-100
6	12.5	f	20	101-120
7	50	f	20	121-140
8	200	f	20	141-160

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**Route, form, volume, and infusion rate:** The drug in Solutol/tap water (20/80) vehicle was administered at 10 ml/kg volume.

**Observations and times:**

**Clinical signs:** The animals were observed twice daily for mortality and morbidity and once daily in cage for abnormal clinical signs. Open field observation and physical examination were done weekly.

**Body weights:** Body weights were measured prior to initiation of dosing, and weekly thereafter and immediately before necropsy.

**Food consumption and water intake:** Food and water consumptions were measured once a day and from the weekly data, the daily food and water intake per animal was computed.



**Hematology:** Blood samples for hematological examinations were collected from animals of all groups twice during the study. Hepato Quick (HQUICK) and differential hematology data were collected from main groups' males and females.

**Clinical chemistry:** Clinical chemistry investigations on blood samples were performed on 10 animals per group twice during the study. The blood samples for determination of glucose concentrations were taken from the caudal vein of fasted (for about 16 hrs), non-anesthetized animals. Urinalysis was done on urine samples collected for 16 hr on day 180/181.

**Gross pathology:** The surviving animals were subjected to complete necropsy on day 182/183 and examined for pathological changes. Animals that died or sacrificed moribund, were also subjected to necropsy examinations.

**Organs weighed:** The weights of the following tissues were recorded: adrenals, brain, heart, kidney, liver, spleen, ovary/testes, epididymides, prostate/uterus, seminal vesicles with coagulation glands and thymus. At the end of the study, liver samples were collected from 10 animals/main study group for the determination of hepatic enzymes aminopyrine-N-demethylase, p-nitroanisole-0-demethylase, cytochrome P-450 content and triglyceride content.

**Ophthalmoscopic Examination:** All animals of the study groups were subjected to ophthalmological inspection before the study start and tested again at the end of the treatment period. The pupillary reflex of both eyes was first tested in a darkened room and the anterior regions of the eye were inspected after dilating the pupils with Mydriaticum stulin drops.

**Histopathology:** Histopathological examinations of the following organs from the animals of control and high dose treatment groups of the main study groups were collected. The liver, kidneys, lungs, pancreas, eyes and optic nerves of the animals of all animals of the study were examined. Adrenal gland, aorta, epididymides, esophagus, eyes, extra orbital lacrimal glands, femur, heart, head, kidneys, larynx, liver and gall bladder, lungs, mesenteric lymph node, mandibular and popliteal lymph nodes, optic nerve, ovaries, oviducts, pancreas, salivary glands, skeletal muscle, spleen, sternum, testes, thymus, thyroid glands, trachea, urinary bladder, uterus and vagina of all animals collected but were not examined in the study.

**Toxicokinetics:** Blood samples for toxicokinetic analyses were collected from retro-orbital venous plexus from the satellite group animals (3 animals/time point) on Day 1, 29 and 176 at 0.5, 1, 2, 4, 7 and 24 hr post dose in animals of each of drug treatment groups and, at 2 hr post dose in control group animals.

**Results:**

**Mortality:** Six rats died during the study. These were 1 and 2 females out of 10 in each of 0 and 50 mg/kg/day groups, respectively, that died of blood sampling error. One female of 12.5 mg/kg/day and 1/sex of 200 mg/kg/day groups were euthanized due to moribund condition. A female rat of 200 mg/kg group was found dead.

**Clinical signs:** In males, hair loss in 0, 1, 1 and 2 of 10 males and blood in eyes of 0, 0, 2 and 1 males was observed in 0, 12.5, 50 and 200 mg/kg/day groups, respectively. In females, the hair loss incidences were 0, 1, 3 and 3 and blood in eye incidences were 0, 1, 1 and 0 in the 0, 12.5, 50 and 200 mg/kg/day groups, respectively.

**Body weights:** A reduction in the body weights of males of 12.5, 50 and 200 mg/kg/day groups were observed. The reductions were 7.8, 11.9 and 15.2% in 12.5, 50 and 200 mg/kg/day males, respectively. The reduction in body weight gain in 12.5 mg/kg/day males was not significant. The body weight gain in females was comparable to the respective control groups.

Parameter	Males				Females			
	Control	12.5 mg/kg	50 mg/kg	200 mg/kg	Control	12.5 mg/kg	50 mg/kg	200 mg/kg
Body Wt. (g) Day 1	166	172	163	168	130	130	128	128
Body Wt. (g) Day 183	435	420	400	396	264	260	256	264
Body Wt. gain (g)	269	248	237	228	134	130	128	138
Body Wt. gain (%control)	100%	92.2%	88.1%	84.8%	100%	97.0%	95.5%	103.0

**Food/Water consumption:** On week 26, the mean food intake in the control male and female rats were 21.3 and 16.0 g/animal/day, respectively. There was no change in the food consumption of study animals. An increase of 10 and 17% in water intake in males and, 12 and 15% in females of 50 and 200 mg/kg/day groups, respectively, was seen.

**Hematology:** Coagulation time (HQUICK) was significantly increased in a dose dependant manner in males and in females from day 88 to 178 as shown in the table 6.4. The coagulation parameters were similarly affected on day 89, i.e., by 16.1 and 35.9% in male and female animals, respectively. The other hematology changes observed were insignificant and not of any relevance. The changes are shown in the following table of sponsor:

**Table 6-4: Hematology**

	ERY	HB	HCT	MCV	MCH	MCHC	RET	THRO	HQUICK
Dose mg/kg	10E12/l	g/l	l/l	fl	pg	g/l ERY	o/oo	10E9/l	sec
m	Day 88								
0	9.11	157	0.474	52.1	17.3	332	22	1149	31.7
12.5	8.92	155	0.462	51.9	17.4	335	24	1125	31.2
50	9.26	159	0.471	50.9	17.1	337 +	20	1111	36.2 +
200	9.29	161	0.476	51.3	17.3	337 +	20	1050	55.7 ++
m	Day 179								
0	9.14	153	0.484	52.9	16.8	317	22	1096	28.7
12.5	8.72 +	150	0.465	53.3	17.2	322 +	22	1124	29.3
50	9.01	150	0.471	52.3	16.6	319	19	1063	35.5 ++
200	8.96	150	0.467	52.1	16.8	322	19	1089	47.8 ++
f	Day 86								
0	8.82	157	0.465	52.8	17.8	337	13	1148	25.9
12.5	8.64	155	0.458	53.0	17.9	338	15	1120	29.1 ++
50	8.55	154	0.451 +	52.8	18.0	341	14	1094	36.6 ++
200	8.63	157	0.459	53.2	18.2	343	15	1076	65.4 ++
f	Day 178								
0	8.42	149	0.466	55.4	17.7	319	15	1115	26.5
12.5	8.39	149	0.461	55.0	17.7	322	13	1078	29.4 +
50	8.24	147	0.453	55.1	17.8	324	13	1101	34.6 ++
200	8.41	150	0.462	55.0	17.9	326	15	1059	55.3 ++

**Clinical chemistry:** On day 183, a slight increase in bilirubin concentration in both males and females of 200 mg/kg/day group (females on day 86:  $p > 0.05$ ) and males of 50 mg/kg/day group was seen. Serum calcium concentration was increased in only males at 200 mg/kg but in an insignificant manner and of no clinical importance. Urinary volume and urea excretion was highest at 200 mg/kg/day group. The urinary creatinine was significantly increased in 200 mg/kg/day females.

**Organ weights:** The mean absolute weight of heart ( $p < 0.01$ ), and thymus and spleen was decreased ( $p < 0.05$ ) in males of 200 mg/kg/day group. The relative brain and kidney weights in males of 200 mg/kg/day group were increased ( $p > 0.05$ ) only slightly among males and none of the organ weights among females were affected.

**Gross pathology:** No treatment related pathological changes were seen in any of the animals of 300, 600 and 1500 mg/kg/day treatment groups.

**Histopathology:** No treatment related histopathological changes were seen in the males and females included in the treatment groups

#### **Liver Enzymes:**

No changes in the activities of liver enzymes aminopyrine-N-demethylase, p-nitroanisole-0-demethylase, cytochrome P-450 content, triglyceride content were observed after 6 months of treatment.

#### **Ophthalmoscopy:**

At the termination (day 180), no treatment related changes in ophthalmoscopic examination of the control and of the high-dose group animals were reported.

**Toxicokinetics:** On day 176, a dose proportional increase in peak plasma concentration was seen up to 50 mg/kg/day in males and females within 0.5 to 1 hr of the administration of the compound. The exposure was not dose proportional at 200 mg/kg/day group. The reduced plasma concentration was noted at 24 hr (0.5% of C<sub>max</sub>) and exposure of the compound was increased on day 176 in all treatment group animals. The C<sub>max</sub> was reduced by 5.5 % in animals of 200 mg/kg/day. The toxicokinetic parameters for BAY 59-7939 in male and female rats at steady state (week 13) were shown in sponsor's summary Table below.

Sex		Male			Female		
Dose	[mg/kg]	12.5	50	200	12.5	50	200
AUC(0-24)	[mg·h/L]	18.0	75.6	137	32.4	114	280
AUC(0-24) <sub>norm</sub>	[kg·h/L]	1.44	1.51	0.685	2.59	2.28	1.40
C <sub>max</sub>	[mg/L]	5.41	16.7	25.5	10.8	26.3	41.8
C <sub>max, norm</sub>	[kg/L]	0.433	0.334	0.128	0.866	0.526	0.209
C(24)/C <sub>max</sub>	[%]	0.232	0.454	1.75	0.124	0.387	5.54
t <sub>max</sub>	[h]	1.00	0.500	0.500	0.500	1.00	1.00
R <sub>A1</sub> *	[%]	164	263	236	242	208	159
R <sub>A3</sub> *	[%]	173	227	146	155	144	164

R<sub>A1</sub>\* = ratio of C<sub>max</sub> from Day 176 to Day 1

R<sub>A3</sub>\* = ratio of AUC(0-24) from Day 176 to Day 1

#### Summary of the study findings:

In summary, Bay 59-7939 from 12.5 to 200 mg/kg/day for 6 months in rats produced a treatment related increase in the plasma concentration within 60 minutes of the administration. Sponsor did not conduct the histopathology examination of all the treated animals. The target organs of toxicity, NOAEL could not be identified.

#### DOGS:

#### 11. Study Title: Four-Week Oral Toxicity Study with BAY 59-7939 in Dogs: Study no: T 7070631

**Key Study Findings:** 4-Week oral gavage toxicity study with BAY 59-7939 in beagle dogs (3/sex/group) was performed at 0, 15, 50 and 150 mg/kg/day doses. White fluid/foam and vomiting was reported in animals of 50 and 150 mg/kg/day treatment groups and discolored (green/bright) feces were observed in 150 mg/kg/day animals. Slightly increased reticulocyte levels were observed at all doses (141.7%, 46.2% and 63.6% in males and 100%, 120% and 290% in females of low, mid and high dose groups, respectively in week 2). Subcutaneous treatment related hemorrhage was observed in all groups including the control (one control, all low dose animals, 1 male and all females at mid dose, and all high dose animals). The hemorrhages at the blood pressure measurement sites and, hematomas at necropsy were reported. Extramedullary hematopoiesis in the spleen was observed in treated males and females and spleen was identified as the

target organ of toxicity. The 'no effect dose' was not established and 150 mg/kg/day considered as the tolerated dose. This provided 37 times the exposure of the clinical dose, in this study.

**Conducting Laboratory:** Department of Toxicology/Pharma, Institute of Toxicology, BAYER AG, 42096 Wuppertal, Germany.

**Dates of Study Initiation and Completion:** June 05, 2001 and Feb 25, 2002.

**GLP Compliance:** yes

**QA Report:** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939, Batch no. 507 046

**Formulation/vehicle:** The test substance, formulated as a co-precipitate with PEG 6000, was used as a suspension in tap water.

**Methods:** There were two control groups in the study. The control group I received tap water only and the control II group received PEG 6000/tap water and the amount of PEG was same as that used in the drug formulation.

**Dosing Information:**

**Species/strain:** Beagle dogs

**#/sex/group or time point:** 3/sex/group

**Satellite groups used for toxicokinetics or recovery:** none

**Age:** 30 – 31 weeks

**Weight:** 6.7 kg – 9.0 kg.

**Doses in administered units:** 0, 15, 50 and 150 mg/kg

**Route, form, volume and infusion rate:** The drug was administered by oral gavage (dose volume = 10 ml/kg).

**Observations and times:**

**Clinical signs** – once daily

**Body weights** – once a week during the dosing period.

**Food and water consumption** – measured daily.

**Ophthalmoscopy-** Ophthalmoscopic examinations were conducted once prior to initiation of dosing and at week 4 of the dosing period.

**EKG-** Electrocardiograms (Leads I, II, III, aVR, aVL and aVF) and blood pressure measurements were conducted once prior to dosing and in weeks 1 and 4 of dosing (before and 2 hours after administration).

**Hematology-** prior to initiation of dosing during week 2 and 4 of the dosing period.

**Clinical Chemistry-** prior to initiation of dosing and in weeks 2 and 4 of the dosing period.

**Urinalysis** - prior to initiation of dosing and in week 1 and 3 of the dosing period.

**Gross pathology** – At the end of the dosing period, the animals were sacrificed and necropsies performed.

**Organs weighed-** the organs separated, cleaned and weighed were: heart, lung, liver, spleen, kidneys, testes, prostate, ovaries, thyroid, adrenals, thymus, brain, pituitary, pancreas, empty gall bladder, epididymides and uterus/oviduct.

**Histopathology-** Histopathological examinations of the following organs of the control and the high dose group animals were conducted.

Adrenal glands, aorta, bone marrow, brain, cecum, colon, duodenum, eyes, femur, gall bladder, heart, jejunum, ileum, lens, liver, lymph nodes (mandibular and mesenteric), esophagus, optic nerves, ovaries, oviducts, pancreas, parathyroid glands, rectum, salivary glands, sciatic nerve, skeletal muscle, spinal cord, sternum, stomach, thyroid glands, tongue, tonsils, ureters, urinary bladder, uterus and vagina.

**Toxicokinetics-** Blood samples for toxicokinetic analysis were collected on dosing days 1 and 24 at 0.5, 1, 2, 4, 6 and 24 hours after dosing.

## **Results:**

**Mortality-** There was no mortality in any group.

**Clinical signs-** Vomiting of whitish fluid/foam were observed in mid and high dose animals. In addition, the high dose animals had discolored feces. The treatment group animals had increased incidences of hematoma at the blood sampling sites and inguinal swellings at the blood pressure measurement sites.

**Body weights-** The mean body weights of the control males and females prior to beginning of dosing (Day -1) were 8.6 and 7.9 kg and at the end of the dosing were 9.2 and 8.9 kg, respectively. No treatment related changes in the body weights were observed in any group.

**Food consumption-** The sponsor did not provide the food consumption values of individual animals. However, the data shows that there were no treatment-related changes in the food consumption in any group.

**Ophthalmoscopy-** No treatment-related ophthalmologic changes were observed in any group.

**Electrocardiography-** No treatment related changes in the ECG parameters or the blood pressure were observed in any group. The heart rates of the 50 and 150 mg/kg animals, measured 2 hours after dosing at weeks 1 and 4, were slightly decreased, as compared with that before dosing (at 50 mg/kg, 5% and 18%, and at 150 mg/kg, 16% and 15% at weeks 1 and 4, respectively).

**Hematology-** The mean reticulocyte levels were increased by 142%, 46% and 64% in males, and 100%, 120% and 190% in females of low, mid and high dose treatment groups, respectively. The increase in PT and aPTT of the mid and high dose animals from weeks 2-4 of the treatment was pharmacologic effect of the compound. The increases in PT and PTT were related to the overt pharmacologic effects of the drug. The pretreatment PT was 6.5 to 7.1 sec, and there were 298% and 203% increases in males and 89% and 272% increases in females in week 4 at mid and high doses, respectively. The pretreatment PTT was 11.4 to 12.3 sec, and there were 83% and 54% increases in males and 24% and 82% increases in females in week 4 at mid and high doses, respectively.

**Clinical Chemistry-** No treatment related changes in the clinical chemistry parameters were observed in any group.

**Urinalysis-** No treatment-related changes were observed in any group.

**Gross pathology-** Hematomas in the subcutis were observed at all doses, including one control (PEG group) animal (one of 3 control II male, 3/3 males

and 3/3 females at low dose, 1/3 male and 3/3 females at mid dose, and 3/3 males and 3/3 females at high dose). The lesions were observed in the inguinal region at the blood pressure measurement sites.

**Organ weights-** Males receiving the mid and the high dose had higher spleen weights (absolute, 30.8% and 88.0%; relative, 35.2% and 110.1% at mid and high doses, respectively) and the females receiving the high dose had higher adrenal weights (absolute, 41.5%; relative, 45%), as compared with the controls.

**Histopathology-** Subcutaneous hemorrhage was observed at all doses (one control, all low dose animals, 1 male and all females at mid dose, and all high dose animals). The hemorrhages were observed at the blood pressure measurement sites, where hematomas were observed at necropsy. Centrilobular fat in the liver was observed in one low, 2 mid and one high dose females. Minimal to slight extramedullary hematopoiesis were observed in the spleen of the treated animals (2/3 males, 2/3 females at 15 mg/kg, 1/3 male and 2/3 females at 50 mg/kg, 1/3 male and 2/3 females at 150 mg/kg).

**Toxicokinetics:** After oral administration to dogs, BAY 59-7939 was rapidly absorbed from the GI tract with the  $T_{max}$  ranging from 1.0 to 1.4 hours. The  $C_{max}$  and AUC values on Days 1 and 24 increased with increasing dose. Unlike rats, no differences in the plasma exposure levels were observed between males and females. The pharmacokinetic parameters in dogs on Days 1 and 24 are summarized in the Table below.

	Male		Female
	15 mg/kg	50 mg/kg	150 mg/kg
<b>Day -1</b>			
AUC <sub>0-24</sub> (µg.h/L)	9616	21333	27147
C <sub>max</sub> (µg/L)	2590	5303	7183
T <sub>max</sub> (h)	1.12	1.00	1.12
<b>Day- 28</b>			
AUC <sub>0-24</sub> (µg.h/L)	8914	27112	36979
C <sub>max</sub> (µg/L)	2135	5331	6709
T <sub>max</sub> (h)	1.41	1.00	1.12

In summary, in the 4-week oral gavage toxicity study with BAY 59-7939 in beagle dogs, groups of animals (3/sex/group) received 0, 15, 50 and 150 mg/kg/day doses of the drug. Animals treated with 50 and 150 mg/kg/day doses had vomiting of white fluid/foam; in addition, the 150 mg/kg/day group had discolored (green/bright) feces. Slightly treatment related increased reticulocyte levels were observed in all doses (141.7%, 46.2% and 63.6% in males and 100%, 120% and 290% in females at low, mid and high doses, respectively in week 2). Subcutaneous hemorrhage was observed in all groups including the control (one control, all low dose animals, 1 male and all females at mid dose, and all high dose animals). The hemorrhages were observed at the blood pressure measurement sites, where hematomas were observed at necropsy. Extramedullary hematopoiesis in the spleen was observed in treated males and females and spleen was identified as the target organ of toxicity. 'No effect dose' was not established and 150 mg/kg/day dose was identified as the tolerated dose in this study and this produced about 37 times the plasma exposure.

**12. Study Title: 13-Week Subchronic Oral Gavage Toxicity Study With BAY 59-7939 in Dogs:**

Study no: AT00861/PH-33056/T 9072703/T 7070631

**Key Study Findings:** Thirteen-week oral gavage toxicity study from 15 to 150 mg/kg BAY 59-7939 produced white fluid/foam vomiting, discolored (green/bright) feces and subcutaneous hemorrhage in tissues and, extramedullary hematopoiesis in the spleen. A dose of 150 mg/kg/day provided 37 times plasma exposure of the clinical dose and was identified as the tolerated dose.

**Conducting Laboratory:** Department of Toxicology/Pharma, Institute of Toxicology, BAYER AG, 42096 Wuppertal, Germany.

**Dates of Study Initiation and Completion:** May 19, 2003 and December 11, 2003.

**GLP Compliance:** yes

**QA Report:** Yes (X) No ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939, Batch no. 200303 14  
Content of active ingredient: 9.4 %

**Formulation/vehicle:** The test substance, formulated as a co-precipitate with PEG 6000, was used as a suspension in tap water.

**Methods:** There were two control groups in the study. The control group I received tap water only and the control II group received PEG 6000/tap water and the amount of PEG was same as that used in the drug formulation.

**Dosing Information:**

**Species/strain:** Beagle dogs

**#/sex/group or time point:** 4/sex/group

**Satellite groups used for toxicokinetics or recovery:** none

**Age:** 30 – 31 weeks

**Weight:** 6.7 kg – 9.0 kg.

**Doses in administered units:** 0, 15, 50 and 150 mg/kg for 13 weeks and the doses were based on the 4 week study with BAY 59-7939 (Report no. PH 3 1848).

**Route, form, volume and infusion rate:** The drug was administered by oral gavage (dose volume = 10 ml/kg).

**Observations and times:**

**Clinical signs** – once daily/week

**Body weights** – once a week during the dosing period of 13 weeks.

**Food and water consumption** – measured daily.

**Ophthalmoscopy-** Ophthalmoscopic examinations were conducted once prior to initiation of dosing and at week 13 of the dosing period.

**EKG-** Blood-pressure measurements were performed once before the start of the study (week -1). Electrocardiograms (ECG) were performed once before the start of the study (week -1) and before and 2h after administration in week 2, 6, and 13 of the study.



**Hematology-** The blood samples collected prior to initiation of dosing and during week 13 after 1 h of administration.

**Clinical Chemistry-** prior to initiation of dosing and in week 13 of the dosing period.

**Urinalysis** – Samples collected prior to initiation of dosing and in week 13 of the study.

**Gross pathology** – At the end of the dosing period, the animals were sacrificed and necropsies performed.

**Organs weighed-** the organs separated, cleaned and weighed were: heart, lung, liver, kidneys, spleen, testes, prostate, ovaries, thyroid/parathyroid, adrenals, thymus, brain, pituitary, pancreas, empty gall bladder, epididymides, and uterus/oviduct. The organs that were fixed and subjected to a histopathological examination and the histological technique are shown in the pathology report in the appendix of the report.

#### **Collection of samples for toxicokinetic measurements**

On day 1 and in week 12 of the study, plasma for toxicokinetic measurements was collected before and 0.5, 1, 2, 4, 6 and 24 h after administration in all groups.

**Histopathology-** Histopathological examinations of the following organs of the control and the high dose group animals were conducted.

Adrenal glands, aorta, bone marrow, brain, cecum, colon, duodenum, eyes, femur, gall bladder, heart, jejunum, ileum, lens, liver, lymph nodes (mandibular and mesenteric), esophagus, optic nerves, ovaries, oviducts, pancreas, parathyroid glands, rectum, salivary glands, sciatic nerve, skeletal muscle, spinal cord, sternum, stomach, thyroid glands, tongue, tonsils, ureters, urinary bladder, uterus and vagina.

#### **Results:**

**Mortality-** There was no mortality in any group.

**Clinical signs-** The high dose treated animals had discolored feces. The treatment group animals had increased incidences of hematoma at the blood sampling sites and, severe bleeding episodes (hemorrhages) in GI-tract, liver, lungs, spinal cord, and thymus were seen.

**Body weights-** The mean body weights of the control males and females prior to beginning of dosing (Day -1) were 8.6 and 7.9 kg and at the end of the dosing were 9.2 and 8.9 kg, respectively. No treatment related changes in the body weights were observed in any group.

**Food consumption-** The sponsor did not provide the food consumption values of individual animals. However, the data shows that there were no treatment-related changes in the food consumption in any group.

**Hematology:** A marked prolongation of prothrombin time, activated partial thromboplastin time was seen from the lowest dose tested. A significant

reduction in red blood cell count, hemoglobin and hematocrit was seen in males and females of 150 mg/kg/day group.

**Ophthalmoscopy-** No treatment-related ophthalmologic changes were observed in any group.

**Organ Weight Changes:** No relevant changes were detected in mean organ weights up to and including 150 mg/kg/day group.

**Pathology Finding:** Treatment-related bleeding in the tooth sockets during dentition, intestinal bleedings, and vaginal bleeding was reported.

**Histopathology:** At necropsy, isolated acute tissue hemorrhage, i.e., gastric, intestinal bleedings, vaginal bleeding etc., were seen. The incidences of hemorrhages or hematomas in different tissues including gastrointestinal tract, liver, lungs, spinal cord and thymus were treatment related. The inflammatory infiltration in the brain, spinal cord and pituitary gland in one group III-female was attributed to a bacterial infection.

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TEST ARTICLE : BAY 59-7939 PATHOL. NO.: 06443 HAR  
TEST SYSTEM : DOG, SUBCHRONIC, ORAL, GAVAGE DATE : 28-NOV-03  
SPONSOR : BAYER AG PathData® System V5.1c

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: TERMINAL SACRIFICE GROUP (K0)					
Sex	Males				
Dose Group No. Animals per Dose Group	01 4	02 4	03 4	04 4	05 4
CEREBELLUM No.Examined	4	4	4	4	4
- Extramedullary hematopoiesis plexus	-	-	1	1	-
Grade 1	-	-	1	1	-
MEDULLA OBLONGATA No.Examined	4	4	4	4	4
- Perivascular infiltration	-	-	-	1	-
Grade 1	-	-	-	1	-
LUNGS No.Examined	4	4	4	4	4
- Granuloma/s	3	2	4	3	3
Grade 1	2	2	3	2	3
Grade 2	1	-	1	1	-
- Hemosiderin deposits	1	1	1	1	1
Grade 1	1	1	-	1	1
Grade 2	-	-	1	-	-
- Alveolar macrophages	1	-	-	1	2
Grade 1	-	-	-	1	2
Grade 2	1	-	-	-	-
- Septal thickening	1	-	-	-	1
Grade 1	1	-	-	-	1
- Foreign body granuloma/s	1	-	-	-	-
Grade 2	1	-	-	-	-
- Focal inflammation	-	-	-	-	1
Grade 1	-	-	-	-	1
- Hemorrhage	-	-	-	1	1
Grade 2	-	-	-	-	1
Grade 3	-	-	-	1	-
TONGUE No.Examined	4	4	4	4	4
- Mononuclear cell infiltration	1	-	-	1	2
Grade 1	-	-	-	1	-
Grade 2	1	-	-	-	2
STOMACH No.Examined	4	4	4	4	4
- Mineralization	1	-	-	-	1
Grade 2	1	-	-	-	1
- Giant cells	1	-	1	-	-
Grade 1	1	-	1	-	-
- Helicobacter spp.	1	1	1	-	-
Grade 1	-	1	-	-	-
Grade 2	1	-	1	-	-
- Erosion/s	-	1	-	-	-
Grade 2	-	1	-	-	-
- Hemorrhage	-	1	-	-	-
Grade 2	-	1	-	-	-

000362

**PATHOLOGY REPORT :**  
**SUMMARY TABLES**

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TEST ARTICLE : BAY 59-7939  
TEST SYSTEM : DOG, SUBCHRONIC, ORAL, GAVAGE  
SPONSOR : BAYER AG

PATHOL. NO.: 06443 HAR  
DATE : 28-NOV-03  
PathData® System V5.1c

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX Necropsy Status: TERMINAL SACRIFICE GROUP (KO)					
Sex	Females				
Dose Group No. Animals per Dose Group	01 4	02 4	03 4	04 4	05 4
THYROID GLAND No. Examined	4	4	4	4	4
- Increased colloidal vacuolation	2	3	3	3	1
Grade 1	1	-	1	1	1
Grade 2	1	3	2	2	-
PARATHYROID GLANDS No. Examined	4	4	4	4	4
- Cyst/s	-	1	-	2	1
Grade 2	-	1	-	1	-
Grade 3	-	-	-	1	1
ADRENAL GLANDS No. Examined	4	4	4	4	4
- Vacuolation Zona glomerulosa	1	-	-	-	-
Grade 1	1	-	-	-	-
- Vacuolation Zona fasciculata	-	-	-	-	1
Grade 1	-	-	-	-	1
SPLEEN No. Examined	4	4	4	4	4
- Hematomas	-	-	1	-	-
Grade 2	-	-	1	-	-
THYMUS No. Examined	4	4	4	4	4
- Involution/Atrophy	-	-	1	-	-
Grade 1	-	-	1	-	-
- Organized hematoma	-	-	-	-	1
Grade 4	-	-	-	-	1
- Hemosiderin deposits	-	-	-	-	1
Grade 3	-	-	-	-	1
PRESENT. LYMPH NODE No. Examined	4	4	4	4	4
- Brown pigment	1	-	-	-	-
Grade 2	1	-	-	-	-
- Lymphangiectasia	-	1	-	-	-
Grade 2	-	1	-	-	-
PAROTID GLAND No. Examined	4	4	4	4	4
- Only site present	-	-	-	2	-
SUBLINGUAL GLAND No. Examined	4	4	3	4	4
- Mononuclear cell infiltration	-	1	-	-	-
Grade 1	-	1	-	-	-
- Only site present	-	-	1	-	-
SKIN (MAMMARY REGION) No. Examined	3	3	3	4	3
- Chronic inflammation	-	-	-	1	-
Grade 2	-	-	-	1	-

000369

**Toxicokinetics:** The plasma concentration [AUC<sub>(0-24)</sub>] was increased dose-dependently but not dose-proportionally in 1 to 2 h and on day 81 at 24 h after dose administration, the trough concentrations were 6.2, 3.5 and 16 % of the peak. The TK data is shown below:

Dose:	15 mg/kg		50 mg/kg		150 mg/kg	
	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.
AUC(0-24) [mg·h/L]	8.59	1.43	16.1	1.72	37.5	1.67
AUC(0-24) <sub>norm</sub> [kg·h/L]	0.573	1.43	0.322	1.72	0.250	1.67
C <sub>max</sub> [mg/L]	1.88	1.69	2.99	1.35	5.38	1.47
C <sub>max, norm</sub> [kg/L]	0.125	1.69	0.0598	1.35	0.0358	1.47
C(24)/C <sub>max</sub> [%]	6.23	1.77	3.46	3.47	15.9	2.70
t <sub>max</sub> [h]	1.09	1.28	1.30	1.67	1.09	1.28
R <sub>A1</sub> [%]	62.2	1.73	58.2	1.38	62.3	1.53
R <sub>A3</sub> [%]	46.7	1.46	58.9	1.68	77.5	1.72

n.c. = not calculated  
R<sub>A1</sub> = C<sub>max, Day 81</sub> / C<sub>max, Day 1</sub>  
R<sub>A3</sub> = AUC(0-24)<sub>Day 81</sub> / AUC(0-24)<sub>Day 1</sub>

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In the 13-week oral gavage toxicity study with BAY 59-7939 in beagle dogs, the doses up to 150 mg/kg/day produced a non-dose proportional plasma increase, caused vomiting, slight increase in reticulocytes and subcutaneous hemorrhage and hematomas. The 'no effect dose' was not established and 150 mg/kg/day was the tolerated dose and, the exposure of animals of this group was 37 times the exposures of the clinical dose.

### 13. Study title: Chronic Oral Gavage Toxicity Study of 52 Weeks in Dogs

**Key study findings:** BAY 59-7939 administration from 5 to 50 mg/kg/day in dogs produced a treatment related but non-dose proportional increase in peak plasma concentrations in 1.5 to 3 hr. Alveolar macrophages in lungs and hemosiderin deposits in lymph node were produced in greater intensity in animals of 50 mg/kg/day group. The identified NOEL was 5 mg/kg/day and the highest tolerable dose was 15 mg/kg/day in the study and the target organs of toxicity were lungs and lymph nodes.

**Study no.:** T 4073149/AT02684/Report #PH-34235

**Volume #, and page #:** Volume 2.3 Appendix 3; pp 1

**Conducting laboratory and location:** International Bayer HealthCare AG, D-42096 Wuppertal (Germany)

**Date of study initiation:** October 13, 2003 and December 9, 2005

**GLP compliance:** A statement of compliance was provided in the report

**QA report:** yes (X) no ()

**Drug, lot #, and % purity:** 030723-100, (a co-precipitate 9.2%; purity not defined)

### Methods

Doses: 0 (tap water), 5, 15 and 50 mg/kg/day in tap water

Species/strain: Beagle dogs (b) (4)

Number/sex/group: 4

Route, formulation, volume, and infusion rate: Oral gavage in 10 ml/kg volume.

Satellite groups used for toxicokinetics or recovery: No separate group was included

Age: 31 to 33 weeks

Weight: Mean body weight of 11.0 to 11.25 kg (males) and 10.7 to 11.03 kg (females)

Sampling times: The blood samples for TK analysis were collected on day 1 and, study week 25, 38 and 51 from jugular vein from study dogs at 1, 2, 4, 7 and 24 hr post dosing

Basis of Dose Selection: The doses were selected on 13-week toxicity study in beagle dogs (study # T90722703)

Unique study design or methodology (if any): --

Clinical signs and mortality: Clinical signs were observed twice daily during randomization, twice daily at the start and end of each treatment day, twice during the 1<sup>st</sup>, 3 to 4 hr post dosing. The detailed observations were made once weekly through out the study.

Body weights: Once prior to treatment during the study and determined on weekly basis for the duration of the study.

Food consumption: Once daily during the study.

Physical Examination & EKG measurements: The testing of the reflexes was carried out in week -2, 6, 13, 26, 39 and 52.

Ophthalmoscopy: Once prior to dosing (week -2), and in week 6, 13, 26, 39 and 52.

Hematology & Blood Chemistry: Blood samples from jugular vein in week -2, 6, 13, 26, 39 and 52.

Urinalysis: Overnight urine samples from main study group animals in metabolic cage in week -2, 6, 13, 26, 39 and 52.

TK: Blood samples (1 ml/animals) were collected during day 1, in week 25, 35 and 51 at 0, 1, 2, 4, 7 and 24 hr after the treatment for the estimation of the TK parameters of the compound.

Gross pathology: All of the study animals were killed and subjected to the gross pathology examination.

Organ weights: All of the tissues and organs separated, cleaned and the organ weights were recorded for liver, kidneys, heart, kidneys, spleen, testes, prostate, and thyroid with parathyroids, adrenal, thymus, brain, pituitary, pancreas, empty gall bladder, epididymides and uterus/oviduct. Liver samples from the study group animals were taken at necropsy and Phase I and II enzymes were estimated at sponsor's facility.

Histopathology: The organs/tissues of all the study group animals were separated, cleaned, fixed with 10% formalin and stained with hematoxylin and eosin. The tissues included were: adrenal, aorta, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, eye/optic nerve, femur, gallbladder, heart, ileum, jejunum, kidneys, larynx, liver, lung, lymph nodes (mesenteric, mandibular), macroscopic lesions, mammary glands, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, rib, salivary glands (mandibular, parotid), sciatic nerve, skeletal muscle, spinal cord (cervical and lumber) , skin, spleen,

sternum, stomach, testes, thymus, thyroid, trachea, tongue, urinary bladder, uterus, vagina and lumbar vertebra.

## **Results:**

Mortality: One female dog of the high dose group died in week 27 due to excessive abdominal bleeding.

Clinical signs: Increased incidences of emesis (food mush), swelling/hematoma at the left hind leg and both eyes were reported in 2-3 of 4 males of 50 mg/kg/day treatment group. Mushy feces were seen 0, 0, and 1 of 4 males and 0, 0 and 1 of 4 females in 0, 5, 15 and 50 mg/kg/day treatment groups, respectively. Red and discolored feces were noted in 1, 3, 3 and 4 males and, 1, 2, 3 and 1 females in 0, 5, 15 and 50 mg/kg/day treatment groups, respectively. Liquid diarrhea was noted in 0, 2, 0 and 2 males and, 1, 2, 1 and 2 females in 0, 5, 15 and 50 mg/kg/day treatment groups, respectively. The rectal temperature of the treated animals was not different from the control group male and female dogs.

Body weight and Food consumption Changes: The body weight increase of the treated animals was similar to the control group. The food and water intake of these animals was not affected during the study.

Ophthalmoscopy: No treatment or dose related effects were seen in males or females of the study.

EKG: The heart rate of the treated animals was similar to the control group. The QT intervals of the treated animals were not affected in any significant manner and QTc was therefore not determined.

Hematology: At week 52, a treatment or dose related increase in PT (2 to 4 times) and APTT (1 to 2 times of control) was observed and these effects were due to the overt pharmacologic activity of the compound. Because of bleeding a significant decrease in Hct and low reticulocytes values were observed.

Clinical chemistry: The chemistry parameters in the animals were not affected up to the dose of 50 mg/kg/day excepting in 1 and 2 males of 15 and 50 mg/kg/day treatment groups, respectively, that showed a slight clinically insignificant effect in serum glutathione, i.e., an increase of 1.5 times in males and decrease in females of 1.5 times in 50 mg/kg/day treatment group. The other serum parameters were not affected in a dose or time related manner. In male dogs of 15 mg/kg/day group, the ECOD activity was increased by 1.5 times whereas in females of the same group, it was decreased by 65 to 70%. The phase II enzyme GS-T increased in males and decreased in females. The changes were slight and inconsistent.

Urinalysis: Triple phosphate was noted in the urinary sediment in 50 mg/kg/day treatment group animals.

Gross pathology: Duodenal lymph node discoloration was reported in 0, 2, 2 and 3 males of 0, 5, 15 and 50 mg/kg/day treatment groups, respectively, but not in mesenteric lymph node. One female of 50 mg/kg/day treatment group that died at week 25 showed abdominal bleeding.

Organ weights: No changes of statistical or clinical importance were noted in organ weights of treated animals when compared with the control group animals.

Histopathology: Alveolar macrophage in lungs of 1, 1, 1 and 2 out of 4 males (grade 2 in 1 male of control and 50 mg/kg/day group and 1 male of 50 mg/kg/day group had marked grade 3/4 - moderate). The histopathological changes of peribronchial inflammation in 0, 2, 1 and 1 males and, 0, 2, 1 and 1 females as well as lymph node hemosiderin in 0, 2, 2 and 2 males were noted in 0, 5, 15 and 50 mg/kg/day treatment groups, respectively.

Toxicokinetics: A dose related increase in plasma concentration was observed during the study and the plasma concentrations in male and female dogs were similar and the systemic exposure was similar in male and female dogs on week 51 of the study compared to Day 1. The data is shown below in the table.

Dose		5 mg/kg		15 mg/kg		50 mg/kg	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
		geom.	geom.	geom.	geom.	geom.	geom.
AUC(0-24)	[mg-h/L]	5.99	1.57	10.2	1.57	22.4	1.62
AUC(0-24) <sub>norm</sub>	[kg-h/L]	1.20	1.57	0.681	1.57	0.447	1.62
C <sub>max</sub>	[mg/L]	1.29	1.53	1.92	1.38	3.10	1.35
C <sub>max, norm</sub>	[kg/L]	0.257	1.53	0.128	1.38	0.0621	1.35
C(24)/C <sub>max</sub>	[%]	2.08	5.04	1.75	4.18	13.17	3.35
t <sub>max</sub>	[h]	1.68	1.38	1.54	1.67	1.49	1.73
R <sub>A1</sub>	[%]	57.4	2.21	64.9	1.73	70.1	1.64
R <sub>A3</sub>	[%]	70.3	1.92	53.0	2.06	63.1	1.89

$R_{A1} = C_{max, resp, Day} / C_{max, Day 1}$   
 $R_{A3} = AUC(0-24)_{resp, Day} / AUC(0-24)_{Day 1}$

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In 52-week chronic study, the oral doses of 5 to 50 mg/kg/day BAY 59-7939 produced treatment but non-dose proportional increase in plasma drug concentrations within 1.75 to 2.25 hr in males and, 1 to 3 hr in females. Alveolar macrophages and hemosiderin deposits in lymph node (deposits of greater intensity) suggest lungs and lymph nodes as target organs of toxicity in males. A dose of 15 mg/kg/day was the NOEL and the highest tolerable dose in the study gives 22 times the plasma exposure of the clinical dose.

**Toxicology summary:**



Acute tolerance of BAY 59-7939 was determined in rats and mice after a single oral dose of 500 mg/kg. None of the animals died and, the minimal lethal dose (MLD) was not identified in either of the species. In acute intravenous study in mice, a 25 mg/kg dose produced treatment related clinical signs of decreased motility, lying on abdominal position, labored breathing, narrowed palpebral fissure and piloerection. The 13-week repeat dose toxicity was assessed in mice at oral gavage doses of 50, 100 and 200 mg/kg/day. The target organs of toxicity were the heart, kidneys, adrenal and spleen in males, and the liver and kidneys in females. A dose of 100 mg/kg/day was well tolerated and it provided 20 and 29.5 times the plasma exposure of the proposed human dose in male and female animals, respectively. 13-week oral dietary toxicity study in CD-1 mice performed at 0 (diet only), 0 (diet plus PEG), 1250, 2500 and 5000 ppm concentrations of the compound caused dose dependent increase in coagulation time and, increase in liver enzymes in males and females. Increased incidences of focal tubular hypertrophy of the kidney in males and focal hepatic necrosis in female suggested that kidney and liver as the target organs of toxicity in males and females. The high dose was identified as an MTD. The plasma exposure levels in male and female mice at the high dose were about 1.2 and 1.7 times the human exposures at the proposed clinical dose of 10 mg/day. In the 13-week oral gavage toxicity study in CD-1 mice, micronized form of BAY 59-7939 up to 1500 mg/kg/day doses produced a non-dose proportional increase of plasma concentrations. The coagulation times in males and females were changed and no other treatment related changes were seen. The target organs of toxicity and MTD were not identified. In the 4-week oral gavage toxicity study in rats, the high dose of 200 mg/kg/day was highest tolerable dose and target organs were not identified. In the 13-week oral gavage toxicity study in Wistar rats, the micronized BAY 59-7939 at 60 to 1500 mg/kg/day doses produced an erratic and, non-dose or treatment related increase in the plasma concentrations. No treatment related histopathological changes were seen. The target organs of toxicity and MTD were not identified. In 13 weeks dietary study, BAY 59-7939 from 75 to 300 mg/kg/kg doses in diet caused a non-dose proportional plasma concentration, reduced plasma glycogen in the males and females of mid and high dose treatment groups and hyperpigmentation in periductal pancreatic Islets, focal fibrosis and/or inflammation of the islet of Langerhans in males of 150 and 300 mg/kg/day treatment groups and not in females. BAY 59-7939 from 12.5 to 200 mg/kg/day doses for 6 months in rats produced a treatment related increase in the plasma concentration within 60 minutes of the administration. Sponsor did not conduct the histopathology examination of all the treated animals. The target organs of toxicity, NOAEL could not be identified and the study was considered incomplete. In the 4-week oral gavage toxicity study in beagle dogs, a slight increase in reticulocyte levels and splenic extramedullary hematopoiesis was observed in both males and females and 150 mg/kg/day was the tolerated dose in the study. In the 13-week oral gavage toxicity study in beagle dogs, the doses of up to 150 mg/kg/day produced a non-dose proportional plasma increase, caused vomiting, slight increase in reticulocytes and subcutaneous hemorrhage and hematomas. 'No effect dose' was not established and 150 mg/kg/day dose was

highest tolerable dose and animals treated at the dose had plasma exposures of 37 times the clinical dose. Chronic treatment of BAY 59-7939 from 5 to 50 mg/kg/day oral dose for 52 weeks produced a non-dose proportional increase in dog plasma concentrations within 1 to 3 hr in males and females. In males, alveolar macrophages and hemosiderin deposits in lymph node (deposits of greater intensity) were suggestive of lungs and lymph nodes being the target organs of toxicity in males. A dose of 15 mg/kg/day was the NOEL and the highest tolerable dose in the study.

### **2.6.6.3 GENETIC TOXICOLOGY:**

#### **1. Study title: Bacterial Reverse Mutation Assay with BAY 59-7939 in Strains of Salmonella typhimurium (Ames Test).**

**Key findings:** BAY 59-7939 was not mutagenic in the Ames test in the presence or absence of metabolic activation.

**Study no:** T 1070545

**Study type:** Ames test

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, Freidrich-Ebert-Straße 217-33, D-42096 Wuppertal, Germany.

**Date of study initiation:** July 12, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939; Batch No. 010621, purity 98.8%.

**Formulation/vehicle:** DMSO in water.

#### **Methods:**

**Strains/species/cell line:** Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 102 and TA 100.

#### **Dose selection criteria:**

**Basis of dose selection:** The doses used were 16, 50, 158, 500, 1581 and 5000 µg/plate. The sponsor used the maximum concentration (5 mg/plate) recommended by the OECD guideline (Guideline for the testing of chemicals-proposals for replacement of guidelines 471; Draft Feb. 97). The drug was not bacteriostatic; however, as precipitation started at 1581 µg/plate, the 5000 µg/plate could not be used.

**Test agent stability:** The sponsor checked the stability of BAY 59-7939 in solution (at 0.01 and 50 mg/ml) at 0 and 24 hours after formulation. The concentrations ranged from 90% to 110% of the nominal concentrations.

**Metabolic activation system:** Male Sprague Dawley rat liver S9 fraction was used as the metabolic activation system. The animals were given single IP injections of Aroclor 1254 (500 mg/kg, in corn oil) five days before sacrifice.

#### **Controls:**

**Vehicle:** BAY 59-7939 was dissolved in DMSO and diluted in distilled water.

**Negative controls:** DMSO was used as a negative control.

**Positive controls:** Sodium azide (for TA 1535), nitrofurantion (for TA 100), 4-nitro-1, 2-phenylene diamine (4-NPDA, for TA 1537 and TA 98), Cumene hydrochloride (for TA 102) were used as positive controls in the absence of metabolic activation. 2-aminoanthracene (1-AA) was used as the positive control for all strains in the presence of metabolic activation.

**Incubation and sampling times:** The test article solutions or the positive and negative controls were mixed with soft agar to which suspensions of the bacterial strains were added. The mixtures were poured onto agar plates and incubated at 37°C for 48 hours before counting the colonies.

**Doses used in definitive studies:** The doses used were 16, 50, 158, 500, 1581 and 5000 µg/plate.

**Study design:** The test article solutions or the positive and negative controls (0.1 ml) were mixed with 0.5 ml of S9 mix or buffer to which the suspensions of the bacterial strains (0.1 ml) were added. The mixtures were preincubated for 20 minutes before adding to soft agar. The soft agar mixtures were poured onto agar plates and incubated at 37°C for 48 hours.

**Number of replicates:** Triplicate plates were used for each concentration of the test substance or the positive and negative controls.

**Counting methods:** The numbers of revertant colonies were counted using an automatic counter. The mean number of revertant colonies at each concentration was calculated and compared with the positive and negative controls.

**Criteria for positive results:** The test article was considered positive if the number of revertant colonies were increased dose-dependently at least in one strain.

**Study validity:** The study was considered valid if the negative controls fell within the expected range (historical control data from the conducting laboratory, or published data), the positive controls caused significant increase in the revertant colonies, and the titer determination showed sufficient bacterial density in the suspension. Based on the above criteria, the study was considered valid.

**Study outcome:** There were no significant increases in the number of revertant colonies for any strains at any concentrations in the presence or absence of metabolic activation. The positive controls, on the other hand, caused significant increase in the number of revertant colonies as compared with the negative controls. BAY 59-7939 was negative in the Ames test under the conditions of the experiment.

## **2. Study title: Salmonella Microsome Test Plate Incorporation and Reincubation Method**

(Study #: AT01556 /T1070545/PH31770)

**Key findings:** BAY 59-7939 up to 5000 ug/plate was negative in the plate incorporation test for point mutagenic on LT2 Salmonella typhimurium mutants with and without S9 mix in the plate incorporation as well as in the preincubation modification.

**Study no:** T 1070545

**Study type:** Ames test

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology International, 42096 Wuppertal, Germany.

**Date of study initiation & completion:** July 12, 2001 & February 13, 2003

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939; Batch No. 010621, purity 98.8%.

**Formulation/vehicle:** Water/DMSO in water.

**Methods:**

Strains/species/cell line: Salmonella typhimurium TA 1535 and TA 1537, as well as Salmonella typhimurium TA 100, TA 98 and TA 102 developed by McCann et al. (1975b) and Levin et al. (1982), respectively. Histidine-deficient mutants of Salmonella typhimurium LT2 served as indicators to demonstrate point mutagenic effects and were selected specifically for the base pair substitution and frame shift mutations.

Dose selection criteria: The doses (ug/plate) of the preincubation test were selected on the basis of the results of the plate incorporation assay.

Basis of dose selection:

For the mutant count, three plates, both with and without S9 mix, for each strain and dose were used. Three plates filled with the solvent, comprised the negative control. Each of the positive controls (0.1 ml/plate) was also done in triplicate/strain. The doses for the first trial were determined by choosing 5000 ug or 5 u1 per plate as the highest concentration. Each positive control also contained three plates per strain. Concentrations are given as ug/tube for better separation of plate incorporation and preincubation trials.

**Test agent stability:** The stability of BAY 59-7939 in solution (at 0.01 and 50 mg/ml) at 0 and 24 hours after formulation. The concentrations ranged from 90% to 110% of the nominal concentrations.

**Metabolic activation system:** Male Sprague Dawley rat liver S9 fraction was used as the metabolic activation system. The animals were given single IP injections of Aroclor 1254 (500 mg/kg, in corn oil) five days before sacrifice.

**Controls:**

**Vehicle:** BAY 59-7939 was dissolved in DMSO and diluted in distilled water.

**Negative controls:** DMSO was used as a negative control.

**Positive controls:** Sodium azide (Na-azide, (b) (4)), order no. 30175 (Control: D), a direct-acting mutagen used as specific positive control for TA 1535. 2.

Nitrofurantoin (b) (4) lot no. 67F0765, a direct-acting mutagen used as specific positive control for TA 100. 3. 4-Nitro-1,2-phenylene diamine (4-NPDA, (b) (4)), batch no. S2077271 1, a direct-acting mutagen used as specific positive control for TA 1537 and TA 98. 4. Cumene hydroperoxide (Cumene (b) (4)), lot. no. 78H0879, a direct-acting mutagen used as specific positive control for TA 102 in preincubation trials.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C and cumene hydroperoxide were only used without S9 mix; the positive control 2-aminoanthracene was only used with S9 mix.

**Incubation and sampling times:** The test article solutions or the positive and negative controls were mixed with soft agar to which suspensions of the bacterial strains were added. The mixtures were poured onto agar plates and incubated at 37°C for 48 hours before counting the colonies.

**Doses used in definitive studies:** The concentrations used were 3, 50, 0.2, 0.6, 10, 16, 50, 150, 500, 1581 and 5000 µg/plate. The sponsor used the maximum concentration (5 mg/plate) recommended by the OECD guideline (Guideline for the testing of chemicals- proposals for replacement of guidelines 471; Draft Feb. 97).

**Study design:** The test article solutions or the positive and negative controls (0.1 ml) were mixed with 0.5 ml of S9 mix or buffer to which the suspensions of the bacterial strains (0.1 ml) were added. The mixtures were preincubated for 20 minutes before adding to soft agar. The soft agar mixtures were poured onto agar plates and incubated at 37°C for 48 hours.

Number of replicates: Triplicate plates were used for each concentration of the test substance or the positive and negative controls with or without S9 mix.

Counting methods: The numbers of revertant colonies were counted using an automatic counter. The mean number of revertant colonies at each concentration was calculated and compared with the positive and negative controls.

Criteria for positive results: The test article was considered positive if the number of reproducible dose related revertant colonies were increased at least in one strain. For TA 1535, TA 100 and TA 98 this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached.

**Study validity:** The study was considered valid if the negative controls fell within the expected range (historical control data from the conducting laboratory, or published data), the positive controls caused significant increase in the revertant colonies, and the titer determination showed sufficient bacterial density in the suspension. Based on the above criteria, the study was considered valid.

**Study outcome:** The compound was not bacteriotoxic at doses of up to and including 5000 µg/plate. No inhibition of growth was noted as well and a precipitate was seen at 1581 µg/plate. None of the five strains showed in a dose related and biologically relevant manner increase in mutant counts in the tests with and without S9 mix and was confirmed by the results of the preincubation trials. The positive controls - sodium azide, nitrofurantoin, 4-nitro-1, 2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus showed that the test was conducted within system's sensitivity.

No significant increases were observed in the number of revertant colonies for any strains at any concentrations in the presence or absence of metabolic

activation. Thus, BAY 59-7939 was negative in the Ames test under the conditions of the experiment.

**3. Study title: Bacterial Reverse Mutation Assay of Anilino-morpholinone (a by product of BAY 59-7939 co-precipitate)**

**Key findings:** The compound showed an increase in the revertants and was mutagenic in the assay.

**Study no:** T 1076143/PH-34344

**Study type:** Ames test Plate Incorporation Screening

**Conducting laboratory and location:** Bayer Health CareAG, 42096 Wuppertal (Germany)

**Date of study initiation & completion:** February 15, 2006 & March 8, 2006

**GLP compliance:** A GLP compliance certification attached

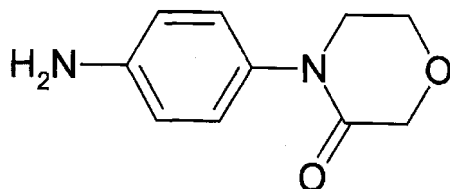
**QA reports:** yes (x) no ( )

**Drug, lot #, radiolabel, and % purity:** BXR387U (Sample BGQ 0210-85 rein) and 100%

**Chemical Name:** 4-Aminophenyl-3-morpholin

**Mol Wt:** 192.2

**Structure:**



**Formulation/vehicle:** DMSO

**Methods:**

Strains/species/cell line: TA-1535, TA-1537, TA-98, TA-100 and TA 102.

Dose selection criteria: The highest concentration was the maximum soluble concentration achieved in DMSO.

Basis of dose selection: A preliminary concentration range finding study.

Range finding studies: doses of the compound used were 16, 50, 160, 500, 1600 and 5000 ug/plate with and without S9 activation.

Test agent stability: The test article solution was used within 24 hr of its preparation

Metabolic activation system: No S9 fraction groups included

Controls:

Vehicle: Diluted DMSO

Negative controls: No untreated negative control was included

Positive controls: sodium azide (10 ug/plate for TA-1535/5 ug/ml), 4-NPDA 10 ug/plate TA137), 4-NPDA (10 g/plate, TA1537), 4-NPDA (0.5 ug/plate, TA98), nitrofluorene (0.2 ug/ml TA-100), MMC (0.2 ug/plate in water TA-102) and 2-AA (3 ug/plate) as a positive control.

Comments:

Exposure conditions:

Incubation and sampling times: Not given in the methods

Doses used in definitive study: 312.5, 625, 1250, 2500 and 5000 ug/plate and 3 plates/concentrations were used and 2 plates acceptable for counting used

Study design:

Analysis:

No. of replicates: 0 to 4 representing growth equivalent to control

Counting method: Automatic plate counter

Criteria for positive results: A reproducible and dose related induction of increased revertants colonies with at least one strain indicated that the compound was positive in the test. For TA-98 and TA-100 or E.coli or 3X the control for TA-1535 or TA-100 or TA98 the increase of at least 2X the respective vehicle control frequency was the criteria of positive test.

## Results:

Anilino-morpholinone up to the dose of 5000 ug/plate was not cytogenic in the test strains. But in 3 of the 5 strains of bacteria cultures, increased mutant colonies as compared with negative controls were noted. The increase in the revertants was noted at the lowest effective concentration of 160, 1600 and 5000 ug/plate for TA100, TA102 and TA98, respectively. Anilino-morpholinone was mutagenic in the test.

### 4. Study Title: BAY 59-7939 In Vitro Chromosomal Aberration Test with Chinese Hamster V79 Cells

Study No.: T 0073244/ AT02611/PH34198

**Key findings:** BAY 59-7939 was not mutagenic in the *in vitro* chromosome aberration assay in CHL cells.

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, D-42096 Wuppertal, Germany.

**Date of study initiation:** March 21, 2005 and November 21, 2005

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, and % purity:** Batch No. BX01SFS, purity 88.4%.

**Formulation/vehicle:** DMSO

**Methods:**

**Strains/species/cell line:** V79 Chinese hamster lung cells were used in the assay. The karyotype of the V79 cells (modal number of chromosomes: 22) was confirmed before initiation and completion of the study.

**Study type:** In vitro chromosome aberration assay in CHL cells.

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, Freidrich-Ebert-Strare 217-33, D-42096 Wuppertal, Germany.

**Date of study initiation:** August 07, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939; Batch No. 010621, purity 98.9%.

**Formulation/vehicle:** DMSO

**Positive Controls:** Mitomycin C ( (b) (4) batch #4541 8812 44903116) without metabolic activator. 0.1 and 0.03 ug/ml for 4 and 18 hr treatment time. It was dissolved and diluted in Hanks' balanced salt solution,

ii. Cyclophosphamide batch #3B 102 was dissolved and diluted in Hanks' balanced salt solution (b) (4) and used in a volume of 0.2 ml/culture.

**Methods:**

**Strains/species/cell line:** V79 Chinese hamster lung cells were used in the assay. The karyotype of the V79 cells (modal number of chromosomes: 22) was confirmed before initiation and completion of the study.

**Basis of dose selection:** The concentrations were selected on the basis of a preliminary toxicity study, in which BAY 59-7939 concentrations of 1, 10, 25, 50 and 100, 125, 250 and 500 µg/ml were used in the presence and absence of metabolic activation with a 4-hour incubation period. In the absence of metabolic activation, with an 18-hour incubation period, BAY 59-7939 concentrations of 1.0 to 180 µg/ml were used. Precipitation of the test compound was observed at 60 µg/ml and above in the absence of metabolic activation, and at 90 µg/ml and above in the presence of metabolic activation. In the absence of S9 mix, the survival index was lower than 50% at 120 µg/ml and higher concentrations (42.4% and 48.8% at 120 and 180 µg/ml, respectively). So, with 4.0-hour incubation period, the sponsor used 60, 90 and 120 µg/ml concentrations and with 18-hour incubation period, 30, 60 and 90 µg/ml concentrations for the evaluation of the test result.

Cytotoxicity determined in the pre-test and in the main-study and cell survival as well as mitotic index was determined in the presence and absence of S9 mix. The mitotic index and number of mitotic cells/1000 cells per culture was determined. In the main study, cultures with a total incubation period of 8 hours were additionally and exclusively used to determine the cytotoxicity of BAY 59-7939. For 4-hour incubation period, BAY 59-7939 concentrations used are shown in the table:



**Table 4-1: Concentrations for 4 Hours Treatment**

test groups	S9 mix	concentration in culture medium as µg/ml	treatment time in hours	harvest time in hours
solvent control	-/+	0	4	18
BAY 59-7939	-/+	30	4	18
BAY 59-7939	-/+	60	4	18
BAY 59-7939	-/+	90	4	18
BAY 59-7939	-/+	120	4	18
BAY 59-7939	-/+	180	4	18
positive controls				
mitomycin C	-	0.1	4	18
cyclophosphamide	+	2.0	4	18
solvent control	-/+	0	4	30
BAY 59-7939	-/+	90	4	30
BAY 59-7939	-/+	120	4	30
BAY 59-7939	-/+	180	4	30

The concentrations and schedule for 18 hr treatment is shown below:

**Table 4-2: Concentrations for 18 Hours Treatment**

test groups	S9 mix	concentration in culture medium as µg/ml	treatment time in hours	harvest time in hours
solvent control	-	0	18	18
BAY 59-7939	-	15	18	18
BAY 59-7939	-	30	18	18
BAY 59-7939	-	60	18	18
BAY 59-7939	-	90	18	18
BAY 59-7939	-	120	18	18
positive controls				
mitomycin C	-	0.03	18	18

**Evaluation Criteria:** Coded slides were evaluated using a light microscope (magnification of 630), the mitotic index was determined by counting 1000 cells per culture. Using a light microscope at a magnification of about 1000, chromosomes of approximately 200 metaphases per concentration (100 metaphases/each of two parallel cultures) were examined.

The classes of structural chromosome damage were recorded. Both chromatid and chromosome-type aberrations were assessed. Chromatid-type aberrations are clastogenic effects restricted to one of the two corresponding chromatids.

The mitotic index was statistically analyzed using the one-sided chi2-test and numbers of metaphases with aberrations (including and excluding gaps) and of metaphases with exchanges were compared with respective to solvent and positive control.

**Positive Test Criteria:** A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate and it was negative, if there was no such increase at any time interval. Test was also considered negative, if there were statistical significant values, which were, however, within the range of historical negative controls.

A test was considered equivocal, if there was an increase above the range of historical negative controls which was statistically significant.

**Results:**

No statistically significant increases of numbers of metaphases with aberrations were detected after 4 hours treatment and total culture times of 18 or 30 hours. The same was true for a treatment period and total culture time of 18 hours. A statistically significant increase of metaphases with aberrations was reported in the positive control mitomycin C that demonstrated the sensitivity of the test.

**5. Study title: *In Vitro* Chromosome Aberration Assay with BAY 59-7939 in V79 Chinese Hamster Lung (CHL) Cells.**

**Key findings:** BAY 59-7939 was not mutagenic in the *in vitro* chromosome aberration assay in CHL cells.

**Study no:** T 2070546/PH31537

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, D-42096 Wuppertal, Germany.

**Date of study initiation:** August 07, 2001 & November 23, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939; Batch No. 010621, purity 98.9%.

**Formulation/vehicle:** DMSO

**Methods:**

**Strains/species/cell line:** V79 Chinese hamster lung cells were used in the assay. The karyotype of the V79 cells (modal number of chromosomes: 22) was confirmed before initiation and completion of the study.

**Study type:** In vitro chromosome aberration assay in CHL cells.

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, Freidrich-Ebert-Straße 217-33, D-42096 Wuppertal, Germany.

**Date of study initiation:** August 07, 2001

**GLP compliance:** Yes

**QA reports:** yes (X) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 59-7939; Batch No. 010621, purity 98.9%.

**Formulation/vehicle:** DMSO

**Methods:**

**Strains/species/cell line:** V79 Chinese hamster cell was used in the assay. The karyotype of the V79 cells (modal number of chromosomes: 22) was confirmed before initiation and completion of the study.

**Basis of dose selection:** The doses were selected on the basis of a preliminary toxicity study, in which BAY 59-7939 concentrations of 1, 10, 25, 50 and 100, 125, 250 and 500 µg/ml were used in the presence and absence of metabolic activation with a 4-hour incubation period. In the absence of metabolic activation,

with an 18-hour incubation period, BAY 59-7939 concentrations of 1.0 to 180 µg/ml were used. Precipitation of the test compound was observed at 60 µg/ml and above in the absence of metabolic activation, and at 90 µg/ml and above in the presence of metabolic activation. In the absence of S9 mix, the survival index was lower than 50% at 120 µg/ml and higher concentrations (42.4% and 48.8% at 120 and 180 µg/ml, respectively). So, with 4.0-hour incubation period, the sponsor used 60, 90 and 120 µg/ml concentrations and with 18-hour incubation period, 30, 60 and 90 µg/ml concentrations for the evaluation of the test result.

**Test agent stability:** The sponsor checked the stability of BAY 59-7939 in solution (at 0.01 and 50 mg/ml) at 0 and 24 hours after formulation. The concentrations ranged from 110% to 90% of the nominal concentrations.

**Metabolic activation system:** Male Sprague Dawley rat liver S9 fraction was used as the metabolic activation system. The animals received Aroclor 1254 (500 mg/kg, in corn oil) by single IP injections, five days before sacrifice.

**Controls:**

**Vehicle:** The test agent was dissolved in DMSO and diluted in Eagles minimal essential medium (MEM, (b) (4)).

**Negative controls:** DMSO was used as a negative control.

**Positive controls:** As positive controls, mitomycin C and cyclophosphamide were used in the absence and presence of metabolic activation, respectively.

**Exposure conditions:**

**Incubation and sampling times:** In the preliminary toxicity test, the cells were exposed to BAY 59-7939 for 4 and 18 hours in the absence and 4 hours in the presence of metabolic activation. For the mutagenicity assay, the cells were incubated with the negative control, BAY 59-7939 or the positive controls for 4 hours or 18 hours in the absence or presence of metabolic activation, and the harvest time was 18 or 30 hours.

**Doses used in definitive studies:** For 4.0-hour incubation period, 60, 90 and 120 µg/ml and, for 18-hour incubation period (without S9 mix), 30, 60 and 90 µg/ml concentrations were used.

**Study design:** Chinese hamster V79 cells were cultured in Minimal Essential Medium (MEM) containing amino acids, vitamins, antibiotics and fetal calf serum. After a passage on the day before treatment, the cells were treated (approximately 1x10<sup>6</sup> cells/75 cm<sup>2</sup> flask) with the positive or the negative controls and the test article for 4 or 18 hours (- S9), in the absence or presence of metabolic activation. The cells were washed and incubated in the fresh medium until harvested at 18 or 30 hours.

Colcemid solution (40 ng/ml) was added to each flask two hours prior to harvesting to arrest the metaphase. The cells were fixed, stained and the chromosomes of approximately 200 metaphases per concentration were examined.

**Analysis:**

**Number of replicates:** Duplicate samples were used for each concentration of the test substance or the positive and negative controls.

**Counting methods:** The chromosomes of approximately 200 metaphases at each concentration (100 metaphases from each parallel culture) were examined under light microscope for both chromatid and chromosome type aberrations.

**Criteria for positive results:** The test was considered positive, if there was a relevant and statistically significant increase in the aberration rate. The test was considered negative if there was no such increase in the aberration rate at any time.

**Study validity:** The assay was considered valid if there were significant increases in the number chromosome aberrations by the positive controls, and the number of aberrations by the positive controls was within the range of historical controls from the conducting laboratory or from the published studies. Based on these criteria, the assay was considered valid.

**Study outcome:** Treatment with BAY 59-7939 did not cause an increase in the number of chromosome aberrations in V79 CHL cells in the presence or absence of metabolic activation. The positive controls, on the other hand, caused significant increases in the number of chromosome aberrations both in the presence or absence of metabolic activation. The chromosomal aberrations data for 4-hour (+S9 and -S9) and 18-hour (-S9) treatment periods are summarized in the Table below.

Experimental Group	Harvest Time (Hr)	Cells Scored	Metaphases with Aberrations (%)		
4 hours treatment (-S9)			Incl. gaps	Excl. Gaps	Exchanges
DMSO	18	100	0.0	0.0	0.0
		100	2.0	2.0	0.0
		200 (Total)	1.0	1.0	0.0
BAY 59-7939 60 µg/ml	18	100	3.0	3.0	1.0
		100	0.0	0.0	0.0
		200 (Total)	1.5	1.5	0.5
BAY 59-7939 90 µg/ml	18	100	0.0	0.0	0.0
		100	0.0	0.0	0.0
		200 (Total)	0.0	0.0	0.0
BAY 59-7939 120µg/ml	18	100	0.0	0.0	0.0
		100	2.0	2.0	1.0
		200 (Total)	1.0	1.0	0.5
Mitomycin C 0.1 µg/ml	18	100	42.0	42.0	26.0
		100	34.0	34.0	21.0
		200 (Total)	38.0*	38.0*	23.5*
4 hours treatment (+S9)					
DMSO	18	100	4.0	4.0	0.0
		100	6.0	6.0	1.0
		200 (Total)	5.0	5.0	0.5
BAY 59-7939 60 µg/ml	18	100	2.0	2.0	1.0
		100	6.0	5.0	2.0
		200 (Total)	4.0	3.5	1.5
BAY 59-7939 90 µg/ml	18	100	6.0	4.0	0.0
		100	2.0	2.0	1.0
		200 (Total)	4.0	3.0	0.5
BAY 59-7939 120µg/ml	18	100	3.0	3.0	0.0
		100	2.0	2.0	0.0
		200 (Total)	2.5	2.5	0.0
Cyclophosphamide 2.0 µg/ml	18	100	52.0	52.0	25.0
		100	59.0	59.0	40.0
		200 (Total)	55.5 *	55.5*	32.5*
18 hours treatment (-S9)					
DMSO	18	100	0.0	0.0	0.0
		100	1.0	1.0	0.0
		200 (Total)	0.5	0.5	0.0
BAY 59-7939 30 µg/ml	18	100	1.0	1.0	0.0
		100	0.0	0.0	0.0
		200 (Total)	0.5	0.5	0.0
BAY 59-7939 60 µg/ml	18	100	0.0	0.0	0.0
		100	1.0	1.0	1.0
		200 (Total)	0.5	0.5	0.5
BAY 59-7939 90µg/ml	18	100	0.0	3.0	0.0
		100	1.0	1.0	1.0
		200 (Total)	0.5	0.5	0.5
Mitomycin C 0.03 µg/ml	18	100	18.0	18.0	10.0
		100	16.0	16.0	6.0
		200 (Total)	17.0 *	17.0*	8.0*

**6. Study title: Micronucleus Test in the Mouse Bone Marrow.**  
(Study #PH31536/T 3070547)

**Key findings:** BAY 59-7939 was not clastogenic in the mouse bone marrow micronucleus assay by the intraperitoneal (IP) route. However, the intended route of administration of the drug is the oral route. The sponsor selected 140 mg/kg as the MTD for the male mice. Though sponsor did not give any reason for selecting IP route, however, the plasma concentration ( $AUC_{0-24hr}$ ) by 140 mg/kg, ip dose was 25.7 mg.h/l at 30 min. The extrapolated AUC value of 140 mg/kg in mice was calculated and peak concentrations were 5.07 and 8.94 mg.h/l in males and females, respectively.

**Study no:** T 3070547

**Study type:** *In vivo* bone marrow micronucleus test.

**Conducting laboratory and location:** Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, Freidrich-Ebert-Stra@e 217-33, D-42096 Wuppertal, Germany.

**Date of study initiation:** August 22, 2001

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug, lot #, and % purity:** BAY 59-7039; Batch No. 010621, purity 98.9%.

**Formulation/vehicle:** The test substance was suspended in aqueous Cremophor (b) (4), batch #398261/1).

**Methods:**

**Strains/species:** Male Hsd/Win: NMRI mice, 6-12 weeks old.

**Dose selection criteria:**

**Basis of dose selection:** The dose was selected based on a preliminary toxicity study in mice. Adult male and female mice (3/sex/group) were treated with IP injections of 100, 400 and 1000 mg/kg of BAY 59-7939. There were deaths at 400 (1/3 M and 2/3 F) and 1000 (1/3 M and 1/3 F) mg/kg doses. Treatment related clinical signs were observed at all doses, and included apathy, roughened fur, weight loss, spasm, scratching of body, difficulty in breathing and closed eyes. Based on these findings, the sponsor selected 140 mg/kg as the MTD for the male mice. The sponsor did not give any reason for selecting IP route; however, the plasma concentration achieved by 140 mg/kg, ip was 25.7 mg.h/l in 30 min. In a single dose study in mice (study #T5076282), an oral dose of 60 mg/kg achieved the peak concentration of 5.07 and 8.94 mg.h/l in males and females, respectively. These were approximately 5 and 3 times the plasma concentration achieved by an i.p dose of 140 mg/kg.

**Test agent stability:** The sponsor determined the stability of 1.0 and 15 mg/ml of the formulation at 0 and 4 hours after preparation. The concentrations were within 98% and 102% of the expected concentrations.

**Controls:**

**Vehicle:** Aqueous Cremophore (0.5%) was used as the vehicle for test substance.

Cyclophosphamide was dissolved in deionized water.

**Negative controls:** 0.5% aqueous Cremophore was administered as a negative control.

**Positive controls:** Cyclophosphamide (at an IP dose of 20 mg/kg) was used as the positive control.

**Exposure conditions:**

**Doses used in definitive study:** BAY 59-7939 was used at 35, 70 and 140 mg/kg IP doses.

**Study design:** Groups of male mice received the vehicle (negative control), BAY 59-7939 (35, 70 and 140 mg/kg) or the positive control (cyclophosphamide), administered by IP injections. BAY 59-7939 was administered twice (separated by 24 hours) and cyclophosphamide was administered once only (administration volume, 10 ml/kg). A replacement group received the 140 mg/kg dose to replace animals in case there were deaths at the high dose. The animals were sacrificed, the femurs removed immediately and the slides of the bone marrow smears prepared. The slides were stained, coded and the number of polychromatic erythrocytes counted.

About 2000 polychromatic erythrocytes per animal were counted using a light microscope. In addition, the number of normochromatic erythrocytes per 2000 polychromatic erythrocytes was counted and the number of normochromatic erythrocytes with micronuclei was established. The statistical significance for the difference between the negative controls and the treatment groups was determined by one-sided chi<sup>2</sup> test.

**Counting method:** The number of polychromatic and normochromatic erythrocytes was counted manually using a light microscope.

**Criteria for positive results:** The result was considered positive if the frequency of micronucleated polychromatic erythrocytes was significantly increased in the treatment group as compared with the negative control. The test was considered negative if the increase in the number of polychromatic erythrocytes was within the historical control values of the conducting laboratory.

**Results:**

**Study validity:** The dose selection for the in vivo mouse micronucleus test was based on a preliminary IP toxicity study. However, the intended route of administration of the drug is the oral route.

**Study outcome:** Intraperitoneal treatment with BAY 59-79 in male mice did not induce any significant increase in the frequency of micronucleated polychromatic erythrocytes, as compared with the control. The positive control (cyclophosphamide), on the other hand, caused significant increase in the number of micronucleated polychromatic erythrocytes. Thus, the results suggest that BAY 59-7939 had no clastogenic effect in mice under the conditions of the experiment. The micronucleus test data is summarized in the sponsor's Table 6 and scanned below.

Table 6

Summary of Results of the Micronucleus Test with  
BAY 59-7939

experimental groups	number of evaluated PCE	number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
negative control	10,000	1445 ± 589	2.0 ± 1.6	2.6 ± 1.1
BAY 59-7939 2x 35 mg/kg	10,000	1719 ± 549	3.0 ± 2.2	2.8 ± 1.5
BAY 59-7939 2x 70 mg/kg	10,000	2086 ± 607	1.2 ± 1.2	4.2 ± 2.4
BAY 59-7939 2x 140 mg/kg	10,000	1897 ± 515	1.7 ± 1.8	2.4 ± 1.3
positive control CP 20 mg/kg	10,000	1441 ± 164	2.5 ± 2.3	25.6* ± 7.9

\*P < 0.01 in non-parametric Wilcoxon ranking test

**Genetic toxicology summary:** The genotoxic potential of BAY 59-7939 was examined in the Ames test, the chromosome aberrations assay in V79 Chinese hamster lung cells and the mouse bone marrow micronucleus test by the intraperitoneal route. BAY 59-7939 was not mutagenic in any of the abovementioned tests under the experimental conditions. However, although the intended route of administration of the drug is by the oral route, in the mouse bone marrow micronucleus assay, the drug was administered by the IP route which is acceptable.

#### 2.6.6.4 CARCINOGENICITY STUDIES:

Sponsor did not submit the full reports of the carcinogenicity studies with the submission. The protocols for these studies were submitted and were assessed by Ex-CAC. In a recent communication, sponsor indicated that the full reports of the mouse and rat studies will be submitted in 2009.

#### 2.6.6.5 Reproductive and Developmental Toxicity



## 6.1 Study of Fertility and Early Embryonic Development in Rats after Oral Administration (Study Number: T2062789)

Key findings: BAY-59-7939 at oral doses of 12.5 to 200 mg/kg/day in male and female rats during the fertility and reproductive performance period produced a reduction in number of dams (90.5%) with viable fetuses and a slight increase in post implantation loss. A dose related reduction in ovarian weight by 8.8% in the dams of the high dose group also occurred. The NOAEL was 50 mg/kg/day and based on the surface area, and it has 41 times safety ratio for the clinical dose.

Study no.: T2062789/AT01125/PH-33273

Conducting laboratory and location: The Department of Experimental Toxicology of BHC-PH-PD-T, 42096-Wuppertal, Germany.

Date of study initiation and completion: August 19, 2002 and April 7, 2004

GLP compliance: A statement of compliance was attached.

QA reports: yes (X) no ( )

Drug, lot #, and % purity: J20020528, 9.5 % BAY 59-7939 coprecipitate; batch #F033082 and 99.3% pure, suspension made using 20 % Solution HS 15 and 80 % dermineralized water in addition with PEG 6000 according to the maximum PEG 6000 content of the high dose group formulation.

### Methods

Doses: Male and female animals (12 week old; 24/sex/dose group) were randomly assigned to 4 groups using a randomization list by a computer program. BAY 59-7939 was administered by oral gavage, the intended route in humans in 10 ml/kg volume. Male rats were treated for 4 weeks prior to mating and during the subsequent mating period up to necropsy. Female rats were treated for 2 weeks prior to mating, during the mating period and to gestation day 7. The dose selection was based on a subacute toxicity study in rats (T7070622) and two kinetic studies with doses of 300 mg/kg and 400 mg/kg (T5068560 and T4062790). No meaningful higher exposure was achieved. The doses and the concentrations of the compound in each dose group are shown in sponsor's Table 7-2 of the submission:

Table 7-2		BEST POSSIBLE COPY	
	Dose (mg/kg bw/day)	Test compound concentration (mg/ml)	
Group 1 (Control)	0	0	(vehicle only)
Group 2	12.5	1.25	
Group 3	50	5	
Group 4	200	20	

Species/strain: SPF-bred Wistar rats (strain: Hsd Cpb:WU),

(b) (4)

Route, formulation, volume: Oral, suspension made using 20 % Solution HS 15 and 80 % demineralized water in addition with PEG 6000; 10 ml/kg.

Groups used for toxicokinetics: TK was not done

Parameters and endpoints evaluated: The general observations, appearance, behavior, excretion (feces and urine) and mortality were monitored twice daily during the pretreatment period (estrus determination days -6 to 0) in the female animals, during the entire treatment

period in male and female animals. Females were observed up to the time of cesarean section (days 14 to 16 p.c.).

Body weight/Food Consumption: Body weights were taken twice a week during the entire treatment period in the male and female animals up to (and on) the day of necropsy. In inseminated females, daily body weight was monitored till the day of cesarean section. The food consumption changes were recorded during treatment up to the start of the mating period (weekly evaluation in males and females) as well as for inseminated females on day 0-7, and 7-14 p.c. The water consumption was estimated daily by visual examination of the water bottles.

Gross pathological examination: The animals were killed using deep carbon dioxide anesthesia and males were killed between study days 45 to 51 and, females on days 14 to 16 p.c. The gross examination of live and dead fetuses was done and, number of corpora lutea, implantation sites, resorptions, live and dead fetuses was counted and, placenta of each of the live and dead fetus was examined for gross changes. The reproductive organs (testes, epididymides, prostate, seminal vesicles, uterus, vagina, ovaries and pituitary gland) from all animals were separated, preserved in Davidson' solution (testes and epididymides) or in 10 % neutral buffered formalin solution. The implantation sites in non-pregnant animals without visible implantations were counted after staining with a solution of 10 % ammonium sulfide.

## Results

Mortality: Four animals died and these were 2 males (1 of 50 and 200 mg/kg/day groups) were sacrificed in moribund condition on day 29 and day 18, respectively. Two females in 200 mg/kg/day treatment group died on premating day 10 and on gestation day 6. These animals showed sunken flanks, respiratory sounds, piloerection, and increased salivation after administration, and decreased water consumption. One male of control group also died on day 3. This animal showed hypoactivity, respiratory sounds, gasping breathing, reduced amount of feces, and salivation. The deaths were due to dosing errors.

Clinical signs: Increased incidence and duration of salivation in males of 50 and 200 mg/kg/day groups were noted.

Body weight changes: A reduction of 17.1% in the body weight gain was observed in females of 200 mg/kg/day group during treatment period and, 15.8% reduction was seen during gestation period. It was related to the reduction of food intake. The initial and final body weight of control females was 259 and 423 g.

Food and water consumptions: The food consumption of the 200 mg/kg/day treatment group males and females was significantly ( $p < 0.01$ ) less than the control from premating to mating and gestation periods. The food consumption in different study groups are shown in the sponsors table 8-1 and scanned below:

**Table 8-1: Mean feed consumption (g/day) in the male animals during the premating period:**

Dose (mg/kg bw/day)	week 1	week 2	week 3	week 4
0	29.14	28.12	27.03	27.08
12.5	28.98	26.95	26.70	27.55
50	28.04	27.61	27.43	27.67
200	24.99**	26.99	27.34	27.29

\*\* significantly different from control,  $p < 0.01$

**Table 8-2: Mean feed consumption (g/day) in the female animals during the premating period and during gestation:**

Dose (mg/kg bw/day)	week 1	week 2	days 0 – 7 p.c.	days 7 – 14 p.c.
0	17.11	17.67	21.41	23.55
12.5	16.89	16.95	21.18	25.10
50	16.33	17.20	20.39	22.97
200	14.90**	17.66	20.46	22.93

\*\* significantly different from control,  $p < 0.01$

There was no treatment related effects on water intake and excretory products in any animal of study dose groups among both genders.

Toxicokinetics: Not done

Necropsy:

One male of the 200 mg/kg/day group showed a treatment related black-brownish hematoma between testis and epididymides. No notable pathology was observed during necropsy in study dams included in the study.

### **Insemination Index, Fertility Index, Gestation Index**

The insemination index, females with implantations and with embryonic viability was similar in treated groups compared to controls. One of 21 females of high dose group was sacrificed because of moribund condition and another female showed reduced viable embryos. These females were excluded. The females with live embryos were 100, 100, 100 and 90.5% among 0, 12.5, 50 and 200 mg/kg/day groups, respectively, with viable embryos significantly less in the 200 mg/kg/day group. The data is shown in the following sponsor's table:

**Table 8-6: Insemination index, fertility index and gestation index:**

Dose (mg/kg bw/day)	used	inseminated		with implantations		with viable embryos	
		n	% of those paired	n	% of those inseminated	n	% of those with implantations
0	23	22	95.7	19	86.4	19	100.0
12.5	24	24	100.0	20	83.3	20	100.0
50	24	24	100.0	21	87.5	21	100.0
200	23	23	100.0	21	91.3	19 <sup>+</sup>	90.5

+ one female with sacrifice in moribund condition on day 6 p.c. was excluded

The mean number of estruses/female during 14 days of premating period were 2.9, 3.2, 3.5 and 3.3 in 0, 12.5, 50 and 200 mg/kg/day groups, respectively, therefore, treatment did not affect the number of estruses.

**Cesarean Section Observations:**

The number of corpora lutea, preimplantation and post implantation sites are shown in sponsor's table 8-9 and scanned here:

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**Table 8-9: Results obtained at cesarean section (mean values per female):**

Dose (mg/kg bw/day)	0	12.5	50	200
Number of females	19	20	21	20
Corpora lutea	13.9	14.3	14.2	13.8
Implantations	11.5	13.0	12.4	11.9
Preimplantation loss	2.5	1.3	1.8	2.0
Postimplantation loss	0.2	0.4	0.7	0.8
Viable embryos	11.3	12.6	11.7	11.1

The mean number of corpora lutea was similar in treated animals compared to the control group animals.

Pre- and Post- implantation losses:

A dose related decrease in the mean number of post-implantation losses per female was observed in treated animals, i.e., number of late resorptions were 0.2, 0.4, 0.7 and 0.8 per female in study group animals. The number of matings, fertility index and number of corpora lutea were similar in treated groups compared to control group females.

Weight of the Testes and Ovaries

The mean testicular and mean combined weights of the ovaries are given in the following table of sponsor and scanned below:

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**Table 8-5: Mean absolute combined testes and ovaries weights (g):**

Dose (mg/kg bw/day)	0	12.5	50	200
Testes	3.693	3.701	3.647	3.710
Ovaries	0.114	0.106	0.106	0.104

BAY 59-7939 treatment up to 200 mg/kg/day produced a reduction in the mean ovarian weight (in comparison to control) of 5.3, 7.0 and 8.8% in females belonging to 0, 12.5, 50 and 200 mg/kg/day groups. The testes weight was not affected in males of the study.

In summary, BAY-59-7939 from the oral doses of 12.5 to 200 mg/kg/day in male and female rats during the fertility and reproductive performance period produced a reduction in number of dams (90.5%) with viable fetuses and a slight increase in post implantation loss and a dose related reduction in ovarian weight by 8.8% in the dams of the high dose group. The NOAEL was 50 mg/kg/day and based on the surface area, the safety ratio to the clinical dose was 41 times.

**B. Study title: Developmental Toxicity Study in Rats after Oral Administration**  
(Study #T3063590/PH-33582)

Key findings: BAY 59-7939 when administered in pregnant rats from 0, 10, 35 and 120 mg/kg/day doses from day 6 to 17 postcoitum produced dose related increase in plasma concentrations and vaginal bleeding, piloerection, hypo-activity and reduced feed intake. A severe body weight loss, uterine bleeding, pale liver, kidneys and enlarged adrenal glands was reported in the animals. Dose related adverse effects of necrotic placental borders, necrotic, engorged and/or pale placentas were observed in fetuses of dams treated from 10 mg/kg/day or greater dose. BAY 59-7939 was not teratogenic in pregnant rats. Based on body surface exposure (mg/mm<sup>3</sup>), it provides 97 times greater exposure than the proposed clinical dose.

Study no.: Study Number: T3063590/PH-33582

Conducting laboratory and location: Bayer HealthCare AG, PH-PD Toxicology International, Wuppertal (Germany)

Date of study initiation and completion: July 09, 2002 and November 18, 2004

GLP compliance: A statement of compliance with the OECD Principles of Good Laboratory Practice and with the revised German Principles of Good Laboratory Practice (German Chemicals Act (Bundesgesetzblatt Part I, No. 40, issued June 27, 2002) was attached.

QA reports: yes (X) no ( )

Drug, lot #, and % purity: J20020528 – Coprecipitate 10%, 101

#### Methods

Doses Used: 0, 10, 35 and 120 mg/kg/day (vol = 10 ml/kg) once daily beginning from days 6 to 17 p.c.

Species/strain: The SPF-bred Wistar rats (strain – Hsd Cpb:WU, (b) (4))

Number/sex/group: 22 pregnant females/group

Route, formulation, volume, and infusion rate: Oral gavage suspension (prepared in demineralized water blended with PEG (polyethylene glycol) 6000, 10 ml/kg and the suspension made every two weeks.

Satellite groups used for toxicokinetics: 5 females/treated group treated and blood samples collected under light ether anesthesia on day 18 p.c. 1, 3, 7, and 24 hours after administration. The animals were killed on day 18 p.c. and plasma samples stored deep frozen (< - 15°C) till sent for plasma concentrations.

Study design: Selected females were assigned to 4 treatment groups as shown below.

**Table 5-1**

	mg/kg body weight/day	concentration in mg/ml
Group 1 (Control)	0	0.0
Group 2 (Low dose)	10	1.0
Group 3 (Medium dose)	35	3.5
Group 4 (High dose)	120	12.0

On day 20 p.c., the general observations were recorded and the animals were c-sectioned and fetal intrauterine development was observed.

Clinical observations: All females of main study were examined twice/day excepting on weekends and holidays when examined once/day. The satellite groups were also examined twice/day and killed on days 18 p.c. (TK groups) and 20 p.c. (main groups). All findings related to changes in the general conditions of the rats and changes in feces and urine excretion were noted.

Body Weight & Feed and Water Intake of Females: Dams were weighed on day 0 p.c. and then daily from days 6 to 18 p.c. (satellite groups) or from days 6 to 20 p.c. (main groups). The corrected body weight gain calculated by subtracting the weight of the uterus on day 20 p.c. from the body weight gain over the period from day 0 to day 20 p.c. The food consumption was estimated for gestation days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20 (in main groups). Water consumption was determined once daily by visual examination of remaining quantities of water in the bottles.

Toxicokinetic Investigations:

Venous blood samples were collected from study females of 10, 35 and 120 mg/kg/day satellite groups under light ether anesthesia on day 17 p.c. 1, 3, 7, and 24 hours after administration. The plasma from the blood samples and the samples were deep frozen (<-15°C) and sent for analysis and the toxicokinetic evaluations.

Necropsy of Females:

On day 18 and 20 p.c., the satellite and main study group animals, respectively were subjected to cesarean section. The main study group uterine contents of females were examined for number of implantations (in females without visible implantation sites after staining of the uterus with a solution of 10 % ammonium sulfide). The uterus and placenta were weighed (individual weight and appearance), number of early resorptions (only implantation site visible) and late resorptions (fetal or placental remnant visible), and dead fetuses (fetuses without signs of life, but without maceration), number of live fetuses, fetal sex and their weights were taken.

External, visceral malformations and minor adverse abdominal, pelvic and thoracic organs abnormalities were evaluated in about half of the fetuses. The remaining half fetuses were used for skeletal abnormalities/ malformations after staining by the modified Dawson technique.

Results

Maternal observations:

Mortality: One female of 120 mg/kg/day dose group was sacrificed as it showed severe body weight loss, reddish vaginal discharge, piloerection, sunken flanks and hypoactivity. A reddish-brown fluid (blood) in the uterus and, pale liver, kidneys and enlarged pale adrenal glands was seen.

**Clinical signs:** Bloody vaginal discharge and piloerection were seen in 1 and all females belonging to 10 and 120 mg/kg/day groups, respectively. The bleeding in the 10 mg/kg/day group was claimed incidental since it was not present in 35/kg/day group dams. The treatment related effects in 120 mg/kg/day group were reduced food intake during treatment period (10.2, 16.0 and 24.8% on study days 9-12, 12-16 and 15-18 p.c, respectively), and water intake and reduced fecal contents. On necropsy of these animals, an enlarged spleen and pale liver were seen in 1 female of 120 mg/kg/day group.

**Toxicokinetics:** Rat plasma concentrations of blood samples collected after 1, 3, 7 and 24 h of the dosing were determined on day 17 post coiturn (p.c.). AUC (0-24) was increased in a treatment related manner. The peak concentration reached in about 1-3 hours. The ratio of the trough to peak concentration increased markedly from 0.7 to 5.7 and 29.2% with increasing doses (saturated and/or protracted absorption) as shown in the table below.

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Dose [mg/kg]	10	35	120
AUC(0-24) [mg·h/L]	18.9	77.7	188
AUC(0-24) <sub>norm</sub> [kg·h/L]	1.89	2.22	1.57
C <sub>max</sub> [mg/L]	2.55	6.59	12.9
C <sub>max, norm</sub> [kg/L]	0.255	0.188	0.108
C(24)/C <sub>max</sub> [%]	0.713	5.70	29.2
t <sub>max</sub> [h]	3.00	3.48	2.08

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The plasma exposures in animals of the high dose group of 120 m/kg/day were 184.3 times the exposure of the clinical dose, Sponsor did not conduct TK estimations in other reproduction studies, the exposures estimated based on the surface area were considered for the sake of uniformity. Based on this, the plasma exposure in animals treated with 120 mg/kg/day were 97.3 times the recommended clinical dose.

**Embryo-Fetal Survival, Fetal Weight and Gravid Uterine Weight:** The mean number of implantations, numbers of corpora lutea, preimplantation losses and implantation sites in the treatment group females were similar to control group animals (shown in sponsor's table 6-3).

**Table 6-3**

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Dose [mg/kg bw/day]	0	10	35	120
inseminated females	22	22	22	22
inseminated females evaluated	22	22	22	22
females with implantations	19	22	20	19 <sup>1</sup>
in % of those inseminated	86.4	100.0	90.9	90.5
mean values per female with implantation sites				
corpora lutea	12.5	13.5	13.1	13.5
preimplantation loss	0.9	1.2	1.6	0.8
implantations	11.6	12.3	11.6	12.7

<sup>1</sup> one female with implantation sites was sacrificed in moribund condition on day 16 p.c.; excluded from calculations

## Effect of Test Compound on Intrauterine Development Gestation Rate

The gestation rate (number of females with viable fetuses as a percentage of the number of females with implantations) was not affected by up to the high dose of 120 mg/kg/day BAY 59-7939 (see Table 6-4 below)

**Table 6-4**

Dose [mg/kg bw/day]	Females with viable fetuses N	Females with viable fetuses in % of females with implantations	Females with total resorption
0	19	100.0	0
10	22	100.0	0
35	20	100.0	0
120	19	100.0	0

The mean values for the parameters of intrauterine development were unaffected by up to 120 mg/kg/day BAY 59-7939 treatment groups (shown below in sponsor table 6-5).

**Table 6-5**

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Dose [mg/kg bw/day]	0	10	35	120
number of females				
with implantations (a)	19	22	20	19
with viable fetuses (b)	19	22	20	19
means per female				
placental weight in g (b)	0.65	0.62	0.67	0.61
number of live fetuses (b)	11.2	11.3	10.9	12.1
postimplantation loss (a/b)	0.4	1.0	0.7	0.6
% males (b)	56.6	48.7	50.1	43.2
fetal weight in g (b)	3.59	3.57	3.70	3.48

Statistically significant increase in incidences of necrotic placental borders in animals of 10, 35 and 120 mg/kg/day groups were seen. An increased number of engorged placentas and, pale colored and necrotic placentas were found in 120 mg/kg/day group animals. The mean placental weight was not affected in 120 mg/kg/day group.

#### Postimplantation Loss, Number & sex of Fetuses

One female of 10 mg/kg/day group had reddish vaginal discharge and late resorptions of 5 of 10 implantation sites at the cesarean section time and no postimplantation loss in 120 mg/kg/day group. Mean litter size was in the treated groups were not different from control group. The mean percentage of male fetuses/litter was slightly low (43.2%) in 120 mg/kg/day group.

#### Fetal Observations:

Mean fetal weights were 3.598, 3.578, 3.70 and 3.48 g and, mean litter size were 11.2, 11.3, 10.9, and 12.1 in the 0, 10, 35 and 120 mg/kg/day groups, respectively. There were no treatment or dose related external, skeletal and visceral malformations among viable fetuses up to 120 mg/kg/day treatment group.



#### Fetal External and Visceral Deviations

No external and visceral deviations (findings other than malformations) in live fetuses (%) or litters were detected. A treatment-related effect for the occurrence the incidence of hemorrhages at different organs (mandible, thyroid, pericardium, abdominal cavity and liver) was seen. These findings were not dose dependent and were in the range of historical control data of sponsor submitted with the document.

BAY 59-7939 administered during the period of organogenesis in pregnant rats caused no developmental abnormalities and it was not teratogenic up to study high dose. Based on body surface exposure ( $\text{mg}/\text{mm}^3$ ), it provides 97 times greater exposure than the proposed clinical dose.

#### **C. Study title: BAY 59-7939 Developmental Toxicity Study in Rabbits after Oral Administration (Study # TO062930/PH-33380/AT01303)**

Key findings: Orally administered BAY 59-7939 produced a linear increase in systemic exposure in 0.5 to 2 h on day 20 p.c. The treatment produced an increased incidence in cold ears at all dose levels. The treatment with BAY 59-7939 produced abortions at all doses and doses of 40 and 160 mg/kg/day were maternally lethal. BAY 59-7939 was not teratogenic in rabbits of the study. The exposures at the estimated NOAEL of 2.5 mg/kg/day, based on the body surface area were 4 times the exposure of the clinical dose.

Study no.: TO062930/PH33380/AT-01303

Conducting laboratory and location: BHC-PH-PD Toxicology International, Bayer HealthCare AG, 42096 Wuppatal, Germany.

Date of study initiation and Completion: June 12, 2002 and July 6, 2004

GLP compliance: A statement that the study was conducted in compliance with ICH guideline "Detection of Toxicity to Reproduction for Medicinal Products" (EU 1993, Japan MHLW 1994, US-FDA 1994).

QA reports: yes (X) no ( )

Drug, lot #, and % purity: J20020430, 9.5 % BAY 59-7939

#### Methods

Animal, Strain: Twenty female Himalayan rabbits/group (between 120 and 274 days old) weights ranged from 2104 to 3361 g on day 0 p.c.

Doses:  
i. Main study group: 0, 2.5, 10, 40, and 160 mg/kg/day BAY 59-7939 Coprecipitate 10 % 100 in demineralized water.

ii. Toxicokinetics group: 3/sex/group animals

The daily oral dose was administered (gavage) as in addition with PEG 6000 from days 6 to 20 p.c. as shown following table (sponsor's table):

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**Table 7-1**

	mg/kg body weight/day	concentration in mg/ml
Group 1 (Control)	0	0
Group 2 (Additional low dose)	2.5	0.5
Group 3 (Low dose)	10	2
Group 4 (Medium dose)	40	8
Group 5 (High dose)	160	32

The dose selection was based on a pilot developmental toxicity study in pregnant rabbits (study #T1071003) with 0, 10, 30, 100 and 200 mg/kg/day (dose volume 5 ml/kg) BAY 59-7939 Coprecipitate 10 % 100 from day 6 to day 20 p.c. One animal of 200 mg/kg/day had reduced gestation rate and aborted on day 20 p.c. Body weight loss was seen in all treatment group animals and necropsy showed an enlarged caecum. The dose of 100 mg/kg/day produced post implantation loss and decreased fetal weights. The dose between 100 and 200 mg/kg/day was identified as the high dose for the present study and sponsor selected 160 mg/kg/day as the high dose in the present study.

Parameters Evaluated:

Clinical observations: All females of main study were examined twice/day from days 0-29 p.c. and satellite group from days 0-21. All findings related to changes in the general conditions of the rabbits (appearance, behavior) and changes in amounts of feces and urine excretions were noted.

Body Weight & Feed and Water Intake of Females: The animals were weighed on day 0 p.c. and daily from days 6 to 21 p.c. (satellite groups) or from days 6 to 29 p.c. (main groups). The food consumption was estimated for gestation Days 0-3, 3 - 6, 6 -9, 9-12, 12-15, 15-18, 18-20, 20-21, 21-24, 24-27, and 27-29 p.c. for the main groups, and days 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20, and 20 -2 1 p.c. for the satellite groups. Water consumption was determined once daily by visual examination of remaining quantities of water in the bottles.

Toxicokinetic Investigations:

Venous blood samples were collected from study females of 2.5, 10 and 40 mg/kg/day satellite groups under light ether anesthesia on day 6 and 20 p.c. at 0.5, 1, 2, 4, 6 and 24 hours after administration. Venous blood samples (extremity veins) were collected from three females of the 160 mg/kg/day main group on day 20 p.c. at 30 minutes as well as 1, 2, 4, 6, and 24 h after administration, because three females were replaced in satellite group. The plasma samples were deep frozen (< - 15°C) and sent for analysis and the toxicokinetic evaluations.

Necropsy of Females:

The females of main and satellite group were dissected on day 29 and 21 p.c., respectively, and uterine contents examined for number of implantations (in females without visible implantation sites after staining of the uterus with a solution of 10 % ammonium sulfide). The uterus and placenta were weighed (individual weight and appearance), number of early (only implantation site visible) and late resorptions (fetal or placental remnant visible), and dead fetuses

(fetuses without signs of life, but without maceration), number of live fetuses, fetal sex and their weights were determined. External, visceral malformations and minor adverse abdominal, pelvic and thoracic organs abnormalities were evaluated in about half of the fetuses. The remaining half fetuses were used for skeletal abnormalities/ malformations after staining by the modified Dawson technique.

## **Results:**

### **A. Maternal Dam Data:**

The treatment related incidences of cold ears were increased in 11, 23, 15, and 23 animals in the 0, 2.5, 10, 40 and 160 mg/kg/day groups, respectively, from day 6 p.c. Daily water intake and urine excretion were decreased in animals of 40 and 160 mg/kg/day treatment groups

Mortality: Two of 24 females in each of 40 and 160 mg/kg/day groups were found dead.

### **Body Weight and Food consumption Changes:**

A severe reduction in the body weight gain in animals of 10, 40 and 160 mg/kg/day groups was seen from days 6-20 p.c. as shown below in the Table 8-2.

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<b>Table 8-2</b>					
Dose (mg/kg b.w./day)	0	2.5	10	40	160
absolute body weight gain (g) days 6 - 20 p.c.					
	46.5	102.1	29.1	9.9	11.8
absolute body weight gain (g) days 0 - 29 p.c.					
	206.6	316.6	151.6	157.2	144.2
corrected body weight gain (g) days 0 - 29 p.c.					
	- 146.0	- 62.3	- 197.7	- 168.3	- 119.7

### **Fetal Observations & Evaluation:**

A marginal reduction was seen in the gestation rate in the 2.5 mg/kg/day group and, total resorption was seen in 1, 1, 2 and 2 females of the 2.5, 10, 40 and 160 mg/kg/day groups, respectively, on days 18 to 26 p.c. One, 2 and 2 females in each of 10, 40 and 160 mg/kg/day groups, respectively, aborted between day 18 and 26 p.c. The females that aborted or all females in these groups showed decrease in feed intakes and body weight loss since beginning of treatment on day 6 p.c. The females which had total resorptions showed slight to marked body weight loss during treatment (-40 g to -194 g), cold ears, and reddish excretion.

### **Gross Pathology Changes:**

Enlarged and gaseous contents in cecum was observed in 4 and 1 females of the 40 and 160 mg/kg/day groups, respectively and only enlarged cecum was in females of the 2.5 and 10 mg/kg/day groups. Pale liver, enlarged gall bladder, hardened fatty tissue, mottled and smaller in size spleen, and pale kidneys were the other observation in the 160 mg/kg/day group.

### General Reproduction Data:

The fertility data including mated females and, number of implantations, corpora lutea and preimplantation losses were similar in treatment group animals compared to the control group animals (as shown in the following table scanned from sponsor's submission). Three and 2 females of 40 and 160 mg/kg/day groups, respectively, were withdrawn and excluded from the study.

**Table 8-3**

Dose (mg/kg b.w./day)	0	2.5	10	40	160
mated females	20	20	20	20	12
mated females evaluated	20	20	20	18 <sup>+</sup>	9 <sup>++</sup>
females with implantations	20	20	20	18	9
in % of those mated	100.0	100.0	100.0	100.0	100.0
mean values (without females displaying abortions) per female with implantation sites					
corpora lutea	8.0	8.3	7.5	8.0	8.9
preimplantation loss	1.4	0.9	0.5	1.0	1.1
implantations	6.7	7.3	7.0	7.0	7.7

+ two females with death were excluded

++ two females with death were excluded, and one female was excluded due to withdrawal of this dose group

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The total resorption was observed in each dose group animals of 2.5, 10, 40 and 160 mg/kg/day and, 1, 2 and 2 females of 10, 40 and 160 mg/kg/day groups aborted. Thus, BAY 59-7939 treatment produced abortions in rabbits from 10 mg/kg/day dose. This should be described in the label. Additionally, increased incidences of coarse grained placentas were noted at 10 mg/kg/day and above study doses.

**Table 8-4**

Dose mg/kg b.w./day	Females with			total resorption n
	viable fetuses on day 29 p.c. n	in % of females with implantations	abortion n	
0	20	100.0	0	0
2.5	19	95.0	0	1
10	18	90.0	1	1
40	15	83.3	2	1
160	6	66.7	2	1

### Appearance and Weight of Placentas

The increased incidences of course/rough grained placentas were from the 10 mg/kg/day and higher dose treatment groups. Significant increased number of necrotic placentas was reported in 40 and 160 mg/kg/day group animals. The placental weights were decreased in females of 40 and 160 mg/kg/day groups. Therefore, the low dose 10 mg/kg/day produced the changes in external appearance of placentas and increased incidence of coarse grained placentas.

### Postimplantation Loss, Number of Fetuses

There was an increase in the mean postimplantation loss in females with viable fetuses at cesarean section in 10, 40 and 160 mg/kg/day treated groups but a statistical significant increase was found in 160 mg/kg/day group animals. The numbers of late resorptions were the consequence of the increased postimplantation loss. The mean number of fetuses was slightly decreased in the 160 mg/kg/day group.

### Sex/Weight of Fetuses:

BAY 59-7939 treatment affected the fetal growth and the fetuses from 40 and 160 mg/kg/day treatment group dams weighed significantly lesser than controls (lower range of sponsor's historical control data). Thus, the fetal growth was affected from 40 mg/kg (slightly) group and the NOAEL was 10 mg/kg/day.

### Fetal Malformations:

The treatment with the compound during organogenesis did not produce external and visceral deviations up to 160 mg/kg/day. The retardation of the vertebral ossifications and infusion of sternbrae (variation) was reported in 40 and 160 mg/kg/day groups. The total numbers of fetuses or litters with malformations was not increased in a dose related manner up to 40 mg/kg/day group. The incidences of fused caudal vertebral bodies at the 40 mg/kg dose were above the historical control data (up to 2.02 %). The incidences of major ventricular septal defect of the heart with/without enlarged pulmonary artery was found in 1 litter of 160 mg/kg/day group, that was 6.6 % and it was greater than the incidences of up to 1.85% in historical control data.

### Toxicokinetics:

Plasma concentrations of BAY 59-7939 were determined after oral administration to pregnant rabbits on day 6 and day 20 p.c. Blood samples were collected at 0.5, 1, 2, 4, 7 and 24 h after administration. The exposure on day 20 (means, n = 3) was as follows:

		Dose [mg/kg]			
		2.5	10	40	160*
AUC(0-24)	[mg·h/L]	0.736	2.78	13.1	23.9
AUC(0-24) <sub>norm</sub>	[kg·h/L]	0.294	0.278	0.329	0.150
C <sub>max</sub>	[mg/L]	0.142	0.294	0.881	1.54
C <sub>max, norm</sub>	[kg/L]	0.0568	0.0294	0.0220	0.00964
C(24)/C <sub>max</sub>	[%]	5.36	12.3	46.2	59.2
t <sub>max</sub>	[h]	0.794	2.00	1.00	1.00
R <sub>A1</sub>	[%]	79.1	66.4	68.7	n.c.
R <sub>A2</sub>	[%]	n.c.	328	283	n.c.
R <sub>A3</sub>	[%]	105	128	132	n.c.

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n.c. = not calculated

\* = data for this dose group only available from Day 20

R<sub>A1</sub> = C<sub>max, Day 20</sub> / C<sub>max, Day 6</sub>

R<sub>A2</sub> = C<sub>(24), Day 20</sub> / C<sub>(24), Day 6</sub>

R<sub>A3</sub> = AUC<sub>(0-24), Day 20</sub> / AUC<sub>(0-24), Day 6</sub>

On day 20 p.c., a linear increase in systemic exposure (AUC<sub>0-24hr</sub>) was seen in study animals. The maximum concentrations were observed around 0.5 to 2 h on day 20 p.c. Data from the 160 mg/kg/day dose group were only available on day 20 p.c. The plasma exposures were not considered because of limited data. The exposures of the animals at the identified no adverse effect dose of 2.5 mg/kg/day was 4 times the plasma exposure of the clinical dose.

In summary, orally administered BAY 59-7939 in pregnant rabbits during organogenesis produced a linear increase in rabbit plasma concentration (AUC<sub>0-24hr</sub>) in 0.5 to 2 h and the doses of 40 and 160 mg/kg/day were maternally lethal. No treatment related external and visceral deviations or malformations were seen

up to 160 mg/kg/day and BAY 59-7939 was not teratogenic in rabbits. Systemic maternal NOEL and intrauterine development safe dose was 2.5 mg/kg/day and the plasma exposures were 4 times the exposure of the human dose.

**D. Study for Effects on Pre- and Postnatal Development in Rats Including Maternal Function after Oral Administration** (Study No. : T9062957)

**Key study findings:** Wistar rats were treated BAY 59-7939 Coprecipitate 10 % 100 at oral gavage doses of 0, 2.5, 10, and 40 mg/kg/day in pregnant females produced overt pharmacologic effect of generalized tissue bleeding in 10 and 40 mg/kg/day treatment group animals and the 40 mg/kg/day dose was maternally lethal. Still births and empty stomach/intestines were reported in pups born to 10 and 40 mg/kg/day treated group dams. The other postnatal developmental adverse effects in pups were hypoactivity, pale skin and cold to touch surface. The NOAEL for dams (FO) and, pre- and postnatal development of the F1 generation was 10 mg/kg/day and based on body surface area it was 8 times the exposures of the proposed human dose.

**Study no.:** T9062957/PH34608

**Conducting laboratory and location:**

**Date of study initiation & completion:** January 14, 2004 & September 27, 2006

**GLP compliance:** The study was conducted in compliance with ICH guideline "Detection of Toxicity to Reproduction for Medicinal Products" (EU 1993, Japan MHLW 1994, US-FDA 1994).

**QA reports:** yes (X) no ( )

**Drug, lot #, and % purity:** #030723-100 BAY 59-7939 Coprecipitate 10 % 100

**Methods**

Doses: 0, 2.5, 10, and 40 mg/kg/day BAY 59-7939 Coprecipitate 10 % 100 formulated in 20 % Solutol HS 15 and 80 % demineralized water in addition with PEG 6000 (volume = 10 ml/kg)

Species/strain: SPF-bred Wistar rats (strain Hsd Cpb:WU)

Number/sex/group: **F0** - 25/inseminated females/group; **F1** - one male and one female per litter/study treatment group to test the fertility of the F1 generation

Route, formulation, volume: Oral gavage

Study design: The dams treated by oral gavage once daily on Day 6 pc through Day 20 pp with an aim to determine the effects of BAY 59-7939 on pregnancy, parturition, lactation and on pre- and postnatal survival and also on neurobehavioral growth and, the development of reproductive parameters of F1 and F2 generations. The females were allowed to deliver and rear their offspring up to day 21 p.p. During, at the end or after the rearing period the physical and functional development of the F1 pups was monitored. One F1 female/litter was reared up to maturity for reproductive and fertility testing.

**Postweaning Evaluations**

F1 Pups were tested for auditory startle reflex (postnatal day 27 to 31), passive avoidance test for learning/retention once between postnatal Day 40 and 50 using an automated system and, motor activity (postnatal day 54 to 61).

**Fertility, Reproductive performance and parturition observations of F1 rats:**

The estrous cycle of F1 females was determined from postnatal day 64 to 73 (beginning on postnatal day 73 to 80) until mated. Mated females (F1) were allowed to deliver their F2 litters. The dams were weighed on day 0, 7, 14 and 21 pc and day 1 and 7 pp and, checked frequently between 0800 and 1800 hours to record the date and time of parturition. Number of pups, the day parturition (pp day 0) were noted and pups counted and weighed on day 0, 7, 14 and 21 pc and day 1 and 7 pp. The test to determine 'stillborn'/'born alive' pup was performed. F1 females were euthanized on post natal 23 and uterine contents examined for implantations (stained with ammonium sulfide if apparently not pregnant), resorptions and live and dead fetuses were counted.

**F2 pup evaluations and termination:** The pups were examined by general observation, number, weights and sex determined. The pups were weighed on postnatal days 1 and post-partum day 7. The cause of death (still born/born dead) of F2 was determined and surviving F2 pups were euthanized by carbon dioxide asphyxiation on Day 7 pp and discarded.

**Results:**

**F<sub>0</sub> Dams:**

**General Observations:** The fertility index of study groups F0 dams up to 40 mg/kg/day was comparable to control and in the range of historical control data. But the rearing index of dams treated with 40 mg/kg/day group was decreased. The gross necropsy in one 40 mg/kg/day female revealed a cervix tightly filled with greenish muddy fluid and 14 dead fetuses in the uterus. An additional female of 40 mg/kg/day group showed a preterm delivery and all pups died. In 40 mg/kg/day group females, treatment related hypoactivity, high stepping gait, piloerection, cold skin, pale eyes, and salivation were noted. Increased incidences of light colored feces during gestation (0-21 p.c.) period were reported. During the lactation period, discolored feces were seen in 10 and 40 mg/kg/day groups.

**Mortality:** Seven of 25 females of 40 mg/kg/day group were sacrificed in moribund condition. Salivation, hypoactivity, high stepping gait, piloerection, cold skin, reddish vaginal discharge, preterm delivery (female no. 51, only), pale eyes, and salivation were observed. Gross necropsy of the dams showed a pale liver and spleen. All of the pups of these animals died.

**Body weight/Food consumption:** A 16.8% reduction in the body weight was reported in the females of 40 mg/kg/day treatment group during the treatment period between days 6-20 p.c. The body weight reduction was 20.6% during the

gestation period (days 0-20 p.c.). The food consumption was reduced significantly in animals of 40 mg/kg/day group as shown below in the table:

**Table 6-1: Mean Feed Intake of the F0 Females**

Dose [mg/kg bw/day]	0	2.5	10	40
Mean feed consumption during gestation [g/female/day]				
days 0 - 6 p.c.	21.4	21.0	21.5	21.0
days 6 - 11 p.c.	21.7	21.7	22.1	19.9*
days 11 - 16 p.c.	23.2	23.1	23.2	21.0**
days 16 - 20 p.c.	26.6	25.4	26.1	20.9**
Mean feed consumption during lactation [g/female/day]				
days 0 - 7 p.p.	42.4	40.5	41.6	40.1
days 7 - 14 p.p.	65.5	62.3	61.7	61.6

Statistically significant difference to control \* =  $p < 0.05$

Statistically significant difference to control \*\* =  $p < 0.01$

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**C-Section Observations of (F0) rats:** Placentas with greenish/yellowish borders were seen and dark red mass (clotted blood) most likely due to an impaired delivery in lung and heart was reported in pups born to 40 mg/kg/day treatment group animals. A treatment related enlarged spleen was noted in two females of the 10 mg/kg/day.

**Table 6-3: Fertility, Gestation, and Rearing Indices of the F0 Females**

Dose [mg/kg bw/day]	females used		Fertility index females with implantations	Gestation index females which delivered		Rearing index females which reared pups	
	n	n	% of those used	n	% of those with implantations	n	% of those which delivered
0	25	19	76.0	18	94.7	18	100.0
2.5	25	21	84.0	21	100.0	21	100.0
10	25	23	92.0	23	100.0	23	100.0
40	25	24	96.0	22	91.7	17	77.3

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The gestation index was similar in treated and control group animals but the rearing index was decreased at the 40 mg/kg/day group. Gestation period was unaffected by BAY 59-7939 but impaired delivery was noted in 2 of 24 females of the 40 mg/kg/day group. One of these animals was killed in moribund condition and the other animal had a preterm delivery and all pups died thereafter. The viability index (up to day 4 p.p.) was statistically significantly decreased in pups of 40 mg/kg doses. The lactation index (up to day 21 p.p.) was unaffected by treatment up to 40 mg/kg.

#### **F1 Generation Physical & Behavioral Development Evaluation:**

A statistically significant increase in pup mortality (including pups found dead, missing, sacrificed in moribund condition, and cannibalized) occurred at the 40 mg/kg level from days 0-4 p.p. Increased incidences of hypoactivity, pale skin, cold to touch surface in 40 mg/kg/day group pups was reported. An increased incidence of pale skin was also seen in pups of 10 mg/kg/day group. At necropsy on day 42 p.p. a treatment related pale liver and increased incidences of pups with no milk spots of 40 mg/kg group animals was reported. The reflex and behavioral tests showed adverse toxic effects on sucking reflexes of pups in 40 mg/kg/day group and no effect on sensory functions up to 40 mg/kg/day dose. The necropsy



data of F1 pups is shown in the following table (sponsor's table on page 121 of eTCD). On day 42 p.p., an increase in the incidences of pups with empty stomach and intestines at 40 mg/kg/day and, stillborn pups occurred at 10 mg/kg/day or higher dose.

T9062957		( F1 GENERATION ) SUMMARY OF PUP NECROPSY OBSERVATIONS				BAY 59-7939	
		0 MG/KG		2.5 MG/KG	10 MG/KG	40 MG/KG	
Litters Evaluated	N	18		21	23	21	
Pups Evaluated	N	167		195	199	168	
Live	N	167		195	199	168	
Stillborn	N	0		0	0	0	
LIVER							
LIVER PALE							
Pup Incidence	N	0 f		0	0	1	
	%	0.0		0.0	0.0	0.6	
	p-value	0.342					
Litter Incidence	N	0 f		0	0	1	
	%	0.0		0.0	0.0	4.8	
	p-value	0.393					
Affected Pups/Litter	MEAN%	0.00 k		0.00	0.00	0.68	
	S.D.	0.000		0.000	0.000	3.117	
	p-value	0.399					
LIVER BLACKISH DISCOLORED							
Pup Incidence	N	0 f		2	4	3	
	%	0.0		1.0	2.0	1.8	
	p-value	0.313					
Litter Incidence	N	0 f		1	2	1	
	%	0.0		4.8	8.7	4.8	
	p-value	0.645					
Affected Pups/Litter	MEAN%	0.00 k		0.79	1.93	2.04	
	S.D.	0.000		3.637	7.226	9.352	
	p-value	0.658					
STOMACH							
STOMACH EMPTY							
Pup Incidence	N	0 f		0	0	16**	
	%	0.0		0.0	0.0	9.5	
	p-value	0.000				0.000	
Litter Incidence	N	0 f		0	0	2	
	%	0.0		0.0	0.0	9.5	
	p-value	0.109					
Affected Pups/Litter	MEAN%	0.00 k		0.00	0.00	7.01	
	S.D.	0.000		0.000	0.000	22.866	
	p-value	0.113					
Statistical key: f=Fisher's Exact k=Kruskal-Wallis ** = p<0.01							

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The clinical findings of head tilted, respiratory sounds, piloerection, wound, hematoma, blackish discolored or missing tip of tail and/or bent tail, restricted motility of right forelimb) were observed in all treated group pups. One pup with tilted neck was also seen in the control group. Increased pup mortality occurred in 40 mg/kg/day treated dams in the study.

### F1 Generation Fertility & Reproductive Development Evaluation

The feed and water consumption, excretions, body weights of the F1 generation after weaning, and gross pathological findings showed no treatment related effects in F1 males and females up to 40 mg/kg/day dose. The insemination, fertility, and gestation indices of the F1 generation as well as time to insemination, number of implantation sites, duration of gestation, litter size (number of viable pups) and number of stillborn pups, sex ratio of F2 pups, clinical findings including

malformations, and body weights of the F2 pups were also unaffected by treatment with BAY 59-7939 at doses up to and including 40 mg/kg. But an increased incidence of stillborn F2 pups and a slightly increased incidence of pups with pale skin occurred at the 10 and 40 mg/kg/day group. Increased % mortality occurred among F2 of 40 mg/kg/day group from days 0-4 p.p. The other effects of increased incidences of pups with hypoactivity, pale skin, cold to touch surface, and not detectable milk spots were also seen in these pups.

The number of viable pups/litter were similar between the control and was decreased in 10 and 40 mg/kg/day groups due to the increased number of stillborn and deaths of pups in 40 mg/kg/day group. After litter reduction on day 4 p.p. a treatment related effect on litter size was not seen up to 40 mg/kg/day. The litter size of F1 generation is shown below:

**Table 6-8: Mean Litter Sizes of the F1 Generation**

Dose [mg/kg bw/day]	0	2.5	10	40
Day p.p.	Number of viable pups (mean values, male and female pups combined)			
0	12.39	11.71	10.91	11.00
4 before reduction	11.83	11.62	10.48	10.59
4 after reduction	7.94	7.67	7.52	7.53
7	7.94	7.67	7.52	7.47
14	7.94	7.57	7.48	7.41
21	7.94	7.52	7.39	7.41

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The lactation index (up to day 21 p.p.), sex ratio, mean body weight of F1 Pups during of rearing was similar in treated group animals compared to controls.

**Table 6-12: Physical Development of the F1 Pups**

Parameter of physical development	Days after birth	Dose [mg/kg bw/day]			
		0	2.5	10	40
Pinnae detachment	2.28	2.28	2.44	2.24	2.28
Development of fur	9.49	9.49	9.47	9.72	9.50
Incisor eruption	9.48	9.48	9.62	9.60	9.50
Eyes opened	16.32	16.32	16.40	16.50	16.10
Normal gait	16.61	16.61	16.63	16.93	16.37
Balanopreputial separation	46.0	46.0	46.0	46.7	46.1
Body weight at balanopreputial separation [g]	219.8	219.8	223.5	230.2	215.2
Vaginal opening	32.8	32.8	33.2	33.2	33.5
Body weight at vaginal opening [g]	103.5	103.5	106.9	107.9	106.5

### Reflex and Behavioral Tests on the F1 Pups Reflex Testing

The surface righting reflex, negative geotaxis, hearing test and pupillary reflex tests showed no relevant drug related aberration/change in these tests.

**Table 6-13: Reflex Tests of the F1 Pups**

	Dose [mg/kg bw/day]			
	0	2.5	10	40
Tests for reflexes and sensory functions				
Surface righting (days p.p.)	1.03	1.03	1.04	1.02
Negative geotaxis (days p.p.)	5.15	5.13	5.14	5.04
Hearing test (positive in %)	100.00	100.00	100.00	100.00
Pupillary reflex (positive in %)	100.00	100.00	100.00	100.00

### Motor Activity & Water-M-Maze Testing

The results of the motor activity testing with the F1 animals showed no treatment related difference between control and treatment group F2 animals. No treatment related adverse development motor effects were seen in F2 pups.

**Table 6-13: Reflex Tests of the F1 Pups**

	Dose [mg/kg bw/day]			
	0	2.5	10	40
Tests for reflexes and sensory functions				
Surface righting (days p.p.)	1.03	1.03	1.04	1.02
Negative geotaxis (days p.p.)	5.15	5.13	5.14	5.04
Hearing test (positive in %)	100.00	100.00	100.00	100.00
Pupillary reflex (positive in %)	100.00	100.00	100.00	100.00

### Gross Pathological Findings in the F1 Pups up to the End of Rearing

The gross pathological necropsy findings of the F1 pups up to day 42 p.p. were autolytic pups, reduced eye ball size or missing, spleen blackish discolored, liver yellowish or blackish discolored, stomach distended or tightly filled with feed paste, dilation of renal pelvis, contents of intestines blackish discolored, testes missing or reduced in size, abdominal cavity contains dark red to blackish mass, and shortening of skull bones after skeletal staining. Significantly increased incidences of pups with empty stomach and intestines were observed in animals included in the 40 mg/kg/day group.

### Pre- and postnatal Development

The gestation duration and index in the treated and control group was unaffected by 40 mg/kg/day BAY 59-7939. The rearing index was slightly decreased at the 40 mg/kg/day dose level. The parturition was affected in two females of the 40 mg/kg group which were sacrificed in moribund condition and these had impaired delivery. One female of 40 mg/kg/day group had a preterm delivery with all pups died. Increased incidences of pups without milk spots and stillborn pups and, an increased incidence of pups with pale skin occurred at the 10 mg/kg/day dose level, for which a treatment related effect cannot be excluded. Increased pup mortality occurred at the 40 mg/kg/day group from days 0-4 p.p. together with increased incidences of pups with hypoactivity, pale skin, cold to touch surface, and not detectable milk spots. The increased pup mortality was in a dose related manner during the study. There was decreased number of viable pups/litter in dams of 10 and 40 mg/kg/day groups. An increased number of stillborn pups were born to 40 mg/kg/day group dams. A treatment related increased incidence of empty stomach and intestines was noted in pups born to 40 mg/kg treated dams and these showed pale liver which suggested possible liver toxicity. Thus,

treatment increased the incidences of developmental adverse effect on sucking reflex in pups.

### Development of the F1 Generation after Weaning:

#### Appearance, Behavior, and Mortality:

One female and 1 male of 2.5 and 40 mg/kg/day groups were killed on day 108 p.p. and day 0 after delivery. Treatment related effects on feed and water consumption were not evident in males and females of the F1 generation up to 40 mg/kg/day group. Fecal and urine excretion were not affected by the compound.

#### Gross Pathological Findings

One F1 male had a left testis lying in a pocket of the abdominal wall, and an additional F1 male of the 40 mg/kg dose group had dilation of the right renal pelvis. The incidence was within the historical data sent by sponsor. Thus, no treatment related gross pathological findings occurred in the F1 males and females. No treatment related change in the mean values for feed consumption during pre-mating and gestation (females, only) period was seen.

#### Fertility Testing of F1 Generation (Insemination, Fertility, and Gestation Indices):

The insemination index [% = Number of females inseminated x 100 ÷ Number of females paired] were similar in the treated and control group animals and shown below.

**Table 6-15: Insemination, Fertility, and Gestation Indices of the F1 Females**

Dose [mg/kg bw/day]	Used [n]	Inseminated [n]	% of those mated	Number of F1 Females			
				with I.S. [n]	% of those inseminated	which delivered [n]	% of those with I.S. / used
0	24	23	95.8	19	82.6	19	100.0
2.5	24	24	100.0	23	95.8	22	95.7
10	24	23	95.8	21	91.3	21	100.0
40	24	24	100.0	23	95.8	23	100.0

I.S. = implantation sites

The insemination, fertility and gestation days were not affected in F1 generation rats. The time of insemination in the pups of treated dams showed that the insemination rate and other fertility data in treated and control dams were similar.

**Table 6-17: Mean Values of the Parameters of Intrauterine Development of the F1 Females, Weights and Sex ratio of F2 Pups**

Dose [mg/kg bw/day]	0	2.5	10	40
		Mean value per female		
Number of implantation sites	12.05	13.14	13.00	12.48
Duration of gestation [days]	22.06	21.95	22.05	22.05
Number of living pups	11.05	12.57	11.76	11.91
Pups stillborn [% per group]	1.9	1.5	0.8	1.8
Pups found dead [N]	0	0	2 (1)	0
Weight of pups [g]	6.05	5.93	6.07	6.07
Sex ratio [%]	50.83	51.19	53.99	47.25
male pups : total pups				

(1) number of litters affected

The number of implantations, duration of gestation, # of living and dead F2 fetuses (litter size), and sex-ratio of F2 fetuses of the treated groups were not affected by BAY 59-7939 treatment. There were no changes in clinical findings of F2 pups. The body weight/growth of F2 fetuses was similar in control and treated groups. Other intrauterine development parameters are given in table above.

In conclusion, BAY 59-7939 Coprecipitate 10 % 100 treatment in pregnant dams produced maternal preterm delivery and, increased incidences of still births, empty stomach and intestines in pups of 10 and 40 mg/kg/day groups treated dams. The developmental adverse effects of hypoactivity, pale skin, cold to touch surface, deficient sucking developmental reflex (no detectable milk spots in pups) were reported in F1 pups of dams of 10 and 40 mg/kg/day groups. The identified NOEL for the F1 generation was 2.5 mg/kg/day. The NOAEL for dams (FO) and, pre- and postnatal development of the F1 generation was 10 mg/kg/day and based on body surface area it was 8 times the exposures of the proposed human dose.

## 2. 6.6.6 SPECIAL TOXICOLOGY:

### 1. BAY 59-7939 Nanosuspension 2% (w/v) (IFT 163 Nanosuspension 2% (w/v) Subacute Toxicity Study in Wistar Rats (2 Weeks Administration by Intravenous Administration): Study Number: T7076284/PH34646

Name of the Conducting Laboratory: Bayer HealthCare AG, 42096 Wuppertal (Germany)

Study initiation & completion dates: November 24, 2005 & October 19, 2006

GLP Compliance: A statement that the study was conducted in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 (ENV/MC/CHEM(98) 17) and with the revised German Principles of Good Laboratory Practice (according to Annex I German Chemicals Act, Bundesgesetzblatt Part I, No. 40, issued June 27,2002) was enclosed.

Test Substance: BAY 59-7939 Nanosuspension 2% (w/v)/ [IFT 163 Nanosuspension 2% (w/v)]; batch # - BXOIGFL

Synonym used: BAY 59-7939 SUSP/Active ingredient: BAY 59-7939 (IFT 163)  
Manufacturer of nanosuspension: (b) (4)

Methods: The study was conducted in 5 male and female rats (strain Wistar Hsd Cpb:W) to determine the possible local and systemic toxic effects of iv dosing in rats. The study aim was also to support a total bioavailability of the clinical dose formulation in man (i.e., 20 mg/ml) and, to assess the local tolerance and possible systemic toxic effects associated with repeated intravenous exposure of rats to the anticipated clinical formulation. The animals were treated with either 0 (0.9% aqueous sodium chloride solution; dose volume 5 ml/kg), at dose volumes of 0.5, 1.5 or 5 ml/kg (0, 10, 30 and 100 mg/kg/day) in new formulation vehicle respectively for 14 days. Blood samples for the determination of hematology and clinical chemistry parameters were collected on day 11 of the study from all non-fasted animals of the main groups. The animals in each of satellite groups (6/sex), BAY 59-7939 SUSP was administered at dose volumes similar to the main study group animals. Three of the animals/sex/group were treated with 5 ml/kg/day (100 mg/kg/day BAY 59-7939) of 0.9% aqueous NaCl solution. Blood samples were collected 5 min., 0.5, 2, 4, 7 and 24 hours after the dosing (dosed groups) or 0.5 hours after administration (0.9% NaCl group).

Table 8-1

main groups			satellite groups (for kinetics)			
dose (ml/kg bw)	group no.	no. of animals	animal no.	group no.	no. of animals	animal no.
Males						
5.0*	1	5	1-5	9	3	41-43
0.5	2	5	11-15	10	6	47-52
1.5	3	5	21-25	11	6	59-64
5.0	4	5	31-35	12	6	71-76
Females						
5.0*	5	5	6-10	13	3	44-46
0.5	6	5	16-20	14	6	53-58
1.5	7	5	26-30	15	6	65-70
5.0	8	5	36-40	16	6	77-82

\* 0.9% aqueous NaCl solution

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## Results:

1. General Observations: Severe morbidity (piloerection, paleness, hypothermia, partly hypoactivity, alteration of gait and/or continuous bleeding from the injection site) resulting in preterm sacrifice of 2 males of the main group and one female of the satellite group. The bleeding was dose related and one male of the 10 mg/kg/day satellite group died before drawing the last blood sample at 24 hr. Piloerection, paleness, hypoactivity, and hypothermia were as well observed in one female of 100 mg/kg/day group surviving up to term. Furthermore, local effects of blue discoloration at injection site of tail were observed at all dose levels tested.

2. Body weight/Food consumption: Reduced feed intake and increased water consumption were observed in males of the 100 mg/kg/day dose group and in females of the 30 mg/kg/day and 100 mg/kg/day dose groups. Transient body

weight loss occurred in both gender after start of treatment at a dose level of 1.5 ml/kg/day and above; in males related to impaired body weight gain.

**Table 9-1**

Mean daily feed consumption								
Dose [ml/kg]	5 <sup>a</sup>	0.5	1.5	5	5 <sup>a</sup>	0.5	1.5	5
	g / animal / day				g/kg body weight / day			
Males								
Week								
1	20.0	18.3	18.1	16.4**	121.5	116.9	117.6	111.3
2	20.7	19.7	20.3	20.9	102.5	100.3	105.6	111.3
Females								
1	18.2	16.5	14.8*	12.7**	133.7	128.5	121.8	104.5**
2	17.9	17.3	16.4	15.9	113.2	115.6	113.7	111.1

<sup>a</sup> 0.9% aqueous NaCl solution

\* statistically significantly different with  $p < 0.05$

\*\* statistically significantly different with  $p < 0.01$

### 3. Hematology:

A dose related increase was observed in number of reticulocytes (48, 68, 81 and 118/dl in males and, 40, 60, 73 and 203 in females of 0, 20, 30 and 100 mg/kg/day, respectively). A clinically insignificant increase in monocytes of 100 mg/kg/day group animals of both genders and, decrease in number of thrombocytes in males treated with mid dose (30 mg/kg/day) was observed. The prolongation of blood coagulation time in females and males and, decrease in hemoglobin and hematocrit in both genders (due to bleeding) of 100 mg/kg/day treatment group animals was reported.

### 4. Clinical Chemistry

No significant changes in blood plasma enzymes were observed in study animals. A treatment- related slight insignificant decrease in potassium concentration in males of the 100 mg/kg/day was reported and was not seen in females.

### 5. Necropsy Findings:

On necropsy at termination, pale discoloration of liver in males of 10 mg/kg/day and, 30 and 100 mg/kg/day females was observed. Swelling or enlargement of spleen in both sexes was noted and the incidences were 0, 0, 1 and 2 in males and, 1, 3, 4 and 2 females included in 0, 10, 30 and 100 mg/kg/day treatment groups, respectively.

### 6. Organ Weight:

A dose related increase in spleen weights was seen, i. e., these were 524, 797, 916 and 1167 mg in males and, 498, 633, 743 and 986 mg in females of 0, 10, 30 and 300 mg/kg/day groups, respectively. The spleen weights could be related to histopathological findings of increased hematopoiesis and hence secondary to treatment-related hemorrhages.

### 7. Histopathology:

Reduced sperm counts were reported in epididymides of animals treated with 30 and 100 mg/kg/day. Increased number of germinal centers, increased lymphocytolysis, increased hematopoiesis, and vacuolated or foamy macrophages in spleen were seen in animals of 10, 30 and 100 mg/kg/day treatment groups. The incidences are shown in the table below (part of the table):

PATHOLOGY REPORT :  
SUMMARY TABLES

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TEST ARTICLE : BAY 59-7939 SUSP  
TEST SYSTEM : RAT, SUBACUTE, INTRAVENOUS  
SPONSOR : BAYER HEALTHCARE AG

PATHOL. NO.: 06984 HAR  
DATE : 16-OCT-06  
PathData® System V5.1c

---

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX						
Necropsy Status: TERMINAL SACRIFICE GROUP (R0), Incl. Deaths						
Sex		Females				
Dose Group		01	02	03	04	
No. Animals per Dose Group		5	5	5	5	
SPLEEN		No. Examined	5	5	5	5
- Increased germinal centers/lymphocytolysis			-	5	5	5
	Grade 1		-	3	1	-
	Grade 2		-	2	3	-
	Grade 3		-	-	1	5
- Hematopoiesis			5	5	5	5
	Grade 1		2	3	-	-
	Grade 2		3	2	4	-
	Grade 3		-	-	1	5
- Vacuolation			-	-	-	4
	Grade 1		-	-	-	4
APPL SITE TAIL		No. Examined	5	5	5	5
- Perivascular inflammation			5	5	5	5
	Grade 1		3	1	1	-
	Grade 2		2	2	2	2
	Grade 3		-	2	2	3

NAD = Nothing abnormal discovered

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The increased and intense hematopoiesis and the increased number of germinal centers/lymphocytolysis megacaryocytes could be compensatory to the hemorrhages. The perivascular inflammation at the injection site (tail) was dose related and was intense in rats of high dose.

#### 7. Toxicokinetics:

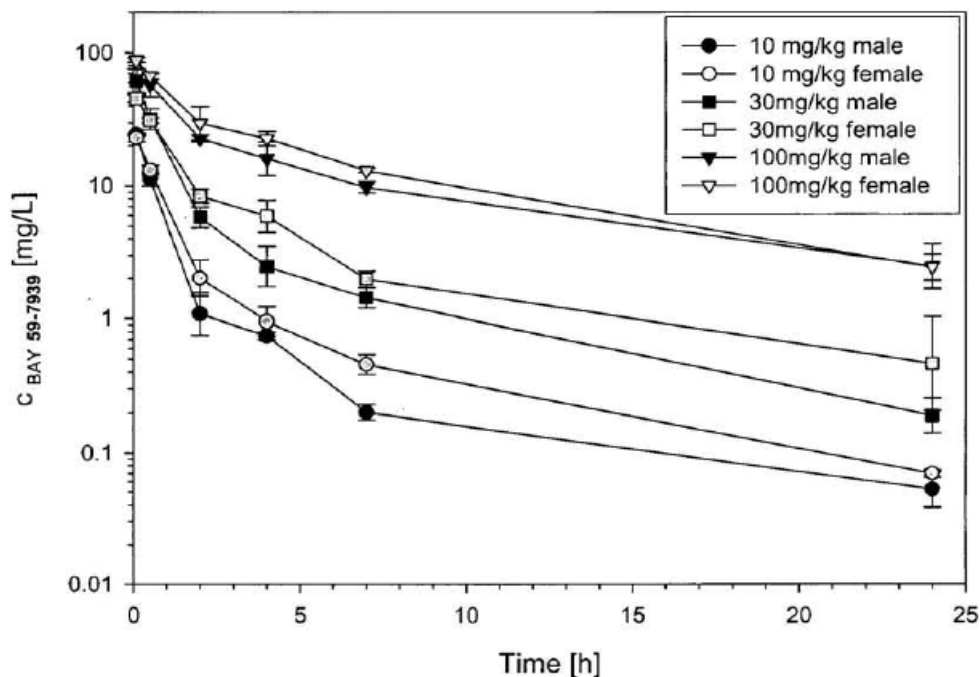
The plasma concentrations in male and female animals increased linearly in non-dose proportional manner and the exposure in females was slightly higher than in males. On Day 11, exposure AUC<sub>0-24hr</sub> was increased in non-dose proportional manner. A comparison of AUC<sub>0-24hr</sub> values from Day 1 and Day 11 showed a decrease in females of 30 and 100 mg/kg/day groups. The data is shown below in sponsor's short summary Table 7 and figure 1.



	Gender	male				female	
	Dose [mg/kg]	10*	30	100	10	30	100
AUC(0-24)	[mg·h/L]	20.7	71.2	260	26.6	88.5	320
AUC(0-24) <sub>norm</sub>	[kg·h/L]	2.07	2.37	2.60	2.66	2.95	3.20
AUC(0-t <sub>i</sub> )	[%]	10.4	7.53	2.66	7.41	4.26	2.20
C <sub>max</sub>	[mg/L]	24.4	61.7	88.3	22.9	45.6	87.9
C <sub>max, norm</sub>	[kg/L]	2.44	2.06	0.883	2.29	1.52	0.879
C(24)/C <sub>max</sub>	[%]	0.215	0.309	2.81	0.304	1.02	2.77
t <sub>max</sub>	[h]	0.083	0.083	0.083	0.083	0.083	0.083
R <sub>A1</sub>	[%]	73.2	104	69.1	47.2	61.6	69.7
R <sub>A3</sub>	[%]	81.2	102	116	86.4	70.8	66.2

$$\begin{aligned} R_{A1} &= C_{\max, \text{Day 11}} / C_{\max, \text{Day 1}} \\ R_{A3} &= \text{AUC}(0-24)_{\text{Day 11}} / \text{AUC}(0-24)_{\text{Day 1}} \\ * &= n=2/3 \end{aligned}$$

**Figure 1:** Plasma concentrations vs. time curve of BAY 59-7939 on Day 11.



A linear but non-dose proportional increase in males and females was achieved after intravenous doses of BAY 59-7939 Nanosuspension 2% (w/v) on the first and on day 11. The increased intense hematopoiesis and the number of germinal centers/ lymphocytolysis megacaryocytes were considered as compensatory to the hemorrhages. A dose related perivascular inflammation at the injection site (tail) indicated that the compound was vaso-irritant in rats.

### **Local Irritation Study**

#### **2. BAY 59-7939: Local Tolerability Study in Beagle Dogs after Paravasal, Intravenous and Intra-arterial Injections.**

(Study No. T4073400/AT01346/PH33414)

Sponsor: Bayer Healthcare AG, 42096 Wuppertal, Germany

Starting & completion dates: September 13, 2003 & November 18, 2003

Six groups of 1/sex Beagle dog were treated with BAY 59-7939 according to the following design:

Group I - paravascular injection: 5 ml BAY 59-7939 near right vena cephalica antebrachii, 5 ml Saline near left vena cephalica antebrachii; number of injections: one with a follow-up period: 6 days

Group II - intraarterial injection: 5 ml BAY 59-7939 into right Arteria femoralis; 5 ml saline into left arteria femoralis. Number of injections: one. Follow-up period: 6 days.

Group III - intravenous infusion: 75 ml Saline into right vena cephalica antebrachii; Number of infusions: 10; Follow-up period: 6 days

Group IV - intravenous infusion: 75 ml BAY 59-7939 into right vena cephalica antebrachii; Number of infusions: 10; Follow-up period: 6 days

Group V - intravenous infusion: 75 ml Saline into right Vena cephalica antebrachii; Number of infusions: one; Follow-up period: 6 days

Group VI - intravenous infusion: 75 ml BAY 59-7939 into right Vena cephalica antebrachii; Number of infusions: one; Follow-up period: 6 days

Injection volume used: BAY 59-7939 = 0.0657 mg/ml.

In group I and II animals, the dose of 0.33 mg/animal of BAY 59-7939 was administered. In group IV and VI the total amount given by short term infusion refers to 4.93 mg/animal per day.

Table 9-1: Group allocation (week -1) including administration days and sites

Group	Animal No.	Sex	Body weight [kg]	Administration sites									
				Study day									
				1	2	3	4	5	6	7	8	9	10
I	K 161	♂	10.9	-	-	-	-	-	-	-	-	-	LF-NaCL
				-	-	-	-	-	-	-	-	-	RF-Verum
	K 146	♀	9.5	-	-	-	-	-	-	-	-	-	LF-NaCL
				-	-	-	-	-	-	-	-	-	RF-Verum
II	J 889	♂	14.9	-	-	-	-	-	-	-	-	-	LA-NaCL
				-	-	-	-	-	-	-	-	-	RA-Verum
	J 874	♀	11.7	-	-	-	-	-	-	-	-	-	LA-NaCL
				-	-	-	-	-	-	-	-	-	RA-Verum
III	K 171	♂	12.4	R	R	R	R	R	R	R	R	R	R-NaCL
	K 148	♀	10.0	R	R	R	R	R	R	R	R	R	R-NaCL
IV	K 181	♂	11.3	R	R	R	R	R	R	R	R	R	R-Verum
	K 162	♀	11.1	R	R	R	R	R	R	R	R	R	R-Verum
V	J 875	♂	12.0	-	-	-	-	-	-	-	-	-	R-NaCL
	J 852	♀	9.2	-	-	-	-	-	-	-	-	-	R-NaCL
VI	J 879	♂	12.5	-	-	-	-	-	-	-	-	-	R-Verum
	J 866	♀	10.6	-	-	-	-	-	-	-	-	-	R-Verum

Body weight: week -2

LF = left forelimb  
 RF = right forelimb  
 LA = left Arteria femoralis  
 RA = right Arteria femoralis  
 R = right Vena cephalica antebrachii  
 - = no treatment  
 ♂ = male  
 ♀ = female  
 Verum = BAY 59-7939

Necropsy and Histopathology: All animals were sacrificed under deep barbiturate anesthesia. Systematic gross examination of each animal was performed and general physical condition noted and, body orifices, external and internal organs and tissues were examined. Organs and tissues collected were fixed in 10 % neutral buffered formalin or Davidson's solution. Bone marrow smears and liver were stained according to the method of May-Gruenwald and with Oil-Red-O, respectively but not evaluated.

Table:

Adrenal glands	Pharynx
Aorta	Pituitary Gland
Brain (cerebrum, cerebellum, brain stem, medulla oblongata*)	Parotis
Epididymides	Prostate
Esophagus	Sciatic nerve
Eyes	Skeletal muscle (thigh)
Femur	Skin (mammary region)
Heart (Papillary muscles, Remaining heart tissue*)	Spleen
Intestine	Sternum
- Duodenum	Stomach
- Jejunum	Sublingual Gland
- Ileum	Testes
- Cecum	Thymus
- Colon	Thyroid glands
- Rectum	(with parathyroid glands)
Kidneys**	Tongue
Larynx	Tonsils
Liver**/Gallbladder	Trachea
Lungs**	Urinary Bladder/Ureters
Lymph nodes, mandibular	Uterus (with cervix)
Lymph nodes, mesenteric	Vagina
Mandibular gland	Organs and tissues with macroscopic findings
Nose (nasal cavity)	Physical identifier*
Optic nerves	Veins/Arteries
Ovaries/Oviducts	V.ceph.ant. right+left
Pancreas	or A.femoralis right+left

\* fixation in 10 % neutral buffered formalin

\*\* additional specimen fixed in 10 % neutral buffered formalin

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## Results

General Observations: None of the animals died and no changes in clinical observations were seen. No adverse effects including irritation attributable to the treatment with the test compound were observed at the injection sites. Body weights and food consumption were not affected in the treated animals. The free hemoglobin and plasma potassium were not altered thus showing BAY 59-7939 may not produce hemolysis. Potassium values are not influenced.

### Histopathology Changes:

#### Paravascular Injection / V. cephalica antebrachii:

1. Focal inflammatory infiltrations of the skin were observed at the saline and the BAY 59-7939 application site and no differences of reactions between the saline treated left vein and the BAY 59-7939 treated right vein were observed.

2. Intraarterial Injection / A. femoralis: A slight periarterial inflammation was seen after saline injection and, a slight hemorrhage and fibrin exsudation at the right A. femoralis vein in male dog treated with BAY 59-7939 injection showed the treatment with the compound produced inflammation in the artery.

3. Intravenous Infusion (10x1 / V. cephalica antebrachii: The infusion containing the compound produced a slight epidermal ulceration in the treated right vein (dog no. 8) and only focal inflammatory infiltration of the skin was found in the left V. cephalica in control.

4. Intravenous Infusion (Ix) / V. cephalica antebrachii:

No differences concerning local tolerance were also seen after single intravenous infusion of either placebo or BAY 59-7939.

In this local tolerance study of BAY 59-7939 solution, a slight hemorrhage and fibrin exudation at the right A. femoralis was seen after intra-arterial dose. A slight epidermal ulceration in the treated right vein (dog no. 8) after an IV injection and an intravenous infusion (Ix) / V. cephalica antebrachii of BAY 59-7939 produced no reaction at the local site.

3. **In vitro 3T3 NRU (neutral red uptake) phototoxicity assay**

Study No. T9075016/AT02074/ PH-33880

Name of the Conducting Laboratory: Bayer HealthCare AG, 42096 Wuppertal (Germany)

Study initiation & completion dates: March 21, 2005 & May 25, 2005

GLP Compliance: A statement that the study was conducted in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 (ENV/MC/CHEM(98) 17) and with the revised German Principles of Good Laboratory Practice (according to Annex I German Chemicals Act, Bundesgesetzblatt Part I, No. 40, issued June 27, 2002) was enclosed.

Test Substance:

a. BAY 59-7939 – Batch #BXOINB2, 99.9% used at the concentrations of 0.1 ug/ml, 1 ug/ml, 3 ug/ml, 10 ug/ml, 30 ug/ml, 50 ug/ml, 100 ug/ml and 250 ug/ml.

b. Positive control: chlorpromazine hydrochloride

Test System: Permanent cell line mouse fibroblasts (Balb/c 3T3.A31; (b) (4)) were propagated in Dulbecco's Modified Eagle's Medium supplemented with 5% FBS (Fetal Bovine Serum), 5 % NCS (Newborn Calf Serum), 1 % glutamine and 1% penicillin/streptomycin. The cells were cultivated in a humidified incubator at 37°C and 5% CO<sub>2</sub>.

Methods:

The stock solution (100 mg/ml) of the test substance prepared in DMSO was diluted in PBS at concentrations from 0.1 - 250 ug/ml. The cell count was done with a cell counter; cells were seeded in 96-wells microtiter plates in a concentration of  $3 \times 10^4$  cells/well for 24h. The cells were treated with either test drug, positive control and, irradiated with a UV-lamp equipped with a H1 filter (b) (4) with an intensity of 1.7 mw/cm<sup>2</sup> (5 J/cm<sup>2</sup>) for 50 min. The irradiated and non-irradiated plates washed to remove the compound and cells were incubated for the next 24h and viability determined by neutral red uptake (viable cells). Each assay was performed three times in triplicate and the solutions were protected from light. For the calculation of a

phototoxic potential a formula of Photo-irritation-factor (PIF) was used (OECD draft TG 432, 15 March 2002):

$PIF = EC_{50}(-UV)/EC_{50}(+UV)$ . It was the ratio of the  $EC_{50}$  values for cytotoxicity without and after the UVA-irradiation. Based on the validation study (Spielmann et al. 1998), a test substance with a  $PE < 2$  predicts "no phototoxicity", a  $PIF > 2$  and  $< 5$  predicts "probable phototoxicity", and a  $PIF \geq 5$  predicts "phototoxicity".

#### Results:

In the prescreening the highest concentration of 100 ug/ml and 250 ug/ml showed slight precipitation. Chlorpromazine hydrochloride (from 0.01 - 100 pg/ml) was used as a positive control.

After treatment of cells with BAY 59-7939 the  $EC_{50}$  value was  $> 250$  ug/ml for control cells and for UVA - irradiated cells. BAY 59-7939 was non-phototoxic with  $PIF = 1$ . Chlorpromazine hydrochloride estimated PIF was 13 as shown below.

**Tab. 6-1. Determination of phototoxic potential in mouse fibroblasts (3T3.A31 cells) measured after UVA-irradiation by cell viability (neutral red uptake): PIF was determined from extrapolated  $EC_{50}$  values.**

	Phototoxicity ( $EC_{50}$ $\mu$ g/ml)		PIF
	UVA-irradiation 0 min.	UVA-irradiation 50 min.	
<b>BAY 59-7939</b>	<b>250</b>	<b>250</b>	<b>1</b>
<b>Chlorpromazine Hydrochloride</b>	<b>75</b>	<b>6</b>	<b>13</b>

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BAY 59-7939 solution treatment produced slight hemorrhage and fibrin exudation at the right A. femoralis was seen after intra-arterial dose and epidermal ulceration was seen after an IV injection. An intravenous infusion (Ix) / V. cephalica antebrachii of BAY 59-7939 produced no reaction at the local site. BAY 59-7939 Nanosuspension 2% (w/v) produced a perivascular inflammation at the injection site and the compound was vaso-irritant in rats.

#### **2.6.6.7 Labeling:**

The proposed label of sponsor will be reviewed separately.

#### **2.6.7 DETAILED CONCLUSIONS AND RECOMMENDATIONS:**

BAY 59-7939 an S-enantiomer and directly acting specific competitive oral inhibitor of Factor Xa, was demonstrated to inhibit the thrombus formation in a concentration dependent manner in vitro and, in rats, dogs, rabbits and human (Ki

of  $0.5 \pm 0.02$  nM for human FXa activity). Its  $IC_{50}$  for thrombin generation was 2.1 nM and no significant platelet aggregation was seen by collagen, ADP or thrombin receptor-activated peptide (TRAP-6). BAY 59-7939 inhibited thrombus formation in arteriovenous shunt model in rats and rabbits and, injury induced venous thrombus formation in rats. BAY 59-7939 had insignificant secondary effects on the cardiovascular function, EKG and respiration in anesthetized beagle dogs. It had no effects on the central nervous system, normal behavior, renal function, hematology and gastrointestinal motility in rats at oral doses of up to 30 mg/kg. Based on these effects, sponsor has developed the compound for its use in the prevention of arterial and venous thrombosis in man.

In support of the present application, sponsor submitted preclinical pharmacology; pharmacokinetics; acute toxicity studies in rats and mice; 2-week iv study in rats, 4-week oral subacute toxicity studies in mice, rats and dogs; 13-week oral gavage/feeding studies in mice, rats and dogs, 26-week chronic toxicity study in rats, 52-week chronic toxicity study in dogs, reproductive and developmental toxicity studies on the fertility & early developmental toxicity study in rats, embryo-fetal development in rats and rabbits and, ore-and post-natal development toxicity study in rats, mutagenicity studies of the Ames assay, chromosome aberrations assay in Chinese hamster lung cells and *in vivo* mouse bone marrow micronucleus assay were submitted.

An oral dose of 3 mg/kg in rats produced an AUC of 6.03 mg.h/ml, an absolute oral bioavailability of 60%, and  $T_{1/2}$  values from 1.2 to 2.3 hours (oral) and 0.9 hr (IV) in rat.  $T_{1/2}$  values of the radioactivity were prolonged (18.8 and 42.1 hrs by the IV and oral routes, respectively) suggesting the tagging of the radioactivity to the metabolites for a prolonged period. Plasma concentration at the steady state (AUC) of IV dose of 0.3 mg/kg was 1.0 mg.h/ml and  $t_{1/2}$  of 0.95 hour. BAY 59-7939 was seen to bind up to 97.1%, 99.1%, 93.4%, 71.8% and 96.1% to plasma proteins from human, rats, dogs, rabbits and mice, respectively. The radioactivity was distributed heterogeneously in different organs and tissues of rats and showing no affinity for any specific tissues/organs. The transport (efflux) of the compound in Caco-2 cell was inhibited by P-gp inhibitor Ivermectin hinting the involvement of P-gp and the bidirectional permeability across P-gp. The efflux ratios of BAY 59-7939 were (molar concentration) 15.9 (0.5  $\mu$ M), 13.1 (1  $\mu$ M), 9.78 (10  $\mu$ M) and 10.6 (100  $\mu$ M) indicating a partial blocking by P-gp inhibitors (Ivermectin and LY 335979). In vitro studies in human and rat hepatocyte cultures, M-1 was the major metabolite in the rat urine, bile and feces. It was catalyzed by CYP3A4 to its morpholino compound. Orally administered radioactivity was excreted by 24.7% and 66.7% in rat urine and feces, respectively, in 7 days and about 0.2% of the administered radioactivity remained unaccounted. In man, BAY 59-7939 after an oral dose of 10 mg/kg [ $^{14}$ C]BAY 59-7939 was excreted by 36% as an unchanged compound in urine. The four hydroxylated metabolites, M-2, M-7, M-8 and M-9 were confirmed in man and dogs. In man, M-5 and M-7 minor metabolites were absent.

Single oral dose tolerance study was performed in mice and rat and, an acute intravenous tolerance study in mice was done. In acute oral studies, a single dose of 500 mg/kg in rats and mice did not produce any treatment-related clinical signs or macroscopic abnormalities and none of the animals died. In IV dose study, only a dose was 25 mg/kg was used and it was not lethal although treatment related adverse effects of decreased motility, abdominal position, labored breathing, narrowed palpebral and piloerection were seen. The minimum lethal oral or intravenous doses in rats and mice were not determined.

The 4-week oral gavage treatment with BAY 59-7939 in CD-1 mice at the doses of 0, 12.5, 50 and 200 mg/kg/day (as PEG-6000 co-precipitate in solutol/water, 20/80 v/v) produced no treatment related changes in the body weight gains, hematology or enzyme contents in any of the animals of treatment groups. The histopathology of the tissues was not done during the study and the MTD was not identified in this study.

BAY 59-7939 (as PEG-6000 co-precipitate in solutol/water, 20/80 v/v) at the oral gavage doses of 0, 50, 100 and 200 mg/kg/day in CD-1 mice for 13 weeks produced a linear non-dose proportional plasma concentration. The adverse effects of higher incidences of fibrosis of the heart, mononuclear cell infiltration in the kidneys, hyperplastic spindle cells in the adrenal, and increased cellularity of marginal zones of the spleen were seen in males of high dose group. Females treated with the high dose showed increased incidences of Kupffer cell foci in the liver and mononuclear cell infiltration in the kidneys. The kidneys and liver were the target organs of toxicity in both sexes and, adrenal and spleen were the additional targets in males. A dose of 100 mg/kg/day was identified as the MTD in this provided plasma exposure of 20 and 29.5 times the exposure of the clinical dose in male and female animals, respectively.

In the 13-week oral dietary toxicity study in CD-1 mice, BAY 59-7939 was given at 0 (diet only), 0 (diet plus PEG), 1250, 2500 and 5000 ppm concentrations in dietary admixture (10% PEG-6000 coprecipitate) in 5 groups of mice. The mean drug intake with the diet for low, mid and high doses were 237, 476 and 1007 mg/kg/day for males, and 237, 476 and 1007 mg/kg/day for females. Dose dependent increase in coagulation time (HQUICK) in males and females and, increase in liver enzymes were seen in treatment related manner in males and females. Increased incidences of focal tubular hypertrophy of the kidney (PEG control, 1 of 10; high dose group 3 of 10) suggested the kidneys as target organ of toxicity in males. In female mice, focal necrosis in liver indicated the liver as the target organ of toxicity. Based on these findings, the high dose appears to be the MTD. The plasma exposure levels (AUC<sub>0-24h</sub>) at low, mid and high doses in male and female mice were 14.4, 21.3 and 31.3 mg.h/L, and 20.1, 31.8 and 43.0 mg.h/L, respectively. 13-week oral dietary toxicity study in CD-1 mice performed at 0 (diet only), 0 (diet plus PEG), 1250, 2500 and 5000 ppm concentrations of the compound caused dose dependent increase in coagulation time and, increase in liver enzymes in males and females. Increased incidences of focal tubular



hypertrophy of the kidney in males and focal hepatic necrosis in female suggested the kidney and liver as the target organs of toxicity in males and females. The high dose was identified as an MTD. The plasma exposure levels in male and female mice at the high dose were about 1.2 and 1.7 times the human exposures at the proposed clinical dose of 10 mg/day.

The 13-week oral gavage toxicity study in CD-1 mice performed by using micronized form of BAY 59-7939 at 0, 60, 300 and 1500 mg/kg/day (in ethanol/Solutol/tap water) doses demonstrated that there was a non-dose proportional increase in plasma concentration of the compound and increase in coagulation times in males and females. The plasma concentration increase seen in 60 mg/kg/day group was erratic and non-dose proportional or not treatment related in other groups. The target organs of toxicity and MTD were not identified.

In 14-day intravenous toxicity study in rats, the doses of 0.0657, 0.1971 and 0.657 mg/kg/day BAY 59-7939 produced a dose proportional increase in plasma concentration and dose related reaction of foamy macrophages in lung parenchyma (enlarged cytoplasm with eosinophilic granules), swelling/vacuolation of proximal convoluted renal tubules and, extramedullary hematopoiesis of spleen. A dose of 0.1971 mg/kg/day was considered as a 'maximal tolerable dose' and lungs, kidneys and spleen were identified as the target organs of toxicity in this study.

In 4-week oral gavage toxicity study conducted at doses of 0, 12.5, 50 and 200 mg/kg/day, 12% decrease in body weight of high dose males and, treatment related increase in several liver enzyme activities in treated animals were noted. The insignificant decrease in CD45<sub>total</sub>, and slight increase in IgA levels females and IgG levels in males were of not clinical significant. The plasma exposure of animals treated with high dose was 162 times the exposure achieved in man treated with a proposed clinical dose. A slight decrease in the body weight and treatment related increase in several liver enzyme activities in males was not associated with histopathological changes in the liver. The target organs of toxicity were not identified and 200 mg/kg/day dose was the maximum tolerated dose.

In the 13-week oral gavage toxicity study with 4 weeks recovery period in rats, BAY 59-7939 was administered at oral doses of 12.5, 50 and 200 mg/kg/day. A slight increase in ALT and decrease of GLDH and LDH enzymes were seen in high dose group animals during the study and, slightly insignificant high ALT levels, and low GLDH and LDH levels were still seen in recovery group. The increase in the plaque cell counts at high dose treated animals, a slight increase IgG in males of all treatment groups and no consistent effect in females was seen. The IgA and IgM antibodies were increased in the males and females of high dose group. The highest tolerable dose was 200 mg/kg/day (provides 162 multiples exposure of the clinical dose).

In another 13-week study in rats, diet containing 0.5% of BAY 59-7939 was administered for 13 weeks at the doses of 75 to 300 mg/kg/day. A non-dose proportional increase in plasma concentration was achieved in rats and minor change of hyper pigmentation in periductal pancreatic islets was noted in males. The target organs of toxicity were not identified from these clinical insignificant changes. Sponsor should have employed high doses of the compound in the study. Sponsor submitted this study in support of dose selection assessment of 2-year carcinogenicity study in rats and CAC-Ex committee concluded that the proposed MFD in diet was not identified and the maximum amount of the active compound was 0.5% and not 5% as required by ACH Guidelines 51C.

A 13-week oral gavage toxicity study with the micronized form of BAY 59-7939 in Wistar rats was performed at the doses of 0, 60, 300 and 1500 mg/kg/day for 91/92 (M/F) days and study included satellite groups animals treated for 81 days. A dose-dependent increase in the coagulation time in males and females rats was observed and no treatment related adverse effects were seen. The plasma concentration measurement indicated that the absorption of the compound was erratic and the observed effects were neither dose nor treatment related. The target organs of toxicity and MTD were not identified in the study.

In 6-Month Chronic Toxicity Study in Rats, Bay 59-7939 at oral doses of 12.5 to 200 mg/kg/day for 6 months produced treatment related increase in the plasma concentration of animals within 60 minutes of the administration. The histopathology changes for all the treated study group animals were not examined. The target organs of toxicity, NOAEL could not be identified. The study was incomplete and inconclusive.

4-Week oral gavage toxicity study with BAY 59-7939 in beagle dogs (3/sex/group) was performed at 0, 15, 50 and 150 mg/kg/day doses. Animals treated with 50 and 150 mg/kg/day doses had vomiting of white fluid/foam and the 150 mg/kg/day group had discolored (green/bright) feces. Slightly increased reticulocyte levels were observed at all doses (141.7%, 46.2% and 63.6% in males and 100%, 120% and 290% in females of low, mid and high dose groups, respectively in week 2). Subcutaneous treatment related hemorrhage was observed in all groups including the control (one control, all low dose animals, 1 male and all females at mid dose, and all high dose animals). Extramedullary hematopoiesis in the spleen was observed in treated males and females and spleen was identified as the target organ of toxicity. 'No effect dose' was not established and 150 mg/kg/day identified as a well tolerated dose. This provides 37 times the exposure of the clinical dose, in this study.

13-Week subchronic oral gavage toxicity study in dogs was conducted at oral gavage doses from 15 to 150 mg/kg. BAY 59-7939 treatment produced white fluid/foam vomiting, discolored (green/bright) feces and subcutaneous

hemorrhage in tissues and, extramedullary hematopoiesis in the spleen. A dose of 150 mg/kg/day was the highest tolerated dose in this study.

In 52-week toxicity study in dogs, the oral gavage doses were 0, 5, 25 and 50 mg/kg/day BAY 59-7939. A treatment but non-dose proportional increase in plasma concentrations was seen from 1.75 to 2.25 hr after administration in males and, 1 to 3 hr in females. The NOEL was 5 mg/kg/day and the highest tolerable dose was 15 mg/kg/day in the study and the target organs of toxicity were lungs and lymph nodes. Sponsor did not conduct the histopathology examination of all the treated animals. The target organs of toxicity and NOAEL could not be identified. The study was incomplete.

In fertility and reproductive performance study, BAY-59-7939 from the oral doses of 12.5 to 200 mg/kg/day in male and female rats produced a reduction in number of dams with viable fetuses and a slight increase in post implantation loss and a dose related reduction in ovarian weight by 8.8% in the dams of the high dose group. It was embryo- and fetotoxic NOAEL was 50 mg/kg/day. Based on body surface area ( $\text{mg}/\text{mm}^2$ ), it provides 41 times greater plasma exposure than the proposed clinical dose exposure.

BAY 59-7939 when administered in pregnant rats from 0, 10, 35 and 120 mg/kg/day doses from day 6 to 17 postcoitum produced no teratogenic adverse effects in rats. Based on body surface exposure ( $\text{mg}/\text{mm}^2$ ), the exposure in the high dose group was 97 times the exposure of proposed clinical dose. BAY 59-7939 administration in pregnant rabbits produced increased incidences abortions at all dose levels and 40 and 160 mg/kg/day were maternally lethal. BAY 59-7939 was not teratogenic in rabbits of the study. Systemic maternal NOEL and intrauterine development safe dose was 2.5 mg/kg/day in the study and the exposure in animals was 4 times the exposure of the clinical dose.

BAY 59-7939 Coprecipitate 10 % 100 treatment in pregnant rat dams produced maternal preterm delivery and, dose related increased incidences of still births, empty stomach and intestines in pups of 10 and 40 mg/kg/day groups treated dams. The developmental adverse effects of hypoactivity, pale skin, cold to touch surface, deficient sucking developmental reflex (no detectable milk spots in pup stomach) were reported in F1 pups of dams of 10 and 40 mg/kg/day groups. The identified NOEL for the physical development and reflex and behavioral testing of the F1 generation was 10 mg/kg/day. Based on body surface area, this produced 8 times greater plasma exposure than the proposed human dose of 0.2 mg/kg/day.

Genotoxic potential of BAY 59-7939 was examined by the Ames assay, the *in vitro* chromosome aberrations assay using Chinese hamster lung (CHL) cells, and the *in vivo* mouse bone marrow micronucleus test. BAY 59-7939 showed no genotoxic potential in any of these tests. However, the *in vivo* mouse bone marrow micronucleus assay the drug was administered by the IP route instead of the intended oral route. Anilino-morpholinone (a by product of BAY 59-7939 coprecipitate, was mutagenic in 3 of the 5 strains of Salmonella in Ames test.

In a vascular irritation toxicity study, BAY 59-7939 solution caused slight hemorrhage and fibrin exudation at the right A. femoralis after intra-arterial dose and, epidermal ulceration was seen after an IV injection. An intravenous infusion (Ix) / V. cephalica antebrachii of BAY 59-7939 produced no reaction at the local site. BAY 59-7939 Nanosuspension 2% (w/v) produced a perivascular inflammation and increased number of lymphocytolysis megacaryocytes as a compensatory to bleeding at the injection site. The nanosuspension formulation of the compound was vaso-irritant in rats.

From the preclinical standpoint, the sponsor submitted adequate preclinical studies to demonstrate that BAY-59-7939 at oral doses inhibit the thrombus formation and inhibitor of Factor Xa in a concentration dependent manner. The compound is approvable from a preclinical standpoint, if the sponsor revised the proposed label as suggested in the review.

#### **CONCLUSIONS:**

a. In conclusion from a preclinical standpoint, the sponsor has demonstrated that the compound at oral doses inhibits the thrombus formation and acts as an inhibitor of Factor Xa in a concentration dependent manner. The compound is approvable from a preclinical standpoint. Sponsor should revise the proposed label as suggested in the review.

b. Recommendations on labeling

A separate labeling review will be written.

Signatures (optional):

Reviewer Signature \_\_\_\_\_  
Yash M. Chopra, M. D., Ph. D.

Supervisor Signature \_\_\_\_\_  
Adebayo Lanionu, Ph.D.

Concurrence Yes \_\_\_\_ No \_\_\_\_

**Appendix/attachments**

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

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Yash Chopra  
5/12/2009 10:50:19 AM  
PHARMACOLOGIST

Adebayo Laniyonu  
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