CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
22-512

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
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NDA: 22-307
Submission date: 4/19/2010
Drug: Pradaxa™ (Dabigatran etexilate mesylate)
Sponsor: Boehringer Ingelheim Pharma GmbH & Co. KG
Indication: Prevention of stroke and systemic embolism in patients with atrial fibrillation

Reviewing Division: Division of Cardiovascular and Renal Products

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

The reviewer proposes pregnancy category C for the labeling. Dabigatran induced some reproductive toxicity in rats manifest as a decreased number of implantations, decreased number of viable fetuses, increase in the resorption rate, increase in post-implantation loss, and an increase in the number of dead offspring when given at doses of 70 mg/kg (about 2.6 to 3 times the MRHD of 300 mg/day on a mg/m^2 basis). Dabigatran also induced some fetal structural variations but did not induce fetal malformations in rats or rabbits.

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

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

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/s/

PAUL C BROWN
10/14/2010

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22-512
Supporting document/s:	EDR
Applicant's letter date:	4/19/10 (Initial 9/17/09)
CDER stamp date:	4/19/10 (Initial 9/17/09)
Product:	Pradaxa™ (Dabigatran etexilate mesylate)
Indication:	Prevention of stroke and systemic embolism in patients with atrial fibrillation
Applicant:	Boehringer Ingelheim Pharma GmbH & Co. KG
Review Division:	Division of Cardiovascular and Renal Products
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Template Version: December 7, 2009

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

NDA 22-512 is approvable from a nonclinical perspective for the proposed indication. Most of the toxicities identified in the non-clinical studies are either attributable to the pharmacodynamic effect of dabigatran (BIBR 953 ZW), the active form of the pro-drug, dabigatran etexilate mesylate (BIBR 1048 MS) or satisfactory safety margins have been demonstrated. However, the label needs to warn women of child-bearing potential of BIBR 1048 MS's embryo/fetal and peri-natal toxicity to offspring.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

[Note: All oral doses of BIBR 1048 MS are expressed as the free base equivalent.]

Sponsor's proposal:

8.1 Pregnancy
Teratogenic Effects, Pregnancy Category C
There are no adequate and well-controlled studies in pregnant women (b) (4)

Reviewer's recommendation:

Teratogenic Effects, Pregnancy Category C
There are no adequate and well-controlled studies in pregnant women.
(b) (4)

Sponsor's proposal:

8.2 Labor and Delivery

(b) (4)

Reviewer's recommendation:

(b) (4)

Sponsor's proposal:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Reviewer's recommendation:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Dabigatran was not carcinogenic when administered by oral gavage to mice and rats for up to 2 years. The highest doses tested (200 mg/kg/day) in mice and rats were approximately 3.6 and 6 times. respectively, the human exposure at MRHD of 300 mg/day based on AUC comparisons).

Sponsor's proposal:

(b) (4)

Reviewer's recommendation:

Dabigatran was not mutagenic in *in vitro* tests, including bacterial reversion tests, mouse lymphoma assay, and chromosomal aberration assay in human lymphocytes, and the *in vivo* micronucleus assay in rats.

Sponsor's proposal:

(b) (4)

Reviewer's recommendation:

In the rat fertility study with oral gavage doses of 15, 70, and 200 mg/kg, males were treated for 29 days prior to mating and during mating (approximately 7 weeks total) and females were treated 15 days prior to mating through gestation Day 6. No adverse effects on male and female fertility were observed at 200 mg/kg or 9 to 12 times the human exposure at MRHD of 300 mg/day based on AUC comparisons. However, the number of implantations decreased in females receiving 70 mg/kg or 3 times the human exposure at MRHD of 300 mg/day based on AUC comparisons.

Sponsor's proposal:

(b) (4)

Reviewer's recommendation:

Section 13.2 can be omitted.

1.2 Brief Discussion of Nonclinical Findings

[Note: Unless otherwise indicated all oral doses of BIBR 1048 MS are expressed as the free base (BIBR 1048 BS).]

Dabigatran etexilate mesylate (BIBR 1048 MS) is the pro-drug of dabigatran (BIBR 953 ZW), a synthetic, direct inhibitor of thrombin (Factor IIa, FIIa). BIBR 953 ZW inhibited human thrombin with a Ki of 4.5 nM. BIBR 953 ZW showed little inhibition of other serine proteases involved in coagulation or fibrinolysis with selectivities greater than 500 fold, except for trypsin (11-fold). BIBR 1048 BS (free base) only minimally inhibited thrombin at l00 µM and its inhibition of trypsin was not studied. BIBR 1048 MS is hydrolysed by carboxyl esterases on microsomes and in plasma to two mono pro-drugs, BIBR 951 BS and BIBR 1087 SE, which are 4 and 400-fold less potent, respectively, in inhibiting thrombin than BIBR 953 ZW.

In vitro binding of BIBR 953 ZW and BIBR 1048 MS to a panel of 80 physiologically important receptors showed little binding of BIBR 953 ZW; however, BIBR 1048 MS inhibited binding of the chloride channel more than the reference compound.

BIBR 953 ZW inhibited thrombin-induced platelet aggregation with a concentration at 50% inhibition (IC_{50}) of 10.4 nM, but showed little effect on platelet aggregation induced by other agonists tested. BIBR 953 ZW prolonged the activated partial thromboplastin time (aPTT), the ecarin clotting time, and the prothrombin time (PT) in a dose dependent manner in plasma from several species. Intravenous administration of BIBR 953 ZW or oral administration of BIBR 1048 MS induced a dose- and time-dependent prolongation of ex vivo aPTT in several species. The anticoagulant activity of BIBR 953 ZW was greater in human plasma than in rat and monkey plasma and even greater than in dog or rabbit plasma.

In rat and rabbit models of venous thrombosis, intravenous BIBR 953 ZW induced a dose-dependent decrease in clot weight with IC_{50}'s of 33 and 66 µg/kg, respectively, concomitant with prolongation of ex vivo aPTT. Oral BIBR 1048MS inhibited clot formation induced by tissue factor/stasis in rats for at least 2 hours after administration. Doses of BIBR 953 ZW at least 9-fold higher than the dose needed to inhibit venous thrombosis were needed to significantly prolong bleeding time. Doses of NovoSeven and Feiba used therapeutically in hemophilic patients were shown to reverse in rats the 10-fold prolongation of bleeding time induced by administration of a supra-therapeutic dose of BIBR 953 ZW.

The sponsor conducted eleven studies with BIBR 953 ZW and eleven studies with BIBR 1048 MS to examine acute neurological, cardiovascular, renal, gastrointestinal and respiratory effects. Most of these studies were conducted as secondary pharmacology studies and were not conducted in compliance with GLP principles.

BIBR 953 ZW even at 30 uM had no effects on hERG potassium current. BIBR 953 ZW up to 10 µM did not affect action potential duration in guinea pig papillary muscle. Significant effects on diastolic blood pressure, maximal left ventricular pressure, maximal left ventricular dP/dt, and femoral artery blood flow, but not QT interval, were observed following intravenous administration of 10 and 30 mg/kg BIBR 953 ZW to pigs. Additionally, no effects on electrocardiogram parameters, including the QT interval, were observed in the 26- and 52 week toxicology studies in monkeys.

Effects on central nervous system parameters were minimal. Slight, reversible effects on grasping and landing reflex, pain sensitivity and grip strength were observed in mice at the first observation timepoint following administration of doses ≥100 mg/kg BIBR 1048 MS or ≥10 mg/kg BIBR 953 ZW. Slight decreases in body temperature were observed in rats at 4 and 24 hours after treatment with dose ≥300 mg/kg BIBR 1048 MS.

Respiratory function was not significantly affected In rabbits by intravenous doses of BIBR 953 ZW up to 10 mg/kg and in rats by oral doses of BIBR 1048 MS at 300 mg/kg. Although intravenous BIBR 953 ZW at 3 mg/kg to dogs produced a slight increase in renal microprotein excretion, oral administration of 10 mg/kg BIBR 1048 MS produced no renal effect in dogs.

Gastric secretion and gastrointestinal transit were not significantly affected by either BIBR 1048 MS or BIBR 953 ZW. However, intravenous doses of 0.1 to 1.0 mg/kg of BIBR 953 ZW produced 18 to 20% decreases in gastric emptying. Moreover, a statistically significant dose-dependent inhibition of gastric emptying was observed with oral administration of 300 mg/kg BIBR 1048 MS.

Absorption of BIBR 1048 MS/BIBR 951 ZW is low in all species. Oral bioavailability of BIBR 1048 MS/BIBR 951 ZW was 12 % in rats, 10% in mice, more than 5.4% in rabbits and 7.7% in monkeys. Consequently, most (87-95%) of a BIBR 1048 MS dose is excreted into the feces in all species and the remainder (2.4-11%) with the urine. Urinary excretion after intravenous administration of BIBR 953 ZW was 13% and 20% of the dose in rats and monkeys, respectively. After intravenous administration, BIBR 953 ZW excretion is primarily through the bile in rats. Monkeys and rats excreted over 50 and 95%, respectively, of the dose within the first 24 hours after administration.

The volume of distribution of BIBR 953 ZW in rats, rabbits, and monkeys of 0.63, 1.2 and 1.34 L/kg is slightly greater than that of total body water (0.55 L/kg). Total plasma clearance for rats, rabbits and monkeys is 11.5, 11.5, and 8.9 mL/(min·kg), respectively. The elimination half-life of BIBR 953 ZW is shortest in rats and mice (1-1.5 hr) and longest in monkeys (4-7 hr).

In vitro cell culture studies showed that BIBR 953 ZW has low permeability and is not a substrate for P-glycoprotein (P-gp). In contrast, BIBR 1048 MS is a P-gp substrate and is highly permeable, although its permeability depends on drug concentration. Neither BIBR 1048 MS nor BIBR 953 ZW inhibited digoxin transport indicating they are not inhibitors of P-gp.

The plasma protein binding of [^{14}C] BIBR 953 ZW was low in all species ranging from about 30% in rats and humans to 39% in rhesus monkeys. The ratio of radioactivity in blood cells and plasma of rats indicated that [^{14}C] BIBR 953 ZW was preferentially distributed in plasma. Whole body autoradiography of rats showed that [^{14}C] BIBR 953 ZW distributed into all organs with the lowest concentration in the central nervous system and the highest concentration in liver and kidney. A low level of placental transfer of [^{14}C] BIBR 953 ZW was demonstrated using whole body autoradiography in pregnant albino rats after subcutaneous administration.

In monkeys, glucuronide conjugates of BIBR 953 ZW represent more than half of the total BIBR 953 ZW in plasma and are also found in urine and feces. The glucuronide conjugates of BIBR 953 ZW consist of the β-anomer of the 1-O-acylglucuronide and α, β-anomers of the 2-O-, 3-O- and 4-O-acylglucuronides of BIBR 953 ZW. In contrast to monkeys, only traces of glucuronide conjugate were found in mouse plasma. In rat and rabbit plasma, glucuronide conjugated BIBR 953 ZW was 2.8 to 8% and 10% of total BIBR 953 ZW, respectively. Each of the four acylglucuronides of BIBR 953 ZW was as active as the parent BIBR 953 ZW in inhibiting thrombin.

In vitro glucuronidation of BIBR 953 ZW was higher using human liver microsomes than human intestinal microsomes. Incubations with microsomes from 16 different individual liver donors indicated at least a 10-fold difference in glucuronidation rate of BIBR 953 ZW. Incubations with individual recombinant human UGT enzymes expressed on

microsomes indicated that BIBR 953 ZW is a non-specific, low-affinity substrate of (b) (4) with the later having the highest activity for the glucuronidation of BIBR 953 ZW.

BIBR 1048, BIBR 1087, BIBR 951, and BIBR 953 at concentrations up to 10 μM did not inhibit any of nine cytochrome P450 catalyzed test reactions using pooled human microsomes. BIBR 1048 MS-treated rats exhibited little induction of hepatic cytochrome P450 enzyme activities or amounts. In vitro metabolite formation of [^{14}C] BIBR 1048 MS was not affected by erythromycin concentrations up to 100 μM.

The approximate oral lethal single dose was above 2000 mg/kg BIBR 1048 MS for both rats and mice, whereas the approximate intravenous lethal single dose was greater than 100 mg/kg BIBR 953 ZW in mice and slightly less in rats. In dogs and Rhesus monkeys, the oral lethal single doses were above 692 and 600 mg/kg, respectively, and the intravenous lethal single doses were above 20 and 40 mg/kg, respectively.

In the repeated dose studies in rats and monkeys, most of the treatment-related deaths and findings were attributable to excessive pharmacodynamic effects. Decreases in serum hemoglobin, hematocrit and red blood cells were sometimes accompanied by increases in reticulocyte counts. In both rats and monkeys, significant prolongation of PT and aPTT occurred in the high dose animals even when blood samples were collected 24 hours after dose administration. In rats, the pancreas and thymus showed a dose-related increase in incidence and severity of hemosiderosis that resulted from recurrent hemorrhages during the studies. Bruising and focal hemorrhages in monkeys were also related to the exaggerated pharmacodynamic effect.

In the chronic 52-week toxicology study in Rhesus monkeys oral dosages of 0, 12, 36 and 200 mg/kg/day BIBR MS resulted in mean $AUC_{(0\text{-}24h)}$ values for total BIBR 953 ZW (BIBR 953 ZW plus glucuronides) of 2692, 6438, and 15475 ng.hr/mL in males and 1973, 3710, and 15300 ng.hr/mL in females, respectively. Prolongation of coagulation times and decreases in hematocrit, hemoglobin concentrations and red blood cell counts noted in the high dose animals were attributed to the pharmacodynamic effect. Changes in triglyceride and albumin concentrations and increased incidence of thymic involution/atrophy in the high dose females may be attributable to infections. Because of the deaths at the high dose due to aspiration of the acidic formulation, the NOAEL of the chronic monkey study was considered to be 36 mg/kg

Following intravenous administration of BIBR 953 ZW to rats and monkeys for four weeks, pharmacodynamic effects of prolongation of coagulation times and decreases in hematocrit, hemoglobin concentrations and red blood cell counts were noted as observed in the oral toxicology studies. However, increases in serum potassium were also observed in the high dose male and female monkeys and the high dose female rats. The NOAEL of 4 mg/kg in monkeys corresponded to mean $AUC_{(0\text{-}24h)}$ values for total BIBR 953 ZW of 13050 and 14400 ng.hr/mL in males and females, respectively. The NOAEL was 0.5 mg/kg in female rats and 5 mg/kg in male rats corresponding to $AUC_{(0\text{-}24h)}$ values of 616 and 7082 ng.hr/mL, respectively.

In two valid in vitro neutral red phototoxicity assays using BALB/c 3T3 cells, BIBR 1048 MS in Earles's Balance Salt Solution at concentrations ≥ 15.6 µg/mL induced a slight phototoxic effect.

BIBR 1048 MS and BIBR 953 ZW were not genotoxic. Neither BIBR 1048 MS nor BIBR 953 ZW induced excess reverse mutations in five recommended bacterial strains in the absence or presence of metabolic activation at maximum doses limited by toxicity or precipitation. BIBR 1048 MS and BIBR 951 ZW did not induce excess gene mutations or chromosomal damage in mouse lymphoma cells with and without metabolic activation. BIBR 1048 MS alone and BIBR 1048 MS spiked with impurities did not induce excess micronucleus formation in rats after two daily doses of 2000 mg/kg/day.

The CMC specified impurities (b) (4) did not induce excess reverse mutations in five recommended bacterial strains in the absence or presence of metabolic activation. BIBR 1048 MS spiked with impurities (b) (4) did not induce an increase of structural chromosomal aberrations in human lymphocytes when tested in the presence and absence of metabolic activation. Bacterial reversion assays for fourteen additional compounds that are either potential impurities in the starting materials or chemical intermediates were negative, except for (b) (4) which were positive in at least one strain. However, (b) (4) did not induce an increase in micronuclei formation in male rats.

In the mouse carcinogenicity study, CD-1 mice received oral doses of BIBR 1048 MS for at least 102 weeks. At dosages of 30, 100 or 200 mg/kg/day the mean $AUC_{(0\text{-}24h)}$ was 1160, 3830, and 5830 ng.hr/mL in males and 1290, 5400, and 9520 ng.hr/mL in females, respectively. Many of the non-neoplastic macroscopic and microscopic findings were related to the pharmacodynamic action of BIBR 1048 MS. However, the incidence of focal hepatocellular necrosis increased in both males and females receiving 200 mg/kg. In addition, the incidence and severity of glandular dilatation of the uterus increased in females given 200 mg/kg, and the incidence of luminal dilatation of the uterus increased in females given 100 or 200 mg/kg. The increased incidences of fibrosarcoma, bronchioalveolar adenocarcinoma and Harderian gland adenocarcinoma observed in the high dose males and increased incidence of mammary gland adenocarcinoma in all female treated groups did not meet the criteria to be considered positive tumor findings. The Executive CAC concluded that the mouse bioassay was adequate and negative for any drug related statistically significant neoplasms.

In the rat carcinogenicity study, Han Wistar rats received oral doses of BIBR 1048 MS for up to 104 weeks. At dosages of 30, 100 or 200 mg/kg, the mean $AUC_{(0\text{-}24h)}$ was 2490, 7270, and 12200 ng.hr/mL in males and 1960, 6460, and 11800 ng.hr/mL in females, respectively. Hematology findings and most of the macroscopic findings were related to the pharmacodynamic action of BIBR 1048 MS. Likewise, the increased incidence of alveolar hemorrhage, pigmented alveolar macrophages, prostate interstitial hemorrhage and prostate pigmented macrophages in mid- and high dose males could be attributed to the pharmacodynamic effect. However, the incidence of liver necrosis increased in all treated groups in both sexes, although statistical significance was not

attained. The increased incidence of testicular Leydig cell adenomas and thyroid C-cell adenoma and follicular cell adenoma in males and stromal cell tumors (ovarian granulosa cell tumors and Sertoli cell tumor) in females did not meet the criteria to be considered positive tumor findings. The Executive CAC concluded that the rat bioassay was adequate and negative for any drug related statistically significant neoplasms.

In an adequate rat fertility and early embryo development (FEED) study, BIBR 1048 MS treatment at doses of 0, 15, 70, 200 mg/kg was started 29 and 15 days before mating of Han Wistar males and females, respectively, and was continued in females to implantation (Gestation Day 6). The males were sacrificed after mating and the females were sacrificed on Gestation day 14. No compound-related mortality was observed. Paternal toxicity occurred in the high dose males during the pre-mating phase based on decreased body weight gain. Maternal toxicity also occurred in the high dose group during gestation based on decreased body weight gain accompanied by decreased food consumption. Treatment with BIBR 1048 MS did not significantly affect the copulation, fertility and gestation indices. However, treatment with BIBR 1048 MS significantly reduced the mean number of implantations in a dose-dependent manner. Also, pre-implantation loss increased and number of viable fetuses decreased in the mid and high dose groups. The sponsor concluded the NOAEL for embryo toxicity was 70 mg/kg. However, the reviewer concludes the NOAEL for embryo toxicity was 15 mg/kg based on the significant decrease in implantations and number of viable fetuses at 70 mg/kg. The corresponding exposure levels (AUC) of BIBR 1048 BS at the no observed adverse effect levels (NOAEL) were based on toxicokinetic data in an embryo-fetal development study (U03-1284). For paternal and maternal toxicity the NOAEL was 70 mg/kg with a maternal exposure ($AUC_{(0-24h)}$) of 7320 ng•h/mL; for mating and fertility the NOAEL was 200 mg/kg with a maternal exposure of 22500 ng•h/mL; and for early embryonic toxicity the NOAEL was 15 mg/kg with a maternal exposure of 1560 ng•h/mL.

In an adequate rat embryo-fetal development (EFD) study, BIBR 1048 MS was administered orally at 0, 15, 70 and 200 mg/kg from gestation day 7 to 16 to Han Wistar female rats that were sacrificed on gestation day 22. A significant decrease in body weight gain and food consumption was observed in the mid and high dose groups indicating maternal toxicity. Excessive toxicity in the high dose group was indicated by two deaths, one of which accompanied an abortion, and resorptions only in two dams. The total, early and late resorptions increased significantly in the high dose group, with values outside the historical control range. Consequently, the resorption rate significantly increased and the mean number of viable fetuses decreased. In the mid dose group, increased resorption rate and decreased number of viable fetuses also occurred; although the values were not statistically significant, they were outside the mean historical control range. The sex ratio was significantly altered and outside the control range. Fetal body weight in the high dose group was significantly decreased and was below the historical control range.

A rare malformation (cleft thoracal vertebral body) was observed only in the treated groups and not in the concurrent control group; the incidence increased with dose and was above the historical control range. The sponsor maintains that another malformation (flat and thickened ribs) occurred with no relation to dose because the incidences in the control, the low, and high dose groups were similar to the

spontaneous background and only the incidence in the mid dose group was increased. However, the lack of a dose relationship may have been due to the excessive maternal toxicity (deaths and fetal resorptions) in the high dose group. Although most of the variations were either singular or distributed similarly across all groups, the incidences of ossification delay of the supraoccipital bone, the occipital bone, cervical vertebral bodies, and calcaneum were increased in the BIBR 1048 MS treated groups. These variations could be the result of either fetal developmental delay resulting from maternal toxicity or a pharmacodynamic effect of BIBR 1048 MS on thrombin, which is important for osteoblast function and proliferation.

The sponsor concluded that BIBR 1048 MS is not teratogenic in rats and that the NOAELs for maternal and embryo/fetal toxicity were 15 mg/kg and 70 mg/kg, respectively. Based on the findings of decreased number of viable fetuses, increased number of resorptions and resorption rate in the mid dose group, the reviewer concludes that BIBR 1048 MS was clearly embryo toxic in rats and the NOAELs for both maternal and embryo/fetal toxicity were 15 mg/kg corresponding to a maternal exposure ($AUC_{(0\text{-}24h)}$) of 1560 ng.hr/mL.

In an acceptable rabbit EFD study, BIBR 1048 MS was administered orally at 0, 15, 70 and 200 mg/kg from gestation day 6 to 18 to presumptive pregnant Himalayan rabbits (Chbb:HM) that were sacrificed on gestation day 29. Mean body weight gain decreased in the high dose group. Although treatment with BIBR 1048 MS did not significantly affect most of the litter parameters, the percentage of males in the high dose group was below the minimum of the historical range. Additionally, the number of total resorptions, number of early resorptions and the resorption rate were increased in the high dose group compared to the concurrent control group and were above the historical control mean. Based upon submission of a report for an additional control study in the NDA, the findings of missing gall bladder, dilated cerebral ventricle, additional vessels at the aortic arch and hypoplasia of gall bladder in the treated fetuses can be attributed to normal spontaneous variation in rabbits. The maternal NOAEL is 70 mg/kg based on decreased body weight gain and the fetal NOAEL is also 70 mg/kg based on increased resorption. These NOAELS correspond to maternal exposures ($AUC_{(0\text{-}24h)}$) of 2770 ng.hr/mL determined in the dose-range finding study.

In an acceptable pre and postnatal development (PPND) study, pregnant Han Wistar rats received 0, 15, 30, 70 mg/kg of BIBR 1048 MS daily from gestation day 6 to lactation day 21. Exposure to BIBR 951 ZW was similar on gestation day 7 and lactation day 5 with a mean $AUC_{(0\text{-}24h)}$ of 1450, 2770, and 6390 ng.hr/mL in the 15, 30 and 70 mg/kg dosage groups, respectively. Treatment related maternal F_0 mortalities were associated vaginal bleeding and occurred primarily during parturition with one and four deaths in the low and high dose groups, respectively. Vaginal bleeding also occurred in other high dose dams. The percentage of dams with delayed labor increased in the treated groups compared to the percentage in the control group. Maternal F_0 body weight decreased in the high dose group during gestation and in both the mid and high dose groups during lactation. These decreases were associated with decreases in food consumption.

The mean post-implantation loss and mean number of dead F_1 offspring were increased in the high dose group resulting in a significantly decreased birth index. Survival of

offspring after birth to LD 4 was lower in the mid and high dose groups compared to that in the low dose and control groups. Although four high dose F_0 litters had offspring with abnormalities and only one control litter had an offspring with abnormalities, these abnormalities were considered incidental.

Treatment of F_0 dams with BIBR 1048 MS during gestation and lactation did not significantly affect sex ratio, body weight at birth, body weight development, and postnatal survival after lactation day 4 or delay general physical development of the F_1 offspring. Neurological assessments showed no significant effect of BIBR 1048 MS treatment on F_1 reflexes, sensory functions, learning ability, memory and explorative behavior. Treatment of F_0 dams with BIBR 1048 MS during pregnancy and lactation did not impair the fertility of the F_1 animals or the intrauterine survival of the F_2 embryos pre- and post implantation. The NOAEL for maternal (F_0) toxicity was 30 mg/kg, the NOAEL for pre/perinatal (to lactation day 4) toxicity in F_1 offspring was 30 mg/kg, the NOAEL for postnatal toxicity, development and fertility in F_1 offspring was 200 mg/kg. These NOAELs corresponded with BIBR 953 ZW $AUC_{(0\text{-}24h)}$ values of 2760, 2760, and 6390 ng.hr/mL.

Administration of BIBR 1048 MS resulted in significant embryo toxicity in all three developmental toxicity studies in rats. The NOAELs for embryo/fetal toxicity in the FEED study, the EFD study, and the PPND study in rats were 15 mg/kg, 15 mg/kg and 30 mg/kg (or 0.5, 0.5 and 1 times the MRHD of 300 mg/day on a mg/m^2 basis), respectively, with embryo/fetal toxicity observed at 70 mg/kg (or 2.3 times the MRHD of 300 mg/day on a mg/m^2 basis) in all three studies. The maternal exposure ($AUC_{(0\text{-}24h)}$) at the NOAEL of 15 mg/kg in the rat FEED and EFD studies was 1560 ng.hr/mL, respectively. Embryo-fetal toxicity was observed at a maternal exposure of 7320 ng•h/mL in the FEED and EFD studies. In the rat PPND study, the NOAEL for offspring (F_1) pre/perinatal toxicity corresponded to a maternal exposure of 2760 ng.hr/mL and toxicity was observed at a maternal exposure of 6390 ng.hr/mL.

In the rabbit EFD study the NOAEL was higher 70 mg/kg (or 4.5 times the MRHD of 300 mg/day based on mg/m^2) and corresponded to maternal exposures ($AUC_{(0\text{-}24h)}$) of 2770 ng.hr/mL. Embryo-fetal toxicity in the rabbit occurred at 200 mg/kg (13 time the MRHD of 300 mg/day based on mg/m^2) and corresponded to maternal exposures of 8210 ng.hr/mL. The higher NOAEL for embryo-fetal toxicity in the rabbit is consistent with the lower anticoagulant activity of BIBR 953 ZW in rabbit plasma compared to rat plasma.

Since pharmacokinetic studies in non-pregnant human females indicate a steady state $AUC_{(0\text{-}24h)}$ of about 2466 ng.hr/mL for the 150 mg BID dose, little or no safety margin exists either for maternal or fetal toxicity, especially during the peri-natal period.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

211915-06-0

2.1.2 Generic Name

Dabigatran etexilate mesylate (Pradaxa™)

2.1.3 Code Name

BIBR 1048 MS

2.1.4 Chemical Name

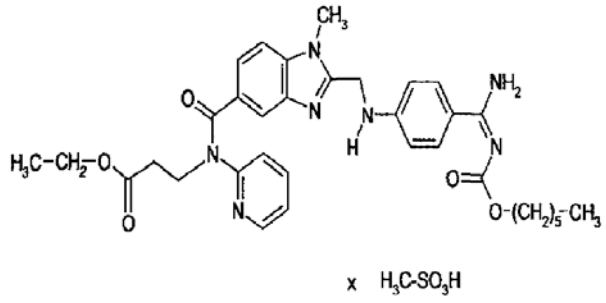
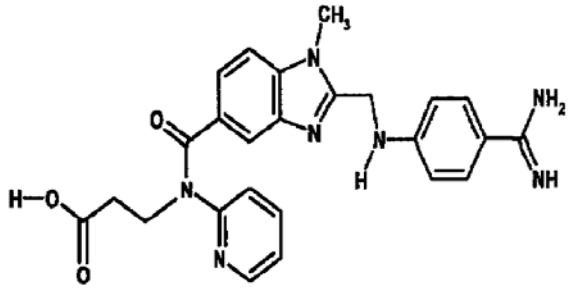
IUPAC name:
Ethyl N-{[2-({[4-((E)-amino{[(hexyloxy)carbonyl]imino}methyl)phenyl]amino}methyl)-1-methyl-1H-benzimidazol-5-yl]carbonyl}-N-pyridin-2-yl-β-alaninate methanesulfonate

2.1.5 Molecular Formula/Molecular Weight

Molecular formula: $C_{34}H_{41}N_70_5$ x CH_3O_3S
Molecular weight: free base: 627.7 g/mole; mesylate salt: 723.8 g/mole

2.1.6 Structure

Figure 1: Structures of BIBR 1048 MS and BIBR 953 ZW

BIBR 1048 MS (pro-drug) (Dabigatran etexilate mesylate)	**BIBR 953 ZW (active drug)** (Dabigatran)
	

2.1.7 Pharmacologic class

BIBR 1048 MS is a double pro-drug that is hydrolyzed to BIBR 953 ZW, a direct thrombin (Factor IIa) inhibitor.

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 65,813 (BIBR 1048 MS, Boehringer Ingelheim)
IND 102,821 (BIBR 1048 MS, Boehringer Ingelheim)
IND 63,267 (BIBR 1048 MS, Boehringer Ingelheim)

2.3 Drug Formulation

Pradaxa™ capsules for oral administration contain either 86.48, (b) (4) or 172.95 mg dabigatran etexilate mesylate (as salt, BIBR 1048 MS) equivalent to 75, 110 or 150 mg of free base (BIBR 1048 BS), respectively, and the following inactive ingredients: acacia, dimethicone, hypromellose (b) (4) hydroxypropyl cellulose, talc, tartaric acid, and dimethicone. The capsule shells are composed of carageenan, FD&C Blue No. 2, FD&C Yellow No. 6, hypromellose, potassium chloride, titanium dioxide, and black edible ink.

2.4 Comments on Novel Excipients

All of the excipients are commonly used in oral commercial pharmaceutical dosage forms and are compendial materials. The CMC Review of NDA 22-512 indicates that the formulation contains no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

Nine impurities that require CMC specifications are listed in Table 1. Based on this table, the nine impurities are considered qualified at the proposed specification level. The lots of BIBR 1048 MS used in the various toxicology studies are listed in Appendix 1.

Two impurities, BIBR 951 and BIBR 1087 are mono-prodrug metabolites formed in the conversion of BIBR 1048 to BIBR 953 ZW, the active moiety. Two impurities, (b) (4) are derived from impurities in the starting material (b) (4) and are related alkyl homologues of the drug substance. (b) (4) was directly tested for genotoxicity in an Ames assay, whereas (b) (4) was not. To evaluate the need to test (b) (4) in a direct Ames assay, a Computational Toxicology Evaluation was requested. The ICSAS prediction for (b) (4) in Salmonella mutagenicity was negative (Appendix 2). Also, based on the presence of (b) (4) in batches 8050461 and 8250250, this impurity, although not specifically measured, was likely present in batch RAL 98 and other batches (RAL 70) used in initial genetic toxicology assays. Therefore, a request to have (b) (4) directly tested in an Ames assay has not been made.

Genetic toxicology study reports for process intermediates and starting materials that could be potential impurities were also submitted. These study reports are summarized in Table 92. Of these fourteen compounds, four (b) (4)) were positive in the Ames assay. (b) (4) was also positive in a mouse lymphoma assay, whereas (b) (4) was not. Both (b) (4) and (b) (4) were negative in a rat micronucleus assay.

Since BIBR 1048 MS is a methanesulfonate salt, additional potential genotoxic impurities include (b) (4) such as (b) (4) are known to be genotoxic and carcinogenic. The sponsor proposed criterion for each (b) (4) impurity is (b) (4) µg/day/individual corresponding to a 1-ppm limit for a 300 mg daily dose of BIBR

1048 MS. The limit of (b) (4) µg/day/individual (b) (4) is below the threshold of toxicological concern (TTC) value of 1.5 µg/day proposed in the guidelines on the limits of genotoxic impurities (FDA Draft Guidance for Industry “Genotoxic and carcinogenic impurities in drug substances and products: recommended approaches” and CPMP/SWP/5199/02). If all four potential (b) (4) are present at (b) (4) µg/day, the sum of all (b) (4) is expected to be less than the (TTC) value of 1.5 µg/day. The reviewer notes that the CMC review recommend additional controls on the reagent (b) (4) used in the manufacture of drug substance to better control for (4) impurities.

1 page withheld in full immediately following this page as (b)(4) CCI/TS.

2.4 Proposed Clinical Population and Dosing Regimen

The proposed indication is for the prevention of stroke and systemic embolism in patients with atrial fibrillation and for the reduction of vascular mortality in patients with atrial fibrillation.

2.5 Regulatory Background

As discussed above, the potential for [redacted] impurities has been a concern from the submission of the original IND. The sponsor was strongly encouraged to limit total [redacted] to [redacted]ppm. Although this required development of more sensitive assays, the sponsor has complied with this request.

Bacterial reversion, mouse lymphoma and rat micronucleus assays were submitted with the original IND; however, the mouse lymphoma and micronucleus assays had issues, such as unacceptable high doses, existed that required repetition of the assays for acceptance. The sponsor conducted additional assays and submitted the study reports.

In the 26-week toxicology study in monkeys, histopathology findings of atrophy of the testes, prostate, seminal vesicles and epididymides were described as "physiological," because Rhesus monkeys are seasonal breeders (Wickings et al 1980; Herndon et al 1996). However, the discrepancy in the incidence between control and treated groups was not fully explained. Therefore, the sponsor was asked include measurements of testosterone in the chronic toxicology study in monkeys to provide support for the physiological state of the male monkeys (Wickings and Nieschlag, 1977).

3 Studies Submitted

3.1 Studies Reviewed

The following study reports included in the NDA were not previously reviewed and are reviewed in this document.

Document Number	Study Title	Report Number
General pharmacology		
U07-1984	The effect of dabigatran on thrombin Inhibition in the clot-bound and fluid phase	2007/LUI/Lab 1/Report1
U00-1351, AM1	The selectivity and specificity of BIBR 953 ZW and BIBR 1048 MS towards purified human serine proteases in vitro. Amendment No. 1	[redacted]3-2000-01
U02-1700	BIBR 1048 BS/BIBR 1048 MS: Comparative investigation of blood coagulation parameters in rats after single oral (gavage) administration of BIBR 1048 BS and BIBR 1048 MS	98B055
U06-1567	Antithrombotic and anticoagulant effects of BIBR 1048 MS (dabigatran etexilate), the double prodrug of the direct thrombin inhibitor BIBR 953 ZW (dabigatran) after single oral administration in a rabbit venous thrombosis model	2006/LUI/Lab 1/Report1
U05-2157	Pharmacological activity and pharmacokinetic exposure in humans of BIBR 951 and BIBR 1087, the two semiprodrugs of dabigatran etexilate (BIBR 1048)	B2648

Document Number	Study Title	Report Number
U06-1711	Pharmacological activity and pharmacokinetic exposure in humans of BIBR 951 and BIBR 1087, the two semi-prodrugs of dabigatran etexilate (BIBR 1048)	2006/LUI/Lab 1/Report2
U09-1332-02	Reversal of dabigatran induced bleeding with activated Factor VII and activated prothrombinase complex	2009-LUI-lab1-report1
U09-1126-01	In Vitro Pharmacology – (b) (4) (receptor) screens – Study of BIBR 1048 MS/ Study of BIBR 1048 MS, BIBR 953 ZW and Quinidine	8810448/MN – 8810441/CP
Safety pharmacology		
U05-1533	BIBR 1048 MS: Modified Irwin test in the male and female rat, including body temperature and short-term locomotor activity – single oral (by gavage) administration	04B082
U06-1056	BIBR 1048 MS: Evaluation of respiratory parameters in the conscious male rat, using whole body bias flow plethysmography – single oral (by gavage) administration	04B112
Pharmacokinetics, Absorption, Distribution, Metabolism, and Excretion Studies		
U03-1061	BIBR 953 ZW: Intravenous maximum tolerated dose study in rhesus monkey	(b) (4) 572268 B1237, B1229
U08-2324-01	Stability testing of BIBR 1048 BS, BIBR 1087 SE and BIBR 951 BS in mice plasma and processed samples	SA145
U06-1706-01	Stability of BIBR 953 ZW 1-O-acyl glucuronide in animal plasma	301_06RM
U06-1211	Protein binding of [^{14}C] BIBR 953 ZW in mouse and rabbit plasma (A130/06GR, A066/04UB)	B2798
U06-0214	Determination of the partition coefficient (n-octanol/water) of BIBR 953 ZW including effect of pH	RCC A27404
U07-3554-02	P-gp-based drug-drug interaction of BIBR 1048 MS using the Caco-2 cell in vitro absorption model	PK0736T
U05-2159	Investigation in the permeability and interaction with P-glycoprotein of BIBR 1048 MS and BIBR 953 ZW including the intermediate pro-drugs using the Caco-2 cell in vitro absorption model	A047/03AV (B2464)
U07-3036	In vitro evaluation of the transport and the interaction of BIBR 1048 MS and its metabolite BIBR 953 ZW with human P-glycoprotein (P-gp/MDR1)	PK0641T
U05-1025	Comparison of the metabolite pattern between the Chbb:THOM- and the CrlGlxBrlHan:WI- rat following oral administration of BIBR 1048 MS and intravenous administration of BIBR 953 ZW	A407/03BC (B2359)
U05-1249	Comparison of the metabolite pattern between the NMRI- and the CD-1- mouse following oral administration of BIBR 1048 MS and intravenous administration of BIBR 953 ZW	A409/03BC (B2260)
U04-2009-01	Metabolism of BIBR 1048 MS and BIBR 953 ZW in rhesus monkeys	A392_03BC
U04-2115	Metabolism of BIBR 1048 MS and BIBR 953 ZW in rabbits	A393_03BC
U09-1024-01	Determination of in vitro stability of BIBR 1048 MS in plasma of mice	A032/08JS (B3520)
U05-1339	Effect of erythromycin on the in vitro metabolism of [^{14}C] dabigatran etexilate	A170_04LU
U05-2451	Plasma level and excretion balance after oral administration of [^{14}C] BIBR 1048 MS in female rabbits.	A065/04UB (B2426)
U08-1941-01	Comparison of pharmacokinetics of BIBR 953 ZW and its glucuronide after single oral administration of BIBR 1048 MS in NMRI and CD-1 mice	A062_03UB (B3493)
U07-1421	In vitro glucuronidation of dabigatran	A260_07TE
U98-2709	BIBR 953 ZW: Basic ADME of [^{14}C] BIBR 953 ZW in the rat	B984
U06-1725	BIBR 1048 MS: Statistical evaluation of toxicokinetic data from rat study BOI/266: relative amount of acyl glucuronides of BIBR 953 ZW	B2913
U06-1046-01	Comparison of pharmacokinetics after oral administration of BIBR 1048 MS in Chbb:THOM and Crl:WI(Han) rats (B2728, B2170)	A061/03UB

Document Number	Study Title	Report Number
U06-1452	Metabolism and excretion of [^{14}C] BIBR 1048 MS in milk after oral administration to lactating rats	A150/06RB (B2962)
U04-1531	BIBR 1048 MS: Oral (gavage) maximum tolerated dose study in rhesus monkeys - Analyst report Toxicokinetics	(b) (4) 568623 (B881)
Repeated Dose Toxicology		
U01-1115	BIBR 953 7W: Intravenous maximum tolerated dose study in Rhesus monkeys.	(b) (4) 17338/572268
Sub-chronic Toxicology		
U00-1180	BIBR 953 ZW: 4 week intravenous toxicity study in rhesus monkeys with a 4 week recovery period	(b) (4) 572619
U03-1157	BIBR 953 ZW: 4 week intravenous toxicity study in rhesus monkeys with a 4 week recovery period – TK report	(b) (4) 572619 (B1328)
U00-1128	BIBR 953 ZW: Repeated dose toxicity study in rats by intravenous administration (bolus) over a period of 4 weeks	99B024
U07-1693	BIBR 1048 MS (dabigatran etexilate) and impurities: 13-week oral (gavage) toxicity study in rats	06B122
Chronic Toxicology		
U05-1557	BIBR 1048 MS: Toxicity Study by Oral Gavage Administration to Rhesus Monkey for 52 Weeks Followed by a 6 Week Recovery Period	BOI 252/ 032248
Genotoxicity		
U05-2047,a1	BIBR 1048 MS: Mutagenicity study using micronucleus analysis in rat bone marrow after oral treatment (retest). Amendment No. 1	04B285
U07-1747	BIBR 953 ZW: Mutagenicity study using the mouse lymphoma (L5178Y) assay	07B082
U07-1489	BIBR 1048 MS (dabigatran etexilate) and impurities: Mutagenicity study using micronucleus analysis of rat bone marrow - Part of the 13-week oral (gavage) toxicity study No. 06B122	06B209
U07-1813	BIBR 1048 MS (Dabigatran etexilate) with Impurities (b) (4) : Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro	07B096
U09-1125-01- AM1	(b) (4) (Impurity of BIBR 1048 MS): Mutagenicity Study for Chromosomal Aberrations in Human Lymphocytes in vitro Amendment 1	08B125
U98-2195	Mutagenicity Study with (b) (4) in the S. typhimurium/ mammalian-microsome assay (Ames test)	97B172
U07-1828	(b) (4) (Impurity of BIBR 1048 MS): Mutagenicity study using the S. typhimurium/mammalian microsome assay (Ames test	07B086
U07-1725	(b) (4) (Impurity of BIBR 1048 MS): Mutagenicity study using the S. typhimurium/mammalian microsome assay (Ames test)	07B087
U07-1699,a1	(b) (4) (Impurity of BIBR 1048 MS): Mutagenicity study using the S. typhimurium/mammalian microsome assay (Ames test)	07B088
U06-2022	Mutagenicity study with (b) (4) (impurity of BIBR 1048) using the S. typhimurium/mammalian microsome assay (Ames II)	06B186
U06-2087	Mutagenicity study with (b) (4) (impurity of BIBR 1048) using the S. typhimurium/mammalian microsome assay (Ames II)	06B187
U06-2088	Mutagenicity study with (b) (4) (impurity of BIBR 1048) using the S. typhimurium/mammalian microsome assay (Ames II)	06B188
U08-2223	(b) (4) (Impurity of BIBR 1048 MS): Mutagenicity study using the S. typhimurium/mammalianmicrosome assay (Ames test)	08B124
U02-1342	BIBR 1048 (b) (4) Mutagenicity study in the mouse lymphoma L5178Y tk$^{+/-}$ assay	02B042
U02-1276	BIBR 1048 (b) (4) Mutagenicity mouse lymphoma L5178Y tk$^{+/-}$ assay	01B173
U03-1154	BIBR 1048 (b) (4) Mutagenicity study using the rat bone marrow micronucleus assay (intraperitoneal)	02B192

Document Number	Study Title	Report Number
U02-1174	BIBR 1048 (b) (4) Mutagenicity study in the rat bone marrow micronucleus assay following intraperitoneal exposure	01B175
U98-2314	BIBR 1048/ (b) (4) - Ames test	97B143
U07-1722	(b) (4) Ames test	07B089
U07-1731	Ames test	07B090
U09-1486-01	(b) (4) - Ames test	08B200
U98-2554	BIBR 1048/ (b) (4) - Ames test	97B146
U98-2562	BIBR 1048/ (b) (4) - Ames test	97B147
U98-2561	BIBR 1048 (b) (4) - Ames test	97B148
U98-2152	BIBR 1048/ (b) (4) - Ames test "	97B081
U98-2150	BIBR 1048/ (b) (4) - Ames test	97B083
U98-2151	BIBR 1048/ (b) (4) - Ames test	97B082
U07-1732	(b) (4) – Ames test	07B091
U04-1334	BIBR1048 (b) (4) - Ames test	04B063
u09-1136-01	(b) (4) Ames test	08B201
Carcinogenicity		
U07-2084	Carcinogenicity Study by Oral Gavage Administration to Han Wistar Rats for 104 Weeks	BOI288/ 042959
U07-2181	Carcinogenicity study by oral gavage administration to CD-1 mice for 104 weeks	BOI287/ 042668
Reproductive toxicology		
U05-1550, a1	BIBR 1048 MS: Study of fertility and early embryonic development to implantation in rats by oral administration, gavage	02B025
U04-1440	Evaluation of the rat strain CrlGlxBrlHan:WI in a study of fertility and early embryonic development to implantation by oral administration of Natrosol® 250 HX, gavage	00B204 (Control data)
U05-1493	Evaluation of the rat strain CrlGlxBrlHan:WI in a study of fertility and early embryonic development to implantation by oral administration of Natrosol® 250 HX, gavage	02B128 (Control data)
U03-1284 (re-evaluation)	BIBR 1048 MS: Study for effects on embryo-fetal development in rats by oral administration (gavage).	00B056 amendment
U02-1648 (re-evaluation)	BIBR 1048 MS: Study for effects on embryo-fetal development in rabbits by oral administration (gavage)	00B058, amendment
U05-1804	Evaluation of the rabbit strain Chbb:HM in a study for effects on embryo-fetal development by oral administration of Natrosol 250 HX, gavage	02B189 (Control data)
U05-2396-01	BIBR 1048 MS: Dose range finding study with rats - pre- and postnatal development to lactation day 4 by oral (gavage) administration	05B020
U06-1586	BIBR 1048 MS: Study for effects on pre- and postnatal development including maternal function in rats by oral administration, gavage	04B182
Other studies		
U07-1799	Cytotoxicity Assay In Vitro with BALB/C 3T3 Cells: Neutral Red (NR) Test with BIBR 1048 MS During Simultaneous Irradiation with Artificial Sunlight	1110200
U00-1025	BIBR 953 ZW: Local tolerance after single intravenous injection to rabbits	99B152
U00-1026	BIBR 953 ZW: Local tolerance after single intra-arterial injection to rabbits	99B153
U00-1027	BIBR 953 ZW: Local tolerance after single paravenous injection in rats	99B154
U99-1743	Hemolysis test with injectable solutions of BIBR 953 ZW (5 mg/ml, calculated as base) and placebo	99B158

3.2 Studies Not Reviewed

The following study reports included in the NDA were not reviewed.

(b) (4)

(b) (4)

3.3 Previous Reviews Referenced

The following study reports included in the NDA were reviewed previously under either IND 65, 813 or IND 63, 267.

Document Number	Study Title	Report Number
Reviewed under IND 65, 813, N000 (submitted 07/03/00)		
General pharmacology		
P03 - 00770	Structure —Based Design of Novel Potent Thrombin Inhibitors. J. Med. 2002; 45:1757-1766	Literature Reference
U98-2873	BIBR 953 ZW: isolation, structure elucidation, chemical stability and pharmacological activity of BIBR 953 ZW 1-O-acylglucuronide and its isomeric 2-0, 3-0- and 4-0-acylglucuronides.	B1018
U00-1229	Anti-thrombotic and anticoagulant effects of the direct thrombin inhibitor BIBR 953 ZW in comparison to refludan, melagatran, argatroban, napsagatran and heparin after i.v. administration in a rat venous thrombosis model.	W05042000
U00-1288	Anti-thrombotic and anticoagulant effects of the direct thrombin inhibitor BIBR 953 ZW in comparison to refludan, melagatran, argatroban, napsagatran and heparin after i.v. administration in a rabbit venous thrombosis model.	W20042000
U00-1351	The selectivity and specificity of BIBR 953 ZW towards purified human serine proteases in vitro.	(b) (4) 3-2000-01
U00-1457-AM1	The in vitro effects of increasing concentrations of BIBR 953 ZW on the activated partial thromboplastin time, ecarin clotting time and prothrombin time in plasma from different animal species.	(b) (4) 3-2000-02
U00-1588	Effect of BIBR 953 ZW on tail bleeding time and activated partial thromboplastin time in the anaesthetized rat.	W18082000
U00-1601	The effects of intravenous and intraduodenal administration of BIBR 953 ZW and oral administration of BIBR 1048 MS to rats on the ex vivo activated partial thromboplastin time.	(b) (4) 3-2000-03
U00-1780	The effects of intravenous administration of BIBR 953 ZW and oral administration of BIBR 1048 BS on the ex vivo activated partial thromboplastin time in conscious dogs.	(b) (4) 3-2000-04
U00-1785	Antithrombotic and anticoagulant effects of BIBR 1048 MS, the double prodrug of the direct thrombin inhibitor BIBR 953 ZW, after oral administration in a rat venous thrombosis model.	W11102000

Document Number	Study Title	Report Number
U00-1836	The effects of intravenous administration of BIBR 953 ZW and oral administration of BIBR 953 ZW and BIBR 1048 MS to rhesus monkeys on the ex vivo activated partial thromboplastin time.	(b) (4) 3-2000-05
U0I-1178	Effects of BIBR 953 ZW on human in vitro platelet aggregation	(b) (4) 3-2001-02
Safety pharmacology		
U97-2804	General pharmacology: BIBR 1048 MS: effects on gastrointestinal transit.	GP97-083-PH4
U98-2030	General pharmacology: BIBR 1048 MS: effects on gastric emptying.	GP97-084-PH4
U98-2397	BIBR 1048 MS/general pharmacology: effects of orally administered 30, 100 and 300 mg/kg BIBR 1048 MS on exploratory motility in conscious rats.	GP97/08/PH5
U98-2408	BIBR 1048 MS / general pharmacology: effects of orally administered 30, 100 and 300 mg/kg BIBR MS on hexobarbitone-induced sleeping time in rats.	GP97/082/PH 5
U98-2414	Influence of BIBR 953 ZW (0.3 to 30 mg/kg iv) on cardiovascular function in anesthetized pigs.	GP1998/031/P H2
U98-2501	General pharmacology: BIBR 1048 MS, effects on renal function in conscious female dogs.	GP98-036-PH4
U98-2502	BIBR 1048 MS: general pharmacology: effects on general behavior in mice after oral administration of 30, 100, 300 and 1000 mg/kg BIBR 1048 MS.	GP97-079-PH5
U98-2503	BIBR 953 ZW / general pharmacology: effects on general behavior in mice after intravenous administration.	GP96-134-PH5
U98-2534	BIBR 953 ZW / general pharmacology: effects of BIBR 953 ZW (3,10 and 30 mg/kg, iv) on exploratory motility in conscious rats	GP96-106-PH5
U99-1211	General pharmacology: BIBR 953 ZW, effects of 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg intravenously on cardiovascular and respiratory function in anaesthetized rabbits.	GP98-019-PH4
U99-1220	General pharmacology: BIBR 953 ZW, effects on gastric secretion in conscious rats.	GP98-112-PH4
U99-1273	General pharmacology: BIBR 953 ZW, effects on renal function in conscious female dogs.	GP98-096-PH4
U99-1344	Effects of BIBR 1048 MS (30, 100 or 300 mg/kg, po) on heart rate and blood pressure in conscious rats.	GP-1997-080-PH5
U99-1371	General pharmacology: BIBR 1048 MS. Effects on gastric secretion in conscious rats.	GP98-114-PH4
U99-1380	General pharmacology: BIBR 953 ZW, effects on gastrointestinal transit in rats.	GP1998-106-PH4
U99-1382	General pharmacology: BIBR 953 ZW, effects on gastric emptying in rats.	GP1998-105-PH4
U99-1658	BIBR 953 ZW/ general pharmacology: effects of 10^{-9} to 10^{-5} mol/l on histamine, acetylcholine, barium chloride and serotonin induced contractions in a smooth muscle preparation (isolated ileum) of the guinea pig.	GP1997/024/P H5
U99-1732	Effects of BIBR 953 ZW (0.01 to 10 µmol/l) on action potential configuration in isolated guinea pig papillary muscle.	GP1999/073/P H2
Pharmacokinetics, Absorption, Distribution, Metabolism, and Excretion Studies		
U98-2257	Basic ADME of [^{14}C] BIBR 1048 MS in the rat.	B903 – AM1
U00-1096	Whole body autoradiography after oral administration of [^{14}C] BIBR 1048 MS and intravenous administration of [^{14}C] BIBR 953 ZW in male albino and male pigmented rats.	B1348
U02-1372	Whole body autoradiography after oral administration of [^{14}C] BIBR 1048 MS and subcutaneous administration of [^{14}C] BIBR 953 ZW in pregnant albino rats.	A036/01UB (B1885)
U99-1092	The disposition of [^{14}C] BIBR 1048 MS in the rhesus monkey following oral administration.	(b) (4) 162713 (B1055)

Document Number	Study Title	Report Number
U01-1761	The disposition of [^{14}C] BIBR 953 ZW in the rhesus monkey following intravenous administration.	(b) (4) 164291 (B1228)
U00-1294	Protein Binding of [^{14}C] BIBR 953 ZW in human, rat and Rhesus plasma.	B1357
U01-1602	Investigation of the human cytochrome P450 enzymes involved in the metabolism of [^{14}C] BIBR 1048 MS and [^{14}C] BIBR 953 ZW.	B1765
U99-1134	BIBR 1048 MS: Enzyme induction of cytochrome P450 enzymes in the rat.	B1113
U98-2873	BIBR 953 ZW: Isolation, structure elucidation, chemical stability and pharmacological activity of BIBR 953 ZW 1-O-acylglucuronide and its isomeric 2-O-, 3-O- and 4-O-acylglucuronides	B1018
U99-1767	BIBR 1048 MS, BIBR 953 ZW, (b) (4) BIBR 1087 SE: In vitro inhibition studies on cytochrome P450 dependent metabolic reactions by BIBR 1048 MS, its pharmacologically active principle BIBR 953 ZW and two intermediate metabolites, (b) (4) and BIBR 1087 SE.	B1345
Acute toxicity		
U98-2722	BIBR 1048 MS: Oral (gavage) maximum tolerated dose study in Rhesus monkeys.	(b) (4) 16146
U01-1428	BIBR 1048 MS: Single dose toxicity study by oral administration in rats.	01B018
U01-1429	BIBR 1048 MS: Single dose toxicity study by oral administration in mice.	01B032
U01-1605	BIBR 953 ZW: Single close toxicity study in mice by intravenous administration.	01B034
U01-1606	BIBR 953 ZW: Single dose toxicity study in rats by intravenous administration.	01B033
U04-1530	BIBR 1048 MS: Maximum tolerated oral (gavage) study of BIBR 1048 MS in rhesus monkeys – Toxicokinetics. Draft (B927)	(b) (4) 568623
U04-1531	BIBR 1048 MS: Maximum tolerated oral (gavage) study of BIBR 1048 MS in rhesus monkeys – Toxicokinetics. Analysts Draft (B881)	(b) (4) 568623
Subchronic toxicity		
U98-2720	BIBR 1048 MS: Dose range finding study by oral administration (gavage) to rats over a period of 2 weeks.	97B106
U98-2729	BIBR 1048 MS: Repeated dose toxicity study in rats by oral administration (gavage) over a period of 4 weeks.	98B024
U04-3478	BIBR 1048 MS: Dose range finding study by oral gavage administration to Han Wistar rats for 2 weeks. (Draft report, no summary tables or line data)	BOI 266/ 020214
U04-3479	BIBR 1048 MS: Dose range finding study by oral gavage administration to CD-1 mice for 2 weeks. (Draft report, no summary tables or line data)	BOI 267/ 020215
U98-2799	BIBR 1048 MS: Dose range finding study in dogs by oral administration (gavage) over a period of 2 weeks.	97B108
U98-2723	BIBR 1048 MS: 4 Week oral (gavage) toxicity study in Rhesus monkeys with a 4 week recovery period.	(b) (4) 570098
U98-2866	BIBR 1048 MS: Four week oral (gavage) toxicity study in Rhesus monkeys with a four week recovery period: toxicokinetics.	(b) (4) 570098 (B990, B966)
Chronic toxicology studies		
U03-1310	BIBR 1048 MS: 26-week oral (gavage administration) toxicity study in the rat with a 6-week treatment-free period.	979/5-D6154
U02-1367	BIBR 1048 MS: 26-week oral (gavage administration) toxicity study in the rat with a 6-week treatment free period; Toxicokinetics.	979/5 (B1799)
U03-1208	BIBR 1048 MS: 26-week oral (gavage) toxicity study in Rhesus monkeys with a 6-week recovery period.	1813-011
Genotoxicity studies		
U98-2147	Mutagenicity study with BIBR 1048 MS in the S. typhimurium/mammalian-microsome assay (Ames test).	00B100
U98-2789	Mutagenicity study with BIBR 953 ZW in the S. typhimurium/mammalian-microsome assay (Ames test).	04B063

Document Number	Study Title	Report Number
U99-1023	Mutagenicity study in the rat bone marrow micronucleus assay after oral treatment with BIBR 1048 MS.	98B101
U99-1063	Mutagenicity study with BIBR 1048 MS/mannitol in the S. typhimurium/ mammalian microsome assay (Ames test).	98B065
U01-1161	Mutagenicity study in the mouse lymphoma L5178Y tk+/- assay with BIBR 1048MS	04B063
Reproductive toxicology		
U01-1820	BIBR 1048 MS: Dose range finding study for effects on embryo-fetal development in rabbits by oral administration (gavage).	00B057
U02-1648	BIBR 1048 MS: Study for effects on embryo-fetal development in rabbits by oral administration (gavage).	00B058
U03-1284	BIBR 1048 MS: Study for effects on embryo-fetal development in rats by oral administration (gavage).	00B056
Other studies		
U01-1430	BIBR 1048 MS: Dermal tolerance after single administration to rabbits.	01B016
U01-1458	BIBR 1048 MS: Acute eye irritation test after single administration to rabbits.	01B017
U02-1429	BIBR 1048 MS: Skin Sensitization study in the guinea pig.	1813/027-D6144

Reviewed under IND 65, 813, N028SX and N029SX (submitted 2/19/04)		
U05-1378	BIBR 1048MS: Maximum tolerated dosage study by oral gavage administration to Han Wistar rats for 13 weeks	BOI 277/ 032919
U05-1377	BIBR 1048MS: Maximum tolerated dosage study by oral gavage administration to CD-1 mice for 13 weeks	BOI-276/ 032942
U04-3479	BIBR 1048 MS: Dose range finding study by oral gavage administration to CD-1 mice for 2 weeks. (Draft report)	BOI-267/ 020215

Reviewed under IND 63, 267, N009 (submitted 1/18/05); N0021 (submitted 5/12/05)		
U04-1606	Influence of dabigatran (BIBR 953 ZW) on hERG-mediated potassium current in HEK293 cells	GP2004-0341-PH2
U04-1254	Plasma level and excretion balance after oral administration of [^{14}C] BIBR 1048 MS to the male mouse	A040/01UB (B1876)
U04-1913	Metabolism of BIBR MS and BIBR 953 ZW in mice	A394-03BC
U04-1627-01	Metabolism of BIBR MS 1048 MS and BIBR 953 ZW in rats (A289/01BC, A344/02BC)	B2314
U03-1157	Toxicokinetics of 4-week iv toxicokinetic study in Rhesus monkeys (B1328)	(b) (4) I572619

Reviewed under IND 63, 267, N040 (submitted 8/17/05)		
U05-1295	BIBR 1048 MS: Mutagenicity study using the mouse lymphoma (L5178Y) assay (supplementary study)	04B068
U05-1803	BIBR 1048 MS: Mutagenicity study using micronucleus analysis in rat bone marrow after oral treatment (supplementary study)	04B065

[Note: Unless otherwise indicated all oral doses of BIBR 1048 MS are expressed as the free base (BIBR 1048 BS).

4 Pharmacology

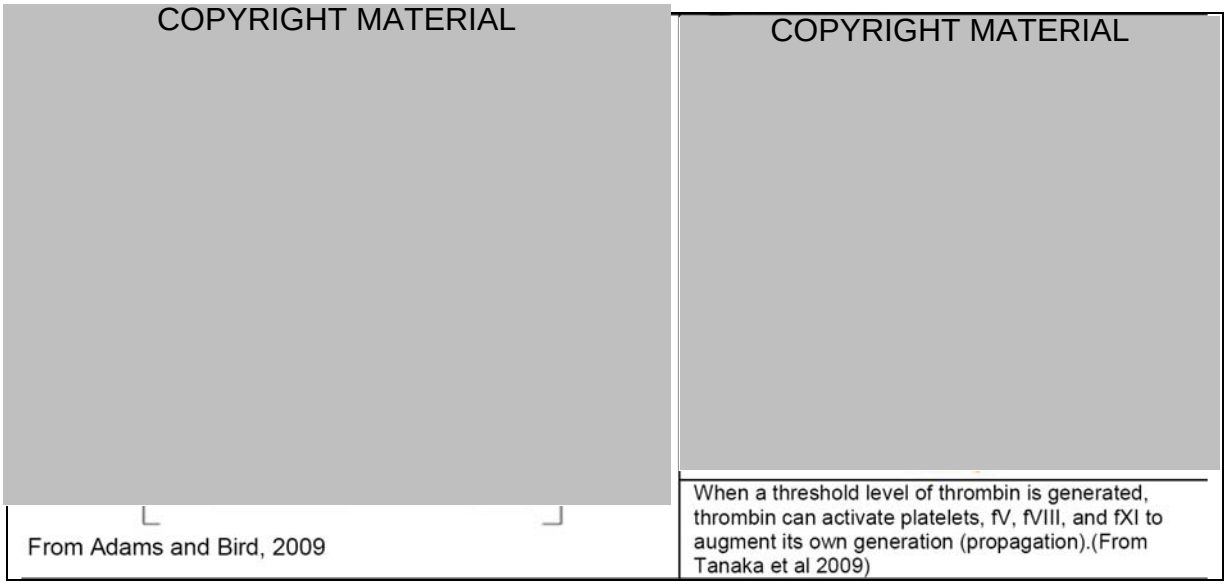
4.1 Primary Pharmacology

Target and mechanism of action:

BIBR 953 ZW is a synthetic, direct thrombin (Factor IIa, FIIa) inhibitor, which interacts with the active site in the catalytic domain of thrombin enzyme (Study P03-00770). Because BIBR 953 ZW has limited absorption and systemic availability after oral administration, its prodrug BIBR 1048 MS was developed for oral treatment.

Thrombin, a serine protease, cleaves fibrinogen to fibrin monomers, which results in blood coagulation. Thrombin, generated by the action of Factor Va on prothrombin, is central to both the extrinsic and intrinsic pathways of coagulation (Figure 2). Furthermore, when a threshold level of thrombin is reached, thrombin can activate platelets, FV, FVIII, and FXI to augment or propagate its own generation (Tanaka et al 2009).

Figure 2: Central Role of Thrombin in Hemostasis



However, thrombin also produces multiple effects on a variety of cell types, including platelets, endothelial and smooth muscles cells, primarily through protease-activated receptors (PARs) (Figure 3). Thrombin activates PAR-1 and PAR-4 with EC_{50} concentrations of 50 pM and 5 nM, respectively (Hirano 2007). FIIa has also been proposed to play a role in a variety of physiological and pathological processes (Couglin, 2005), including inflammation (Vergnolle et al 2001; Leung et al 2008), tissue repair (Ryaby et al 2006, Strukova 2001), neurite outgrowth (Suo et al 2004), atherosclerosis (Martorell et al 2008), and tumor cell metastasis (Nierodzik and Karpatkin 2006).

Figure 3: Cellular Effects of Thrombin

Receptor	Cells	Physiological role
PAR-1	Platelets* Endothelium SMCs Leukocytes	Platelet activation and thrombosis Embryogenic development Vasoregulation Tissue remodelling
PAR-2	Endothelium SMCs Leukocytes	Inflammation Vasoregulation
PAR-3	Platelets# Endothelium	Platelet activation and thrombosis in mice
PAR-4	Platelets Endothelium SMCs Leukocytes	Platelet activation and thrombosis Inflammation

From Matorelli et al 2008
mouse platelets

Thrombin's Effects on Endothelial and Smooth Muscle Cells

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From Hirano, 2007

Relationship of target to proposed indication:
Thrombin is the most potent activator of platelets (Davey and Luscher, 1967, Martin et al 1975). Platelet activation by thrombin is necessary for normal hemostasis (Sambrano et al 2001). Under pathophysiological conditions, thrombin-mediated platelet activation plays an important role in arterial thrombogenesis, which can trigger acute coronary syndromes and stroke. Additionally, cellular effects of thrombin are mediated through PAR-1, whose increased expression has been observed in atherosclerotic plaques from human arteries (Nelken et al 1992; Stoop et al 2000). The pathology of intimal hyperplasia in acute coronary syndrome patients following percutaneous coronary intervention is characterized by extensive smooth muscle cell proliferation.

Drug activity related to proposed indication:

In vitro studies

Specificity for the proposed target:
BIBR 953 ZW inhibited human thrombin (Factor IIa) with a Ki of 4.5 nM in *in vitro* assays [Study U00-1351]. To determine the specificity of BIBR 953 ZW, the Ki values for the human enzymes listed in Table 2 were determined by evaluating the inhibition of each enzyme at three substrate concentrations and three concentrations of BIBR 953 ZW. In contrast to the inhibition of thrombin, BIBR 953 ZW showed little inhibition of most other serine proteases involved in coagulation or fibrinolysis with selectivity of over 500-fold. However, the selectivity over trypsin with a Ki of 50 nM was only 11-fold. The amendment to Study U00-1351 included data indicating that BIBR 1048 BS (free base) at l00 µM only minimally inhibited thrombin (24.7%) and FXa (7.9%). However, the selectivity of BIBR 1048 MS against trypsin was not provided.

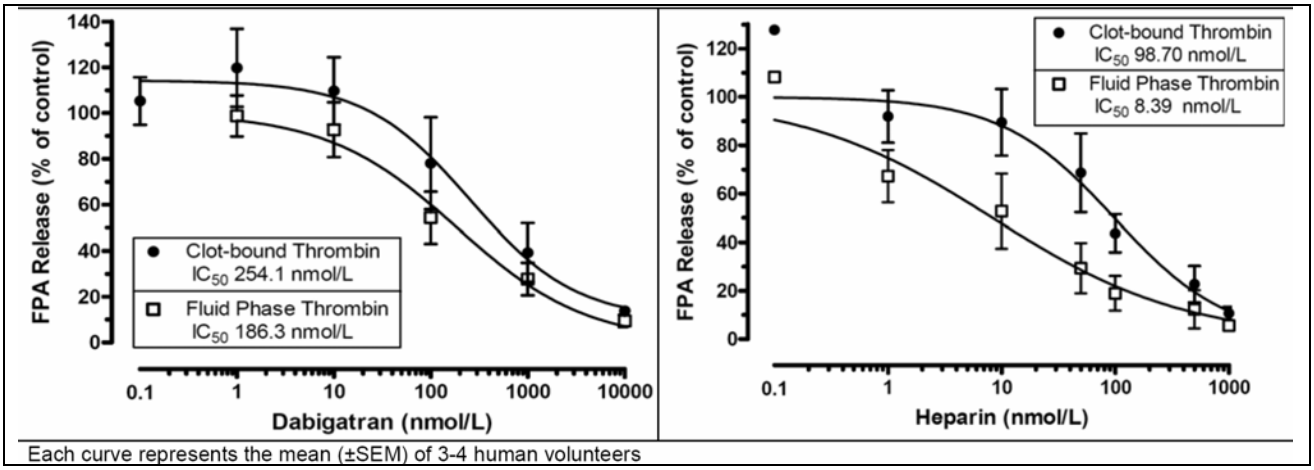
Table 2: Sponsor's Summary of Selectivity for Thrombin - Study U00-1351

Selectivity of BIBR 953 ZW toward purified human serine proteases *in vitro*			
Enzyme	**Ki ± SEM x10^{-9}mol/l**	**N**	**Ki ratio Enzyme/thrombin**
Trypsin	50.3±2.3	12	11
Thrombin	4.5±0.2	12	1
Factor Xa	3760±220	12	838
FactorVIIa/tissue factor complex	>66670	6	>14815
Factor XIa	3520±70	12	785
Plasma kallikrein	25890±500	12	5775
Plasmin	16950±650	12	3780
Two-chain urokinase	12580±170	12	2806
Tissue-type plasminogen activator	45360±910	12	10117
Activated protein C	20930±410	12	4668
Granulocyte elastase	>100000	6	>22222
C1s esterase	20200±1080	12	4506

The specificity of BIBR 953 ZW toward thrombin and trypsin is between that observed for other thrombin inhibitors. Gustafsson et al (1998) reported Ki values for melagatran of 2 and 4 nM for thrombin and trypsin, respectively. Teger-Nilsson et al (1997) reported a specificity profile towards trypsin of 45-fold for inogatran, with a Ki of 15 nM toward thrombin.

Study U07-1984 examined the ability of BIBR 953 ZW to inhibit thrombin located within a clot. When thrombin binds to fibrin strands in a clot via its exosite, the active site of thrombin is exposed. The high local thrombin concentrations within a clot can result in thrombus propagation. Fibrin bound thrombin is not available to inhibition by large heparin/anti-thrombin III complexes. The conversion of fibrinogen into fibrin by thrombin results in the release of fibrinopeptide A (FPA), which can be used as an indicator of the level of thrombin activity present. In Study U07-1984, increasing concentrations of inhibitor in triplicate were added to a fluid phase thrombin (a mixture of 20 pM thrombin in platelet poor plasma) or in clot-bound thrombin (platelet poor plasma containing a thrombus that was previously formed in 500 µL platelet rich plasma and washed 6 times over 20 hr to remove FPA). Three to four experiments with different human volunteers were performed per compound. After 1 hr incubation, plasma was removed without the clot. Uncleaved fibrinogen in the plasma was removed using bentonite and FPA levels were then assayed by ELISA. The results (Figure 4) indicate that the IC_{50} value of heparin for clot-bound thrombin (98.7 nM) was 10-fold higher than that for fluid phase thrombin (8.4 nM). In contrast, BIBR 953 ZW inhibited both clot-bound and fluid phase thrombin with similar IC_{50} values of 254 nM and 186 nM, respectively. It is unclear how the density or porosity of these in vitro clots compares to clots formed in vivo in veins and arteries.

Figure 4: Sponsor's Figures – Study U07-1984



Each curve represents the mean (±SEM) of 3-4 human volunteers

Effect on platelet aggregation

With human gel filtered platelets, BIBR 953 ZW inhibited thrombin-induced platelet aggregation with an IC_{50} of 10.4 nM [Study U01-1178]. Collagen-, ADP- or arachidonic acid-induced platelet aggregation was not inhibited by concentrations of up to 100 µM of BIBR 953 ZW. However, high concentrations (30 and 100 µM) of BIBR 953 ZW inhibited botrocetin-induced platelet aggregation by 33 and 57%, respectively. The sponsor concluded that BIBR 953 ZW inhibition of platelet aggregation was selective for thrombin, although its effect on platelet aggregation induced by other agonists, such as platelet activating factor, was not evaluated.

Effect on coagulation

The anticoagulant potential of BIBR 953 ZW was evaluated in three types of *in vitro* coagulation assays [Study U00-1457]. The ED_{200} or concentrations of BIBR 953 ZW required to double the clotting time in each assay was determined using plasma from seven species. BIBR 953 ZW prolonged the activated partial thromboplastin time (aPTT), the ecarin clotting time (ECT) and the prothrombin time (PT) in a dose dependent manner in human plasma with ED_{200} values of 230, 184, and 825 nM, respectively. The lowest ED_{200} values were obtained for human, rat and monkey plasma; the highest ED_{200} values were obtained for dog and rabbit plasma.

Table 3: From Sponsor's Tables – Study U00-1457 – Coagulation Assays

Calculated ED_{200} values of BIBR 953 ZW when added to plasma of different species

Species	ED 200 (µmole/L) of BIBR 953 ZW					
	APTT		ECT		PT	
	Mean	SEM	Mean	SEM	Mean	SEM
Human	0.230	0.021	0.184	0.004	0.825	0.066
Rat	0.460	0.023	0.098	0.021	0.557	0.058
Rhesus monkey	0.586	0.033	0.199	0.004	0.988	0.057
Guinea pig	0.906	0.159	0.090	0.008	3.110	1.238
Pig	1.008	0.035	0.030 *	0.011	1.450	0.030
Dog	1.816	0.112	0.111	0.006	2.462	0.321
Rabbit	1.905	0.312	0.155	0.068	4.571	0.387

* : ED100 because the ED200 was outside the tested dose range.

Pharmacologically active form

BIBR MS (BIBR BS) is a double pro-drug that is first hydrolysed by carboxyl esterases to two mono pro-drugs, BIBR 951 BS and BIBR 1087 SE and then subsequently hydrolyzed to BIBR 953 ZW as shown in Figure 5. Studies U05-2157 and U06-1711 evaluated the ability of BIBR 951 BS and BIBR 1087 SE to inhibit human thrombin and FXa and to prolong aPTT and thrombin times in human plasma. Table 4 summarizes the results in comparison to those of BIBR 953 ZW and BIBR 1048 BS. BIBR 1087 SE does not inhibit FXa and is a weak inhibitor of thrombin activity, since its IC_{50} is at least 400-fold less than BIBR 953 ZW. In contrast, BIBR 951 BS is only 4-fold less active against thrombin than BIBR 953 ZW and is only slightly less active in prolonging aPTT measurements. However, in the human ADME study U04-1378, only trace amounts (<0.3%) of BIBR 1087 SE and BIBR 951 BS were detected in plasma. Therefore, the sponsor concludes that BIBR 953 ZW is the active form in human plasma.

Figure 5: Sponsor's Diagram – Conversion of BIBR 1048 BS to BIBR 953 ZW

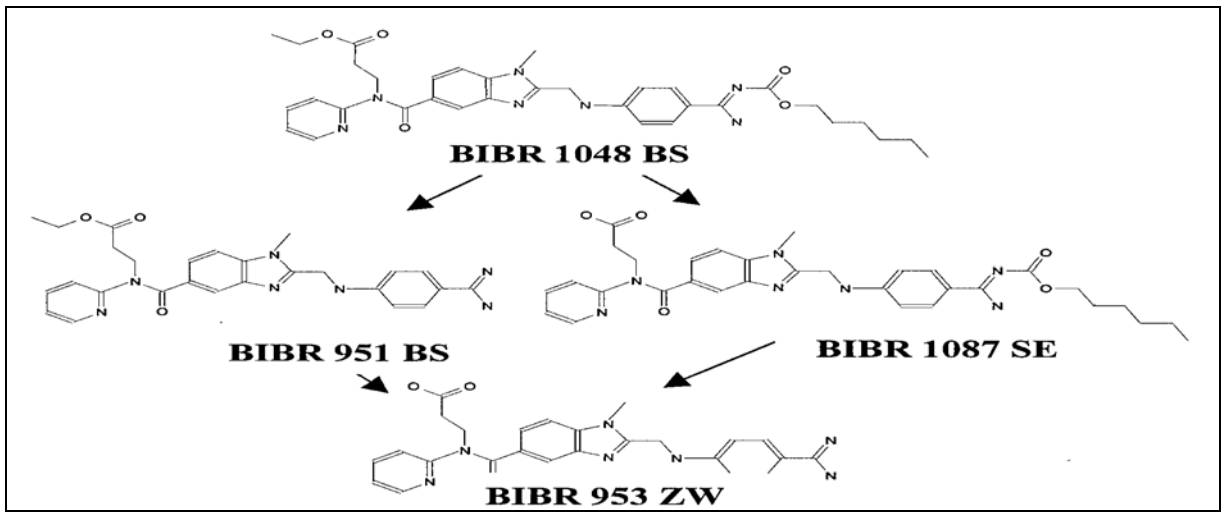


Table 4: Reviewer's Summary - Activity of Forms of Dabigatran

	Thrombin IC_{50}	FXa IC_{50}	ED_{200} aPTT	ED_{200} TT
BIBR 1048 BS	>100 µM	>>100 µM		
BIBR 951 BS	20.7 nM	1.36 µM	0.37 µM	0.04 µM
BIBR 1087 SE	1.96 µM*	> 100 µM		
BIBR 953 ZW	4.5 nM	3.76 µM	0.23 µM	

ED_{200} = effective dose to increase aPTT 200%
* Both reports are confusing because each indicates IC_{50} values of 1.96 and 2.96 µM for BIBR 1087 SE

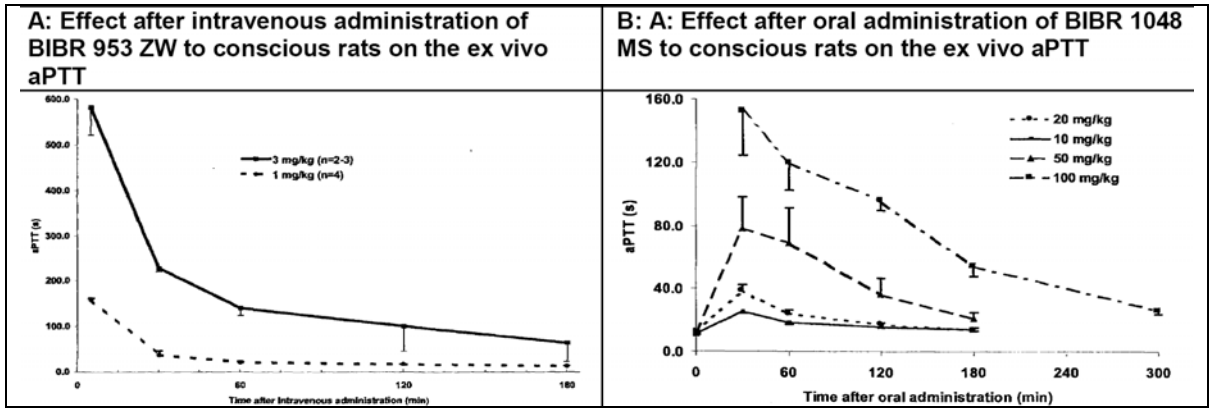
In vivo studies:

Pharmacodynamic profile of BIBR 953 ZW and BIBR 1048 MS

The oral bioavailability of BIBR 953 ZW is very low compared with that of BIBR 1048 MS. In rats intravenous administration of 1 mg/kg of BIBR 953 ZW induced a 15-fold prolongation of aPTT [Figure 6A], whereas intraduodenal administration of 20 mg/kg BIBR 953 ZW induced only a 1.4-fold prolongation of aPTT. However, oral

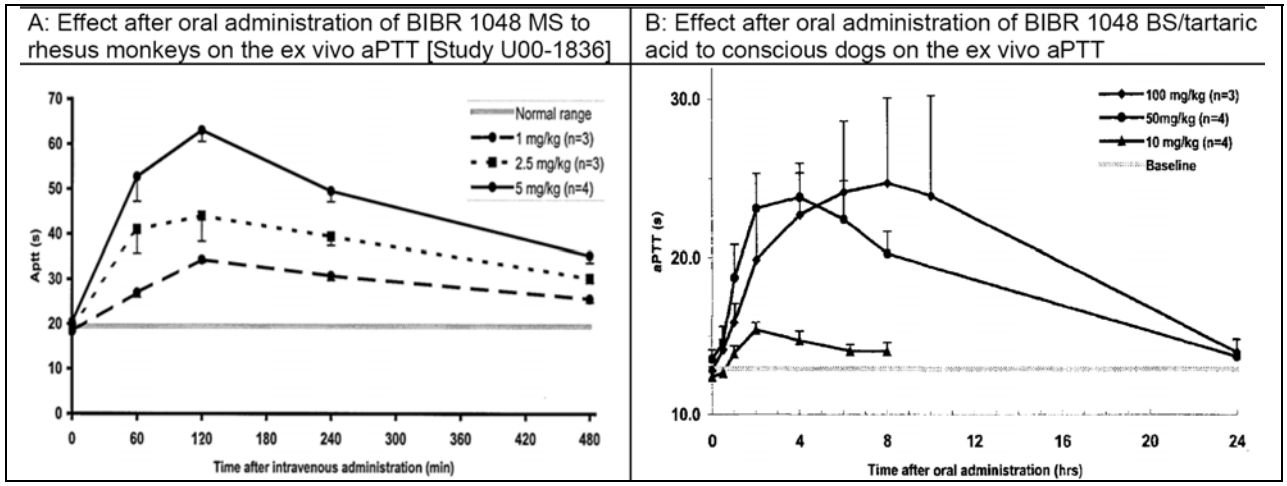
administration of 20 mg/kg BIBR 1048 MS induced a 3-fold increase in aPTT [Figure 6B, Study U00-1601].

Figure 6: Sponsor's Figures - Study U00-1601



Similar to the findings in rats, oral administration of BIBR 1048 MS, but not BIBR 953 ZW, induced a dose- and time-dependent prolongation of aPTT in Rhesus monkeys [Figure 7, Study U00-1836]. Oral administration of 5 and 20 mg/kg of BIBR 1048 MS induced a 3-fold prolongation of aPTT in Rhesus monkeys and rats, respectively. In contrast, oral administration of 50 or 100 mg/kg BIBR 1048 BS to dogs induced only a two-fold increase in aPTT relative to pre-dose values [Figure 7B, Study U00-1780]. Therefore, Rhesus monkeys were chosen as the non-rodent species to evaluate BIBR 1048 MS/953 ZW toxicity

Figure 7: Sponsor's Figures - Study U00-1836 and U00-1780



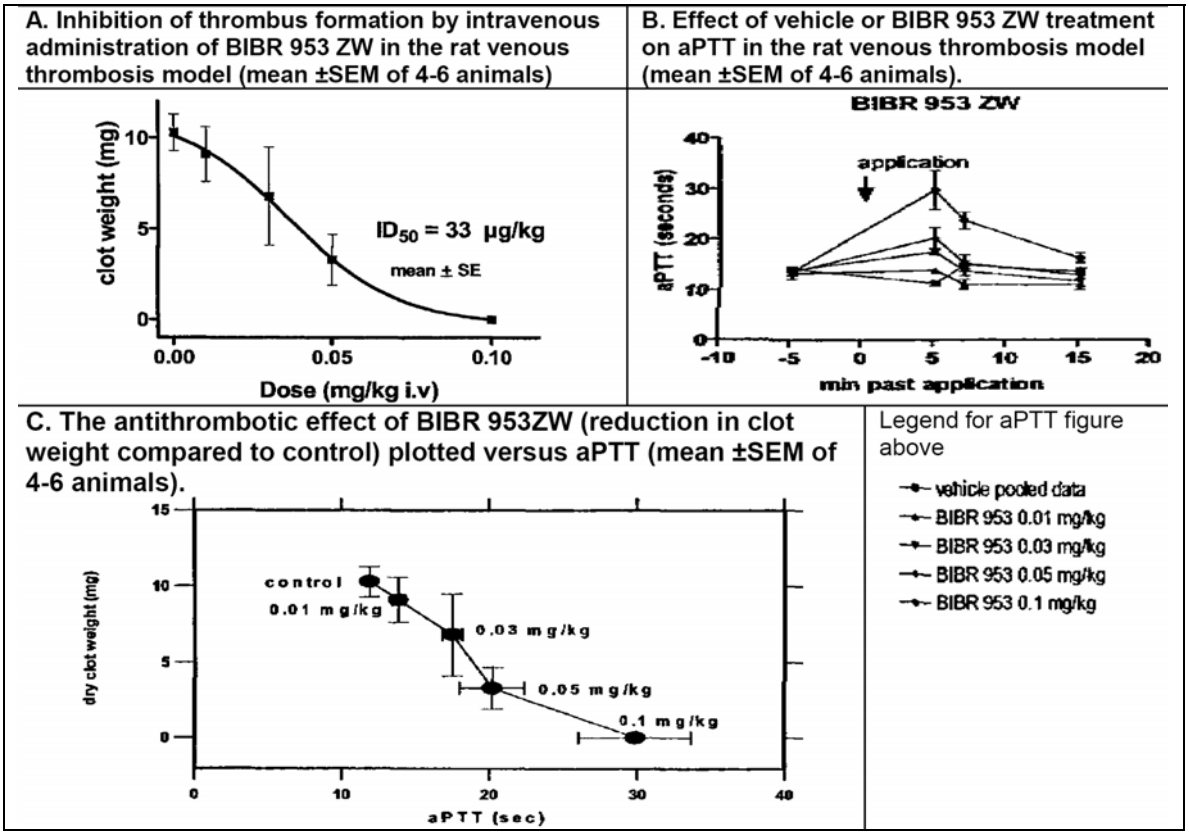
Antithrombotic efficacy of BIBR 953 ZW

The antithrombotic efficacy of BIBR 953 ZW was evaluated two animal models of venous thrombosis, but not arterial thrombosis, in rats and rabbits.

In a rat model of thromboplastin/stasis induced thrombosis (Herbert et al 1992, Berry et al 1998), BIBR 953 ZW was intravenously administered to anesthetized rats five minutes prior to injection of thromboplastin followed by ten minutes of stasis of the

abdominal vena cava (Study U00-1229). The thrombus was removed, dried and weighed. Figure 8A shows that BIBR 953 ZW induced a dose-dependent decrease in clot weight with an ID_{50} of 33 µg/kg. For comparison, other thrombin inhibitors, melagatran and argatroban showed ID_{50}'s of 122 and 586 µg/kg, respectively, for inhibition of clot weight in this model. Blood samples were collected immediately prior to and 5, 7 and 15 min after administration of BIBR 953 ZW for the measurement of activated partial thromboplastin time (aPTT). Figure 8B shows aPTT values were increased more than 2-fold at the dose of 0.1 mg/kg, a dose that completely inhibited clot formation. Figure 8C shows the inverse correlation observed between the increase in aPTT and clot weight with increasing doses of BIBR 953 ZW. The sponsor concluded that a correlation exists between the anticoagulant and the antithrombotic effect of BIBR 953 ZW in this rat model of venous thromboembolism.

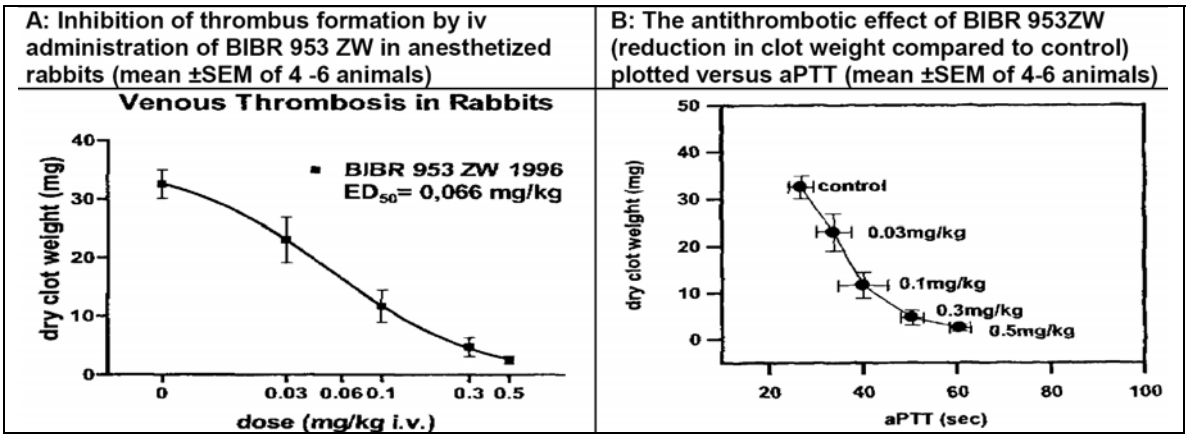
Figure 8: Sponsor's Figures – Study U00-1229



A rabbit model of thrombosis induced by endothelial damage combined with blood flow reduction (Matthiasson et al 1995) was used in Study U00-1288 to evaluate the in vivo effects of BIBR 953 ZW. Compounds were intravenously administered to anesthetized rabbits 2.5 minutes after endothelial damage to the isolated vein with 0.5% policanol. Following removal of policanol 2.5 minutes later, the veins were declamped and narrowed by means of a ligature. Twenty-five minutes after the veins were decamped, the veins were opened and the clots removed and weighed. Figure 9A shows that BIBR 953 ZW induced a dose-dependent decrease in clot weight with an ED_{50} of 66 µg/kg. For comparison, melagatran, argatroban showed ED_{50}'s of 58, 247 µg/kg, respectively.

Blood samples for aPTT measurement were collected prior to and 2, 5, 15 and 30 min after compound or vehicle administration. As observed with the rat venous thrombosis model, an inverse correlation was obtained between aPTT and the clot weight (Figure 9B) in the rabbit model of venous thrombosis.

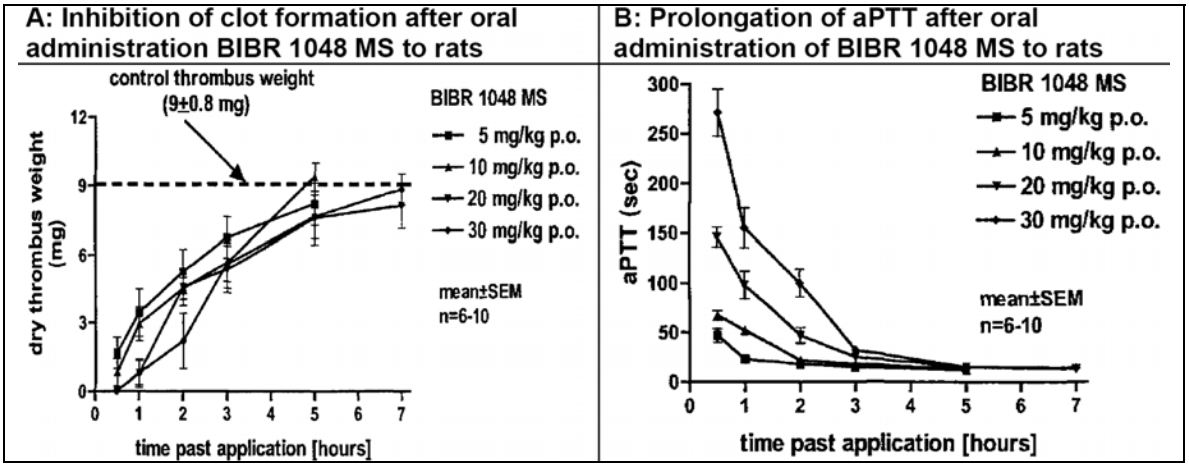
Figure 9: Sponsor's Figures - Study U00-1288



Oral antithrombotic efficacy of BIBR 1048 MS

In Study U00-1785, BIBR 1048 MS was administered orally to conscious rats as single doses ranging from 5 to 30 mg/kg. At various times after administration, thrombosis was induced in the abdominal vena cava with tissue factor and stasis (Herbert et al 1992; Berry et al 1998). Figure 10A shows that inhibition of clot formation was observed 30 min after administration of all 4 doses of BIBR 1048 MS. Significant inhibitions of clot formation was observed for at least 2 hours after dosing. Subsequently, clot formation gradually approached that in control animals between 5 to 7 hours after dosing. Coagulation times (aPTT) were prolonged fold in blood samples collected immediately prior to thrombus induction from 0.5 to 2 hours after dosing (Figure 10B). BIBR 1048 MS induce a dose and time dependent antithrombotic effect that inversely correlated with anticoagulation after oral administration in a rat model of venous thrombosis.

Figure 10: Sponsor's Figures – Study U00-1785



In Study U06-1567, BIBR 1048 MS was administered orally as single doses ranging from 1 to 20 mg/kg to conscious rabbits. Two hours after administration the rabbits were anesthetized and venous thrombosis was induced in the isolated jugular veins by endothelial damage with policanol followed by blood flow reduction (Matthiasson, et al 1995). Blood was collected for aPTT and thrombin time measurements at the time of thrombus collection 150 min after drug administration. Dose-dependent inhibition thrombus formation was accompanied by a prolongation of the thrombin time (TT) and activated partial thromboplastin time (aPTT). At the dose of 10 mg/kg clot formation was inhibited by 97% corresponded to a 2.2 fold prolongation of aPTT values.

In a second set of experiments, rabbits were pre-treated with 10 mg/kg BIBR 1048 MS for varying periods (1, 2, 3, 5, 7 and 24 hr) prior to induction of venous thrombosis. Clot formation was inhibited more than 70% up to 5 hours after drug administration when aPTT values were prolonged 1.5 fold. Clot formation gradually restored with time and was comparable to the vehicle treated animals 24 hours after drug administration.

Table 5: Sponsor's Summaries – Rabbit Venous Thrombosis – Study U06-1567

A. Effect of BIBR 1048 MS on thrombus weight and ex vivo thrombin time and aPTT at 2 hours after a single oral administration with different doses.

	BIBR 1048 MS (oral administration)					
	Vehicle	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg
thrombus dry weight [mg]	38.2 ± 2.3 (9)	40.0 ± 8.4 (4)	21.5 ± 7.3 (4)	16.2 ± 5 (6)*	0.9 ± 0.7 (4) *	0.0 ± 0.0 (3) *
Thrombin time [seconds]	13.8 ± 0.7 (9)	14.2 ± 1.2 (4)	75.3 ± 11.2 (4)	77.6 ± 21.6 (6)	639 ± 128 (4)*	794 ± 66 (3)*
aPTT [seconds]	20.8 ± 1.0 (9)	27.6 ± 2.6 (4)	29.8 ± 2.5 (4)	31.1 ± 3.9 (6)	46.2 ± 4.8 (4)*	55.0 ± 8.8 (3)*

B. Time-dependent effect of BIBR 1048 MS on thrombus weight and ex vivo thrombin time and aPTT after a single oral dose of 10 mg/kg.

	BIBR 1048 MS [10 mg/kg p.o]					
Vehicle 2 hours	1 h	2 h	3 h	5 h	7 h	24 h
38.2 ± 2.3 (9)	1.9 ± 1.3 (4)*	0.9 ± 0.7 (4)*	2.7 ± 1.8 (4)*	10.4 ± 2.3 (5)*	18.5 ± 4.4 (6)*	32.8 ± 2.7 (4)
13.8 ± 0.7 (9)	438 ± 81 (4)*	639 ± 128 (4)*	272 ± 64 (4)*	222 ± 39 (5)*	189 ± 43 (6)*	14.2 ± 1.2 (4)
20.8 ± 1.0 (9)	40.4 ± 4.2 (4)*	46.2 ± 4.8 (4)*	28.8 ± 3.2 (4)	32.4 ± 2.5 (5)*	33.0 ± 3.2 (6)*	22.5 ± 2.0 (4)

Values are presented as the mean + SE with number of animals in parentheses. * denotes significantly different ($p<0.05$, Dunnett´s test) thrombus dry weight, thrombin time or aPTT compared with the vehicle treated group.

Comparison of BIBR 1048 MS and BIBR 1048 BS

The pharmacodynamic activity of the free base (BIBR 1048 BS) and the methanesulfonate salt (BIBR 1048 MS) was compared after oral administration to rats (Study U02-1700). Single doses of 70 mg/kg BIBR 1048 BS and 80.5 mg/kg BIBR 1048 MS (equivalent to 70 mg/kg free base) were administered as suspensions solution in phosphate-buffered 0.5% hydroxyethylcellulose. Blood was collected 24 hours after dosing, plasma prepared and assayed for thrombin time, aPTT and PT. Although only slight effects were observed on APTT and PT, thrombin times increased 2.2 and 3.3-fold following BIBR 1048 BS administration and 1.3 and 2.5-fold following BIBR 1048 MS administration in males and females, respectively (Table 6). These results suggest higher absorption of BIBR 1048 BS compared to BIBR 1048 MS; however, plasma levels of BIBR 953 ZW were not measured to verify absorption.

Table 6: Sponsor's Summary - Study U02-1700

Comparison of BIBR 1048 MS and BIBR 1048 BS

Male					Female				
Parameter		TT [s]	APTT [s]	PT [s]	Parameter		TT [s]	APTT [s]	PT [s]
Group					Group				
control	n	6	6	6	control	n	6	6	6
	mv	38.88	14.83	16.33		mv	38.60	15.00	15.33
	sd	1.08	1.79	0.95		sd	1.12	0.51	0.39
BIBR 1048 MS	n	6	6	6	BIBR 1048 MS	n	6	6	6
80.5	mv	51.63	15.28	16.15	80.5	mv	95.83	17.10	16.12
[mg/kg]	sd	18.83	1.53	1.11	[mg/kg]	sd	39.86	2.20	1.04
	p	0.5762	0.6491	0.7552		p	* 0.0191	* 0.0463	0.1155
BIBR 1048 BS	n	6	6	6	BIBR 1048 BS	n	6	6	6
70	mv	87.08	15.97	16.97	70	mv	126.83	17.83	16.77
[mg/kg]	sd	64.23	1.71	0.93	[mg/kg]	sd	51.84	1.82	0.87
	p	* 0.0474	0.2605	0.2900		p	* 0.0011	* 0.0103	* 0.0080

Effect of BIBR 953 ZW on the template bleeding time in rats *in vivo*

Template bleeding time was assessed in anesthetized rats using a spring-loaded blade device for standardized induction of bleeding by means of a defined incision of the tail (Study U00-1588). Each rat served as its own control and bleeding time was determined at different intervals after intravenous administration of BIBR 953 ZW. Blood samples for aPTT determination were collected from the same animals prior to and 15, 30, 60 and 120 min after compound or vehicle administration. Table 7A shows that template bleeding time was prolonged in a dose dependent manner with statistical significance first reached at 0.5 mg/kg. At 15 minutes after administration, 0.3 mg/kg BIBR 953 ZW induced both a 2-fold prolongation of bleeding time and a 2-fold prolongation of aPTT. However, since Study U00-1229 (Figure 8) showed that intravenous BIBR 953 ZW inhibited clot formation in a rat venous thrombosis model with an ED_{50} 0.033 mg/kg, the sponsor maintains that significant prolongation of the bleeding time was observed only at doses more than 9 times higher than the ED_{50} dose for antithrombotic efficacy in the rat venous thrombosis model.

Table 7: Sponsor's Tables - Study U00-1588

A: Effect of vehicle or BIBR 953 ZW on tail bleeding time (seconds after tail incision) in anesthetized rats at different time points after administration.

Bleeding time [seconds]	Pre-dose	15min	30min	45min	60min	90min	120min
Vehicle	110±6 (11)	113±9 (11)	115±11 (11)	113±8 (11	108±7 (11)	108±5 (11)	107±7 (10)
0.1 mg/kg	108±7 (5)	114±14 (5)	117±6 (5)	102±9 (5)	81±6 (5)	117±20 (5)	75±8 (5)
0.3 mg/kg	135±8 (8)	223±37 (8)	178±21 (8)	111±7 (8)	114±13 (7)	96±9 (7)	107±9 (7)
0.5 mg/kg	109±6 (8)	653±164 *(8)	152±9 (7)	133±20 (8)	135±25 (7)	111±22 (7)	92±4 (7)
1.0 mg/kg	108±8 (11)	*4125 ±735 (3)	*3341 ±881 (4)	840±144 *(4)	336±77 *(5)	141±8 (7)	132±13 (11)

* denotes p<0.05 vs the time-matched vehicle-treated group (Dunnett´s test).

B: Effect of vehicle or BIBR 953 ZW on aPTT in anesthetized rats at different time point after administration.

aPTT [seconds]	Pre-dose baseline	15min	30min	60min	120min
Vehicle	17±0.5 (9)	13±1.0 (10)	12±0.7 (9)	11±0.7 (8)	11±0.7 (8)
0.1 mg/kg	15±1.1 (5)	18±1.3 (5)	13±1.2 (5)	13±0.5 (5)	13±0.5 (5)
0.3 mg/kg	16±1.0 (8)	*31±2.7 (8)	*23±1.9 (8)	*18±1.6 (7)	15±1.3 (7)
0.5 mg/kg	16±0.5 (8)	*34±2.2 (8)	*21±1.1 (8)	16±0.8 (7)	14±0.6 (7)
1.0 mg/kg	§13±0.5 (11)	*76±2.6 (11)	*38±1.9 (11)	*21±1.6 (11)	13±1.2 (11)

§ denotes p<0.05 in pre-dose value vs the other experimental groups (Tukey´s test)
* denotes p<0.05 vs the time matched vehicle treated group (Dunnett´s test).

Values are presented as mean ±SE with the number of animals at a given time point in parentheses.

4.2 Secondary Pharmacology

BIBR 953 ZW has high selectivity over almost all of the serine proteases examined, except for trypsin. However, interaction between BIBR 953 ZW and trypsin is not likely to have an adverse effect. Under normal physiological conditions, trypsin is enzymatically active only in the upper part of the small intestine and not in plasma, where BIBR 953 ZW exerts its antithrombotic effect. However, the pro-drug, BIBR 1048 MS, will be present in the gastrointestinal tract, where trypsin acts on chymotrypsinogens, pro-elastase and pro-carboxypeptidases. However, the selectivity of BIBR 1048 MS for trypsin or other intestinal peptidases has not been evaluated.

In vitro binding of BIBR 953 ZW and BIBR 1048 MS to a panel of 80 physiologically important receptors (U09-1126) was tested at a single concentration of 10 µM (b) (4) study 8810441). Binding of reference compounds to these receptors was minimally inhibited by BIBR 953 ZW with the greatest inhibition being 30 and 29% to the 5HT7 and 5HT2b receptors, respectively. In contrast, BIBR 1048 MS inhibited the binding of reference compounds to the 17 receptors listed in Table 8. A second study ((b) (4) Study 8810448) was conducted to determine the IC_{50} values for BIBR 1048 MS binding. All of the IC_{50} values are 0.67 µM or higher. Since little BIBR 1048 MS (up to 10 nM) was observed in human plasma (Study U09-1052), the sponsor maintains that interactions of BIBR 1048 MS with these receptors are not physiologically relevant. However, calcium and chloride channels are involved in the function of the gastrointestinal tract (Curro 2010; Fry et al 2006; Ambizas and Ginzburg 2007; Lacy and Levy 2007), where high concentrations of BIBR 1048 MS will exist at least transiently. These interactions may be related to the significantly decreased gastric emptying in rats following oral administration of 300 mg/kg BIBR 1048 MS (see Section 4.3 Safety Pharmacology).

Table 8: From (b) (4) Study 8810448

(b) (4)

The direct inhibition of thrombin by BIBR 953 ZW will result not only in inhibition of clot formation, but also will prevent the activation of the various thrombin sensitive protease activated receptors (PAR-1, PAR-3, PAR-4) present not only in the membranes of platelets, but also membranes of endothelial cells, smooth muscle cells and monocytes. Inhibition of PAR-1 activation could be beneficial in inhibition of intimal hyperplasia in atherosclerotic plaques. However, the sponsor has not presented any data either in vitro or in vivo to address this possibility. Local administration of another thrombin inhibitor, argatroban, was found to inhibit restenosis after ballon angioplasty (Imanishi et al 1997; Itoh et al 2004).

4.3 Safety Pharmacology

Reports for a total of 19 secondary pharmacology studies were submitted and reviewed with the original submission (N000) to IND 65,813. Detailed reviews of ten studies with BIBR 953 ZW and nine studies with BIBR 1048 MS examining acute neurological, cardiovascular, renal, gastrointestinal and respiratory effects are provided in the IND review dated 9/24/03. Subsequently, a study report for the evaluation of BIBR 953 ZW on hERG-mediated potassium current (U04-1606) was submitted and reviewed under IND 63, 267 (N009, 1/18/05); N0021, 5/12/05). The following section summarizes the previously reviewed studies by physiological system and includes reviews of the two safety pharmacology studies in the NDA that were not previously reviewed.

Central nervous system*:*

General behavior in mice assessed with a modification of the Irwin system was only slightly affected (e.g. decrease of grip strength in males) 20 minutes after intravenous doses of 10 and 30 mg/kg BIBR 953 ZW [U98-2503]. Intravenous administration of BIBR 953 ZW (3, 10 or 30 mg/kg) or oral administration of BIBR 1048 MS (30, 100 and 300 mg/kg) had no significant effect on exploratory motility in conscious rats [U98-2534, U98-2397]. BIBR 1048 MS when given orally in doses of 100 or 1000 mg/kg was associated with slight, reversible effects on the grasping and landing reflex and a decreased reaction time in the hot plate test [U98-2502]. In addition, oral administration of BIBR 1048 MS (30, 100 and 300 mg/kg) had no effect on hexobarbital-induced sleeping time in rats [U98-2408].

Study title: BIBR 1048 MS: Modified Irwin test in the male and female rat, including body temperature and short-term locomotor activity – single oral (by gavage) administration

Study no.:	04B082 (U05-1533)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	May 6, 2004
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, purity: 98.094 %

Methods:
The effects of BIBR 1048 MS on the central nervous system were evaluated following gavage administration of single oral doses of 34.59, 115.3 and 345.9 mg/kg BIBR 1048 MS (equivalent to 30, 100 and 300 mg/kg BIBR 1048 BS, respectively) to 4 male and 4 female Han Wistar rats. General behavior, physiological state and neurological observations were made in a modified Irwin test (Irwin, 1968) along with evaluations of body temperature and short-term locomotor activity. On the day of treatment, these observations were made before and at 2, 4, and 24 hours after treatment. Specific assessments included cage observations for skin color, explorative behavior, respiration, piloerection, body position, and stereotypy as well as general observations for appearance, increased secretion, defecation and urination, vocalization, aggression, ptosis, convulsions and tremor. Assessments outside the cage included the pinna reflex, corneal reflex, flexor reflex, foot and tail pinch tests, landing reflex, grasping reflex, traction test, two equilibrium tests, rigidity, righting reflex, gait/assessment and abdominal tone.

Results:
The low and mid doses of BIBR 1048 MS did not induce significant effects on behavior, reflexes, and physiological state including body temperature and short-term locomotor activity. However, body temperature was slightly, but significantly, decreased in animals at 4 and 24 hours after treatment with 300 mg/kg BIBR 1048 MS.

Table 9: From Sponsor's Body Temperature Tables – Study U05-1533

Absolute values - comparison of treatment values to control values

Time [Day before adm.]	Treatment	n	median	Min	Max	comparison versus Control [p value]
1d before	Control	8	37.9	37.0	38.3	-
1d before	Low-Dose	8	38.3	36.9	38.6	0.2219
1d before	Mid-Dose	8	38.4	37.5	39.3	**0.0455**
1d before	High-Dose	8	38.6	38.0	38.8	**0.0113**

Change from baseline values - comparison of treatment values to control values

Time [Day hours after adm.]	Treatment	n	median	Min	Max	comparison versus Control [p value]
1d 120 min	Control	8	0.40	-0.90	1.30	-
1d 120 min	Low-Dose	8	0.10	-0.60	0.50	0.3117
1d 120 min	Mid-Dose	8	-0.20	-1.00	0.30	0.0674
1d 120 min	High-Dose	8	-0.20	-0.50	0.40	0.0841
1d 240 min	Control	8	0.15	-1.00	1.00	-
1d 240 min	Low-Dose	8	-0.20	-1.50	1.10	0.4263
1d 240 min	Mid-Dose	8	-0.20	-1.30	1.60	0.3657
1d 240 min	High-Dose	8	-0.45	-1.20	0.10	**0.0348**
1d 24h	Control	8	-0.05	-0.50	1.80	-
1d 24h	Low-Dose	8	0.10	-1.40	0.80	0.6645
1d 24h	Mid-Dose	8	-0.35	-1.40	0.80	0.1691
1d 24h	High-Dose	8	-0.90	-1.70	0.70	**0.0224**

Cardiovascular effects:

In vitro effects on the HERG channel

Study report U04-1606 was reviewed under IND 63, 267, N009 and N021). The in vitro effect of BIBR 953 ZW on potassium ionic currents was evaluated in human embryonic kidney (HEK-293) cells that express the human gene hERG. Four concentrations (1, 3, 10, and 30 uM) of BIBR 953 ZW had no effects on hERG potassium current; however, the positive control, dofetilide, inhibited the hERG current with IC_{50} of 0.023 μM.

In vitro effects on action potential

BIBR 953 ZW had no effect on action potential duration and contractile force in isolated guinea pig papillary muscles at concentrations up to 10 μM [U99-1732]. However, no positive control was used to demonstrate the laboratory's ability to detect changes.

In vivo cardiovascular effects

BIBR 953 ZW at intravenous doses up to 3 mg/kg had no influence on cardiovascular parameters in anesthetized pigs and BIBR 953 ZW at intravenous doses of 10 and 30 mg/kg produced significant effects on diastolic blood pressure, maximal left ventricular pressure, maximal left ventricular dP/dt, and femoral artery blood flow, but not QT or PQ intervals in pigs [U98-2414]. However, intravenous administration of BIBR 953 ZW up to 10 mg/kg had no effect on systolic arterial pressure and heart rate in rabbits [U99-1211]. Furthermore, oral administration of BIBR 1048 MS (30, 100 or 300 mg/kg) did not affect heart rate and blood pressure in conscious rats [U99-1344]. ECG parameters, including the QT interval, were observed in the 26-week toxicology study in monkeys.

Respiratory effects:
Intravenous administration of BIBR 953 ZW up to 10 mg/kg to anesthetized rabbits did not influence respiratory function as assessed by respiratory rate, peak inspiratory flow, peak expiratory flow, and tidal volume [U99-1211].

Study title: BIBR 1048 MS: Evaluation of respiratory parameters in the conscious male rat, using whole body bias flow plethysmography - single oral (by gavage) administration

Study no.:	04B112 (U06-1056)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	July 16, 2004
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, purity: 98.094 %

Methods:
Doses of 0, 34.59, 115.3 and 345.9 mg/kg BIBR 1048 MS (equivalent to 0, 30, 100 and 300 mg/kg BIBR 1048 BS, respectively) in 0.5% hydroxyethylcellulose were administered to 8 male rats per group. Based on the pretest respiratory rates prior to the day of dosing, rats were allocated into study groups so the mean respiratory rate was approximately equal in the four groups. On the test day animal were placed in the plethysmograph for habituation for 60 minutes prior to acquisition of pre-dose respiratory rate, tidal volume and minute volume values for 15 minutes. The animals were removed from the plethysmograph, dosed with the appropriate formulation by oral gavage, and returned to the plethysmograph for measurement of respiratory rate, tidal volume and minute volume for a period of at least 7.5 hours. Median values were calculated for consecutive 5 minute intervals. After taking into account individual pre-dose baseline levels, a standardized area under the curve (AUC divided by interval length) was calculated for three periods (0.75-3, 3-5, and 5-7.5 hours after dosing). The first 45 minutes after administration were excluded from evaluation to eliminate an effect of the administration procedure.

Results:
The sponsor concluded that BIBR 1048 MS up to 300 mg/kg as free base equivalent did not exhibit any consistent effect on respiratory rate, tidal volume and minute volume in comparison to the control group. However, several statistically significant changes in each parameter were noted, when the data were evaluated relative to the pre-dose mean for each parameter (Table 10).

First, the low dose group showed a significant increase in tidal volume at 0.75 to 3 hours post dosing. Since this increase in tidal volume in the low dose group did not show either a dose dependency or time persistence, this change was not considered toxicologically relevant.

Second, the high dose group showed a significant increase in respiratory rate at 3 to 5 hours post dosing, a period when the mean increase in respiratory rate exhibited a dose

dependency. During the first two time periods (0.75 to 3 hours and 3 to 5 hours post dosing), one and two animals in the mid and high dose groups, respectively, had mean values exceeding the maximum value in the control group (116.5, 114.8% of pre-dose).

Third, a significant increase in minute volume was observed in the mid and high dose groups at 3 to 5 hours post dosing. The sponsor argues that this increase in minute volume is 1) a consequence of calculating the minute volume from tidal volume and respiratory rate where the latter was increased at both doses and 2) the lack of a dose-dependency on tidal volume indicates this change was not considered toxicologically relevant.

To evaluate these results, the reviewer examined the individual animal values. Table 11 summarizes the ranges of the absolute values for each parameter by dose group. In general, the ranges of values in the treated groups were within or close to either the range of pre-dose values or the range in the control group. However, one low dose animal, number 202, had values outside these reference ranges at the end of the monitoring period (26700 – 27600 sec). This event in this animal involved first increases in respiratory rate and minute volume around 7.5 hr after dosing, followed by decreases in tidal volume and minute volume. However, the event occurred long after the Tmax of 0.5 hr in rats. Since no animals in the mid and high dose groups exhibited these changes, the event is not dose-related and appears to be an isolated incident.

Table 10: Modification of Sponsor's Tables – Study U06-1056

Values are % of pre-dose value			Dose of BIBR 1048 MS [mg/kg] as base			
			0	30	100	300
Respiratory rate	0.75-3 hr	n	8	8	8	8
		Mean	97.66	93.23	108.94	101.78
		SD	18.35	16.05	8.45	12.94
		Max.	116.7	108.4	118.4	129.7
	3-5 hr	n	8	7	8	8
		Mean	89.74	93.59	104.78	107.78
		SD	16.82	17.09	13.81	15.85
		P value	-	0.6432	0.0692	**0.0314**
		Max.	114.8	117.3	122.2, 123.2	118.5, 136.5
	5-7.5 hr	n	8	7	8	8
		Mean	96.13	93.58	102.77	104.49
		SD	21.96	23.66	13.81	10.30
		Max.	124.5	133.5	123.8	122.8
Tidal volume	0.75-3 hr	n	8	8	8	8
		Mean	94.19	101.46	100.31	99.27
		SD	6.06	6.27	5.72	8.49
		P value	-	**0.0392**	0.0791	0.1417
	3-5 hr	n	8	7	8	8
		Mean	93.17	102.05	98.39	97.31
		SD	4.31	9.00	6.20	14.62
	5-7.5 hr	n	8	7	8	8
		Mean	93.20	100.50	101.96	96.81
		SD	5.27	7.78	7.36	20.89

Values are % of pre-dose value			Dose of BIBR 1048 MS [mg/kg] as base			
			0	30	100	300
Minute volume	0.75-3 hr	n	8	8	8	8
		Mean	90.55	92.50	103.46	98.80
		SD	18.88	16.83	13.01	8.09
		Min.	61.5	77.2	86.0	89.2
		Max.	112.9	113.2	114.0, 124.6	110.9
	3-5 hr	n	8	7	8	8
		Mean	81.83	93.55	97.41	98.83
		SD	15.90	21.18	13.55	6.91
		P value	-	0.1429	**0.0475**	**0.0317**
		Min.	57.9	75.5	76.5	89.5
		Max.	99.3	105.9, 131.3	110.6, 114.2	101.8, 113.2
	5-7.5 hr	n	8	7	8	8
		Mean	86.59	92.41	98.80	97.30
		SD	17.96	26.76	14.74	15.35
		Min.	57.8	62.1	82.8	67.1
		Max.	107.	138.7	123.8	113.2

Table 11: Reviewer's Summary of Ranges of Individual Values – Study U06-1056

		Dose of BIBR 1048 MS			
Parameter	Time period	0	30	100	300
Minute Volume (mL/min)	Pre-dose	182-604	161-446	159-643	164-435
	0.75-3 hr	126-525	159-464	140-455	156-459
	3-5 hr	131-424	168-513	152-444	154-485
	5-7.5 hr	141-475	151-416 (871*)	151-396	156-406
Tidal Volume (mL)	Pre-dose	1.56-5.16	1.17-4.66	1.72-4.38	1.41-2.81
	0.75-3 hr	1.48-2.81	1.64-2.81	1.56-2.73	1.33-2.58
	3-5 hr	1.41-2.73	1.48-2.73	1.56-2.66	1.02-2.81
	5-7.5 hr	1.41-2.73	0.94-2.73	1.56-2.73	0.94-2.73
Respiratory rate (breaths/min)	Pre-dose	89-266	89-252	87-218	91-227
	0.75-3 hr	89-230	86-229	84-228	84-232
	3-5 hr	85-223	84-227	76-222	83-229
	5-7.5 hr	89-217	81-247 (277*)	83-215	80-223
	Time relative to dosing				
* Animal 202	Pre-dose	26700 sec	27000 sec	27300 sec	27600 sec
Minute volume	161-260	625	871	31	31
Tidal volume	1.33-2.19	1.80	0.94	0.23	0.23
Respiratory rate	96-165	247	277	167	192

Renal effects:

Intravenous administration of BIBR 953 ZW doses up to 1 mg/kg did not affect renal function in conscious female dogs. However, an intravenous dose of 3 mg/kg was associated with slightly decreased excretion of sodium and chloride and slightly increased excretion of potassium, glucose and microprotein [U99-1273]. In contrast, the oral administration of BIBR 1048 doses up to 10 mg/kg did not affect renal function in

dogs [U98-2501]. However, oral absorption of BIBR 1048 MS in dogs was very low (U98-2799].

Gastrointestinal effects:
All intravenous doses of BIBR 953 ZW from 0.1 to 1 mg/kg slightly, but not significantly decreased gastric emptying in rats [U99-1382]. Additionally, gastric emptying in rats was decreased in a dose dependent manner by single oral administration of BIBR 1048 MS doses from 30 up to 300 mg/kg. However, the inhibition was only statistically significant at the highest dose [U98-2030]. The mechanism of inhibition of gastric emptying was not investigated. However, the effects on gastric emptying may be related to BIBR 1048 MS inhibition of chloride and calcium channels (Table 8).

Gastric secretion in the conscious rat was not significantly affected by intravenous administration of BIBR 953 ZW up to 1 mg/kg [U99-1220]. However, intra-duodenal administration BIBR 1048 MS slightly increased gastric secretion at 10 mg/kg, but not at 30 or 100 mg/kg (U99-1371).

Gastrointestinal transit was not significantly affected after intravenous administration of 0.1 to 1 mg/kg of BIBR 953 ZW [U99-1380] or oral administration of 30 to 300 mg/kg of BIBR 1048 MS in rats [U97-2804]. In addition, in the isolated guinea pig ileum, BIBR 953 ZW (10^{-9} to 10^{-7} mol/L) did not affect contractions in the absence of agonists or contractions induced by histamine, acetylcholine, serotonin, and barium chloride [U99-1658].

Abuse liability:
No specific studies addressed abuse liability. However, the abuse liability for BIBR 1048 MS/953 is expected to be low, since it has limited distribution to the brain and minimal CNS effects as described above.

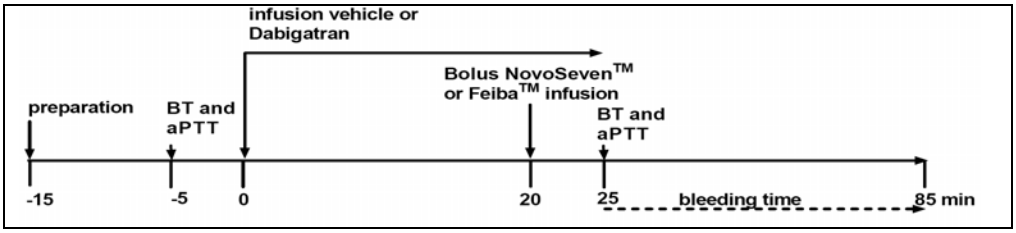
Other potential adverse effects:
As discussed previously in Section 4.1 [Study U00-1588], the sponsor maintains that the concentration of BIBR 1048 MS needed to prolong the bleeding time is greater than the concentration needed to prevent thrombosis.

Study title: Effect of Recombinant Factor VIIa (NovoSeven) or Activated Prothrombin Complex Concentrate (Feiba) on the Bleeding Time in Anesthetized Rats during High Dose Anticoagulant Treatment with the Direct Thrombin Inhibitor Dabigatran

Study no.:	2009/LUI/Lab1/Report1 (U09-1332-02)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study report:	July 17, 2009
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch RAL 102

Methods:
An activated prothrombin complex concentrate purified from human plasma, containing FII, FIX and FX, (APCC, Feiba) or recombinant factor VIIa (r-FVIIa, NovoSeven) were evaluated for their ability to reverse the activity of BIBR 953 ZW using the study design in Figure 11. Anesthetized male rats received intravenous administration of vehicle or BIBR 953 ZW administered as an initial bolus of 1 µmol/kg followed by a continuous infusion at a rate of 0.5 µmol/kg/h in the right jugular vein and 20 minutes later either NovoSeven (0.1 or 0.5 mg/kg) or Feiba (50 or 100 U/kg) was administered as a bolus or as a slow infusion over 4.5 min, respectively. Bleeding time was assessed using a standardized incision in the tail. Blood samples for aPTT determination were collected from the cannulated right carotid artery prior to vehicle or BIBR 953 ZW administration and at the end of the infusion of NovoSeven or Feiba.

Figure 11: Sponsor's Figure of Study Design – Study U09-1332-02



Results:
Infusion of the high dose of NovoSeven (range bleeding time, aPTT: 105-180 sec, 6.4-16.7 sec) or Feiba (75-150 sec, 7.2-32.1 sec) did not significantly affect either bleeding time or aPTT in vehicle treated animals (75-165 sec, 4.2-9.5 sec).

Infusion of BIBR 953 ZW increased the bleeding time (range 330 - >3600 sec) from 2.4 to >30-fold compared to pre-infusion values. Administration of either NovoSeven (75-195 sec) or Feiba (105-270 sec) at the end of BIBR 953 ZW infusion significantly decreased bleeding time. Infusion of BIBR 953 ZW increased the aPTT (24.9 – 113.2 sec) from 2.2 to 10-fold compared to pre-infusion values. Infusion of NovoSeven (19.8-39 sec) reduced the mean aPTT compared to that with infusion of BIBR 953 ZW alone. However, infusion of Feiba (63.4-82.5 sec) did not reduce the mean aPTT.

Doses of NovoSeven and Feiba used therapeutically in hemophilic patients can reverse the prolongation of bleeding time in rats induced by administration of a supra-therapeutic dose of BIBR 953 ZW. These results suggest that NovoSeven and Feiba may useful in reversing overdoses of BIBR 953 ZW.

Table 12: Sponsor's Summary Tables – Study U09-1332-02

	Prior to BIBR 1048 MS infusion		After infusions (see diagram)	
experimental group (n)	**bleeding time [sec]***	**aPTT [sec] $**	**bleeding time [sec]***	**aPTT [sec] $**
a) vehicle (11)	138 ± 7	12 ± 0.4	125 ± 8 §	7 ± 0.5
b) dabigatran [1 µmol/kg/h + 0.5 µmol/kg/h] (13)	125 ± 6	12.1± 0.5	1455 ± 352#	58.1± 8.2#
c) NovoSeven alone [1mg/kg] (8)	141 ± 9	12.4 ± 0.6	131 ± 9 §	10 ± 1.1§
d) Feiba alone [100 U/kg] (8)	122 ± 7	12 ± 0.8	113 ± 12 §	13.7 ± 3.3§
e) dabigatran [1 µmol/kg + 0.5 µmol/kg/h] + low dose NovoSeven [0.1 mg/kg] (7)	126 ± 10	11.6 ± 0.7	186 ± 49 §	31.2 ± 1.9
f) dabigatran [1 µmol/kg + 0.5 µmol/kg/h] + high dose NovoSeven [0.5 mg/kg] (8)	114 ± 6	11.8 ± 0.5	135 ± 13 §	26.8 ± 2
g) dabigatran [1 µmol/kg + 0.5 µmol/kg/h] + low dose Feiba [50U/kg] (8)	120 ± 10	12.7 ± 0.2	146 ± 11 §	52.8 ± 2.8#
h) dabigatran [1 µmol/kg + 0.5 µmol/kg/h] + high dose Feiba [100U/kg] (8)	139 ± 14	12.4 ± 0.4	174 ± 18	72.1 ± 3#

Data as mean + SEM; number of animals in parentheses
* data are rounded to full seconds
S data are rounded to 1 decimal point

#: p<0.05 vs vehicle (group a)
§: p<0.05 vs dabigatran alone (group b)
(Kruskal-Wallis (non-parametric ANOVA) followed by Dunn´s multiple comparison test)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Single Dose Studies

The main pharmacokinetic parameters observed in rats, rabbits, and monkeys after oral administration of [^{14}C] BIBR 1048 MS and intravenous administration [^{14}C] BIBR 953 ZW are shown in Table 13 and Table 14. To determine the concentration of glucuronide conjugates, plasma was subjected to alkaline hydrolysis and the total BIBR 953 ZW determined. In monkeys, glucuronide conjugates represented more than half of the total BIBR 953 ZW in plasma. Although only 80-90% of the total radioactivity in rat plasma was present as BIBR 953 ZW, the level of glucuronide conjugates was not determined in either Study U98-2257 or Study U98-2709.

Absorption of BIBR 1048 MS/BIBR 951 ZW is low in all species. For rats, comparison of plasma AUC values by both administration routes in Study U98-2257 resulted in an estimated oral absorption of 12%, a value consistent with urinary excretion after oral administration of [^{14}C] BIBR 1048 MS (11%) and after intravenous administration of [^{14}C] BIBR 953 ZW (13%) and biliary excretion of 10% following both routes of administration. Mice also had an oral absorption for BIBR 953 ZW of about 10%, based on urinary excretion. Bioavailability for BIBR 953 ZW of 5.4% in rabbits was considered an underestimate, because of the additional plasma metabolites, such as M630 identified in Study U04-2115 following oral administration. The bioavailability in

monkeys for total BIBR 953 ZW was also only 7.7%. Consequently, most of a BIBR 1048 MS dose is excreted into feces in all species.

The volume of distribution of BIBR 953 ZW in rats, rabbits, and monkeys of 0.63, 1.2 and 1.34 L/kg is slightly above that of total body water (0.55 L/kg). Total plasma clearance for rats, rabbits and monkeys of 11.5, 11.5, and 8.9 mL/(min·kg), respectively, is considered moderate and is equivalent to 20 - 25% of hepatic blood flow. The elimination half-life of BIBR 953 ZW was shortest in rats and mice (1-1.5 hr) and longest in monkeys (4-7 hr).

Table 13: Sponsor's PK Overview Tables - Rats and Rabbits

Rats

Report	U98-2257			U98-2709
Drug administered	[^{14}C]dabigatran etexilate mesilate		dabigatran	[^{14}C]dabigatran
Route	oral gavage		intravenous bolus	intravenous bolus
Dose (mg/kg)	3.0		3.0	0.3
Matrix	plasma		plasma	blood
Analyte	radioactivity	free dabigatran	free dabigatran	radioactivity
C_{max} [ngeq/mL]	305.4	236.2	6919	189.0
t_{max} [h]	0.50	0.50	(not calculated)	(not calculated)
$t_{½}$ [h]	1.65	1.12	0.95	1.96
MRT_{tot} [h]	(not calculated)	1.58	0.92	1.36
$AUC_{0-\infty}$ [ngeq·h/mL]	569.7	464.6	4372	168.2
V_{ss} [L/kg]	(not applicable)	(not applicable)	0.63	(not applicable)
CL [mL/(min·kg)]	(not applicable)	(not applicable)	11.49	(not applicable)

Female rabbits

Report	U05-2451			
Drug administered	[^{14}C]dabigatran etexilate mesilate		dabigatran etexilate mesilate	dabigatran
Route	oral gavage		oral gavage	intravenous bolus
Dose (mg/kg)	17.3		17.3	0.3
Matrix	plasma		plasma	plasma
Analyte	radioactivity	free dabigatran	free dabigatran	free dabigatran
C_{max} [ngeq/mL]	1120	226	241	556
t_{max} [h]	1.0	1.33	1.6	0.0831
$t_{½}$ [h]	6.32	2.70	2.54	1.94
MRT_{tot} [h]	4.15	3.70	3.66	1.75
$AUC_{0-\infty}$ [ngeq·h/mL]	3950	910	892	444
V_{ss} [L/kg]	(not applicable)	(not applicable)	(not applicable)	1.20
CL [mL/(min·kg)]	(not applicable)	(not applicable)	(not applicable)	11.5

Table 14: Sponsor's PK Overview Tables - Monkeys and Mice

Rhesus monkey

Report	U99-1092		U01-1761	
Drug administered	[^{14}C]dabigatran etexilate mesilate		[^{14}C]dabigatran	
Route	oral gavage		intravenous bolus	
Dose (mg/kg)	2.7 mg/kg [^{14}C]dabigatran etexilate		0.3 mg/kg [^{14}C]dabigatran	
Matrix	plasma		plasma	
Analyte	free dabigatran	sum dabigatran	free dabigatran	sum dabigatran
C_{max} [ngeq/mL]	54.8	151.8	(not calculated)	(not calculated)
t_{max} [h]	1.25	1.25	(not calculated)	(not calculated)
$t_{½}$ [h]	7.27	5.81	1.72*	4.54
MRT_{tot} [h]	7.92	7.19	2.05*	3.97
$AUC_{0-\infty}$ [ngeq·h/mL]	318.8	974.3	577.3	1712
V_z [L/kg]	(not applicable)	(not applicable)	1.34	(not investigated)
CL [mL/(min·kg)]	(not applicable)	(not applicable)	8.9	(not investigated)

* underestimated due to BLQ of values at 24 hours

Mice

Report	U04-1254		
Drug administered	[^{14}C]dabigatran etexilate mesilate		
Route	oral gavage		
Dose (mg/kg)	3.5		
Matrix	plasma		
Analyte	radioactivity	free dabigatran	sum dabigatran
C_{max} [ngeq/mL]	120	107	108
t_{max} [h]	0.50	0.50	0.50
$t_{½}$ [h]	1.31	1.19	1.22
MRT_{tot} [h]	2.00	1.56	1.56
AUC_{0-t} [ngeq·h/mL]	265	206	204

In vitro Studies

BIBR 1048 MS and BIBR 953 ZW were evaluated in experiments in vitro using the human colon carcinoma derived cell line, Caco-2, to determine permeability and interaction with the efflux transporters P-glycoprotein (P-gp) and Multi-drug Resistance associated Protein (MRP2) [Study U05-2159]. Transport of radiolabelled compounds across Caco-2 cell monolayers from apical to basolateral (AtoB = absorptive) or from basolateral to apical (BtoA = secretory) over a 90 minute incubation was assessed by measuring the concentration of radiolabeled compounds from both apical and

basolateral compartments and determining the apparent permeability coefficients (Papp) with and without the P-gp inhibitors (cyclosporine A, verapamil and zosuquidar), and the MRP inhibitor MK571. The results were compared to passive permeability values for reference compounds (mannitol, atenolol and propranolol), and the P-gp substrate digoxin (with and without the P-gp inhibitors cyclosporine A and verapamil).

BIBR 1048 MS was found to be highly permeable (Table 15); however, permeability depends on drug concentration, because BIBR 1048 MS is a P-glycoprotein substrate based on inhibition of transport in the presence of P-gp inhibitors cyclosporin A and zosuquidar, but maintenance of transport in the presence of the MRP inhibitor, MK571. In contrast, BIBR 953 ZW has low permeability that was independent of concentration and in the range of the low permeable reference substance, mannitol. BIBR 953 ZW transport was not affected by the presence of P-gp inhibitors and BIBR 953 ZW is not a substrate for P-gp.

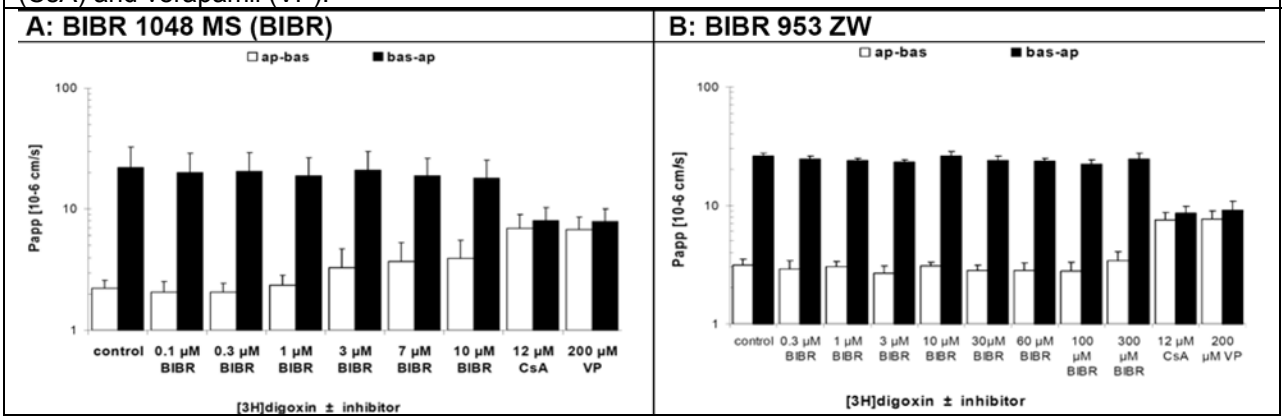
Table 15: Sponsor's Summary – Study U05-2159

Substrate	mean Papp AtoB [1x10^{-6} cm/sec] mean (± SD)	mean Papp BtoA [1x10^{-6} cm/sec] mean (± SD)	Efflux ratio (Papp BtoA / Papp AtoB) mean (± SD)
Test compound BIBR 1048 MS:			
[^{14}C]BIBR 1048 MS (0.6 µmol/L)	**10.45** ± 1.00	**62.47** ± 9.91	**5.96** ± 0.38
[^{14}C]BIBR 1048 MS (2 µmol/L)	**12.17** ± 1.50	**54.07** ± 10.25	**4.53** ± 1.40
[^{14}C]BIBR 1048 MS (7 µmol/L)	**14.86** ± 3.06	**54.97** ± 4.01	**3.75** ± 0.50
[^{14}C]BIBR 1048 MS (20 µmol/L)	**19.03** ± 3.30	**37.59** ± 7.28	**1.97** ± 0.04
[^{14}C]BIBR 1048 MS (40 µmol/L)	**21.65** ± 1.13	**27.80** ± 7.30	**1.28** ± 0.27
Test compound BIBR 953 ZW:			
[^{14}C]BIBR 953 ZW (3 – 300 µmol/L)	**0.35 – 0.31**	**0.36 – 0.30**	**1.09 – 0.95**
Test compound BIBR 1048 MS + inhibitors:			
[^{14}C]BIBR 1048 MS (2 µmol/L) + Cyclosporin A (12 µmol/L)	**23.98**	**29.03**	**1.21**
[^{14}C]BIBR 1048 MS (2 µmol/L) + Verapamil (200 µmol/L)	**35.38**	**49.29**	**1.39**
[^{14}C]BIBR 1048 MS (2 µmol/L) + Zosuquidar (5 µmol/L)	**23.61**	**19.49**	**0.83**
[^{14}C]BIBR 1048 MS (2 µmol/L) + MK571 (25 µmol/L)	**19.89**	**62.64**	**3.15**
Reference substrates of passive permeability:			
[^{14}C]Mannitol	**0.42** ± 0.13	**0.48** ± 0.15	**1.25** ± 0.51
[^{3}H]Atenolol	**1.59** ± 0.60	**2.02** ± 0.83	**1.26** ± 0.21
[^{3}H]Propranolol	**88.34** ± 9.83	**93.28** ± 8.59	**1.07** ± 0.15
Reference substrates of P-glycoprotein:			
[^{3}H]Digoxin	**1.90** ± 0.71	**28.38** ± 5.64	**17.17** ± 7.32
[^{3}H]Digoxin + Cyclosporin A (12 µmol/L)	**7.42** ± 1.30	**8.24** ± 1.18	**1.12** ± 0.08
[^{3}H]Digoxin + Verapamil (200 µmol/L)	**7.72** ± 1.40	**8.75** ± 1.55	**1.14** ± 0.08

Transport studies in Caco-2 cells using radiolabeled digoxin as substrate were performed in the absence or presence of BIBR 1048 MS (up to 10 µM) and BIBR 953 ZW (up to 300 µM) to determine their ability to inhibit P-gp activity (Study U05-2159). BIBR 953 ZW did not inhibit digoxin transport mediated by P-gp at the highest concentration examined. However, BIBR 1048 MS appears to be both a medium-affinity substrate (apparent Km: 14 µM) and inhibitor (apparent ap-bas IC_{50}: 25 µM) for the drug efflux transporter P-gp, although digoxin transport was incompletely inhibited.

Figure 12: Sponsor's Figures – Study U05-2159

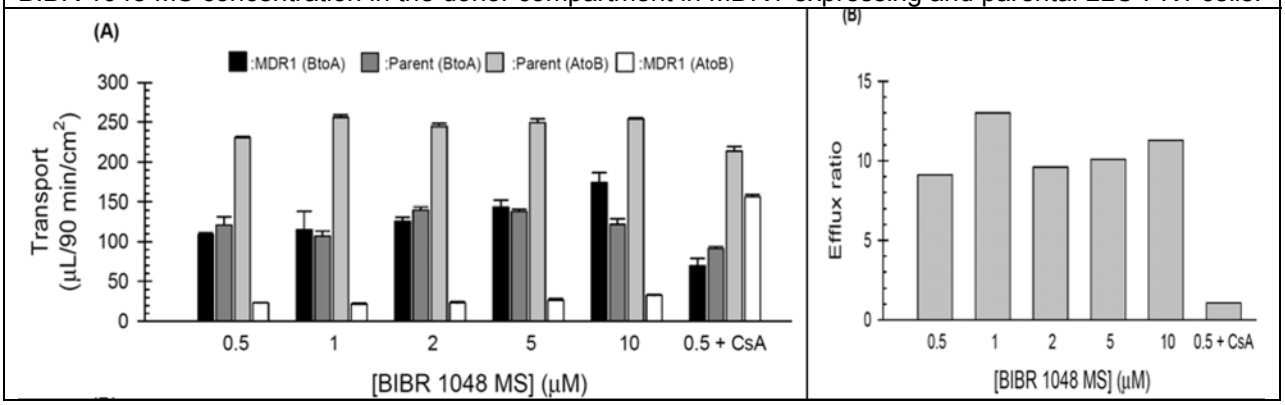
Inhibition of the apical-to-basal (ap-bas) and basal-to-apical (bas-ap) apparent permeability coefficients of [^{3}H]digoxin (1 µM) in the absence (control) and presence of increasing concentrations of BIBR 1048 MS (A) or BIBR 953 ZW (B) and of fully blocking concentrations of the known P-gp inhibitors cyclosporin A (CsA) and verapamil (VP).



BtoA transport and AtoB transport of radiolabeled BIBR 1048 MS and BIBR 953 ZW was evaluated in cell monolayers of parental Lewis-lung cancer porcine kidney 1 (LLC PK1) cells and LLC-PK1 cells expressing recombinant MDR1 [Study U07-3036]. Asymmetric MDR1-mediated drug efflux transport was expected due to the localization of MDR1 in the apical membrane. Compared with transport in parental LLC-PK1 cells, the BtoA transport and AtoB transport of BIBR 1048 MS increased and decreased, respectively in MDR1-expressing LLC-PK1 cells (Figure 2Figure 13). The MDR1 inhibitor, cyclosporin A (CsA), increased the AtoB transport of BIBR 1048 MS indicating involvement of MDR1 in the transport of BIBR 1048 MS. However, the efflux ratio for BIBR 1048 MS was not significantly changed at concentrations up to 10 µM suggesting that the Km of BIBR 1048 MS for MDR1 is higher than 10 µM. In contrast, the transport of BIBR 953 ZW in MDR1-expressing and parental LLC-PK1 cells was similar to or less than the transport of the passive permeability reference, mannitol, suggesting MDR1 has little involvement in the transport of BIBR 953 ZW.

Figure 13: Sponsor's Figures – Study U07-3036

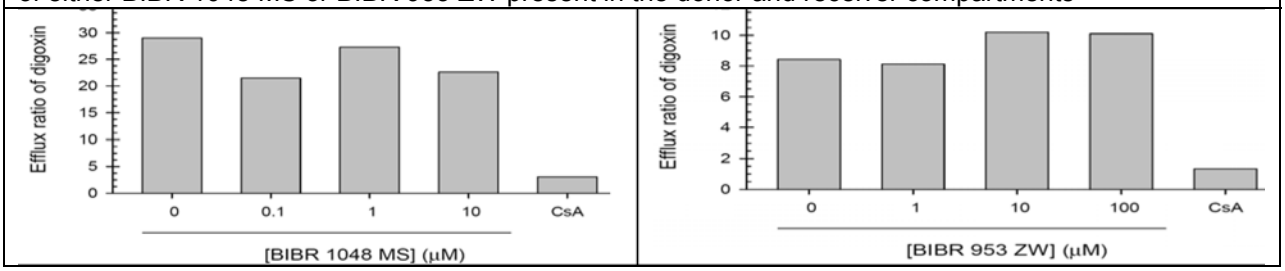
Measured BtoA and AtoB transport (A) and efflux ratios (B) of BIBR 1048 MS as a function of a target BIBR 1048 MS concentration in the donor compartment in MDR1-expressing and parental LLC-PK1 cells.



The bi-directional transport of [^{3}H]-digoxin was measured using parental LLC-PK1 cells and LLC-PK1 cells expressing recombinant MDR1 in the absence or presence of BIBR 1048 MS or BIBR 953 ZW at concentrations up to 10 µM and 100 µM, respectively. Compared to the MDR inhibitor cyclosporine, neither BIBR 1048 MS nor BIBR 953 ZW significantly inhibited digoxin efflux, indicating that BIBR 1048 MS and BIBR 953 ZW are not MDR1 inhibitors.

Figure 14: Sponsor's Figures – Study U07-3036

Efflux ratios in MDR1-expressing and parental LLC-PK1 cells of [^{3}H]-Digoxin (0.1 µM) in the donor compartment in the absence or presence of the MDR1 inhibitor CsA (3 µM) and increasing concentrations of either BIBR 1048 MS or BIBR 953 ZW present in the donor and receiver compartments



Using polarized monolayers of Caco-2 cells, the P-gp mediated transport of BIBR 1048 MS was determined in the absence or presence of increasing concentrations of several P-gp modulators [Study U07-3354]. The IC_{50} values on P-gp-mediated BIBR 1048 MS transport were determined to be >10 µM for amiodarone, 17.1 µM for clarithromycin, 74.5 µM for digoxin, 0.47 µM for itraconazole, 33.4 µM for quinidine and 13.2 µM for ritonavir. Although drug-drug interactions based on the inhibition of P-gp-mediated BIBR 1048 MS transport can not be excluded when the concentrations of these P-gp modulators exceed the above IC_{50} values, the sponsor believes such interactions are unlikely, because of the high permeability of BIBR 1048 MS.

Metabolism:

The in vitro metabolism of [^{14}C] BIBR 1048 MS by plasma esterases was determined in mouse plasma by generation of plasma concentration-time profiles of double prodrug BIBR 1048 BS, mono-prodrugs, BIBR 1087 SE and BIBR 951 BS, as well as the active moiety, BIBR 953 ZW (Study U09-1024-01). The double prodrug BIBR 1048 BS was completely metabolized to its mono-prodrug BIBR 1087 SE within 1.5 minutes after addition of BIBR 1048 MS to murine plasma. Further metabolism to BIBR 953 ZW represented 14.6% and 13.6% of total drug within 45 minutes after addition of BIBR 1048 MS to plasma of male and female mice, respectively.

The metabolite pattern of the double pro-drug, BIBR 1048 MS, and its active form, BIBR 953 ZW, was evaluated in male and female mice of the NMRI and CD-1 strains following administration of a single oral dose of [^{14}C] BIBR 1048 MS and a single intravenous dose of [^{14}C] BIBR 953 ZW (Study U05-1249). The metabolism of BIBR 1048 MS and BIBR 953 ZW was considered essentially identical in the two strains. Only BIBR 953 ZW was identified after oral dosing of BIBR 1048 MS in plasma of male and female NMRI- and female CD-1 mice. However, 30 % of sample radioactivity was assigned to metabolites M369, M324, M579, and M355/M325 which accounted for 6.2

%, 7.3 %, 9.2 % and 6.9 % of the sample radioactivity, respectively, in plasma of male CD-1 mice.

The metabolite pattern of the double pro-drug, BIBR 1048 MS, and its active form, BIBR 953 ZW, was evaluated in male and female rats of the Chbb:THOM and CrlGlxBrlHan:WI strains following administration of a single oral dose of [^{14}C] BIBR 1048 MS and a single intravenous dose of [^{14}C] BIBR 953 ZW (Study U05-1025). The metabolism of BIBR 1048 MS and BIBR 953 ZW was considered essentially identical in the two strains. BIBR 953 ZW was identified as the principal metabolite after oral dosing of BIBR 1048 MS in plasma of male and female Chbb:THOM and Han Wistar rats.

The metabolite pattern of the double pro-drug, BIBR 1048 MS, and its active form, BIBR 953 ZW, was evaluated in female rabbits (Chbb:HM (SPF) strain) following administration of a single oral dose of [^{14}C] BIBR 1048 MS and a single intravenous dose of [^{14}C] BIBR 953 ZW (Study U04-2115). Essentially only BIBR 953 ZW was identified in plasma after intravenous dosing of BIBR 1048 MS. However, following intraduodenal administration of BIBR 1048 MS, BIBR 953 ZW and the mono-prodrug BIBR 1087 accounted for 52.0 % and 4.7 % of the sample radioactivity, respectively. Metabolite M630, formed by hydrolysis of the ethylester-moiety and oxidation of the hexyloxycarbonyl-side chain of BIBR 1048 MS, and metabolite M602, formed by subsequent ß-oxidation of M630, represented 34.2 % and 9.1 % of the sample radioactivity, respectively, in rabbit plasma.

The metabolite pattern of the double pro-drug, BIBR 1048 MS, and its active form, BIBR 953 ZW, was evaluated in male and female Rhesus monkeys following administration of a single oral dose of [^{14}C] BIBR 1048 MS and a single intravenous dose of [^{14}C] BIBR 953 ZW (Study U04-2009-01). Following intravenous administration of BIBR 953 ZW, acylglucuronides of BIBR 953 ZW (M648) accounted for 59.4, 67.3, and 69.2 % of the sample radioactivity and BIBR 953 ZW itself accounted for 40.6, 32.7, and 30.8 % of the sample radioactivity in plasma at 2, 4, and 6 h post dosing. Following oral administration of BIBR 1048 MS, acylglucuronides of BIBR 953 ZW (M648) accounted for 56.7, 69.9, and 71.4 % of the sample radioactivity and BIBR 953 ZW itself accounted for 43.3, 30.1, and 28.6 % of the sample radioactivity in plasma at 2, 4, and 6 h post dosing.

The sponsor provided the following tables (Table 16, Table 17) and figure (Figure 15) that overview the metabolism of [^{14}C]-BIBR 1048 MS in animals compared to humans. After oral administration of [^{14}C] BIBR 1048 MS, BIBR 953 ZW (M472) was the principal form of radioactivity in the plasma of mice, rats, rabbits and man. In monkeys the principal form was the acyl-glucuronide forms of BIBR 953 ZW (M648). Mice have a taurine conjugate (M579) instead of glucuronide conjugates. Rabbits have high amounts of metabolite M630 in their plasma after oral administration of BIBR 1048 MS.

Table 16: Sponsor's Tables – Overview of Metabolites in Plasma

Single intravenous dose of [^{14}C] BIBR 1048 MS – Metabolites in plasma

Metabolite	Synthetic Reference	Mouse		Rat (0.5 h)		Rabbit		Rhesus monkey			Man		
		1 h	3 h	m	f	2 h	4 h	2 h	4 h	6 h	0.67 h	2 h	4 h
M369											0.7		
M324	BIBR1151			0.2	+	0.5		+				+	
M325	CD00000338			+	+	+							
M355	BI00003521			0.1	+	0.7							
M500(1)				+	+								
M411*					+	1.3	0.7						
M472	**BIBR953**	**93.7**	**90.3**	**99.7**	**100**	**97.0**	**99.3**	**40.6**	**32.7**	**30.8**	**99.3**	**98.0**	**95.7**
M648		+		+	+	+		59.4	67.3	69.2		2°	4.3°
M579		6.3	9.7										
M486(1)					+	0.5							
Total		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

* structure unknown
\+ traces, detected by mass spectrometry, not by radioactivity detection
° underestimated due to very low concentration of radioactivity and hydrolysis of acyl-glucuronides

Single oral dose of [^{14}C] BIBR 1048 MS – Metabolites in plasma

Metabolite	Synthetic Reference	Mouse		Rat (0.5 h)		Rabbit	Rhesus monkey			Man
		1 h	3 h	male	female	pool of 2 h + 4 h	2 h	4 h	6 h	pool of 2 h + 4 h + 6 h
M396	BI00003522									+
M324	BIBR1151			+	+		+			+
M355	BI00003521			2.2	+					+
M325	CD00000338			+	+					
M500(1)				+	+		+			+
M411*				+	+					
M472	**BIBR953**	**91.3**	**100**	**94.5**	**84.7**	**52.0**	**43.3**	**30.1**	**28.6**	**100**
M648		+		2.4	5.2	+	56.7	69.9	71.4	+°
M579		5.4								
M602						9.1				
M630						34.2				
M486(1)		3.3		+	+					
M488										+
M600	BIBR1087			0.9	10.1	4.7				
Total		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

* structure unknown
\+ traces, detected by mass spectrometry, not by radioactivity detection
° underestimated due to very low concentration of radioactivity and hydrolysis of acyl-glucuronides

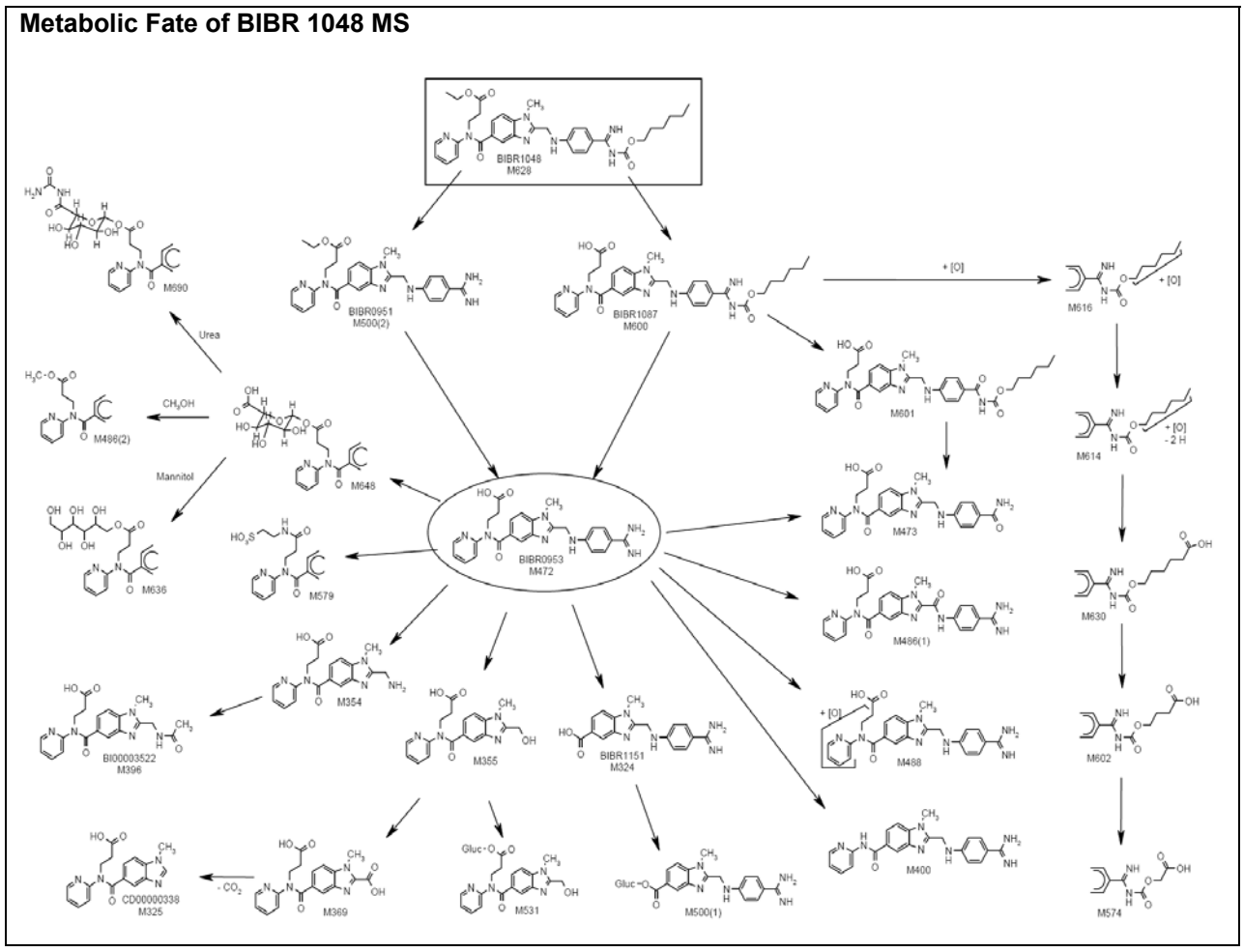
Table 17: Sponsor's Overview of Metabolites in Urine and Feces

Single oral dose of [^{14}C] BIBR 1048 MS – Metabolites in urine and feces

Metabolite	Synthetic Reference	Mouse (0 h - 48 h)		Rat (0 h - 48 h)		Rabbit (0 h - 72 h)		Rhesus monkey (0 h - 48 h)		Man (0 h - 48 h)	
		Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
M354								< 0.1	0.4	+	
M369		0.2	< 0.1	< 0.1		0.3	0.2	+	0.2	0.5	0.4
M324	BIBR1151	< 0.1	+	+	+	0.1	0.3	< 0.1	0.6	0.4	2.3
M355	BI00003521	0.2	< 0.1	0.3	0.1	0.7	0.4	0.1	0.4	0.6	0.6
M325	CD00000338							< 0.1	< 0.1	0.3	
M396	BI00003522					+	+	+		0.1	0.1
M500(1)		0.1	< 0.1	+				0.1		+	
M531										+	
m5*		0.1									
m12*								< 0.1			
M472	**BIBR953**	**6.6**	**75.1**	**8.3**	**75.5**	**2.3**	**59.3**	**1.2**	**69.9**	**4.3**	**66.1**
M473			+	+	+	0.1	1.2		0.7		
M574						0.3				+	
M648 + M690		+§		0.4§		+§		1.4		0.4°	
M579		0.2	0.1								
m6*			1.4								
m14*								< 0.1			
M602		+			0.7	0.3	0.3	+			0.5
M636										< 0.1	
M630						0.2	1.1		0.3	0.1	0.2
M486(1)				+							
M486(2)								< 0.1			
M488		+		+			+	< 0.1	0.4	0.1	1.2
M614							+		+	+	+
M400	BI00003543	< 0.1	2.0		+			< 0.1	1.1	0.2	6.0
M616					2.0	+	+		0.7	< 0.1	2.0
M601					+		+	+	0.7		
M600	BIBR1087	0.4	8.4		10.4	0.1	0.8	0.1	2.9	+	0.3
M500(2)	BIBR951				+	< 0.1	11.8	< 0.1	3.2		
M628	BIBR1048	0.2	1.9		0.2		0.1	0.2	2.6	+	
Subtotal		8.0	89.0	9.0	88.9	4.4	75.6	3.3	83.9	7.1	79.8
Total		**97.0**		**97.5**		**80.0**		**87.2**		**86.9**	

* structure unknown
\+ traces, detected by mass spectrometry, not by radioactivity detection
§ only M648, M690 not present
° underestimated due to very low concentration of radioactivity and hydrolysis of acyl-glucuronides

Figure 15: Sponsor's Diagram of BIBR 1048 MS Metabolism



Distribution:

Plasma Protein Binding (Study U00-1294, Study U06-1211)

The plasma protein binding of [^{14}C]-BIBR 953 ZW was low in all species (humans, rats, mice, rabbits, and monkeys) as determined by ultracentrifugation and/or ultrafiltration. The plasma protein binding ranged between 22% and 39% of the added radioactivity for all concentrations and species with monkeys showing the highest protein binding.

Table 18: Sponsor's Summary - Protein Binding – Studies U00-1294 and U06-1211

Plasma concentration [ng/mL]	Mouse	Rat	Rabbit	Rhesus monkey	Man
50		32.04	31.51	38.06	29.39
100	26.22				
500		30.53	32.02	38.64	28.53
1,000	25.62				
5,000		28.05	31.85	38.60	28.24
10,000	22.26				

Distribution between Red Blood Cells and Plasma (Study U00-1096)

After oral administration of [^{14}C] BIBR 1048 MS or intravenous administration of [^{14}C]-BIBR 953 ZW to rats, the ratio of radioactivity in blood cells to that in plasma was between 0.01 and 0.74 with the highest values 24 hours after dosing when blood concentrations were very low [U00-1096]. These results indicate that [^{14}C] BIBR 953 ZW preferentially distributes to the plasma. Similar results were observed after oral administration of [^{14}C]-BIBR 1048 MS or intravenous administration of [^{14}C] BIBR 953 ZW to humans with cell to plasma ratios of 0.1 to 0.4 [U04-1378].

Whole Body Autoradiography in the Rat (Study U00-1096)

The distribution of drug related radioactivity was investigated after oral dosing of [^{14}C] BIBR 1048 MS and after intravenous administration of [^{14}C] BIBR 953 ZW in albino and pigmented rats. The following tables (Table 19, Table 20) summarize the distribution to the major tissues evaluate either in punched tissue samples or by whole body autoradiography. Following either oral or intravenous dosing the radioactivity distributed into all organs, except the central nervous system where the level of radioactivity in brain was 2 and 3% of that in plasma in albino and pigments rats, respectively. The highest concentration of radioactivity was found in liver and kidney. In pigmented rats, little radioactivity was detectable in the melanin containing parts of the eye and skin. The concentration of radioactivity in all tissues decreased rapidly with time with little or no tissue retention at 24 hours post-dose.

Table 19: Sponsor's Tables – Punched Samples – Study U00-1096

Concentration of total radioactivity (ng-eqv. BIBR 1048 BS/g tissue or ml fluid) in tissues of male albino and pigmented rats after oral administration of 11.53 mg/kg of [^{14}C] BIBR 1048 MS.

punched samples (measured by LSC)					
	albino rats			pigmented rats	
tissue	0.5 h	2.0 h	24 h	0.5 h	24 h
brain	21.4	6.9	1.5	25.9	1.1
lungs	628.3	122.3	2.1	505.5	5.0
liver	3887.5	816.4	13.0	5445.3	60.6
muscle	335.3	72.6	2.5	110.7	3.3
blood (heart)	450.7	80.8	3.2	348.0	2.7
thymus	128.4	24.3	1.9	156.7	9.8
salivary gland	292.3	76.2	n.d.	291.3	4.7
testis	157.7	54.8	1.2	124.7	2.5
fat	262.7	383.8	5.6	234.7	6.4
blood (retro.)	564.3	93.1	0.7	467.5	0.7
plasma	1006.5	160.9	1.1	874.0	1.1
erythrocytes	23.8	6.7	0.3	9.0	0.2
hematocrit (%)	45	44	47	47	45
C_C/C_P (quotient)	0.02	0.04	0.24	0.01	0.15

Concentration of total radioactivity (ng-eqv. BIBR 953 ZW/g tissue or ml fluid) in tissues of male albino and pigmented rats after intravenous administration of 5.0 mg/kg of [^{14}C] BIBR 953 ZW.

punched samples (measured by LSC)					
	albino rats			pigmented rats	
tissue	4 min	1 h	24 h	3 min	24 h
brain	205.6	28.4	n.d.	393.1	1.3
lungs	5056.8	587.6	1.5	6821.7	4.6
liver	12227.4	4816.4	7.4	23214.7	55.7
muscle	3301.9	449.4	2.2	2614.4	3.1
blood (heart)	4797.3	490.6	1.0	6096.8	3.6
thymus	1361.9	154.5	n.d.	1929.9	2.3
salivary gland	3078.0	309.8	25.5	4725.1	2.7
testis	1135.8	239.3	1.4	1106.0	1.7
fat	2094.2	1132.3	4.0	1927.4	n.m.
blood (retro.)	5839.3	576.0	0.7	8217.7	1.3
plasma	10359.7	1018.7	0.8	15350.8	2.0
erythrocytes	86.2	12.5	0.6	174.1	0.5
hematocrit (%)	44	44	46	47	46
C_C/C_P (quotient)	0.02	0.01	0.74	0.01	0.28

Table 20: Sponsor's Tables – Radioluminography - Study U00-1096

Concentration of total radioactivity (ng-eqv. BIBR 953 ZW/g tissue or ml fluid) by radioluminography in tissues of male albino and pigmented rats at various time points after iv administration of 5.0 mg/kg of [^{14}C] BIBR 953 ZW.

	Albino rats								Pigmented rats					
	4 min			1 h			24 h		3 min			24 h		
tissues	N	mean	CV (%)	N	mean	CV (%)	N	mean	N	mean	CV (%)	N	mean	CV (%)
brain	6	227	83.4	4	7	22.7	0	n.d.	4	130	14.2	0	n.d.	
Harder's gland	2	1504	2.2	2	187	5.2	0	n.d.	2	2277	3.1	0	n.d.	
salivary gland	4	2582	14.7	6	235	6.2	0	n.d.	6	4184	16.5	0	n.d.	
thymus	8	903	8.1	6	113	5.4	0	n.d.	3	1790	4.4	0	n.d.	
lungs	11	4427	7.8	9	505	5.6	0	n.d.	8	5822	11.3	0	n.d.	
heart muscle	8	2444	10.3	9	286	8.4	0	n.d.	7	3400	11.2	0	n.d.	
blood (heart)	12	4748	5.9	12	493	6.9	0	n.d.	8	6462	7.0	0	n.d.	
artery wall	4	12618	4.1	5	2823	29.2	0	n.d.	1	21711	0.0	0	n.d.	
liver	16	12176	8.0	16	4731	5.5	0	n.d.	16	23409	8.5	11	45	25.0
spleen	2	1943	5.9	3	186	4.1	0	n.d.	5	2571	2.6	0	n.d.	
pancreas	3	2140	7.0	3	303	22.7	0	n.d.	5	2652	6.1	0	n.d.	
kidney (total)	3	39360	17.7	3	1723	10.7	0	n.d.	2	30074	2.9	1	16	
kidney pelvis	4	66735	41.7	2	1959	0.9	0	n.d.	2	42712	10.0	0	n.d.	
kidney cortex	10	33916	12.8	8	1503	5.6	0	n.d.	8	26136	6.2	5	25	14.3
adrenal cortex	1	1751	0.0	2	171	0.0	0	n.d.	2	3082	6.9	0	n.d.	
adrenal medulla	1	2780	0.0	1	210	0.0	0	n.d.	1	4246	0.0	0	n.d.	
testis	5	1123	14.8	6	174	4.7	0	n.d.	7	1336	23.5	0	n.d.	
epididymis	3	3662	5.9	2	933	18.7	0	n.d.	6	3864	21.0	0	n.d.	
fat	8	437	23.5	5	46	29.0	0	n.d.	7	498	43.6	0	n.d.	
skin (total)	12	5946	11.7	12	1412	18.4	0	n.d.	13	6500	18.8	0	n.d.	
skin (epidermis)	11	8242	5.6	5	2221	11.5	0	n.d.	9	8073	19.3	12	16	47.3
bone marrow	7	1745	17.7	4	222	9.5	0	n.d.	4	1831	5.3	0	n.d.	
tongue	5	2889	27.5	4	302	14.6	0	n.d.	4	4534	8.4	0	n.d.	
muscle (head)	11	1743	8.4	11	180	21.2	0	n.d.	13	2652	24.6	0	n.d.	
muscle (back)	10	1669	12.6	12	167	31.0	0	n.d.	15	2078	13.7	0	n.d.	

Placental Transfer in the Rat (Study U02-1372)

Using whole body autoradiography, the placental transfer of total radioactivity was investigated in pregnant albino rats at days 15 to 19 of pregnancy after oral administration of [^{14}C] BIBR 1048 MS and subcutaneous administration of [^{14}C] BIBR 953 ZW. After oral administration, little radioactivity was distributed at 0.5 hour to the fetus, whereas a low concentration of radioactivity was distributed at 2 hours post dose to the fetus. Following subcutaneous administration of [^{14}C] BIBR 953ZW, little radioactivity was distributed at 5 minutes post dose to the fetus, but at 2 hours post dose the level of radioactivity in the fetus was similar to that in caudal muscle. Therefore, some BIBR 953 ZW crosses the placental barrier and distributes to the fetus.

Table 21: Sponsor's Tables – Placental Transfer - Study U02-1372

Concentration of total radioactivity (ng-.eqv. BIBR 1048 BS/g tissue or ml fluid) by radioluminography in pregnant rats at various time points after oral administration of 11.53 mg/kg of [^{14}C] BIBR 1048 MS or subcutaneous administration of 5.0 mg/kg of [^{14}C] BIBR 953 ZW.

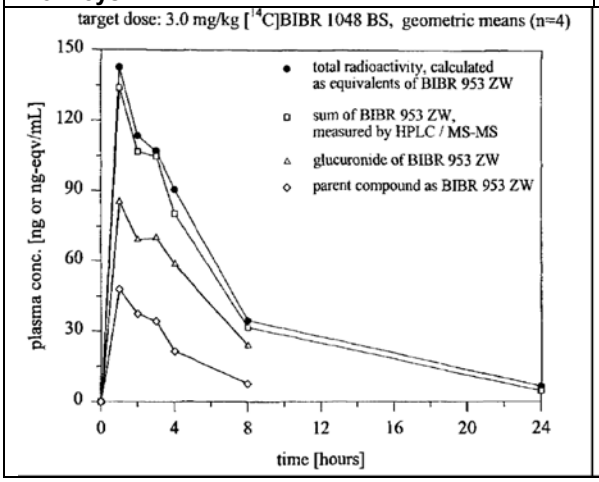
	Oral								Subcutaneous							
	0.5 h				2 h				5 min				2 h			
tissue	N	mean	SD	CV (%)	N	mean	SD	CV (%)	N	mean	SD	CV [%]	N	mean	SD	CV [%]
brain	8	20	9	46.1	8	10	6	62.5	9	28	6	20.6	7	18	3	19.5
pituitary	3	333	75	22.7	2	256	46	18.1	2	507	142	27.9	2	238	3	1.3
Harder's gland	2	452	184	40.8	2	92	6	6.5	2	342	5	1.5	1	271	n.c.	n.c.
salivary gland	5	497	56	11.3	5	132	22	16.4	6	613	37	6.1	3	403	55	13.6
thymus	4	207	23	11.0	4	84	12	14.0	5	325	17	5.1	3	244	15	6.0
lungs	11	932	107	11.5	11	357	37	10.4	15	1735	100	5.7	9	897	119	13.3
heart muscle	9	405	79	19.4	6	173	32	18.7	14	763	101	13.3	9	392	59	15.1
blood (heart)	12	894	47	5.3	12	366	23	6.2	12	1780	201	11.3	11	915	26	2.9
artery wall	6	4598	2442	53.1	6	1872	324	17.3	6	5133	1591	31.0	4	4860	1479	30.4
liver	23	4676	264	5.7	24	2909	171	5.9	24	1772	84	4.7	24	4253	297	7.0
spleen	4	420	110	26.3	5	150	20	13.4	4	479	18	3.7	4	312	28	8.9
pancreas	2	340	8	2.5	3	159	18	11.4	2	449	18	3.9	6	311	32	10.2
kidney (total)	4	2525	305	12.1	3	924	58	6.3	2	6736	65	1.0	3	2631	291	11.1
kidney pelvis	2	3404	78	2.3	2	792	133	16.7	2	20192	2626	13.0	3	6271	1155	18.4
kidney medulla	4	2801	157	5.6	5	1027	47	4.5	6	4856	436	9.0	6	2233	71	3.2
kidney cortex	5	2554	151	5.9	7	899	79	8.8	6	4469	127	2.8	7	1901	50	2.6
adrenal total	2	371	7	2.0	2	201	19	9.4	2	829	144	17.4	1	366	n.c.	n.c.
bone marrow	8	194	13	6.8	12	98	16	16.3	8	286	18	6.3	9	239	23	9.7
tongue	4	444	70	15.8	4	229	32	14.0	5	761	110	14.5	6	463	59	12.7
muscle (cranial)	21	230	45	19.6	23	78	14	17.9	21	317	32	10.1	21	225	30	13.4
muscle (caudal)	18	160	21	13.1	23	66	10	14.8	21	268	40	14.9	21	168	27	16.3
skin (epidermis)	22	1428	207	14.5	16	2028	199	9.8	21	756	94	12.5	16	4574	645	14.1
fat	9	33	8	25.5	14	15	6	39.6	8	52	14	27.7	14	32	10	29.4
placenta	15	594	34	5.7	17	270	23	8.7	20	1087	80	7.4	18	731	42	5.8
fetus total	15	17	4	25.5	19	53	7	13.9	17	19	3	15.2	10	158	17	10.8

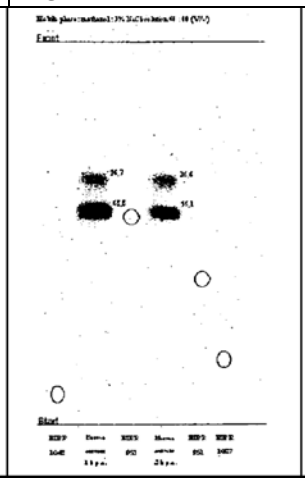
Metabolism:

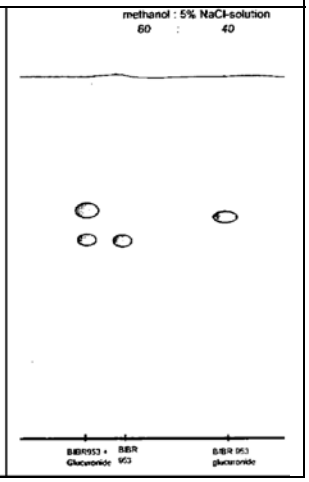
After single oral administration of [^{14}C] BIBR 1048 MS to rhesus monkeys, the radioactivity found in plasma is represented by BIBR 953 ZW and a more polar metabolite, subsequently identified as the glucuronide of BIBR 953 ZW. Since the sum of BIBR 953 ZW and its glucuronide was essentially equivalent to the total radioactivity, the sponsor concluded that no additional metabolites circulated in plasma (Study U99-1092). The sponsor explained that difference in concentration of the glucuronide between HPLC/MS/MS and thin layer chromatography was due to freezing and thawing of the plasma samples prior to the later analysis.

Figure 16: Sponsor's Figures – Study U99-1092

Plasma concentration-time profiles after single oral administration of [^{14}C]-BIBR 1048 MS to monkeys	Metabolite patterns in monkey plasma 1 and 3 hr post administration of 3 mg/kg [^{14}C]-BIBR 1048 MS



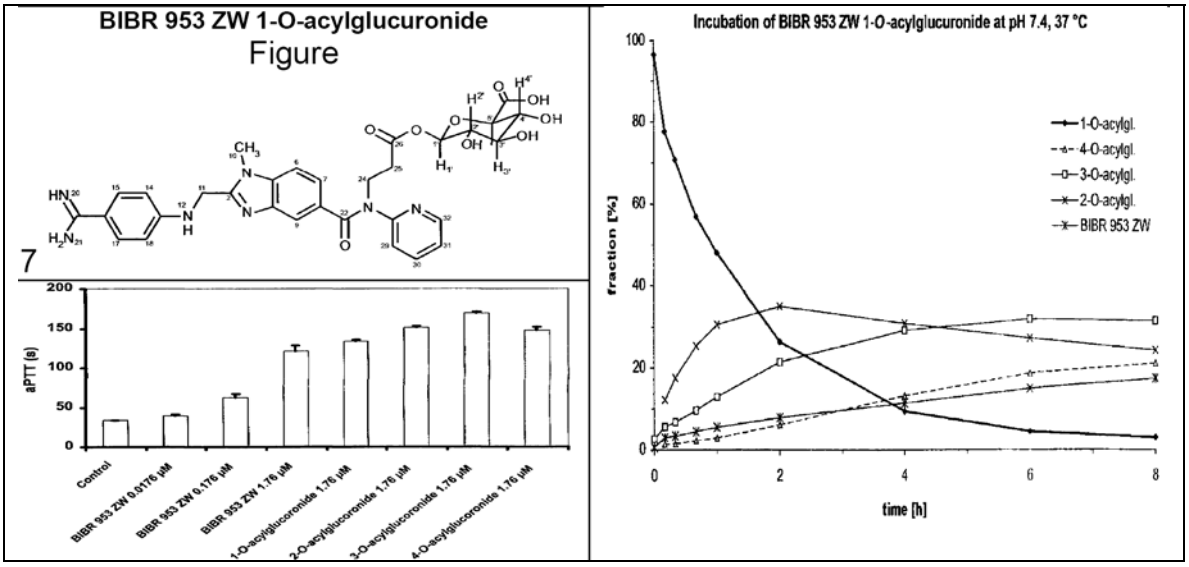




In rhesus monkeys, thin layer chromatography of fecal extracts showed four components identified as the parent (BIBR 1048 BS), BIBR 1087, BIBR 951, and BIBR 953. The large proportion of BIBR 1048 BS indicated incomplete oral absorption. Analysis of urine from monkeys indicated two components. One component had a relative mobility (Rf) similar to that of BIBR 953 ZW, whereas the other component was more polar.

To elucidate the chemical structure of the metabolites of BIBR 953 ZW (Study U98-2873, U99-1767), the urine of rhesus monkeys that were dosed with 500 mg/kg BIBR 1048 MS was analyzed by HPLC-MS/MS. This analysis indicated several metabolites exhibiting masses consistent with the mass of glucuronide conjugates of BIBR 953 ZW. Four metabolite fractions were isolated, purified and analyzed by proton NMR. The deduced chemical structures indicated these metabolites are the β-anomer of the 1-*O*-acylglucuronide of BIBR 953 ZW and α, β-anomers of the 2-*O*-, 3-*O*- and 4-*O*-acylglucuronides. In vitro studies showed that microsomes from Rhesus monkeys could form small amounts of the β-anomer of the 1-*O*-acylglucuronide of BIBR 953. Subsequent in vitro studies showed that the β-anomer undergoes intramolecular rearrangement to form isomeric acylglucuronides as shown below.

Figure 17: Sponsor's Figures – Studies U98-2873, U99-1767, and U98-2873

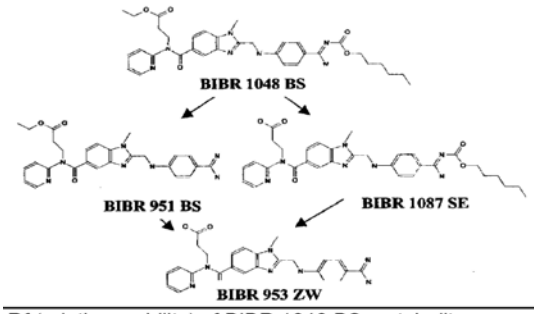


To determine the pharmacological activity of the four isomeric acylglucuronides of BIBR, the sponsor first purified the individual acylglucuronides and showed less than 1.1% unconjugated BIBR 953 ZW. The abilities of the parent compound BIBR 953 ZW and its four isomeric acylglucuronides to prolong aPTT were then compared in vitro using equivalent concentrations of each acylglucuronide form. The histogram in Figure 17 shows that each of the four acylglucuronides was at least as potent as the parent compound, BIBR 953 ZW, in prolonging the aPTT in human plasma (Study U98-2873). Therefore, the acylglucuronides of BIBR 953 ZW are pharmacologically active in inhibiting thrombin.

Following oral administration of [^{14}C] BIBR 1048 MS to rats, total radioactivity in plasma was primarily (80-90 %) in the form of [^{14}C] BIBR 953 ZW (Study U98-2257). However, the plasma components that were not BIBR 953 ZW were not identified in this study. The forms of BIBR 953 ZW in the bile and urine of rats were examined after intraduodenal or intravenous dosing of [^{14}C] BIBR 1048 MS, as shown in the figure below. In both bile and urine, most (85 - 91 %) of the radioactivity was in the form of BIBR 953 ZW. At 30 minutes after dosing, two less polar intermediates (BIBR 1087 and BIBR 951) were detected in bile, but not urine. No BIBR 1048 BS was detected in plasma, bile or urine. At 30 minutes after dosing, low levels of two more polar metabolites were present in bile. By 1 hour after dosing the level of the intermediates decreased, and the level of the more polar components increased in bile. The sponsor proposed the following scheme (Figure 18) for conversion of BIBR 1048 BS to BIBR 953 ZW. The sponsor did not identify the more polar components of rat bile or urine. However, the relative mobility of this polar component in rats is similar to the relative mobility of the polar component in monkeys. The similarity suggests that the glucuronide of BIBR 953 ZW may be present in rat plasma.

Figure 18: Sponsor's Figures - Study U98-2257

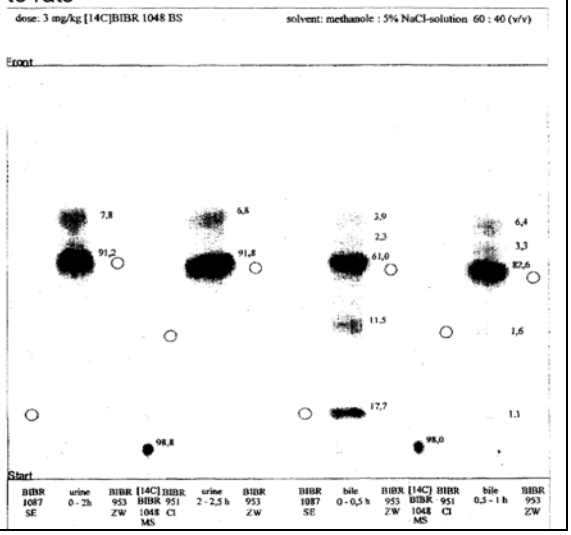
Conversion of BIBR 1048 BS to BIBR 953 ZW



Rf (relative mobility) of BIBR 1048 BS metabolites

Compound	Rat	Monkey
BIBR 1048 BS	0.089	0.086
BIBR 1087 SE	0.17	0.19
BIBR 951 Cl	0.40	0.40
BIBR 953 ZW	0.53, 0.54	0.56, 0.57
Most polar 1	0.64, 0.65	0.66
More polar 2	0.58, 0.59	
Monkey, glucuronide		0.63, 0.65

Metabolic pattern of urine and bile after p.o. and i.d. administration of 3.0 mg/kg [^{14}C] BIBR 1048 BS to rats



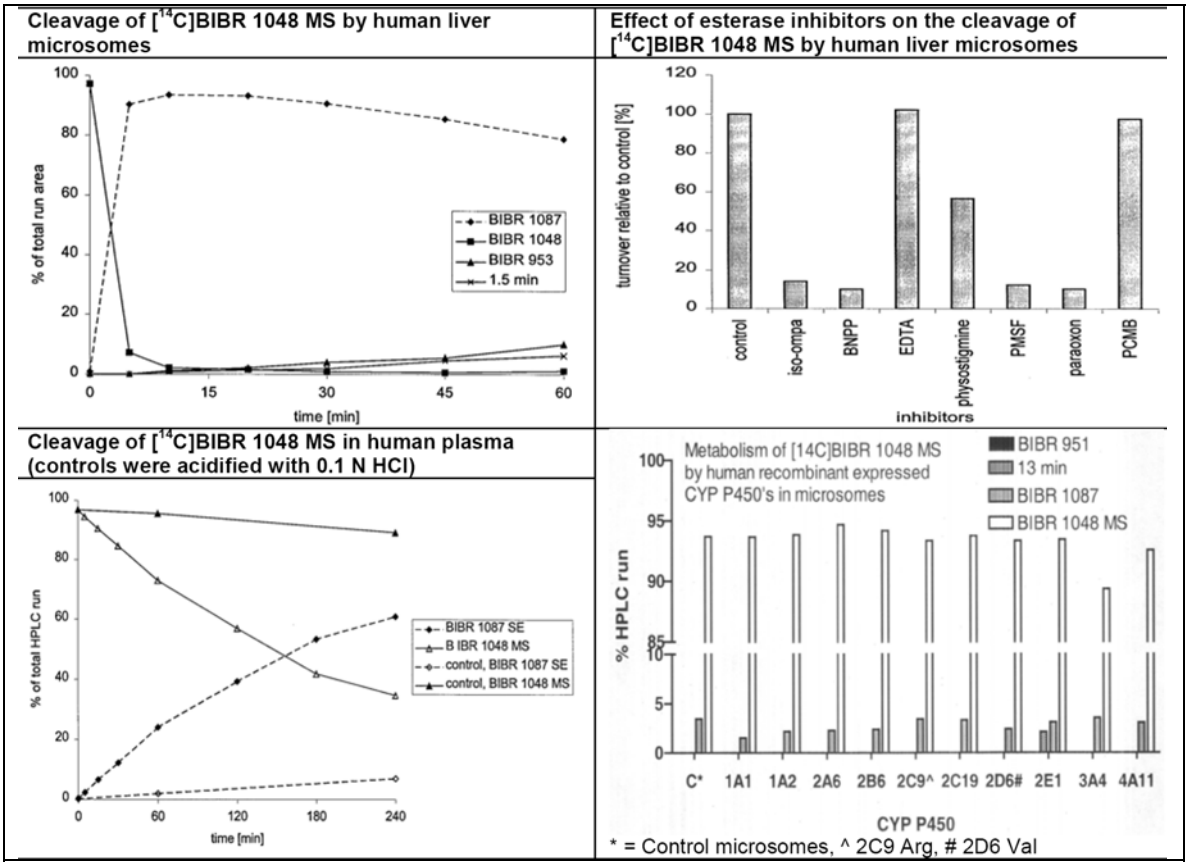
Subsequently, Study U06-1725 evaluated statistically the PK data from Study U04-3478 (BOI/266) in which BIBR 1048 MS had been administered orally to Han Wistar rats for 2 weeks. The analysis indicated that glucuronic acid conjugated BIBR 953 ZW represented 2.8 % of the total BIBR 953 ZW. Subsequently, a comparison of Chbb:THOM and Crl:WI(Han) rats indicated the mean relative percentage of acyl-glucuronides was about 8 % in plasma from both strains following oral administration of BIBR 1048 MS (Study U06-1046-01).

Only traces of glucuronidated BIBR 953 ZW could be detected in plasma from NMRI or CD1 mice following oral administration of BIBR 1048 MS (Study U08-1941-01). Furthermore, the BIBR 1048 BS, BIBR 1087 SE, and BIBR 951 BS forms were found to be unstable in mouse plasma and processed samples (Study U08-2324-01). Although the BIBR 953 ZW 1-O-acyl glucuronide is very unstable in animal plasma, the 2-O- and 3-O-isomers are more stable (Study U06-1706-01).

In vitro studies

Esterases found in human liver microsomes and human plasma rapidly hydrolyzed the prodrug BIBR 1048 MS to the mono-pro drugs BIBR 1087 and BIBR 951 (Study U01-1602) (Figure 19). The hydrolysis of [^{14}C] BIBR 1048 MS or BIBR 1087 SE was inhibited by the esterase inhibitors, tetraisopropyl pyrophophoramide (iso-OMPA), bis-(p-nitrophenyl) phosphate sodium (BNPP), phenylmethylsulfonyl fluoride (PMSF) and paraoxon, but not p-chloro mercuribenzoate (PCMB) indicated the involvement of microsomal carboxylesterases (serine-esterases). In contrast, cleavage of [^{14}C] BIBR 1048 MS by esterases in human plasma was only inhibited by 10 mM EDTA, but not 1 mM EDTA, or the other esterase inhibitors. Recombinant human cytochrome P450 enzymes did not significantly effect the metabolism of [^{14}C] BIBR 1048 MS, although the amount of BIBR 1048 MS slightly decreased in the presence of CYP 3A4.

Figure 19: Sponsor's Figures – Study Study U01-1602



Using pooled human microsomes, the sponsor evaluated the effect of BIBR 1048 MS, BIBR 953 ZW, BIBR 951 CL and BIBR 1087 SE on the cytochrome P450 catalyzed test reactions indicated in Table 22 (Study U99-1767). At a concentration of 100 µM BIBR 1048 MS showed maximal inhibition (50%) of the CYP2E1 and CYP3A4 reactions, but slight inhibition (35%) of the CYP2C9 reaction. BIBR 953 ZW did not inhibit any of the test reactions; however, BIBR 953 ZW and BIBR 951/1087 slightly activated (< 2-fold) the CYP2E1 and CYP4A11 reactions. Although BIBR 1087 SE slightly inhibited 1A1/1A2 reactions at concentrations of 1, 10 and 100 µM, the sponsor concluded that no form of BIBR at concentrations up to 10 µM exhibited relevant inhibition of any of the test reactions. The sponsor concluded that clinically relevant drug-drug interactions are unlikely to occur between any BIBR form and cytochrome P450 metabolized drugs.

Table 22: Reviewer's Summary - Study U99-1767

In vitro inhibition of CYP P450 reactions.

CYP P450	Test reaction	% of control at 10, 100 µM				
		BIBR 1048 MS	BIBR 953 ZW	BIBR 951 CL	BIBR 1087 SE	Control compounds
1A1 and 1A2	phenacetin O-dealkylation	111, 79	91, 89	103, 91	***70, 64**	A: 49% at 2 µM
2B6	*S*-mephenytoin N-dealkylation	111, 123	103, 99	98, 83	91, 75	
2C9	tolbutamide hydroxylation	102, **66**	103, 101	111, 112	98, 78	B: 25% at 2 µM
2C19	*S*-mephenytoin 4'-hydroxylation	135, 127	94, 105	106, 89	95, 102	
2D6	bufuralol 1'-hydroxylation	101, 86	100, 100	100, 98	98, 93	
2E1	lauric acid 11-hydroxylation	125, **50**	***178, 180***	***182, 158***	***171, 145***	C: 55% at 400 µM
3A	nifedipine oxidation	108, **69**	124, 130	111, 83	120, 96	D: 43% at 0.05 µM
3A	testosterone 6ß-hydroxylation	97, **49**	110,109	97, 81	104, 97	
4A11	lauric acid 12-hydroxylation	151, 130	***204, 188***	***187, 176***	***181, 162***	

Control compounds: A, furafylline; B, sulphaphenazole; C: diethyldithiocarbamate ; D, ketoconazole.
* 75% of control at 1 µM

To investigate effects on the hepatic expression of cytochrome P450 enzymes, BIBR 1048 MS was dosed at 3 or 100 mg/kg to two groups of male rats for 4 days, along with positive and negative control groups (U99-1134). Liver microsomes were prepared and used *in vitro* for cytochrome P450 enzyme assays and for immunoblotting of cytochrome P450 isoenzymes after normalization for total protein concentration. Table 23 indicates that relative to the positive controls, BIBR 1048 BS did not induce any cytochrome P450 enzymes tested based on enzyme activity. However, the dose of 100 mg/kg BIBR 1048 MS resulted in a 60 % decrease in CYP2E1 and CYP4A1 activities. Using immunoblotting, some increase of the amounts of CYP1A2, CYP2E1 and CYP4AI protein concentrations was observed. However, these increases were less than 20% of the increases for the respective positive controls. Thus, the sponsor concluded that BIBR 1048 MS-treated rats exhibited no relevant cytochrome P450 enzyme induction.

Table 23: Reviewer's Modification of Sponsor Tables - Study U99-1134

Effect of oral BIBR 1048 MS on hepatic cytochrome P450 enzymes

A: Effect on *in vitro* hepatic cytochrome P450 enzyme activities

Test reaction	Marker for	Fold change versus vehicle control		Positive control compound	Positive control Fold change versus vehicle control
		3 mg/kg BIBR 1048 BS	100 mg/kg BIBR 1048 BS		
ethoxyresorufin O-deethylation	CYP1A1/1A2	1.4	1.0	β-napthoflavone	17.2
pentoxyresorufin O-dealkylation	CYP2B1/2B2	1.2	1.0	phenobarbital	45
testosterone 6ß-hydroxylation	CYP3A	0.8	0.9	Dexamethasone, phenobarbital	4.8 3.4
lauric acid 11-hydroxylation	CYP2E1	0.9	0.4	(acetone)	- (Not done)
lauric acid 12-hydroxylation	CYP4A1	0.8	0.4	clofibrate	2.7

B: Effect on hepatic cytochrome P450 enzyme amounts assayed by immunoblotting

Cytochrome P450 Enzyme	Result		Positive control compound	Positive control Fold change versus vehicle
	dose 3.46 mg/kg	dose 115 mg/kg		
CYP1A1	-	-	β-napthoflavone	>7.3
CYP1A2	-	+/-	β-napthoflavone	
CYP2B1/2B2	-	-	phenobarbital	>>10
CYP2E1	-	+/-	Acetone*	15.8
CYP3A	-	-	Dexamethasone	>>10
CYP4A1	+/-	+/-	clofibrate	23.9

Legend:
- not relevant induction
+/- minor induction, less than model inducer
+ induction similar to model inducer
++ strong induction, exceeding model inducer

* From a previous experiment.

The effect of erythromycin, a well known inhibitor of CYP 3A4, on the activity of the esterases that metabolize BIBR 1048 MS was examined in vitro using human liver microsomes (Study U05-1339). As shown in Table 24, even after pre-incubation of erythromycin with human liver microsomes in the presence of NADPH, no significant effect was observed on the conversion of BIBR 1048 MS to BIBR 1087 SE, BIBR 951 CL and BIBR 953 ZW in vitro. An unknown metabolite was present at about 2 % of total peak area in the presence of NADPH formation and at 0.3% in the absence of NADPH. The human liver microsomes were shown to be active with a CYP 3A4/5 activity of 2718 pmol/min/mg protein for testosterone 6ß-hydroxylation. Ester cleavage and metabolite formation of [^{14}C] BIBR 1048 MS were not affected by erythromycin in vitro.

Table 24: Reviewer's Summary - Study U05-1339

Erythromycin was preincubated with human liver microsomes (0.25 mg protein/mL) in the presence of NADPH for 20 min before the addition of [^{14}C]BIBR 1048 MS and subsequent incubation for 30 min. Values are mean % of total peak area.				
	Erythromycin concentration (µM)			
Compound	0	1	10	100
BIBR 1087 SE	82.8	81.6	82.0	82.8
BIBR 953 ZW	4.47	4.75	5.19	4.57
BIBR 951 CL	0.58	0.42	0.49	0.41
BIBR 1048 MS	5.75	6.98	6.38	7.04
unknown	1.92	1.88	1.57	1.42
Not resolved	4.43	4.34	4.33	3.80

The glucuronidation of BIBR 953 ZW was investigated in vitro using human liver or intestinal microsomes or expressed recombinant UDP-glucuronosyltransferases in the presence of the co-substrate UDP-glucuronic acid to yield the formation of the 1-O-acylglucuronide of BIBR 953 ZW (Study U07-1421). The glucuronidation rate of BIBR 953 ZW in incubations with 16 different individual liver donors varied more than 10-fold (Figure 20A). No obvious relationship was found between the glucuronidation rate and the gender of the individual donors. However, the high rate of glucuronidation of one donor was attributed to that donor receiving phenobarbital, a known enzyme inducer. Using a range of substrate concentrations, enzyme kinetic parameters were calculated based on Michaelis-Menten kinetics. Comparison of microsomes from liver and intestine indicated a lower Vmax of the intestinal glucuronidation of BIBR 953 ZW (Table 25).

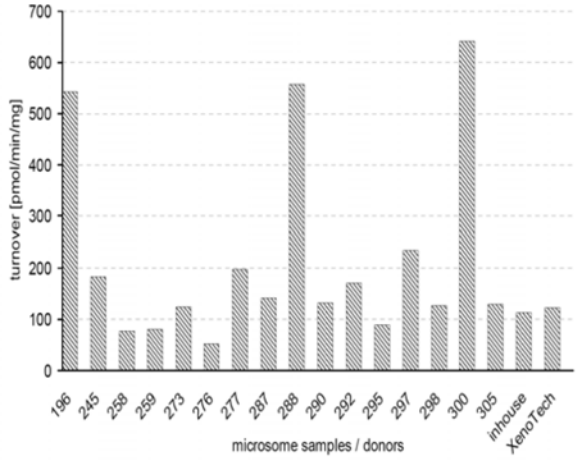
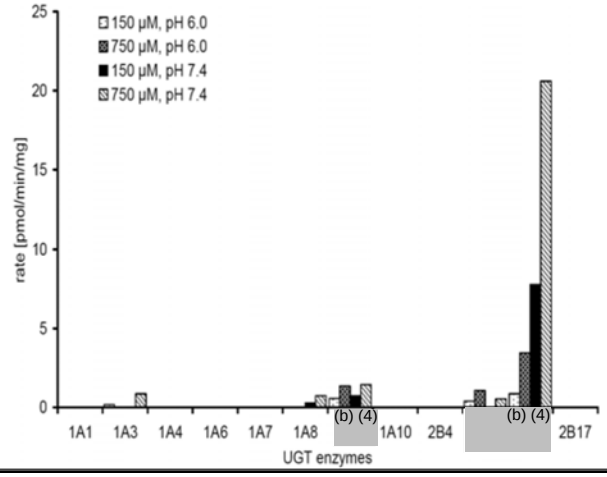
Table 25: Reviewer's Summary – Enzyme Kinetics – Study U07-1421

Sample	N	Km (µM)	Vmax (pmol/min/mg)
Individual liver microsome	6	Range 180 – 240	Range 92.3 - 981
Liver, XenoTech pool	50	503	215
Liver, in-house pool	10	438	186
Ileum microsomes	2	411, 454	8.6, 0.7
Jejunum microsomes	2	299, 759	2.2, 12.9
UGT 1A9 Supersome	NA	371	1.7
UGT 2B7 Supersome	NA	987	2.0
UGT 2B15 Supersome	NA	512	31.8

BIBR 953 ZW was also incubated with commercially available recombinant human UGT enzymes expressed on microsomes from baculovirus infected insect cells expressing

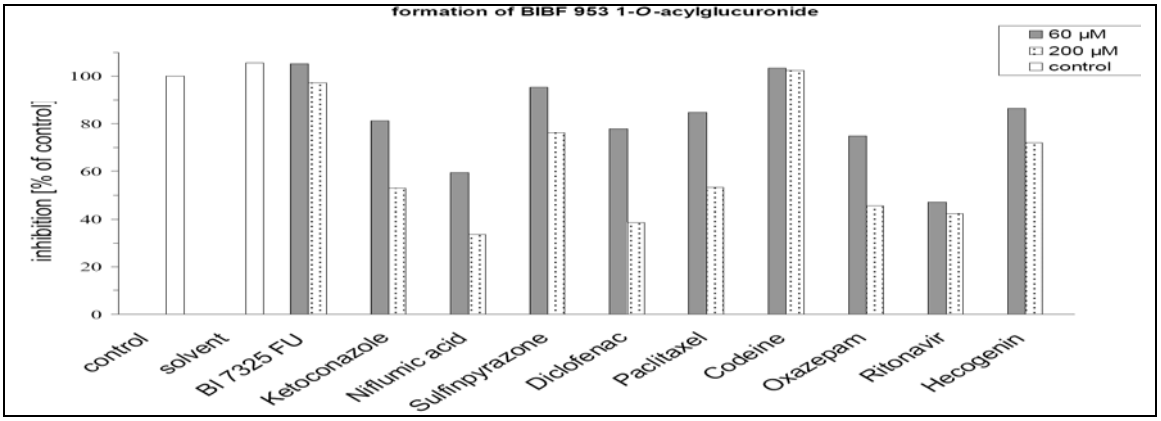
UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, (b) (4), 1A10, 2B4 (b) (4), and 2B17. Of the 12 human UGT enzymes, only (b) (4) exhibited reproducible glucuronidation activity (Figure 20B). The intrinsic clearance of (b) (4) (expressed as Vmax/Km) was 0.004, 0.002 and 0.062 µL/mg/min, respectively, indicating that (b) (4) has the highest activity for the glucuronidation of BIBR 953 ZW (Table 25).

Figure 20: Sponsor's Figures – Study U07-1421

A. Glucuronidation of BIBR 953 ZW by a panel of individual human liver microsomes and two samples of pooled human liver microsomes.	B. Glucuronidation of BIBR 953 ZW by expressed human recombinant UGT enzymes on microsomes from baculovirus infected insect cells
	

To further assess the UGT enzyme involved in the glucuronidation of BIBR 953 ZW, eleven inhibitors were evaluated for the ability to inhibit BIBR 953 ZW glucuronidation by pooled human liver microsomes. Diclofenac, amiodarone, niflumic acid, ketoconazole and ritonavir inhibited BIBR 953 ZW glucuronidation by human liver microsomes with IC_{50} values of 155 µM, 107 µM, 55.7 µM, 47.8 µM and 10.4 µM, respectively (Figure 21). The sponsor concluded that BIBR 953 ZW is a non-specific, low-affinity substrate of (b) (4). Also, (b) (4) is the major catalyst for the formation of the 1-O-acylglucuronide of BIBR 953 ZW.

Figure 21: Sponsor's Figure – Study U07-1421



Excretion:

In rats, most (83%) of an intravenous dose of [^{14}C] BIBR 1048 MS is eliminated via the bile within 6 hours (Study U98-2257). Excretion balance demonstrated that 87-89 % of the dose is excreted with the feces and 13-11% with the urine after either intravenous administration of BIBR 953 ZW or oral administration of BIBR 1048 MS. Most (> 95%) of the total radioactivity is excreted within the first 24 hours after administration. Biliary excretion accounted for 8% of the dose after intraduodenal administration and 83% of the dose after intravenous administration. The resulting oral absorption of 10% based on biliary excretion is consistent with the 12% oral absorption based upon comparison of oral and intravenous AUC values. The degree of the enterohepatic recirculation was estimated to be only 0.4 % of the dose.

In rabbits, most of the radioactivity (87%) was excreted with feces after oral administration of [^{14}C] BIBR 1048 MS and a small fraction (2.4 %) was excreted via urine (Study U05-2451). Similarly, in mice, most of the radioactivity (93%) was excreted with feces after oral administration of [^{14}C] BIBR 1048 MS and a fraction (6 %) was excreted via urine (Study U04-1254).

Monkeys also excreted most (90 %) of the orally dosed radioactivity into the feces and only 3% into the urine (Study U99-1092). Figure 22 below shows more than 50% of the total radioactivity is excreted within 24 hours and 93-97% is excreted within 120 hours. In contrast, following intravenous administration, 20% of the dose was excreted into the urine (Study U01-1761 and U99-1092).

Figure 22: Sponsor's Figures – Studies Study U01-1761

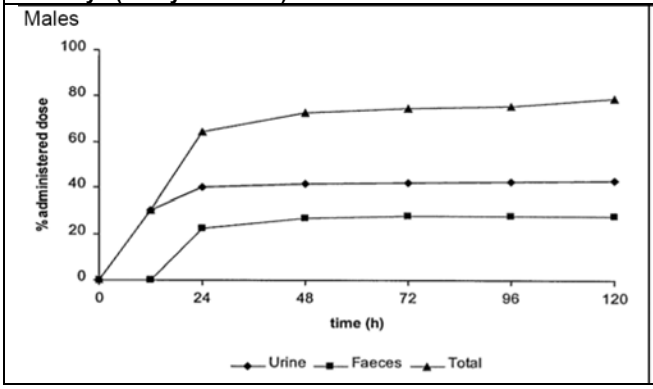
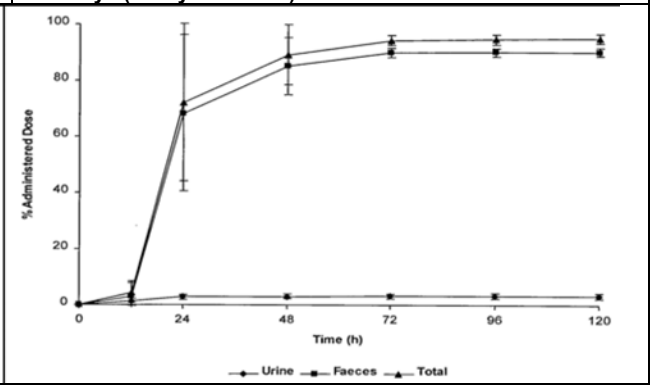
Cumulative Recovery of Total Radioactivity Following Single Intravenous Administration of 0.3 mg/kg [^{14}C]-BIBR 953 ZW to Monkeys (Study U01-1761)	**Cumulative Recovery of Total Radioactivity Following Single Oral Administration of 3 mg/kg [^{14}C]-BIBR 1048 to Monkeys (Study U99-1092)**
	

Table 26 shows that the parent compound, BIBR 1048 MS, accounted for a large proportion of the radioactivity extracted from monkeys feces. This is consistent with the low absorption of 5-7% based upon comparison of oral and intravenous AUC values. As previously discussed, the unidentified component was subsequently determined to be a mixture of acylglucuronides of BIBR 953 ZW.

Table 26: Sponsor's Tables – Study U99-1092

Quantitative Distribution of Metabolites in Monkey Feces Extract and Urine Pools Following TLC Analysis

					% radioactivity applied to plate		% dose applied to plate	
Sample	Animal Number	Extraction Efficiency (%TRR)	% dose	Component	R_f	%	R_f	%
Faeces 0-48 h	1♂-2♂	74.6	81.22	1[a]	0.11	40.1	0.11	24.9
				2[b]	0.21	12.2	0.21	7.4
				3[c]	0.44	9.3	0.44	5.6
				4[d]	0.59	31.1	0.59	18.8
	3♀-4♀	74.5	87.53	1[a]	0.11	24.5	0.11	16.0
				2[b]	0.22	14.3	0.22	9.3
				3[c]	0.45	7.9	0.45	5.1
				4[d]	0.60	44.7	0.60	29.1
Urine 0-24 h	1♂-2♂	NA	3.25	4[d]	0.54	22.9	0.54	0.7
				5[e]	0.63	67.7	0.63	1.8
	3♀-4♀	NA	2.70	4[d]	0.58	43.1	0.58	1.2
				5[e]	0.66	52.2	0.66	1.4

Reference Standards (R_f):

[a] BIBR1048 (0.11)
[b] BIBR1087 (0.22)
[c] BIBR951 (0.44)
[d] BIBR953 (0.59)
[e] Unidentified component

NA = Not applicable
% TRR = % Total Radioactive Residue

The sponsor provided the following overview of excretion and mass balance in various animal species. Urinary excretion was highest in the rat and lowest in the rabbit.

Table 27: Sponsor's Overview of Excretion in Animals

Overview of the excretion routes and balances after oral administration of [^{14}C] BIBR 1048 MS in animal species [mean percent of dosed radioactivity]

Species	Mouse	Rat	Rabbit	Rhesus monkey
Sampling period (maximum)	0 h – 72 h	0 h – 72 h	0 h – 72 h	0 h – 120 h
Sampling period (for ≥ 95% of dose)	0 h – 48 h	0 h – 24 h	0 h – 72 h*	0 h – 120 h*
Urinary excretion (maximum)	6.4%	11.1%	2.4%	3.4%
Fecal excretion (maximum)	92.6%	87.6%	87.3%	89.8%
Total excretion (maximum)	101.0%	98.7%	89.7%	94.6%

* recovery of dosed radioactivity < 95% within the sampling time

Metabolism and excretion of [^{14}C] BIBR 1048 MS in milk was evaluated following administration of a single oral dose of 10 mg/kg [^{14}C] BIBR 1048 MS to lactating Han Wistar rats on Day 11 or 12 of lactation (Study U06-1452). At 1 hour after dosing, the mean concentration of radioactivity in milk (132 ng-eq/mL) was 11% of the mean concentration in plasma of five dams (1160 ng-eq/mL). The $AUC_{(0\text{-}24h)}$ of radioactivity in milk was calculated to range from 1060 to 1940 ng-eq.h/g. The average concentration of radioactivity in milk ranged from 44.2 to 80.8 ng-eq/mL or 0.0021 to 0.0035% of the dose per gm of milk. Only 0.076 to 0.125% of the dose administered to the dams was excreted into milk within 24 hr. After administration of BIBR 1048 MS, the milk samples contained primarily BIBR 953 ZW, which represented 69 to 100% of sample radioactivity. However, at the 1 and 6 hour timepoints a new metabolite (M385), accounted for 30.7% and 21.2% of sample radioactivity, respectively. M385, whose highly lipophilic structure could not be determined by mass spectrometry, was not detected in previous metabolism studies of BIBR 1048 MS in rats [U04-1627-01]. A dose of BIBR 1048 MS was administered to a dam at 3 h postpartum and at 2 to 3 h post dose 0.002 to 0.003% of the dose was found in two pups. Since most of the

recovered radioactivity was located in the carcass rather than the gastrointestinal tract, radioactivity passed across the gastrointestinal wall of the pups. However, the structure of the radioactive material in the pups was not determined.

Table 28: Sponsor's Tables – Study U06-1452

Metabolite pattern in milk from lactating female rats after oral administration

time [h]	metabolite	[ngeq/g]	[µmol/L]	(% of sample radioactivity)
1	M385	40.5	64.6	30.7
	BIBR 953 ZW	91.5	145.7	69.3
6	M385	22.1	35.2	21.2
	BIBR 953 ZW	82.2	130.9	78.8
24	M385	-	-	-
	BIBR 953 ZW	4.6	7.4	100.0

Concentration of radioactivity in dams

	plasma			milk	
time	n	mean	CV	mean	CV
[h]		[ngeq/g]	(%)	[ngeq/g]	%
1	5	1160	15.3	132	38.7
6	5	44.1	49.3	104	23.2
24	5	3.45	24.4	4.62	14.7

Distribution of BIBR 953 ZW radioactivity in pups

	pup 156-1 2.33 h post dose		pup 156-2 3 h post dose	
	ng-eq	% of dose	ng-eq	% of dose
GI tract	6.81	0.00032	4.16	0.00019
Carcass	64.71	0.00301	45.5	0.00212
Total	71.52	0.00333	49.7	0.00231

5.2 Toxicokinetics

Table 29 summarizes the toxicokinetic parameters in multiple toxicology studies. Glucuronide conjugates accounted for 7-10% of the total BIBR 953 ZW in rabbit plasma, but more than 50% of the total BIBR 953 ZW in monkey plasma. Glucuronide conjugates were present at low levels (2.8 – 7%) in rat plasma and were not measured in rat toxicology studies.

In general, gender did not consistently affect exposure to BIBR 953 ZW in rats and monkeys. However, in the 26-week study in rats, females tended to have a lower AUC than males. Although repeat dosing did not consistently affect exposure, both male rats and male monkeys tended to have a lower exposure with repeat dosing.

Table 29: Reviewer's Summary of TK from Multiple Toxicology Studies

Summary of toxicokinetic studies				**Male**		**Female**	
Species/ Study	**Study length**	**Dose* mg/kg**	**Study day**	**Cmax ng/mL**	**AUC (0-t) ng.hr/mL**	**Cmax ng/mL**	**AUC (0-t) ng.hr/mL**
Rat (Chbb: THOM strain)	**14 days (3/sex/ group)**	34.6 (30)	1	792	2762	1200	2846
			15	465	1757	739	2565
		115.3 (100)	1	3150	15550	5767	14110
			15	792	4385	2310	8076
		346 (300)	1	3944	37360	4684	23380
			15	2345	13490	2447	8955
Rat (Chbb: THOM strain)	**28 days (3-4/ sex/ group**	17.3 (15)	1	370	1134	382	971
			25	269	836	528	1156
		80.5 (70)	1	1363	6098	1129	4742
			25	1195	5016	1315	4642
		346 (300)	1	2476	22190	3156	18010
			25	1442	13350	1945	12840
Rat (Chbb: THOM strain)	**26 weeks N = 3-4/ sex/ group**	11.5 (10)	1	160	600	65	211
			90	134	583	195	648
			181	137	739	129	545
		46.1 (40)	1	643	1950	315	912
			90	682	2540	634	1960
			181	488	2640	430	1420
		230.6 (200)	1	4710	14700	2720	6150
			90	985	6240	1200	4420
			181	1260	8780	1280	6640

Summary of toxicokinetic studies				Male		Female	
Species/ Study	**Study length**	**Dose* mg/kg**	**Study day**	**Cmax ng/mL**	**AUC (0-t) ng.hr/mL**	**Cmax ng/mL**	**AUC (0-t) ng.hr/mL**
Rat, Carcino-genicity (Wistar strain)	**104 weeks**	34.6 (30))	1	772	2310	502	1470
			181	544	2470	681	2550
			371	381	2720	473	2000
		115.3 (100)	1	3920	11100	1730	5760
			181	823	4290	1600	8870
			371	1130	8040	1140	5270
		230.6 (200)	1	4840	14400	2940	11200
			181	1130	9380	2100	13400
			371	1930	13400	2620	13300
Mouse	**104 weeks**	34.6 (30))	181	422	1160	827	1290
		115.3 (100)	181	1460	3830	2330	5400
		230.6 (200)	181	2510	5830	3220	9520
Rat, pregnant	**GD 7 to GD 16**	17.3 (15)	GD 16	-	-	633	1470
		80.7 (70)	GD 16	-	-	2560	7200
		230.6 (200)	GD 16	-	-	4280	20800
Rat, Pregnant or lactating	**GD 6 LD 21**	17.3 (15)	GD 7			294	1520
		17.3 (15)	LD 5			258	1380
		34.6 (30)	GD 7			451	2470
		34.6 (30)	LD 5			593	3110
		80.7 (70)	GD 7			1220	6280
		80.7 (70)	LD 5			1180	6500
Sum = BIBR 953 ZW + glucuronide conjugates				**953 ZW Sum**	**953 ZW Sum**	**953 ZW Sum**	**953 ZW Sum**
Rabbit, pregnant	**GD 6 to GD 18**	17.3 (15)	GD 13	-	-	162 178	940 1020
		80.7 (70)	GD 13	-	-	498 536	2550 2760
		230.6 (200)	GD 13	-	-	1790 1590	10900 7700
Monkey (Rhesus)	**28 days N = 3-6/ sex/ group**	34.6 (30)	1	127 445	1086 4526	74 311	418 2009
			28	44 306	510 4160	18 162	186 1710
		117.5 (100)	1	181 846	2470 11580	155 542	1170 4849
			28	308 1319	1608 12610	97 760	1164 9499
		346 (300)	1	231 934	2790 11810	302 1734	3688 22020
			28	196 1483	2881 23880	218 2285	2629 29700
Monkey (Rhesus)	**26 weeks N = 4-6/ sex/ group**	13.8 (12)	1	132 322	1011 3040	138 357	673 2260
			86	89 280	508 2160	143 343	799 2380
			179	47 231	390 2050	98 505	613 2910
		41.5 (36)	1	216 675	2120 7250	179 407	1160 3110
			86	246 739	1780 5950	299 669	1630 4590
			179	232 719	1540 5910	389 1040	2490 8130
		230.6 (200)	1	318 664	2210 5860	431 1150	4160 12000
			86	476 1140	4920 13900	1000 2420	10400 0500
			179	452 1260	4370 14500	672 1570	5640 19000
Monkey (Rhesus)	**52 weeks N = 4-6/ sex/ group**	13.8 (12)	1	148 535	713 3350	86 405	454 2660
			114	98 340	458 2170	49 330	245 2110
			210	204 475	987 2600	104 245	470 1300
			391	124 389	632 2650	54 290	287 1820
		41.5 (36)	1	241 895	1480 6820	176 772	1010 5530
			114	316 1120	1450 7320	136 591	693 4170
			210	506 858	2230 5880	184 332	913 2190
			391	230 898	1120 5730	104 417	527 2950
		230.6 (200)	1	821 1640	5130 16000	647 1470	4620 16300
			114	904 2780	4110 17600	979 2720	4710 18300
			210	1070 2030	4880 13400	915 1630	4860 10700
			391	645 1610	3980 14900	672 1780	4740 15900

* Dose as BIBR 1048 MS (BIBR 1048 BS, the base), GD = gestation day LD = Lactation day

6 General Toxicology

6.1 Single-Dose Toxicity

After a single oral administration of BIBR 1048 MS, the approximate oral lethal dose was above 2000 mg/kg for both rats and mice [Studies U01-1428 and U01-1429]. After a single intravenous administration of BIBR 953 ZW, the approximate intravenous lethal dose was above 100 mg/kg for mice [Study U01-1605], but between 75 mg/kg and 100 mg/kg for rats [Study U01-1606].

Acute toxicity was also evaluated in dose range-finding studies in dogs [Study U98-2799] and Rhesus monkeys [Study U98-2722]. Oral administration of 692 mg/kg BIBR 1048 BS to dogs only produced the expected pharmacodynamic effect on coagulation parameters. However, oral administration of 500 and 600 mg/kg BIBR 1048 MS to monkeys also produced positive fecal occult blood. However, no deaths occurred either in the dog or in the monkey study. Therefore, the approximate oral lethal dose of BIBR1048 MS in dogs and monkeys is above 600 mg/kg.

In the oral dose range finding study in dogs, BIBR 953 ZW was also given intravenously to one male dog over seven consecutive days [Study U98-2799]. Since a dose of 20 mg/kg on day 1 caused severe hemorrhages, but no deaths, the approximate lethal dose in dogs after a single intravenous administration was considered to be above 20 mg/kg.

In an intravenous maximum tolerated dose study in Rhesus monkeys, BIBR 953 ZW doses of up to 40 mg/kg were administered for seven consecutive days [Study U01-1115]. Although bruising and dark feces were observed, the approximate lethal dose in monkeys after intravenous administration was considered to be above 40 mg/kg.

6.2 Repeat-Dose Toxicity

Study reports for repeat dose toxicology studies were submitted and reviewed with the original submission (N000) to IND 65813. The detailed reviews of these studies are provided in the IND review dated 9/24/03. In addition, the study reports for 13-week maximum tolerated dosage studies in rats and mice were submitted with the proposed protocols (N028SX and N029SX) for the rat and mouse carcinogenicity studies. The detailed reviews of these 13-week studies are provided in the IND reviews dated 2/19/04. Table 30 below provides a summary of the previously reviewed studies in rats and monkeys.

Table 30: Reviewer's Summary of Longest Toxicology Studies Previously Reviewed

Species	**Rat**			**Rat**			**Monkey**		
Strain	**Han Wistar**			**Chbb:THOM**			**Rhesus**		
Study length	13 weeks			26 weeks			26 weeks		
Study code	BOI 277/032919			979/5-D6154			1813-011		
Document code	U05-1378			U03-1310			U03-1208		
Administration	Oral gavage			Oral gavage			Oral gavage		
Doses, mg/kg (base)	0, 30, 100, 300 of 1048 BS			0, 10, 40, 200 of 1048 BS			0, 12, 36, 200 of 1048 BS		
Number/sex/group	10 for main ; 10 for TK			20 for main (10 low and high dose recovery			4 for main (2 low and high dose recovery)		
Treatment –related Mortality/morbidity	In HD, 3 main, 4 TK deaths drug related			None (Two non-treatment related deaths)			1 HD female (Treatment–related anemia) (Two non-treatment related deaths)		
Adverse clinical signs	In HD, relate to bleeding			No treatment-related effects			No treatment-related effects		
Body weight gain	No treatment-related effects			No treatment-related effects			Overall 14% reduction in high dose males		
Food consumption	No treatment-related effects			No treatment-related effects			Reduced in mid- & high dose F only week 1		
Ophthalmoscopy:	Not monitored			No treatment-related effects			No treatment-related effects		
Electrocardiography	Not monitored			Not conducted			No treatment-related effects		
Hematology	Not monitored			Increased PT, APTT of >13% in high dose all timepoints, low and mid dose at 26 weeks			Increased APTT. Increased PT time HD. Decreased Hb, increased reticulocyte HD		
Blood chemistry	Not monitored			Decreased AST and ALT in HD M Week 4, MD & HD M & F Week 13, & MD& HD F Week 26. Slight increased bilirubin in high dose F			No significant treatment-related effects		
Urinalysis	Not monitored			No treatment-related effects			Increased protein in high dose F		
PK/TK at study end Cmax = ng/mL AUC = ng.hr/mL * BIBR 953 ZW + glucuronide † 1048 MS + 1%1157	Dose Mg/kg	Male	Female	Dose Mg/kg	Male	Female	Dose Mg/kg	Male	Female
	30	2600	2810	10	739	545	12	2050	2910
	100	6260	10500	40	2640	1420	36	5910	8130
	300	15200	26400	200	8780	6640	200	14500	19000
	300†	17100	24300						
Relative organ weight	Increased testes & prostate			No treatment-related effects			38% decrease in testes/epididymides relative weight in mid- and high dose M		
Macroscopic pathology	Dark colored thymus in high dose			No treatment-related effects			No treatment-related effects, but reduced testes size in some M		
Microscopic pathology	HD: epithelial vacuolation and hyperplasia in limiting ridge of the stomach, increased incidence of hemorrhage in multiple tissues			Dose-related increase in hemosiderosis with fibrosis in pancreas and thymus. Thymic atrophy in 4 HD males & 1 LD female Overall incidence of hemorrhage similar in control and high dose groups			Hemorrhage in various organs in high dose M&F. Atrophy of testes, prostate, seminal vesicle, epididymides increased in drug-treated males. Sponsor maintains atrophy is physiological due to seasonality of testicular function in rhesus monkeys		
Sponsor's NOAEL	100 mg/kg			40 mg/kg			36 mg/kg		

6.2.1 Repeat-Dose Toxicity in Rodents

Study title: BIBR 1048 MS (dabigatran etexilate) and impurities: 13-week oral (gavage) toxicity study in rats

Study no.:	06B122 (U07-1693)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	July 6, 2006
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch number: 1024879SPK, purity 96.7%. This batch of BIBR 1048 MS was spiked with the following impurities (target concentrations/concentrations in certificate): (b) (4)

Key Study Findings

Han Wistar rats received BIBR 1048 MS spiked with five specified impurities (b) (4) by oral gavage for 13 weeks followed by a recovery period of 4 weeks in the control and high dose animals. At dosages of 0, 15, 55, and 200 mg/kg/day BIBR 1048 MS, the mean $AUC_{(0-24h)}$ for BIBR 953 ZW was 1260, 2420, and 10900 ng•hr/mL in males and 1050, 3690, and 11400 ng.hr/mL in females, respectively. The activated partial thromboplastin time (aPTT) values were prolonged at the mid and high dosages and the thrombin time prolonged at all dosages. Treatment-related histopathological changes found only in the thymus included recurrent hemorrhages and hemosiderosis in the thymus of high dose animals, but not in the thymus of control animals. Because these findings were attributed to the pharmacodynamic effect of BIBR 1048 MS, the NOAEL of the study was considered to be 200 mg/kg. This NOAEL is consistent with the prior 13-week study in Han Wistar rats (U05-1378) in which the NOAEL was 100 mg/kg because of deaths at 300 mg/kg. Therefore, the spiked impurities are considered qualified.

Methods

Doses:	0, 15, 55, 200 mg/kg/day BIBR 1048 MS
Frequency of dosing:	Daily for 91 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)

Species/Strain:	Rat (Crl:WI(Han)
Number/Sex/Group:	Main: 10/sex/group
Age:	55-59 days
Weight:	M: 189.5 to 244.9 g, F: 147.1 to 194.8 g
Satellite groups:	Recovery: 10/sex for control and high dose only; TK: 5/sex/group
Unique study design:	This study was to determine the effects of BIBR 1048 MS batch spiked with impurities ([redacted] (b) (4) At necropsy, bone marrow samples were taken from the first five main male animals of the control), mid and high dose groups for analysis of micronuclei. The results of this analysis were reported separately in the report for 06B209 [U07-1489] and are reviewed in Section 7 of this document.
Deviation from study protocol:	High dose animals received 230.0 mg/kg BIBR 1048 BS on Day 29-32 instead the intended dose of 230.6 mg/kg BIBR 1048 BS. A mid dose animal (359) received 0.08 mL of formulation less than intended on Day 78. On Day 13, a control female (153) was injured at the tail and was sacrificed immediately for animal welfare reasons; however no samples were taken for clinical pathology or histopathology.

Observations and Results

Mortality

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

No treatment-related mortality occurred.

Clinical Signs

In addition to a more detailed weekly physical examination, detailed observations during treatment were made at least twice daily on regular work days. During acclimation and recovery observations were made once a day.

Other than bleeding after blood sampling on Day 3 in one high dose male and three high dose females, no clinical observations were considered related to treatment.

Body Weights

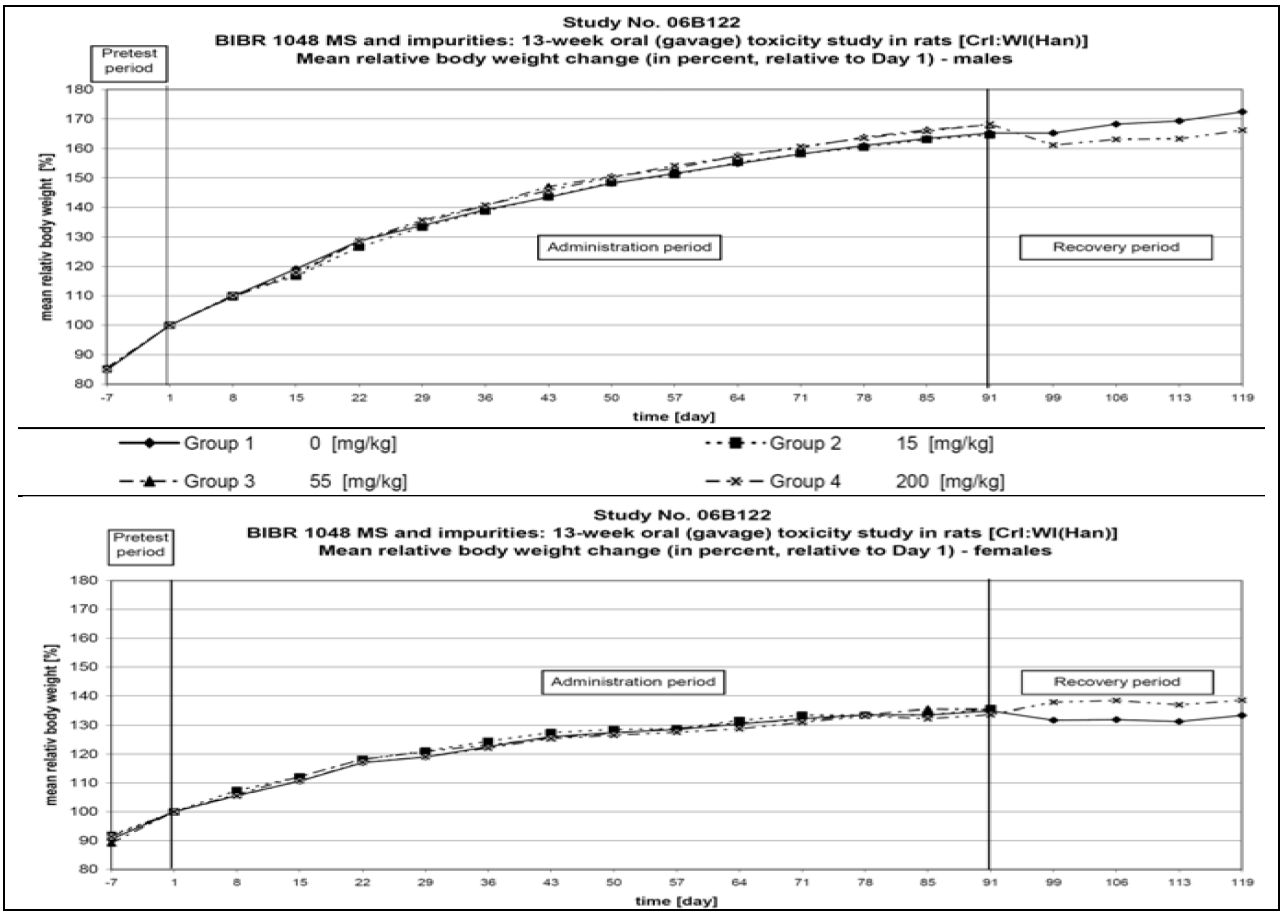
The animals were weighed weekly during acclimation, on Day 1 of treatment, weekly until study termination and before necropsy.

The test article did not significantly affect body weight either at the end of treatment or recovery (Table 31). However, during recovery, the body weight gain of the high-dose males was below and the body weight gain of high dose females was above those in the respective control groups (Figure 23).

Table 31: From Sponsor's Tables – Mean Body Weight - Study U07-1693

		Males (gm)				Females (gm)			
[day]		1	50	91	119	1	50	91	119
Group									
Group 1 0 [mg/kg]	n	20	20	20	10	20	19	19	10
	mv	222.54	330.46	368.67	375.68	170.42	216.94	229.55	230.30
	sd	14.45	39.25	45.75	49.16	12.29	18.06	18.16	19.17
Group 2 15 [mg/kg]	n	10	10	10		10	10	10	
	mv	218.56	325.28	360.83		162.17	208.44	219.97	
	sd	17.61	38.74	44.80		9.86	18.84	20.63	
	p	0.4328	0.6989	0.6098		0.0706	0.1757	0.1538	
Group 3 55 [mg/kg]	n	10	10	10		10	10	10	
	mv	216.90	326.86	364.73		169.13	215.02	228.56	
	sd	13.16	31.70	33.11		11.92	12.47	12.55	
	p	0.2674	0.7881	0.7975		0.7742	0.7577	0.8820	
Group 4 200 [mg/kg]	n	20	20	20	10	20	20	20	10
	mv	217.14	326.48	365.63	358.73	166.47	210.43	222.31	221.89
	sd	7.71	27.47	31.86	26.54	11.36	13.33	15.60	16.23
	p	0.1944	0.7160	0.8082	0.3500	0.2837	0.2055	0.1882	0.3038

Figure 23: Sponsor's Figures - Relative Body Weight Change - Study U07-1693

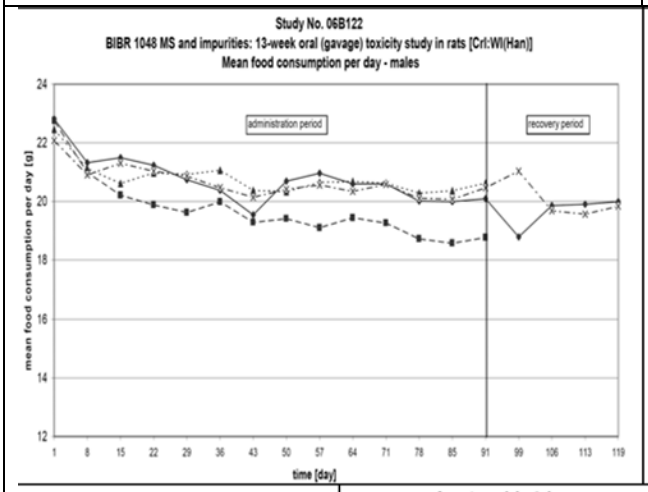
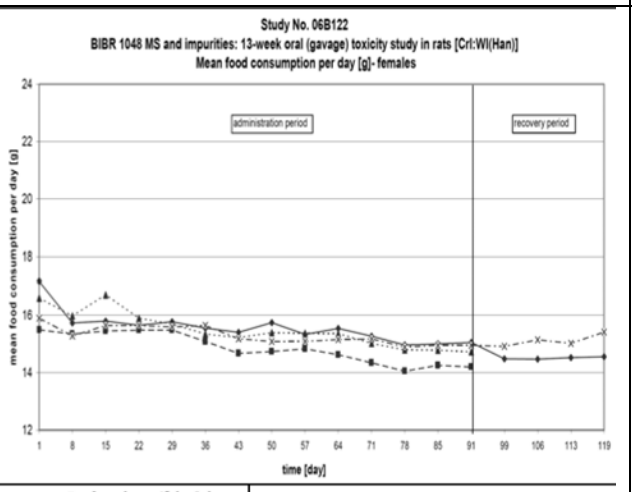


Food Consumption

The mean weekly food consumption per animal was estimated during pretest, treatment and recovery period on a cage basis by weighing of the remaining food (versus the pre-weighed food amount) on the same days as body weight measurements. Water consumption was checked daily by visual inspection.

Although slight decreases in food consumption were observed in the low dose males and females (Figure 24), a treatment-related effect was not observed.

Figure 24: Sponsor's Figures of Food Consumption - Study U07-1693

Males	Females
	

Ophthalmoscopy

After dilation with a mydriatic agent, the eyes of all main study and recovery animals were examined pretest on Day -5. The eyes of the control and high dose groups were examined during treatment on Day 83 and the eyes of the recovery animals were examined on Day 118. A slit lamp was used for ophthalmological examination of conjunctiva, sclera, cornea, anterior chamber of the eye, iris, lens and anterior part of the vitreous body, and an ophthalmoscope was used to examine the fundus.

The report stated that some findings (focal opacities in lens cortex and other locations) were observed. However, no individual animal data was provided. The sponsor concluded the findings were spontaneous, because such findings commonly occur in untreated animals of this strain and were similar in incidence in the control and the high dose group.

Hematology

Blood samples were obtained from all animals after fasting from the retrobulbar venous plexus under isoflurane anesthesia at the end of treatment on Day 92/93 and at the end of recovery on Day 120. The following hematology parameters were measured:

hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, morphology, platelet count, total white cell count, normoblasts (from blood smear) and differential white cell count, including neutrophils, lymphocytes, eosinophils, basophils, monocytes, and large unstained cells. Additional samples were taken for measurement of thrombin time, prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen concentration.

The sponsor noted statistically significant changes in hemoglobin, white blood cell count, lymphocytes and neutrophils as indicated in Table 32. However, these changes were not considered toxicologically significant because all individual values were within or close to the range of individual values for the concurrent control group or a clear dose relationship was lacking.

In contrast, the 14% and 22% increases in aPTT for the high dose groups were due to many values for individual males and all values for individual females being above the maximum value in the respective control group. The greater than 2-fold increase in thrombin time resulted from all individual values in the high dose groups being above the maximum in the respective control group. The increases in aPTT and thrombin time also showed a dose relationship.

Table 32: Sponsor's Table of Hematology Changes - Study U07-1693

Parameter [unit]	Day	Group Gender	Daily dose of BIBR 1048 BS [mg/kg]			
			0	15	55	200
			Group 1	Group 2	Group 3	Group 4
			mean	*mean*	*mean*	*mean*
HGB [g/dL]	92	M	16.67	16.45	16.43	16.40
	93	F	15.70	15.30↓	15.41	15.31↓
	120	M	16.60	---	---	16.45
	120	F	16.12	---	---	15.91
WBC [10^3/µL]	92	M	5.39	5.45	5.49	4.66
	93	F	3.62	2.83↓	3.32	2.92↓
	120	M	5.17	---	---	4.17
	120	F	3.45	---	---	3.19
Lympho [10^3/µL]	92	M	4.167	4.103	4.196	3.509↓
	93	F	2.884	2.234↓	2.715	2.357↓
	120	M	3.941	---	---	3.039
	120	F	2.736	---	---	2.399
PLT [10^3/µL]	92	M	844.7	880.4	817.1	849.3
	93	F	887.4	847.3	962.8↑	925.1
	120	M	760.3	---	---	738.6
	120	F	779.9	---	---	765.1
Neut.ce [10^3/µL]	92	M	1.001	1.144	1.053	0.957
	93	F	0.566	0.469	0.470	0.434↓
	120	M	0.948	---	---	0.868
	120	F	0.531	---	---	0.604
APTT [s]	92	M	16.90	17.15	18.65↑	19.22↑
	93	F	15.96	16.54	18.07↑	19.41↑
	120	M	15.70	---	---	16.17
	120	F	14.84	---	---	16.19
TT [s]	92	M	42.33	45.03	53.21	95.77↑
	93	F	39.03	46.79	82.70↑	79.63↑
	120	M	39.97	---	---	40.65
	120	F	38.11	---	---	37.81

↑, ↓: significantly increased, decreased compared with Control; p< = 0.05, many-to-one t-test, two-sided
M, F: males, females

Clinical Chemistry

At the same time as the collection for peripheral hematology, additional blood was obtained for the preparation of plasma and measurement of the following parameters:

alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase, glutamate dehydrogenase (GLDH), glucose, total bilirubin, total cholesterol, triglycerides, creatinine, urea, sodium, potassium, chloride, magnesium, calcium, inorganic phosphate, total protein and protein fractions (albumin, globulin, α-globulin, β-globulin, γ-globulin, and albumin/globulin ratio). Additionally, blood was collected for analysis of liver enzyme activities on Day 3 from non-fasted main and recovery animals.

On day 3, AST decreased in both males and females. However, the slight decreases in ALT and GLDH that occurred in females did not occur in males. At the end of treatment (Day 92), ALT, but not AST and GLDH, decreased in the high dose males and all three enzymes (AST, ALT and GLDH) decreased in the high dose females. However, a clear dose relationship was not evident.

At the end of treatment, the group mean for glucose was increased in the high dose males (+10.0 %, p = 0.05) and the high dose females (+9.4%, p = 0.02) compared to the respective group means in the control groups. However, a clear dose relationship was not evident for either gender. Furthermore, all values for individual treated animals were within or close to the maximum individual values in the respective control groups. At the end of the recovery period, the group mean for the high dose males remained higher (+10%, p = 0.15) than that for the control males; however, the group means for the high dose females and control females were almost identical.

Table 33: From Sponsor's Tables - Clinical Chemistry - Study U07-1693

Males		Day 3		Day 92				Day 120
Parameter		AST [U/L]	ALT [U/L]	AST [U/L]	ALT [U/L]	GLDH [U/L]	Glucose [mmol/L]	Glucose [mmol/L]
Group								
Group 1	n	20	20	20	20	20	20	10
0	mv	101.40	67.68	58.66	30.04	5.53	8.357	7.647
[mg/kg]	sd	30.04	7.58	15.48	3.96	0.92	1.194	0.872
Group 2	n	10	10	10	10	10	10	
15	mv	98.58	78.26	64.06	29.67	6.07	8.982	
[mg/kg]	sd	9.47	5.96	20.65	4.44	2.62	1.741	
	p	0.7214	* 0.0006	0.4080	0.8599	0.5640	0.2257	
Group 3	n	10	10	10	10	10	10	
55	mv	88.44	70.50	65.60	31.06	6.53	8.909	
[mg/kg]	sd	10.65	9.31	19.39	7.20	2.30	1.335	
	p	0.1051	0.3372	0.2887	0.6204	0.2891	0.2839	
Group 4	n	20	20	20	20	20	20	10
200	mv	85.34	64.78	59.07	26.58	6.37	9.192	8.407
[mg/kg]	sd	14.76	7.17	14.36	5.80	3.28	1.191	1.346
	p	* 0.0154	0.2271	0.9378	* 0.0444	0.2751	0.0501	0.1514

Female		Day 3			Day 92			
Parameter		AST [U/L]	ALT [U/L]	GLDH [U/L]	AST [U/L]	ALT [U/L]	GLDH [U/L]	Glucose [mmol/L]
Group								
Group 1	n	20	20	20	19	19	19	19
0	mv	79.59	53.61	4.98	73.05	28.50	13.97	6.267
[mg/kg]	sd	22.77	8.53	0.66	26.55	10.34	20.85	0.821
Group 2	n	10	10	10	10	10	10	10
15	mv	79.46	53.20	5.03	62.93	23.87	8.21	6.472
[mg/kg]	sd	26.56	4.75	0.81	9.44	3.49	4.43	0.880
	p	0.9869	0.9010	0.8316	0.1884	0.0928	0.2980	0.5047
Group 3	n	10	10	10	10	10	10	10
55	mv	71.56	45.97	4.39	68.54	27.62	9.81	6.313
[mg/kg]	sd	13.04	6.80	0.70	19.06	5.91	10.62	0.753
	p	0.2937	* 0.0236	* 0.0269	0.5554	0.7464	0.4512	0.8805
Group 4	n	20	20	20	20	20	20	20
200	mv	62.45	47.20	4.28	59.90	23.30	8.31	6.853
[mg/kg]	sd	13.90	10.31	0.56	14.58	3.92	9.75	0.704
	p	* 0.0076	* 0.0201	* 0.0015	* 0.0394	* 0.0227	0.2133	* 0.0229

Urinalysis

Urine was collected from main study and recovery animals on Day 85 (males), Day 86 (females) and on Day 112 (recovery animals, males and females) using metabolic cages. Prior to a 5 hour collection period, the animals received 20 mL/kg of drinking water by gavage 30 minutes after administration of the BIBR 1048 MS daily dose. The measured parameters included: color, turbidity, volume, pH, specific gravity, protein, nitrate, urobilinogen, glucose, bilirubin, ketone bodies, leucocytes, and erythrocytes. The urine sediment was examined microscopically for epithelial cells, leucocytes, erythrocytes, crystals, casts (hyaline, granulated, erythrocyte, leukocyte), kidney cells, bacteria and inorganic material.

During treatment, no treatment related changes were present at any dose group compared with the control group.

Gross Pathology

Animals were euthanized using ketamine/xylazine, exsanguinated, and subjected to detailed necropsy at the end of treatment or the end of recovery. The whole or a sample of the tissues listed below (Table 34) from all animals was preserved in 4% neutral buffered formalin, except for the following tissues. The eyes, optic nerves, testes and epididymides were fixed first in Davidson's fluid prior to fixation in 4% neutral buffered formalin. Bone marrow samples obtained from the sternum during necropsy were used to prepare smears that were dried, fixed, and then stained using a modified Pappenheim method. However, the smears were not examined because the peripheral blood results did not indicate such evaluation was needed.

Table 34: Reviewer's Table of Tissues Collected - Study U07-1693

Abnormal tissues	Kidneys	Seminal vesicles
Adrenal glands	Knee joint (with femur)	Sciatic nerve
Aorta	Larynx	Seminal vesicles
Bone (sternum)	Liver	Skeletal muscle
Bone marrow (sternum)	Lungs	Skin
Brain	Lymph nodes (axillary, mesenteric)	Spinal cord (cervical, thoracic and lumbar)
Cecum	Mammary gland (females)	
Cervix	Optic nerves	Spleen
Colon	Ovaries	Stomach
Duodenum	Oviducts	Testes
Epididymides	Pancreas	Thymus
Esophagus	Parathyroid glands	Thyroid
Extraorbital lacrimal glands	Peyer's patches	Tongue
Eyes	Pituitary	Trachea
Harderian glands	Prostate	Ureters
Heart	Rectum	Urinary bladder
Ileum	Salivary gland (parotid, sublingual submandibular)	Uterus
Jejunum		Vagina

The only macroscopic lesion attributable to treatment with BIBR 1048 MS was discoloration of the thymus in two males and two females in the high dose group.

Table 35: Sponsor's Summary of Macroscopic Findings - Study U07-1693

Group	1		3		4	
Group name	*Group 1*		*Group 3*		*Group 4*	
Dose level [mg/kg]	0		55		200	
	m	f	m	f	m	f
Animals in group :	10	9	10	10	10	10
Animals examined :	10	9	10	10	10	10
thymus	**(10)**	**(9)**	**(10)**	**(10)**	**(10)**	**(10)**
-discoloration	0	0	0	0	2	2
-reduced in size	0	0	0	0	1	0

Organ Weights

From each animal at necropsy, the following organs were excised and weighed: adrenal glands, brain, heart, kidneys, liver, lungs, mesenteric lymph node, axillary lymph node, ovaries, pituitary, prostate, spleen, testes, thymus, and thyroids with parathyroids.

The sponsor noted a decrease in the mean absolute and relative (to brain weight) organ weight of the prostate and an increase in the absolute and relative (to brain weight) organ weight of the testes of high dose males at the end of treatment. However, the changes relative to body weight were not statistically significant. A decrease in absolute and relative (to body weight) weight of the thymus in the high dose males was not statistically significant and did not occur in females. The high dose females showed an increase in absolute and relative (to body weight) weight of the heart, whereas the high dose males did not. None of these findings correlated with any histopathology finding.

Table 36: From Sponsor's Tables of Organ Weights - Study U07-1693

		Male					Female		
Parameter		Body wt. [g]	Prostate [g]	Testes [g]	Heart [g]	Thymus [g]	Body wt. [g]	Heart [g]	Thymus [g]
Group									
Group 1	n	10	10	10	10	10	9	9	9
0	mv	351.93	0.8397	3.2150	1.0091	0.2614	215.49	0.6832	0.2605
[mg/kg]	sd	43.05	0.1807	0.3655	0.1310	0.0363	15.33	0.1147	0.0776
Group 2	n	10	10	10	10	10	10	10	10
15	mv	336.64	0.7794	3.2975	0.9716	0.2467	203.61	0.6789	0.2072
[mg/kg]	sd	41.61	0.1153	0.2732	0.0921	0.0467	18.60	0.0528	0.0340
	p	0.3478	0.2704	0.5502	0.4416	0.4900	0.0945	0.9043	* 0.0461
Group 3	n	10	10	10	10	10	10	10	10
55	mv	341.53	0.8096	3.2395	1.0350	0.2447	211.34	0.7059	0.2377
[mg/kg]	sd	30.52	0.0682	0.2839	0.1101	0.0365	12.82	0.0748	0.0520
	p	0.5217	0.5801	0.8583	0.5942	0.4334	0.5522	0.5245	0.3821
Group 4	n	10	10	10	10	10	10	10	10
200	mv	361.58	0.7221	3.4935	1.0370	0.2393	211.00	0.7356	0.2590
[mg/kg]	sd	25.51	0.0856	0.2922	0.0934	0.0629	12.69	0.0548	0.0547
	p	0.5520	* 0.0355	* 0.0491	0.5663	0.3003	0.5203	0.1477	0.9555
Parameter		Body wt. [g]	Prostate [%]	Testes [%]	Heart [%]	Thymus [%]	Body wt. [g]	Heart [%]	Thymus [%]
Group									
Group 1	n	10	10	10	10	10	9	9	9
0	mv	351.93	0.2394	0.918	0.2877	0.0756	215.49	0.3167	0.1194
[mg/kg]	sd	43.05	0.0484	0.081	0.0255	0.0153	15.33	0.0474	0.0287
Group 2	n	10	10	10	10	10	10	10	10
15	mv	336.64	0.2369	0.991	0.2921	0.0742	203.61	0.3349	0.1025
[mg/kg]	sd	41.61	0.0586	0.127	0.0420	0.0165	18.60	0.0292	0.0186
	p	0.3478	0.9023	0.1288	0.7653	0.8301	0.0945	0.2419	0.1696
Group 3	n	10	10	10	10	10	10	10	10
55	mv	341.53	0.2395	0.954	0.3046	0.0714	211.34	0.3338	0.1126
[mg/kg]	sd	30.52	0.0374	0.102	0.0370	0.0063	12.82	0.0269	0.0257
	p	0.5217	0.9982	0.4448	0.2583	0.5104	0.5522	0.2702	0.5779
Group 4	n	10	10	10	10	10	10	10	10
200	mv	361.58	0.2011	0.970	0.2873	0.0659	211.00	0.3492	0.1235
[mg/kg]	sd	25.51	0.0304	0.106	0.0234	0.0152	12.69	0.0267	0.0303
	p	0.5520	0.0645	0.2682	0.9779	0.1298	0.5203	* 0.0404	0.7305

Histopathology

Adequate Battery

Tissue samples from all main study animals were dehydrated, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. In addition tissue slides from kidneys, testes and epididymides were stained according to the Periodic Acid Schiff's (PAS) method. The indicated tissues listed in Table 34 from the control and high dose groups and gross abnormalities identified at macroscopic examination from all animals sacrificed at the end of the scheduled treatment period were examined by histology. Because histopathological changes (hemorrhages) were noted in the thymus of the main study animals, a histopathological examination of the thymus of all animals in the low and mid dose groups and of the recovery animals was conducted. Changes of the mesenteric lymph nodes were also recorded, because thymus and mesenteric lymph nodes were embedded in the same paraffin block.

Peer Review

A reviewing pathologist in the conducting laboratory conducted a peer review.

Histological Findings

Treatment-related histopathological changes were only found in the thymus. Although no unequivocal increase in the incidence of acute hemorrhages was treatment related, recurrent hemorrhages (i.e., presence of hemosiderin together with acute hemorrhages) and hemosiderosis (i.e., presence of hemosiderin without evidence of acute hemorrhages) were present in the thymus of high dose animals and not in the thymus of control animals. These findings were attributed to the pharmacodynamic effect of BIBR 1048 MS.

Table 37: Reviewer's Modification - Sponsor's Table - Thymus Histopathology - Study U07-1693

Histopathological finding	Group 1 [0 mg/kg]		Group 2 [15 mg/kg]		Group 3 [55 mg/kg]		Group 4 [200 mg/kg]	
	M	F	M	F	M	F	M	F
End of treatment period								
Number evaluated	9	9	10	10	10	10	10	10
Thymus, acute hemorrhages	4	4	5	5	6	2	3	5
Thymus, recurrent hemorrhages	0	0	0	0	0	1	2	2
Thymus, hemosiderosis	0	0	0	0	0	0	2	3
End of recovery period								
Number evaluated	10	10					10	10
Thymus, acute hemorrhages	6	4	-	-	-	-	6	5
Thymus, recurrent hemorrhages	0	0	-	-	-	-	1	2
Thymus, hemosiderosis	0	0	-	-	-	-	1	3

M = maleF = female

Special Evaluation

The results of the analysis of micronuclei in half of the males in the control, mid and high dose groups are reported separately under Study No. 06B209 [U07-1489] in Section 7.

Toxicokinetics

Blood samples were collected from the toxicokinetic animals via the retrobulbar venous plexus under isoflurane anesthesia at 1, 3, 8 and 24 hours after dosing on Day 1 and on Day 90. Since a high dose male (421) was found dead on Day 90 after the 3 hour time point, the 8 and 24 hour samples could not be taken from this animal. BIBR 953 ZW was measured using a validated HPLC-MS/MS assay with [$^{13}C_6$]-BIBR 953 ZW as an internal standard. The assay with an inaccuracy (b) (4) and imprecision of (b) (4) was valid in the concentration range of (b) (4).

Systemic exposure to BIBR 953 ZW based on C_{max} and $AUC_{(0-24h)}$ increased with dose of BIBR 1048 MS; however, the increases were slightly less than proportional with dose. Repeated dosing or gender did not significantly change the C_{max} and $AUC_{(0-24h)}$ values.

Table 38: Reviewer's Modification - Sponsor's TK Parameters - Study U07-1693

parameter	day	gender	15 mg/kg	55 mg/kg	200 mg/kg
C(max) [ng/mL]	1	m	379	1340	2430
		f	407	1310	1970
	90	m	419	833	1880
		f	417	1250	2970
AUC(0-24h) [(ng·h)/mL]	1	m	1160	3880	11500
		f	980	3400	8730
	90	m	1260	2420	10900
		f	1050	3690	11400
Mean ratio Day 90/Day1,		m & f	1.09	0.89	1.30
Ratio M/F		Day 1	1.19	1.14	1.32
		Day 90	1.21	0.66	0.95

Formulation Analysis

The test article formulations were prepared daily on the day before being administered. Aliquots were taken on Day 1 and Day 90 of treatment and analyzed for achieved concentration and homogeneity. Stability of BIBR 1048 MS in the vehicle was previously assessed by the sponsor.

On Day 1 and Day 90, the mean assayed concentrations of BIBR 1048 BS in the formulations were within acceptable limits. The concentrations of all individual samples were within the range of (b) (4) of the nominal value and the mean values were within the range of 92.1 % to 95.0%. The formulation contained individual impurities in the following ranges: (b) (4) The assayed concentrations of the impurities were close to the concentrations on the certificate of analysis, except for (b) (4) which was (b) (4) times above the concentration listed in the certificate (b) (4).

Study title: BIBR 953 ZW: Repeated dose toxicity study in rats by intravenous administration (bolus) over a period of 4 weeks

Study no.:	99B024 (U00-1128)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	March 22, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch 8830251, 98.5%

Key Study Findings

Male and female Chbb:THOM rats received intravenous dose of 0, 0.05, 0.5 and 5 mg/kg of BIBR 953 ZW daily for 4 week. On day 28, doses of 0.5 and 5 mg/kg corresponded to AUC values of 516 and 7082 ng.hr/mL in males and 616 and 6737 ng.hr/ml in females. Slightly lower group mean values for hematocrit, hemoglobin concentrations and red blood cell counts were noted in the high dose males, but not the high dose females, in comparison with those of the respective controls. However, a significant increase in reticulocyte counts was observed in the high dose females but not the high dose males. PT values did not change; however, the mean aPTT values increased in the high dose females (16%). Thrombin times increased in the mid (8%) and high (10%) dose males and the high (12%) dose females. Fibrinogen concentrations increased in the mid (12%) and high (16%) dose males and the high (16%) dose females. Increases in mean potassium levels were statistically significant only in the high dose females. The NOAEL was 0.5 mg/kg in females and 5 mg/kg in males.

Methods

Doses:	0, 0.05, 0.5, 5 mg/kg
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Intravenous at 4 mL/minute
Dose volume:	10 mL/kg
Formulation/Vehicle:	20% propylene glycol, 5% mannitol
Species/Strain:	Rat (Chbb: THOM)
Number/Sex/Group:	10/sex/group
Age:	57 days
Weight:	M: 269.7-326.8, F: 173.4-217.4 gm
Satellite groups:	TK: 4/sex/group; Recovery: 10/sex/group for 0 and 5 mg/kg;
Unique study design:	Hemodynamic measurements (blood pressure and heart rate
Deviation from study protocol:	Control animals were dosed with vehicle instead of Natrosol 250 HX, as indicated in the protocol

Observations and Results

Mortality

The animals were examined visually at least twice daily for mortality, morbidity and reaction to treatment.

No unscheduled deaths occurred.

Clinical Signs

Detailed observations during treatment were made at least twice daily. Detailed physical examinations were made once per week during pre-test, treatment and recovery periods.

Some high dose animals had red urine during weeks 3 and 4.

Body Weights

The animals were weighed weekly during acclimation, on Day 1 of treatment, weekly until study termination and before necropsy.

Body weight was not affected by treatment.

Food Consumption

The mean weekly food consumption per animal was estimated from the daily weights of food supplied, food remaining, and spillage per cage housing 5 animals.

Food consumption was not significantly affected by treatment, although food consumption decreased by 5% in the mid and high dose female groups.

Ophthalmoscopy

The eyes of all main study animals were examined after dilation with a mydriatic agent using a slit lamp and fundus camera before treatment initiation, and during week 4 of treatment and week 4 of recovery.

Treatment related ophthalmoscopic findings were not observed.

Hematology

Blood samples were obtained from all animals (not fasted) from the retrobulbar venous plexus under isoflurane anesthesia at the end of treatment on Day 29/30 and at the end of recovery on Day 66. The following hematology parameters were measured: hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, normoblast count, platelet count, total white cell count, and differential white cell count, including neutrophils, lymphocytes, eosinophils, basophils,, monocytes and large unstained cells. Additional samples were taken for measurement of thrombin time, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, and fibrinogen concentration. The laboratory's normal ranges for this rat strain were not provided.

Slightly lower group mean values for hematocrit, hemoglobin concentrations and red blood cell counts were noted in the high dose males, but not the high dose females, in comparison with those of the respective controls (Table 39). However, a significant increase in reticulocyte counts was observed in the high dose females but not the high dose males. PT values did not change; however, the mean aPTT values increased in the high dose females (16%). Thrombin times increased in the mid (8%) and high (10%) dose males and the high (12%) dose females. Fibrinogen concentrations increased in the mid (12%) and high (16%) dose males and the high (16%) dose females.

Table 39: From Sponsor's Hematology Summary – Study U00-1128

Males

Parameter		HGB [g/dl]	RBC [10^6/ul]	HCT [vol%]	MCHC [g/dl]	Retic [o/oo]	PT [s]	APTT [s]	TT [s]	Fibrinogen [g/l]
Group										
Control	n	20	20	20	20	20	20	20	20	20
	mv	14.04	7.957	40.97	34.26	23.5	16.47	16.76	36.08	2.466
	sd	0.45	0.255	1.24	0.51	5.4	1.17	3.12	4.41	0.497
Low Dose 0.05 [mg/kg]	n	10	10	10	10	10	10	10	10	10
	mv	13.82	7.814	40.79	33.88	23.7	15.93	16.01	37.89	2.653
	sd	0.40	0.284	1.02	0.41	6.8	1.05	0.66	1.58	0.178
	mvdiff	-0.22	-0.143	-0.18	-0.38	0.3	-0.54	-0.75	1.81	0.188
	p%	18.58	12.00	68.03	* 3.73	90.25	19.56	34.33	15.23	28.46
Mid Dose 0.5 [mg/kg]	n	10	10	10	10	10	10	10	10	10
	mv	13.91	7.929	41.20	33.76	23.7	16.37	16.59	39.05	2.765
	sd	0.34	0.219	1.01	0.35	5.1	0.74	0.46	1.63	0.228
	mvdiff	-0.13	-0.028	0.23	-0.49	0.3	-0.10	-0.17	2.97	0.300
	p%	43.94	75.84	59.87	* 0.72	90.25	81.69	83.31	* 2.07	8.99
High Dose 5 [mg/kg]	n	20	20	20	20	20	20	20	20	20
	mv	13.44	7.773	40.08	33.53	22.3	16.62	17.14	39.84	2.874
	sd	0.42	0.187	1.10	0.48	4.3	1.06	1.37	2.95	0.552
	mvdiff	-0.60	-0.184	-0.89	-0.73	-1.2	0.15	0.39	3.76	0.409
	p%	* < 0.01	* 1.59	* 1.50	* < 0.01	49.08	65.46	54.76	* 0.05	* 0.56

Females

Parameter		HGB [g/dl]	RBC [10^6/ul]	HCT [vol%]	MCHC [g/dl]	Retic [o/oo]	PT [s]	APTT [s]	TT [s]	Fibrinogen [g/l]
Group										
Control	n	20	20	20	20	20	20	20	20	19
	mv	12.82	7.210	37.56	34.14	25.4	15.25	14.51	35.42	2.311
	sd	0.25	0.305	0.95	0.49	6.8	1.69	3.49	6.31	0.148
Low Dose 0.05 [mg/kg]	n	10	10	10	10	10	10	10	10	10
	mv	12.91	7.241	37.53	34.40	35.1	15.36	14.66	37.67	2.356
	sd	0.25	0.228	0.79	0.43	9.0	0.57	1.25	2.01	0.166
	mvdiff	0.09	0.032	-0.03	0.26	9.8	0.12	0.15	2.26	0.045
	p%	59.03	78.77	95.88	14.81	* 0.13	79.51	86.87	45.98	70.14
Mid Dose 0.5 [mg/kg]	n	10	10	10	10	10	10	10	10	10
	mv	12.86	7.304	38.00	33.84	26.3	15.29	16.11	37.48	2.421
	sd	0.49	0.266	1.39	0.47	7.6	0.78	1.39	1.93	0.172
	mvdiff	0.04	0.095	0.45	-0.30	1.0	0.05	1.60	2.07	0.110
	p%	81.07	42.03	35.91	9.25	74.32	91.91	8.19	49.84	35.31
High Dose 5 [mg/kg]	n	20	20	20	20	20	20	20	20	20
	mv	12.77	7.157	38.05	33.54	35.1	15.27	16.81	39.68	2.684
	sd	0.58	0.339	1.56	0.42	7.2	0.72	1.47	11.70	0.465
	mvdiff	-0.06	-0.053	0.50	-0.60	9.8	0.02	2.30	4.26	0.373
	p%	68.68	58.28	21.30	* 0.01	* 0.01	95.59	* 0.29	9.06	* 0.03

Clinical Chemistry

At the same time as the collection of blood for hematology, additional blood was obtained for measurement of the following clinical chemistry parameters: alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate dehydrogenase, leucine arylamidase, γ-glutamyl transpeptidase, adolase,

glucose, total bilirubin, total cholesterol, triglycerides, creatinine, urea, total protein, protein fractions (by electrophoresis), sodium, potassium, chloride, calcium, magnesium, and phosphorus.

Statistically significant increases in mean potassium and globulin levels were observed in the high dose females. The sponsor concluded these changes were not related to treatment, because they were restricted to one sex. Liver function enzymes, AST and ALT, did not increase with treatment. In males mean values of AST and ALT decreased with dose. Slight decreases in albumin occurred along with slight increased in globulins in males.

Table 40: From Sponsor's Clinical Chemistry Summary – Study U00-1128

Males

Parameter		AST [U/l]	ALT [U/l]	Aldolase [U/l]	K [mmol/l]	Alb [g/l]	Glob [g/l]
Group							
Control	n	20	20	20	20	20	20
	mv	39.61	55.22	10.13	4.636	34.57	26.77
	sd	4.82	10.96	2.48	0.225	1.99	1.66
Low Dose	n	10	10	10	10	10	10
0.05	mv	35.63	53.52	9.19	4.431	33.86	27.22
[mg/kg]	sd	4.23	7.06	1.47	0.283	0.99	1.46
	mvdiff	-3.98	-1.70	-0.94	-0.205	-0.71	0.44
	p%	* 2.99	59.28	22.26	* 3.85	25.13	49.00
Mid Dose	n	10	10	10	10	10	10
0.5	mv	35.85	51.34	8.54	4.469	33.22	27.78
[mg/kg]	sd	4.04	6.59	1.31	0.297	1.37	0.92
	mvdiff	-3.76	-3.88	-1.59	-0.167	-1.35	1.01
	p%	* 3.98	22.48	* 4.11	8.98	* 3.08	11.94
High Dose	n	20	20	20	20	20	20
5	mv	36.18	50.09	8.73	4.504	32.15	27.92
[mg/kg]	sd	4.80	5.66	1.82	0.232	1.42	1.95
	mvdiff	-3.43	-5.13	-1.40	-0.133	-2.42	1.15
	p%	* 2.21	5.17	* 2.76	9.90	* < 0.01	* 3.16

Females

Parameter		AST [U/l]	ALT [U/l]	Aldolase [U/l]	K [mmol/l]	Alb [g/l]	Glob [g/l]
Group							
Control	n	20	20	20	20	20	20
	mv	37.35	46.85	7.44	4.280	36.50	26.81
	sd	5.02	5.26	1.90	0.327	2.63	1.80
Low Dose	n	10	10	10	10	10	10
0.05	mv	38.18	42.92	6.80	4.494	37.74	26.60
[mg/kg]	sd	6.81	9.34	1.98	0.474	1.99	1.65
	mvdiff	0.83	-3.93	-0.64	0.215	1.24	-0.21
	p%	71.40	17.38	37.71	11.94	20.62	78.63
Mid Dose	n	10	10	10	10	10	10
0.5	mv	36.50	44.43	6.20	4.409	37.57	27.60
[mg/kg]	sd	4.24	5.76	1.27	0.344	2.38	1.31
	mvdiff	-0.85	-2.42	-1.24	0.130	1.07	0.79
	p%	70.74	39.99	9.01	34.38	27.44	32.02
High Dose	n	20	20	20	20	20	20
5	mv	38.05	46.07	7.13	4.544	36.66	28.03
[mg/kg]	sd	6.64	8.67	1.98	0.304	2.67	2.59
	mvdiff	0.70	-0.79	-0.32	0.265	0.15	1.22
	p%	70.70	73.74	59.36	* 2.03	84.60	6.19

Urinalysis

On day 23/24 of treatment and on day 64 (recovery), urine samples were collected for 5 hrs after administration of 20 mL/kg of water. The measured quantitative parameters included: volume, pH, specific gravity, color, turbidity, blood, white blood cells, protein, glucose, urobilinogen, bilirubin, nitrite and ketone bodies. The urine sediment was examined microscopically for epithelial cells, leucocytes, erythrocytes, crystals, casts, bacteria and other abnormal components.

The sponsor concluded that treatment related effects on urinalysis parameters were not observed. However, the reviewer noted the incidence and severity of blood in the urine increased in the mid and high dose males and the high dose females.

Gross Pathology

Animals were euthanized using sodium pentobarbital and subjected to detailed necropsy. The whole or a sample of the tissues listed below (Table 41) from all animals was preserved in 10% neutral buffered formalin, except for the eyes which were fixed in Davidson's fluid.

Low incidences of hemorrhages were present in all dose groups.

Table 41: Reviewer's Summary of Tissues Collected – Study U00-1128

Abnormal tissues	Injection site (tail)	Sciatic nerve†
Adrenal glands	Jejunum	Seminal vesicles
Aorta - thoracic	Kidneys	Skeletal muscle
Bone marrow	Liver	Skin
Brain (cerebellum, cerebrum, midbrain and medulla	Lacrimal glands (extraorbital)	Spinal cord †
Bulbourethral glands	Lungs	Spleen
Cecum	Lymph nodes (submandibular, mesenteric, in neck region, and aorta iliac region)	Sternum
Colon		Stomach
Coagulating glands		Tattoos†
Duodenum	Mammary gland	Testes
Epididymides	Optic nerves	Thymus
Esophagus	Ovaries	Thyroid with parathyroids
Eyes	Pancreas	Tongue
Femur	Pituitary	Trachea
Harderian gland	Prostate	Urinary bladder
Heart	Rectum	Uterus
Ileum	Salivary gland (submandibular, sublingual, parotid)	Vagina

† Collected but not examined,

Organ Weights

From each animal at necropsy, the following organs excised and weighed: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes with epididymides, thymus, and thyroids.

The sponsor noted a slight increase in thymus relative weight in the high dose male group (16%). Relative spleen weight decreased in the mid and high dose males (9-10%) and females (5-6%). The relative thyroid weight decreased in the mid and high dose males (6-7.6%) and females (7-20%). Ovarian relative weights decreased 9-12% in all female groups; however, relative testes weight increase 5-7% in all male groups.

Table 42: From Sponsor's Tables of Relative Organ Weight – Study U00-1128

		Males				Females			
Parameter		Thymus 10 ^ -3	Spleen 10 ^ -3	Thyroid 10 ^ -6	Testes 10 ^ -3	Thymus 10 ^ -3	Spleen 10 ^ -3	Thyroid 10 ^ -6	Ovaries 10 ^ -6
Group									
Control	n	10	10	10	10	10	10	10	10
	mv	1.0309	2.441	53.794	8.854	1.1924	2.670	69.011	474.146
	sd	.1131	.227	12.420	1.202	.2592	.397	16.714	55.164
	mvdiff%								
Low Dose	n	10	10	10	10	10	10	10	10
0.05	mv	1.0867	2.438	54.708	9.355	1.2299	2.631	80.100	430.244
[mg/kg]	sd	.1759	.371	15.211	.720	.2120	.327	13.321	57.387
	mvdiff%	5.4121	-.130	1.700	5.655	3.1473	-1.438	16.068	-9.259
Mid Dose	n	10	10	10	10	10	10	10	10
0.5	mv	.9321	2.176	49.665	9.304	1.2416	2.528	64.378	426.004
[mg/kg]	sd	.1918	.196	12.451	.662	.2732	.287	12.967	87.651
	mvdiff%	-9.5848	-10.841	-7.676	5.080	4.1237	-5.323	-6.713	-10.154
High Dose	n	10	10	10	10	10	10	10	10
5	mv	1.2025	2.219	50.480	9.466	1.1463	2.506	55.104	418.813
[mg/kg]	sd	.2124	.152	11.796	.668	.1565	.291	11.875	48.672
	mvdiff%	16.6385	-9.107	-6.160	6.915	-3.8693	-6.119	-20.151	-11.670

Histopathology

Adequate Battery

Tissue samples from all study animals were dehydrated, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The indicated tissues listed in Table 55 and gross abnormalities identified at macroscopic examination from all animals in the control and high dose groups sacrificed at the end of the scheduled treatment and recovery period. Only macroscopic changes and target organs were examined for the low and mid dose groups.

Peer Review

Not indicated

Histological Findings

The finding of hemosiderosis in the spleen was present on in the high dose animals. Hemorrhage in the thymus was slightly increased in the high dose males, but not the females. Both findings are related to the pharmacodynamic effect of BIBR 953 ZW.

Table 43: From Sponsor's Summary of Microscopic Findings

	findings/organ	control (n= 10)	f	BIBR 953 ZW 0.05 mg/kg (n= 10)	f	BIBR 953 ZW 0.50 mg/kg (n= 10)	f	BIBR 953 ZW 5.00 mg/kg (n= 10)	f
Males	spleen	(10)	0			(1)	0	(10)	2
	hemosiderosis								2
	thymus	(10)	1	(1)	1	(1)	0	(10)	5
	hemorrhage		1		1				3
	hematoma								1
Females	spleen	(10)	1	(3)	0			(10)	5
	hyperemia		1						
	hemosiderosis								5
	thymus	(10)	3	(2)	1	(3)	2	(10)	2
	hemorrhage		2		1		1		
	hematoma								1

Special Evaluation

Hemodynamic measurements (blood pressure and heart rate) were made during study week 3 before dosing and at 0.5 and 2 hours after dosing using a tail cuff and a combined system from (b) (4).

Compared to the pre-dose measurements, systolic blood pressure increased slightly (10%) in the high dose group and diastolic blood pressure increased in the mid dose group at 0.5 hour after dosing. Heart rate decreased in the low dose group at 2 hours after dosing. The sponsor concluded that blood pressure and heart rate were not consistently affected by treatment with BIBR 953 ZW.

Table 44: Sponsor's Summary of Blood Pressure and Heart Rate - Study U00-1128

Mean arterial blood pressure and mean heart rate of conscious rats under daily intravenous treatment with BIBR 953 ZW

Data are expressed as mean ± SD

Study week	Dosage mg/kg	N	T (h)	Blood pressure in mmHg systolic	Δ %#	Δ%§	diastolic	Δ %#	Δ%§	Heart rate in beats/min	Δ%#	Δ%§
3	0	8	0	141.0 14.1			99.0 15.0			367.5 23.9		
		8	0.5	140.7 7.8	-0.2		94.7 9.7	-4.3		364.7 17.3	-0.8	
		8	2	142.7 14.9	1.2		95.6 9.1	-3.4		382.0 27.1	3.9	
	0.05	8	0	132.4 15.8		-6.1	94.8 11.6		-4.2	358.4 35.7		-2.5
		8	0.5	132.0 18.2	-0.3	-6.2	91.7 13.7	-3.3	-3.2	360.8 33.5	0.7	-1.1
		8	2	140.0 10.4	5.7	-1.9	93.2 11.0	-1.7	-2.5	334.8*° 20.4	-6.6	-12.4
	0.5	8	0	139.6 15.6		-1.0	94.6 14.1		-4.4	352.9 16.5		-4.0
		8	0.5	142.1 13.5	1.8	1.0	102.7 9.9	8.6	8.4	373.8 44.7	5.9	2.5
		8	2	131.8 13.0	-5.6	-7.6	89.2 9.2	-5.7	-6.7	367.3 47.1	4.1	-3.8
	5	8	0	129.5 14.9		-8.2	92.2 9.8		-6.9	359.1 23.8		-2.3
		8	0.5	142.1* 13.2	9.7	1.0	94.6 9.0	2.6	-0.1	367.1 14.5	2.2	0.7
		8	2	136.8 11.9	5.6	-4.1	95.3 10.2	3.4	-0.3	356.9° 17.1	-0.6	-6.6
10	0	8		140.1 14.9			94.1 6.3			329.8 21.4		
	5	8		147.4 6.2		5.2	100.3 7.5		6.6	335.8 16.4		1.8

N Number of animals
T Time after administration
% Change versus: # measurements before administration of this dose on that particular day
§ control group
° $p \leq 0.05$ versus control values (unpaired t-test)
* $p \leq 0.05$ versus the value before administration of this dose on that particular day (paired t-test)

Toxicokinetics

Blood samples were collected from all animals before and 15 minutes, 0.5, 1, 4, 7, and 24 hours after dosing on Days 1 and 28 of treatment. Analysis for BIBR 953 ZW used a validated HPLC-MS/MS assay with [$^{13}C_6$]-BIBR 953 ZW as an internal standard. The assay with an inaccuracy (b) (4) and imprecision of (b) (4) was valid in the concentration range of (b) (4)

No clear gender effect was observed. No clear effect of repeated dosing was observed. The increase in plasma concentration and exposure was dose proportional.

Table 45: From Sponsor's Summary of TK Parameters - Study U00-1128

				953 ZW, C_{1h}		953 ZW, $AUC_{(0-7hr)}$		
dose [mg/kg]	day	gender	N	mean [ng/ml]	CV (%)	N	mean [ng·h/ml]	CV (%)
0.05	1	m	4	16.23	11.2	NA	NA	NA
0.05	1	f	4	14.20	25.0	NA	NA	NA
0.05	1	m&f	8	15.21	18.6	NA	NA	NA
0.5	1	m	4	155.3	21.6	4	527.0	16.1
0.5	1	f	4	155.8	9.3	4	592.8	5.7
0.5	1	m&f	8	155.5	15.4	8	559.9	12.4
5.0	1	m	4	2125	5.3	4	6830	10.1
5.0	1	f	4	1965	15.8	4	6334	12.8
5.0	1	m&f	8	2045	11.4	8	6582	11.3
0.05	28	m	4	16.53	8.9	NA	NA	NA
0.05	28	f	4	18.20	13.1	NA	NA	NA
0.05	28	m&f	8	17.36	11.8	NA	NA	NA
0.5	28	m	4	136.0	9.3	4	515.9	8.4
0.5	28	f	4	182.5	15.5	4	616.6	12.0
0.5	28	m&f	8	159.3	20.2	8	566.2	13.8
5.0	28	m	4	1645	16.9	4	7082	16.3
5.0	28	f	4	1858	16.2	4	6737	16.1
5.0	28	m&f	8	1751	16.6	8	6909	15.2

Formulation Analysis

Although samples of formulation were taken for analysis on Day 1 and Day 28, no result of the analysis was provided.

6.2.2 Repeat-Dose Toxicity in Nonrodents

Study title: BIBR 1048 MS: Toxicity Study by Oral Gavage Administration to Rhesus Monkey for 52 Weeks Followed by a 6 Week Recovery Period

Study no.:	BOI 252/032248 (U05-2047)
Study report location:	9/17/09 submission EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 21, 2003
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch number 8250250 (two consignments), purity 97.6 – 98%

Key Study Findings

Rhesus monkeys received BIBR 1048 MS by oral gavage for up to 56 weeks because deaths of two high dose females in the first study week forced stoppage of dosing followed by a period of dose escalation in the high dose animals. These deaths were attributed to aspiration of the highly acidic high dose formulation. Additional deaths of a low dose male and a high dose female later in the study were attributed to infections.

At dosages of 0, 12, 36 and 200 mg/kg/day the mean $AUC_{(0\text{-}24h)}$ for total BIBR 953 ZW (BIBR 953 ZW plus glucuronides) was 2692, 6438, and 15475 ng.hr/mL in males and 1973, 3710, and 15300 ng.hr/mL in females, respectively. As expected, coagulation times were prolonged with the thrombin time prolonged at all doses and timepoints. Slight decreases in hematocrit, hemoglobin concentrations and red blood cell counts were noted in the high dose animals. The high dose females showed higher plasma triglyceride and lower albumin concentrations. At necropsy the high dose females had an increased incidence of thymic involution/atrophy. Repeated measurements of testicular volume and plasma testosterone levels during the in-life phase and at necropsy as well as repeated analyses of did not reveal any effects of treatment. A diagnosis of testicular atrophy was not reported in this 52-week study. Because of the deaths at the high dose, the NOAEL of the study was considered to be 36 mg/kg.

Methods

Doses:	0, 12, 36, 200 mg of base/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Natrosol® 250 HX 0.5% aqueous hydroxyethylcellulose solution.
Species/Strain:	Rhesus monkeys (Macaca mullata)
Number/Sex/Group:	Main: 4/sex/group; Recovery: 2/sex in the low and high dose groups
Age at treatment start:	Males: 38-44 months; Females: 40-43 months
Weight at treatment start:	Males: 2.93-4.0 kg; Females: 2.89 – 4.1 kg
Satellite groups:	None
Unique study design:	Study measurements included plasma testosterone and testicular volume
Deviation from study protocol:	1. The animals were older than the 24 months of age specified in the protocol. 2. Following the death of the second Group 4 female on Day 7 of the study, dosing of the group 4 animals was stopped until Day 9 when dosing recommenced at 36 mg/kg. These animals underwent dose escalation (Day 16: 100 mg/kg; Day 22: 150 mg/kg; and Day 29: 200 mg/kg). Day 29 became the start of Week 1 of 52 weeks of treatment for all groups followed by 6 weeks of recovery. The study report stated that these periods were differentiated as follows.

Week designation	Period
-2 to -1	Pretreatment period
A-D	Week of primary start and experimental incremental dosing and introduction of flush/refinement of procedure (grp 4)
1 to 52	Restart of treatment all groups to termination of main phase animals
R0	Day 1 of the recovery period (bodyweights only)
R1-R6	Recovery period Weeks 1 to 6

However, some of the provided data tables (e.g. bodyweight) did not follow the above convention, but used weeks 1 to 56 for the treatment period.

Observations and Results

Mortality

The animals were examined visually at least twice daily for mortality, morbidity and reaction to treatment.

Four animals died or were euthanized prematurely as outlined in Table 46. None of the deaths was directly related to systemic toxicity of BIBR 1048 MS.

Although the dosage of 200 mg/kg was used in a previous 26-week monkey study, two deaths during the first week of the current study. These deaths were attributed to aspiration of the high dose formulation, which had a pH value of 2.55. To prevent further deaths, the sponsor initiated a period of dose escalation for the high dose animals and incorporated a 3 mL water flush immediately following dose administration.

Two additional deaths were attributed to infection. During week 38, a low dose male (M151) died from Shigellosis. As a result, all animals were treated with an antibiotic (Baytril) for ten days. Because of consistent weight loss (0.84 kg), F172 was euthanized during week 51. Histology indicated that this animal had lymphocytic infiltration of intestinal mucosa consistent a chronic enteric infection. The report was unclear if the infection was due to Shigellosis. Another high dose female (F174) also continually lost weight (1 kg), but was not euthanized. This female also had a chronic enteric infection based on histology at study termination.

Table 46: Reviewer's Summary of Unscheduled Deaths – Study U05-2047

Animal	Dose, mg/kg	Date of death	Comments	Cause of death
F170	200	Day 7	Both females had gasping and labored respiration, and then died within 15 minutes of dose administration. F176 also had red-stained vomit. Histology indicated edema in lungs and sloughing of bronchial epithelium	Aspiration of the acidic dose formulation
F176	200	Day 5		
M151	12	Week 38	Diarrhea, weight loss (0.5 kg), then excessive watery feces with some blood resulted in unscheduled sacrifice. Hematology showed elevated WBC and neutrophils. Histology showed marked ulcerative typhlitis, colitis and an encysted necrotic parasite in the sub-renal region of the abdomen	Shigellosis, confirmed with tests of a fecal sample
F172	200	Week 51	Consistent weight loss (0.84 kg over 50 weeks) resulted in unscheduled sacrifice. Enlarged mesenteric lymph nodes in GI tract. Histology showed diffuse lymphocytic infiltration of intestinal mucosa	Chronic enteric infection.

Clinical Signs

In addition to a more detailed weekly physical examination, observations during treatment were made immediately before dosing, between 0.5 and 2 hours after dosing on return of the animal to its cage, and as late as possible in the working day. During acclimation and recovery observations were made once a day. The sponsor considered only clinical signs related to bleeding as treatment-related and only reported these findings. The reviewer requested a complete listing of clinical signs observed.

The sponsor asserted that the control and low dose groups and the mid-dose males showed no remarkable signs. However, the cage housing the mid-dose females was found to have a large amount of red staining during week 28, but the finding was not associated with injury. The sponsor noted the clinical signs summarized in Table 47 in six of the high dose animals and attributed them to the pharmacodynamic effect of BIBR 1048 MS.

Table 47: Reviewer's Summary -Clinical signs, Original Report - Study U05-2047

Animal	Week	Finding	Dosing stopped
M139	19	Prolonged bleeding from cuts on hand	Week 19
M141	21-24	Hematoma on head requiring veterinary attention	9 days, Week 21-23
M167	1	Hematoma in femoral area	
	12	Red staining in cage and pale gums	
	25	Rectal prolapse and hemorrhage	Week 25
	46	Aural hematoma	
M173	37	Lower gum abrasion requiring veterinary treatment	Week 37
F168	11	Bruise and cut to foot	
F178	13	Facial bruise	1 day in Week 13

Based on the supplemental complete listing of clinical signs, the reviewer observed a tendency for loose or liquid feces to increase with dose of BIBR 1048 MS with correlation being better in males than females (Table 48). In addition, the observation of

red staining in cages tended to increase with dose with the correlation better in females than males. In females, the red staining generally occurred during weeks when menses were observed. To minimize excessive bleeding, females treated with BIBR 1048 MS were not dosed during menses, with the exception of Weeks 51 and 52 in which all animals were treated regardless of bleeding status.

Table 48: Reviewer's Summary of Clinical Signs – Study U05-2047

Number of weeks sign observed/animal – Supplement (N065, dated 5/13/2010)								
	Males				Females			
Clinical sign	**0**	**12**	**36**	**200**	**0**	**12**	**36**	**200**
Loose feces	1.3	2.2	3	5.7	2.3	5.7	1	8
Liquid feces	0	0.67	0	1	0	3	0.5	3
Loose and/or liquid feces	1.3	2.8	3	6.7	2.3	6	1.5	11
Red stain in cage	0	0.7	0	2.8	0	1.2	2.5	5

Body Weights

The animals were weighed weekly during acclimation, on Day 1 of treatment, weekly until study termination and before necropsy.

The sponsor maintains that bodyweight was unaffected by treatment, despite observing lower mean bodyweight gains in the mid- and high dose males and in the high dose females in comparison with the gains in the control groups. The sponsor attributed the differences in weight gain to large weight gain in two control males (No. 143 and 145) and an overall weight loss in one high dose female (No. 174). Although the differences were not statistically significant, the reviewer noted 1, 2 and 3 males and 2, 1 and 1 females in the low, mid, and high dose groups, respectively, with body weight gains less than the lowest control body weight gain. A definitive trend attributable to a treatment effect may be absent amongst the groups; however, one-third of the individual treated animals had bodyweight gains less than the minimum in the control groups.

Figure 25: Sponsor's Mean Bodyweight Graphs – Study U05-2047

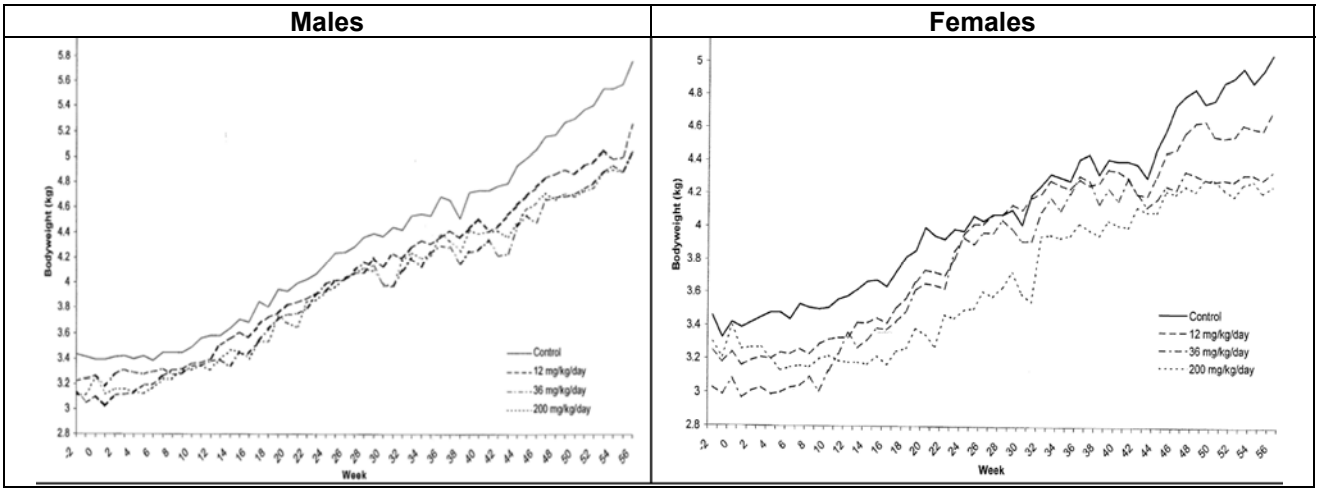


Table 49: Reviewer's Summary of Bodyweight Changes – Study U05-2047

	Males					Females				
	Animal	Week 0	Week 56	Gain	Δ*	Animal	Week 0	Week 56	Gain	Δ*
1	175	3.12	4.82	**1.70**	Lowest	142	3.18	4.72	1.54	
	177	2.91	4.66	1.75		144	2.98	4.01	**1.03**	Lowest
	143	3.51	6.53	3.02		146	4.10	6.44	2.34	
	145	4.00	7.06	3.06						
	Mean	3.385	5.768	2.383			3.420	5.057	1.637	
	SD	0.479	1.208	0.760			0.597	1.249	0.660	
2	147	3.07	4.55	*1.48*	-0.22	148	3.21	4.68	1.47	
	149	3.18	5.25	2.07		150	3.10	4.06	*0.96*	-0.07
	151	3.52			Death	152	3.15	5.17	2.02	
	153	3.32	6.25	2.93		154	3.12	3.91	*0.79*	-0.24
	155	3.03	5.63	2.60		156	3.63	5.19	1.56	
	157	2.89	4.62	1.73		158	3.20	5.24	2.04	
	Mean	3.168	5.260	2.162			3.235	4.708	1.473	
	SD	0.225	0.713	0.600			0.198	0.598	0.521	
% control		**93.60**	**91.20**	**90.75**			**94.59**	**93.11**	**90.02**	
3	159	3.40	5.95	2.55		160	3.11	4.04	*0.93*	-0.10
	161	3.21	4.74	*1.53*	-0.17	162	3.00	4.25	1.25	
	163	3.49	5.53	2.04		164	3.03	4.18	1.15	
	165	2.93	3.95	*1.02*	-0.68	166	3.16	4.94	1.78	
	Mean	2.807	4.571	1.835			3.075	4.353	1.278	
	SD	0.248	0.884	0.658			0.073	0.401	0.361	
% control		**96.23**	**87.43**	**74.92**			**89.91**	**86.07**	**78.05**	
4	167	3.09	4.27	*1.18*	-0.52	168	3.57	5.14	1.57	
	169	3.19	5.36	2.17		172	3.15	(2.26†)	*(-0.89†)*	Death
	171	3.06	5.10	2.04		174	3.79	2.81	*-0.98*	-1.14
	173	3.83	5.99	2.16		178	3.34	4.61	1.27	
	139	3.25	4.85	*1.60*	-0.10	140	2.89	4.48	1.59	
	141	3.07	4.64	*1.57*	-0.13					
	Mean	3.248	5.035	1.787			3.398	4.260	0.863	
	SD	0.295	0.600	0.400			0.385	1.008	1.237	
% control		**95.96**	**87.30**	**74.99**			**99.34‡**	**84.25‡**	**52.70‡**	

Δ* Difference of body weight gain from lowest control body weight gain. † Data on Week 50, ‡ Excludes Female 172, Underlined values indicate the maximum individual gain in each group. Bold values indicate the minimum gain in the control groups. Italic text indicates gains in the treated groups that are below the minimum in the control group.

Food Consumption

The mean weekly food consumption per animal was estimated from the daily weights of food supplied, food remaining, and spillage.

Food consumption in the low and mid dose groups was generally similar to that in the control groups. The sponsor noted slightly lower food consumption in the high dose males and females relative to the concurrent control animals. However, the weekly values for all treated groups were within the range of values during acclimation or in the respective control groups during treatment.

Ophthalmoscopy

The eyes of all main study animals were examined after dilation with a 0.5% tropicamide ophthalmic solution using a handheld direct ophthalmoscope before treatment initiation, and during weeks 25 and 51 of treatment. .

No treatment related findings were observed.

ECG and blood pressure

Electrocardiograms were recorded using six leads (I, II, III, aVR, aVL and aVF) from all animals during acclimation and during Weeks 13, 26, 39 and 52 of treatment, at approximately 2 and 24 hours after dose administration. The ECG parameters included abnormalities of the electrical complexes, heart rate, and the P, PR, QRS, ST, and QT wave intervals. QTc was calculated using the methods of Bazett (1920), Fridericia (1920) and Matsunaga et al (1997). At the same time as electrocardiography, indirect blood pressure tracings were obtained from each animal using a pressure cuff, secured at the base of the tail, connected to a physiological pressure transducer and chart recorder. From five measurements on each occasion, the mean systolic pressure, mean arterial pressure and pulse rate were calculated.

The range of values for systolic blood pressure and pulse rate in the treated animals were similar to the range of values for these parameters in control animals or in all animals prior to initiation of treatment. The ranges of values for ECG parameters, including the QT interval and QTc, in the treated animals were generally similar to the range of values for these parameters in control animals or in all animals prior to initiation of treatment. One exception was a high dose male (M139) whose QT and QTcM values were outside the 95% confidence limit at two hours after dosing in Week 39; however, this animal displayed bradycardia on this and other occasions. All of the ECG observations, including ventricular premature beats, sinus tachycardia (heart rate >280 beats/minute), sinus bradycardia (heart rate <160 beats/minute}, and changes in high voltage complexes, were considered normal spontaneous changes seen in rhesus monkeys, because no association with treatment was evident.

Hematology

Blood samples were obtained via venipuncture from all animals after fasting during acclimation, prior to dose administration during weeks 13, 26, 42 and 52 of treatment and in Week 6 of recovery. A blood smear was prepared for all animals. The following hematology parameters were measured: hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, morphology, platelet count, total white cell count, and differential white cell count, including neutrophils, lymphocytes, eosinophils, basophils, monocytes, and large unstained cells. Additional samples were taken for measurement of thrombin time, prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen concentration.

In comparison to the concurrent control groups during the treatment phase, aPTT values were prolonged 28-65% and 41-81% in the high dose males and females respectively, and PT values were prolonged 10-12% in the mid-dose groups and 8-22% in the high dose groups. Despite sample collection when BIBR 1048 MS plasma concentrations were at trough levels, thrombin times were prolonged in all treated groups with a minimum increase in group mean of 60% in the low dose females (Table 50). Most of individual thrombin times in the high dose animals were greater than 150 sec. or prolonged more than 5-fold throughout the study. Following the recovery period, all values for coagulation times were within the range of values for control animals.

Table 50: Reviewer's Summary of Coagulation Times – Study U05-2047

			Male						Female					
Group		Study Week	Pre-	13	26	42	52	Rec.	Pre-	13	26	42	52	Rec.
PT, sec	1	Mean	11.7	11.5	11.7	11.7	11.1		11.9	11.3	12.1	11.3	11.0	
		SD	0.4	0.1	0.3	0.4	0.3		0.4	0.3	0.5	0.8	0.5	
	2	Mean	11.4	11.8	11.7	11.5	11.2	10.6 #	11.5	11.8	11.8	11.6	11.1	10.5 #
		SD	0.4	0.3	0.3	0.2	0.2		0.6	0.7	0.6	0.6	0.7	
	3	Mean	11.5	12.1	12.6	**12.2**	**12.4**		11.9	12	12.1	11.7	12.2	
		SD	1	1	1.1	0.9	0.8		0.6	1	0.8	0.9	0.8	
	4	Mean	11.4	**13.7**	**13.1**	13.7	**13.8**	10.9 #	11.7	**13.7**	13.1	12.7	**13.5**	12.0 #
		SD	0.5	1	0.8	1	0.5		0.4	0.8	1.4	1.5	1.7	
aPPT, sec	1	Mean	36.8	44.5	39.7	46.7	35.9		36.9	42.8	32.6	40.7	36.3	
		SD	4.8	5.9	4.4	10.6	4.6		1.4	4.5	3	3.3	1	
	2	Mean	34.5	44.5	39.3	44.9	41.6	33.5 #	35.6	48.4	39.2	58.4	42.4	36.8 #
		SD	3.8	10	10.1	8.6	6.1		6.7	8.6	5.8	23.3	7.2	
	3	Mean	32.6	43.4	40.9	46.6	44.8		33.7	44	38.7	45.3	42.4	
		SD	2.8	8.1	*5*	4.3	8		2.8	5.9	3	6.1	5.8	
	4	Mean	31.6	**57.1**	46.1	63.8	**59.4**	32.9 #	35.4	**60.5**	49.6	56.7	**65.3**	33.5 #
		SD	4.6	6	6.2	22.7	6.5		7.4	8.8	22.7	3.7	14.8	
TT, sec	1	Mean	23.3	26.2	25.6	25.4	22.7		26.3	26.7	27.4	26.9	26.4	
		SD	1.6	2.2	2.4	1.6	1.8		1.4	2.6	1	1.4	1.1	
	2	Mean	24.6	**78.5**	**60.2**	67.9	**65.7**	24.4 #	25.1	**55.2**	43.8	**49.4**	**64.5**	26.8 #
		SD	1	6.2	24	14.8	17.9		1.2	7.5	13.6	3.9	24.4	
	3	Mean	23.5	**85.0**	**74.1**	**88.0**	**80.0**		23.9	**86.0**	**62.0**	**95.2**	**66.9**	
		SD	1.4	54.7	*17.2*	26.9	*		1.2	35	22.8	33.6	22.5	
	4	Mean	31.6	**>150**	**>115**	**>150**	**>150**	26.2 #	24.5	**>90.1**	**>88.0**	**>150**	**>150**	30.3 #
		SD	4.6	*	*	*	*		0.8	*	*	*	*	

Rec. = recovery; Bold text indicates statistically significance, p<0.05, # Only 2 animals, no SD calculated
Underlined mean value indicates one or more values >150 sec, * No SD calculated

The sponsor noted slightly higher fibrinogen concentrations in the high dose groups, especially in the females. During Week 13 the increases were attributable to 45 and 58% increases in fibrinogen in females 174 and 172, respectively, compared to their individual pre-dose values. During Week 42, female 172 again exhibited a 45% increase in fibrinogen compared to her pre-dose value. During Week 52, two females (140 and 168) and two males (139 and 167) exhibited 22-25% increases in fibrinogen compared to their individual pre-dose values. However, statistical significance was not attained by any treated group at any sampling occasion.

Slightly lower group mean values for hematocrit, hemoglobin concentrations and red blood cell counts were noted in the high dose males in comparison with those of the controls. The values for hemoglobin concentrations in the high dose males were significantly lower than that in the control group during Weeks 26 and 52 (Table 51). Although the group means for the high dose females were not decreased relative to the control group, all of the high dose females showed a decrease of ≥1 gm/dL on at least one occasion compared to their individual pre-dose value. Following the recovery period, all values for hematology parameters were within the range of values for control animals.

Table 51: Reviewer's Table of Hemoglobin Values (gm/dL) – Study U05-2047

			Study Week						Study Week			
Group	Male	Pre-	13	26	42	52	Female	Pre-	13	26	42	52
1	175	13.3	12.4	13.1	12.4	13.1	142	13.6	13.4	12.9	13.7	13.3
	177	14.0	13.5	13.2	13.4	14.1	144	13.6	13.6	12.2	11.9	13.1
	143	13.3	13.2	13.5	13.3	13.2	146	12.1	11.9	11.7	11.4	11.8
	145	14.3	14.5	13.8	13.9	14.0						
	Mean	**13.73**	**13.40**	**13.40**	**13.25**	**13.60**		**13.10**	**12.97**	**12.27**	**12.33**	**12.73**
	SD	0.51	0.87	0.32	0.62	0.52		0.87	0.93	0.60	1.21	0.81
2	147	13.3	12.9	12.8	12.5	12.9	148	14.4	12.3	12.9	13	13.8
	149	12.7	12.6	12.2	12.2	11.5	150	14.7	14.4	13.8	14.2	13.9
	151	14.6	13.8	13.1	(13.3*)		152	13.2	13.2	12.2	12.3	13.0
	153	11.5	12.7	11.5	12.1	12.4	154	13.6	14.0	13.3	13.2	13.8
	155	13.4	13.9	13.5	13.4	13.1	156	14.0	13.5	12.6	12.4	12.7
	157	13.0	13.0	13.1	13.3	13.4	158	13.3	13.3	13.1	13.2	12.9
	Mean	**13.08**	**13.15**	**12.70**	**12.70**	**12.66**		**13.87**	**13.45**	**12.98**	**13.05**	**13.35**
	SD	1.01	0.56	0.73	0.61	0.74		0.61	0.72	0.56	0.69	0.54
3	159	14.3	13.7	13.5	13.3	14.0	160	14.4	*12.4*	*12.6*	13.7	13.0
	161	14.3	13.9	13.5	14.0	13.3	162	14.0	12.1	12.3	13.0	13.1
	163	13.2	12.6	12.3	11.5	12.2	164	15.3	14.2	14.1	13.3	14.1
	165	13.6	13.9	13.8	13.2	13.4	166	12.9	12.2	12.3	12.5	12.7
	Mean	**13.85**	**13.53**	**13.28**	**13.0**	**13.23**		**14.15**	**12.73**	**12.83**	**13.13**	**13.23**
	SD	0.54	0.62	0.67	1.06	0.75		0.99	0.99	0.86	0.51	0.61
4	139	13.8	11.7	12.5	12.6	12.7	140	15.3	15.4	14.4	14.7	14.9
	141	13.7	13.1	13.3	13.9	12.0	168	14.5	13.4	13.3	13.3	13.5
	167	13.3	12.4	12.6	13.9	12.5	172	13.1	13.7	11.8	13.0	(11.1*)
	169	13.1	12.2	12.9	12.9	12.6	174	14.1	15.1	12.9	12.5	12.2
	171	12.6	13.0	11.8	12.2	12.1	178	13.1	10.5	11.8	11.3	12.2
	173	12.2	11.1	10.4	10.9	11.1						
	Mean	**13.12**	**12.25**	**12.25**	**12.73**	**12.17**		**14.02**	**13.62**	**12.84**	**12.96**	**13.20**
	SD	0.62	0.77	1.03	1.13	0.59		0.94	1.95	1.10	1.24	1.29

Bold text indicates mean values; Yellow highlight indicates statistical significance, p<0.05, Underlined values indicates decrease ≥ 1 gm/dL from pre-dose value. * Blood sample collected at unscheduled sacrifice

Apparent reductions of lymphocyte counts in the high dose males were noted during treatment compared with pretreatment and control values. However, two to three control males showed increases in lymphocyte counts during treatment compared to their pre-treatment values. Furthermore, all values for the individual high dose males were within the range of pre-dose values. The reviewer does not consider these reductions in lymphocyte counts toxicologically significant.

Clinical Chemistry

At the same time as the collection of blood for hematology, additional blood was obtained for measurement of the following clinical chemistry parameters: alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase, leucine aminopeptidase, lactate dehydrogenase, aldolase, glucose, total bilirubin, total cholesterol, triglycerides, creatinine, urea, total protein, albumin/globulin ratio (A/G ratio), sodium, potassium, chloride, magnesium, calcium, phosphorus, and uric acid. Albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin

were evaluated with a protein electrophoretogram. The sponsor did not provide the laboratory's normal range for any parameter.

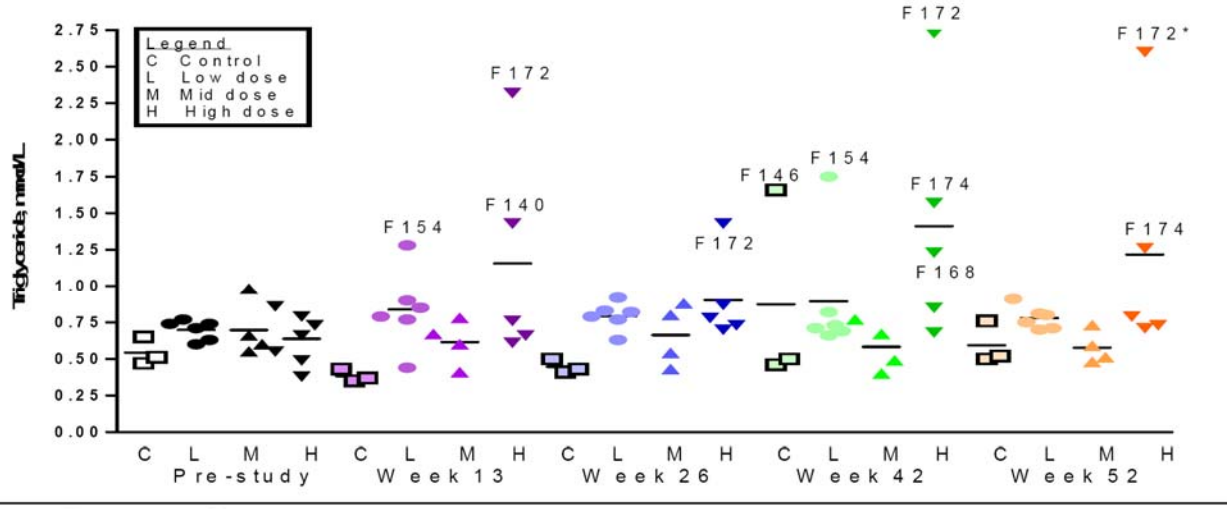
The high dose females, but not males, exhibited increased mean triglyceride values compared to the control group with statistical significance being attained in Weeks 13, 26 and 52 (Table 52). The sponsor attributed the increase in triglycerides to a high value in a high dose female (172), who was later prematurely sacrificed during week 51 and found to have diffuse lymphocytic infiltration of the intestinal mucosa. The reviewer plotted individual triglyceride values for females in the pre-study and treatment phases (Figure 26). Female 172 had triglyceride values above the maximum pre-study value (0.98 mmole/L) during Weeks 13, 26, 42 and 51. However, other treated females (154 from low dose group and 140, 174, 168 from the high dose group) also had triglyceride values above this maximum. The triglyceride value for control female 146 (1.66 mmol/L) during Week 42 appears anomalous compared to the other triglyceride values for this animal of 0.51, 0.43, 0.50 and 0.52 mmol/L) and is higher than the maximum pre-study value (0.98 mmol/L) as well as the mean+2SD (0.94 mmol/L) of the pre-study values for all females. The triglyceride values for M151 during week 26 and at unscheduled termination during week 38 were also high.

Table 52: Reviewer's Summary of Triglyceride Values – Study U05-2047

			Male						Female					
Study	Week		Pre-	13	26	42	52	Rec.	Pre-	13	26	42	52	Rec.
Triglycerides,	1	Mean	0.54	0.69	0.56	0.54	0.48		0.54	0.38	0.45	0.87	0.59	
		SD	0.117	0.232	0.092	0.087	0.085		0.095	0.042	0.047	0.682	0.145	
	2	Mean	0.59	0.57	0.71	0.56	0.67	0.56	0.7	0.84	0.79	0.89	0.78	0.78
		SD	0.093	0.154	0.369	0.174	0.379		0.068	0.27	0.095	0.423	0.078	
	3	Mean	0.71	0.75	0.7	0.61	0.58		0.7	0.62	0.66	0.58	0.58	
		SD	0.13	0.176	0.097	0.099	0.099		0.194	0.155	0.212	0.168	0.112	
	4	Mean	0.63	0.74	0.67	0.6	0.59	0.76	0.64	**1.16**	**0.9**	1.41	**0.87***	0.54
		SD	0.126	0.204	0.08	0.042	0.064		0.172	0.73	0.302	0.813	0.261	
		M151	0.50	0.57	1.35	2.36†								

Bold text indicates statistical significance, * Mean without F172 † Value at unscheduled termination in week 38

Figure 26: Reviewer's Plot – Individual Triglyceride Values - Study U05-2047



Control: □; Low dose: ○; Mid dose: △; High dose: ▽; Pre-study: white & black; Week 13: purple; Week 26: blue; Week 42: green; Week 52: orange; Individuals with triglyceride values greater than the maximum value (0.98 mmol/L) observed pre-study are labeled with their animal number. * F172 was prematurely sacrificed during Week 51. Mid-dose Female 164 value of 0.98 pre-study

The sponsor noted significantly decreased mean sodium values during Week 13 for the mid- and high dose female groups in comparison with the control group. The decrease of 2.7% in the group mean sodium for the mid-dose females (144 mmol/L) is not toxicologically significant, since the lowest sodium value of 141 mmol/L for mid-dose female 160 is very close to the minimum sodium value (142 mmol/L) for all control female values. However, the decrease of 4.8% in group mean sodium for the high dose female (139 versus 148 mmol/L in controls) is attributable due to an abnormally low sodium value of 115 mmol/L for female 174, who also had very low values for chloride, calcium, magnesium, urea nitrogen, creatinine, ALT, AST and alkaline phosphatase, but increases in potassium, albumin, β-globulin, and total protein. Interestingly, male 151, who was prematurely sacrificed in week 38 due to Shigellosis, and female 172, who was sacrificed during week 51, also had low sodium values of 125 and 138, respectively, at sacrifice.

The sponsor noted lower albumin mean values and consequently lower total protein mean values in the high dose females In Weeks 26, 42 and 52 in comparison with those of the controls. These decreases were attributable to significantly lower values in Females 172 and 174 as indicated in Table 53. Slightly lower group mean glucose values were noted in Weeks 42 and 52 in the high dose females in comparison with those of the controls. Again these changes were attributable to females 172 and 174.

Table 53: Reviewer's Table - Albumin and Glucose Values - Study U05-2047

		Study Week Albumin , gm/L						Study Week Glucose, mmol/L					
Group		Pre-	13	26	42	52	Rec	Pre-	13	26	42	52	Rec
1F	Mean	43	47	41	40	44		3.84	4.39	3.91	3.83	3.50	
	SD	2.3	3.5	2.1	3.1	1.5		0.61	0.94	0.51	0.41	0.76	
2F	Mean	47	46	40	41	44	49	3.93	3.49	3.67	3.07	3.51	3.11
	SD	2.7	3.2	2.3	2.2	2.2		0.37	0.67	0.27	0.81	0.49	
3F	Mean	49	45	41	40	45		3.66	2.95	3.47	2.89	3.14	
	SD	2.5	2.9	2.5	3.9	2.4		0.32	0.32	0.35	0.62	0.42	
4F	Mean	45	45	34	32	41	50	3.30	3.55	3.45	**2.54**	3.09	2.70
	SD	3.1	14.0	8.7	11.2	8.7		0.82	0.82	0.33	0.37	0.23	
4	F172	42	22	25	16	18*		2.09	4.91	3.26	1.89	3.90*	
	F174	50	59	25	25	28		3.59	2.94	3.53	2.72	2.86	
2	M151	47	49	27	12*			4.83	3.13	2.59	5.42*		

* Value at premature sacrifice, Rec = recovery

The sponsor noted that the high dose females in Week 26 had statistical significant higher group mean values for AST and LDH in comparison with those of the controls (Table 54). However, the increases in AST were attributed to females 172 and 174. Moreover, all ALT values even those for females 172 and 174 were within the range of individual control values. Since males did not exhibit an increase in AST, the sponsor concluded that these increases do not result from administration of BIBR 1048 MS, but from non treatment-related clinical abnormalities in a few animals.

Table 54: Reviewer's Summary of AST and ALT Values – Study U05-2047

		Study Week AST, U/L						Study Week ALT, U/L					
Group		Pre-	13	26	42	52	Rec	Pre-	13	26	42	52	Rec
1M	Mean	34	43	48	48	38		34	39	62	47	42	
	SD	7.1	10.7	14.5	12.3	9.8		10.8	9.6	17.3	12.6	6.3	
	Max	51*	57	65	59	50		63*	52	84	64	49	
2M	Mean	38	43	47	42	33	33	47	49	57	50	38	43
	SD	2.7	5.9	9.4	6.8	4.7		8.6	10.0	19.4	5.7	3.6	
	M151	29	47	53	27†	-		33	32	24	10†	-	
3M	Mean	35	45	43	44	35		38	44	49	45	40	
	SD	6.1	10	13.1	13.3	9.6		4.5	2.4	9.0	9.0	4.7	
4M	Mean	40	37	49	39	36	36	50	42	50	44	38	40
	SD	10	7.9	13.1	7.1	8.3		10.4	5.0	8.3	2.0	4.2	
1F	Mean	31	33	33	33	25		38	39	47	39	35	
	SD	7.5	5.9	2.1	2.9	1.5		11.2	5.9	16.1	5.6	3.2	
	Max	53*	40	35	36	27		87*	46	65	44	37	
2F	Mean	32	36	35	33	30	28	35	37	41	33	38	38
	SD	6.1	5.0	6.0	3.2	5.5		10.4	6.0	10.7	7.7	10.8	
3F	Mean	36	32	47	39	34		59	39	48	45	35	
	SD	5.9	3.4	4.8	14.4	8.1		32	18.6	21.2	28.1	16.1	
4F	Mean	38	35	**49**	38	35	28	42	28	38	28	37	36
	SD	8.8	14.7	13.6	9.8	14.1		19.8	13.3	4.4	8.6	21.9	
	F172	38	51	58	40	46†		28	32	39	21	32†	
	F174	53	13	67	51	54		40	8	38	28	34	

* All animals pre-study; †Value at premature sacrifice; Bold text with highlight = statistically significant, $p<0.05$, Rec = recovery

Urinalysis

Once during acclimation and during Week 12, 25, 38 and 51 of treatment, urine samples were collected overnight from all animals deprived of water and food. The measured quantitative parameters included: volume, pH, specific gravity, protein, sodium, potassium, and chloride. The measured semi-quantitative parameters included: appearance, glucose, ketones, bile pigments, blood pigments. The urine sediment was examined microscopically for epithelial cells, leucocytes, erythrocytes, crystals, casts, spermatozoa and other abnormal components.

Although changes in some urinalysis parameters (e.g. urine volume) occurred during treatment compared to acclimation, these changes occurred similarly in both the control groups and the treated groups. Thus, no effects on urinary parameters could be attributed to BIBR 1048 MS treatment.

Gross Pathology

Animals were euthanized using sodium pentobarbitone and subjected to detailed necropsy. The whole or a sample of the tissues listed below (Table 55) from all animals was preserved in 10% neutral buffered formalin, except for the following tissues. Testes and epididymides were fixed in Bouin's solution prior to transfer to 70% industrial methylated spirit. The eyes were fixed in Davidson's fluid prior to transfer to 70% industrial methylated spirit. Bone marrow samples obtained from the sternum during necropsy were used to prepare smears that were dried, fixed, and then stained using a Romanowsky procedure. However, the smears were not examined because the peripheral blood results did not indicate such evaluation was needed.

No macroscopic lesions identified at termination or following six weeks of the recovery period were attributable to treatment with BIBR 1048 MS.

Table 55: Sponsor's List of Tissues Collected - Study U05-2047

Abnormal tissues Adrenal glands Aorta - thoracic Brain (cerebellum, cerebrum, midbrain and medulla Cecum Colon Duodenum Epididymides Esophagus Eyes Femur‡ (articular surface, epiphysial plate, bone marrow) Gall bladder Heart Ileum Jejunum	Kidneys (cortex, medulla and papilla) Liver Lungs with mainstem bronchi Lymph nodes (mandibular, mesenteric and regional to masses) Mammary area - caudal Optic nerves Ovaries Pancreas Pituitary Prostate Rectum Salivary gland (submandibular)† Seminal vesicles Sciatic nerve† Seminal vesicles	Skeletal muscle - thigh† Skin Spinal cord (cervical, thoracic and lumbar) Spleen Sternum Stomach Testes Thymus Thyroid with parathyroids Tongue Trachea Ureters Urinary bladder Uterus and cervix Vagina
† Only one processed for examination; ‡ Both hindlimbs, one sectioned where appropriate.		

Organ Weights

From each animal at necropsy, the following organs excised and weighed: adrenal glands, brain, epididymides, heart, kidneys, liver, lungs with mainstem bronchi, ovaries, pituitary, prostate, salivary glands, spleen, testes, thymus, thyroids with parathyroids and uterus with cervix.

The sponsor noted non-statistically significant lower absolute group mean heart, lungs, thymus, testes, epididymides and prostate weights in some treated male groups, especially the high dose group, compared to those in the control group. Of these only the testes, epididymides and prostate weights relative to body weight were lower than those in the control group. These findings were attributed to the high values in one control male (143), whose values were above those of other control males.

The non-statistically significant increases in absolute (20%) and relative (30%) adrenal weights in the mid and high dose males compared to the controls did not occur in females and had no histopathology correlate.

The sponsor noted lower absolute group mean heart, kidney, lung, ovaries, thymus, uterus, and spleen weights in the high dose female group compared to those in the control group. The reviewer also noted decreases in the liver, thyroids, salivary gland, and pituitary absolute weights. Of these changes, only the pituitary, lung, uterus and ovary weights relative to body weight were more than 10% lower than those in the control group. The sponsor attributed the low absolute organ weights to female 174, whose body weight was only 2.78 kg compared to a range of 3.88 to 6.30 kg for all other females. Relative to bodyweight only the decreases in the thymus, ovary and uterus weights relative to body weight could be attributed to female 174.

The sponsor did not note the statistically significant decreases in absolute (37 to 48%) and relative (30 to 43%) spleen weight in all male treated groups (Table 56). However, no clear histopathology correlate was observed. Although the mean absolute spleen weight in the high dose females was 13% lower than that in the control group, the mean relative spleen weight was 16% higher. Likewise, the statistically significant decreases in absolute (47%) and relative (29%) pituitary weight in the high dose females had no histopathological correlate and similar decreases were not observed in males.

Following the recovery period, lower absolute and relative heart weights were observed in the high dose females compared to the weights obtained in the control females at the end of the treatment period. Similar to the end of the treatment period lower absolute, but not relative, heart weights were observed in the high dose males following recovery. In contrast, the absolute and relative pituitary weights in the high dose females following the recovery period were similar to the weights obtained in the control females at the end of the treatment period. However, the absolute and relative pituitary weights in the low dose and high dose males following the recovery period were 38-70% higher than the weights obtained in the control males at the end of the treatment period. The absolute and relative spleen weights in the low and high dose males after recovery were lower than the weights in the low and high dose males after treatment. In contrast, the absolute and relative spleen weights in the low and high dose females were higher than the weights in the low and high dose females after treatment.

Table 56: Reviewer's Compilation of Selected Organ Weights – Study U05-2047

Organ	Type		SEX:	MALE				FEMALE			
			GROUP:	---1---	---2---	---3---	---4---	---1---	---2---	---3---	---4---
			NUMBER:	4	3	4	4	3	4	4	2
Spleen	Absolute	Main	N :	4	3	4	4	3	4	4	2
			MEAN :	10.15	6.36*	6.23*	5.24**	5.22	6.76	5.45	4.56
			sd :	1.67	1.67	3.00	1.17	1.15	0.70	1.46	0.15
		Rec.	Mean§		5.54		4.26		9.58		7.21
	Relative *	Main	N :	4	3	4	4	3	4	4	2
			MEAN :	0.182	0.120	0.127	0.104*	0.109	0.156	0.126	0.127
			sd :	0.029	0.012	0.061	0.020	0.031	0.022	0.033	0.057
		Rec.	Mean§		0.104		0.088		0.188		0.160
Pituitary	Absolute	Main	N :	4	3	4	4	3	4	4	2
			MEAN :	0.057	0.070	0.061	0.056	0.081	0.071	0.078	0.043**
			sd :	0.014	0.026	0.016	0.011	0.003	0.010	0.003	0.006
		Rec.	Mean§		0.087		0.079		0.080		0.081
	Relative *	Main	N :	4	3	4	4	3	4	4	2
			MEAN :	0.0010	0.0013	0.0013	0.0011	0.0017	0.0016	0.0018	0.0012*
			sd :	0.0002	0.0003	0.0003	0.0003	0.0004	0.0001	0.0001	0.0003
		Rec.	Mean§		0.0017		0.0016		0.0016		0.0018
Heart	Absolute	Main	N :	4	3	4	4	3	4	4	2
			MEAN :	28.04	24.43	23.07	24.46	22.27	19.58	18.26	16.51
			sd :	4.76	6.91	5.12	4.57	4.15	3.61	2.68	6.28
		Rec.	Mean§		24.04		23.45		23.37		18.20
	Relative *	Main	N :	4	3	4	4	3	4	4	2
			MEAN :	0.497	0.459	0.464	0.482	0.455	0.447	0.422	0.422
			sd :	0.020	0.061	0.055	0.047	0.030	0.036	0.032	0.018
		Rec.	Mean§		0.458		0.484		0.462		0.404

Rec. = following 6 weeks of recovery; * Relative to body weight; § Number of recovery animals was 2.

Histopathology

Adequate Battery

Tissue samples from all study animals were dehydrated, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The indicated tissues listed in Table 55 and gross abnormalities identified at macroscopic examination from all animals sacrificed at the end of the scheduled treatment period and from all animals killed or dying during the study were examined by histology.

Peer Review

A reviewing pathologist in the conducting laboratory conducted a peer review.

Histological Findings

The sponsor maintains that histopathology changes related to treatment were confined to the thymus and all other findings were incidental and not toxicologically significant. However, interpretation of the data is complicated by limited numbers of animals per group especially having only two females in the high dose group at the end of treatment. No histopathology findings correlated with the changes in absolute or relative organ weight differences as discussed above.

An increased incidence and severity of thymic involution/atrophy was observed in the high dose females compared with incidence in the control females. The two most severely affected animals were male 151 (low dose) and female 172 (high dose); these animals were euthanized during weeks 38 and 51, respectively, due to infections. The thymic atrophy may relate more to the clinical condition of the animals rather than to a toxicological effect.

Table 57: Sponsor's Table: Histopathology in Thymus – Study U05-2047

		Male				Female			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dosage (mg base/kg/day)		**0**	**12**	**36**	**200**	**0**	**12**	**36**	**200**
Involution/atrophy	Total	1	2	0	0	1	1	0	3
	Minimal	1	1	0	0	0	1	0	0
	Slight	0	0	0	0	1	0	0	2
	Moderate	0	1*	0	0	0	0	0	0
	Marked	0	0	0	0	0	0	0	1**
Number of animals examined		**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**

* Decedent No.151 (2M, 12 mg base/kg/day), ** decedent No.172 (4F, 200 mg base/kg/day), the 2 early decedents (Nos.170, 176: 4F, 200 mg base/kg/day) are not included in this table.

Because of the increased incidence in liver necrosis observed in the mouse and rat carcinogenicity studies (Table 102, Table 115), the histopathology findings in the liver are presented below (**Error! Reference source not found.**) for the monkeys in the 52-week study. No finding of liver necrosis was reported.

Table 58: Sponsor's Summary of Liver Histopathology - Study U05-2047

	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	4	3	4	4	3	4	4	2
After 52 weeks treatment									
LIVER ..... NUMBER EXAMINED:		4	3	4	4	3	4	4	2
--PORTAL INFLAMMATORY CELLS		1	0	0	1	0	1	2	1
--INFLAMMATORY CELL FOCI		4	3	4	4	0	4	2	2
--HEPATOCYTE VACUOLATION - MEDIAN CLEFT		0	0	0	1	0	1	1	1
--CENTRILOBULAR HEPATOCYTE DISSOCIATION (ARTEFACTUAL)		0	0	0	0	0	0	0	1
--HEPATOCYTE RAREFACTION		0	1	0	0	0	0	0	0
--AREA OF SUBCAPSULAR HEPATOCYTE ATROPHY AND FIBROSIS		0	0	1	0	0	0	0	0
--HEPATOCYTE VACUOLATION		0	0	0	0	0	1	0	0
--CENTRILOBULAR HEPATOCYTE RAREFACTION		0	0	0	0	0	0	2	0
After 6 weeks recovery									
LIVER ..... NUMBER EXAMINED:		0	2	0	2	0	2	0	2
--PORTAL INFLAMMATORY CELLS		0	0	0	0	0	1	0	1
--INFLAMMATORY CELL FOCI		0	2	0	2	0	1	0	1
--CENTRILOBULAR HEPATOCYTE RAREFACTION		0	1	0	0	0	0	0	0

Table 59: Reviewer's Table - Liver Histopathology in Decedents - Study U05-2047

Animal	Dose, mg/kg	Date of death	Liver Findings	Cause of death
F170	200	Day 7	Inflammatory cell foci – minimal Centrilobular hepatocyte dissociation (artefactual) - present	Aspiration into the lung of the acidic dose formulation
F176	200	Day 5	Inflammatory cell foci – minimal	
M151	12	Week 38	Portal inflammatory cells – minimal, multi-focal	Shigellosis, confirmed with tests of a fecal sample
F172	200	Week 51	Portal inflammatory cells – minimal Inflammatory cell foci – minimal	Chronic enteric infection.

An increased incidence of cardiomyopathy diagnosed by histopathology in the female, but not the male, treated groups in the two-year rat carcinogenicity study. In addition, cardiomyopathy was found at a high incidence in both control and treated groups of the mouse carcinogenicity study with a slight increase in incidence in the low and high dose males and the high dose females. However, no diagnosis of cardiomyopathy was reported either in the 26 or the 52-week monkey studies.

Special Evaluation

In the 26-week studies, a finding found in monkeys, but not in rats, was an effect on the male reproductive system. The number of male monkeys with atrophy of the testes, prostate, seminal vesicles and epididymides increased in all the drug-treated groups compared to the control group. The sponsor described these histopathological findings of atrophy as "physiological" resulting from the seasonality of testosterone production and breeding in rhesus monkeys even in a controlled environment. Therefore, in the 52-week monkey study the sponsor evaluated the effects of BIBR 1048 MS on plasma testosterone and testicular volume prior to termination of the main and recovery study periods.

Blood samples were collected prior to daily dosing during Weeks 42, 43, 51, 52, and Recovery Weeks 5 and 6. Plasma was prepared and assayed for testosterone using a commercial testosterone radioimmunoassay kit.

The volume of each testicle was determined by measuring the dimensions (length and width) of both testes within the abdominal cavity of each male using calipers in Weeks 34, 38, 42, 46, 50 52 and Recovery Weeks 3 and 6 and calculated using the formula: Volume = 4/3 (л)(length/2)(width/2)^2 (after Bercovitch, 1993).

Most of the males in this study were immature. Based on testicular histopathology only one control male (M143) was considered fully mature and three males (low dose: M155, mid-dose: M159; high dose: M169) were considered not fully mature. Consequently, these males displayed higher testicular weights, testicular volumes and plasma testosterone values in comparison with the remaining animals. To better correlate these parameters, the reviewer generated Table 60.

Consistent with the findings for the testicular absolute and relative weights, the group-mean testicular volume was lower in all treated groups in comparison with that for the

control group from Week 42 to termination. However, the sponsor maintains that no effect of BIBR 1048 MS treatment was observed in testicular volume during the in-life portion of the study or at necropsy. This conclusion is based on the lack of a dosage relationship, relative consistency of the volumes over time from Week 42 to termination and the overlap of the individual values in the treated groups with those in the control group, especially when the most mature males are omitted from consideration. Because control male 143 was the most mature animal, his large testicular volume compared to the less fully mature males in the treated groups makes it appear as though there was an effect of treatment.

Similarly, the sponsor maintains that no effect of BIBR 1048 MS treatment was observed on plasma testosterone concentrations. Again, this conclusion is based on the lack of a dosage relationship, relative consistency of the values from Week 42 to termination and the overlap of the individual values in the treated groups with those in the control group, especially when the most mature males are omitted from consideration. With the exception of the mid-dose male 159, whose plasma testosterone fell from 21.0 nmol/L in Week 42 to 1.1 nmol/L in Week 52, the variation of testosterone concentrations for individual males was generally low over time.

Testicular volume and weights tended to correlate overall with testosterone concentrations in that the most mature males (males 143, 155, 159 and 169) had higher testosterone concentrations, testicular volumes and testicular weights compared to the other males in their respective groups. However, over time the changes in testosterone concentrations in these males did not directly correlate with changes in testicular volume. The sponsor attributed differences seen in testicular weight, volume and testosterone concentration and sexual maturity of the testes to normal biological variation in adolescent rhesus monkeys and not an effect of treatment with BIBR 1048 MS. In contrast to the 26-week study, a diagnosis of testicular atrophy was not reported in the 52-week study. Because no male monkey in the treated groups was sexually mature, it is difficult to assess the effect of BIBR 1048 MS on male reproductive organs.

Table 60: Reviewer's Summary of Testicular Parameters – Study U05-2047

ORGAN AND FINDING DESCRIPTI					
		--- N U M B E R - (			
		SEX: --------MALE--------			
		GROUP: -1- -2- -3- -4-			
		NUMBER: 4 3 4 4			
TESTES	NUMBER EXAMINED:	4 3 4 4			
--IMMATURE		3 3 3 3			
--NOT FULLY MATURE		0 0 1 1			
--TUBULAR HYPERTROPHY		0 1 0 0			
--TUBULAR DILATATION		0 0 1 0			
--INTERSTITIAL FIBROSIS		0 0 2 0			
	SEX:	----------MALE----------			
	GROUP:	---1--- ---2--- ---3--- ---4--			
	NUMBER:	4 3 4 4			
Testes – Absolute weight	N :	4 3 4 4			
	MEAN :	12.063 2.506 5.794 4.757			
	sd :	18.421 1.624 5.853 5.892			
† At end of recovery	Highest individual	M143: 39.7	M155: 12.6†	M159: 14.5	M169: 13.6
	Range of remaining males	2.0-3.3	1.3-4.3	2.5-3.5	1.3-2.3
Testes – Relative to body weight	N :	4 3 4 4			
	MEAN :	0.1960 0.0455 0.1105 0.0923			
	sd :	0.2860 0.0226 0.0971 0.1123			
† At end of recovery	Highest individual	M143: 0.62	M155: 0.22†	M159: 0.26	M169: 0.26
	Range of remaining males	0.04-0.07	0.03-0.07	0.05-0.07	0.03-0.05

			Group	1	2	3	4
Testicular volume (cm^3)	**Week 42**		Mean	7.9	4.6	5.8	3.6
		Highest individual		M143: 18.2	M155: 10.2	M159: 14.6	M169: 10.2
		Range of remaining males		3.3-6.2	1.8-8.4	2.3-4.0	2.1-2.7
	Week 48		Mean	7.8	4.6	4.9	4.0
		Highest individual		M143: 20.2	M155: 8.7	M159: 11.3	M169: 12.1
		Range of remaining males		2.7-5.1	1.5-6.9	2.2-3.8	1.6-3.2
	Week 50		Mean	8.5	5.2	5.2	4.5
		Highest individual		M143: 22.2	M155: 9.3	M159: 12.0	M169: 14.6
		Range of remaining males		2.9-5.2	1.7-7.4	2.4-4.1	1.8-3.6
	Week 52		Mean	8.5	5.3	5.3	4.6
		Highest individual		M143: 21.7	M155: 9.5	M159: 12.0	M169: 14.8
		Range of remaining males		3.0-5.3	1.7-7.6	2.4-4.2	1.8-3.6
	Necropsy		Main Mean	5.1	1.3	3.1	2.3
		Highest individual		M143: 16.2	M155: §	M159: 8.0	M169: 6.4
		Range of remaining males		1.1-1.6	0.6-2.1	1.2-1.9	0.8-1.1
Testosterone (nmol/L)	**Week 42**		Mean (SD)	2.1 (2.9)	0.5* (0.18)	5.5 (10.3)	1.0 (0.59)
		Highest individual		M143: 6.4	M155: 0.7	M159: 21.0	M169: 2.1
		Range of remaining males		0.3-1.0	<0.2-0.7	0.2-0.5	0.5-1.2
	Week 43		Mean (SD)	2.1 (3.2)	0.7* (0.44)	1.2 (1.72)	1.4 (1.82)
		Highest individual		M143: 6.9	M155: 1.3	M159: 3.8	M169: 5.1
		Range of remaining males		0.4-0.6	<0.2-0.5	<0.2-0.5	<0.2-0.9
	Week 51		Mean (SD)	3.6* (5.5)	0.5* (0.06)	0.7 (0.64)	0.6* (0.33)
		Highest individual		M143: 9.9	M155: 0.5	M159: 1.6	M169: 1.1
		Range of remaining males		<0.2-0.5	<0.2–0.5	0.3-0.4	<0.2-0.7
	Week 52		Mean (SD)	1.9* (2.5)	0.6* (0.13)	0.6* (0.47)	0.6* (0.26)
		Highest individual		M143: 4.8	M155: 0.7	M159: 1.1	M169: 1.0
		Range of remaining males		<0.2-0.5	<0.2-0.6	<0.2=0.4	<0.2=0.6

§ M155 was in the recovery group. No measurement of testicular volume for M155 was presented in the report

* Animals with values below limit of detection are omitted from group means

Toxicokinetics

Blood samples were collected from all animals before and 2, 4, 8 and 24 hours after dosing on Day 1 (Week A) and on one day during weeks 13, 26 and 52. Females

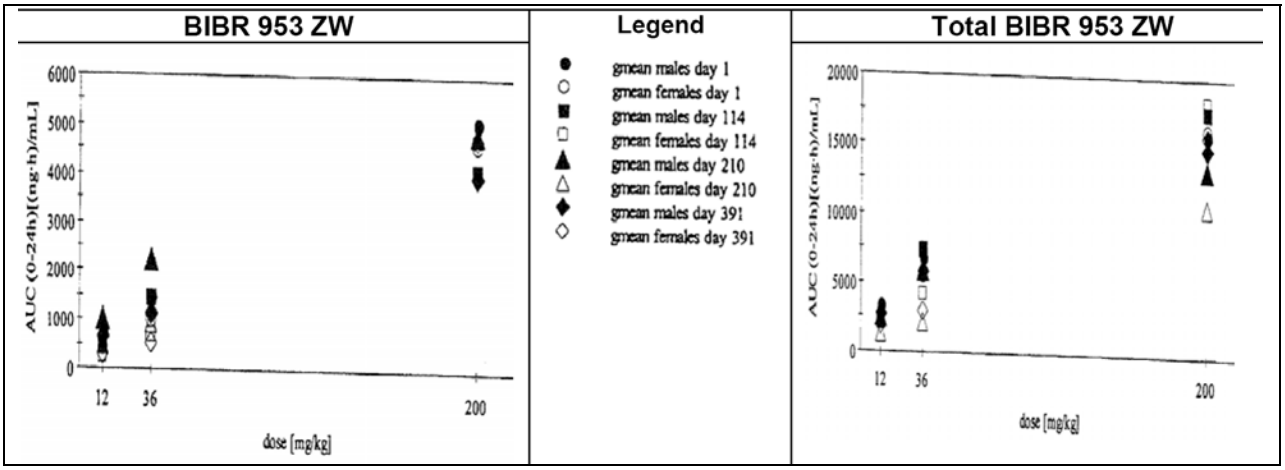
undergoing menstruation receiving treatment with BIBR 1048 MS were excluded from the toxicokinetic sampling. Analysis for BIBR 953 ZW used a validated HPLC-MS/MS assay with [$^{13}C_6$]-BIBR 953 ZW as an internal standard. To quantify the total of BIBR 953 ZW and its glucuronide (SUM BIBR 953 ZW), the glucuronide was cleaved by alkaline hydrolysis prior to analysis. The assay with an inaccuracy (b) (4) and imprecision of (b) (4) was valid in the concentration range o (b) (4).

The mean $C_{(max)}$ and $AUC_{(0\text{-}24h)}$ data are shown in Table 61. Exposure to BIBR 953 ZW and Total BIBR 953 ZW increased with dose (Figure 27); however, the increase was less than dose proportional. Exposure to BIBR 953 ZW alone represented (b) (4) of the Total BIBR 953 ZW plus glucuronides. After repeated dosing at day 391, lower plasma concentrations, especially in the two lower dose groups, were observed in both genders. In the two lower doses, but not the highest dose, exposures to Total BIBR 953 ZW were up to two-fold higher in males than in females. At dosages of 0, 12, 36 and 200 mg/kg/day the mean $AUC_{(0\text{-}24h)}$ for Total BIBR 953 ZW was 2692, 6438, and 15475 ng.hr/mL in males and 1973, 3710, and 15300 ng.hr/mL in females, respectively. These results differ from those at the highest dose of 200 mg/kg in the 26-week study in which males ($AUC_{(0\text{-}24h)}$ 24750 ng.hr/mL) had higher exposures than females ($AUC_{(0\text{-}24h)}$ 14200 ng.hr/mL).

Table 61: Reviewer's Compilation from Sponsor's Tables – Study U05-2047

			BIBR 953 ZW				Total BIBR 953ZW			
dose	day	gender	C(max) gmean [ng/mL]	C(max) gCV (%)	AUC(0-24h) gmean [(ng·h)/mL]	AUC(0-24h) gCV (%)	C(max) gmean [ng/mL]	C(max) gCV (%)	AUC(0-24h) gmean [(ng·h)/mL]	AUC(0-24h) gCV (%)
12	1	m	148	55.1	713	58.0	535	42.5	3350	45.7
12	1	f	86.3	80.2	454	92.0	405	59.4	2660	74.7
12	114	m	98.3	38.8	458	31.0	340	37.7	2170	35.3
12	114	f	48.6	43.5	245	33.7	330	26.0	2110	27.2
12	210	m	204	51.8	987	47.0	475	49.6	2600	58.9
12	210	f	104	26.8	470	21.4	245	57.5	1300	47.3
12	391	m	124	68.3	632	62.3	389	81.2	2650	64.8
12	391	f	53.6	70.2	287	61.5	290	63.0	1820	58.0
36	1	m	241	27.5	1480	37.0	895	40.2	6820	42.9
36	1	f	176	8.8	1010	23.2	772	10.0	5530	20.7
36	114	m	316	52.3	1450	63.1	1120	54.2	7320	61.4
36	114	f	136	37.3	693	31.9	591	17.4	4170	13.1
36	210	m	506	32.4	2230	41.2	858	41.3	5880	26.3
36	210	f	184	12.8	913	7.2	332	44.1	2190	22.5
36	391	m	230	98.3	1120	102.8	898	68.0	5730	76.2
36	391	f	104	20.5	527	21.5	417	34.0	2950	29.7
200	1	m	821	34.5	5130	54.0	1640	32.2	16000	44.1
200	1	f	647	139.8	4620	101.1	1470	116.7	16300	75.6
200	114	m	904	50.1	4110	54.9	2780	39.0	17600	47.8
200	114	f	979	106.4	4710	120.0	2720	75.9	18300	77.6
200	210	m	1070	42.7	4880	43.3	2030	59.8	13400	49.3
200	210	f	915	73.7	4860	120.0	1630	133.6	10700	128.8
200	391	m	645	32.4	3980	39.8	1610	37.3	14900	36.8
200	391	f	672	76.3	4740	88.6	1780	40.4	15900	53.7

Figure 27: Sponsor's Figures of AUC Dose Dependence - Study U05-2047



Formulation Analysis

The test article formulations for Groups 1-3 were prepared daily on the day before being administered and analyzed during Weeks 1, 17, 30, 43, and 55 of treatment for achieved concentration. The test article formulations for Group 4 were prepared daily and analyzed during Weeks 1, 13, 26, 39, and 51 of treatment for achieved concentration. Homogeneity of the test substance in the vehicle was evaluated prior to study start. Stability of BIBR 1048 MS in the vehicle was previously assessed by the sponsor.

All dose formulations were homogenous throughout the study. The concentrations of all individual samples were within the range of (b) (4) of the nominal value and the mean values were within the range of 93.2 – 98.8 %.

Following the death of Group 4 animals in the first week of treatment, pH measurements of the formulations were taken during Week 1 and 2 following the recommencement of administration of 200 mg base/kg/day to the Group 4 animals. The control formulation had a pH value of 7.5, whereas the formulations for Groups 2, 3, and 4 with concentrations of 1.38, 4.15, and 23.06 mg/mL had pH values of 3.45, 3.05, and 2.55, respectively.

Study title: BIBR 953 ZW: Intravenous Maximum Tolerated Dose Study in Rhesus Monkey

Study no.:	(b) (4) 572268, B1237, B1229 (U03-1061)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
For TK analysis	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	February 10, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch ZB 1398,

Key Study Findings

The objective of this study was to determine an appropriate maximum dose of BIBR 953 ZW for a 4 week study. In Part A, two Rhesus monkeys received daily intravenous administration of 5 mg/kg for 3 days, followed by 10 mg/kg for 3 days, 20 mg/kg for 3 days and 40 mg/kg BIBR 953 ZW for 6 days. Progressive decreases in hemoglobin, red blood cells and hematocrit were accompanied by increases in reticulocytes and APTT values in the male and female. At 40 mg/kg, clinical signs of bruising, swelling of the lips, and dark feces were observed. In Part B, two Rhesus monkeys received 20 mg/kg BIBR 953 ZW daily for two weeks. Decreases in hemoglobin, red blood cells and hematocrit were accompanied by increases in reticulocytes, potassium, and APTT values in the males and females. At necropsy some organs showed reddening and hemorrhaging. The mean $AUC_{(0\text{-}24h)}$ values for total BIBR 953 ZW (BIBR 953 ZW plus glucuronides) of 94460 and 86880 ng.hr/mL in the male and female, respectively. The maximum tolerated intravenous dose of BIBR 953 ZW in monkeys was determined to be 20 mg/kg/day, which was used as the high dose in the following 4-week toxicology study.

Study title: BIBR 953 ZW: 4 Week Intravenous Toxicity Study in Rhesus Monkeys with a 4 Week Recovery Period

Study no.:	(b) (4) 572619 (U00-1180) (b) (4) 572619 (U03-1157) for TK analysis
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
For TK analysis	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	May 27, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch ZB1411, 99%

Key Study Findings

Rhesus monkeys received daily intravenous administration of 0, 0.8, 4 and 20 mg/kg BIBR 953 ZW for 4 weeks. These dosages resulted in a mean $AUC_{(0\text{-}24h)}$ values for total BIBR 953 ZW (BIBR 953 ZW plus glucuronides) of 3240, 13050, and 83300 ng.hr/mL in males and 3230, 14400, and 96100 ng.hr/mL in females, respectively. Prolongation of aPTT values was observed in the high dose animals. Potassium values increased 8.8 and 18.8% in the high dose males and females, respectively. The NOAEL is considered to be 4 mg/kg based on the increases in serum potassium.

Methods

Doses:	0, 0.8, 4, 20 mg/kg
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Intravenous
Dose volume:	4 mL/kg
Formulation/Vehicle:	20% propylene glycol, 5% mannitol
Species/Strain:	Rhesus monkey
Number/Sex/Group:	3/sex/group
Age:	
Weight:	2.0-3.3 kg
Satellite groups:	Recovery: 2/sex/group for 4 weeks
Unique study design:	None
Deviation from study protocol:	The week 4 blood samples from all animals for coagulation were incorrectly stored and handled, resulting in high PT and aPTT values above the laboratory normal range.

Observations and Results

Mortality

The animals were examined visually at least twice daily for mortality, morbidity and reaction to treatment.

No unscheduled deaths occurred.

Clinical Signs

Detailed observations during treatment were made at least three times per day, particularly during the first hour after dosing on return of the animal to its cage, and as late as possible in the working day.

The clinical signs of red and swollen lips, anus, penis and vagina were commonly observed in the high dose groups. Bruising was also frequently observed in the high dose group and occasionally in the mid-dose groups.

Body Weights

The animals were weighed weekly during acclimation, on Day 1 of treatment, weekly until study termination and before necropsy.

Body weight was not affected by treatment.

Food Consumption

The mean weekly food consumption per animal was estimated from the daily weights of food supplied, food remaining, and spillage.

Food consumption was not affected by treatment.

Ophthalmoscopy

The eyes of all main study animals were examined after dilation with a 0.5% tropicamide ophthalmic solution using an indirect ophthalmoscope before treatment initiation, and during week 4 of treatment and week 4 of recovery.

Treatment related findings were not observed.

Hematology

Blood samples were obtained via venipuncture from all animals after fasting during acclimation, prior to dose administration during week 4 of treatment and week 4 of recovery. A blood smear was prepared for all animals. The following hematology parameters were measured: hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, platelet count, total white cell count, and differential white cell count, including neutrophils, lymphocytes, eosinophils, basophils, and monocytes. Additional samples were taken for measurement of thrombin time, prothrombin time (PT), activated partial thromboplastin time (aPTT), D-Dimer, and fibrinogen concentration. Laboratory control values (2.5 to 97.5%) were 10-14 sec for PT and 23-49 sec for aPTT.

Slightly lower group mean values for hematocrit, hemoglobin concentrations and red blood cell counts were noted in the high dose females, but not the high dose males, in comparison with those of the controls (Table 39). However, increases in reticulocyte counts were observed in the high dose females and all male treated groups. Because blood samples from all animals for coagulation were incorrectly stored and handled, the resulting in high PT and aPTT values for the control groups were above the laboratory normal range. Although this error affected interpretation of the PT results, the aPTT values were prolonged 21-75% and 15-71% in the high dose males and females respectively, in comparison to the concurrent control groups during week 4 of treatment. The prolongation of coagulation times is an expected pharmacodynamic effect. The measured values represent minimum increases because blood was probably collected for hematology prior to administration of the daily dose.

Table 62: From Sponsor's Hematology Tables – Study U00-1180

Males

Dose Group/Treatment ($mg.kg^{-1}.day^{-1}$)		Hb	RBC	Hct	MCH	MCV	MCHC	Reti	WBC	PT	APTT	Fib
1 (0)	Number	5	5	5	5	5	5	5	5	5	4	5
	Mean	12.8	5.52	0.425	23.2	77.1	30.1	1.8	7.99	42	70	209
	SD	0.7	0.19	0.020	0.5	1.7	0.6	0.6	2.80	27	30	35
2 (0.8)	Number	3	3	3	3	3	3	3	3	3	3	3
	Mean	13.4	5.73	0.445	23.3	77.6	30.1	3.1	8.86	24	85	222
	SD	0.1	0.06	0.020	0.3	3.1	1.6	1.5	2.01	7	21	46
	Prob.											
3 (4)	Number	3	3	3	3	3	3	3	3	3	3	3
	Mean	13.3	5.71	0.434	23.2	76.0	30.5	2.0	5.30	20	85	207
	SD	0.5	0.34	0.026	0.7	0.1	0.9	0.9	1.51	5	8	40
	Prob.											
4 (20)	Number	5	5	5	5	5	5	5	5	5	4	5
	Mean	12.6	5.39	0.412	23.5	76.4	30.7	2.4	6.22	28	123	225
	SD	0.8	0.23	0.025	1.4	3.3	1.2	1.5	1.39	7	34	45
	Prob.											

Females

Dose Group/Treatment ($mg.kg^{-1}.day^{-1}$)		Hb	RBC	Hct	MCH	MCV	MCHC	Reti	WBC	PT	APTT	Fib
1 (0)	Number	5	5	5	5	5	5	4	5	5	5	5
	Mean	13.4	5.79	0.425	23.2	73.7	31.5	1.8	8.96	16	52	213
	SD	0.8	0.48	0.024	1.4	2.9	1.0	0.7	4.96	2	10	19
2 (0.8)	Number	3	3	3	3	3	3	3	3	3	3	3
	Mean	13.4	5.69	0.441	23.5	77.4	30.4	1.6	6.81	16	60	200
	SD	1.0	0.34	0.029	0.7	2.4	0.3	0.8	0.56	2	13	44
	Prob.						*					
3 (4)	Number	3	3	3	3	3	3	3	3	3	3	3
	Mean	13.5	5.82	0.431	23.3	73.9	31.5	1.6	6.66	17	69	220
	SD	1.3	0.40	0.037	1.2	3.4	0.4	1.0	0.52	1	11	23
	Prob.											
4 (20)	Number	5	5	5	5	5	5	5	5	5	5	5
	Mean	11.7	4.90	0.386	23.8	78.7	30.2	4.2	10.47	17	89	275
	SD	1.8	0.72	0.059	0.9	1.9	0.5	2.5	6.77	2	18	74
	Prob.					*	**				**	

Clinical Chemistry

At the same time as the collection of blood for hematology, additional blood was obtained for measurement of the following clinical chemistry parameters: alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase, lactate dehydrogenase, glucose, total bilirubin, total cholesterol, creatinine, urea, total protein, albumin, globulin, albumin/globulin ratio (A/G ratio), sodium, potassium, chloride, calcium, and phosphorus.

Statistically significant increases in mean potassium and globulin levels were observed in the high dose females. The sponsor concluded these changes were not related to treatment, because they were restricted to one sex. However, the reviewer notes that

the mean potassium value also increased in the high dose males, although the value was not statistically different from the mean in the control group.

Table 63: From Sponsor's Clinical Chemistry Summary - Study U00-1180

Males

Dose Group/Treatment ($mg.kg^{-1}.day^{-1}$)		Urea	Glu	AST	ALT	AP	LDH	Na	K	Cl	TP	Alb	Glob	AG-R	Chol	Crea	Ca	Phos	T.Bi	CPK
1 (0)	Number	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	7.5	3.26	36	41	607	674	151	3.4	112	80	46	34	1.4	4.4	72	2.43	2.16	3.5	111
	SD	1.3	0.92	6	5	113	127	3	0.2	1	4	1	3	0.1	0.8	11	0.03	0.11	0.9	14
2 (0.8)	Number	3	3	1	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	7.3	3.83	26	31	652	735	155	3.5	114	85	46	39	1.2	4.3	79	2.52	2.08	3.1	121
	SD	2.1	0.34		11		240	3	0.3	5	7	2	8	0.3	0.7	4	0.05	0.31	1.4	25
	Prob.																			
3 (4)	Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	7.4	2.95	37	38	631	688	152	3.7	112	79	45	34	1.3	3.9	73	2.48	2.15	3.4	118
	SD	0.8	0.37	6	10	186	50	2	0.1	1	4	1	3	0.1	0.6	7	0.05	0.25	0.7	8
	Prob.																			
4 (20)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	7.4	3.06	31	36	636	587	153	3.7	114	84	48	36	1.3	3.7	68	2.44	2.21	3.0	122
	SD	1.8	0.56	4	7	81	158	2	0.2	2	5	2	4	0.1	0.6	17	0.20	0.33	0.6	25
	Prob.																			

Females

Dose Group/Treatment ($mg.kg^{-1}.day^{-1}$)		Urea	Glu	AST	ALT	AP	LDH	Na	K	Cl	TP	Alb	Glob	AG-R	Chol	Crea	Ca	Phos	T.Bi	CPK
1 (0)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	6.8	2.79	33	36	806	680	153	3.2	114	81	47	34	1.4	3.8	69	2.44	2.13	3.5	138
	SD	1.0	0.47	5	5	196	162	1	0.2	3	2	2	2	0.1	1.0	6	0.08	0.21	0.6	30
2 (0.8)	Number	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	5.1	3.10	31	47	561	541	154	3.5	116	83	49	33	1.5	3.5	71	2.52	1.88	3.0	108
	SD	1.0	0.27	6	9	169	145	2	0.1	1	6	3	4	0.1	0.3	5	0.11	0.35	0.8	16
	Prob.																			
3 (4)	Number	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	6.3	3.52	30	27	800	635	155	3.3	114	84	47	37	1.2	4.4	72	2.52	1.99	3.8	124
	SD	0.6	0.48	5	6	298	61	4	0.3	3	3	1	2	0.1	0.9	4	0.09	0.20	1.0	6
	Prob.													*						
4 (20)	Number	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	6.6	2.77	39	34	702	791	153	3.8	116	83	45	38	1.2	3.4	71	2.44	2.14	4.8	134
	SD	1.3	0.38	9	10	122	228	3	0.2	4	3	3	1	0.1	0.4	7	0.09	0.19	1.6	13
	Prob.								**				**	***						

Urinalysis

Once pretrial, during Week 4 of treatment and during the last week of recovery, urine samples were collected overnight from all animals deprived of water and food. The measured quantitative parameters included: volume, pH, specific gravity, protein, glucose, urobilinogen, bilirubin, and blood pigments. The urine sediment was examined microscopically for epithelial cells, leucocytes, erythrocytes, crystals, casts, spermatozoa and other abnormal components.

Treatment related effects on urinalysis parameters were not observed.

Gross Pathology

Animals were euthanized using sodium pentobarbitone and subjected to detailed necropsy. The whole or a sample of the tissues listed below (Table 64) from all animals was preserved in 10% neutral buffered formalin, except for the eyes which were fixed in Davidson's fluid. Bone marrow samples, obtained from the sternum during necropsy, were used to prepare smears that were dried, and fixed. However, the smears were not examined because the peripheral blood results did not indicate such evaluation was needed.

Table 64: Reviewer's Table of Tissues Collected - Study U00-1180

Abnormal tissues	Kidneys	Skeletal muscle
Adrenal glands	Liver	Skin
Aorta - thoracic	Lungs	Spinal cord †
Brain (cerebellum, cerebrum, midbrain and medulla	Lymph nodes (submandibular and mesenteric)	Spleen
Cecum	Mammary area	Sternum
Colon	Optic nerves	Stomach
Duodenum	Ovaries	Tattoos†
Epididymides	Pancreas	Testes
Esophagus	Pituitary	Thymus
Eyes	Prostate	Thyroid with parathyroids
Gall bladder	Rectum	Tongue
Heart	Salivary gland (submaxillary, parotid)	Trachea
Ileum	Sciatic nerve†	Urinary bladder
Jejunum		Uterus
		Vagina

† Collected but not examined,

In almost all high dose animals and one control female, the areas around the injection sites were red in color.

Organ Weights

From each animal at necropsy, the following organs excised and weighed: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pancreas, parotid and submaxilliary salivary glands, pituitary, prostate, spleen, testes with epididymides, thymus, thyroids with parathyroids and uterus.

Of the differences in organ weights observed among groups, only the decrease in heart weight in the high doses groups was consistent between males (-7%) and females (-12%). However, there was no histology correlate to this finding.

Table 65: Sponsor's Organ Weight Summary – Study U00-1180

Males

Dose Group/Treatment ($mg.kg^{-1}.day^{-1}$)		Body Weight (kg)	Adrenal Glands	Brain	Heart	Kidneys	Liver	Lung	Pancreas
1	Number	3	3	3	3	3	3	3	3
(0)	Mean	2.7	0.680	97.11	13.40	14.99	79.15	17.94	5.96
	SD	0.3	0.147	11.17	3.52	2.34	13.99	2.63	1.05
2	Number	3	3	3	3	3	3	3	3
	Mean	2.7	0.661	92.04	12.89	15.28	71.99	18.77	5.59
(0.8)	SD	0.5	0.139	6.36	3.26	4.25	22.07	2.51	1.33
	Prob.								
3	Number	3	3	3	3	3	3	3	3
	Mean	2.7	0.647	80.94	12.75	14.09	73.05	18.10	6.08
(4)	SD	0.4	0.125	5.22	0.77	1.97	13.64	1.05	0.84
	Prob.								
4	Number	3	3	3	3	3	3	3	3
	Mean	2.6	0.651	87.22	12.45	13.70	71.29	18.04	5.51
(20)	SD	0.7	0.022	10.21	3.36	2.72	17.01	3.66	2.84
	Prob.								

Pituitary Gland	Prostate	Spleen	Salivary Glands	Testes	Thymus	Thyroid Glands
3	3	3	3	3	3	3
0.037	0.20	4.39	1.8397	1.35	2.64	0.268
0.006	0.12	1.92	0.7027	0.33	1.17	0.081
3	3	3	3	3	3	3
0.037	0.24	3.77	1.9260	1.28	2.57	0.268
0.016	0.09	1.46	0.1008	0.31	0.82	0.096
3	3	3	3	3	3	3
0.034	0.29	2.67	1.6390	1.06	2.30	0.270
0.019	0.04	0.91	0.4552	0.16	1.32	0.066
3	3	3	3	3	3	3
0.029	0.30	3.08	1.9247	1.17	2.07	0.277
0.002	0.02	1.05	0.2333	0.29	0.87	0.129

Females

Dose Group/Treatment ($mg.kg^{-1}.day^{-1}$)		Body Weight (kg)	Adrenal Glands	Brain	Heart	Kidneys	Liver	Lung	Ovaries
1	Number	3	3	3	3	3	3	3	3
(0)	Mean	2.9	0.674	89.67	14.92	15.93	79.90	20.93	0.154
	SD	0.6	0.136	5.73	0.88	2.56	14.30	5.77	0.017
2	Number	3	3	3	3	3	3	3	3
	Mean	3.1	0.902	88.82	15.14	17.12	81.64	19.59	0.236
(0.8)	SD	0.4	0.094	2.73	1.65	1.68	8.00	3.37	0.060
	Prob.		*						
3	Number	3	3	3	3	3	3	3	3
	Mean	2.6	0.705	87.50	12.98	13.35	65.27	18.96	0.198
(4)	SD	0.4	0.031	3.95	0.96	2.45	12.51	4.00	0.068
	Prob.								
4	Number	3	3	3	3	3	3	3	3
	Mean	2.9	0.691	81.59	13.08	16.49	78.16	17.79	0.168
(20)	SD	0.2	0.031	6.61	0.72	2.39	7.44	1.37	0.158
	Prob.								

Pancreas	Pituitary Gland	Spleen	Salivary Glands	Thymus	Thyroid Glands	Uterus
3	3	3	3	3	3	3
5.76	0.036	3.59	1.6347	2.72	0.437	0.63
2.08	0.013	0.49	0.1300	1.09	0.109	0.36
3	3	3	3	3	3	3
7.39	0.042	2.77	1.7377	2.81	0.360	1.97
1.80	0.007	0.40	0.2961	0.77	0.163	2.05
3	3	3	3	3	3	3
6.08	0.034	3.04	1.5830	2.36	0.259	1.23
1.70	0.006	0.29	0.2723	1.11	0.009	1.08
3	3	3	3	3	3	3
6.19	0.048	3.84	1.8693	3.29	0.250	1.46
0.55	0.011	0.81	0.4611	0.20	0.105	1.53

Histopathology

Adequate Battery

Tissue samples from all study animals were dehydrated, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The indicated tissues listed in Table 55 and gross abnormalities identified at macroscopic examination from all animals sacrificed at the end of the scheduled treatment period.

Peer Review

Not indicated

Histological Findings

Findings of perivascular hemorrhage and inflammation were noted at the injections sites in all animals. This was considered a non-specific reaction to intravenous injection.

Special Evaluation

Fecal samples were collected at the same times as the urine collection. The samples were assayed for occult blood.

All samples were negative for occult blood.

Toxicokinetics

Blood samples were collected from all animals before and 0.25, 1, 2, 4, and 8 hours after dosing on Days 1 and 28 of treatment. Analysis for BIBR 953 ZW used a validated HPLC-MS/MS assay with [$^{13}C_6$]-BIBR 953 ZW as an internal standard. To quantify the total of BIBR 953 ZW and its glucuronide (SUM BIBR 953 ZW), the glucuronide was cleaved by alkaline hydrolysis prior to analysis. The assay with an inaccuracy (b) (4) and imprecision of (b) (4) was valid in the concentration range of (b) (4).

Table 66 shows that no clear gender effect was observed, although females had higher exposures at the high dose on both day 1 and 28. No clear effect of repeated dosing was observed. The increase in exposure at the mid dose was dose proportional and the increase in exposure at the high dose was more than dose proportional.

Table 66: Reviewer's Summary of Exposure – Study U00-1180

			Male – AUC (ng.hr/mL)				Female AUC (ng.hr/mL)			
Dose			BIBR 953 ZW		SUM BIBR 953 ZW		BIBR 953 ZW		SUM BIBR 953 ZW	
(mg/kg)	Day	N	Mean	CV*	Mean	CV*	Mean	CV*	Mean	CV*
0.8	1	3	1660	18.5	3070	7.2	1720	15.6	3070	15.5
	28	3	1690	28.1	3240	10.5	1450	17.4	3230	4.7
4	1	3	7990	25.0	13000	27.4	7960	30.6	13900	23.7
	28	3	6450	12.9	13100	20.3	6690	26.8	14900	15.8
20	1	5	62900	13.4	83300	7.1	75800	16.7	98700	12.4
	28	5	58200	13.5	84500	7.9	67100	18.9	96100	20.7
CV* - % CV										

Formulation Analysis

Although samples of formulation were taken for analysis on Day 1 and Day 28, no results of the analysis was provided.

7 Genetic Toxicology

Study reports for genetic toxicology studies for BIBR 1048 MS, or BIBR 953 ZW were submitted and reviewed with the original submission to IND 65813. The detailed reviews of these studies are provided in the IND review dated 9/24/03. Subsequently, studies U05-1295 and U05-1803 were submitted and reviewed under IND 63, 267. Table 67 below provides a summary of the previously reviewed studies.

Table 67: Reviewer's Summary - Genetic Toxicology Studies Previously Reviewed - IND 65, 813 and IND 63, 267

	In vitro					*In vivo*	
IND review	65, 813	65, 813	65, 813	65, 813	63, 267	65, 813	63, 267
Assay	Ames	Ames	Ames	Mouse lymphoma	Mouse lymphoma	Micronucleus	Micronucleus
Drug lot/purity	BIBR 1048 MS, Lot RAL 70/purity 97.3%	BIBR1048MS/mannitol 23.4%/76.6%, Lot ZB1361	BIBR 953 ZW, Lot RAL 102/purity 94.7%	BIBR 1048 MS, Lot 8050461, purity not indicated	BIBR 1048 MS, Lot 8250250, 98.6%	BIBR 1048 MS, Lot RAL 70/purity 97.3%	BIBR 1048 MS, Lot 8250250, 98.6%
Study code	97B110	99B065	98B037	00B100	04B068	98B101	04B065
Document code	U98-2147	U99-1063	U98-2789	U01-1161	U05-1295	U99-1023	U05-1803
Conducting laboratory and location	Boehringer Ingelheim Pharma KG, B berach an der Riss, Germany	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany
Study dates	9/23/97 – 11/10/97	9/15/98 – 12/07/98	6/16/98 – 8/11/98	7/11/00 - 11/8/00	03/17/04 – 03/1/05	11/16/01 - 6/12/02	3/2004-6/2005
GLP/QA	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Vehicle	DMSO	DMSO	DMSO	DMSO	DMSO	0.5% hydroxyl-ethylcellose	0.5% hydroxyl-ethylcellose
Metabolic activation	Yes, induced rat liver S9	Yes, induced rat liver S9	Yes, induced rat liver S9	Yes, induced rat liver S9	Yes, induced rat liver S9	Not applicable – in vivo	Not applicable – in vivo
Maximum dose	1000 µg/plate	2500-5000 µg/plate	Both assay, 5000 µg/plate	200 µg/mL	-S9: 500 µg/mL +S9: 200 µg/mL	Two doses of 454 mg/kg/day	Two doses of 1000 & 2000 mg/kg
Appropriate replication	Triplicate plates – each dose in two assays	Triplicate plates -each dose in two assays	Triplicate plates - each dose in two assays	Duplicates for each dose in two assays	Duplicates for each dose in two assays	5 Males/group - Only 4 evaluable in high dos	5 Males/group - Only 4 evaluable in high dose
Toxicity or exposure	Toxicity or ppt. in both assays-S9, and one assay +S9	Toxicity or ppt in Assay 2, but not assay 1.	Toxicity or ppt in both assays +/- S9	Ppt +S9 both assays, ppt –S9 assay 1, but problem with toxicity level	Ppt: -S9 – 100, 200 RTG at 50 = 44% +S9: Toxic at 500, 32% RTG at 400	Minimal clinical signs (reduced motor activity & hunching) after 2nd high dose	One death and bleeding at 2000 mg/kg. aPTT increased 2 & 6-fold at 1000 & 2000
Appropriate controls	Without S9, yes With S9, No	Without S9, yes With S9, No	Without S9, yes With S9, No	Yes, with and without S9	Yes, with and without S9	Yes	Yes
	Only used 2-AA as positive control. Need characterization of S9 lots.						
Study Comments	Five recommended strains - Background TA100, TA102 outside historical	Five recommended strains	Five recommended strains. Background TA1537, TA100 outside historical	- S9; 4 hr & 24 hr + S9, 4 hr exposure in two assays.	conversion rate to ZW of 39% and 22% for concentrations of 100 and 500 µg/ml	% PCE< 20% Cellulose column fractionation – No validation	% PCE < 20% for all groups
Study result	**Negative**	**Negative**	**Negative**	**Negative/Equivocal**	**Negative**	**Negative**	**Negative**
Study evaluation	Without S9 acceptable With S9 unacceptable	Without S9 acceptable With S9 unacceptable	-S9 acceptable +S9 unacceptable [2-AA as contro]	Assay 1 ± S9 acceptable, Assay 2 unacceptable, appropriate toxicity not achieved. Unclear if BIBR 953 ZW formed	Acceptable Some BIBR 953 ZW was shown to be formed.	Unacceptable Dose not high enough % PCE < 20%	Acceptable; however, % PCE abnormally low
	2-AA as control, and no ppt or tox in one assay +S9. However, the two studies confirm each other						

Reports of additional genetic toxicology studies using BIBR 953 ZW or BIBR 1048 MS were submitted with NDA along with reports for assays of drug impurities. The reports reviewed in detail in Section 7.1 are listed in Table 68. The study reports for the Ames assays of drug intermediates and starting materials that are only potential impurities are reviewed in Section 7.4 (Table 92).

Table 68: Reviewer's Summary of Genetic Toxicology Studies in Section 7.1

Study Number	Compound	Assay	Result
[redacted] (b) (4)		Ames	Negative
		Ames	Negative
		Ames	Negative
		Ames	Negative
		Ames II	Negative
		Ames II	Negative
		Ames II	Negative
		Ames	Negative
U07-1747	BIBR 953 ZW	Mouse lymphoma	Negative
[redacted] (b) (4)		Mouse lymphoma	Negative
		Mouse lymphoma	Positive
U07-1813	BIBR 1048 MS spiked with (b) (4)	Chromosomal aberration	Negative
U09-1125-01	(b) (4)	Chromosomal aberration	Negative
U05-2047		Rat micronucleus	Negative
U07-1489	BIBR 1048 MS spiked with (b) (4)	Rat micronucleus	Negative
U03-1154	(b) (4)	Rat micronucleus	Negative
U02-1174		Rat micronucleus	Negative

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

Study title: Mutagenicity Study with (b) (4) in the S. typhimurium/ Mammalian-Microsome assay (Ames test)

Study no.:	97B172 (U98-2195)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	December 16, 1997
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	(b) (4) (chemical intermediate), Batch SCH 199, 92.1%

Key Study Findings

In a single valid assay, (b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or

TA102) either in the absence or presence of metabolic activation at a maximum doses limited by toxicity or precipitation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537, TA102
Concentrations:	0, 100, 500, 1000, 2500, 5000 µg/plate
Basis of concentration selection:	A dose-range finding study indicated slight toxicity at the limit dose of 5000 µg/plate
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (10 µg/plate) TA100: Sodium azide (5 µg/plate) TA1535: Na azide (5 µg/plate) TA1537: 9-Aminoacridine (50 µg/plate) TA102: Mitomycin C (0.5 µg/plate) With metabolic activation TA98, TA100, TA1535, TA1537: 2-Aminoanthracene (2 µg/plate) TA102: 2-Aminoanthracene (10 µg/plate)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Standard protocol indicated. Details not provided.

Study Validity

Criteria for a positive result included a reproducible, concentration-dependent increase in the number of revertants of at least one tester strain over the vehicle control value and/or outside the historical [background] control range is indicative of genotoxic activity. The negative and positive controls should be within the laboratory historical range.

The results (Table 69) indicated that the negative and positive controls were within the laboratory historical range. However, the reviewer notes that 2-aminoanthracene was the only positive control for all strains in the presence of metabolic activation. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens.

Results

(b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) either in the absence or presence of metabolic activation at a maximum doses limited by toxicity or precipitation. The sponsor indicated only a single assay was performed because the results were unequivocal.

Table 69: Reviewer's Compilation of Sponsor's Tables – Study U98-2195

No metabolic activation

COMPOUND (µg/plate)	MEAN REVERTANTS/PLATE S. TYPHIMURIUM				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
CONTROLS NEGATIVE DMSO	8	6	24	71	230
POSITIVE					
NaN_3 5	1021	-	-	1175	-
9-AA 50	-	396	-	-	-
2-NF 10	-	-	686	-	-
MMC 0.5	-	-	-	-	809
(b) (4)					
100	9	8	25	77	237
500	9	8	31	84	242
1000	10	7	22	68	224
2500	7	4 T	24	77	214
5000	7 T	5 T	22	58 T	151 T

With metabolic activation

COMPOUND (µg/plate)	MEAN REVERTANTS/PLATE S. TYPHIMURIUM				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
CONTROLS NEGATIVE DMSO	14	10	34	97	264
POSITIVE					
2-AA 4	117	272	1727	1711	-
2-AA 10	-	-	-	-	1128
(b) (4)					
100	9	6	34	107	256
500	13	6	33	91	269
1000	10	8	39	98	220
2500	10	9	36	98	210
5000	7	6 T	33	86	216

P: Precipitation T: Toxicity Underlined values are regarded as increased

Historical controls [The sponsor's table did not indicate which range corresponded to which strain.]

BI Pharma KG	6 - 14	2 - 10	10 - 13	15 - 52	59 - 130	159 - 252
AMES	3 - 37	4 - 31	15 - 35	15 - 60	75 - 200	200 - 360
Reviewer's guess based on other reports from the same laboratory	TA1537	TA1535	TA98		TA100	TA102

Study title: (b) (4) (Impurity of BIBR 1048 MS): Mutagenicity Study using the S. typhimurium/Mammalian Microsome assay (Ames test)

Study no.:	07B086 (U07-1828)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	June 13, 2007
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	(b) (4) (BIBR 1048 MS impurity), Batch RCC 0423A, 96.8%

Key Study Findings

In two valid assays, (b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) either in the absence or presence of metabolic activation at a maximum doses limited by precipitation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537 and TA102
Concentrations in definitive study:	0, 30, 100, 300, 1000, 3000 µg/plate (5000 µg/plate was used in the first experiment)
Basis of concentration selection:	A dose-range finding study indicated precipitation occurred at ≥300 µg/plate in the absence of metabolic activation
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (10 µg/plate) TA100: Sodium azide (5 µg/plate) TA1535: Na azide (5 µg/plate) TA1537: 9-Aminoacridine (50 µg/plate) TA102: Mitomycin C (0.5 µg/plate) With metabolic activation TA98, TA100, TA1535, TA1537: 2-Aminoanthracene (2 µg/plate) TA102: 2-Aminoanthracene (10 µg/plate)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation: Test article was mixed with cells and top agar and immediately poured onto plates. Preincubation: Test article was mixed with cells and shaken for 20 minutes prior to addition of top agar and pouring onto plates All plates were incubated at 37°C for 2 days (TA 102: 3 days) prior to counting revertants.

Study Validity

The assay was considered valid because all strains exhibited the number of spontaneous revertants per plate characteristic of the strain based the historical control ranges. In addition, the positive controls induced a significant increase in the number of revertants. The only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens. However, since a commercial preparation of S9 was used, the reviewer assumes that the preparation was appropriately characterized.

Results

(b) (4) precipitated at ≥300 µg/plate. The concentration of test article was not limited by bacteriotoxicity as indicated by a reduced background lawn and/or a decrease of absolute revertants.

(b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) compared to the

vehicle control either in the absence or presence of metabolic activation at a maximum doses limited by precipitation (Table 70). All revertant counts for test article samples were within the historical negative control range. A repeat experiment using the preincubation method confirmed the results using the plate incorporation method.

Table 70: Sponsor's Summary Tables – Study U07-1828

No metabolic activation

Experiment 1 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	8	5	41	120	371
(b) (4)					
100	7	5	44	119	344
300	11 P	6 P	47	108	328
1000	9 P	7 P	43 P	110 P	300
3000 P	7	6	40	119	389
5000 P	12	4	36	124	355
Positive Controls					
NaN_3 5	1091	-	-	1203	-
9-AA 50	-	445	-	-	-
2-NF 10	-	-	892	-	-
MMC 0.75	-	-	-	-	1274

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	9	6	32	107	356
(b) (4)					
30	10	4	35	99	331
100	12	8	29	111	319
300 P	12	7	29	97	295
1000 P	10	5	29	97	287
3000 P	10	4	27	106	314
Positive Controls					
NaN_3 5	1021	-	-	1175	-
9-AA 50	-	425	-	-	-
2-NF 10	-	-	703	-	-
MMC 0.75	-	-	-	-	1815
Historical Range	6 – 22	3 - 30	16 - 68	49 - 150	249 - 458

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

With metabolic activation

Experiment 1 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	9	50	123	408
(b) (4)					
100	12	8	56	111	380
300	10 P	10 P	60	121	387
1000	9 P	7 P	57 P	129 P	332
3000 P	11	6	46	127	428
5000 P	10	8	59	140	425
Positive Controls					
2-AA 4	208	195	1316	1563	-
2-AA 10	-	-	-	-	1383

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	5	30	114	429
(b) (4)					
30	9	7	33	82	433
100	8	7	35	95	468
300	11	4	33	101	432
1000	10 P	5 P	32 P	104 P	393
3000 P	8	11	32	108	388
Positive Controls					
2-AA 4	168	246	1552	1413	-
2-AA 10	-	-	-	-	1703
Historical Range	10 - 22	3 - 39	20 - 61	74 - 164	279 - 531

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Study title: (b) (4) (Impurity of BIBR 1048 MS): Mutagenicity Study using the S. typhimurium/Mammalian Microsome assay (Ames test)

Study no.:	07B087 (U07-1725)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	June 13, 2007
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	(b) (4) (BIBR 1048 MS impurity), Batch RCC 0409A, 99.4%

Key Study Findings

In two valid assays, (b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) either in the absence or presence of metabolic activation at a maximum doses limited by precipitation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537 and TA102
Concentrations in definitive study:	Plate method: 0, 100, 300, 1000, 3000, 5000 µg/plate Repeat TA100 plate: 0, 18.75, 37.5, 75, 150, 300 µg/plate Pre-incubation: 0, 10, 30, 100, 300, 1000 µg/plate
Basis of concentration selection:	A dose-range finding study indicated precipitation occurred at ≥300 µg/plate
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (10 µg/plate) TA100: Sodium azide (5 µg/plate) TA1535: Na azide (5 µg/plate) TA1537: 9-Aminoacridine (50 µg/plate) TA102: Mitomycin C (0.5 µg/plate) With metabolic activation TA98, TA100, TA1535, TA1537: 2-Aminoanthracene (2 µg/plate) TA102: 2-Aminoanthracene (10 µg/plate)

Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation: Test article was mixed with cells and top agar and immediately poured onto plates. Preincubation: Test article was mixed with cells and shaken for 20 minutes prior to addition of top agar and pouring onto plates. All plates were incubated at 37°C for 2 days (TA 102: 3 days) prior to counting revertants.

Study Validity

The assay was considered valid because all strains exhibited the number of spontaneous revertants per plate characteristic of the strain based the historical control ranges. In addition, the positive controls induced a significant increase in the number of revertants. The only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens. However, since a commercial preparation of S9 was used, the reviewer assumes that the preparation was appropriately characterized.

Results

(b) (4) precipitated at ≥300 µg/plate. The concentration of test article was not limited by bacteriotoxicity as indicated by a reduced background lawn and/or a decrease of absolute revertants.

(b) (4) did not induce excess reverse mutations in S. typhimurium TA1535, TA1537, TA98 and TA102 compared to the vehicle control either in the absence or presence of metabolic activation at a maximum doses limited by precipitation (Table 71). A slight increase (1.6-fold) in revertants was observed in TA100 with metabolic activation in the first plate incorporation assay. However, the results were not confirmed in a repeat assay (Assay 3) using more closely spaced concentrations. The negative results for all strains were confirmed in an experiment using the preincubation method. All revertant counts were within the historical control range, except for experiment 1 plate test for strain TA100 only.

Table 71: Sponsor's Summary Tables – Study U07-1725

No metabolic activation

Experiment 1 and 3 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium*					
	TA 1535	TA 1537	TA 98	TA 100	TA 100 Exp. 3	TA 102
Negative Control						
DMSO	8	5	41	120	120	371
(b) (4)						
18.75	-	-	-	-	116	-
37.5	-	-	-	-	118	-
75	-	-	-	-	114	-
100	13	7	48	147	-	416
150	-	-	-	-	115	-
300 P	12	7	51	161	123	427
1000 P	9	4	58	164	-	364
3000 P	5	6	54	141	-	364
5000 P	4	4	42	146	-	305
Positive Controls						
NaN_3 5	1091	-	-	1203	1271	-
9-AA 50	-	445	-	-	-	-
2-NF 10	-	-	892	-	-	-
MMC 0.75	-	-	-	-	-	1274

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	9	6	32	107	356
(b) (4)					
10	11	6	30	119	404
30	8	7	34	108	412
100	10	8	39	107	405 P
300 P	11	5	30	112	424
1000 P	9	6	33	121	396
Positive Controls					
NaN_3 5	1021	-	-	1175	-
9-AA 50	-	425	-	-	-
2-NF 10	-	-	703	-	-
MMC 0.75	-	-	-	-	1815
P: Precipitation T: Toxicity -: Not tested	Underlined values are regarded as increased				
Historical Range	6 – 22	3 - 30	16 - 68	49 - 150	249 - 458

With metabolic activation

Experiment 1 and 3 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium*					
	TA 1535	TA 1537	TA 98	TA 100	TA 100 Exp. 3	TA 102
Negative Control						
DMSO	12	9	50	123	110	408
(b) (4)						
18.75	-	-	-	-	119	-
37.5	-	-	-	-	106	-
75	-	-	-	-	109	-
100	11	7	46	197	-	446
150	-	-	-	-	117	-
300 P	12	7	43	193	107	443
1000 P	11	8	49	180	-	401
3000 P	11	7	48	158	-	318
5000 P	6	5	45	188	-	349
Positive Controls						
2-AA 4	208	195	1316	1563	449	-
2-AA 10	-	-	-	-	-	1383

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	5	30	114	429
(b) (4)					
10	12	7	38	122	448
30	14	9	32	141	485
100	11	11	31	125	440
300	11	4	28	123	485
1000 P	11	4	35	131	464
Positive Controls					
2-AA 4	168	246	1552	1413	-
2-AA 10	-	-	-	-	1703
P: Precipitation T: Toxicity -: Not tested	Underlined values are regarded as increased				
Historical Range	10 - 22	3 - 39	20 - 61	74 - 164	279 - 531

Study title: (b) (4) (Impurity of BIBR 1048 MS): Mutagenicity Study using the S. typhimurium/Mammalian Microsome assay (Ames test)

Study no.:	07B088 (U07-1699) Amendment 1
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	June 13, 2007
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	(b) (4) (BIBR 1048 MS impurity), Batch RCC 0414A, 94.2%

Key Study Findings

In two valid assays, (b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) either in the absence or presence of metabolic activation at a maximum doses limited by precipitation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537 and TA102
Concentrations in definitive study:	Plate method: 0, 100, 300, 1000, 3000, 5000 µg/plate Pre-incubation: 0, 100, 300, 1000, 3000, 5000 µg/plate
Basis of concentration selection:	A dose-range finding study indicated precipitation occurred at ≥3000 µg/plate.
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (10 µg/plate) TA100: Sodium azide (5 µg/plate) TA1535: Na azide (5 µg/plate) TA1537: 9-Aminoacridine (50 µg/plate) TA102: Mitomycin C (0.5 µg/plate) With metabolic activation TA98, TA100, TA1535, TA1537: 2-Aminoanthracene (2 µg/plate) TA102: 2-Aminoanthracene (10 µg/plate)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation: Test article was mixed with cells and top agar and immediately poured onto plates. Preincubation: Test article was mixed with cells and shaken for 20 minutes prior to addition of top agar and pouring onto plates. All plates were incubated at 37°C for 2 days (TA 102: 3 days) prior to counting revertants.

Study Validity

The assay was considered valid because all strains exhibited the number of spontaneous revertants per plate characteristic of the strain based the historical control ranges. In addition, the positive controls induced a significant increase in the number of revertants. The only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens. However, since a commercial preparation of S9 was used, the reviewer assumes that the preparation was appropriately characterized.

Results

(b) (4) precipitated at ≥300 µg/plate. The concentration of test article was not limited by bacteriotoxicity as indicated by a reduced background lawn and/or a decrease of absolute revertants.

(b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) compared to the vehicle control either in the absence or presence of metabolic activation at a maximum doses limited by precipitation (Table 72). All revertant counts for test article samples were within the historical negative control range. A repeat experiment using the preincubation method confirmed the results using the plate incorporation method.

Table 72: Sponsor's Summary of Study U07-1699

No metabolic activation

Experiment 1 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	8	5	41	120	371
(b) (4)					
100	6	7	45	120	411
300	7	6	39	110	426
1000	11	7	44	115	384
3000 P	10	10	53	113	340
5000 P	11	8	38	105	389
Positive Controls					
NaN_3 5	1091	-	-	1203	-
9-AA 50	-	445	-	-	-
2-NF 10	-	-	892	-	-
MMC 0.75	-	-	-	-	1274

With metabolic activation

Experiment 1 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
DMSO	12	9	50	123	408
(b) (4)					
100	12	7	40	113	430
300	10	7	42	134	428
1000	11	10	46	121	434
3000	10	10	54 P	125 P	481 P
5000 P	13	7	44	137	420
Positive Controls					
2-AA 4	208	195	1316	1563	-
2-AA 10	-	-	-	-	1383

No metabolic activation

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	9	6	32	107	356
(b) (4)					
100	9	3	37	113	405
300	10	5	35	100	428
1000	11	5	32	102	392 P
3000 P	9	5	36	113	354
5000 P	8	3	38	112	375
Positive Controls					
NaN_3 5	1021	-	-	1175	-
9-AA 50	-	425	-	-	-
2-NF 10	-	-	703	-	-
MMC 0.75	-	-	-	-	1815
Historical Range	6 – 22	3 - 30	16 - 68	49 - 150	249 - 458

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

With metabolic activation

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	12	5	30	114	429
(b) (4)					
100	8	5	37	128	429
300	11	6	36	112	469
1000	10	7	42	116	432
3000	7	8	28	116	497
5000 P	11	5	36	124	505
Positive Controls					
2-AA 4	168	246	1552	1413	-
2-AA 10	-	-	-	-	1703
Historical Range	10 - 22	3 - 39	20 - 61	74 - 164	279 - 531

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Study title: Mutagenicity Study with (b) (4) (impurity of BIBR 1048) using the S. typhimurium/Mammalian Microsome assay (Ames II)

Study no.:	06B186 (U06-2022)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	September 5, 2006
GLP compliance:	Not indicated
QA statement:	Not indicated
Drug, lot #, and % purity:	(b) (4) (BIBR 1048 MS impurity), Batch PR2GFK01970A1, purity not indicated

Key Study Findings

In one valid Ames II assay, (b) (4) did not induce excess reverse mutations in a mixture of S. typhimurium strains (TA7001, TA7002, TA7003 or TA7004, TA7005, TA7006) and TA98 either in the absence or presence of metabolic activation at doses up to a maximum dose of 5000 µg/mL.

Methods

Strains:	S. typhimurium TA98 and mixture of TA7001, TA7002, TA7003 or TA7004, TA7005, TA7006 (Gee et al. 1998)
Concentrations:	0, 1, 4, 20, 100, 500, 2500, 5000 µg/mL
Basis of concentration	No precipitation or toxicity was observed with

selection:	concentrations up to 5000 µg/mL (b) (4)
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (0.5 µg/mL) TA mixture: 4-Nitroquinoline-N-oxide (0.5 µg/mL) With metabolic activation 2-Aminoanthracene (1 or 5 µg/mL)
Vehicle:	DMSO
Incubation & sampling time:	The assay was performed according to the instruction manual for the Ames II (Xenometrix, Boulder/USA). Vehicle, test substance or positive control (0.01 mL) was mixed with 0.24 mL bacterial overnight culture (10^7 cells/mL) in 24-well plates for 90 min at 37°C. For metabolic activation 0.2 mL bacterial culture and 0.04 mL S9-mix (30%) were used. After 90 min incubation, the exposed cultures were diluted with pH indicator medium lacking histidine and aliquoted into 48 wells of a 384-well plate (3 replicates) using an 8-channel pipette. The plates were incubated for 48 hrs at 37°C. The wells in each 48-well section were scored for the number of positive revertant wells (yellow); the mean value of the triplicates was calculated and was compared to the number of spontaneous revertant wells of the solvent control. Cultures containing the pH indicator bromocresol purple turn from blue to yellow as the pH drops due to the accumulation of catabolites from the metabolic activity of revertant cells.

Study Validity

The experiment is valid, if the vehicle control showed the normal spontaneous revertant frequency in the historical range and the positive mutagens caused the expected increase in the mutation rate. A concentration-dependent increase of revertant wells (mean of triplicate) over the vehicle control is indicative of genotoxic activity. The individual test chemicals were classified according to the following criteria:
Negative: ≤8/48 wells Equivocal: 9-12/48 wells Positive: ≥13/48 wells
Negative historical control range: 0-7/48 wells in ca 500 experiments (1999 to date).

The current experiment is valid because the vehicle controls were within the historical control range and the positive controls showed an unequivocal positive mutagenic response.

However, the only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens.

Results

In one experiment, (b) (4) at concentrations up to a maximum dose of 5000 µg/mL did not increase the number of positive wells in the different tester strains either in presence or absence of a metabolic activation compared to the negative-vehicle control (≤8/48 wells). All revertant counts for test article samples were within the historical negative control range.

Table 73: Sponsor's Summary – Study U06-2022

Strain: **TA 98**

Compound	Conc. (µg/mL)	Nonactivation RCSP-Replicates 1	2	3	Mean	Activation (S9) RCSP-Replicates 1	2	3	Mean
Neg. control (DMSO)		2	0	2	**1**	3	2	0	**2**
(b) (4)	**1**	0	1	0	**0**	3	1	3	**2**
	4	1	1	1	**1**	2	2	3	**2**
	20	2	1	0	**1**	1	1	3	**2**
	100	2	0	3	**2**	4	2	3	**3**
	500	1	0	1	**1**	1	1	0	**1**
	2500	0	0	2	**1**	0	4	2	**2**
	5000	2	1	2	**2**	1	4	7	**4**
Pos. control (2-NF)	**0.5**	37	28		**33**				
Pos. control (2-AA)	**1**					28	17		**23**

Strain: **TA Mix**

Compound	Conc. (µg/mL)	Nonactivation RCSP-Replicates 1	2	3	Mean	Activation (S9) RCSP-Replicates 1	2	3	Mean
Neg. control (DMSO)		2	1	0	**1**	1	1	1	**1**
(b) (4)	**1**	1	3	0	**1**	1	1	2	**1**
	4	2	0	1	**1**	1	1	0	**1**
	20	0	1	2	**1**	2	0	1	**1**
	100	1	1	2	**1**	0	3	2	**2**
	500	0	0	1	**0**	0	3	0	**1**
	2500	0	1	3	**1**	0	0	0	**0**
	5000	1	1	0	**1**	0	0	1	**0**
Pos. control (4-NQO)	**0.5**	48	48		**48**				
Pos. control (2-AA)	**5**					29	27		**28**

RCSP: Revertant Colony Selection Plate (384 wells) T: Toxicity P: Precipitation
Negative: ≤8 per 48 wells, Equivocal: 9-12 per 48 wells and Positive: ≥13 per 48 wells
Underlined values are regarded positive

Study title: Mutagenicity Study with (b) (4) (Impurity of BIBR 1048) using the S. typhimurium/Mammalian Microsome assay (Ames II)

Study no.:	06B187 (U06-2087)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	September 6, 2006
GLP compliance:	Not indicated
QA statement:	Not indicated
Drug, lot #, and % purity:	(b) (4) (impurity of BIBR 1048) Batch: PR1BIR04194A2, purity not indicated

Key Study Findings

In one Ames II valid assay, (b) (4) did not induce excess reverse mutations in a mixture of S. typhimurium strains (TA7001, TA7002, TA7003 or TA7004, TA7005,TA7006, and TA98) either in the absence or presence of metabolic activation at doses up to a maximum dose of 5000 µg/mL.

Methods

Strains:	S. typhimurium TA98, and mixture of TA7001, TA7002, TA7003 or TA7004, TA7005, TA7006 (Gee et al. 1998)
Concentrations:	0, 1, 4, 20, 100, 500, 2500, 5000 µg/mL
Basis of concentration selection:	No toxicity was observed with concentrations up to 5000 µg/mL; however, (b) (4) precipitated at 5000 µg/mL with metabolic activation
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (0.5 µg/mL) TA mixture: 4-Nitroquinoline-N-oxide (0.5 µg/mL) With metabolic activation 2-Aminoanthracene (1 or 5 µg/mL)
Vehicle:	DMSO
Incubation & sampling time:	The assay was performed according to the instruction manual for the Ames II (Xenometrix, Boulder/USA). Vehicle, test substance or positive control (0.01 mL) was mixed with 0.24 mL bacterial overnight culture (10^7 cells/mL) in 24-well plates for 90 min at 37°C. For metabolic activation 0.2 mL bacterial culture and 0.04 mL S9-mix (30%) were used. After 90 min incubation, the exposed cultures were diluted with pH indicator medium lacking histidine and aliquoted into 48 wells of a 384-well plate (3 replicates) using an 8-channel pipette. The plates were incubated for 48 hrs at 37°C. The wells in each 48-well section were scored for the number of positive revertant wells (yellow); the mean value of the triplicates was calculated and was compared to the number of spontaneous revertant wells of the solvent control. Cultures containing the pH indicator bromocresol purple turn from blue to yellow as the pH drops due to the accumulation of catabolites from the metabolic activity of revertant cells.

Study Validity

The experiment is valid, if the vehicle control showed the normal spontaneous revertant frequency in the historical range and the positive mutagens caused the expected increase in the mutation rate. A concentration-dependent increase of revertant wells

(mean of triplicate) over the vehicle control is indicative of genotoxic activity. The individual test chemicals were classified according to the following criteria:
Negative: ≤8/48 wells Equivocal: 9-12/48 wells Positive: ≥13/48 wells
Negative historical control range: 0-7/48 wells in ca 500 experiments (1999-up to date).

The current experiment is valid because the vehicle controls were within the historical control range and the positive controls showed an unequivocal positive mutagenic response.

However, the only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens.

Results

In one experiment (Table 74), (b) (4) at concentrations up to a maximum dose of 5000 µg/mL did not increase the number of positive wells in the different tester strains either in presence or absence of a metabolic activation compared to the negative-vehicle control (≤8/48 wells). All revertant counts for test article samples were within the historical control range.

Table 74: Sponsor's Summary – Study U06-2087

Strain: **TA 98**

Compound	Conc. (µg/mL)	Nonactivation RCSP-Replicates				Activation (S9) RCSP-Replicates			
		1	2	3	Mean	1	2	3	Mean
Neg. control (DMSO)		0	1	1	**1**	1	1	4	**2**
(b) (4)	1	1	1	0	**1**	0	1	3	**1**
	4	3	0	1	**1**	3	2	2	**2**
	20	1	0	2	**1**	0	2	3	**2**
	100	0	0	0	**0**	1	2	2	**2**
	500	3	2	4	**3**	1	1	3	**2**
	2500	1	1	1	**1**	3	5	3	**4**
	5000	1	1	2	**1**	1 P	2 P	0 P	**1**
Pos. control (2-NF)	0.5	31	27		**29**				
Pos. control (2-AA)	1					16	17		**17**

Strain: **TA Mix**

Compound	Conc. (µg/mL)	Nonactivation RCSP-Replicates				Activation (S9) RCSP-Replicates			
		1	2	3	Mean	1	2	3	Mean
Neg. control (DMSO)		1	1	1	**1**	1	0	0	**0**
(b) (4)	1	3	1	1	**1**	0	2	1	**1**
	4	4	2	2	**3**	0	1	1	**1**
	20	3	0	2	**2**	1	2	2	**2**
	100	1	3	1	**2**	3	0	2	**2**
	500	0	2	0	**1**	0	0	0	**0**
	2500	0	2	3	**2**	1	2	0	**1**
	5000	2	1	0	**1**	0 P	1 P	1 P	**1**
Pos. control (4-NQO)	0.5	48	48		**48**				
Pos. control (2-AA)	5					20	28		**24**

RCSP: Revertant Colony Selection Plate (384 wells) T: Toxicity P: Precipitation
Negative: ≤8 per 48 wells, Equivocal: 9-12 per 48 wells and Positive: ≥13 per 48 wells
Underlined values are regarded positive

Study title: Mutagenicity Study with [redacted] (b) (4) (Impurity of BIBR 1048) using the S. typhimurium/Mammalian Microsome assay (Ames II)

Study no.:	06B188 (U06-2088)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	September 6, 2006
GLP compliance:	Not indicated
QA statement:	Not indicated
Drug, lot #, and % purity:	[redacted] (b) (4) (impurity of BIBR 1048) Batch: PR1GTL05912A2, purity not indicated

Key Study Findings

In one valid Ames II assay, [redacted] (b) (4) did not induce excess reverse mutations in a mixture of S. typhimurium strains (TA7001, TA7002, TA7003 or TA7004, TA7005, and TA7006) and TA98 either in the absence or presence of metabolic activation at doses up to a maximum dose of 5000 µg/mL.

Methods

Strains:	S. typhimurium TA98, and mixture of TA7001, TA7002, TA7003 or TA7004, TA7005, TA7006 (Gee et al. 1998)
Concentrations:	0, 1, 4, 20, 100, 500, 2500, 5000 µg/mL
Basis of concentration selection:	No toxicity was observed with concentrations up to 5000 µg/mL; however, [redacted] (b) (4) precipitated at 2500 µg/mL with metabolic activation
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (0.5 µg/mL) TA mixture: 4-Nitroquinoline-N-oxide (0.5 µg/mL) With metabolic activation 2-Aminoanthracene (1 or 5 µg/mL)
Vehicle:	DMSO
Incubation & sampling time:	The assay was performed according to the instruction manual for the Ames II (Xenometrix, Boulder/USA). Vehicle, test substance or positive control (0.01 mL) was mixed with 0.24 mL bacterial overnight culture (10^7 cells/mL) in 24-well plates for 90 min at 37°C. For metabolic activation 0.2 mL bacterial culture and 0.04 mL S9-mix (30%) were used. After 90 min incubation, the exposed cultures were diluted with pH indicator medium lacking histidine and aliquoted into 48 wells of a 384-well plate (3 replicates) using an 8-channel pipette. The plates were incubated for 48 hrs at 37°C. The wells in each 48-

well section were scored for the number of positive revertant wells (yellow); the mean value of the triplicates was calculated and was compared to the number of spontaneous revertant wells of the solvent control. Cultures containing the pH indicator bromocresol purple turn from blue to yellow as the pH drops due to the accumulation of catabolites from the metabolic activity of revertant cells.

Study Validity

The experiment is valid, if the vehicle control showed the normal spontaneous revertant frequency in the historical range and the positive mutagens caused the expected increase in the mutation rate. A concentration-dependent increase of revertant wells (mean of triplicate) over the vehicle control is indicative of genotoxic activity. The individual test chemicals were classified according to the following criteria:
Negative: ≤8/48 wells Equivocal: 9-12/48 wells Positive: ≥13/48 wells
Negative historical control range: 0-7/48 wells in ca 500 experiments (1999-up to date).

The current experiment is valid because the vehicle controls were within the historical control range and the positive controls showed an unequivocal positive mutagenic response.

However, the only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens.

Results

In one experiment (Table 75), (b) (4) at concentrations up to a maximum dose of 2500 µg/mL did not increase the number of positive wells in the different tester strains either in presence or absence of a metabolic activation compared to the negative-vehicle control (≤8/48 wells). All revertant counts for test article samples were within the historical control range.

Table 75: Sponsor's Summary – Study U06-2088

Strain: **TA 98**

Compound	Conc. (µg/mL)	Nonactivation RCSP-Replicates 1	2	3	Mean	Activation (S9) RCSP-Replicates 1	2	3	Mean
Neg. control (DMSO)		1	0	1	**1**	2	3	2	**2**
[redacted] (b) (4)	**1**	2	1	0	**1**	1	1	1	**1**
	4	1	3	0	**1**	2	6	1	**3**
	20	3	1	1	**2**	3	0	2	**2**
	100	1	1	1	**1**	1	1	1	**1**
	500	1	4	0	**2**	3	1	0	**1**
	2500	2 P	0 P	1 P	**1**	4	1	3	**3**
	5000 P	2	0	0	**1**	1	2	1	**1**
Pos. control (2-NF)	**0.5**	31	27		**29**				
Pos. control (2-AA)	**1**					16	17		**17**

Strain: **TA Mix**

Compound	Conc. (µg/mL)	Nonactivation RCSP-Replicates 1	2	3	Mean	Activation (S9) RCSP-Replicates 1	2	3	Mean
Neg. control (DMSO)		2	1	1	**1**	1	0	2	**1**
[redacted] (b) (4)	**1**	2	1	0	**1**	0	0	0	**0**
	4	1	1	0	**1**	0	1	1	**1**
	20	0	0	3	**1**	1	0	1	**1**
	100	0	0	1	**0**	1	0	1	**1**
	500	0	0	3	**1**	2	2	0	**1**
	2500	0 P	0 P	0 P	**0**	0	1	2	**1**
	5000 P	0	0	0	**0**	1	1	0	**1**
Pos. control (4-NQO)	**0.5**	48	48		**48**				
Pos. control (2-AA)	**5**					20	28		**24**

RCSP: Revertant Colony Selection Plate (384 wells) T: Toxicity P: Precipitation
Negative: ≤8 per 48 wells, Equivocal: 9-12 per 48 wells and Positive: ≥13 per 48 wells
Underlined values are regarded positive

Study title: [redacted] (b) (4) (Impurity of BIBR 1048 MS): Mutagenicity Study using the S. typhimurium/Mammalian Microsome Assay (Ames test)

Study no.:	08B124 (U08-2223-01)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	July 13, 2008
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	[redacted] (b) (4) (BIBR 1048 MS impurity), Batch 0002 (RCC0477A), purity 95.1% Batch PR1GTL05912A2, purity 99.38%

Key Study Findings

In two valid assays, purified [redacted] (b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) either in the absence or presence of metabolic activation at a maximum doses limited by precipitation.

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537 and TA102
Concentrations in definitive study:	Plate method: 0, 100, 300, 1000, 3000, 5000 µg/plate Pre-incubation: 0, 100, 300, 1000, 3000, 5000 µg/plate
Basis of concentration selection:	No toxicity of [redacted] (b) (4) occurred at 5000 µg/plate; however, precipitation occurred at ≥3000 µg/plate in the absence of metabolic activation.
Negative control:	DMSO
Positive control:	No metabolic activation: TA98: 2-Nitrofluorene (10 µg/plate) TA100: Sodium azide (5 µg/plate) TA1535: Na azide (5 µg/plate) TA1537: 9-Aminoacridine (50 µg/plate) TA102: Mitomycin C (0.5 µg/plate) With metabolic activation TA98, TA100, TA1535, TA1537: 2-Aminoanthracene (2 µg/plate) TA102: 2-Aminoanthracene (10 µg/plate)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation: Test article was mixed with cells and top agar and immediately poured onto plates. Preincubation: Test article was mixed with cells and shaken for 20 minutes prior to addition of top agar and pouring onto plates All plates were incubated at 37°C for 2 days (TA102: 3 days) prior to counting revertants.

Study Validity

The assay was considered valid because all strains exhibited the number of spontaneous revertants per plate characteristic of the strain based the historical control ranges. In addition, the positive controls induced a significant increase in the number of revertants. The only positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. No information was provided demonstrating that the activity of the S9 preparation was characterized with appropriate indirect mutagens. However, since a commercial preparation of S9 was used, the reviewer assumes that the preparation was appropriately characterized.

Results

In presence of metabolic activation, Batch 0002 of [redacted] (b) (4) induced less than 2-fold increase in the number of revertant colonies in TA 98 at 3000 and 5000 µg/plate compared to the negative control in the plate test (Table 76). Both these mean values were minimally above the historical ranges for TA98 (20-61). However, Batch 0002 did not increase the number of revertant colonies in any other strain or test conditions and all values were clearly within the historical background data range. Furthermore, a repeat experiment using the preincubation method using Batch 0002 did not confirm the results in TA98 using the plate incorporation method.

Because the purity of Batch 0002 was only 95%, the batch was purified to >99%. The purified batch of [redacted] (b) (4) (PR1GTL05912A2) did not increase the number of revertant colonies with TA 98, TA100 or the other strains in presence of metabolic activation in a plate test. All revertant counts for the purified [redacted] (b) (4) were within the historical negative control range. These results indicate an impurity was responsible for the equivocal result for [redacted] (b) (4) in the first plate test with TA98 and [redacted] (b) (4) itself is not genotoxic.

[redacted] (b) (4) did not induce excess reverse mutations in five recommended bacterial strains (S. typhimurium TA98, TA100, TA1535, TA1537 or TA102) compared to the vehicle control either in the absence or presence of metabolic activation at a maximum doses limited by precipitation.

Table 76: Sponsor's Summary - Study U08-2223

No metabolic activation

Batch 0002

Experiment 1 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	8	4	40	137	395
(b) (4)					
100	9	6	36	156	390
300	7	4	48	143	380
1000	8	4	46	141	396
3000	10	5	46	136	399
5000	6 P	4 P	36 P	140 P	391 P
Positive Controls					
NaN_3 5	867	-	-	841	-
9-AA 50	-	344	-	-	-
2-NF 10	-	-	760	-	-
MMC 0.5	-	-	-	-	1094

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	8	6	42	100	323
(b) (4)					
100	6	3	39	91	342
300	10	3	40	121	321
1000	10	4	34	110	316
3000	6 P	3 P	31 P	128 P	356
5000	7 P	7 P	33 P	136 P	353
Positive Controls					
NaN_3 5	985	-	-	915	-
9-AA 50	-	327	-	-	-
2-NF 10	-	-	657	-	-
MMC 0.5	-	-	-	-	1610
P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased					
Historical Range	5 - 22	3 - 30	16 - 68	49 - 156	249 - 458

With metabolic activation

Experiment 1 (Plate test)

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	11	7	38	150	423
(b) (4)					
100	7	7	41	148	450
300	10	6	49	147	438
1000	11	4	46	136	402
3000	10	3	71	141	426
5000	9	4	64	137	412
Positive Controls					
2-AA 4	88	63	481	856	-
2-AA 10	-	-	-	-	892

Experiment 2 (Preincubation)

µg/plate	Mean Revertants/Plate S. typhimurium TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	9	7	35	115	330
(b) (4)					
100	9	5	33	118	336
300	11	6	31	120	351
1000	8	7	37	107	314
3000	11	6	36	116	346
5000	10	7	31	110	307
Positive Controls					
2-AA 4	155	188	1221	882	-
2-AA 10	-	-	-	-	885
P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased					
Historical Range	8 - 22	3 - 39	20 - 61	74 - 164	279 - 531

Batch PR1GTL05912A2 (plate)

No metabolic activation

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	8	5	46	144	347
(b) (4)					
100	8	3	55	158	346
300	10	5	73	139	343
1000	8	7	58	148	317
3000	9 P	9 P	48 P	164 P	372
5000	12 P	10 P	42 P	178 P	370 P
Positive Controls					
NaN_3 5	751	-	-	922	-
9-AA 50	-	294	-	-	-
2-NF 10	-	-	626	-	-
MMC 0.5	-	-	-	-	1042

With metabolic activation

µg/plate	Mean Revertants/Plate *S. typhimurium* TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control DMSO	10	7	32	141	361
(b) (4)					
100	13	6	32	150	349
300	10	5	36	148	367
1000	8	8	50	136	339
3000	10	7	46	145	377
5000	9	7	53	127	344
Positive Controls					
2-AA 4	85	101	760	645	-
2-AA 10	-	-	-	-	736
P: Precipitation T: Toxicity	-: Not tested	Underlined values are regarded as increased			
Historical Range	5 - 22	3 - 39	16 - 68	49 - 164	249 - 531

7.2 *In Vitro* Assays in Mammalian Cells

Study title: BIBR 953 ZW: Mutagenicity Study using the Mouse Lymphoma (L5178Y) Assay

Study no.:	07B082 (U07-1747)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	June 13, 2007
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch RCC0432A, 98.4%

Key Study Findings

BIBR 953 ZW, the active form of BIBR 1048 MS, did not induce excess gene or chromosomal mutations at the thymidine kinase (tk) locus of mouse lymphoma L5178Y ($tk^{+/-}$) cells when tested in the presence and absence of metabolic activation at concentrations that produced precipitation.

Methods

Cell line:	Mouse lymphoma L5178Y ($tk^{+/-}$) cells
Concentrations in definitive study:	50, 100, 200 and 400 µg/mL
Basis of concentration selection:	The solubility and cytotoxicity of BIBR 953 ZW were determined in a non-GLP range-finding experiment using concentrations between 50 and 500 µg/mL. At the end of the 4 hour treatment a concentration dependent precipitation was present at concentrations ≥300 µg/mL
Negative control:	Dimethylsulfoxide (DMSO) +10% 1N HCL

Positive control:	4-nitroquinoline-*N*-oxide (4-NQ), 0.1 µg/mL Benzo(*a*)pyrene (B(*a*)P), 2 µg/mL
Formulation/Vehicle:	Dimethylsulfoxide (DMSO) +10% 1N HCL
Incubation & sampling time:	After exposure to BIBR 953 ZW for 3 hr in the presence and absence of S9, and 24 hr in the absence of S9, the expression period was for 2 days. Mutant frequency was determined by plating on microtiter plates in the presence of 3 µg/mL trifluorothymidine for at least 6 days.

Study Validity

The sponsor considered the assay valid if the vehicle control data were within historical ranges and if the positive control showed a clear increase in mutant frequency for both small and large clones as compared to the concurrent negative vehicle control. Demonstration of acceptable cell growth and maintenance throughout the experiment, the absolute plating efficiency for the solvent control should be >60% for survival and 70-130% for viability. The sponsor defined a positive response as a concentration-related and/or reproducible increase in the mutant frequency the average of which should be at least 2-fold higher than the mean control value (Mitchell et al., 1997).

The plating efficiencies of the negative vehicle control were acceptable with 96-123% for survival and 103-118% for viability. At the end of the 4 and 24-h treatment periods, the test substance precipitated at 400 µg/mL in the presence and absence of metabolic activation. The mutant frequencies of the concurrent negative controls were close to or within the recommended range of 50 to 170 X 10^{-6}, and within the provided laboratory historical range (80-297 X 10^{-6}), Table 78. The appropriate positive controls in the presence and absence of metabolic activation produced clear increases in the induced mutant frequency. The mutant frequencies of the concurrent positive control were within the provided historical laboratory range. Sizing of colonies for all samples showed a large increase in the percentage of small colony mutants only for the positive controls. The recommended range of cytotoxicity (RTG = 10-20%) was not reached for the BIBR 953 ZW cultures. However, the maximum dose was limited by the solubility of BIBR 953 ZW in the cell culture medium.

Results

Using the criteria of Moore et al (2006), the mutant frequency for all doses of BIBR 953 ZW was less than the sum of the Global Evaluation Factor (GEF, 126 X 10^{-6}) and the mutant frequency of the negative control in each assay (Table 77). Therefore, BIBR 953 ZW is classified as a negative in these assays.

Table 77: Sponsor's Summary of Study U07-1747

4 hours, no activation

Test Substance	Culture	Cytotoxicity		Mutant frequencies/10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: DMSO/HCL	1	100	100	40	114	163
	2	100	100	48	125	188
	Mean	**100**	**100**	**44**	**120**	**176**
BIBR 953 ZW (µg/mL) 50	1	121	98	34	102	145
	2	97	102	41	112	161
	Mean	**109**	**100**	**38**	**107**	**153**
100	1	109	87	46	130	192
	2	108	121	48	111	173
	Mean	**109**	**104**	**47**	**121**	**183**
200	1	112	97	39	126	175
	2	108	110	54	91	158
	Mean	**110**	**104**	**47**	**109**	**167**
400 P	1	87	80	36	148	199
	2	77	97	37	108	156
	Mean	**82**	**89**	**37**	**128**	**178**
Positive Control: 4-NQO 0.1	1	72	72	219	443	1030

4 hours, with activation

Test Substance	Culture	Cytotoxicity		Mutant frequencies/10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: DMSO/HCL	1	100	100	36	69	109
	2	100	100	29	67	99
	Mean	**100**	**100**	**33**	**68**	**104**
BIBR 953 ZW (µg/mL) 50	1	85	93	37	61	104
	2	98	104	36	69	112
	Mean	**92**	**99**	**37**	**65**	**108**
100	1	96	84	37	69	111
	2	107	99	27	84	116
	Mean	**102**	**92**	**32**	**77**	**114**
200	1	100	93	41	73	120
	2	85	100	34	54	94
	Mean	**93**	**97**	**38**	**64**	**107**
400 P	1	88	108	30	41	74
	2	107	93	26	71	100
	Mean	**98**	**101**	**28**	**56**	**87**
Positive Control: 4-NQO 0.1	1	66	70	216	503	909

24 hours, no activation

Test Substance	Culture	Cytotoxicity		Mutant frequencies/10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: DMSO/HCL	1	100	100	34	114	159
	2	100	100	40	107	157
	Mean	**100**	**100**	**37**	**111**	**158**
BIBR 953 ZW (µg/mL) 50	1	115	100	40	127	179
	2	81	91	37	85	141
	Mean	**98**	**96**	**39**	**106**	**160**
100	1	102	103	37	119	172
	2	93	98	33	116	159
	Mean	**98**	**101**	**35**	**118**	**166**
200	1	93	85	42	120	175
	2	74	85	32	102	142
	Mean	**84**	**85**	**37**	**111**	**159**
400 P	1	80	92	40	143	201
	2	74	77	45	118	178
	Mean	**77**	**85**	**43**	**131**	**190**
Positive Control: B(*a*)P 2	1	38	27	362	628	1677

Table 78: Sponsor's Summary of Historical Control Data – Study U07-1747

Negative vehicle controls

	Mutant frequency/10^6 cells		
	Non-activation 4 h	Non-activation 24 h	Activation 4 h
Range Total Clones Mean (SD)	84-297 156 (43)	80-250 143 (38)	88-261 149 (45)
Range Small Clones Mean (SD)	23-90 49 (17)	27-55 37 (8)	29-79 44 (13)
Range Large Clones Mean (SD)	50-212 99 (39)	48-191 99 (35)	49-179 98 (32)
N	27	22	28

Positive controls

	Mutant frequency/10^6 cells		
	Non-activation 4 h 4-NQO (0.1 µg/mL)	Non-activation 24 h 4-NQO (0.1 µg/mL)	Activation 4 h B(*a*)P (2 µg/mL)
Range Total Clones Mean (SD)	548-1129 793 (162)	501-1170 749 (194)	963-2365 1539 (281)
Range Small Clones Mean (SD)	160-342 248 (47)	132-290 194 (55)	303-848 511 (116)
Range Large Clones Mean (SD)	201-550 361 (99)	237-629 370 (96)	320-1201 585 (197)
N	24	20	29

Study title: BIBR 1048 (b) (4): Mutagenicity Study in the Mouse Lymphoma L5178Y tk Assay

Study no.:	01B173 (U02-1276)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	December 04, 2001
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 (b) (4) (chemical intermediate of BIBR 1048 MS-synthesis), Batch T4/01, purity not indicated

Key Study Findings

In valid assays, (b) (4) (BIBR 1048 (b) (4) (chemical intermediate of BIBR 1048 MS-synthesis), did not induce excess gene and chromosomal mutations at the tk locus of mouse lymphoma L5178Y ($tk^{+/-}$) cells when tested in the presence and absence of metabolic activation at concentrations that produced precipitation.

Methods

Cell line:	Mouse lymphoma L5178Y ($tk^{+/-}$) cells
Concentrations in definitive study:	No activation: 4 hr: 0, 500, 1000, 2500 and 4000 µg/mL 24 hr: 0, 250, 500, 1000, 2000 µg/mL Activation: 4 hr: 500, 1000, 2500, 5000 µg/mL
Basis of concentration selection:	The solubility and cytotoxicity of (b) (4) were determined in a non-GLP range-finding experiment using concentrations between 100 and 5000 µg/mL. At the end of the 4 hour treatment no precipitation was present at concentrations ≥2500 µg/mL; however, strong toxicity was observed.
Negative control:	dimethylsulfoxide (DMSO)
Positive control:	4-nitroquinoline-*N*-oxide (4-NQ), 0.1 µg/mL Benzo(*a*)pyrene (B(*a*)P), 2 µg/mL
Formulation/Vehicle:	dimethylsulfoxide (DMSO)
Incubation & sampling time:	After exposure to BIBR 953 ZW for 4 hr in the presence and absence of S9, and 24 hr in the absence of S9, the expression period was for 2 days. Mutant frequency was determined by plating on microtiter plates in the presence of 3 µg/mL trifluorothymidine for at least 6 days.

Study Validity

The sponsor considered the assay valid if the vehicle control data were within historical ranges and if the positive control showed a clear increase in mutant frequency for both small and large clones as compared to the concurrent negative vehicle control. Demonstration of acceptable cell growth and maintenance throughout the experiment, the absolute plating efficiency for the solvent control should be >60% for survival and 70-130% for viability. The sponsor defined a positive response as a concentration-related and/or reproducible increase in the mutant frequency the average of which should be at least 2-fold higher than the mean control value (Mitchell et al., 1997).

The plating efficiencies of the negative vehicle controls were acceptable with 108-135% for survival and 112-133% for viability. The mean mutant frequencies for the solvent controls were between 84 and 110 mutants/10^{-6} cells, respectively, and are within the laboratory historical control range (80 – 357/10^{-6} cells).

Appropriate concurrent positive controls (4-NQO and benzo(a)pyrene/) in the presence and absence of metabolic activation, respectively, produced clear increases in the induced mutant frequency. However, the mutant frequencies of the positive controls were just below the provided historical laboratory ranges.

Results

In the absence of metabolic activation, the recommended range of cytotoxicity (RTG = 10-20%) was not reached at the top concentration of [redacted] (4000 µg/mL, 35% RS) for the 4 hour exposure, but was exceeded at the highest concentration (2000 µg/mL, 0% RS) and approached at 1000 µg/mL (22%) for continuous treatment for 24 hours. Precipitation was observed at the two highest concentrations for the 4 hour exposure. In the presence of metabolic activation, the recommended range of cytotoxicity was not reached at the highest concentration of [redacted] (5000 µg/mL, 88% RS), although precipitation was observed at the two highest concentrations.

After 4 hours of treatment with [redacted] in the absence of activation, the total mutant frequency (102-148/10^{-} cells) was comparable with that for the vehicle control (110/10^{-6} cells). After 24 hrs exposure with [redacted] the total mutant frequency (115-140/10^{-6} cells) was comparable with that for the vehicle control (106/10^{-6} cells). Using the criteria of Moore et al (2006), the mutant frequency for the highest dose of [redacted] (140-148/10^{-6}) was less than the sum of the Global Evaluation Factor (GEF, 126/10^{-6}) and the mutant frequency of the negative control for this assay (266-274/10^{-6}). Furthermore, the mutant frequencies for the highest doses of [redacted] are within the negative control range (80-315/10^{-6}). Therefore, [redacted] is classified as a negative in the absence of metabolic activation.

After 4 hours of treatment with [redacted] in the in the presence of metabolic activation, the total mutant frequency (98-120/10^{-6} cells) was only slightly above that for the vehicle control (84/10^{-6} cells). The mutant frequency at the highest concentration was within the historical control range (88-357/10^{-6} cells). Using the criteria of Moore et al (2006), the mutant frequency for the highest dose of [redacted] (658/10^{-6}) was less than the sum of the Global Evaluation Factor (GEF, 126/10) and the mutant frequency of the negative control for this assay (210/10^{-6}). Therefore, [redacted] is classified as a negative in the presence of metabolic activation. However, precipitation was observed at both 2500 and 5000 µg/mL.

Table 79: Sponsor's Summary – Study U02-1276

	Test Article (µg/ml)	Culture	Survivor		Viability	Mutant frequencies/ 10^6 cells		
			PE%	RS%	PE%	small clones	large clones	total
4 hours, no activation	Controls Negative DMSO	1	123	100	127	40	59	105
		2	135	100	133	35	73	114
		Mean		**100**		**38**	**66**	**110**
	Positive 4-NQO 0.1	1	98	**76**	79	**271**	**201**	**572**
	BIBR 1048 [redacted]							
	500	1	133	108	135	31	58	94
		2	120	89	116	35	71	111
		Mean		**98**		**33**	**64**	**102**
	1000	1	138	112	127	32	69	107
		2	112	83	114	33	81	122
		Mean		**98**		**32**	**75**	**114**
	2500 P	1	84	68	123	40	57	103
		2	105	78	127	51	51	113
		Mean		**73**		**46**	**54**	**108**
	4000 P	1	48	39	79	57	71	135
		2	42	31	77	89	64	160
		Mean		**35**		**73**	**68**	**148**

24 hours, no activation

Test Article (µg/ml)	Culture	Survivor		Viability	Mutant frequencies/ 10^6 cells		
		PE%	RS%	PE%	small clones	large clones	total
Controls Negative DMSO	1	118	100	112	54	58	118
	2	118	100	116	41	50	94
	Mean		**100**		**48**	**54**	**106**
Positive 4-NQO 0.1	1	79	**67**	110	**175**	**251**	**532**
BIBR 1048 (b) (4) (b) (4)							
250	1	100	85	116	56	67	130
	2	120	102	114	46	51	100
	Mean		**94**		**51**	**59**	**115**
500	1	60	51	103	56	53	117
	2	62	53	85	58	56	120
	Mean		**52**		**57**	**54**	**118**
1000	1	23	19	78	70	69	147
	2	30	25	73	68	58	132
	Mean		**22**		**69**	**64**	**140**
2000 P	1	0	0	4	**not evaluable**		
	2	0	0	5			
	Mean		**0**				

4 hours, with activation

Test Article (µg/ml)	Culture	Survivor		Viability	Mutant frequencies/ 10^6 cells		
		PE%	RS%	PE%	small clones	large clones	total
Controls Negative DMSO	1	108	100	116	31	51	85
	2	114	100	133	31	49	83
	Mean		**100**		**31**	**50**	**84**
Positive B(a)P 2	1	32	**29**	73	**445**	**365**	**1114**
BIBR 1048 (b) (4) (b) (4)							
500	1	114	106	127	31	62	98
	2	106	93	125	34	69	110
	Mean		**100**		**32**	**66**	**104**
1000	1	108	100	112	26	57	86
	2	110	96	94	33	71	109
	Mean		**98**		**30**	**64**	**98**
2500 P	1	106	98	116	35	87	128
	2	108	95	116	36	71	111
	Mean		**96**		**36**	**79**	**120**
5000 P	1	108	100	127	29	65	98
	2	88	77	116	33	69	108
	Mean		**88**		**31**	**67**	**103**

Table 80: Sponsor's Summary of Historical Control Data – Study U02-1276

	Total mutant frequency/10^6 cells		
	Non-activation 4 hr	Non-activation 24 hr	Activation 4 hr
	Vehicle control		
Range	106-315	80-250	88-357
Mean (SD)	207 (74)	155 (52)	196 (78)
N	12	9	11
	Positive controls		
	4-NQO (0.1 µg/ml)	4-NQO (0.1 µg/ml)	B(a)P 2 (µg/ml)
Range	577-936	548-744	1116-1727
Mean (SD)	739 (114)	632 (98)	1496 (176)
N	8	5	11

Study title: BIBR 1048 [redacted]: Mutagenicity Study in the Mouse Lymphoma L5178Y tk Assay

Study no.:	02B042 (U02-1342)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	February 26, 2002
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 [redacted] (chemical intermediate of BIBR 1048 MS-synthesis), Batch T20003, purity not indicated

Key Study Findings

In valid assays, [redacted] (BIBR 1048 [redacted], a chemical intermediate of BIBR 1048 MS-synthesis), induced excess gene and chromosomal mutations at the tk locus of mouse lymphoma L5178Y ($tk^{+/-}$) cells when tested in the presence and absence of metabolic activation at concentrations that produced precipitation.

Methods

Cell line:	Mouse lymphoma L5178Y ($tk^{+/-}$) cells
Concentrations in definitive study:	50, 100, 200 and 400 µg/mL
Basis of concentration selection:	The solubility and cytotoxicity of [redacted] were determined in a non-GLP range-finding experiment using concentrations between 250 and 5000 µg/mL. At the end of the 4 hour treatment precipitation was present at concentrations ≥2500 µg/mL
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	4-nitroquinoline-*N*-oxide (4-NQ), 0.1 µg/mL Benzo(*a*)pyrene (B(*a*)P), 2 µg/mL
Formulation/Vehicle:	Dimethylsulfoxide (DMSO)
Incubation & sampling time:	After exposure to BIBR 953 ZW for 4 hr in the presence and absence of S9, and 24 hr in the absence of S9, the expression period was for 2 days. Mutant frequency was determined by plating on microtiter plates in the presence of 3 µg/mL trifluorothymidine for at least 6 days.

Study Validity

The sponsor considered the assay valid if the vehicle control data were within historical ranges and if the positive control showed a clear increase in mutant frequency for both small and large clones as compared to the concurrent negative vehicle control. Demonstration of acceptable cell growth and maintenance throughout the experiment, the absolute plating efficiency for the solvent control should be >60% for survival and 70-130% for viability. The sponsor defined a positive response as a concentration-related and/or reproducible increase in the mutant frequency the average of which should be at least 2-fold higher than the mean control value (Mitchell et al., 1997).

The plating efficiencies of the negative vehicle controls were acceptable with 89-138% for survival and 103-118% for viability. The mean mutant frequencies for the solvent controls were between 100 and 154 mutants/10^{-6} cells, respectively, and are within the laboratory historical control range (80 – 357/10^{-6} cells).

Appropriate concurrent positive controls (4-NQO and benzo(a)pyrene) in the presence and absence of metabolic activation, respectively, produced clear increases in the induced mutant frequency. The mutant frequencies of the positive controls were within the provided historical laboratory ranges.

Results

In the absence of metabolic activation, the recommended range of cytotoxicity (RTG = 10-20%) was exceeded at the top concentration of (b) (4) (1000 µg/mL, 3% RS) for the 4 hour exposure and not reached at the highest concentration (800 µg/mL, 34% RS) for continuous treatment for 24 hours. In the presence of metabolic activation, the recommended range of cytotoxicity was not reached at the highest concentration of (b) (4) (2500 µg/mL, 40% RS).

After 4 hours of treatment with (b) (4) in the absence of activation, the total mutant frequency (136-165/10^{-} cells) was comparable with that for the vehicle control (154/10^{-6} cells). However, after 24 hrs exposure with (b) (4) the total mutant frequency (256/10^{-6} cells) at the highest concentration of 800 µg/mL increases 2.5 fold compared with the vehicle control (100/10^{-6} cells). The number of small clones increased about 4-fold compared with number of large clones (1.6 fold) relative to the number of small and large clones for the negative control. At 600 µg/mL the small clones increased 1.5 fold compared with 1.1 fold for the large clones. Using the criteria of Moore et al (2006), the mutant frequency for the highest dose of (b) (4) (256/10^{-6}) was greater than the sum of the Global Evaluation Factor (GEF, 126/10^{-6}) and the mutant frequency of the negative control for this assay (100/10^{-6}). Furthermore, the mutant frequency for the highest dose of (b) (4) is outside the negative control range (80-250/10^{-6}) for the 24 hr incubation. Therefore, (b) (4) is classified as a positive in the absence of metabolic activation. However, precipitation was observed at both 600 and 800 µg/mL.

In the presence of metabolic activation, 4 hour exposure to (b) (4) at the highest dose produced an increase in the mutant frequency of total (5-fold), small (7-fold) and large (3.5 fold) mutant clones compared to the number of total, small and large clones for the negative control. The mutant frequency at the highest concentration was clearly outside of the historical control range (88-357/10^{-6} cells). Using the criteria of Moore et

al (2006), the mutant frequency for the highest dose of (b) (4) ($658/10^{-6}$) was greater than the sum of the Global Evaluation Factor (GEF, 126/10) and the mutant frequency of the negative control for this assay ($128/10^{-6}$). Therefore, (b) (4) is classified as a positive in the presence of metabolic activation. However, precipitation was observed at both 1000 and 2500 µg/mL.

Table 81: Sponsor's Summary – Study U02-1342

	Test Article (µg/ml)	Culture	Survivor		Viability	Mutant frequencies/ 10^6 cells		
			PE%	RS%	PE%	small clones	large clones	total
4 hours, no activation	Controls Negative DMSO	1	116	100	118	68	85	162
		2	101	100	110	65	73	145
		Mean		**100**		**66**	**79**	**154**
	Positive 4-NQO 0.1	1	83	**76**	87	**262**	**271**	**648**
	BIBR 1048 (b) (4)							
	100	1	105	91	103	74	106	195
		2	79	78	103	65	62	135
		Mean		**84**		**70**	**84**	**165**
	250	1	120	103	130	55	71	133
		2	103	102	118	55	73	138
		Mean		**102**		**55**	**72**	**136**
	500	1	98	84	141	62	67	139
		2	89	88	120	57	78	146
		Mean		**86**		**60**	**72**	**142**
	1000 P	1	6	5	45	not evaluable		
		2	1	1	4			
		Mean		**3**				

	Test Article (µg/ml)	Culture	Survivor		Viability	Mutant frequencies/ 10^6 cells		
			PE%	RS%	PE%	small clones	large clones	total
4 hours, with activation	Controls Negative DMSO	1	91	100	110	42	77	123
		2	89	100	127	41	72	134
		Mean		**100**		**42**	**74**	**128**
	Positive B(a)P 2	1	24	**27**	67	**539**	**539**	**1512**
	BIBR 1048 (b) (4)							
	250	1	95	104	123	54	94	157
		2	76	85	120	57	80	148
		Mean		**94**		**56**	**87**	**152**
	500	1	89	98	133	75	82	173
		2	84	94	116	79	85	178
		Mean		**96**		**77**	**84**	**176**
	1000 P	1	84	92	101	84	111	210
		2	77	87	123	79	86	183
		Mean		**90**		**82**	**98**	**196**
	2500 P	1	31	34	84	292	297	692
		2	40	45	91	283	226	623
		Mean		**40**		**288**	**262**	**658**

	Test Article (µg/ml)	Culture	Survivor		Viability	Mutant frequencies/ 10^6 cells		
			PE%	RS%	PE%	small clones	large clones	total
24 hours, no activation	Controls Negative DMSO	1	138	100	118	32	64	100
		2	108	100	127	31	67	101
		Mean		100		32	66	100
	Positive 4-NQO 0.1	1	80	65	89	187	285	589
	BIBR 1048 [(b) (4)] 75	1	138	100	112	41	72	118
		2	130	120	94	42	62	106
		Mean		110		42	67	112
	150	1	116	84	133	37	63	107
		2	103	95	116	25	65	92
		Mean		90		31	64	100
	300	1	98	71	108	40	70	117
		2	106	98	110	37	74	115
		Mean		84		38	72	116
	600 P	1	123	89	114	44	75	128
		2	108	100	89	54	76	138
		Mean		94		49	76	133
	800 P		40	29	92	99	99	214
			43	40	70	144	118	297
		Mean		34		122	108	256

Table 82: Sponsor's Summary of Historical Control Data – Study U02-1342

	Total mutant frequency/10^6 cells		
	Non-activation 4 hr	Non-activation 24 hr	Activation 4 hr
	Vehicle control		
Range	84-315	80-250	88-357
Mean (SD)	198 (79)	150 (51)	189 (78)
N	13	10	12
	Positive controls		
	4-NQO (0.1 µg/ml)	4-NQO (0.1 µg/ml)	B(a)P 2 (µg/ml)
Range	577-936	532-744	1114-1727
Mean (SD)	720 (121)	615 (97)	1464 (201)
N	9	6	12

Study title: BIBR 1048 MS (Dabigatran etexilate) with Impurities ([(b) (4)] and [(b) (4)]): Mutagenicity Study for Chromosomal Aberrations in Human Lymphocytes In Vitro

Study no.:	07B096 (U07-1813)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	July 3, 2007
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 1029452, purity 99.8%) spiked with [(b) (4)] (Batch RCC0409A, 99.4%) and [(b) (4)] (Batch RCC0414A, 94.2%)

Key Study Findings

In valid in vitro assays, BIBR 1048 MS spiked with the impurities (b) (4) and (b) (4) did not induce a significant, reproducible increase of structural chromosomal aberrations in human lymphocytes when tested in the presence and absence of metabolic activation. The maximum doses were limited by solubility, toxicity and/or hemolysis. The concentrations of the impurities, (b) (4) and (b) (4), were 4 and 20-fold higher compared with those proposed in the drug product specifications.

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	Nonactivation: 4 h exposure: 10, 50, 100, 250 µg/mL 24 h exposure: 5, 25, 50, 75 µg/mL Delayed harvest: 75, 100 µg/mL Activation: 4 h exposure: 100, 500, 1000 µg/mL
Basis of concentration selection:	Dose selection was based on the concentration-dependent precipitation and/or cytotoxicity (mitotic inhibition, morphological cellular changes and hemolysis) after exposure to test article
Negative control:	DMSO
Positive control:	Adriamycin (0.05 and 0.075 µg/mL) Cyclophosphamide (7 µg/mL)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Heparinized whole blood from healthy volunteers was added to chromosome medium 1A, containing the mitogen phytohaemagglutinin (PHA), and incubated for 48 hrs. Dilutions of the test article were added to duplicate cultures in the presence or absence of a metabolic activation system. In one experiment the cultures were treated for 4 hrs with and without metabolic activation. The lymphocytes were washed and resuspended in fresh growth medium. Twenty-four hours after initiation of treatment, the lymphocytes were harvested. In another experiment, lymphocytes were exposed to test article for 24 hrs without metabolic activation. For the vehicle control, the positive control and four high dose cultures a late sampling at 96 h was included to assess any effects caused by a cell cycle delay (48 h after start of treatment). Two hours prior to harvest, colcemid (0.2 µg/mL) was added to arrest dividing cells in metaphase. Cells were collected by centrifugation and resuspended in 0.4% KCl for hypotonic treatment. After fixation in ethanol/glacial acetic acid, the cells were dropped onto slides, and stained with 7% Giemsa stain.

Table 83: Sponsor's Summary of Treatment Schedule – Study U07-1813

Test	Culture Initiation	Treatment	Colcemid	Harvesting	
				Regular	Delayed
Nonactivation	0	48 - 52	70	72	-
Nonactivation	0	48 - 72	70 (94)	72	96
With Activation	0	48 - 52	70	72	-

Study Validity

Cytotoxicity and mitotic index were determined from 1000 cells per culture. The number of polyploid cells was recorded in 2000 cells. At least, 200 cells per concentration were scored for the test article.

The sponsor considered an assay acceptable if the vehicle control data were within the historical ranges and if positive controls showed significant increases in the number of cells with chromosomal aberrations. In the current experiments, all of the vehicle controls were within the laboratories historical control range and all of the positive controls produced statistically significant increases in the number of cells with chromosomal aberrations.

Results

Exposure to test article for 4 hours in the absence of metabolic activation did not result in a statistically significant increase in mean aberrant cells excluding gaps (0.5-4.0%) when tested up to 100 µg/mL, the highest concentration limited by precipitation. Although the percentage of aberrant cells at 100 µg/mL (4.0%) was slightly above the historical control range (0-2.5%) when gaps were excluded, the percentage of aberrant cells at 100 µg/mL (5.5%) was within the historical control range (0-6%) when gaps were included. The structural aberrations included no chromosomal/chromatid exchanges but primarily gaps and unspecific breaks and acentric fragments.

Continuous exposure to the test article for 24 hours in the repeat experiment did not result in a statistically significant increase in mean aberrant cells. The maximum concentration, 75 µg/mL, had a significantly decreased mitotic index indicative of toxicity. All values for the test article when gaps were included (0-4.0%) or excluded (0-2.0%) were within the historical control ranges (0-6% or 0-2.5%, respectively). These results were confirmed with delayed harvest (96 hours) after 24 hr exposure. Because continuous exposure for 24 hours failed to reproduce the slight increase after 4 hour exposure in the percentage of aberrant cells when gaps were excluded, this increase is not considered biologically relevant.

Exposure to test article for 4 hours in the presence of metabolic activation did not result in a statistically significant increase in mean aberrant cells. The maximum concentration, 1000 µg/mL, was limited by hemolysis. All values for the test article when gaps were included (1.0-4.0%) or excluded (0-3.0%) were within the historical control ranges (0-7% or 0-3%, respectively).

In the absence of metabolic activation, exposure to the test article did not there was no increase in the number of polyploid cells at the regular harvest. Following the delayed harvest in the absence of metabolic activation, a slight increase in polyploid cells (4, 7, and 5/2000 cells at 50, 75 and 100 µg/mL, respectively) was observed compared to control (2/2000 cells); however, no dose dependency was observed. In the presence of metabolic activation, a slight increase (6/2000 cells) was observed at the highest dose, 1000 µg/mL, compared to the control (0/2000 cells). Because of precipitation and hemolysis at this concentration, this finding was not considered biologically relevant.

Table 84: Reviewer's Modification of Sponsor's Tables – Study U07-1813

No activation – Treat 48-52 hr, harvest at 72 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Aberrant Cells Total Excl. Gaps	Aberrant Cells % Incl. Gaps	Aberrant Cells % Excl. Gaps
Controls:							
		A	100	6	2	6.0	2.0
Negative		B	100	5	2	5.0	2.0
DMSO	100	A+B	200	11	4	5.5	2.0
		A	100	20	15	20.0	15.0
Positive		B	100	21	14	21.0	14.0
ADR 0.075	82	A+B	200	41	29	20.5	14.5
BIBR 1048 MS + Impurities [1)]		A	100	3	0	3.0	0
		B	100	2	1	2.0	1.0
10	93	A+B	200	5	1	2.5	0.5
		A	100	1	0	1.0	0
		B	100	3	1	3.0	1.0
50	90	A+B	200	4	1	2.0	0.5
		A	100	4	2	4.0	2.0
		B	100	7	6	7.0	6.0
100	82	A+B	200	11	8	5.5	4.0
		A					
		B					
250 P,T	3	A+B					

No activation – Treat 48-72 hr, harvest at 72 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Aberrant Cells Total Excl. Gaps	Aberrant Cells % Incl. Gaps	Aberrant Cells % Excl. Gaps
Controls:							
		A	100	3	1	3.0	1.0
Negative		B	100	1	0	1.0	0
DMSO	100	A+B	200	4	1	2.0	0.5
		A	100	24	19	24.0	19.0
Positive		B	100	23	18	23.0	18.0
ADR 0.05	69	A+B	200	47	37	23.5	18.5
BIBR 1048 MS + Impurities [1)]		A	100	4	2	4.0	2.0
		B	100	3	2	3.0	2.0
5	105	A+B	200	7	4	3.5	2.0
		A	100	3	0	3.0	0
		B	100	1	0	1.0	0
25	93	A+B	200	4	0	2.0	0
		A	100	0	0	0	0
		B	100	3	1	3.0	1.0
50 T	52	A+B	200	3	1	1.5	0.5
		A	66	0	0	0	0
		B	67	1	1	1.5	1.5
75 T	24	A+B	133	1	1	0.8	0.8

With activation – Treat 48-52 hr, harvest at 72 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Total Excl. Gaps	% Incl. Gaps	% Excl. Gaps
Controls:							
Negative DMSO	100	A	100	1	1	1.0	1.0
		B	100	1	1	1.0	1.0
		A+B	200	2	2	1.0	1.0
Positive CP 7	88	A	100	26	21	26.0	21.0
		B	100	24	21	24.0	21.0
		A+B	200	50	42	25.0	21.0
BIBR 1048 MS + Impurities [1] 100	92	A	100	2	1	2.0	1.0
		B	100	3	2	3.0	2.0
		A+B	200	5	3	2.5	1.5
500	90	A	100	2	1	2.0	1.0
		B	100	1	0	1.0	0
		A+B	200	3	1	1.5	0.5
1000 H	91	A	100	3	2	3.0	2.0
		B	100	4	3	4.0	3.0
		A+B	200	7	5	3.5	2.5

No activation – Treat 48-72 hr, harvest at 96 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Total Excl. Gaps	% Incl. Gaps	% Excl. Gaps
Controls:							
Negative DMSO	100	A	100	3	1	3.0	1.0
		B	100	2	1	2.0	1.0
		A+B	200	5	2	2.5	1.0
Positive ADR 0.05	97	A	100	28	25	28.0	25.0
		B	100	37	31	37.0	31.0
		A+B	200	65	56	32.5	28.0
BIBR 1048 MS + Impurities [1] 75	104	A	100	4	1	4.0	1.0
		B	100	3	1	3.0	1.0
		A+B	200	7	2	3.5	1.0
100 T	63	A	100	2	0	2.0	0
		B	53	1	1	1.9	1.9
		A+B	153	3	1	2.0	0.7

[1]: Test substance: 80% BIBR 1048 MS (b) (4)
T: Toxicity P: Precipitation ne: not evaluable nd: not done
H: Hemolysis

Negative Controls

	Aberrant Cells (%)			
	Without Metabolic Activation		With Activation	
	incl. Gaps	excl. Gaps	incl. Gaps	excl. Gaps
Studies (n)	77	77	48	48
Mean	2.47	0.86	2.61	0.70
Range	0-6	0-2.5	0-7	0-3

Positive Controls

	Aberrant Cells (%)			
	Without Metabolic Activation		With Activation	
	Adriamycin (0.05 µg/mL)		Cyclophosphamide (7 µg/mL)	
	incl. Gaps	excl. Gaps	incl. Gaps	excl. Gaps
Studies (n)	57	57	27	27
Mean	25.2	21.2	31.5	26.4
Range	11-56	4-54	12-55	9-46

Study title: (b) (4) (Impurity of BIBR 1048 MS): Mutagenicity Study for Chromosomal Aberrations in Human Lymphocytes In Vitro, Amendment 1

Study no.: 08B125 (U09-1125-01-AM1)
Study report location: EDR
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation: July 11, 2008
GLP compliance: Indicated
QA statement: Indicated
Drug, lot #, and % purity: (b) (4) (BIBR 1048 MS impurity), Batch no.: 0002 (RCC0477A), 98.9%

Key Study Findings

In valid in vitro assays, (b) (4) a BIBR 1048 MS impurity, did not induce a significant, reproducible increase of structural chromosomal aberrations in human lymphocytes when tested in the presence and absence of metabolic activation. The maximum doses were limited by solubility or toxicity.

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	Nonactivation: 4 h exposure: 100, 300, 1000, 3000 µg/mL 24 h exposure: 10, 30, 100, 300 µg/mL Delayed harvest: 300 µg/mL Activation: 4 h exposure: 300, 1000, 3000 µg/mL
Basis of concentration selection:	Dose selection was based on the concentration-dependent cytotoxicity after exposure to test article
Negative control:	DMSO
Positive control:	Adriamycin (0.05 and 0.075 µg/mL) Cyclophosphamide (7 µg/mL)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Heparinized whole blood from healthy volunteers was added chromosome medium 1A, containing the mitogen phytohaemagglutinin (PHA) and incubated for 48 hrs. Dilutions of the test article were added to duplicate cultures in the presence or absence of a metabolic activation system. In one experiment the cultures were treated for 4 hrs with and without metabolic activation. The lymphocytes were washed and resuspended in fresh growth medium. Twenty-four hours after initiation of treatment, the lymphocytes were harvested. In another experiment, lymphocytes were exposed to test article for 24 hrs without metabolic activation. For the vehicle control, the positive control and four high dose cultures a late sampling at 96 h was included to assess any effects caused by a cell cycle delay (48 h after start of treatment). Two hours prior to harvest, colcemid (0.2 µg/mL) was added to arrest dividing cells in metaphase. Cells were collected by centrifugation and resuspended in 0.4% KCl for hypotonic treatment. After fixation in ethanol/glacial acetic acid, the cells were dropped onto slides, and stained with 7% Giemsa stain.

Table 85: Sponsor's Summary of Treatment Schedule – Study U09-1125

Test	Culture Initiation	Treatment	Colcemid	Harvesting Regular	Harvesting Delayed
Nonactivation	0	48 - 52	70	72	-
	0	48 - 72	70 (94)	72	96
With activation	0	48 - 52	70	72	-

Study Validity

Cytotoxicity and mitotic index were determined from 1000 cells per culture. The number of polyploid cells was recorded in 2000 cells. At least, 200 cells per concentration were scored for the test article.

The sponsor considered an assay acceptable if the vehicle control data were within the historical ranges and if positive controls showed significant increases in the number of cells with chromosomal aberrations. In the current experiments, all of the vehicle controls were within the laboratories historical control range and all of the positive controls produced statistically significant increases in the number of cells with chromosomal aberrations.

Results

Exposure to test article for 4 hours in the absence of metabolic activation did not result in a statistically significant increase in mean aberrant cells excluding gaps (1.5%) when tested up to 1000 µg/mL, the highest concentration limited by toxicity. All values for the test article when gaps were included (2-5%) or excluded (1-2%) were within the historical control ranges (0-6% or 0-2.5%, respectively).

Continuous exposure to the test article for 24 hours in the repeat experiment did not result in a statistically significant increase in mean aberrant cells. The maximum concentration, 300 µg/mL, had a significantly decreased mitotic index indicative of toxicity. All values for the test article when gaps were included (0-2%) or excluded (0-2%) were within the historical control ranges (0-6% or 0-2.5%, respectively). These results were confirmed with delayed harvest (96 hours) after 24 hr exposure. .

Exposure to test article for 4 hours in the presence of metabolic activation did not result in a statistically significant increase in mean aberrant cells. The maximum concentration, 3000 µg/mL, was limited by precipitation. All values for the test article when gaps were included (1-5%) or excluded (0-2%) were within the historical control ranges (0-7% or 0-3%, respectively).

In the absence of metabolic activation, exposure to the test article did not there was no dose-dependent increase in the number of polyploid cells. In the presence of metabolic activation, a slight increase (4/2000 cells) was observed at the highest dose, 1000 µg/mL, compared to the control (2/2000 cells). Because of precipitation at this concentration, this finding was not considered biologically relevant.

Table 86: Reviewer's Compilation from Sponsor's Tables – Study U09-1125

No activation – Treat 48-52 hr, harvest at 72 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Total Excl. Gaps	% Incl. Gaps	% Excl. Gaps
Controls:							
		A	100	2	1	2.0	1.0
Negative		B	100	5	4	5.0	4.0
DMSO	100	A+B	200	7	5	3.5	2.5
		A	100	23	20	23.0	20.0
Positive		B	100	29	26	29.0	26.0
ADR 0.075	88	A+B	200	52	46	26.0	23.0
(b) (4)		A	100	2	1	2.0	1.0
		B	100	5	2	5.0	2.0
100	93	A+B	200	7	3	3.5	1.5
		A	100	2	1	2.0	1.0
		B	100	2	2	2.0	2.0
300	81	A+B	200	4	3	2.0	1.5
		A	100	3	1	3.0	1.0
		B	100	4	2	4.0	2.0
1000	76	A+B	200	7	3	3.5	1.5
		A					
		B					
3000 PT	2	A+B					

No activation – Treat 48-72 hr, harvest at 72 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Total Excl. Gaps	% Incl. Gaps	% Excl. Gaps
Controls:							
		A	100	3	1	3.0	1.0
Negative		B	100	2	1	2.0	1.0
DMSO	100	A+B	200	5	2	2.5	1.0
		A	97	45	43	46.4	44.3
Positive		B	81	36	34	44.4	42.0
ADR 0.05	36	A+B	178	81	77	45.5	43.3
(b) (4)		A	100	2	0	2.0	0
		B	100	1	0	1.0	0
10 T	73	A+B	200	3	0	1.5	0
		A	100	2	1	2.0	1.0
		B	100	2	0	2.0	0
30	91	A+B	200	4	1	2.0	0.5
		A	100	2	1	2.0	1.0
		B	100	1	0	1.0	0
100 T	71	A+B	200	3	1	1.5	0.5
		A	100	2	2	2.0	2.0
		B	44	0	0	0	0
300 T	42	A+B	144	2	2	1.4	1.4

With activation – Treat 48-52 hr, harvest at 72 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Total Excl. Gaps	% Incl. Gaps	% Excl. Gaps
Controls:							
		A	100	2	0	2.0	0
Negative		B	100	2	1	2.0	1.0
DMSO	100	A+B	200	4	1	2.0	0.5
		A	100	30	28	30.0	28.0
Positive		B	100	26	23	26.0	23.0
CP 7	123	A+B	200	56	51	28.0	25.5
(b) (4)		A	100	2	0	2.0	0
		B	100	5	2	5.0	2.0
300	140	A+B	200	7	2	3.5	1.0
		A	100	2	1	2.0	1.0
		B	100	3	2	3.0	2.0
1000	120	A+B	200	5	3	2.5	1.5
		A	100	1	1	1.0	1.0
		B	100	1	1	1.0	1.0
3000 P	101	A+B	200	2	2	1.0	1.0

No activation – Treat 48-72 hr, harvest at 96 hr

Substance (µg/mL)	MI %	Culture No.	Cells scored	Aberrant Cells Total Incl. Gaps	Total Excl. Gaps	% Incl. Gaps	% Excl. Gaps
Controls:							
		A	100	2	1	2.0	1.0
Negative		B	100	1	0	1.0	0
DMSO	100	A+B	200	3	1	1.5	0.5
		A	100	57	55	57.0	55.0
Positive		B	91	39	39	42.8	42.8
ADR 0.05	36	A+B	191	96	94	50.3	49.2
(b) (4)		A	100	2	2	2.0	2.0
		B	100	0	0	0	0
300	80	A+B	200	2	2	1.0	1.0

Negative Controls

	Aberrant Cells (%)			
	Nonactivation		With Activation	
	incl. Gaps	excl. Gaps	incl. Gaps	excl. Gaps
Studies (n)	92	92	52	52
Mean	2.63	0.97	2.55	0.73
Range	0-6	0-3.0	0-7	0-3.0

Positive Controls

	Aberrant Cells (%)			
	Nonactivation		With Activation	
	Adriamycin (0.05 µg/mL)		Cyclophosphamide (7 µg/mL)	
	incl. Gaps	excl. Gaps	incl. Gaps	excl. Gaps
Studies (n)	67	67	28	28
Mean	26.5	22.5	32.1	27.1
Range	11-56	4-54	12-55	9-46

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

Study title: BIBR 1048 MS: Mutagenicity Study using Micronucleus Analysis in Rat Bone Marrow after Oral Treatment (Retest). Amendment 1

Study no.:	04B285 A1 (U05-2047)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	January 18, 2005
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, 99.2%

Key Study Findings

In one valid assay, BIBR 1048 MS did not increase excess micronucleus formation in rats after treatment with a single dose of 2000 mg/kg.

Methods

Doses:	0, 500, 1000, and 2000 mg/kg (BIBR 1048 BS)
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Rat, Han Wistar(Crl:WI(Han))
Number/Sex/Group:	5 males/group
Satellite groups:	One group for positive control
Basis of dose selection:	2000 mg/kg is the limit dose
Negative control:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Positive control:	Cyclophosphamide (20 mg/kg)

Study Validity

BIBR 1048 MS was administered orally, similar to the intended clinical administration. Formulation analysis showed the concentrations of each suspension were greater than 96% of nominal. Although no evidence of exposure was provided by observation of clinical signs, the bone marrow from high dose group harvested at 48 hours after dosing showed an increase in erythropoiesis based on an increase in % PCE compared to the bone marrow from the negative control and BIBR 1048 MS treated groups harvested at 24 hours after dosing. No deaths occurred and no significant clinical signs were observed. Toxicokinetics were not monitored in this study. Since BIBR 1048 BS was tested at 2000 mg/kg, the limit dose according to the ICH S2(R1) Guidance (2008), the sponsor has tested BIBR 1048 MS at acceptable doses.

More than the expected 2000 PCE were scored blindly per animal. At the 24 hour timepoint, the proportion of immature erythrocytes among total erythrocytes was similar

between BIBR1048 MS treated animals (means: 27.4-29.3%) and the vehicle control animals (28.5%). These values are greater than the minimum 20% of control expected and are within the laboratory historical PCE values. Therefore, BIBR1048 MS at the doses used did not induce any bone marrow toxicity over the time of the assay. However, the high dose group harvested at 48 hours after dosing had a mean PCE value (individual range) of 32.3% (25-45.5%). An increased PCE value indicative of increased hematopoiesis is consistent previous micronucleus studies in the Chbb: THOM rats (Table 88)

The positive control, cyclophosphamide, is a known clastogen (EPA OPPTS 870.5375). The percent MNPCE values for the positive control animals were significantly increased over the values for the negative control animals.

Results

No dose of BIBR 1048 MS produced significant decreases in the percentage of PCE, indicative of bone marrow toxicity. The percentages of MN-PCE in the bone marrow of BIBR 1048 MS-treated rats were within the range of that in both the concurrent and historical vehicle control rats (Table 87). In one valid assay, BIBR 1048 MS did not increase excess micronucleus formation in rats after treatment with the limit dose of 2000 mg/kg.

Table 87: Sponsor's Summary - Micronucleus Study U05-2047

Test Article (mg/kg)		Sampl. Time (h)	n	PCE (%) *P-value (%)*	MNE (%) *P-value (%)*
Negative Control 0.5% Hydroxyethylcellulose		24	5	**28.5**	**0.16**
BIBR 1048 MS					
Base	Salt				
500	576.5	24	5	**29.3**/*88.0*	**0.07**/*6.3*
1000	1153	24	5	**27.8**/*85.7*	**0.10**/*18.3*
2000	2306	24	5	**27.4**/*74.7*	**0.10**/*21.5*
		48	5	**32.3**/*43.7*	**0.15**/*100.0*
Positive Control Cyclophosphamide 20		24	2	**17.8***/*4.8*	**0.8***/*4.8*

Negative control values		Historical control values Rat Crl:WI(Han) Male	
		PCE (%)	MNE (%)
	Studies (n)	**28**	
	Mean	**33.2**	**0.15**
	Range	18.3-43.5	0.07-0.20

Table 88: Sponsor's Comparison of BIBR 1048 MS Micronuclei Studies

Study No./ Report No.	Rat Strain	BIBR 1048 free base (mg/kg)	Animal Nos.	PCE %	MNE %
98B101 U99-1023 Experiment 1	Chbb:THOM	Neg. Contr.	5	18.0	0.12
		Pos. Contr. CP 20	5	11.3*	1.52*
		2 x 50	5	22.6	0.10
		2 x 200	5	28.6*	0.14
		2 x 600	4	29.5*	0.14
04B065 U05-1803 Experiment 2	Chbb:THOM	Neg. Contr.	5	10.4	0.12
		Pos. Contr. CP 20	1[2)]	7.0	1.10
		2 x 1000	5	12.0	0.17
		1 x 2000 (48 h)	4[1)]	19.5*	0.19
Historical Range (%)				*18.0-41.1*	*0.10-0.36*
04B285 Experiment 3	Crl:WI(Han)	Neg. Contr.	5	28.5	0.16
		Pos. Contr. CP 20	2	17.8*	0.80*
		1 x 500	5	29.3	0.07
		1 x 1000	5	27.8	0.10
		1 x 2000	5	27.4	0.10
		1 x 2000 (48 h)	5	32.3	0.15
Historical Range (%)				*18.3-43.5*	*0.07-0.20*

PCE: Polychromatic erythrocytes MNE: Micronucleated erythrocytes
[1)]: One intercurrent death [2)]: No statistical analysis
*: Significantly different from the negative vehicle control (P≤ 5%)

Study title: BIBR 1048 MS (Dabigatran Etexilate) and Impurities: Mutagenicity Study using Micronucleus Analysis of Rat Bone Marrow - Part of the 13-week Oral (Gavage) Toxicity Study U07-1693

Study no.:	06B209 (U07-1489)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	July 6, 2006
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch number: 1024879SPK, purity 96.7%. This batch of BIBR 1048 MS was spiked with the following impurities (target concentrations/concentrations in certificate): (b) (4)

Key Study Findings

After repeated dosing for 13-weeks, a BIBR 1048 MS formulation spiked with impurities did not induce an increase of micronucleated polychromatic erythrocytes in male rats compared with the concurrent negative control and the historical control range for this strain.

Methods

Doses in definitive study:	0, 55, 200 mg/kg/day
Frequency of dosing:	Daily for 91 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Rat (Crl:WI(Han)
Number/Sex/Group:	5 males from each main study group
Satellite groups:	Toxicokinetics was monitored in the main study using 5 animals/sex/group.
Basis of dose selection:	Dose levels were selected based on the results of prior 4-week (U98-2729) and 26-week (U03-1310) toxicity studies with BIBR 1048 MS in which the high dose (200 mg/kg) produced clear toxicity.
Negative control:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Positive control:	No separate positive control was used because the laboratory has conducted 44 previous studies.

Study Validity

Based on deaths at 300 mg/kg in another 13-week study (U05-1378) the high dose of 200 mg/kg was chosen for this study, which was conducted as part of the 13-week oral (gavage) toxicity study, U07-1693. The high dose males showed a 14% increase in aPTT and greater than 2-fold increase in thrombin time. Treatment-related histopathological changes found only in the thymus included recurrent hemorrhages and hemosiderosis in the thymus of high dose animals, but not in the thymus of control animals. Thus, the dose used in this 13-week study produced some toxicity in the high dose group.

Results

The BIBR 1048 formulation spiked with impurities did not induce an increase of micronucleated polychromatic erythrocytes in rats (0.04-0.09%) compared with the concurrent negative control (0.09%). All mean and individual values were in the historical control range for this strain (0.07-0.20%). The mean (20.9-23.3%) and individual percentage of polychromatic erythrocytes were within the concurrent control range (12-33%) and the historical control range for this rat strain (18.3-43.5%).

Table 89: Sponsor's Summary – Study U07-1489

Test substance (mg/kg)		Sampl. time (h)	N	PCE (%)	*p-value*	MNE (%)	*p-value*
Negative vehicle control: 0.5% hydroxyethylcellulose		24-30	5	**24.0**		**0.09**	
BIBR 1048 formulation							
free base	salt						
55	63.42	24-30	5	**23.3**	*0.9129*	**0.04**	*0.1907*
200	230.6	24-30	5	**20.9**	*0.4994*	**0.09**	*1.0000*

Historical controls		Male rats, Crl:WI(Han)	
		PCE (%)	MNE (%)
	Studies (n)	44	
	Mean	32.7	0.14
	Range	18.3-43.5	0.07-0.20

Study title: BIBR 1048 (b) (4): Mutagenicity Study using the Rat Bone Marrow Micronucleus Assay (Intraperitoneal)

Study no.:	02B192 (U03-1154)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	November 7, 2002
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 (b) (4) (chemical intermediate of BIBR 1048 MS-synthesis), Batch T20003, 97.4%

Key Study Findings

After two intraperitoneal (ip) doses, (b) (4) did not induce an increase of micronucleated polychromatic erythrocytes in male rats compared with the concurrent negative control and the historical control range for this strain.

Methods

Doses in definitive study:	0, 50, 150, 500 mg/kg/day
Frequency of dosing:	Daily for 2 days
Route of administration:	Intraperitoneal (ip)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% Cremophor EL
Species/Strain:	Rat (Crl:WI(Han)
Number/Sex/Group:	Main: 5 males/group; Positive control: 2 males

Satellite groups:	None
Basis of dose selection:	An acute oral toxicity study with (b) (4) in rats did not show of toxicity at a dose of 5000 mg/kg. Since data on oral absorption is lacking, the animals were treated ip to achieve a maximal systemic exposure. Assuming that an ip dose corresponds to about 10 fold of an oral dose a maximum dose of 500 mg/kg, the high dose exceeds the limit dose of 2000 mg/kg.
Negative control:	0.5% Cremophor EL
Positive control:	Cyclophosphamide, 15 mg/kg

Study Validity

Although no animals died, a dose-related increase occurred in the number of animals with decreased motor activity and with abdominal spasms. All the high dose males showed piloerection. Thus, the dose used in this study was adequate since some toxicity was observed.

The results for all negative control animals were within the historical control range. The positive control induced a significant increase in the percentage of micronucleated polychromatic erythrocytes. Therefore, the study is considered valid.

Results

(b) (4) did not induce an increase of micronucleated polychromatic erythrocytes in rats (0.12%) compared with the concurrent negative control (0.17%). All mean and individual values were in the historical control range for this strain (0.09-0.17%). The mean (28.5-32.0%) and individual percentage of polychromatic erythrocytes were within the concurrent control range (28.5-31.5%) and the historical control range for this rat strain (18.3-43.5%).

Table 90: Sponsor's summary – Study U03-1154

TEST ARTICLE (mg/kg)	SAMPL. TIME (hrs)	N	PE (%) *p-Value (%)*	MNE (%) *p-Value (%)*
Negative control: 0.5% Cremophor	24	5	**29.7**	**0.17**
BIBR 1048 (b) (4) (b) (4)				
50	24	5	**28.5**/*84.30*	**0.12**/*40.35*
150	24	5	**32.0**/*7.26*	**0.12**/*43.51*
500	24	5	**31.6**/*84.94*	**0.12**/*43.83*
Positive control: Cyclophosphamide 15	24	2	**13.3***/*4.65*	**2.85***/*4.65*

* Significantly different from the negative vehicle control ($p \leq 5\%$)

Historical controls

	Historical values: Rat, CrlGlxBrlHan: WI	
	PE (%)	MNE (%)
Studies (n)	13	
Mean	30.3	0.15
Range	18.3-42.5	0.09-0.19

Study title: BIBR 1048 (b) (4): Mutagenicity Study in the Rat Bone Marrow Micronucleus Assay following Intraperitoneal Exposure

Study no.:	01B175 (U02-1174)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	December 6, 2001
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 (b) (4) (chemical intermediate of BIBR 1048 MS-synthesis), Batch T4/01, purity not indicated

Key Study Findings

After two ip doses, (b) (4) did not induce an increase of micronucleated polychromatic erythrocytes in male rats compared with the concurrent negative control and the historical control range for this strain.

Methods

Doses in definitive study:	0, 50, 150, 500 mg/kg/day
Frequency of dosing:	Daily for 2 days
Route of administration:	IP
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% Cremophor EL
Species/Strain:	Rat (Crl:WI(Han)
Number/Sex/Group:	Main: 5 males/group; Positive control: 2 males
Satellite groups:	None
Basis of dose selection:	An acute oral toxicity study with (b) (4) in rats did not show of toxicity at a dose of 5000 mg/kg. Since data on oral absorption is lacking, the animals were treated ip to achieve a maximal systemic exposure. Assuming that an ip dose corresponds to about 10 fold of an oral dose a maximum dose of 500 mg/kg, the high dose exceeds the limit dose of 2000 mg/kg.
Negative control:	0.5% Cremophor EL
Positive control:	Cyclophosphamide, 15 mg/kg

Study Validity

No animals died. No animals treated with (b) (4) exhibited clinical signs of toxicity.

The results for all negative control animals were within the historical control range. The positive control induced a significant increase in the percentage of micronucleated polychromatic erythrocytes. Therefore, the study is considered valid.

Results

(b) (4) did not induce an increase of micronucleated polychromatic erythrocytes in rats (0.08-0.14%) compared with the concurrent negative control (0.18%). All mean and individual values were in the historical control range for this strain (0.09-0.17%). The mean (28.8-43.9%) and individual percentage of polychromatic erythrocytes were within the concurrent control range (15.0-35.0%) and the historical control range for this rat strain (18.3-43.5%).

Table 91: Sponsor's Summary – Study U02-1174

TEST ARTICLE (mg/kg)	SAMPL. TIME (hrs)	MALE N	MALE PE (%) *p-Value (%)*	MALE MNE (%) *p-Value (%)*
CONTROLS: NEGATIVE				
0.5% Cremophor EL®	24	5	**27.9**	**0.18**
POSITIVE				
Cyclophosphamide 2 x 20	24	5	**16.1*/4.1**	**3.98*/0.8**
BIBR 1048 (b) (4) (b) (4)				
50	24	5	**43.9*/1.6**	**0.14/38.0**
150	24	5	**31.7/50.2**	**0.13/37.4**
500	24	5	**28.8/87.1**	**0.08*/4.1**

* Significantly different from the vehicle control ($p \leq 5\%$)

		Historical values: Rat, CrlGlxBrlHan: WI Male	
		PE (%)	MNE (%)
Historical controls	Studies (n)	7	
	Mean	31.6	0.15
	Range	22.2-42.5	0.09-0.18

7.4 Other Genetic Toxicity Studies

The sponsor submitted the results of Ames assays for fourteen additional compounds that are either potential impurities in the starting materials or chemical intermediates. These results are summarized in the following table (Table 92).

Table 92: Reviewer's Summary of Ames Assays for Potential Impurities

Study #	Document	Compound (Batch)	GLP/ QA	Doses (µg/plate)	Neg. Con.	Pos. Con	Result	Comment
97B143	U98-2314	BIBR 1048/ (b) (4) (SCH191)	Yes	Plate: 100-5000	DMSO	1	TA 1537, TA 98 Positive	Toxicity TA100, TA102
07B089	U07-1722	(b) (4)	Yes	Plate: 30-3000 Pre-: 12.5-200	DMSO	1	Negative	No ppt, but toxicity all strain
07B090	U07-1731	(b) (4)	Yes	Plate: 30-3000 Pre-: 12.5-200	DMSO	1	Negative	Toxicity all strain
08B200	U09-1486-01	(b) (4)	Yes	Plate: 100-5000	DMSO	1	Negative	Toxicity all strain
97B146	U98-2554	BIBR 1048/ (b) (4) (SCH 186)	Yes	Plate: 100-5000	DMSO	1	Negative	Toxicity TA102
97B147	U98-2562	BIBR 1048/ (b) (4) (SCH 195)	Yes	Plate: 100-5000	DMSO	1	TA 98 positive + S9	TA 1538 negative confirmed
97B148	U98-2561	BIBR 1048/ (b) (4) (SCH 198)	Yes	Plate: 10-2500	DMSO	1	Negative	Ppt at 2500
97B081	U98-2152	BIBR 1048/ (b) (4) (SCH 170)	Yes	Plate: 100-7500	DMSO	1	TA98, TA1538 Positive	Toxicity all strains, TA98 most sensitive
97B083	U98-2150	BIBR 1048/ (b) (4) (SCH 172)	Yes	Plate: 50-2500	DMSO	1, No TA102	Negative	Toxicity all strains Ppt at 2500
97B082	U98-2151	BIBR 1048/ (b) (4) (SCH 173)	Yes	Plate: 5-500	DMSO	1, No TA102	Negative	Toxicity all strains Confirmed
07B091	U07-1732	(b) (4) (S31459)	Yes	Plate: 30-3000 Pre-: 12.5-200	DMSO	1	TA100, TA98 Positive +S9	Toxicity all strains TA100 + confirmed
04B063	U04-1334	BIBR1048 (b) (4) (hps-103-3)	No	Ames II: 4-5000	DMSO	2	Negative	Not confirmed
08B201	U09-1136-01	(b) (4)	Yes	Plate: 100-5000	DMSO	1	Negative	Toxicity all strain

1: Ames I - No activation: TA98, 2-Nitrofluorene; TA100 and TA1535, Sodium azide; TA102, Mitomycin C; TA1537, 9-Aminoacridine. With Activation: All strains, 2-Aminoanthracene
2: Ames 2 - TA98, TA98, 2-Nitrofluorene; TA mix (TA 7001, TA 7002, TA 7003, TA 7004, TA 7005 and TA 7006), 4-Nitroquinoline oxide
Neg. Con. = Negative control; Pos. Con. = Positive control; Red text = compounds with positive results

8 Carcinogenicity

Study title: Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice for 104 weeks

Study no.:	BOI287/042668 (U07-2181)
Study report location:	9/17/09 submission in EDR
Conducting laboratory and location:	(b) (4) Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	May 12, 2004 (dosing initiated May 26, 2004)
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, purity 99.2 %, 97.8% and 98.5% on March 2005, 2006 and 2007, respectively
CAC concurrence:	Upon review of the study protocol, the Executive CAC recommended doses of 0, 30, 100, and 200 mg/kg/d. The minutes from the meeting on March 16, 2004 are in Appendix 3. The Executive CAC reviewed the study results on February 16, 2010. The meeting minutes are in Appendix 4.

Key Study Findings

CD-1 mice received oral doses of BIBR 1048 MS for up to 104 weeks in males and up to 102 weeks in females. At dosages of 30, 100 or 200 mg/kg/day the mean $AUC_{(0\text{-}24h)}$ was 1160, 3830, and 5830 ng.hr/mL in males and 1290, 5400, and 9520 ng.hr/mL in females, respectively. Many of the non-neoplastic macroscopic and microscopic findings were related to the pharmacodynamic action of BIBR 1048 MS. However, the incidence of focal hepatocellular necrosis increased in both males and females receiving 200 mg/kg/d. In addition, the incidence and severity of glandular dilatation of the uterus increased in females given 200 mg/kg/day, and the incidence of luminal dilatation of the uterus increased in females given 100 or 200 mg/kg/day.

Adequacy of Carcinogenicity Study

The mouse carcinogenicity study used the doses (0, 30, 100 and 200 mg/kg/d) that were recommended by the Exec CAC. The study length was acceptable since the male and female mice were treated for up to 104 and 102 weeks, respectively. Analysis of the mortality indicated that there was no statistically significant difference in mortality between control and treated groups for either sex. However, a slightly higher mortality in

females at 100 mg/kg/d resulted in termination of all female groups during week 102. One factor identified as contributing to the death of female animals treated with BIBR 1048 MS at 100 or 200 mg/kg/day was the presence of large, hemorrhagic ovarian cysts, which are consistent with the pharmacodynamic effect of BIBR 1048 MS.

Appropriateness of Test Models

The Crl: CD-1™ (ICR) BR strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data has been established in the conducting laboratory. The most predominant form of BIBR 1048 MS in both mouse and human plasma was BIBR 953 ZW. However, the further metabolism of BIBR 953 ZW in mice was slightly different, since mice form taurine conjugates whereas humans form acylglucuronides.

Evaluation of Tumor Findings

The increased incidence of fibrosarcoma observed in all male, but not female, treated groups was attributed to trauma. The higher incidence of bronchioalveolar adenocarcinoma and Harderian gland adenocarcinoma observed in the high dose males did not achieve statistical significance and was within the laboratory historical control range. A higher incidence of pleomorphic lymphoma observed in mid dose males was within the laboratory historical control range. The incidence of mammary gland adenocarcinoma increased in all female treated groups compared to controls, but was not statistically significant and was within the laboratory historical control range. The reviewer concurs with the sponsor that no significant evidence of neoplasia related to BIBR 1048 MS treatment was observed in CD-1 mice.

Executive CAC Recommendations and Conclusions:

The Committee concluded that the mouse bioassay was adequate and noted that the sponsor used the doses recommended by the prior Exec CAC protocol agreement. The Committee found that the mouse carcinogenicity study was negative for any drug related statistically significant neoplasms.

Methods

Doses:	0, 30, 100 and 200 mg/kg/day (expressed as the free base)
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Natrosol 250 HX (hydroxyethylcellulose) solution in water
Basis of dose selection:	MTD determined by mortality at 300 mg/kg due to hemorrhage, an expected consequence of the pharmacodynamic action of the drug
Species/Strain:	Mouse (*Mus musculus*)/ Crl: CD-1™ (ICR) BR, (b) (4)

Number/Sex/Group:	54 animals/sex/group for the control, low and mid-dose groups; 63 animals/sex for the high dose group
Age:	At the start of treatment, the mice were 47 to 51 days of age.
Animal housing:	During Weeks 1 to 71 all animals were housed three of one sex per cage unless this number was reduced by mortality or isolation. Because of fighting among the males resulting in deaths or premature sacrifices, male animals were housed individually from Week 72.
Paradigm for dietary restriction:	Not applicable, since the animals had free access to the standard rodent diet.
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetic satellite groups consisted of 24 animals/sex for each of the BIBR 1048 MS treated groups and 8 animals/sex for the control groups.
Deviation from study protocol:	Of the deviations listed in the study report, the following deviations deserve mention. 1. Because of fighting among the males resulting in deaths or premature sacrifices, male animals were housed individually from Week 72 onwards. 2. During Week 81, the formulation for the high dose animals was spilled during preparation. As a consequence, the high dose animals were dosed later than usual, because additional formulation was prepared by stirring the mixture for a minimum of 3 hours. An associated study showed that 3 hours of mixing for this dose level was adequate to achieve homogeneity and the nominal formulation concentration.

Observations and Results

Mortality

The animals were examined visually at least twice daily for mortality, morbidity and reaction to treatment.

Although the analysis of mortality summarized in Table 93 below indicated no statistically significant difference between control and treated groups for either sex ($p>0.05$), the Kaplan-Meier graphs in Figure 28 indicate a decreased survival in the male groups, particularly of the control males, prior to week 70. Some of the deaths in the male groups were associated with fighting prior to week 71. More deaths due to fighting were observed in the control group (8) than in the treated groups (2, 3 and 4, respectively). Consequently, those males with wounds in the treated groups survived

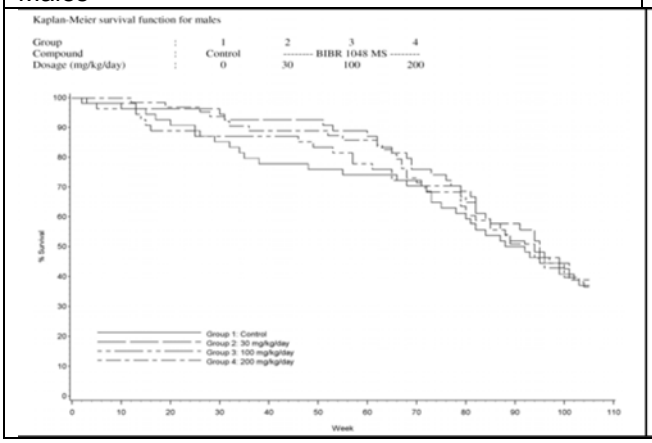
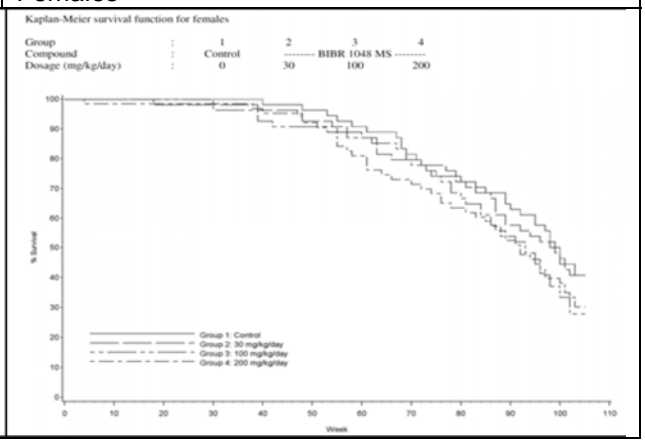
longer than the control males with wounds. To reduce these deaths the males were housed individually beginning in week 72.

Table 93: Sponsor's Summary of Mortality – Study U07-2181

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	**0**	**30**	**100**	**200**	**0**	**30**	**100**	**200**
Group size	54	54	54	63	54	54	54	63
Human kill	7	11	7	12	10	8	14	11
Killed *in extremis*	10	12	10	15	13	14	15	20
Found dead	17	10	17	13	7	10	10	11
Total no. of deaths	34	33	34	40	30	32	39	42
% mortality	63	61	63	63	56	59	72	67
No of fighting deaths @	8	2	3	4				
No. of deaths - non-fighting	26	31	31	36				

Up to and including Week 71

Figure 28: Sponsor's Kaplan-Meier Graphs - Study U07-2181

Males	Females
	

The slightly higher mortality in the mid-dose females resulted in termination of all female groups during week 102. The study pathologist identified large, hemorrhagic ovarian cysts as a factor contributing to the death of the mid- and high dose females during treatment. This factor is consistent with an exaggerated pharmacodynamic effect of BIBR 1048 MS. Table 94 indicates that uterine endometrial polyps also contributed to the deaths of the high dose females.

Table 94: From Sponsor's Table - Factors Contributing to Death - Study U07-2181

	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	34	33	34	41	32	32	39	44
FAC CON TO DEATH	NUMBER EXAMINED:	34	33	34	41	32	32	39	44
--UNDETERMINED		13	5	12	13	8	7	7	13
--MALIGNANT LYMPHOMA		3	6	6	2	5	8	5	9
--SKIN LESIONS		6	8	5	5	3	2	6	1
--UTERINE ENDOMETRIAL POLYP		0	0	0	0	1	1	1	4
--OVARIAN CYST		0	0	0	0	0	0	3	6

Clinical Signs

In addition to a more detailed weekly physical examination, which included palpation of masses, detailed observations were made immediately before dosing, immediately after dosing on return of the animal to its cage, on completion of dosing of each group. These observations were made 1-2 hours after completion of dosing of all groups, and at the end of the work day weekly during Weeks 1 to 13, every two weeks during Weeks 14 to 27, and during weeks 28, 30, 32, 36, 40, 45, 48, 52, 56, 60, 64, 71, 72, 75, 76, 79, 80, 84, 88, 92, 96, 100 and 104.

Table 95 indicates a treatment-related trend in the incidence of penis mutilation in males; however, the incidences of perigenital wet/dry abrasions and encrustations were higher in the control group than in the treated male groups.

Table 95: Sponsor's Summary of Perigenital Findings - Study U07-2181

Sign	1M	2M	3M	4M
Penis mutilation	14	13	3	2
Wet abrasion, perigenital	3	0	0	0
Dry abrasion, perigenital	4	0	0	0
Encrustations, perigenital	10	7	2	1
Number of deaths attributed to fighting (up to Week 71)	8	2	3	4

Body Weights

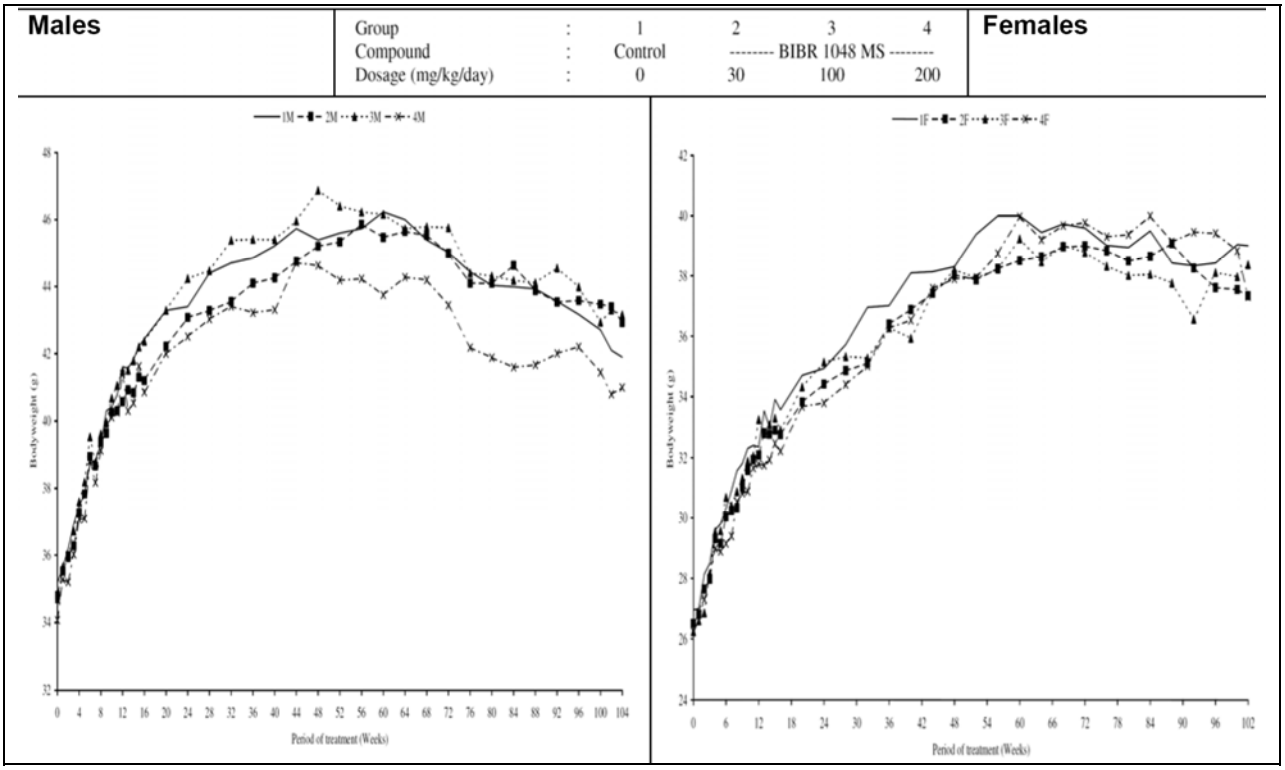
The animals were weighed on Day 1 of treatment, weekly during weeks 1 through 16 weeks of treatment, then every four weeks to study termination and before necropsy.

No treatment-related effect was observed on bodyweight gain as indicated in Table 96. However, the group mean of the high dose males decreased between weeks 72 and 88 as indicated in Figure 29.

Table 96: Sponsor's Summary of Body Weight Gain - Study U07-2181

SEX:		MALE				FEMALE			
GROUP:		1	2	3	4	1	2	3	4
WEEK									
Gain 0-16	N	51	52	48	62	54	54	54	62
	MEAN	7.3	6.4	7.6	6.9	6.6	6.2	6.7	6.0
	S.D.	3.50	2.78	2.58	2.73	3.06	2.87	2.82	2.68
As % of Control		-	88	104	95	-	94	102	91
Gain 0-102	N					23	22	15	21
	MEAN					11.5	11.1	12.4	12.0
	S.D.					5.77	4.46	2.99	7.31
As % of Control						-	97	108	104
Gain 0-104	N	20	21	20	23	-	-	-	-
	MEAN	6.5	8.0	8.3	6.8				
	S.D.	4.89	3.00	3.33	3.70				
As % of Control		-	123	128	105				

Figure 29: Sponsor's Body Weight Graphs - Study U07-2181



Food Consumption

The mean weekly food consumption per animal (g/mouse/week) was determined weekly for the first 16 weeks, and once every four weeks thereafter.

No treatment-related effect of BIBR 1048 MS was observed on food consumption or food conversion efficiency.

Hematology

Blood samples were obtained prior to dosing from all surviving females during Week 102 and surviving males during Week 104 via the retro-orbital sinus. The non-fasted animals were held under light isoflurane anesthesia during blood collection. A blood smear was prepared for all animals. The following parameters were measured in samples from 20 surviving animals from each sex: hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, platelet count, total white cell count, and differential white cell count (neutrophils, lymphocytes, eosinophils, basophils, monocytes, and large unstained cells). Samples from the remaining animals were examined for erythrocyte count, total white cell count, and differential white cell count.

The study report concluded that no treatment related effect of BIBR 1048 MS on hematology parameters was observed. As noted in Table 97, the high dose female group had a non-statistically significant decrease in the group mean for neutrophils, eosinophils, basophils, and red blood cells, but an increase in the percentage of

reticulocytes compared to the control group. The effect on red blood cells and reticulocytes could be a consequence of the exaggerated pharmacodynamic effect of BIBR 1048 MS. The increased group mean for neutrophils in the low dose females results from inclusion of female 309, who had a very high neutrophil value of 63.

Table 97: From Sponsor's Tables - Hematology Parameters - Study U07-2181

Group (Dose, mg/kg)		Males					Females				
		N $x10^9$/L	E $x10^9$/L	B $x10^9$/L	RBC $x10^{12}$/L	Retic %	N $x10^9$/L	E $x10^9$/L	B $x10^9$/L	RBC $x10^{12}$/L	Retic %
1 (0)	Mean	1.89	0.25	0.03	9.06	3.35	1.79	0.18	0.07	8.39	3.73
	SD	0.911	0.203	0.040	1.100	0.660	1.647	0.194	0.092	0.864	1.351
	n	19	19	19	19	19	20	20	20	20	20
2 (30)	Mean	2.28	0.21	0.04	8.98	3.62	4.48	0.19	0.11	8.31	5.35
	SD	0.722	0.084	0.022	0.792	1.125	13.802	0.307	0.274	1.546	4.304
	n	19	19	19	19	19	20	20	20	20	20
3 (100)	Mean	2.02	0.17	0.03	8.43	4.29	1.42	0.16	0.03	8.03	3.76
	SD	1.226	0.105	0.015	0.805	3.852	1.473	0.145	0.011	0.734	1.268
	n	20	20	20	20	20	15	15	15	15	15
4 (200)	Mean	2.07	0.35	0.03	8.91	3.27	1.33	0.12	0.04	7.89	4.71
	SD	1.249	0.458	0.018	0.726	0.601	1.081	0.099	0.059	1.607	3.557
	n	20	20	20	20	20	20	20	20	20	20

Comment: Neutrophil (N) for Group 2 F#309 = 63 and the neutrophil mean without F#309 = 1.40 (0.99)

Organ Weights

From each animal necropsied after 102/104 weeks of treatment, the following organs excised and weighed: brain, epididymides, heart, kidneys, liver, lungs (with mainstem bronchi), ovaries with oviducts, pituitary (after partial fixation), prostate, seminal vesicles, spleen, testes, thymus, and uterus with cervix.

The study report concluded that no changes in organ weight were treatment related. However, the reviewer noted effects in the kidneys and ovaries. Table 98 indicates that the male, but not the female, treated groups showed an 8-12.5% decrease in group mean absolute kidney weight and 10-12% decrease in mean relative kidney weight. Examination of the values for individual animals indicates this finding can be explained by the high kidney weight for control male #52, who not only had multiple large cysts, but also a metastatic kidney tubular carcinoma.

The reviewer also noted a 2-2.9-fold increase in ovary/oviduct absolute weight and relative weight in the treated female groups. Examination of the values for individual animals indicates that 3, 2, and 2 females treated with 30, 100, 200 mg/kg/d, respectively, had ovarian absolute weights that were up to 6-fold higher than the maximum absolute weight in the control group. Although females in all groups had ovarian cysts, these particular females had larger cysts as indicated in Table 98.

Table 98: Reviewer's Modification of Selected Organ Weights - Study U07-2181

	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	NUMBER:	20	21	20	22	22	22	15	19
Absolute kidney weight		KIDNEYS							
	N :	19	21	20	22	22	22	15	19
	MEAN :	0.88	0.81	0.79	0.77 a	0.48	0.48	0.48	0.46
	sd :	0.24	0.12	0.11	0.10	0.08	0.07	0.07	0.07
Relative kidney weight									
	N :	19	21	20	22	22	22	15	19
	MEAN :	2.130	1.912	1.881	1.911	1.299	1.355	1.299	1.276
	sd :	0.571	0.236	0.268	0.192	0.189	0.170	0.189	0.167

	SEX:	FEMALE			
	GROUP:	1	2	3	4
	NUMBER:	22	22	15	19
Absolute ovary* weight	N :	21	20	13	17
	MEAN :	0.3232	0.9192	0.9323	0.6874
	sd :	0.2305	1.5001	1.2569	0.8892
Relative ovary* weight	N :	21	20	13	17
	MEAN :	0.8803	2.6172	2.5410	1.8697
	sd :	0.6670	4.4065	3.3384	2.4502

Group (Dose)	Female number	Ovary Wt.	Cyst diameter, mm
1 (0 mg/kg)	240	0.87	8, 12
	273	0.81	6, 12
2 (30 mg/kg)	285	5.0	15, 24
	303	5.1	30
	314	2.4	13
3 (100 mg/kg)	350	4.7	19, 23
	380	2.2	19, 10
4 (200 mg/kg)	400	2.7	20, 11
	447	3.0	6, 20

* Ovary and oviducts

Gross Pathology

The whole (or a sample of) tissues listed below in Table 99 from all animals was preserved in 10% neutral buffered formalin, except for the following tissues. Testes and epididymides were fixed in Bouin's solution. The urinary bladder was initially inflated with Bouin's solution prior to transfer to 70% industrial methylated spirit. The eyes were fixed in Davidson's fluid.

Table 99: Reviewer's List of Tissues Collected - Study U07-2181

Abnormal tissues	Jejunum	Sciatic nerve+
Adrenals	Kidneys	Seminal vesicles
Aorta - thoracic	Larynx	Skeletal muscle - thigh+
Brain	Liver	Skin
Cecum	Lungs with mainstem bronchi	Spinal cord
Clitoral gland	Lymph nodes (mandibular –	Spleen
Colon	mesenteric)	Sternum
Duodenum	Mammary area - caudal	Stomach
Epididymides	Optic nerves	Testes
Esophagus	Ovaries with oviduct	Thymus
Eyes	Pancreas	Thyroid with parathyroids
Femurs+	Pituitary	Tongue
Gall bladder	Preputial gland	Trachea
Harderian glands	Prostate	Ureters
Head#	Rectum	Urinary bladder
Heart	Salivary glands+ (submandibular,	Uterus and cervix
Ileum including Peyers patches	sublingual and parotid)	Vagina

+ Only one processed for examination; # Not processed for examination

Findings of abnormal contents were observed in a number of tissues, particularly the gastrointestinal tract and the urinary bladder. As indicated in Table 9, a higher number of high dose males and females had abnormal contents of the GI tract with the males attaining statistical significance. Most of the treated animals with this finding had

abnormal contents described as dark, whereas the control animals had abnormal contents described as gas. Most of the animals with this finding died or were euthanized during treatment. In the urinary bladder, the number of males with abnormal contents described as dark, dark fluid or red fluid increased with dose of BIBR 1048 MS compared with the control male group. The incidence was statistically significant in the mid- and high dose male groups. The abnormal contents in the one female, a control animal, with this finding were described as pale, creamy material. Most of the animals with the finding of dark contents in the urinary bladder died or were euthanized during treatment. The findings of dark contents or dark areas (e.g. liver) in various organs are consistent with an exaggerated pharmacodynamic activity of BIBR 1048 MS. Likewise, the modest degree of anemia and reticulocytosis in surviving animals noted in Table 97 is consistent with bleeding.

The incidence of pale skin increased in all treated male groups and in the mid- and high dose female groups compared with the respective control group. Statistical significance was attained in the mid-dose males and the high dose females. All of the animals with this finding died or were euthanized during treatment.

The study report noted the findings of cysts on the kidneys and of thymus masses in females, but considered these findings to be of no toxicological significance. Although the incidences by sex of cysts on the kidneys were similar across all groups of animals that were killed after the treatment period, the incidence in treated males killed during the treatment period was higher than the incidence in control males. In contrast, the incidences of thymus masses in the female treated groups killed during treatment were similar to the incidence in the control female group, whereas the incidences of thymus masses in the female treated groups killed after treatment were higher than the incidence in the control female group. The study report did not note the finding of enlarged hearts which occurred only in the treated groups and in animals killed during the treatment period. This finding is not correlated with excess cardiomyopathy (see Table 102), it is consistent with bleeding-induced anemia, decreased vascular resistance, and an expected increase in cardiac output.

Table 100: Reviewer's Compilation - Gross Pathology Findings - Study U07-2181

Group	SEX: / GROUP:	-------MALE------- -1-	-2-	-3-	-4-	------FEMALE------ -1-	-2-	-3-	-4-
	ORGAN AND KEYWORD(S) OR PHRASE NUMBER:	34	33	34	41	32	32	39	44
All	HEART ... NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	ENLARGED	0	3	1	3	0	1	1	0
During	HEART ... NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	ENLARGED	0	3	1	3	0	1	1	0
All	KIDNEYS ... NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	PALE	3	2	4	5	2	7	5	5
	CYST(S)	11	17	14	19	3	3	3	5
During	KIDNEYS ... NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	PALE	2	2	3	4	2	5	5	3
	CYST(S)	2	7	5	8	2	2	2	3
After	KIDNEYS ... NUMBER EXAMINED:	20	21	20	22	22	22	15	19
	PALE	1	0	1	1	0	2	0	2
	CYST(S)	9	10	9	11	1	1	1	2
All	LIVER ... NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	PALE AREA(S)	2	2	5	5	5	4	1	3
	DARK	0	0	2	1	0	0	0	0
	PALE	1	0	0	2	1	3	5	8 a

Group	ORGAN AND KEYWORD(S) OR PHRASE	NUMBER:	SEX: -------MALE------- GROUP: -1-	-2-	-3-	-4-	------FEMALE------ -1-	-2-	-3-	-4-
During	LIVER	NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	PALE AREA(S)		2	1	5	5	4	3	1	3
	DARK		0	0	2	1	0	0	0	0
	PALE		1	0	0	2	1	3	5	7
All	THYMUS	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	MASS(ES)		2	3	3	0	7	13	8	14
During	THYMUS	NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	MASS(ES)		2	3	1	0	6	9	3	10
After	THYMUS	NUMBER EXAMINED:	20	21	20	22	22	22	15	19
	MASS(ES)		0	0	2	0	1	4	5 a	4
All	URINARY BLADDER	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	ABNORMAL CONTENTS		1	4	7 a	10 b	1	0	0	0
During	URINARY BLADDER	NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	ABNORMAL CONTENTS		1	4	7 a	9 a	1	0	0	0
All	MISCELLANEOUS	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	ABNORMAL CONTENTS G.I TRACT		2	5	7	11 a	5	5	4	10
During	MISCELLANEOUS	NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	ABNORMAL CONTENTS G.I TRACT		2	5	7	10 a	5	5	3	10
All	SKIN/SUBCUTIS	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
	PALE		3	7	10 a	8	7	7	13	18 a
During	SKIN/SUBCUTIS	NUMBER EXAMINED:	34	33	34	41	32	32	39	44
	PALE		3	7	10 a	8	7	7	13	18

Significant when compared with Group 1: a - p<0.05; b - p<0.01

Histopathology

Tissue samples from all main study animals were dehydrated, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Samples of the head [including nasal cavity, paranasal sinuses, nasopharynx and zymbals gland with external ear (ear canal)], and the remaining femur, salivary gland, sciatic nerve and skeletal muscle (thigh) were not examined histologically, but were retained for any future request for microscopic examination. All tissues listed in Table 99 and gross abnormalities identified at macroscopic examination from all animals sacrificed at the end of the scheduled treatment period and from all animals killed or dying during the study were examined by histology.

Peer Review:

A sponsor's pathologist conducted the peer review

Neoplastic findings:

The study pathologist and statistician concluded that no neoplastic findings in the mice were considered to be related to treatment with BIBR 1048 MS, although some neoplastic findings were considered to be incidental. The sponsor's complete summary of neoplasms is located in Appendix 5. These findings included malignant skin fibrosarcomas and sarcomas, bronchioloalveolar adenomas and carcinomas, malignant lymphomas, mammary gland carcinomas, and Harderian gland adenocarcinomas. As summarized in Table 101, only the skin fibrosarcomas, malignant pleomorphic lymphoma, and benign bronchioalveolar adenoma reached statistical significance. The reviewer also noted the presence of kidney tubular adenomas only in treated males, but the presence of kidney tubular carcinoma only in a control male.

Skin fibrosarcomas were present at an incidence of 5.6 to 7.4% in the treated male groups, but not the control group. In contrast, skin fibrosarcomas were present in the control female group, but not the treated female groups. The malignant fibrosarcomas in males were generally present in the sacral posterior region and appeared subsequent to evidence of trauma (scabs, swelling, encrustation or abrasion). These fibrosarcomas were described as being atypical with large pleomorphic tumor cells along with extensive inflammatory cell infiltration, necrosis and skin ulceration. Nine of the eleven males with this tumor died during the treatment period. The sponsor argued that these skin tumors are the result of trauma, not treatment with BIBR 1048 MS and cited literature (Son, 2003; Son and Gopinath, 2004) identifying a relationship between traumatic damage and the development of fibrosarcomas. This argument is consistent with the higher mortality in the control males prior to week 71 and longer survival of the treated males. Historical data (Appendix 6) from nine previous studies in the conducting laboratory indicate a mean incidence for fibrosarcoma of 1.5% with a range of 0 – 3.9% for all males and a mean incidence of 2.2% with a range of 0 – 9.1% for males that died during treatment. The incidence of fibrosarcoma alone was not significantly increased in any of the treated male groups ($p = 0.085$ to 0.130 pairwise test; $p = 0.17$ trend test). However, when the sponsor combined the benign fibroma, malignant fibrosarcoma and malignant sarcoma, the pairwise comparison was statistically significant ($p=0.035$) in the high dose group compared to control group and the trend test was statistically significant ($p = 0.045$) in the males when all groups were included in the analysis. Since the draft FDA Guidance (FDA 2001) indicates lower p values (0.005 and 0.01) are needed to classify a common tumor (>1%) as a positive finding and fibrosarcoma is a common tumor, the attained p values of the sponsor's combination of fibroma, fibrosarcoma and sarcoma do not reach the thresholds to classify the increased incidence of this tumor combination as a positive finding.

Two other neoplastic findings, malignant pleomorphic lymphoma in the mid-dose males ($p = 0.032$) and benign bronchioalveolar adenoma in the mid-dose females ($p = 0.045$), were statistically significant in pairwise comparisons with the respective control group. However, the trend test was not statistically significant either for malignant pleomorphic lymphoma in males ($p = 0.36$) or for bronchioalveolar adenoma in females ($p = 0.349$). Again, since both of these tumors are common tumors, the attained statistical significance did not reach the required threshold to classify these findings as a positive finding.

Although not statistically significant by a pairwise test, higher incidences of bronchioalveolar adenocarcinoma ($p = 0.146$) and Harderian gland adenocarcinoma ($p = 0.321$) were observed in the high dose males. However, the incidence of each tumor was within the laboratory historical control range for that tumor (bronchioalveolar adenocarcinoma: 2.0 - 17.2%; Harderian gland adenocarcinoma: 0 - 4.3%). The incidences of the combination of bronchioalveolar adenocarcinoma + adenoma or the combination of Harderian gland adenocarcinoma + adenoma were not significantly different for the treated groups compared to the control group for both males and females.

The higher incidence of pleomorphic lymphoma observed in mid dose males was statistically significant by the pairwise test ($p = 0.032$) compared to the control group,

but not statistically significant by the trend test (p = 0.36). The incidences of pleomorphic lymphoma in the male groups were within the laboratory historical control range (0 - 7.1%). The incidences of the combination of all malignant lymphomas were similar across all groups for both males and females. The incidences of the combination of all malignant lymphoma in the males were within the laboratory historical range (1.9 – 20.4%).

The incidence of mammary gland adenocarcinoma increased in all treated female groups, but did not statistically significantly exceed that of the control group. All incidences of mammary gland adenocarcinoma are within the laboratory historical control range (1.4 – 14.8%).

Kidney tubular adenoma was present in the low (p = 0.30) and high (p = 0.20) dose male groups, but statistical significance was not attained either in the pairwise test or the trend test (p = 0.125). However, kidney tubular carcinoma was present only in the control group and the incidence of tubular carcinoma combined with tubular adenoma in the treated groups was not statistically significantly different from that in the control group (trend test p = 0.275).

Table 101: Reviewer's Table - Neoplastic Findings - Study U07-2181

Reviewer's table of major neoplastic findings in the mouse carcinogenicity study – All animals		BIBR 1048 MS Dose level (mg/kg/day)							
		0	30	100	200	0	30	100	200
Organ/Tissue Finding		M	M	M	M	F	F	F	F
Harderian glands	#	54	54	54	63	54	54	54	63
Adenoma – B	#	6	4	4	6	3	2	0	2
(Cr†: 0-18.6%, 0-8.3%)	%	11.1	7.4	7.4	9.5	5.6	3.7	0	3.2
Adenocarcinoma – M	#	0	1	0	2	0	0	1	0
(Cr†: 0-8.3%, 0-2.4%)	%	0	1.8	0	3.7	0	0	1.8	0
Adenoma + Adenocarcinoma	#	6	5	4	8	3	2	1	2
	%	11.1	9.2	7.4	12.6	5.6	3.7	1.8	3.2
Kidneys	#	54	54	54	63	54	54	54	63
Tubular adenoma - B	#	0	2	0	3	0	0	0	0
(Cr†: 0-4.0%,0-2.0%)	%	0	3.7	0	4.8	0	0	0	0
Tubular carcinoma - M	#	1	0	0	0	0	0	0	0
(Cr†: 0-2.0%, 0-2.0%)	%	1.8	0	0	0	0	0	0	0
Tubular carcinoma + adenoma	#	1	2	0	3	0	0	0	0
	%	1.8	3.7	0	4.8	0	0	0	0
Lungs and bronchi	#	54	54	54	63	54	54	54	63
Bronchioalveolar adenoma - B	#	8	9	6	10	8	13	**14**	11
(Cr†: 0-42.0%, 0-26.7%)	%	14.8	16.6	11.1	15.8	14.8	24.0	**25.9***	17.5
Bronchioalveolar adenocarcinoma - M	#	2	3	3	7	4	1	2	3
(Cr†: 0-26.0%, 0-18.4%)	%	3.7	5.6	5.6	11.1	7.4	1.8	3.7	4.8
Bronchioalveolar adenocarcinoma + adenoma	#	10	12	9	17	12	14	16	14
	%	18.5	22.2	16.6	27.0	22.2	25.9	29.6	22.2
Mammary gland	#	54	54	54	63	54	54	54	63
Mammary adenocarcinoma - M	#	0	0	0	0	2	5	4	5
(Cr†: F: 0-8.3%)	%	0	0	0	0	3.7	9.2	7.4	7.9
Mammary carcinosarcoma - M	#	0	0	0	0	0	1	0	1
	%	0	0	0	0	0	1.8	0	1.6
Mammary adenocarcinoma + carcinosarcoma	#	0	0	0	0	2	6	4	6
	%	0	0	0	0	3.7	11.1	7.4	9.5
Hematopoietic tumor	#	54	54	54	63	54	54	54	63
Lymphocytic/Lymphoblastic Lymphoma - M	#	3	2	2	3	7	8	7	8
(Cr†: 0-4.1%, 0-27.5%)	%	5.6	3.7	3.7	5.6	13.0	14.8	13.0	12.6
Pleomorphic Lymphoma - M	#	0	3	**5***	2	4	7	2	5
	%	0	5.6	**9.2**	3.7	7.4	13.0	3.7	7.9
Plasma Cell Lymphoma - M	#	0	1	0	0	0	0	0	0
	%	0	1.8	0	0	0	0	0	0
Immunoblast lymphoma - M	#	0	0	0	0	0	1	0	0
	%	0	0	0	0	0	1.8	0	0

Reviewer's table of major neoplastic findings in the mouse carcinogenicity study – All animals

Organ/Tissue	Finding		BIBR 1048 MS Dose level (mg/kg/day) 0 M	30 M	100 M	200 M	0 F	30 F	100 F	200 F
	Malignant Lymphoma (All combined)	#	3	6	7	5	11	16	9	13
	($Cr^{\dagger}$: 0-21.7%, 0-50.0%)	%	5.6	11.1	13.0	7.9	20.4	29.6	16.6	20.6
Skin		#	54	54	54	63	54	54	54	63
	Squamous cell papilloma - B	#	1	0	1	0	0	0	0	0
	($Cr^{\dagger}$: 0-2.0%, 0-2.0%)	%	1.8	0	1.8	0	0	0	0	0
	Fibroma - B	#	0	0	0	1	0	0	0	0
	($Cr^{\dagger}$: M:0-2.1%)	%	0	0	0	1.8	0	0	0	0
	F brosarcoma - M	#	0	4	3	4	2	0	0	0
	($Cr^{\dagger}$: 0-3.3%, 0-6.7%)	%	0	7.4	5.6	6.3	3.7	0	0	0
	Sarcoma - M	#	0	0	0	1	0	0	1	0
	$Cr^{\dagger}$: 0-2.0%, 0-1.7%	%	0	0	0	1.6	0	0	1.8	0
	Fibroma+ fibrosarcoma + sarcoma	#	0	4	3	**6***	2	0	1	0
		%	0	7.4	5.6	**9.5**	3.7	0	1.8	0
	Papilloma+fibroma+ fibrosarcoma + sarcoma	#	1	4	4	6	2	0	1	0
		%	1.8	7.4	7.4	9.5	3.7	0	1.8	0

* Statistically significant, $Cr^{\dagger}$: Incidence range for CD-1 (ICR) male and female mice, [redacted] (b) (4) 2005

Non neoplastic findings:

The study pathologist noted non-neoplastic findings in the liver, lungs, prostate, seminal vesicles and uterus as being related with treatment. These findings are listed in Table 102 along with additional findings noted by the reviewer.

The increased incidence of focal hepatocyte necrosis observed in the high dose males and females did not attain statistical significance. Although this finding occurred primarily at minimal or slight severity in all groups, the severity in two treated males and three treated females, but not in control animals, was moderate or marked.

The incidence of uterine glandular dilatation increased in the high dose female group and the incidences of uterine luminal dilatation increased in mid and high dose female groups compared to the incidence in the control group. Although statistical significance was attained only for the incidence of luminal dilatation in the high dose group, the maximum severity of both glandular and luminal dilatation increased in the high dose group compared to that in the control group.

The study pathologist noted the statistically significant increase in the incidence of eosinophilic inclusions in the larynx epithelium and of inflammation in the gall bladder, as well as a statistically significant decrease in the incidence of inflammation of the pancreas, of osteopetrosis in the femur, and of increased granulopoiesis in the femur and sternum marrow of high dose females. However, the study pathologist concluded that the relationship of these findings to treatment was unclear.

Other findings included an increased incidence of hemorrhage in both sexes. These findings are not unexpected and are related to the pharmacodynamic effect of BIBR 1048 MS. In the lungs, alveolar hemorrhage was present in the treated male groups, but not the control male group; however, a similar incidence of alveolar hemorrhage was present in both control and treated female groups. Hemorrhage was also present in the prostate of some mid- and high-dose males with statistical significance attained in the high dose group. Likewise, hemorrhage was present in the seminal vesicles of some low, mid- and high-dose males with statistical significance attained in the mid and high dose groups.

The reviewer noted increased incidences of acinar necrosis/atrophy/basophilia of the Harderian gland in the high dose males and females, acinar hyperplasia of the Harderian gland in the high dose males, cardiomyopathy in the high dose males and females, and seminiferous tubular atrophy in the testes in the high dose males. In the kidneys, the incidence of cortical tubular dilatation increased in all female treated groups and the incidence of kidney hyperplasia increased in the male treated groups and the mid-dose females.

In the ovaries, the incidence of cystic ovarian bursa and cystic papillary hyperplasia increased in all female treated groups. Although the incidence of ovarian hemorrhage increased in the high dose females, the incidence of hemorrhagic cysts in all females did not increase despite the finding that ovarian cysts contributed to female deaths during treatment. The incidence of hemorrhage also increased in the thymus of all treated female groups.

Table 102: Reviewer's Table - Non-neoplastic Findings - Study U07-2181

Organ/Tissue	Finding		BIBR 1048 BS Dose level (mg/kg/day) 0 M	30 M	100 M	200 M	0 F	30 F	100 F	200 F
Gall bladder		#	42	49	44	53	45	44	48	55
	inflammation	#	0	1	1	0	1	0	1	**8***
		%	0	2	2.2	0	2.2	0	2	**14.5**
Harderian glands		#	54	54	54	63	54	54	54	63
	acinar necrosis/ atrophy/basophilia	#	24	29	29	**33**	37	34	35	*47*
		%	44.4	53.7	53.7	**52.4**	68.5	63.0	64.8	*74.6*
	acinar hyperplasia	#	1	5	0	**7**	3	1	2	1
		%	1.9	9.3	0.0	**11.1**	5.6	1.9	3.7	1.6
Heart		#	54	54	54	63	54	54	54	63
	cardiomyopathy		33	39	35	*46*	37	34	35	*47*
		%	61.1	72.2	64.8	*73.0*	68.5	63.0	64.8	*74.6*
Kidney		#	54	54	54	63	54	54	54	63
	cortical tubular dilatation	#	12	7	13	12	7	*12*	**18***	*15*
		%	22.2	13.0	24.1	19.0	13.0	*22.2*	**33.3**	*23.8*
	atypical hyperplasia	#	0	0	*1*	*2*	0	0	0	0
		%	0	0.0	*1.9*	*3.2*	0.0	0.0	0	0
	tubular hyperplasia, simple	#	3	*6*	*8*	4	6	*8*	*11*	4
		%	5.6	*11.1*	*14.8*	6.3	11.1	*14.8*	*20.4*	6.3
	hyperplasia, papillary epithelium	#	0	0	0	1	0	0	0	0
	hyperplasia, pelvic epithelium	#	0	0	0	1	0	0	0	0
	all kidney hyperplasia	#	3	*6*	**9**	**8**	6	8	**11**	4
		%	5.6	*11.1*	**16.7**	**12.7**	11.1	14.8	**20.4**	6.3
Larynx		#	54	54	54	63	54	54	54	63
	epithelial eosinophilic inclusions	#	0	0	0	0	0	1	1	**6***
		%	0	0	0	0	0	1.8	1.8	**9.5**
Liver		#	54	54	54	63	54	54	54	63
	hepatocyte necrosis, focal	#	6	3	6	**12**	7	8	5	**13**
		%	11.1	5.6	11.1	**19.0**	13.0	14.8	9.3	**20.6**
	centrolobular hepatocyte necrosis	#	0	0	1	0	1	0	0	1
		%	0.0	0.0	1.9	0.0	1.9	0.0	0.0	1.6
	hepatocyte necrosis, combined	#	6	3	7	**12**	8	8	5	**14**
		%	11.1	5.6	13.0	**19.0**	14.8	14.8	9.3	**22.2**
Lungs/Bronchi		#	54	54	54	63	54	54	54	63
	alveolar hemorrhage	#	0	**9***	5	**13***	10	7	8	6
		%	0.0	**13.0**	9.3	**20.6**	18.5	13.0	14.8	9.5
	alveolar epithelial hyperplasia	#	0	**7***	2	1	3	2	3	5
		%	0.0	**13.0**	3.7	1.6	5.6	3.7	5.6	7.9

Organ/Tissue	Finding		BIBR 1048 BS Dose level (mg/kg/day) 0 M	30 M	100 M	200 M	0 F	30 F	100 F	200 F
Ovaries/oviducts		#	0	0	0	0	54	54	54	63
	cystic ovarian bursa	#	0	0	0	0	14	18	17	**24**
		%	0	0	0	0	25.9	33.3	31.5	**38.1**
	hemorrhagic cysts	#	0	0	0	0	12	9	13	**15**
		%	0	0	0	0	22.2	16.7	24.1	**23.8**
	hemorrhage	#	0	0	0	0	0	2	0	**4**
		%	0	0	0	0	0.0	3.7	0.0	**6.3**
	cystic papillary hyperplasia	#	0	0	0	0	0	4	**7***	1
		%	0	0	0	0	0.0	7.4	**13.0**	1.6
Prostate		#	54	54	54	63	0	0	0	0
	hemorrhage	#	0	0	4	**6***	0	0	0	0
		%	0.0	0.0	7.4	**9.5**	0	0	0	0
Seminal vesicles		#	54	54	54	63	0	0	0	0
	hemorrhage	#	0	1	**7***	**6***	0	0	0	0
		%	0.0	1.9	**13.0**	**9.5**	0	0	0	0
Testes		#	54	54	54	63	0	0	0	0
	seminiferous tubular atrophy	#	33	37	34	**47**	0	0	0	0
		%	61.1	68.5	63.0	**74.6**	0	0	0	0
Uterus		#	0	0	0	0	54	54	54	63
	glandular dilatation	#	0	0	0	0	27	31	29	**42**
		%	0	0	0	0	50.0	57.4	53.7	**66.7**
	luminal dilatation	#	0	0	0	0	2	3	7	**11***
		%	0	0	0	0	3.7	5.6	13.0	**17.5***
Femur		#	54	54	54	63	54	54	54	63
	Increased granulopoiesis	#	5	7	4	2	10	7	5	**4**
		%	9.2	13.0	7.4	3.2	18.5	13.0	9.2	**6.3**
Sternum		#	54	54	54	63	54	54	54	63
	Increased granulopoiesis	#	3	6	4	2	11	5	**2***	**3***
		%	5.6	11.1	7.4	3.2	20.4	9.2	**3.7**	**4.8**

* Statistically significant ($p<0.05$) in yellow highlight; Notable change from control in **bold** text

Toxicokinetics

On Day 1 of treatment blood samples were obtained from four satellite animals/sex/group at 1 hour after dosing. During Week 26, blood samples were obtained from 3-4 satellite animals/sex/group at 0.5, 1, 2, 4 and 8 hours after dosing. In addition, blood samples were collected 1 hour after dosing from five main study animals/sex/group at terminal necropsy. Analysis for BIBR 953 ZW was performed using a validated HPLC-MS/MS assay.

After oral administration of the prodrug BIBR 1048 MS, the plasma concentrations of the active moiety BIBR 953 ZW increased rapidly with maximum plasma concentrations reached at the first sampling time points (0.5-1 hour) after dosing. The plasma concentrations of BIBR 953 ZW then decreased rapidly to low levels between 8 and 24 hours after dosing (Figure 30 A). The plasma concentrations of BIBR 953 ZW at 1 hour post dose were generally higher on day 1 than in week 26 or at necropsy in week 103 (females) and 105 (males). Although on Day 1 the plasma concentrations at 1 hour post dose were similar in males and females, the C_{max} and $AUC_{(0\text{-}24\ hr)}$ after repeated dosing for 26 weeks were higher in females than in males, particularly at the two highest doses (Table 103). During week 26, both C_{max} and $AUC_{(0\text{-}24\ hr)}$ of BIBR 953 ZW increased dose-dependently for both genders (Figure 30 B, C).

Table 103: Sponsor's Summary of Toxicokinetics - Study U07-2181

Table of $AUC_{(0\text{-}24hr)}$ and Cmax at 26 weeks

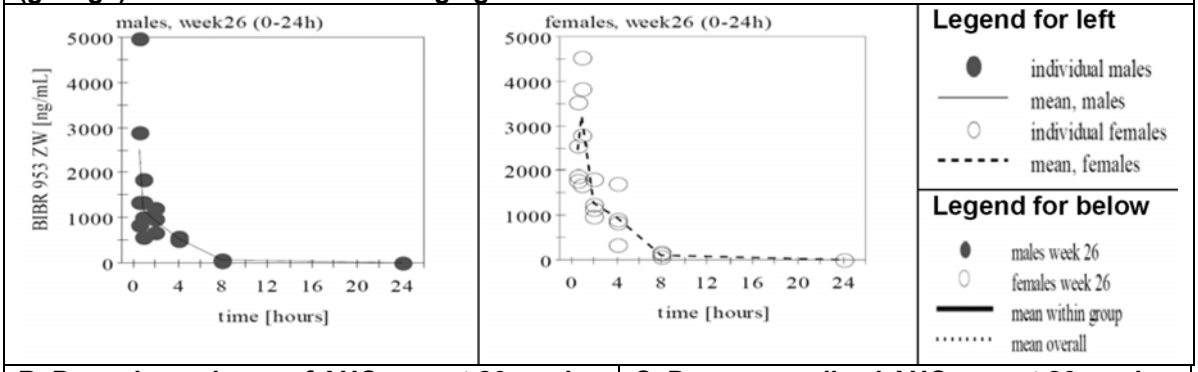
dose [mg/kg]	week	gender	C(max) [ng/mL]	C(max)/ dose [ng/mL]/ [mg/kg]	AUC(0-24h) [(ng·h)/ mL]	AUC(0-24h)/ dose [(ng·h)/mL]/ [mg/kg]
30	26	m	422	14.1	1160	38.8
		f	827	27.6	1290	43.1
100	26	m	1460	14.6	3830	38.3
		f	2330	23.3	5400	54.0
200	26	m	2510	12.6	5830	29.1
		f	3220	16.1	9520	47.6

Concentration of BIBR 953 ZW at 1hr post dose

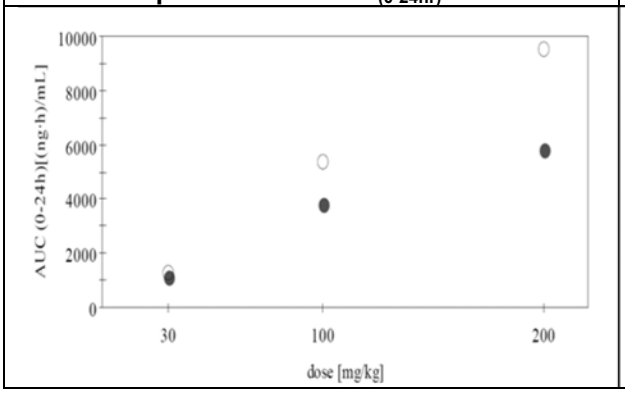
dose [mg/kg]	week	gender	gmean [ng/mL]
30	1	f	414
		m	551
	26	f	412
		m	146
	103	f	240
	105	m	308
100	1	f	1750
		m	1840
	26	f	1160
		m	951
	103	f	922
	105	m	891
200	1	f	3530
		m	3280
	26	f	3010
		m	1090
	103	f	1480
	105	m	981

Figure 30: Sponsor's Figures - Toxicokinetics - Study U07-2181

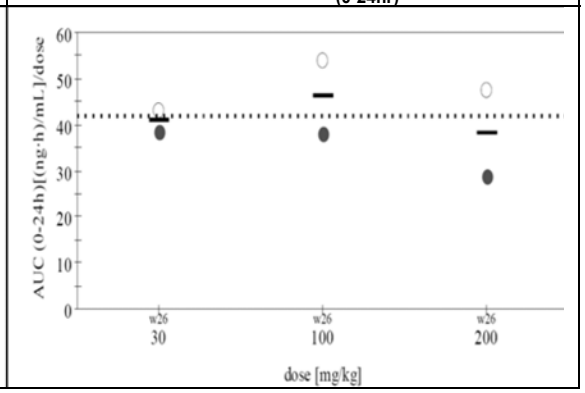
A. Individual and average plasma concentrations of BIBR 953 ZW after once daily oral (gavage) administration of 200 mg/kg of BIBR 1048 BS for 26 weeks



B. Dose dependence of $AUC_{(0\text{-}24hr)}$ at 26 weeks



C. Dose-normalized $AUC_{(0\text{-}24hr)}$ at 26 weeks



Formulation Analysis

The test article formulations were prepared daily and analyzed on Day 1 and during weeks 13, 26, 39, 52, 65, 78, 91, and 103. The analysis data showed that all dose formulations were homogenous and the measured concentrations were 90.5 to 96.9% of nominal.

Study title: Carcinogenicity Study by Oral Gavage Administration to Han Wistar Rats for 104 Weeks

Study no.:	BOI 288/042959 (U07-2084)
Study report location:	9/17/09 submission in EDR
Conducting laboratories and locations:	(b) (4) Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany; (b) (4)
Date of study initiation:	June 29 2004 (dosing initiated July 21, 2004)
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, purity 99.2 %, 97.8% and 98.5% on March 2005, 2006 and 2007, respectively
CAC concurrence:	Upon review of the study protocol, the Executive CAC recommended doses of 0, 30, 100, and 200 mg/kg/d. The minutes from the meeting on March 16, 2004 are in Appendix 3. The Executive CAC reviewed the study results on February 16, 2010. The meeting minutes are in Appendix 4.

Key Study Findings

Han Wistar rats received oral doses of BIBR 1048 MS for up to 104 weeks. At dosages of 30, 100 or 200 mg/kg/day, the mean $AUC_{(0\text{-}24h)}$ was 2490, 7270, and 12200 ng.hr/mL in males and 1960, 6460, and 11800 ng.hr/mL in females, respectively. Hematology findings (decreased hemoglobin concentration and red blood cells along with increased reticulocyte counts and coagulation times) and macroscopic findings were related to the pharmacodynamic action of BIBR 1048 MS. Likewise, the increased incidence of alveolar hemorrhage, pigmented alveolar macrophages, prostate interstitial hemorrhage and prostate pigmented macrophages in mid- and high dose males could be attributed to the pharmacodynamic effect of BIBR 1048 MS. In addition, the incidence of necrosis in the liver increased in all treated groups in both sexes, although statistical significance was not attained.

Adequacy of Carcinogenicity Study

The rat carcinogenicity study used the doses (0, 30, 100 and 200 mg/kg/d) that were recommended by the Exec CAC. The study length was acceptable since the rats were treated for up to 104 weeks. Increased mortality, attributed to the pharmacodynamic

effect of BIBR 1048 MS, was observed at all dose levels in both sexes compared to control groups.

Appropriateness of Test Model

The Han Wistar strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data has been established in the conducting laboratory. The most predominant form of BIBR 1048 MS in both rat and human plasma was BIBR 953 ZW. The plasma protein binding and metabolism of BIBR 1048 MS was similar in rats and humans.

Evaluation of Tumor Findings

Testicular Leydig cell adenomas were observed only in treated males; the pairwise test ($p = 0.032$) and the trend test ($p = 0.020$) for the high dose group were statistically significant. However, neither test attained the threshold significance (p values of 0.01 and 0.005, respectively) needed to classify Leydig cell adenoma, a common tumor (>1%), as a positive finding according to the draft FDA Guidance (2001). Although ovarian granulosa cell tumors were observed only in the mid and high dose female groups, a Sertoli cell tumor, another stromal tumor, was observed in the control group. The incidence of stromal cell tumors was not statistically significant by the pair-wise test. The higher incidence of C-cell adenoma and follicular cell adenoma in the thyroid observed in the treated male groups did not achieve statistical significance and was within the laboratory historical control range.

Executive CAC Recommendations and Conclusions:

The Committee concluded that the rat bioassay was adequate and noted that the sponsor used the doses recommended by the prior Exec CAC protocol agreement. The Committee found that the mouse carcinogenicity study was negative for any drug related statistically significant neoplasms.

Methods

Doses:	0, 30, 100, and 200 mg/kg/d as the free base
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Natrosol 250 HX (hydroxyethylcellulose) solution
Basis of dose selection:	MTD determined by mortality at 300 mg/kg in the 13-week study U05-1378 due to hemorrhage, an expected pharmacodynamic effect of the drug
Species/Strain:	Rat (*Rattus norvegicus*)/Han Wistar (HsdBrl Han:Wist, (b) (4)
Number/Sex/Group:	55 animals/sex/group for the control, low and mid-dose groups; 65 animals/sex for the high dose group

Age:	At the start of treatment, the animals were 39 to 43 days of age.
Animal housing:	5/sex/cage unless number reduced by mortality or isolation
Paradigm for dietary restriction:	Not applicable, since the animals had free access to the standard rodent diet.
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetic satellite groups consisted of 10 animals/sex for each of the BIBR 1048 MS treated groups and 5 animals/sex for the control groups.
Deviation from study protocol:	Because of accidental spillage or premature discarding of dose formulation, replacement formulations were prepared on the same day as dosing for a Group 3 dose in Week 5 and for a Group 4 formulation in Week 6 following three hours stirring. An assessment of concentration and homogeneity was performed on these occasions.

Observations and Results

Mortality

The animals were examined visually at least twice daily for mortality, morbidity or reaction to treatment.

As summarized in Table 104 below, mortality was higher in all treated groups than in the control groups. Although the pairwise comparisons of mortality in the low dose groups with the control groups were not statistically significant, both the trend tests and the pairwise comparisons of mortality in the mid- and high dose groups with the control groups were statistically significant. The Kaplan-Meier survival graphs (Figure 31) below show the higher mortality in males occurred after Week 80. In contrast, the higher mortality in the low, mid and high dose females occurred after weeks 60, 50 and 25, respectively. Due to the pharmacodynamic effect of BIBR 1048 MS on bleeding, a higher mortality in treated animals was anticipated resulting in the larger group size for the high dose group.

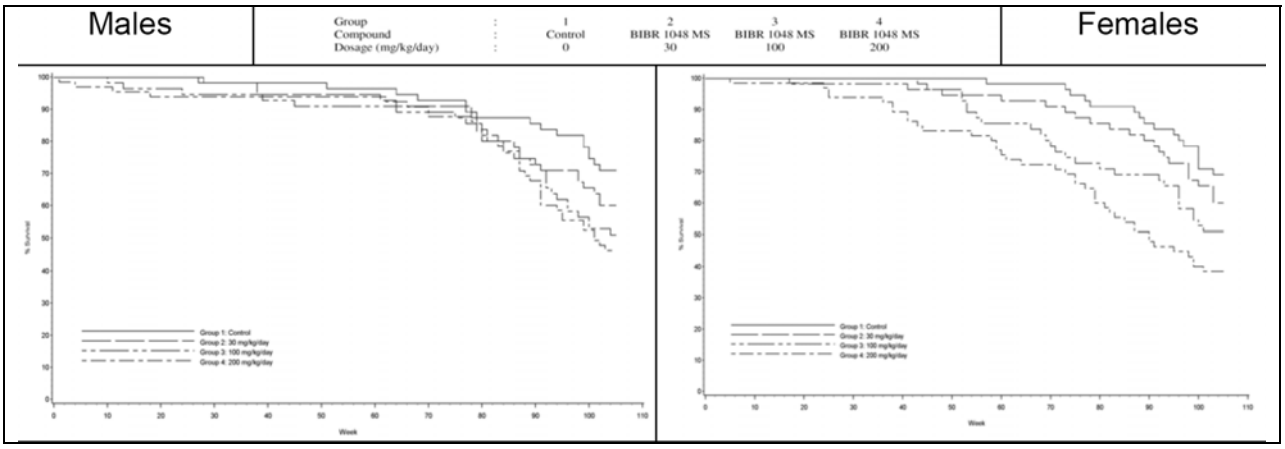
Table 104: Sponsor's Summary of Mortality – Study U07-2084

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	30	100	200	0	30	100	200
Group size	55	55	55	65	55	55	55	65
Total No. of deaths *	16	22	27	35	17	24	27	41
No. of survivors	39	33	28	30	38	31	28	24
% Survival	71%	60%	51%	46%	69%	56%	51%	37%

* Includes animals dying during the necropsy period.

Trend-test with all groups	p = 0.007				p < 0.001			
Pairwise versus control		p >0.05	p = 0.038	p = 0.009		p >0.05	p = 0.035	p< 0.001

Figure 31: Sponsor's Kaplan-Meier Survival Curves - Study U07-2084



As indicated in Table 105, the principal factor contributing to the deaths of both control and treated animals was pituitary adenoma. Many of the deaths of the mid- and high dose animals were the result of poor clinical condition or an undetermined cause. However, an increase in paw lesions contributed to the death of some high dose animals.

Table 105: From Sponsor's Table - Factors Contributing to Death -Study U07-2084

Tissue and Finding	Group/Sex:	1M	2M	3M	4M	1F	2F	3F	4F
	Number:	16	22	27	35	17	24	27	41
Fac Con to Death	Number Examined:	16	22	27	35	17	24	27	41
Pituitary Adenoma		10	11	6	11	10	5	6	12
Paw Lesions		0	0	0	5	0	0	0	2
Poor Clinical Condition		0	3	12	3	1	2	10	8
Undetermined		0	1	1	5	0	1	8	5

Clinical Signs

In addition to a more detailed weekly physical examination, which included palpation of masses, detailed observations were made immediately before dosing, immediately after dosing on return of the animal to its cage, on completion of dosing of each group, 1-2 hours after completion of dosing of all groups, and at the end of the work day weekly during Weeks 1 to 13, every two weeks during Weeks 14 to 26, and once every four weeks to study termination.

A dose-related increase in the incidence of whole body pallor and dark red and/or bloody discharge from the nose or vagina was attributed to the pharmacodynamic effect of BIBR 1048 MS. Some mid- and high dose animals also showed swollen and/or reddened hindlimbs. The clinical signs of inactivity, vocalization, piloerection, dull eyes, irregular, shallow or noisy breathing and reduced body temperature were also observed

at a higher incidence in treated groups than in the control groups; however, these signs were generally found in animals that died prematurely.

Body Weights

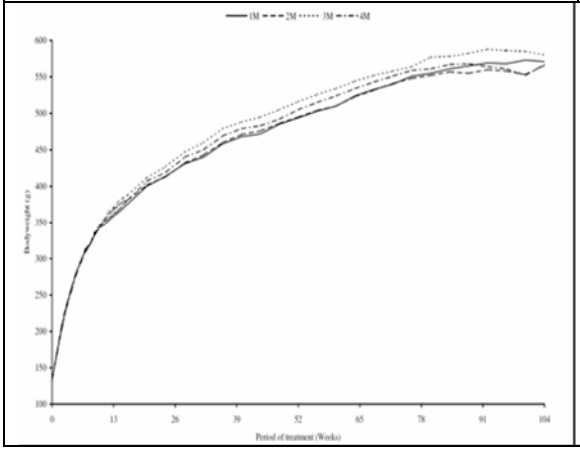
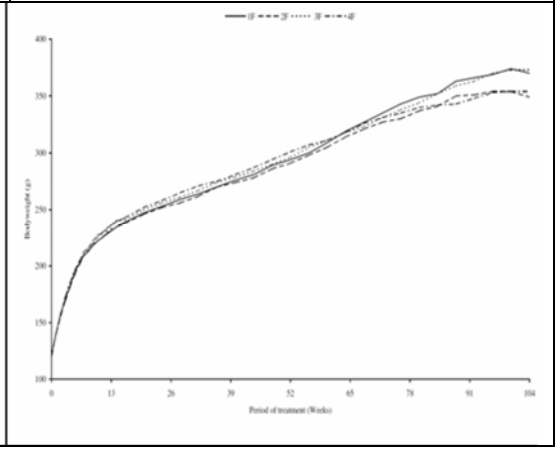
The animals were weighed on Day 1 of treatment, weekly during weeks 1 through 16 weeks of treatment, then every four weeks to study termination and before necropsy.

No significant treatment-related effect of BIBR 1048 MS was observed on body weight or body weight gain as shown in Table 106 and Figure 32.

Table 106: Compilation from Sponsor's Body Weight Tables - Study U07-2084

		Males				Females			
Group (Dose)	Statistic	Week 0	Week 104	Weeks 0-104	As % of Control	Week 0	Week 104	Weeks 0-104	As % of Control
1 (0)	Mean	133	571	437	-	122	370	248	-
	SD	10.8	66.2	65.1		8.8	61.3	60.2	
	N	55	39	39		55	38	38	
2 (30)	Mean	135	566	432	99	120	349	230	93
	SD	9.3	60.4	59.0		9.8	51.1	45.6	
	N	55	33	33		55	32	32	
3 (100)	Mean	131	580	451	103	120	373	254	102
	SD	10.8	78.6	73.7		8.4	45.8	45.1	
	N	55	28	28		55	28	28	
4 (200)	Mean	132	567	434	99	120	354	236	95
	SD	9.5	70.9	70.3		10.5	43.2	39.8	
	N	65	30	30		65	25	25	

Figure 32: Sponsor's Bodyweight Graphs - Study U07-2084

Males	Females
	

Food Consumption

The mean weekly food consumption per animal (g/mouse/week) was determined weekly by cage for the first 16 weeks, and once every four weeks thereafter.

No treatment-related effect of BIBR 1048 MS was observed on food consumption or food conversion efficiency.

Ophthalmoscopy

Before treatment initiation, the eyes of all main study animals were examined after dilation with a 0.5% tropicamide ophthalmic solution using a binocular indirect ophthalmoscope. The eyes of 20 animals/sex from the control and high dose groups were similarly examined during Weeks 52 and 100 of treatment. The examination in Week 100 was subsequently extended to all males of all groups to further evaluate an increased incidence of slight hyperreflection in the high dose males (2/20) compared to the control group (1/20) observed during week 52.

The study report concluded no treatment-related effect of BIBR 1048 MS was observed on ophthalmoscopy. Table 107 shows that the incidence of hyperreflection increased in all the BIBR 1048 MS treated male groups compared to the male control group; however, but the incidence of hyperreflection decreased in the high dose female group compared to that in the female control group.

Table 107: Ophthalmoscopy Results Week 100 - Study U07-2084

Group	:	1	2	3	4		
Compound	:	Control	BIBR 1048 MS	BIBR 1048 MS	BIBR 1048 MS		
Dosage (mg/kg/day)	:	0	30	100	200		
			Group and sex/Number of animals				
Structure	Observation		1M	2M	3M	4M	1F	4F
Fundus	(Slight) hyperreflection		10	11	11	13	14	7
	Blood vessel attenuation		0	2	0	3	1	3
Number of animals examined :			41	36	29	34	20	20
Incidence of hyperreflection, %			24.4	30.6	37.9	38.2	70	35

Hematology

Blood samples were obtained at terminal necropsy from all surviving animals via the retro-orbital sinus. The non-fasted animals were held under light isoflurane anesthesia during blood collection. A blood smear was prepared for all animals. The following parameters were measured in samples from 20 animals from each sex: hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, morphology, platelet count, total white cell count, and differential white cell count, including neutrophils, lymphocytes, eosinophils, basophils, monocytes, and large unstained cells. Additional blood samples were taken for measurement of prothrombin time (PT) and activated partial thromboplastin time (aPTT). Samples from the remaining animals were examined for erythrocyte count, total white cell count, and differential white cell count.

Hemoglobin concentration decreased 4 and 8% respectively in the high dose males and females (Table 108). Similar decreases in red blood cell counts in the high dose males and females were also not statistically significant, but were associated with increases in the percentage of reticulocytes. The number of animals with values for hemoglobin, red blood cell count and reticulocytes outside the concurrent control range tended to

increase with dose. Although the high dose females showed a statistically significant decrease in eosinophils, all values for individual females were within the range of values for the concurrent controls.

Coagulation times were significantly prolonged in the high dose male and female groups with 11-12% increases in PT and 17-34% increases in aPTT. In addition, the group mean for aPTT was significantly increased in the low and mid-dose females. The number of animals with PT and aPTT values above the concurrent normal range increased with dose.

Table 108: From Sponsor's Hematology Tables - Study U07-2084

		Males					Females				
Group (Dose)	Statistic	Hb g/dL	RBC $x10^{12}$/L	Retic %	PT sec	APTT sec	Hb g/dL	RBC $x10^{12}$/L	Retic %	PT sec	APTT sec
1 (0)	Mean	15.2	8.36	2.54	17.6	19.3	14.5	7.50	2.90	16.2	18.3
	SD	0.73	0.543	0.683	1.53	1.77	0.75	0.524	0.591	0.93	2.41
	N	19	19	19	19	19	20	20	20	20	20
2 (30)	Mean	15.3	8.32	2.98	17.5	19.7	14.0	7.18	4.49	16.8	20.6 a
	SD	1.09	0.696	2.331	1.64	2.71	0.87	0.728	4.443	1.01	2.48
	N	20	20	20	19	19	20	20	20	20	20
3 (100)	Mean	14.5	8.19	3.93	18.4	19.4	14.0	7.24	3.02	16.5	20.5 a
	SD	1.79	0.684	3.540	1.51	3.11	0.82	0.435	0.766	1.68	3.52
	N	20	20	20	20	20	20	20	20	19	19
4 (200)	Mean	14.6	7.98	3.19	19.5 b	22.6 b	13.4	6.85	6.35	18.2 b	24.7 b
	SD	1.68	0.996	2.215	3.12	5.16	2.60	1.362	10.068	2.09	5.42
	N	20	20	20	20	20	19	19	19	18	18
Control	minimum	13.7	7.4	1.8	14.1	15.2	13.2	6.49	1.79	14.8	14.6
	maximum	16.2	9.1	4.1	19.3	20.8	15.6	8.65	3.86	18.3	22.4
2	Number of animals out-side control range (most deviant value)	1< (12.2)	1< (6.21)	1> (12.2)	1> (20.3)	3> (26.5)	2< (11.6)	2< (5.16)	7> (22.4)	1> (18.8)	4> (25.7)
3		4< (10.0)	2< (6.28)	4> (15.7)	4> (21.7)	7> (26.5)	2< (12.3)	1< 6.25)	2> (5.4)	3> (19.7)	5> (28.1)
4		5< (9.6)	4< (4.48)	3> (10.2)	7> (29.9)	11> (42.9)	4< (5.1)	4< (2.92)	4> (42.3)	5> (24.1)	8> (35.6)

Clinical Chemistry

Additional blood was obtained from the same 20 animals/sex from each group as for hematology. Plasma was prepared and the following parameters were measured: alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase, total bilirubin, urea, creatinine, glucose, total cholesterol, triglycerides, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, α1 globulin, α2 globulin, β globulin, and γ globulin.

The study report noted decreases in mean ALT values in the mid and high dose groups that reached statistical significance only in the males. However, a decrease in ALT was not considered toxicologically significant.

Mean urea values significantly decreased in the high dose males; however, the mean urea values increased in the low and high dose females without statistical significance (Table 109). In addition, individual serum creatinine values were not associated with changes in individual urea values.

Mean glucose values were significantly decreased in all treated male groups; mean glucose values were also decreased in the mid- and high dose female groups, but did

not reach statistical significance. Mean cholesterol values were significantly decreased in the high dose male group; mean cholesterol values were also decreased in the low and high dose female groups, but did not reach statistical significance. A few individual males and females had glucose or cholesterol values less than the minimum in the respective control groups. However, a clear relationship of these changes to treatment with BIBR 1048 MS is not evident.

Mean potassium values were significantly increased in the low and mid-dose male groups and in all female treated groups. The number of males and females with individual values above the maximum potassium value on the respective control groups increased in the mid-dose groups compared to the number in the low dose groups. The maximum potassium values are 11 and 12% above the maximum in the control male and female groups, respectively. Such increases in potassium could potentially be adverse. However, the group mean and the maximum individual values in the high dose groups are not entirely consistent with a treatment related effect.

Table 109: Modification of Sponsor's Tables - Clinical Chemistry - Study U07-2084

		Males						Females					
Group (Dose)	Statistic	ALT U/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	K mmol/L	ALT U/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	K mmol/L
1 (0)	Mean	63	5.02	37	8.84	3.22	4.0	75	5.05	37	7.89	2.83	3.8
	SD	28.3	0.814	3.9	2.125	0.676	0.23	31.8	1.030	3.8	1.384	0.554	0.38
	N	20	20	20	20	20	20	20	20	20	20	20	20
2 (30)	Mean	57	5.28	35	7.40 a	2.97	4.2 a	73	5.89	37	8.09	2.59	4.0 a
	SD	20.4	0.842	3.1	1.558	0.675	0.32	41.4	1.206	3.1	1.250	0.485	0.38
	N	20	20	20	20	20	20	20	20	20	20	20	20
3 (100)	Mean	49 a	5.06	34	7.56 a	3.14	4.3 b	57	5.16	36	7.52	2.90	4.1 a
	SD	12.5	0.564	4.1	1.046	0.554	0.42	21.5	1.016	3.7	0.976	0.717	0.43
	N	20	20	20	20	20	20	20	20	20	20	20	20
4 (200)	Mean	46 b	4.42 a	39	7.81 a	2.51 b	4.0	58	5.60	39	7.60	2.49	4.0 a
	SD	12.0	0.863	5.8	1.547	0.632	0.30	24.7	0.712	4.4	1.126	0.546	0.35
	N	20	20	20	20	20	20	19	19	19	19	19	19
Control range Minimum		42	3.56	30	5.34	2.02	3.5	36	3.17	28	5.96	2.07	3.1
maximum		155	6.55	44	11.93	4.73	4.4	127	6.93	43	11.77	4.17	4.3
2	Number of animals out-side control range (most deviant value)	1< (40)	0	0	1< (5.0)	0	2> (4.8)	1< (27)	3> (8.2)	0	1< (5.7)	3< (1.93)	2> (4.6)
3		4< (33)	0	3< (29)	1< (4.6)	0	5> (4.9)	2< (22)	1> (7.2)	0	1< (5.5)	2< (1.56)	5> (4.8)
4		8< (19)	1< (1.9)	0	2< (4.85)	5< (1.85)	1> (4.7)	2< (20)	0	2> (47)	1< (4.0)	2< (1.6)	1> (4.5)

Urinalysis

Overnight (16-17 hr) urine samples were collected in metabolism cages without food or water from 20 animals/sex in each group during Week 103. The measured parameters included appearance, glucose, ketones, bile pigments, blood pigments, and microscopic examination of the urine sediment.

The incidence of hemoglobin pigment or red blood cells in the urine of treated males increased with the dose of BIBR 1048 MS (Table 110). In contrast, treated females did not show an increase in incidence, but a slight decrease.

Table 110: Sponsor's Urinalysis Summary Table - Study U07-2084

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	30	100	200	0	30	100	200
Group size	20	20	20	20	20	20	20	20
Blood pigment present	2	4	8	9	3	3	2	1
Red blood cells present	1	4	8	9	2	3	2	1

Organ Weights

From each animal necropsied after 104 weeks of treatment, the following organs excised and weighed: adrenals, brain, epididymides, heart, kidneys, liver, lungs with mainstem bronchi, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid with parathyroids (after partial fixation), uterus with cervix.

No statistically significant effect was observed on organ weights for males killed after 104 weeks of treatment; however, the mean thymus absolute and bodyweight relative weights for the high dose males are 75-80% higher than the respective means for the control males (Table 111). Some of this increase may be explained by an increased number of high dose males (7) with thymic cysts compared to the number of control males (4) with thymic cysts. No statistically significant effect was observed on thymic weights for females. The increase in thymic weights in the low dose female group is associated with a higher severity (moderate) of thymic cysts in some low dose females compared to the maximum severity for control females (slight).

All treated female groups had increased absolute and bodyweight-relative ovary weights. Higher ovary weights for low and mid dose females were attributed to a few animals with the incidental finding of periovarian sac distention and were considered not related to treatment. The higher ovary weights for the high dose females can be attributed to a higher incidence of ovarian cysts or cystic ovaries in the high dose females (22) compared to the number of control females (6).

The high dose females had a statistically significant 14% decrease in absolute kidney weight and a 9% decrease in absolute liver weight; however, the corresponding 12% decrease in relative kidney weight and 5% decrease in relative liver weight were not statistically significant. No effect was observed on kidney and liver weights in the treated males. The decrease in kidney weights in the females is partially explained by control female #260, who had an absolute kidney weight of 6.33 gm and a relative kidney weight of 2.07%. The increased kidney weight in female #260 is attributable to a large cyst on the right kidney and severe hydronephrosis.

Table 111: From Sponsor's Tables of Organ Weights - Study U07-2084

Group (Dose)		Males		Females							
		Abs. wt, gm	Rel.to BW	Absolute wt. (gm)				Relative to BW (%)			
	Statistic	Thymus	Thymus	Kidneys	Liver	Ovaries+ Oviducts	Thymus	Kidneys	Liver	Ovaries+ Oviducts	Thymus
1 (0)	Mean	0.077	0.0136	2.31	11.19	0.133	0.145	0.637	3.03	0.035	0.0401
	SD	0.024	0.0042	0.75	2.10	0.152	0.136	0.255	0.40	0.032	0.0394
	N	38	38	38	38	38	38	38	38	38	38
2 (30)	Mean	0.080	0.0141	2.07	10.39	0.225 A	0.425	0.594	2.96	0.064 A	0.1344
	SD	0.024	0.0038	0.29	1.78	0.347	1.199	0.096	0.40	0.090	0.3933
	N	32	32	31	31	31	31	31	31	31	31
3 (100)	Mean	0.087	0.0149	2.07	11.06	0.221 A	0.138	0.556	2.97	0.062 A	0.0362
	SD	0.030	0.0048	0.26	1.41	0.414	0.141	0.062	0.32	0.119	0.0347
	N	28	28	28	28	27	27	28	28	27	27
4 (200)	Mean	0.138	0.0239	1.98 B	10.14 a	1.290 B	0.253	0.563	2.87	0.362 B	0.0733
	SD	0.327	0.0543	0.28	1.82	1.654	0.681	0.064	0.37	0.448	0.2041
	N	30	30	24	24	23	24	24	24	23	24

A = p < 0.05; B = p < 0.01

Gross Pathology

The whole or a sample of the tissues listed below (Table 112) from all animals was preserved in 10% neutral buffered formalin, except for the following tissues. Testes and epididymides were fixed in Bouin's solution. The urinary bladder was initially inflated with Bouin's solution prior to transfer to 70% industrial methylated spirit. The eyes were fixed in Davidson's fluid.

Table 112: Reviewer's List of Tissues Collected - Study U07-2084

Abnormal tissues	Kidneys	Sciatic nerve+#
Adrenals	Lachrymal glands	Seminal vesicles
Aorta - thoracic	Larynx	Skeletal muscle - thigh+#
Brain	Liver	Skin
Cecum	Lungs with mainstem bronchi	Spinal cord
Clitoral gland	Lymph nodes (mandibular, mesenteric	Spleen
Colon	and regional to masses)	Sternum
Duodenum	Mammary area - caudal	Stomach
Epididymides	Optic nerves	Testes
Esophagus	Ovaries with oviduct	Thymus
Eyes	Pancreas	Thyroid with parathyroids
Femur+#	Pituitary	Tongue
Harderian glands	Preputial gland	Trachea
Head#	Prostate	Ureters
Heart	Rectum	Urinary bladder
Ileum	Salivary gland+# (submandibular,	Uterus and cervix
Jejunum	sublingual and parotid)	Vagina

+ Only one processed for examination; # Retained and not processed for histologic examination, including the head (nasal cavity, paranasal sinuses, nasopharynx and zymbals gland with external ear), and the remaining femur, salivary gland, sciatic nerve and skeletal muscle (thigh).

Many of the treatment-related macroscopic findings listed in Table 113 are consistent with the pharmacodynamic effect of BIBR 1040 MS. The incidence of dark areas was increased in the prostate of all treated male groups. An increased incidence of dark areas was observed on the lungs, skeletal muscle, and ovaries of treated animals, particularly the high dose group. The incidence of fluid and/or abnormal contents described as dark, red and/or clotted blood was observed in a number of tissues, including the nasal turbinates, gastrointestinal tract, oral cavity, urinary bladder, uterus, trachea, thorax and abdomen.

The incidence of cystic ovaries was significantly higher in the high dose females than in the control females. Although the incidence of periovarian sac distention was higher in the low and high dose females than in the control group, this finding was not considered a treatment-related effect because no dose response was seen.

The incidence of thickened limiting ridge of the stomach was higher in the mid- and high dose males and the high dose females than in the control groups; however, no corresponding microscopic findings were observed in these animals. In addition, swollen paws were observed in a few high dose males and females, but not in the control groups.

Table 113: Modification - Sponsor's Table Macroscopic Findings - Study U07-2084

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	**0**	**30**	**100**	**200**	**0**	**30**	**100**	**200**
Group size	55	55	55	65	55	55	55	65
Prostate - masses	1	10 b	12 b	11 b				
Prostate – dark areas	0	14 b	17 b	23 b				
Ovaries - cystic					2	2	3	14 b
Stomach – thickened limiting ridge	2	2	6	10 a	1	2	1	4
Abnormal contents GI tract	1	12b	13b	9a	2	3	8a	7
Lungs + bronchi, dark areas	0	3	4	4	0	0	1	1
Skeletal muscle, dark areas	0	0	0	3	0	0	0	3
Paws, swollen	0	0	0	5a	0	0	0	2
Ovaries, dark areas	-	-	-	-	0	0	1	3
Periovarian sac distention	-	-	-	-	2	10a	4	8
P value - a = p<0.05; b = p<0.01								

Histopathology

Tissue samples from all main study animals were dehydrated, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The indicated tissues listed in Table 112 and gross abnormalities identified at macroscopic examination from all animals sacrificed at the end of the scheduled treatment period and from all animals killed or dying during the study were examined by histology.

Peer Review:

A reviewing pathologist in the conducting laboratory as well as the sponsor's pathologist conducted a peer review.

Neoplastic findings:

The study pathologist and statistician concluded that no neoplastic findings were considered related to treatment with BIBR 1048 MS; however, their reports discussed the statistically significant findings regarding testicular Leydig cell adenomas and ovarian granulosa cell tumors. The reviewer also notes non-statistically significant findings in the thyroid gland, skin and Harderian gland in Table 114 below. The sponsor's complete table of neoplasms is in Appendix 7.

Interstitial (Leydig) cell adenomas were observed in the testes of males in all treated groups. Both the pairwise test (p = 0.032) for the high dose group and the trend test (p = 0.020) are statistically significant. However, neither test attained the threshold significance (p values of 0.01 and 0.005, respectively) needed to classify Leydig cell adenoma, a common tumor (>1%), as a positive finding according to the draft FDA Guidance (2001). Although the study statistician cited a historical incidence of 2.5% for Leydig cell adenoma in the conducting laboratory, the report from the conducting laboratory dated March 2007 (Appendix 8) indicated a mean incidence of 1.8% and range of 0% to 8.0% for Leydig cell adenomas. The incidence for this tumor of 6.3% in the high dose group is above the laboratory historical mean, but is still within the historical control range for the laboratory. The submission also included a report covering 1983-2003 historical data for Leydig cell adenomas in male Wistar rats in the Registry of Industrial Toxicology Animal (RITA) database (Appendix 9). When the data from a breeder with a very high incidence (range 18-60%) of Leydig cell adenoma are excluded, the mean incidence for the remaining breeders is 6.6% with a range of 0% to 22%. The incidence of 6.3% in the high dose group in the current study is similar to the mean incidence for the RITA database and is within the range of 1.67% to 10.91% for testicular interstitial cell adenoma in the (b) (4) listing of spontaneous neoplasms in Wistar Han rats (2003). The study pathologist concluded the presence of Leydig cell adenomas only in the treated groups was incidental and not treatment-related because of the lack of a clear dose-relationship for the incidence of Leydig cell adenomas and the lack of finding either Leydig cell hyperplasia or Leydig cell carcinomas in the high dose males. There was no anterior pituitary hyperplasia to suggest the possibility that increased LH and FSH might underlie the increased Leydig and granulosa cell (below) neoplasia, respectively.

A higher incidence of granulosa cell tumors was found in the ovaries of mid and high dose females. The sponsor's statistical analysis showed that the trend test (p = 0.011) was statistically significant, the pairwise test (p = 0.084) for the high dose group was not. However, the analysis by the CDER statistician, Dr. Steven Thomson, indicated that both the pairwise test (p = 0.033) for the high dose group and the trend test (p = 0.0038) were statistically significant. Since granulosa cell tumor is a common tumor, the threshold significances for the pairwise and trend tests of 0.005 and 0.01, respectively are needed to classify a common tumor (>1%) as a positive finding according to the draft FDA Guidance (2001). Based on the sponsor's analysis, both tests did not attain the critical p values to classify granulosa cell tumors as positive. In contrast, Dr. Thompson's analysis indicates that the trend test attained a p value to classify the granulosa tumors as positive, whereas the pairwise test did not attain a p value to classify the granulosa tumors as positive. Although the study statistician reported a historical incidence of 3.4% for granulosa cell tumors, the report from the conducting laboratory dated February 2007 indicated a mean incidence of 2.7% with a range of 0.0

to 8.2% for granulosa cell tumors. The incidences in the mid and high dose females are within historical control range for the conducting laboratory as well as within the 1983-2003 historical incidence range (0% to 15%) from the RITA database. Furthermore, incidences of granulosa cell hyperplasia were found in the control group as well as the low dose and high dose groups. In addition, a Sertoli cell adenoma, another stromal tumor, was found only in the control group. The p values for the incidence of stromal cell tumors (granulosa plus Sertoli cell) are above the critical p values needed to classify the stromal cell tumors as positive.

The incidence of thyroid C-cell adenoma increased in treated male groups, but did not attain statistical significance either for the pair-wise comparisons (p = 0.109 to 0.331) or the trend test (p = 0.159). Similarly, the combined incidence of thyroid C-cell adenoma and C-cell carcinoma increased in treated male groups, but did not attain statistical significance either for the pair-wise comparisons (p = 0.122 to 0.308) or the trend test (p = 0.122). Additionally, no increase in incidence is observed for the combination of C-cell hyperplasia, C-cell adenoma, and C-cell carcinoma. The incidences of C-cell adenoma and C-cell carcinoma are within the historical incidences for Wistar rats reported by (b) (4) (2003).

The incidence of follicular cell adenoma increased in the treated male groups, but did not attain statistical significance either for the pair-wise comparisons (p = 0.118 to 0.170) or the trend test (p = 0.288). Follicular cell carcinoma was only found in a control male and a control female. The combined incidence of thyroid follicular cell adenoma and follicular cell carcinoma also did not attain statistical significance either for the pair-wise comparisons (p = 0.232 to 0.308) or the trend test (p = 0.389). Additionally, no increase is observed for the combination of follicular cell hyperplasia, adenoma, and carcinoma. The incidences of follicular cell adenoma and follicular cell carcinoma are within the historical incidences for Wistar rats reported by (b) (4) (2003).

Some skin tumors (basal cell, fibrosarcoma (p = 0.495 to 0.51), sarcoma and squamous cell carcinoma) were present only in treated male groups. However, the incidences of neither the individual tumors nor the combination of fibroma plus fibrosarcoma nor the combination of squamous cell papilloma plus sarcoma attained statistical significance either for pair-wise tests or the trend test. Additionally, the incidences were within the historical incidences for Wistar rats reported by (b) (4)

Harderian gland tumors were present only in the high dose males. The incidence (p = 0.185) is not statistically significant in a pairwise comparison with the control group or by the trend test (0.052), although the incidence exceeds the historical incidences for male Wistar rats reported by (b) (4)

Table 114: Reviewer's Summary -Neoplastic Findings - Study U07-2084

All animals		BIBR 1048 MS Dose level (mg/kg/day)							
		0	30	100	200	0	30	100	200
Organ/Tissue	Finding	M	M	M	M	F	F	F	F
Ovaries	#	0	0	0	0	55	55	55	65
Granulosa cell hyperplasia	#					1	1	0	1
	%					1.8	1.8	0	1.5
Granulosa cell tumor - B	#					0	0	2	**4**
Cr†: 0-1.8%	%					0	0	3.6	**6.2***
Sertoli cell adenoma - B						1	0	0	**0**
						1.8	0	0	**0**

All animals		BIBR 1048 MS Dose level (mg/kg/day)							
		0	30	100	200	0	30	100	200
Organ/Tissue Finding		M	M	M	M	F	F	F	F
Granulosa cell tumor + Sertoli cell adenoma	#					1	0	2	4
	%					1.8	1.8	3.6	7.7
Testes	#	55	55	55	64	0	0	0	0
Interstitial cell hyperplasia - B	#	0	1	2	0				
	%	0	1.8	3.6	0				
Interstitial (Leydig) cell adenoma - B	#	0	2	1	**4**				
Cr†: 0-10.9%	%	0	3.6	1.8	**6.3***				
Thyroid	#	55	55	55	65	55	55	55	63
C-cell hyperplasia	#	26	21	19	22	21	24	24	21
	%	47.2	38.2	34.5	33.8	38.2	43.6	43.6	32.3
C-cell adenoma – B	#	1	4	3	4	4	3	1	0
(Cr†: 3.6 – 18.3%, 0-21.8%)	%	1.8	7.2	5.4	6.2	7.2	5.4	1.8	0
C-cell carcinoma - M	#	1	1	1	1	0	2	0	0
(Cr†: 1.8 – 5.45%, 0 – 1.8%)	%	1.8	1.8	1.8	1.5	0	3.6	0	0
C-cell adenoma + C-cell carcinoma	#	2	5	4	5	4	5	1	0
	%	3.6	9.0	7.2	7.6	7.2	9.0	1.8	0
Follicular cell hyperplasia	#	5	0	1	4	1	2	1	0
	%	9.0	0	1.8	6.2	1.8	3.6	1.8	0
Follicular cell adenoma - B	#	1	5	4	4	0	0	1	1
(Cr†: 1.67-12.73%, 0- 9.1%)	%	1.8	9.0	7.2	6.2	0	0	1.8	1.6
Follicular cell carcinoma - M	#	1	0	0	0	1	0	0	0
Cr†: 0 – 3.64%, 0-3.6%	%	1.8	0	0	0	1.8	0	0	0
Follicular cell adenoma + follicular cell	#	2	5	4	4	1	0	1	1
carcinoma	%	3.6	9.0	7.2	6.2	1.8	0	1.8	1.6
Skin	#	55	55	55	65	55	55	55	65
Basal cell tumor-B	#	0	0	2	1	0	1	0	0
Cr†: 0-5.45%, 0-1.8%,	%	0	0	3.6	1.5	0	1.8	0	0
Sarcoma (NOS) - M	#	0	1	0	0	0	0	0	0
	%	0	1.8	0	0	0	0	0	0
Squamous cell papilloma - B	#	1	0	1	1	0	0	1	0
Cr†: 0-3.6%, 0-1.8%	%	1.8	0	1.8	1.5	0	0	1.8	0
Squamous cell carcinoma - M	#	0	0	1	1	0	0	0	0
Cr†: 0-1.8%	%	0	0	1.8	1.5	0	0	0	0
Squamous cell papilloma+carcinoma	#	1	0	2	2	0	0	1	0
	%	1.8	0	3.6	3.1	0	0	1.8	0
Fibroma - B	#	1	2	1	2	0	0	0	0
Cr†: 1.67-10.9%, 0-5.5%,	%	1.8	3.6	1.8	3.0	0	0	0	0
F brosarcoma – M	#	0	1	0	1	0	0	0	0
Cr†: 0-3.6%, 0-1.5%	%	0	1.8	0	1.5	0	0	0	0
F broma+ fibrosarcoma	#	1	3	1	3	0	0	0	0
	%	1.8	5.4	1.8	4.5	0	0	0	0
Harderian glands	#	55	55	55	65	55	55	55	65
Adenoma – B	#	0	0	0	2	0	0	0	0
Cr†: 0-1.8%	%	0	0	0	3.0	0	0		
Hyperplasia	#	1	1	1	1	1	0	0	1
	%	1.8	1.8	1.8	1.5	1.8	0	0	1.5

Statistically significant, Cr†: Incidence range for Wistar males and females [redacted] (b) (4) 2003
Rows highlighted in yellow indicate statistically significant findings

Non-neoplastic findings:

Some of the findings listed in Table 115 below can be attributed to the pharmacodynamic effect of BIBR 1048MS, especially at the high dose levels. In the prostate, an increased microscopic incidence of pigmented macrophages and interstitial and/or intracinar hemorrhage were observed in the mid- and high dose groups. The increased incidence of inflammation/edema in the high dose males was considered secondary to repeated hemorrhages to the prostate. These microscopic findings correlated with the macroscopic findings of masses and dark areas on the prostate of all treated male groups.

Other findings consistent with the pharmacodynamic effect of BIBR 1048MS include the statistically significant increase in incidence of sinus erythrocytosis/erythrophagocytosis in mandibular lymph nodes in the high dose females and non-significant increase in the mid and high dose males. In the lungs, a statistically significant increase in incidence of pigmented alveolar macrophages was observed in mid and high dose males, but not the females. However, the high dose females and the mid dose males showed a non-statistically significant increase in alveolar hemorrhage.

In the ovary, a higher incidence of cystic ovarian bursa was observed in all treated groups and was statistically significant in the low and high dose groups. In addition, the incidence of ovarian cysts was significantly increased in the high dose females. The Increased absolute and bodyweight-relative ovary weights higher ovary weights for the high dose females can be attributed to a higher incidence of ovarian cysts or cystic ovaries in the high dose females (22) compared to the number of control females (6).

The study pathologist also noted the increased incidence of skin lesions in the high dose males and the increased incidence of pododermatitis in the mid and high dose males as well as the high dose females. However, the pathologist did not note the presence of transitional hyperplasia in the urinary bladder of high dose males, the presence of uterine squamous metaplasia in the mid and high dose females and the Increased incidence of periductal pigment in the pancreas of the mid and high dose males. Perhaps because the finely distinguished liver diagnoses (focal hepatocyte necrosis, centrolobular necrosis, and necrosis/hemorrhage/inflammation) were kept separate, the study report did not note necrosis in the liver. However, the incidence of combined liver necrosis slightly increased in all treated groups of both sexes.

Table 115: Reviewer's Summary - Non-neoplastic Findings - Study U07-2084

		BIBR 1048 MS Dose level (mg/kg/day)							
		0	30	100	200	0	30	100	200
Organ/Tissue Finding		M	M	M	M	F	F	F	F
Liver (n)	#	55	55	55	65	55	55	55	65
Hepatocyte necrosis, focal	#	0	2	3	4	1	2	2	2
	%	0	3.6	5.4	6.2	1.8	3.6	3.6	3.1
Centrolobular hepatocyte necrosis	#	1	0	0	1	0	1	0	0
	%	1.8	0	0	1.5	0	1.8	0	0
Necrosis/hemorrhage/inflammation	#	0	0	0	0	0	0	0	2
	%	0	0	0	0	0	0	0	3.1
Liver necrosis, combined	#	1	2	3	5	1	3	2	4
	%	1.8	3.6	5.4	7.6	1.8	5.4	3.6	6.2
Lungs/Bronchi	#	55	55	55	65	55	55	55	65
Alveolar hemorrhage	#	4	3	8	5	2	5	1	9
	%	7.2	5.4	14.5	7.6	3.6	9.0	1.8	13.8
Alveolar epithelial hyperplasia	#	4	2	1	2	0	0	1	1
	%	7.2	3.6	1.8	3.0	0	0	1.8	1.5
Pigmented alveolar macrophages	#	2	5	**10***	**13***	15	15	14	14
(Males increased severity with dose)	%	3.6	9.0	**18.2**	**20.0**	27.2	27.2	25.4	21.5
Lymph node, mandibular	#	54	55	55	64	55	54	48	61
Sinus erythrocytosis/	#	8	8	11	16	9	12	10	**21***
erythrophagocytosis	%	14.8	14.5	20	25	16.4	22.2	20.8	**34.4**
Ovaries/oviducts	#	0	0	0	0	55	55	55	65
Cystic ovarian bursa	#					2	**9***	5	**11***
	%					3.6	**16.4***	9.0	**16.9***
Hemorrhagic cysts	#					0	1	0	1
	%					0	1.8	0	1.5
Cysts	#					8	0	4	**20**
	%					14.5	0	7.2	**30.8**
Pancreas	#	55	55	55	65	55	55	55	65

		BIBR 1048 MS Dose level (mg/kg/day)							
		0	30	100	200	0	30	100	200
Organ/Tissue Finding		M	M	M	M	F	F	F	F
Periductal pigment	#	2	1	**8**	**8**	0	0	0	1
	%	3.6	1.8	**14.5**	**12.3**	0	0	0	1.8
Prostate	#	55	55	55	65	0	0	0	0
Inflammation/edema	#	8	12	13	**22***				
	%	14.5	21.8	23.6	**33.8***				
Interstitial hemorrhage	#	0	1	4	**12***				
(increased severity with dose)	%	0	1.8	7.2	**18.5**				
Pigmented macrophages	#	0	0	7	**17***				
(increased severity with dose	%	0	0	12.7	**26.2**				
Urinary bladder	#	55	55	55	65	55	55	55	65
Transitional hyperplasia	#	0	0	0	2	0	0	0	0
	%	0	0	0	3.0	0	0	0	0
Uterus	#	0	0	0	0	55	55	55	65
Squamous metaplasia	#					0	0	4	4
	%					0	0	7.2	6.2
Skin		55	55	55	65	55	55	55	65
Dermal inflammation/fibrosis	#	1	1	2	**5**	1	0	0	0
		1.8	1.8	3.6	**7.6**	1.8	0	0	0
Epithelial ulceration	#	1	1	2	**7**	1	0	0	1
	%	1.8	1.8	3.6	**10.8**	1.8	0	0	1.5
Paws	#	2	0	9	10	3	0	1	8
Pododermatitis	#	2	0	7	9	3	0	1	7
Maximum severity (0-4), (#)		2 (2)	0	4 (1)	4 (5)	3 (2)	0	2 (1)	4 (1)

* Statistically significant in **bold** text and yellow highlight.

Toxicokinetics

Blood samples were obtained from 2-4 satellite animals/sex/group at 2, 4, 8 and 24 hours after dosing on Day 1 of treatment, during Week 26 and during week 53. In addition, blood samples were collected at two hours after dosing from 5 main study animals/sex/group at terminal necropsy. Analysis for BIBR 953 ZW was performed using a validated HPLC-MS/MS assay.

After oral administration of the prodrug BIBR 1048 MS, the plasma concentrations of the active moiety BIBR 953 ZW increased rapidly with maximum plasma concentrations generally reached at the first sampling time point (2 hour) after dosing. The plasma concentrations of BIBR 953 ZW then decreased rapidly to low levels between 8 and 24 hours after dosing (Figure 33A). In weeks 1, 26, and 53 both C_{max} and $AUC_{(0\text{-}24\ hr)}$ of BIBR 953 ZW increased dose-dependently for both genders (Figure 33 B, C); however the increase was less than dose proportional. The plasma concentrations of BIBR 953 ZW at 2 hour post dose were similar on day 1 and during weeks 26 and 53 and at necropsy in week 104 indicating no effect of repeated dosing. The plasma concentrations at 2 hour post dose and the $C_{(max)}$ and $AUC_{(0\text{-}24h)}$ during weeks 1, 26 and 53 in Table 116 were similar in males and females indicating a lack of a gender effect.

Table 116: Sponsor's Summary of Toxicokinetics - Study U07-2084

Sponsor's Summary Tables

parameter	week	gender	30 mg/kg	100 mg/kg	200 mg/kg
C(max) [ng/mL]	1	m	772	3920	4840
		f	502	1730	2940
	26	m	544	823	1790
		f	681	1600	2100
	53	m	381	1130	1930
		f	473	1140	2620
AUC(0-24h) [(ng·h)/mL]	1	m	2310	11100	14400
		f	1470	5760	11200
	26	m	2470	4290	9380
		f	2550	8870	11100
	53	m	2720	8040	13400
		f	2000	5270	13300

Overall summary

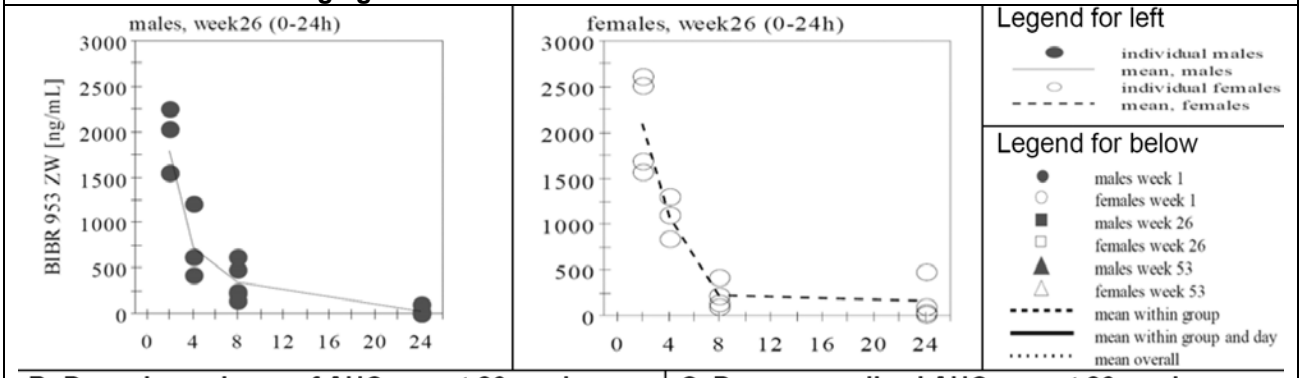
dose [mg/kg]	week	gender	C(max) [ng/mL]	C(max)/dose [ng/mL]/[mg/kg]	AUC(0-24h) [(ng·h)/mL]	AUC(0-24h)/dose [(ng·h)/mL]/[mg/kg]
30	1&26&53	m	543	18.1	2490	83.1
	1&26&53	f	545	18.2	1960	65.2
100	1&26&53	m	1540	15.4	7270	72.7
	1&26&53	f	1470	14.7	6460	64.6
200	1&26&53	m	2560	12.8	12200	60.8
	1&26&53	f	2530	12.6	11800	59.1

Concentrations at 2hr post dose

group	dose [mg/kg]	week	gender	N	mean [ng/mL]	CV (%)
group 2	30	1	m	4	772	28.6
group 2	30	1	f	5	502	22.5
group 2	30	1	m&f	9	622	34.1
group 2	30	26	m	5	544	40.9
group 2	30	26	f	4	681	55.6
group 2	30	26	m&f	9	605	47.8
group 2	30	53	m	5	366	36.5
group 2	30	53	f	5	473	42.9
group 2	30	53	m&f	10	420	40.8
group 2	30	105	m	5	722	49.9
group 2	30	105	f	5	663	34.6
group 2	30	105	m&f	10	693	41.3
group 3	100	1	m	5	3920	23.0
group 3	100	1	f	5	1730	39.3
group 3	100	1	m&f	10	2830	48.7
group 3	100	26	m	4	823	43.9
group 3	100	26	f	5	1600	38.6
group 3	100	26	m&f	9	1250	50.8
group 3	100	53	m	4	1130	31.5
group 3	100	53	f	3	1140	20.4
group 3	100	53	m&f	7	1140	25.1
group 3	100	105	m	5	1920	26.3
group 3	100	105	f	5	2720	51.8
group 3	100	105	m&f	10	2320	46.7
group 4	200	1	m	5	4840	39.6
group 4	200	1	f	4	2940	19.1
group 4	200	1	m&f	9	4000	43.1
group 4	200	26	m	5	1790	18.7
group 4	200	26	f	4	2100	25.8
group 4	200	26	m&f	9	1930	22.8
group 4	200	53	m	5	1930	27.5
group 4	200	53	f	4	2620	12.0
group 4	200	53	m&f	9	2240	25.0
group 4	200	105	m	5	2460	37.8
group 4	200	105	f	5	3780	22.7
group 4	200	105	m&f	10	3120	35.1

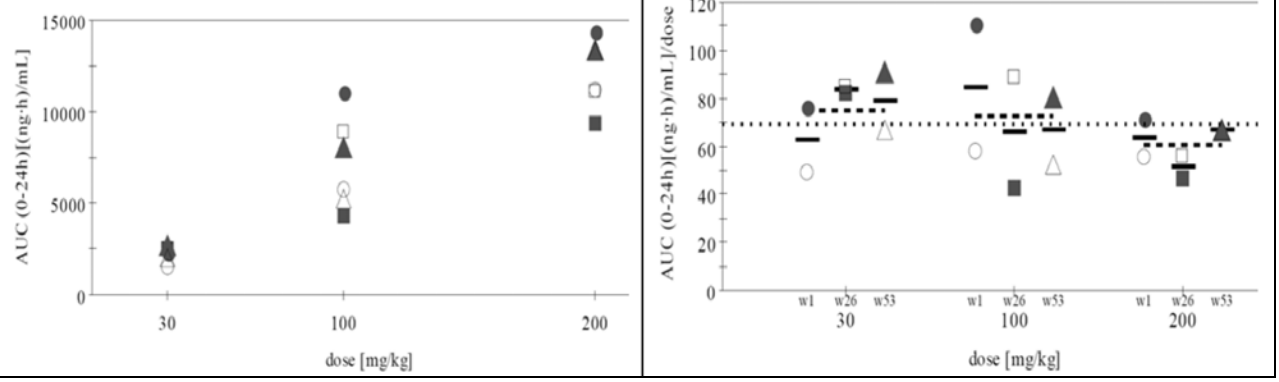
Figure 33: Sponsor's Figures Summarizing Toxicokinetics - Study U07-2084

A. Individual and average plasma concentrations of BIBR 953 ZW after once daily oral (gavage) administration of 200 mg/kg of BIBR 1048 BS for 26 weeks



B: Dose dependence of $AUC_{(0\text{-}24h)}$ at 26 weeks

C. Dose-normalized $AUC_{(0\text{-}24hr)}$ at 26 weeks



Formulation Analysis

The test article formulations were prepared daily and analyzed during Weeks 1, 13, 26, 39, 53, 65, 78, 91 and 103 of treatment for achieved concentration and homogeneity of the test substance in the vehicle. Additional analyses were conducted on the Group 3 formulation during week 5 and the Group 4 formulation during week 6, because replacement formulations were required. Stability of BIBR 1048 MS in the vehicle was previously assessed by the Sponsor.

All dose formulations were homogenous throughout the study. The concentrations of all individual samples were within the range of 86.3 – 97.4 % of nominal and the mean values were within the range of 90.8 – 96.3 %.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: BIBR 1048 MS: Study of Fertility and Early Embryonic Development to Implantation in Rats by Oral Administration, Gavage

Study no.:	02B025 (U05-1550)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	February 17, 2003
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, 98.9%

Key Study Findings

Treatment of Han Wistar males and females with 0, 15, 70, 200 mg/kg BIBR 1048 MS was started 29 and 15 days before mating, respectively, and was continued in females to implantation (Gestation Day 6). The males were sacrificed after mating and the females were sacrificed on Gestation day 14. No compound-related mortality was observed. Paternal toxicity occurred in the high dose males during the pre-mating phase based on decreased body weight gain. Maternal toxicity also occurred in the high dose group during gestation based on decreased body weight gain accompanied by decreased food consumption. Treatment with BIBR 1048 MS did not significantly affect the copulation, fertility and gestation indices. However, treatment with BIBR 1048 MS significantly reduced the mean number of implantations in a dose-dependent manner. Also, pre-implantation loss increased and number of viable fetuses decreased in the mid and high dose groups. The sponsor concluded the NOAEL for embryo toxicity was 70 mg/kg. However, the reviewer concludes the NOAEL for embryo toxicity was 15 mg/kg based on the significant decrease in implantations at 70 mg/kg

The corresponding exposure levels ($AUC_{(0-24h)}$) of BIBR 953 ZW at the no observed adverse effect levels (NOAEL) were based on toxicokinetics data in EFD study U03-1284. For paternal and maternal toxicity the NOAEL was 70 mg/kg with 7320 ng·h/mL); for mating and fertility the NOAEL was 200 mg/kg with a maternal exposure of 22500 ng·h/mL; and for early embryonic development the NOAEL was 15 mg/kg a maternal exposure of 1560 ng•h/mL.

Methods

Doses: 0, 15, 70, 200 mg/kg (BIBR 1048 MS)

Group	m/f per group	Weighed substance	Dose (mg/kg)	Free base equivalent	Dose* (mg/kg)
1	24/24	Natrosol 250 HX (0.5%)	0		0
2	24/24	BIBR 1048 MS	17.7	BIBR 1048 BS	15
3	24/24	BIBR 1048 MS	82.7	BIBR 1048 BS	70
4	24/24	BIBR 1048 MS	236.2	BIBR 1048 BS	200

* free base rounded figures

Dose selection: In a prior embryo-fetal development study (U03-1284), BIBR 1048 MS was dosed orally from Gestation Day 7 (GD) to GD 16 with doses of 15, 70 and 200 mg/kg BIBR 1048 BS. The high dose produced maternal toxicity indicated by two intercurrent deaths, one abortion and increased resorptions associated with slightly decreased body weight, body weight gain and food consumption. Thus, the high dose of 200 mg/kg was expected to produce toxicity in the current study.

Frequency of dosing: Daily

Dose volume: 10 mL/kg

Route of administration: Oral gavage

Formulation/Vehicle: 0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)

Species/Strain: Rat (CrlGlxBrlHan:WI (SPF quality))

Number/Sex/Group: 24/sex/group

Satellite groups: None

Study design: BIBR 1048 MS treatment of males and females was started 29 and 15 days before mating, respectively. After the pre-mating period the females were mated with the males at a 1:1 ratio. Confirmation of mating was performed the following morning by examination of vaginal smears (sperm-positive = GD 1). Mating was limited to a maximum of 20 days. After insemination the pairs were separated and treatment was continued in females to implantation (GD 6). Hysterectomy was performed in the dams on GD14 and parameters and indices of copulation, fertility and gestation determined.

Deviation from study protocol:	For technical reason, three females of the control group were sacrificed on gestation days other than GD 14 (F112 on GD 12, F114 and F119 on GD 16).

Observations

Survival:	Animals were checked twice daily during the work week and once daily during weekends
Clinical signs:	Animals were checked twice daily during the work week and once daily during weekends
Body weight:	The bodyweight of each animal during acclimatization was determined on day 1. During the pre-mating period, the body weight of males was recorded on days 1 to 29 and in females on days 1 to 15. During the mating period the body weight of both genders was recorded on days 1 to 20 and during pregnancy on days GD 1-6 and 14.
Food consumption:	The quantity of food consumed during pre-mating by each animal was determined weekly and during gestation by each female on GD 6 and 14.
Necropsy observations in males:	After the mating period, all males were sacrificed, necropsied and subjected to macroscopic examination. The testes, epididymides, prostate, and seminal vesicles of those males, whose female partners did not become pregnant after the first estrous cycle or whose female partners showed corpora lutea but no implantations, were fixed in Bouin's solution and subjected to histopathological examination. For comparison, the testes, epididymides, prostates and seminal vesicles of ten males who mated during the first estrous cycle were also fixed in Bouin's solution and subjected to histopathological examination.
Necropsy observations in females:	The females were sacrificed on GD 14, necropsied and subjected to macroscopic examination. If necessary, a histological examination followed.
Uterine observations:	The uterus of each female was excised and the number of corpora lutea, number and position of implantation sites, and early or late resorptions were recorded per dam. Uteri without macroscopic implantation sites were stained according to Salewskil [1964, R97-0600].
Toxicokinetics:	TK was not performed in this study because TK results at the same doses were performed in an embryo-fetal development study in rats [U03-1284].
Formulation analysis:	The formulations were prepared daily. Samples were taken for analysis of homogeneity and concentration at the start of dosing in males and at the end of dosing in females.

Other: The estrous cycle was monitored for eight days before and eight days after the start of treatment by daily examination of vaginal secretion and classified as either di-estrous (singular cells), pro-estrous (clumped nucleated epithelial cells), estrous (large cornified epithelial cells), or met-estrous (single cornified and numerous polymorphonuclear cells

Sponsor's calculated indices

$$\text{Pre} - \text{Implatation loss} = 100 \cdot \left(1 - \left(\frac{\text{number of implantations at hysterectomy}}{\text{corpora lutea}}\right)\right)$$

$$\text{Resorption rate} = 100 \cdot \left(\frac{\text{early resorptions} + \text{late resorptions}}{\text{number of implantations at hysterectomy}}\right)$$

$$\text{Copulation index} = 100 \cdot \left(\frac{\text{females successfully mated}}{\text{females paired for mating}}\right)$$

$$\text{Fertility index during mating phase} = 100 \cdot \left(\frac{\text{females successfully mated and pregnant}}{\text{females successfully mated}}\right)$$

$$\text{Gestation index} = 100 \cdot \left(\frac{\text{number of females with live fetuses}}{\text{females pregnant}}\right)$$

Results

Study acceptability

Toxicity was observed and at least 23 litters were available for evaluation. Therefore, the study is acceptable.

Mortality

No mortality occurred in males. One mid-dose female (310) died, because of a dosing error.

Clinical Signs

Several of the high dose males had bloody scabs or wounds. No treatment-related signs were observed in the females

Body Weight

In males during the pre-mating phase, the mean body weight gain was slightly and significantly decreased during the first week and non-significantly decreased at the end of the pre-mating phase. However, during the mating phase the body weight gain of the treated groups increased relative to the control group. In females, the mean body weight gain was significantly decreased in the low and high dose groups and non-significantly decreased in the mid-dose group on GD 6 (Table 117). Although the difference in body weight gain on GD14 in females was not statistically significant, a dose related decrease in body weight gain was evident.

Table 117: Sponsor's Summary - Study U05-1550

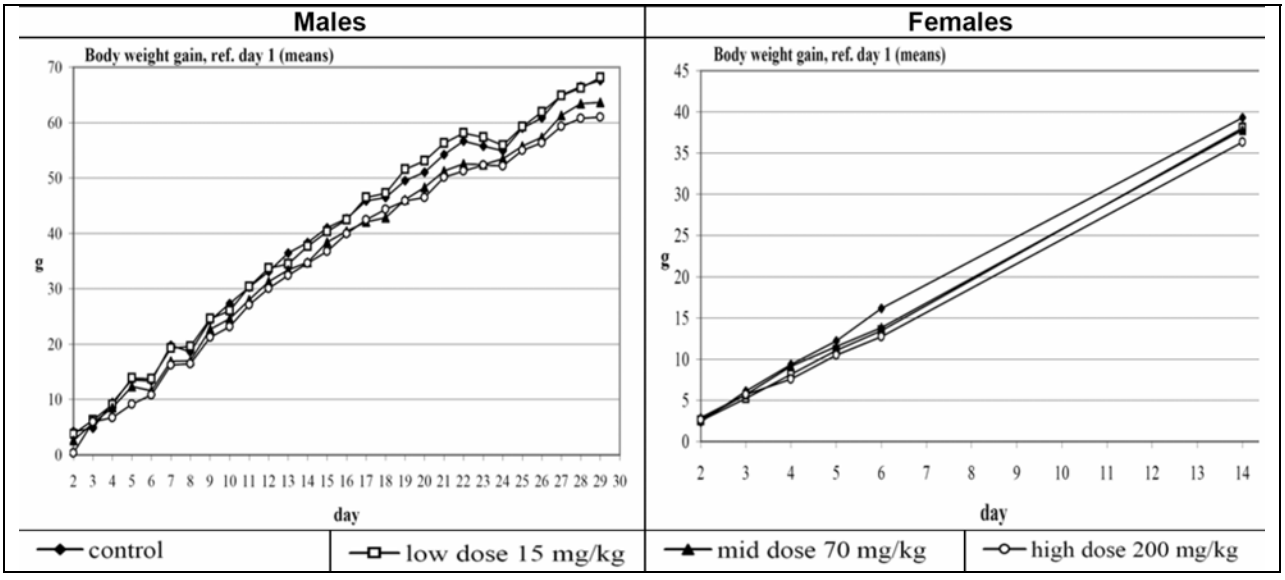
BIBR 1048 BS (mg/kg)	Means of body weight gain in males (administration period) relative to day 1 of the respective phase (g)								
Groups (sample size)	Pre-mating phase							Mating phase	
	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28	Day 29	Day 2	Day 3+
Control (n=24)	4.27	9.48	19.85	38.38	54.28	66.57	67.63	-1.64	-0.54
15 (n=24)	3.82	9.09	19.28	37.72	56.30	66.27	68.17	-2.76	3.01
70 (n=24)	2.68	8.58	16.93	34.81	51.30	63.44	63.67	-0.66	3.98*↑
200 (n=24)	0.37*↓	6.76*↓	16.25*↓	34.62	50.18	60.78	61.03	-0.86	3.20*↑

* significant difference ($p<0.05$)
↓ = decreased; ↑ = increased
\+ = due to mating success decreased sample size, control: n=22; 15 mg/kg: n=12; 70 mg/kg: n=13; 200 mg/kg: n = 17

BIBR 1048 BS (mg/kg)	Mean of body weight gain in females (g)								
Groups (sample size)	Pre-mating phase+				Mating phase		Post-mating phase+++		
	Day 2	Day 4	Day 7	Day 14	Day 2+	Day 4++	GD 2	GD 6	GD 14
Control	-2.16	0.73	4.34	6.96	1.55	5.56	2.37	16.17	39.32
15	0.39*↑	1.60	5.73	9.81*↑	0.58	8.06	2.53	13.47*↓	38.08
70	-1.23	1.07	4.24	7.12	2.63	5.25	2.90	13.86	37.83
200	-2.72	-0.92	2.91	7.19	1.50	4.53	2.67	12.73*↓	36.34

* = significant difference ($p<0.05$)
↑ = increased ; ↓ = decreased ; week = week before mating ; GD = gestation day
\+ = pre-mating phase and Day 2 of mating phase control: n=24;, 15 mg/kg: n=24; 70 mg/kg: n=23; 200 mg/kg: n=24
++ = due to mating success decreased sample size, control: n=17; 15 mg/kg: n=10; 70 mg/kg: n=11; 200 mg/kg: n = 12
+++ = post-mating phase control: n=23(GD 14 = 22); 15 mg/kg: n=18; 70 mg/kg: n=17, 200 mg/kg: n=23

Figure 34: Sponsor's Figures of Body Weight Gain - Study U05-1550



Food Consumption

Food consumption was slightly decreased in the high dose males, although the decrease was not statistically significant. However, a decrease in food consumption was

statistically significant in the low and high dose females on GD 6 and the high dose females on GD14 (Table 118).

Table 118: Sponsor's Summary Tables of Food Consumption - Study U05-1550

Males

BIBR 1048 BS (mg/kg)	Mean food consumption in males (pre-mating administration period) (g)			
Groups (sample size)	Week 1	Week 2	Week 3	Week 4
Control (n=24)	156.69	151.47	149.85	150.99
15 (n=24)	160.50	152.83	152.61	148.22
70 (n=24)	156.48	150.73	147.04	145.85
200 (n=24)	154.89	150.27	148.40	144.76

Week= week before mating

Mean of food consumption in females (g)			
Pre-mating phase		Post-mating phase	
Week 1	Week 2	GD 6	GD 14
104.98 (n=24)	97.49 (n=24)	82.51 (n=23)	149.60 (n=22)
102.38 (n=24)	97.66 (n=24)	78.29 (n=18)*↓	142.84 (n=18)
102.11 (n=22)	96.78 (n=23)	79.65 (n=17)	145.14 (n=17)
97.88 (n=24)*↓	96.80 (n=24)	76.08 (n=23+)*↓	140.48 (n=23+)*↓

↓ = decreased ; GD = gestation day
* = significant difference (p<0.05)
+ = post-mating phase n=23 due to the one pregnant female without sperm

Formulation Analysis

The formulations were homogeneous on both analysis days. The results for all individual formulation samples were within the range of 97.6 - 103.5 % of the nominal concentration. The mean values are in the range of 98.3 – 101.3 %.

Necropsy

One male each in the low and high dose groups was found to have a hematoma on necropsy. The reproductive tracts of all the low and mid-dose males, whose female partners showed corpora lutea, but were non-pregnant, were considered normal by histopathology. No evidence was found to explain their infertility. Histopathology of the reproductive tract could explain the infertility of two of the four other males whose female partners did not become pregnant. One (M274) of two low dose males dose group had vacuolation of Sertoli cells and atrophy of germinal epithelium in small testes and no spermatozoa in the epididymides. The other low dose male (M271) and the one mid-dose male (M351) did not have histopathological changes in their reproductive tracts. The one high dose male (M463) had a small left testis with atrophy of germinal epithelium (Sertoli only) and unilateral severe reduction of the number of spermatozoa along with interstitial fibrosis in the epididymides.

Table 119: Reviewer's Table - Males with Non-Pregnant Partners - Study U05-1550

	Dose, mg/kg			
	0	15	70	200
Number of males with partners that had corpora lutea, but did not become pregnant	0	4	5	
Male ID number		255, 264, 269, 273	355, 359, 364, 366, 368	
Number of other males with partners that did not become pregnant	0	2	1	1
Male ID number		271, **274**	351	**463**
Bold text indicates reproductive histopathology findings				

One low dose female (F224) had elongation of uterine horns and cavum uteri not connected with the vagina. One high dose female (F421) had an enlarged spleen with extramedullary hematopoiesis.

During treatment an irregular estrous cycle was observed in 9, 4, 6 and 7 females in the control, low, mid and high dose groups, respectively.

Mating/Fertility Parameters

Most females (70.8%, 82.6%, 70.8%, and 66.7% in the control, low, mid and high dose groups, respectively) copulated during the first three days of mating. Copulation index was 100% in all groups. All females were pregnant in the control and high dose groups. In the low and mid dose groups, one female each was not pregnant although sperm was found in their vaginal smear. Consequently, the fertility index decreased in these groups, but was within the range of values in the control studies. The gestation index decreased in the control group due to the one female with resorptions only and in the low and mid-dose groups due the 4 and 5 females, respectively, with corpora lutea only. Although the gestation index in the low and mid dose groups is below the minimum of 87.5% observed in the control studies, the gestation index was 100% in the high dose group. Therefore, treatment with BIBR 1048 MS does not affect mating, fertility or gestation.

Table 120: Reviewer's Modification - Mating Outcomes/Fertility - Study U05-1550

	Study U05-1550				Control Studies							
					Study U04-1440				Study U05-1493			
Group	Control	15 mg/kg	70 mg/kg	200 mg/kg	I	II	III	IV	I	II	III	IV
Sample size (n litters)	24	23[+++)]	23	24								
Parameter												
Females with sperm and pregnant	24	22	22	23								
Females without sperm[*)] and pregnant	0	0	0	1								
Females with sperm and not pregnant	0	1	1	0								
Females without sperm and not pregnant	0	1[+++)]	0	0								
Copulation index[#]	100	100[+++)]	100	100[++)]	100	100	100	**83.33**	100	100	100	100
Fertility index[#]	100	95.65	95.65	100	100	100	91.76	**80**	100	100	100	100
Gestation index	95.83	81.82	77.27	100	100	100	100	100	**87.50**	100	95.83	100

[*)] no sperm found in the vaginal smear
[++)] the calculated copulation index was 95.83%. Due to the one pregnant female which did not show sperm in the vaginal smear the copulation index was 100%.
[+++)] female No. 224 showed at necropsy a cavum uteri not connected with the vagina and was, therefore, excluded from further evaluations and from conclusions on effects of the compound on fertility
[#] dams pregnant without sperm found in vaginal smear included

I-IV = control groups
bold letters = indices below 90%
[#] dams pregnant without sperm found in vaginal smear included

Litter Parameters

One control female (124) had resorptions only. No dead fetuses were observed in any group.

The following discussion concerning the litter parameters compares values in the current study (Study U05-1550) with those obtained in two control studies (U04-1440 and U05-1493). Reviews of these control studies follow this review of Study U05-1550.

The mean number of corpora lutea per dam for the high dose group was decreased compared to that in the concurrent control group and was below the range of means in the control studies (Table 121).

Mean numbers of implantations per dam decreased significantly in both the mid and high dose groups compared to those in the concurrent control and low dose groups and were below the range of means in the laboratory's control studies. Since the mean numbers of implantations per dam decreased as the dose of BIBR 1048 MS increased, the sponsor concluded that a relationship of decreased implantation to dose of BIBR 1048 MS was likely.

The mean percentage of pre-implantation loss was significantly increased in the mid dose group and non-significantly increased in the high dose group compared to the control group. The mean percentage of pre-implantation loss for the mid-dose group (10.54%) was above the range of means in the laboratory's control studies. The mean percentage of pre-implantation loss for the high dose group (8.3%) was within the range of means in the laboratory's control studies, but was more than 2 times the concurrent control mean. Comparison of mean percentage of pre-implantation loss in the mid and the high dose groups is confounded by the decrease in the number of corpora lutea in the high dose group.

The mean numbers of viable fetuses per dam decreased in both the mid and high dose groups compared to those in the control and low dose groups and were below the range of means in the laboratory's control studies.

The number of total resorptions, the number of early resorptions, and the resorption rate decreased in the treated groups with the effects greatest in the high dose group compared to those in the concurrent control group. These findings were not considered toxicologically significant because of the significant decrease in implantations.

The reviewer's NOAEL for early embryo toxicity is 15 mg/kg.

Table 121: Sponsor's Summary of Litter Parameters - Study 02B025

Study	(U05-1550)				Control Studies	
Groups	G1: Control	G2: 15 mg/kg	G3: 70 mg/kg	G4: 200 mg/kg	Spontaneous incidences from [U04-1440]	Spontaneous incidences from [U05-1493]
n litters +	23	18	17	24	90	92
Litter parameters						
	Means ranges of individual data				means ranges of means/ ranges of individual data	
Corpora lutea	12.2	11.8	12.0	11.5	12.3	12.1
	10-16	10-14	9-15	9-14	11.9-12.5/ 8-16	11.6-12.5/ 6-16#
Implantations	11.8	11.3	10.7*↓	10.5*↓	11.3	11.3
	9-16	9-14	5-15	7-13	11.0-11.6/ 3-15	10.9-11.5/ 3(3)-16
Viable fetuses	10.3	10.4	9.7	9.8	10.5	10.3
	7-12	8-13	5-14	6-13	10.1-11.0/ 3-14	10.1-10.4/ 1(3)-14
Dead fetuses	0	0	0	0	0	0
Total resorptions	1.43	0.94	1.00	0.75	0.83	0.96
	0[1]-4	0[1]-2	0[1]-5	0[1]-5	0.59-1.15/ 0[1]-3	0.79-1.24/ 0[1]-5
early resorptions	1.17	0.78	0.82	0.54*↓	0.78	0.53
	0[1]-4	0[1]-2	0[1]-4	0[1]-2	0.59-1.15/ 0[1]-3	0.42-0.71/ 0[1]-3
late resorptions	0.26	0.17	0.18	0.21	0.06	0.42
	0[1]-3	0[1]-2	0-1	0[1]-3	0-0.17/ 0-1	0.38-0.52/ 0[1]-3
Pre-implantation loss (%)	3.68	4.02	10.54*↑	8.31	8.01	7.24
	0[7.14]-9.09 (18.18)	0[7.69]-16.67 (25.00)	0[7.14]-28.57 (54.55)	0[7.14]-25.00 (30.00)	7.06-9.50/ 0[6.25]-57.14 (66.67)	5.55-9.07/ 0[7.14]-27.27(100)
Resorption rate (%)	11.82	8.38	8.70	6.87	7.71	9.00
	0[7.69]-27.27 (30.77)	0[7.14]-18.18 (20.00)	0[6.67]-30.77 (38.46)	0[8.33]-33.33 (41.67)	4.88-11.28/ 0[6.67]-22.22 (50.0)	7.40-10.65/ 0[7.14]-33.33(66.67)

+ non pregnant animals excluded
[ ] the lowest number above 0
( ) outlyer included
dam No. 320 with 22 corpora lutea but no implantations
* significant difference ($p<0.05$); ↑ = increased ; ↓ = decreased

Study title: Evaluation of the rat strain CrlGlxBrlHan:WI in a study of fertility and early embryonic development to implantation by oral administration of Natrosol® 250 HX, gavage

Study no.:	00B204 (U04-1440)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	April 2, 2001
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	Natrosol® 250 HX (hydroxyethylcellulose), Batch 101479, purity not indicated

Key Findings:
Because the laboratory converted from the Chbb:THOM strain to the Han Wistar strain of rats, this study provides spontaneous background control data.

Methods

Doses:	No test compound, vehicle only
Dose selection:	Not applicable
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Rat (CrlGlxBrlHan:WI (SPF quality))
Number/Sex/Group:	24/sex/group
Satellite groups:	None
Study design:	BIBR 1048 MS treatment of males and females was started 29 and 15 days before mating, respectively. After the pre-mating period the females were mated with the males at a 1:1 ratio. Confirmation of mating was performed the following morning by examination of vaginal smears (sperm-positive = GD 1). Mating was limited to a maximum of 20 days. After insemination, each pair was separated and treatment was continued in females to implantation (GD 6).. Hysterectomy was performed in the dams on GD14 and parameters and indices of copulation, fertility and gestation determined.
Deviation from study protocol:	At supply the body weight range was 172.1-198.2 g for males and 124.1-146.7 g for females instead of 175-270 g and 130-230 g, respectively as given in the study plan.

Observations

Survival:	Animals were checked twice daily during the work week and once daily during weekends
Clinical signs:	Animals were checked twice daily during the work week and once daily during weekends
Body weight:	The bodyweight of each animal during acclimatization was determined on day 1. During the pre-mating period, the body weight of males was recorded on days 1 to 29 and in females on days 1 to 15. During the mating period the body weight of both genders was recorded on days 1 to 20 and during pregnancy on days GD 1-6 and 14.

Food consumption:	The quantity of food consumed during pre-mating by each animal was determined weekly and during gestation by each female on GD 6 and 14.
Necropsy observations in males:	After the mating period, all males were sacrificed, necropsied and subjected to macroscopic examination. The testes, epididymides, prostate, and seminal vesicles of those males, whose female partners did not become pregnant after the first estrous cycle or whose female partners showed corpora lutea but no implantations, were fixed in Bouin's solution and subjected to histopathological examination. For comparison, the testes, epididymides, prostates and seminal vesicles of ten males who mated during the first estrous cycle were also fixed in Bouin's solution and subjected to histopathological examination.
Necropsy observations in females:	The females were sacrificed on GD 14, necropsied and subjected to macroscopic examination. If necessary, a histological examination followed.
Uterine observations:	The uterus of each female was excised and the number of corpora lutea, number and position of implantation sites, and early or late resorptions were recorded per dam. Uteri without macroscopic implantation sites were stained according to Salewskil [1964, R97-0600].
Other:	The estrous cycle was monitored for 1 week before and 1 week after the start of treatment by daily examination of vaginal secretion and classified as either di-estrous (singular cells), pro-estrous (clumped nucleated epithelial cells), estrous (large cornified epithelial cells), or met-estrous (single cornified and numerous polymorphonuclear cells

Results

Mortality

No mortality occurred.

Clinical Signs

Alopecia and the presence of scabs occurred in some animals.

Body Weight

Body weight gain was comparable among the four groups (Table 122).

Table 122: Sponsor's Summary of Body Weight Gain – Study U04-1440

Groups	Means of body weight gain in males (administration period) relative to Day 1 of the respective phase [g]							
	Premating phase						Mating phase	
	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28	Day 2	Day 5
Control I	3.75* II	11.77* II	22.85	47.58	65.36	77.17	-3.81	-3.95
Control II	1.28* I, III, IV	8.75* I	20.47	44.01	60.38	73.04	-3.75	-1.04
Control III	3.56* II	10.49	19.57	41.23	58.43	70.99	-1.51	3.30
Control IV	3.49* II	10.93	20.43	43.11	61.69	73.85	-2.30	0.62
Overall mean +	3.02	10.48	20.83	44.01	61.50	73.79	-2.86	0.05

male No. 355 excluded
significant difference ($p<0.05$) if compared with Control I = *I , Control II = *II , Control III = *III , Control IV = *IV
+ = calculated from all individual data

Groups	Mean of body weight gain in females [g]								
	Premating phase				Mating phase		Postmating phase		
	Day 2	Day 4	Day 7	Day 14	Day 2	Day 5	GD 2	GD 6	GD 14
Control I	-1.58	1.05* II, IV	6.26	13.29* IV	2.28	8.05	3.59	16.54	45.38
Control II	-0.16* III	3.43* I	6.96	15.74	3.36	9.50	2.28	15.00	42.29
Control III	-2.37* II	2.55	6.38	14.12* IV	3.03	7.73	3.40	15.53	44.26
Control IV	-0.68	3.60* I	6.89	17.50* I, III	2.49	9.06	2.23	14.98	45.17
Overall mean +	-1.20	2.65	6.62	15.16	2.79	8.74	2.92	15.56	44.19

week = week before mating ; GD = gestation day ; + = calculated from all individual data
significant difference ($p<0.05$) if compared with Control I = *I , Control II = *II , Control III = *III , Control IV = *IV

Food Consumption

Food consumption was comparable among the four groups (Table 123).

Table 123: Sponsor's Summary of Food Consumption - Study U04-1440

Groups	Mean food consumption in males (premating administration period) [g]			
	Week 1	Week 2	Week 3	Week 4
Control I	157.55	157.38	160.03	152.50
Control II	151.21	156.24	158.94	153.36
Control III	151.56	155.65	157.55	150.52
Control IV	154.36	156.94	161.23	154.77
overall mean +	153.67	156.56	159.46	152.79

week = week before mating
+ = calculated from all individual data

Groups	Mean of food consumption in females [g]			
	Premating phase		Postmating phase	
	Week 1	Week 2	GD 6	GD 14
Control I	120.83	114.23	84.49* II, IV	151.16* II
Control II	123.97	119.23	90.18* I	164.38*
Control III	120.53	114.43	88.97	160.11
Control IV	120.22	119.03	91.56*	162.79
Overall mean +	121.40	116.75	88.54	159.30

GD = gestation day ; + = calculated from all individual data
significant difference ($p<0.05$) if compared with Control I = *I , Control II = *II , Control III = *III , Control IV = *IV

Necropsy

The most common finding in males was darkened spleen. Histopathology of the reproductive tract could not fully explain the infertility of all males whose female partners showed corpora lutea, but were non-pregnant. However, three out of five of these males had minimal to moderate atrophy of the seminiferous epithelium. Two out of nine males whose female partners became pregnant also showed minimal to slight atrophy of the seminiferous epithelium. One out of nine males whose female partners became pregnant also showed inflammatory cell infiltrates in the prostate.

The most common finding in females (10) was yellowish discolored kidneys which correlated with histology findings of vacuolation in the cortical tubular epithelium in one of these females.

Mating/Fertility Parameters

Most females copulated during the first three days of mating. The minimum copulation index was 83.3% and the minimum fertility index was 80%.

Table 124: Sponsor's Summary of Mating Outcome and Fertility - Study U04-1440

Parameter (%)	Control I	Control II	Control III	Control IV
Sample size (n litters)	24	24	24	24
Females with sperm and pregnant	24	24	22	16
Females without sperm and pregnant	0	0	0	4
Females with sperm and non pregnant	0	0	2	4
Females without sperm and non pregnant	0	0	0	0
Copulation index#	100	100	100	83.33
Fertility index#	100	100	91.67	80
Gestation index	100	100	100	100

dams pregnant without sperm found in vaginal smear included

Litter Parameters

The means of the litter parameters were comparable among the four groups (Table 125). Comparisons were also made between litter parameters in Chbb: THOM and Han Wistar strains. The differences between litter parameters in the two rat strains include decreased mean number of number of corpora lutea, mean numbers of implantations, means of total, early and late resorptions, and number of viable fetuses in the Han Wistar strain than in the Chbb: THOM strain.

Table 125: Sponsor's Summary of Litter Parameters - Study U04-1440

	Control I	Control II	Control III	Control IV	Controls I-IV	Spontaneous incidences from (U03-1549)
n litters *	24	24	22	20	90	85
Litter parameters						
	means ranges of individual data				means ranges of means/ ranges of individual data	
Corpora lutea	11.9 9-15	12.3 9-15	12.5 10-16	12.5 8-15	12.3 11.9-12.5/ 8-16	12.0 11.8-12.3/ 9-15
Implantations	11.0 4-14	11.1 3-15	11.6 8-15	11.5 6-15	11.3 11.0-11.6/ 3-15	11.1 10.9-11.4/ 5-15
Viable fetuses	10.1 3-13	10.5 3-14	11.0 7-14	10.4 3-13	10.5 10.1-11.0/ 3-14	10.5 10.1-10.7/ 5-14
Dead fetuses	0 0	0 0	0 0	0 0	0 0	0 0
Total resorptions	0.96 0[1]-2	0.67 0[1]-2	0.59 0[1]-3	1.15 0[1]-3	0.83 0.59-1.15/ 0[1]-3	0.65 0.27-0.83/ 0[1]-3 (6)
early resorptions	0.79 0[1]-2	0.63 0[1]-2	0.59 0[1]-3	1.15 0[1]-3	0.78 0.59-1.15/ 0[1]-3	0.60 0.27-0.74/ 0[1]-3 (6)
late resorptions	0.17*III, IV↑ 0-1	0.04 0-1	0*I↓ 0	0*I↓ 0	0.06 0-0.17/ 0-1	0.05 0-0.11/ 0-1.0
Pre-implantation loss (%)	7.28 0[6.67]-18.18 (63.64)	9.50 0[7.69]-57.14 (66.67)	7.06 0[6.25]-25.0 (33.33)	8.14 0[7.69]-35.71 (40.0)	8.01 7.06-9.50/ 0[6.25]-57.14 (66.67)	7.57 6.13-10.06/ 0[6.67]-46.15 (61.54)
Resorption rate (%)	9.19*III↑ 0[7.14]-22.22 (25.0)	5.87 0[8.33]-16.67 (18.18)	4.88I↓ 0[6.67]-15.38 (23.08)	11.28 0[7.69]-20.0 (50.0)	7.71 4.88-11.28/ 0[6.67]-22.22 (50.0)	5.70 2.30-7.29/ 0[7.69]-23.08 (54.55)

* non pregnant animals excluded
[ ] the lowest number above 0
( ) outlyer included
significant difference ($p<0.05$) if compared with Control I = *I, Control III = *III, Control IV = *IV

U03-1549 Viertel B, Nolte T. Evaluation of the rat strain CrlGlxBrlHan:WI in a study for effects on embryofetal development by oral administration of Natrosol 250 HX, gavage, 29 July 2003)

Study title: Evaluation of the rat strain CrlGlxBrlHan:WI in a study of fertility and early embryonic development to implantation by oral administration of Natrosol® 250 HX, gavage

Study no.:	02B128 (U05-1493)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany
Date of study initiation:	August 12, 2002
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	Natrosol® 250 HX (hydroxyethylcellulose), Batch 101479, purity not indicated

Key Findings:

Because the laboratory converted from the Chbb:THOM strain to the Han Wistar strain of rats, this study provides spontaneous background control data.

Methods

Doses:	No test compound, vehicle only
Dose selection:	Not applicable
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Rat (CrlGlxBrlHan:WI (SPF quality))
Number/Sex/Group:	24/sex/group
Satellite groups:	None
Study design:	BIBR 1048 MS treatment of males and females was started 29 and 15 days before mating, respectively. After the pre-mating period the females were mated with the males at a 1:1 ratio. Confirmation of mating was performed the following morning by examination of vaginal smears (sperm-positive = GD 1). Mating was limited to a maximum of 20 days. After insemination the pairs were separated and treatment was continued in females to implantation (GD 6).. Hysterectomy was performed in the dams on GD14 and parameters and indices of copulation, fertility and gestation determined.
Deviation from study protocol:	The males and females in Groups I and II were 7 days younger than those in Groups III and IV.

Observations

Survival:	Animals were checked twice daily during the work week and once daily during weekends
Clinical signs:	Animals were checked twice daily during the work week and once daily during weekends
Body weight:	The bodyweight of each animal during acclimatization was determined on day 1. During the pre-mating period, the body weight of males was recorded on days 1 to 29 and in females on days 1 to 15. During the mating period the body weight of both genders was recorded on days 1 to 20 and during pregnancy on days GD 1-6 and 14.
Food consumption:	The quantity of food consumed during pre-mating by each animal was determined weekly and during gestation by each female on GD 6 and 14.

Necropsy observations in males:	After the mating period, all males were sacrificed, necropsied and subjected to macroscopic examination. The testes, epididymides, prostate, and seminal vesicles of those males, whose female partners did not become pregnant after the first estrous cycle or whose female partners showed corpora lutea but no implantations, were fixed in Bouin's solution and subjected to histopathological examination. For comparison, the testes, epididymides, prostates and seminal vesicles of ten males who mated during the first estrous cycle were also fixed in Bouin's solution and subjected to histopathological examination.
Necropsy observations in females:	The females were sacrificed on GD 14, necropsied and subjected to macroscopic examination. If necessary, a histological examination followed.
Uterine observations:	The uterus of each female was excised and the number of corpora lutea, number and position of implantation sites, and early or late resorptions were recorded per dam. Uteri without macroscopic implantation sites were stained according to Salewskil [1964, R97-0600].
Other:	The estrous cycle was monitored for 1 week before and 1 week after the start of treatment by daily examination of vaginal secretion and classified as either di-estrous (singular cells), pro-estrous (clumped nucleated epithelial cells), estrous (large cornified epithelial cells), or met-estrous (single cornified and numerous polymorphonuclear cells

Results

Mortality

No parental mortality occurred.

Clinical Signs

Alopecia and the presence of scabs occurred in some animals.

Body Weight

Body weight gain was lower in groups III and IV than in Groups 1 and II in males during the pre-mating phase and in females on Gestation day 6 (Table 126). This difference was attributed to the older age of Groups III and IV.

Table 126: Sponsor's Summary of Body Weight Gain – Study U05-1493

Groups	Means of body weight gain in males (administration period) relative to day 1 of the respective phase [g]							
	Pre-mating phase						Mating phase	
	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28	Day 2	Day 5
Control I	3.09*III, IV	9.64*III, IV	18.59*III, IV	39.74*III, IV	56.58*III, IV	72.53*III, IV	-5.00	-4.34
Control II	1.80*III, IV	7.86*III, IV	16.68*III, IV	36.76*III, IV	53.30*III, IV	66.69*III, IV	-3.10	-0.71
Overall mean + controls I a.II	2.44	8.75	17.64	38.25	54.94	69.61	-4.05	-2.53
Control III	0.45*I, II	3.70*I, II	11.50*I, II	26.39*I, II	38.33*I, II	49.80*I, II	-3.27	-3.43
Control IV	-0.46*I, II	3.09*I, II	12.00*I, II	26.43*I, II	39.81*I, II	51.55*I, II	-3.13	-3.01
Overall mean + controls III a. IV	-0.01	3.40	11.75	26.41	39.07	50.68	-3.20	-3.22

* = significant difference ($p<0.05$, pooled variances) if compared with Control I = *I , Control II = *II , Control III = *III , Control IV = *IV
+ = calculated from all individual data

Groups	Mean of body weight gain in females [g]								
	Pre-mating phase				Mating phase		Post-mating phase		
	Day 2	Day 4	Day 7	Day 14	Day 2	Day 5	GD 2	GD 6	GD 14
Control I	-2.21	-0.28	2.05*III, IV	9.47	-0.09*III, IV	5.53	4.61*IV	18.21*IV	42.13*IV
Control II	-1.43	0.00	3.68	10.73	0.08*IV	7.25	4.21	17.43*IV	38.86
Overall mean + controls I a. II	-1.82	-0.14	2.86	10.10	0.00	6.39	4.40	17.80	40.39
Control III	-1.32	0.91	5.18*I	9.17	1.75*I	6.49	4.47*IV	15.66	39.49
Control IV	-1.11	0.47	5.44*I	9.44	2.12*I, II	7.24	2.29*I, III	13.97*I, II	37.68*I
Overall mean + controls III a. IV	-1.22	0.69	5.31	9.30	1.93	6.86	3.36	14.79	38.56

* = significant difference ($p<0.05$, pooled variances) if compared with Control I = *I , Control II = *II , Control III = *III , Control IV = *IV
+ = calculated from all individual data

Food Consumption

Food consumption varied among the groups, but was not consistent with the age of the animals. Food consumption was slightly decreased in the high dose males, although the decrease was not statistically significant. However, a decrease in food consumption was statistically significant in the low and high dose females on GD 6 and the high dose females on GD14 (Table 127).

Table 127: Sponsor's Summary of Food Consumption - Study U05-1493

Groups	Mean food consumption in males (pre-mating administration period) [g]			
	Week 1	Week 2	Week 3	Week 4
Control I	154.35	153.67	152.45	152.23
Control II	151.30	152.05	153.88	150.79
Overall mean + controls I and II	152.83	152.86	153.16	151.51
Control III	153.46	151.28	146.34	146.15
Control IV	153.94	150.91	147.18	145.52
Overall mean + controls III and IV	153.70	151.09	146.76	145.84

+ = calculated from all individual data

Groups	Mean of food consumption in females [g]			
	Pre-mating phase		Post-mating phase	
	Week 1	Week 2	GD 6	GD 14
Control I	114.10	112.68*	87.12	151.64
Control II	112.18	109.85*	86.93	149.73
Overall mean + controls I and II	113.14	111.26	87.02	150.64
Control III	111.60	107.39	89.92*	154.72
Control IV	110.03	103.41*	85.29*	147.43
Overall mean + controls III and IV	110.80	105.44	87.56	151.00

GD = gestation day ; + = calculated from all individual data
* = significant difference ($p<0.05$, pooled variances)

Necropsy

One male had chronic progressive nephropathy. Histopathology of the reproductive tract could not explain the infertility of males whose female partners showed corpora lutea, but were non-pregnant. However, one out of four of these males had severe atrophy of the seminiferous epithelium. None of nine males whose female partners became pregnant showed atrophy of the seminiferous epithelium. However, one and four out of nine males whose female partners became pregnant showed inflammatory cell infiltrates in the prostate and the seminal vesicles, respectively. The most common finding in females (2) was yellowish discolored kidneys which had no histopathology correlate.

Mating/Fertility Parameters

Most females copulated during the first three days of mating. The minimum copulation index was 100% and the minimum fertility index was 100%. However, the gestation index was lowered to 87.5% and 95.83% in the Control I and III due to the three and one females with corpora lutea only and no implantations or no viable fetuses.

Table 128: Sponsor's Summary of Mating Outcome and Fertility - Study U05-1493

Parameter (%)	Control I	Control II	Control III	Control IV
Sample size (n litters)	24	24	24	24
Females with sperm and pregnant	24	24	24	24
Females without sperm and pregnant	0	0	0	0
Females with sperm and not pregnant	0	0	0	0
Females without sperm and not pregnant	0	0	0	0
Copulation index	100	100	100	100
Fertility index	100	100	100	100
Gestation index	87.50	100	95.83	100

Litter Parameters

The means of the litter parameters were comparable among the four groups. Comparisons were made between the Han Wistar strain and the Chbb: THOM strain that was previously used in the laboratory. The differences between litter parameters in the two rat strains include decreased mean numbers of corpora lutea, mean numbers of implantations, mean numbers of total, early and late resorptions, and numbers of viable fetuses in the Han Wistar strain than in the Chbb: THOM strain.

Table 129: Sponsor's Summary of Litter Parameters - Study U05-1493

	Control I	Control II	Control III	Control IV	Controls I-IV	Spontaneous incidences from U04-1440
Age (females)	62-66 days		69-73 days		62-73 days	
n litters +	21	24	23	24	92	90
Litter parameters						
	means				means	
	ranges of individual data				ranges of means/ ranges of individual data	
Corpora lutea	12.2	11.6	12.3	12.5	12.1	12.3
	9-16#	6-14	10-16##	10-16	11.6-12.5/ 6-16	11.9-12.5/ 8-16
Implantations	11.5	10.9	11.3	11.3	11.3	11.3
	8-16	3-14	8-14	10(3)-16	10.9-11.5/ 3(3)-16	11.0-11.6/ 3-15
Viable fetuses	10.3	10.1	10.4	10.4	10.3	10.5
	7-13	1-14	7-13	8(3)-14	10.1-10.4/ 1(3)-14	10.1-11.0/ 3-14
Dead fetuses	0	0	0	0	0	0
						0
Total resorptions	1.24	0.79	0.91	0.92	0.96	0.83
	0[1]-5	0[1]-2	0[1]-3	0[1]-4	0.79-1.24/ 0[1]-5	0.59-1.15/ 0[1]-3
early resorptions	0.71	0.42	0.52	0.50	0.53	0.78
	0[1]-2	0[1]-2	0[1]-3	0[1]-2	0.42-0.71/ 0[1]-3	0.59-1.15/ 0[1]-3
late resorptions	0.52	0.38	0.39	0.42	0.42	0.06
	0[1]-3	0[1]-2	0[1]-2	0[1]-3	0.38-0.52/ 0[1]-3	0-0.17/ 0-1
Pre-implantation loss (%)	5.55	6.10	8.05	9.07	7.24	8.01
	0[7.69]-27.27(100)	0[7.14]-16.67(66.67)	0[7.69]-25.0(100)	0[7.14]-23.08(75.0)	5.55-9.07/ 0[7.14]-27.27(100)	7.06-9.50/ 0[6.25]-57.14 (66.67)
Resorption rate (%)	10.65	9.76	8.35	7.40	9.00	7.71
	0[7.14]-27.27(41.67)	0[7.14]-33.33(66.67)	0[7.69]-20.0(27.27)	0[7.69]-25.0(33.33)	7.40-10.65/ 0[7.14]-33.33(66.67)	4.88-11.28/ 0[6.67]-22.22 (50.0)

+ non-pregnant animals excluded
[ ] the lowest number above 0
( ) outlayer included. Animals with 100% pre-implantation loss were excluded from calculation.
dam nos. 111 with 13, 116 with 14 and 121 with 11 corpora lutea but no implantations were excluded from calculation.
320 with 22 corpora lutea but no implantation were excluded from calculation.

The litter parameters in the current EED study (U05-1493) were compared to the litter parameters obtained another EED study (U04-1440) and an EFD study (U03-1549, study not submitted).

Table 130: Sponsor's Comparison of Litter Parameters in Three Studies

	U05-1493 (02B128)		U04-1440 (Table 129)		U03-1549	
Evaluation studies	Data from the present study with the strain CrlGlxBrlHan:WI		Data from `Evaluation of the rat strain CrlGlxBrlHan:WI in a study of fertility and early embryonic development to implantation by oral administration of Natrosol® 250 HX, gavage´ (U04-1440)		Data from `Evaluation of the rat strain CrlGlxBrlHan:WI in a study for effects on embryo-fetal development by oral administration of Natrosol 250 HX, gavage´ (hysterectomy on GD 22; U03-1549)	
	Means ++	Ranges of means/ individual data	Means ++	Ranges of means/ individual data	Means ++	Ranges of means/ individual data
n litters +	92		90		85	
Corpora lutea	12.1	11.6-12.5/6-16§	12.3	11.9-12.5/8-16	12.0	11.8-12.3/9-15
Implantations	11.3	10.9-11.5/3(3)-16	11.3	11.0-11.6/3-15	11.1	10.9-11.4/5-15
Viable fetuses	10.3	10.1-10.4/1(3)-14	10.5	10.1-11.0/3-14	10.5	10.1-10.7/5-14
Dead fetuses	0	0	0	0	0	0
Total resorptions	0.96	0.79-1.24/0[1]-5	0.83	0.59-1.15/0[1]-3	0.65	0.27-0.83/0[1]-3# (6)
early resorptions	0.53	0.42-0.71/0[1]-3	0.78	0.59-1.15/0[1]-3	0.60	0.27-0.74/0[1]-3# (6)
late resorptions	0.42	0.38-0.52/0[1]-3	0.06	0-0.17/0-1	0.05	0-0.11/0-1.0
Pre-implantation loss (%)	7.24	5.55-9.07/0[7.14]-27.27(100)	8.01	7.06-9.50/0[6.25]-57.14 (66.67)	7.57	6.13-10.06/0[6.67]-46.15# # (61.54)
Resorption rate (%)	9.00	7.40-10.65/0[7.14]-33.33(66.67)	7.71	4.88-11.28/0[6.67]-22.22 (50.0)	5.70	2.30-7.29/0[7.69]-23.08# (54.55)

+ non-pregnant animals excluded
++ overall means from the four controls
[ ] the lowest number above 0
( ) outlayer included. Animals with 100% pre-implantation loss were excluded from calculation.
§ dam nos. 111 with 13, 116 with 14, 121 with 11 and 320 with 22 corpora lutea but no implantations were excluded from calculation
# one outlayer (No. 319) excluded (in brackets: No. 319 included)
# # one outlayer (No. 222) excluded (in brackets: No. 222 included)

U03-1549 - Viertel, B., Nolte, T. Evaluation of the rat strain CrlGlxBrlHan:WI in a study for effects on embryo-fetal development by oral administration of Natrosol 250 HX, gavage, 29 July 2003 (Study report was not submitted)

9.2 Embryonic Fetal Development

Study reports for two embryo-fetal development studies, one in rats [U03-1284] and one in rabbits [U02-1648], and a dose range-finding study conducted in rabbits [U01-1820] were submitted and reviewed with the original submission to IND 65813. The detailed reviews of these studies are in the IND review dated 9/24/03.

However, the NDA 22-512 submission contained amendments to these studies. The embryo-fetal development study U02-1648i n rabbits was reinterpreted based on additional control studies submitted with the NDA. The reviewer confirmed that the data did not change, only the interpretation. To facilitate understanding of the revised interpretations, relevant portions of the previous reviews are provided below.

The sponsor and the reviewer differ in the determination of the NOAEL in the rat embryo-fetal development study U03-1284. Therefore, the reviewer requested a consult from the Reproductive and Developmental Toxicology Subcommittee of the Pharmacology and Toxicology Coordinating Committee. The committee responses (Appendix 10) support the reviewer's NOAEL determination.

Study title: BIBR 1048 MS: Study for Effects on Embryo-Fetal Development in Rats by Oral Administration (Gavage).

Study no:	U03-1284 (00B056, amendment date 4/24/03)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany
Date of study initiation:	06/16/00
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, batch 8050461, purity 99.4%

Key Study Findings

In an adequate embryo-fetal development study, BIBR 1048 MS was administered orally at 0, 15, 70 and 200 mg/kg from gestation day 7 to 16 to Han Wistar rats that were sacrificed on gestation day 22.

Two deaths occurred in the high dose group with one of these deaths accompanying an abortion. Two high dose dams showed resorptions only. A significant decrease in body weight gain and food consumption was observed in the mid and high dose groups. In the high dose group, the total, early and late resorptions increased significantly with values outside the control range. Consequently, the resorption rate was significantly increased and the mean number of viable fetuses was decreased at the high dose, which can be considered above a maximally tolerated dose because of the deaths. In the mid dose group, increased resorption rate and decreased number of viable fetuses also occurred; although the values were not statistically significant, they were outside the mean historical range. Fetal body weight in the high dose group was significantly decreased and was below the control range.

A rare malformation (cleft thoracal vertebral body) was observed only in the treated groups and not in the concurrent control group. The incidence increased with dose [(low (1/116, 0.9%), mid (1/91, 1.1%), and high dose group (2/66, 3.0%)] and was above the historical control range (1/465, 0.21%). The sponsor maintains that another malformation (flat and thickened ribs) occurred with no relation to dose because the incidences in the control (9.1%), the low (7.8%), and high (7.6%) dose groups were similar to the spontaneous background (9.7%) and only the incidence in the mid dose group(16.5%) was increased. However, the lack of a dose relationship may have been due to the excessive maternal toxicity (deaths and fetal resorptions) in the high dose group that confounds determination of any dose-relatedness of the vertebral anomaly.

Although most of the variations were either singular or distributed similarly across all groups, the incidences of ossification delay of the supraoccipital bone, the occipital bone, cervical vertebral bodies, and calcaneum were increased in the BIBR 1048 MS treated groups. These variations could be the result of either fetal developmental delay resulting from maternal toxicity or a pharmacodynamic effect of BIBR 1048 MS on thrombin, which is important for osteoblast function and proliferation.

The sponsor concluded that BIBR 1048 MS is not teratogenic in rats and that the NOAELs for maternal and embryo/fetal toxicity were 15 and 70 mg/kg, respectively. Based on the findings of decreased number of viable fetuses, increased number of resorptions and resorption rate in the mid dose group, the reviewer concludes that BIBR 1048 MS was clearly embryo toxic in rats and the NOAELs for both maternal and embryo/fetal toxicity were 15 mg/kg corresponding to a maternal exposure (AUC) of 1560 ng.hr/mL. It is not known whether the embryotoxicity is intrinsic or extra-embryonic.

Observations

For detailed review of Study 00B056, see review of N000 submission to IND 65, 813, dated 9/24/03. The following information from that review is provided to facilitate reading the current document.

Results

Study acceptability

The study was acceptable since the mid and high dose groups demonstrated maternal toxicity and at least 16 litters were evaluable.

Mortality

The sponsor considered the deaths of two high dose dams (F416 on GD16 and F411 on GD17 and the abortion occurring in one of these dams (F411) as compound related. Both deaths were associated with blood in the vagina and in the bedding.

Clinical signs

One high dose dam (F411) that died had an abortion of one fetus and ten resorptions (two early and eight late). Two high dose dams showed resorptions only. Blood in the vagina was observed in toxicokinetic animals, one in the low dose group, one in the mid dose group and five in the high dose group. Blood was observed on the bedding of four high dose dams. Bleeding in this study is consistent with the pharmacodynamic activity of BIBR 1048 MS, but is not related to parturition, as the dams were sacrificed on GD22.

Body Weight

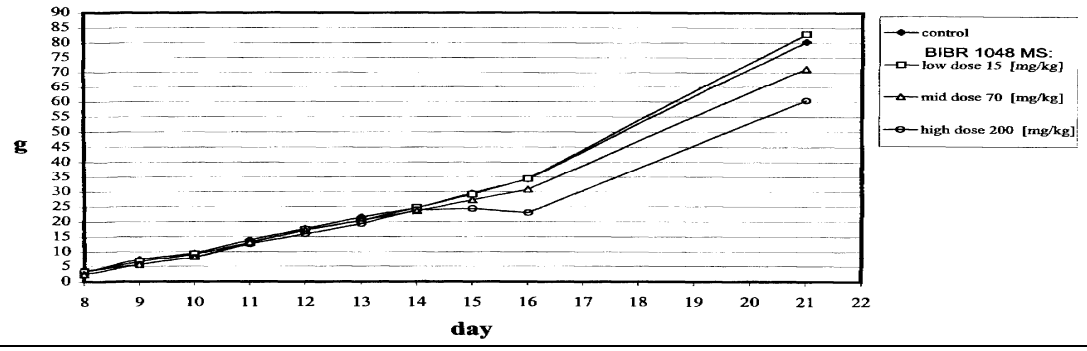
At the end of treatment on gestation day 16, BIBR 1048 MS at 70 and 200 mg/kg produced a 5 and 33% decrease, respectively, in body weight gain in the dams relative to gestation day 7. At study termination, the mid- and high doses decreased body weight gain relative to gestation day 7 by 11 and 25%, respectively.

Table 131: Sponsor's Summary of Body Weight – Study U03-1284

Mean of body weight gain [g] relative to GD 7

Dose BIBR 1048 BS (mg/kg)	Mean of body weight gain (g) relative to GD 7					
	GD 8	GD 9	GD 10	GD 13	GD 16	GD 21
Control	3.17	7.44	9.55	21.56	34.27	80.39
15	3.22	6.58	9.20	20.43	34.40	82.99
70	2.39	5.60↓*	8.10	20.44	30.84	71.30↓*
200	3.56	5.58	8.13	19.33	22.98↓*	60.46↓*

* significant difference ($p<0.05$), ↓ decreased, GD = gestation day



Food consumption

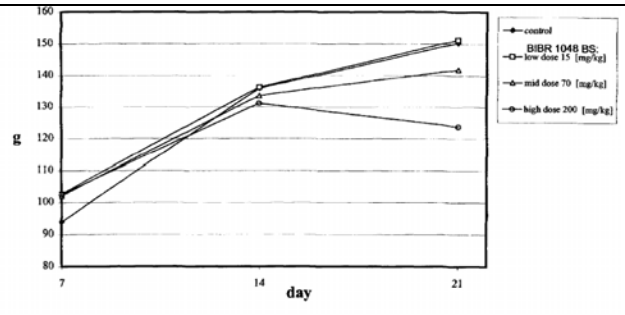
At study termination, the mid- and high doses decreased food consumption in the dams by 6 and 18%, respectively.

Table 132: Sponsor's Summary of Food Consumption – U03-1284

Food consumption, mean gm per week

Dose BIBR 1048 BS (mg/kg)	Food consumption, mean (g)		
	GD 7	GD 14	GD 21
Control	93.93	135.82	150.33
15	102.65	136.17	151.25
70	102.09	133.51	141.87↓*
200	102.56	131.13	123.76↓*

* significant difference ($P<0.05$) ↓ decreased, GD = gestation day



Toxicokinetics

On gestation day 16, blood was collected for determination of the concentration of BIBR 953 ZW. The observed values for the low dose are similar to the values (Cmax, 528 ng/mL; AUC 1156 ng.hr/mL) obtained for the low dose females in a 4-week toxicology study in rats (Study U98-2729). However, the values for the mid- and high doses were almost two-fold higher than those obtained in the 4-week study. This comparison must take into account the fact that the Ch:THOM strain was used in Study U98-2729, whereas Han Wistar rats were used in the current study.

Table 133: Reviewer's Modification of Sponsor's TK Summary - Study U03-1284

Calculated geometric mean (gmean) Cmax and $AUC_{(0-24h)}$ values of BIBR 953ZW					
Gestation day/ treatment day	Dose [mg/kg/day] BIBR 1048 MS (BIBR 1048 BS)	C_{max} [ng/ml]	gCV (%)	AUC_{0-24h} [(ng·h)/ml]	gCV (%)
16/10	17.3 (15)	633	39.0	1470	40.0
16/10	80.7 (70)	2560	9.1	7200	21.1
16/10	230.6 (200)	4280	33.9	20800	47.0

From 4-week study in rats (Study U98-2729)– females only					
Day	Dose	Cmax		$AUC_{(0-24h)}$	
	[mg/kg]	[ng/mL]	gCV%	[ng.hr/mL]	gCV%
1	17.3 (15)	381.6	14.3	971.0	25.3
25	17.3 (15)	528.0	17.7	1156	6.1
1	80.5 (70)	1129	27.7	4742	15.1
25	80.5 (70)	1315	42.5	4642	25.8

Necropsy

Both the dams that died had light brownish livers and dilation of the atria. The uterus of one of these dams was filled with blood. In the dams completing the study, yellowish discolored kidneys were seen in all dose groups including the control. One dam each in the low and high dose groups had light brownish discolored livers. Five high dose dams had enlarged spleens with mild diffuse increase in extramedullary hematopoiesis that is consistent with anemia secondary to blood loss and the pharmacodynamic activity of BIBR 1048 MS. One dam each in the mid and high dose groups had uterine horns filled with blood that again is consistent with the pharmacodynamic activity of BIBR 1048 MS.

Formulation Analysis

Analysis of the formulations at the start of treatment indicated deviations from nominal of no more than 1.7%.

Cesarean Section Data and Litter Parameters

No effects on litter parameters were observed for the low dose group (Table 134). In the mid dose group, the mean fetal body weight was significantly increased, but was within the historical range. The sex ratio was changed in favor of females and was outside the control range. The number of viable fetuses decreased and the resorption rate in the mid dose group increased with the values outside the mean historical range. In the high dose group, the total, early and late resorptions increased significantly with values outside the control range. Consequently, the resorption rate was significantly increased and the mean number of viable fetuses was significantly decreased. Fetal body weight in the high dose group was significantly decreased and was below the control range. Although no dead fetus was found in any group, one runt was found in the low dose group.

Table 134: Sponsor's Summary of Litter Parameters - Study U03-1284

	Control	15 mg/kg	70 mg/kg BIBR 1048 BS	200 mg/kg	Spontaneous incidences from evaluation study (Viertel 2003, Study No. 00B203, Draft) mean/range	
n litters +	27	22	20	16	85	
Litter parameters (means)					means	ranges of means/individual data
Corpora lutea	10.9	11.6	10.8	11.1	12.0	11.8-12.3/9.0-15.0
Implantations	10.1	10.6	9.9	10.6	11.1	10.9-11.4/9.0-15.0
Viable fetuses	9.3	10.0	8.7	7.6*	10.5	10.1-10.7/5.0-14.0
Dead fetuses	0.0	0.0	0.0	0.0	0	0
Sex (%)						
male	58.65	53.90	43.11*	49.01	49.14	46.92-52.18/18.18-80.0
female	41.35	46.10	56.89*	50.99	50.59	47.82-53.08/20.0-81.82
Total resorptions	0.9	0.6	1.3	2.9*	0.6	0.3-0.8/0[1.0]-3.0# (6.0)
early resorptions	0.8	0.6	1.2	2.0*	0.6	0.3-0.7/0[1.0]-3.0# (6.0)
late resorptions	0.1	0	0.1	0.9*	0	0-0.1/0-1.0
Fetal weight (g)	4.96	5.04	5.09*	4.72*	5.03	4.90-5.13/2.3-6.3
Praeimplantation loss (%)	6.99	8.27	8.43	4.52	7.57	6.13-10.06/0[6.67]-46.15## (61.54)
Resorption rate (%)	7.65	5.47	13.15	27.93*	5.70	2.30-7.29/0[7.69]-23.08# (54.55)

* significantly different to control I ($p<0.05$)
\+ non pregnant animals excluded
\# one outlayer (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlayer included
\## one outlayer (No. 222) excluded (in brackets: No. 222 included); mean calculated with the outlayer included
[ ] the lowest number greater than 0

Offspring – Malformations and Variations

The total frequency of malformations was similar in the control, low and high dose groups, but was higher in the mid dose group (Table 135, Table 136). However, given the maternal toxicity, the decreased number of viable fetuses, and the increased resorption rate in the high dose group, the dose administered may have been excessive and above an appropriate level to evaluate the effects of BIBR 1048 MS on embryo-fetal organogenesis.

Most malformations were singular. However, one malformation (cleft thoracal vertebral body) was observed only in the BIBR 1048 MS treated groups and not in the concurrent control group. The frequency of this malformation increased with dose [(low (1/116, 0.9%), mid (1/91, 1.1%), and high dose group (2/66, 3.0%)]. Given the low numbers, this apparent dose related increase may be a spontaneous chance effect. However, the spontaneous background incidence of this malformation is very low (1/465, 0.21%) in the sponsor's historical controls, the reviewer finds it difficult to believe that the 4 fetuses in this study could have this finding by chance alone. It is also possible that cleft thoracal vertebral body is just a more severe form of the delay in ossification

The sponsor maintained that incidence of one malformation (flat and thickened ribs) had no relation to dose. In the control (9.1%), the low, and high dose groups (7.8 and 7.6%), the frequencies on a per fetus basis were below the spontaneous background (9.7%); however, the frequency increased (16.5%) in the mid dose group. When evaluated on a per litter basis (Table 136, see Appendix 11), again only the mid-dose group had a higher frequency of this malformation than the control group. Because of the significant maternal toxicity and increased fetal resorptions at the high dose, the high dose group may not display a higher frequency of this malformation. Therefore, an effect of BIBR 1048 MS on the increased frequency of this malformation in the mid-dose group, which received approximately 1/3 of a maternal lethal dosage, cannot be excluded.

Table 135: Sponsor's Summary of Malformations – Study U03-1284

Findings	Control	BIBR 1048 BS 15 mg/kg	BIBR 1048 BS 70 mg/kg	BIBR 1048 BS 200 mg/kg	Spontaneous incidences+ from validation study (U03-1549)
	n (%)	n (%)	n (%)	n (%)	n (%)
Malformations					
External n fetuses	n = 251	n = 220	n = 173	n = 122	n = 889
Brachygnathia inferior	-	-	1 (0.57)	-	-
Visceral n fetuses	n = 119	n = 104	n = 82	n = 56	n = 424
Proximal double ureter	1 (0.84)	-	-	-	-
Cleft in the soft palate	-	-	1 (1.21)	-	-
Skeletal n fetuses	n = 132	n = 116	n = 91	n = 66	n = 465
2nd thoracal vertebral body asymmetrical	-	1 (0.86)	-	-	-
Flat and thickened ribs	12 (9.09)	9 (7.75)	15 (16.48)	5 (7.57)	45 (9.67)
Wavy ribs	-	1 (0.86)	-	-	-
Cleft thoracal vertebral body	-	1 (0.86)	1 (1.09)	2 (3.03)	1 (0.21)
Z shaped ribs	2 (1.51)	-	-	-	2 (0.43)

Table 136: Reviewer's Summary of Malformations – Study U03-1284

Rat Segment II study Abnormalities in fetuses	Number (%) 0 mg/kg	15 mg/kg	70 mg/kg	200 mg/kg	Historical control§ (%)
Malformations					
Analysis by fetus					
Total number of fetuses	251	220	173	122	889
Total percentage malformations	11.44	10.33	19.35	10.6	
Number of skeletal evaluations	132	116	91	66	465
Total skeletal malformation (% fetuses evaluated)	14 (10.5)	12 (10.3)	16 (17.6)	7 (10.6)	
Flat and thickened r bs (% fetuses evaluated)	12 (9.09)	9 (7.75)	15 **(16.48)**	5 (7.57)	45 (9.67)
Cleft thoracal vertebral body (% fetuses evaluated)	0 (0)	1 (0.86)	1 (1.09)	2 **(3.03)**	1 (0.21)
Analysis by litter					
Total litter	27	22	20	16	88*
Litters with any skeletal malformation (% litter)	5 (18.5)	6 (27)	9 **(45)**	5 (31)	
Litters with Flat and thickened ribs (% litters) [number of fetuses per litter]	5 (18.5) [2.4]	5 (22.7) [1.8]	8 **(40)** **[1.8]**	3 (18.8) [1.7]	22.5^ (25.6) [2]
Litters with Cleft thoracal vertebral body (% litters)	0 (0)	1 (4.5)	1 (5.0)	2 (12.5)	1 (1.1)

§ Historical Controls provided by sponsor, * Assumes 10.1 fetuses per litter, ^ Assumes 2 fetuses per litter displaying this malformation

Most of the variations were either singular or distributed similarly across all groups. However, the incidence of ossification delay of the supraoccipital bone, the occipital bone, cervical vertebral bodies, vertebral arch, forelimbs (paw) and the calcaneum were increased in the BIBR 1048 MS treated groups. The increased incidences of delayed ossification indicate delayed fetal development possibly due to the associated maternal toxicity, a common relationship in such studies (Carney and Kimmel 2007). Alternatively, the delayed ossification may be a pharmacodynamic effect, since thrombin is critical for the function and proliferation of osteoblasts (Pagel et al 2006) and thrombin inhibition by BIBR 953 ZW could affect bone formation.

Table 137: Reviewer's Summary - Variations in Study U03-1284

Rat Segment II study Abnormalities in fetuses	Number (%) 0 mg/kg	15 mg/kg	70 mg/kg	200 mg/kg	Historical control§ (%)
Variations (% fetuses evaluated)					
Number of skeletal evaluations	132	116	91	66	465
Ossification delay supraoccipital bone	5 (3.77)	2 (1.72)	7 (7.68)	11 **(24.63)**	18 (4.08)
Ossification delay cervical vertebral bodies	22 (16.7)	28 (24.1)	27 (29.7)	26 **(39.4)**	76 (16.3)
Split or displace sternebrae	5 (3.78)	7 (6.03)	3 (3.29)	6 **(9.09)**	12 (2.58)
Ossification delay sacral vertebral arch	6 (4.54)	14 (12.08)	12 (13.18)	5 (7.57)	17 (3.65)
Lumbar ribs	75 (56.8)	84 (72.4)	62 (68.1)	53 (80.3)	329 (70.75)
Ossification delay calcaneum	55 (41.66)	68 (58.62)	48 (52.74)	39 (59.09)	225 (48.38)
§ Historical Controls provided by sponsor					

The sponsor concluded that BIBR 1048 MS is not teratogenic. The reviewer agrees that BIBR 1048 MS is not overtly teratogenic in the rat; however, BIBR 1048 MS is clearly embryotoxic in the rat based on the findings of increased resorption rate, increased number of resorptions and decreased number of viable fetuses. The embryotoxicity could be direct and/or extra-embryonic e.g., interference with progestational factors such as prolactin. The sponsor maintains that the NOAELs for maternal and embryo/fetal toxicity were 15 mg/kg and 70 mg/kg corresponding to maternal exposures (AUC) of 1560 and 7320 ng.hr/mL at the dose of 15 and 70 mg/kg, respectively. However, the reviewer concludes that the NOAELs for both maternal and embryo/fetal toxicity in the rat EFD study are 15 mg/kg.

Study title: BIBR 1048 MS: Study for Effects on Embryo-Fetal Development in Rabbits by Oral Administration (Gavage).

Study no:	U02-1648 (00B058, amendment date 4/11/06)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany
Date of study initiation:	09/09/00
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, batch 8050461, purity 99.4%

Key Study Findings

In an adequate embryo-fetal development study, BIBR 1048 MS was administered orally at 0, 15, 70 and 200 mg/kg from gestation day 6 to 18 to presumptive pregnant Himalayan rabbits (Chbb:HM) that were sacrificed on gestation day 29. Mean body weight gain decreased 10% in the high dose group. Although treatment with BIBR 1048 MS did not significantly affect most of the litter parameters, the percentage of males in the high dose group was below the minimum of the historical range. Additionally, the number of total resorptions, number of early resorptions, and the resorption rate were increased in the high dose group compared to the concurrent control group and were above the historical control mean. Based upon submission of a report for an additional

control study in the NDA, the findings of missing gall bladder, dilated cerebral ventricle, additional vessels at the aortic arch and hypoplasia of gall bladder in the treated fetuses can be attributed to normal spontaneous variation in rabbits. The maternal NOAEL is 70 mg/kg based on decreased body weight gain and the fetal NOAEL is also 70 mg/kg based on increased resorption. These NOAELS correspond to exposures of 2770 ng.hr/mL determined in the dose-range finding study.

Observations and Results

The detailed reviews of Study U02-1648 and Study U01-1820 (a dose-range finding study) are in the review dated 9/24/03 of submission N000 of IND 65, 813. The following information from that review is provided to facilitate reading the current document. A review of the additional control study (U05-1804) follows this information.

Study acceptability

The study was acceptable since the high dose group demonstrated maternal toxicity and at least 18 litters were evaluable.

Body weight

Although BIBR 1048 MS did not produce a statistically significant effect on body weight, the gain in body weight in the high dose group was 10% less than that in the control group at the end of treatment.

Table 138: Sponsor's Summary of Body Weight Gain - Study U02-1648

Mean of body weight gain [g] relative to GD 6

Dose (mg/kg BIBR 1048 BS)	GD 7	GD 8	GD 10	GD 14	GD 18	GD 21	GD 28
Control	-6.62	-6.99	5.14	52.73	127.08	146.57	287.87
15	1.23	-2.82	3.77	43.48	112.96	149.48	289.08
70	-8.68	-8.10	-2.17	51.29	125.58	157.94	298.09
200	-7.60	-4.28	-6.00	48.26	123.47	138.71	259.37

Food consumption

The table of food consumption in the review of N000 is incorrect. The correct table (Table 139) is below. Although mean weekly food consumption was not significantly affected by treatment, food consumption during the first week of treatment decreased 7.2% in the high dose group compared to that in the control group.

Table 139: Sponsor's Summary of Food Consumption - Study U02-1648

Dose (mg/kg BIBR 1048 BS)	Week 1	Week 2	Week 3	Week 4
Control	812.68	834.83	823.44	745.93
15	779.31	852.71	848.62	769.11
70	774.52	799.21	850.44	748.02
200	753.71	797.71	814.71	726.65

Formulation Analysis

Analysis of the formulations at the start and end of the treatment period indicated deviations from nominal of no more than 2.2%.

Toxicokinetics

The plasma levels of BIBR 951 ZW and Sum BIBR 951 ZW at 2 hours after dosing on treatment day 8 (GD 13) in the present study are comparable with the Cmax values at 2 hours after dosing in the dose range finding study (U01-1820, 00B057) as demonstrated below (Table 140).

Table 140: Reviewer's Compilation of TK Parameters – Studies U02-1648 and U01-1820

Plasma levels at 2 hours after dose administration on GD 13 in Study U02-1648		BIBR 953 ZW		Sum BIBR 951 ZW	
	Dose, mg/kg	Cmax	AUC (0-24h)	Cmax	AUC (0-24h)
		Mean ng/mL		Mean ng/mL	
	15	128		140	
	70	761		765	
	200	1810		1740	

TK parameters based on plasma levels measured on GD 13 at 0, 2, 4, 8, 24 hours post treatment in Study U01-1820	BIBR 953 ZW				Sum BIBR 951 ZW			
	Cmax		AUC (0-24h)		Cmax		AUC (0-24h)	
	dose [mg/kg]	mean [ng/ml]	%CV	mean ([ng·h]/ml)	%CV	mean [ng/ml]	%CV	mean ([ng·h]/ml)	%CV
	15	165	20.2	976	34.8	180	19.0	1060	35.6
	70	512	27.7	2550	5.0	560	33.9	2770	6.7
	200	2120	66.3	13500	78.2	1750	59.0	8210	49.1

Cesarean Section and Litter Data

Treatment with BIBR 1048 MS did not significantly affect the mean number of corpora lutea, implantations, pre-implantation loss, number of viable fetuses, and fetal weight. However, the percentage of males in the high dose group was below the minimum of the historical range. Additionally, the number of total resorptions/litter, number of early resorptions/litter and the resorption rate were increased in the high dose group compared to the concurrent control group. Although the means for these parameters in the high dose group are within the historical control ranges and are not statistically significant, they are above the historical control mean. The number and percentage of dams displaying resorptions increased in the high dose group (12, 57%) compared to the numbers and percentages in the other groups (8-9, 40-45%).

Table 141: Sponsor's Summary of Litter Parameters - Study U02-1648

Parameter (means)	Control	15 mg/kg BIBR 1048 BS	70 mg/kg BIBR 1048 BS	200 mg/kg BIBR 1048 BS	Historical data (mean/range)
Corpora lutea	8.0	8.1	8.3	8.0	7.5/6.4-8.4
Implantations	7.9	7.7	8.1	7.8	7.0/6.1-7.8
Viable fetuses	7.0	6.9	7.5	6.7	6.5/5.3-7.4
Dead fetuses	0.1[+)]	0.0	0.0	0.0	0.01/0-0.08
Sex (%)					
male	50.92	45.82	47.12	43.36	52/48-59
female	49.08	54.18	52.88	56.64	48/41-52
total resorptions	0.8	0.8	0.7	1.1	0.5/0.2-1.2
early resorption	0.8	0.8	0.6	1.0	0.4/0.1-1.1
late resorption	0.1	0.0	0.1	0.1	0.1/0-0.1
Fetal weight (g)	37.70	38.93	37.66	37.66	38.3/35.9-40.3
Preimplantation loss (%)	1.67	6.02	2.01	2.60	6.5/1.7-13.1
Resorption rate (%)	10.55	9.39	8.88	13.39	7.3/4.2-14.4

[+)] due to one dead fetus each in the litter no. 103 and 110

Offspring Malformations and Variations

The malformation of missing gall bladder was found in one fetus of the control group, in one of the mid-dose group and in two fetuses each in the low and high dose groups. Since a clear dose-dependency was missing, the sponsor previously excluded this malformation as an effect of the compound. The amendment to the study report indicates that missing gall bladder has been observed in other rabbit studies, including one fetus (0.99%) of the control group in Study U05-1726 (03B102) and a singular missing gall bladder without relationship to dose in Study U96-2578. Furthermore, in a control study (U05-1804, 02B189) missing gall bladder occurred in three control groups at spontaneous incidences of 2.08%, 1.47%, and 0.74% with an overall mean of 1.44%. Additionally, a publication by Morita et al. (1987) indicated a frequency of 0.16% (range: 0 - 0.98%) for Japanese White rabbits. These data support the exclusion of missing gall bladder as an effect of the compound in the present study.

The frequencies of most skeletal and visceral variations were equally distributed among the dose groups without any apparent relation to dose or were singular findings. Such variations found in all dose groups included additional vessels at the carotid arteries and common trunk of right and left carotid the artery. However, the incidence of three variations (dilated cerebral ventricle, additional vessel at the aortic arch, hypoplasia of gall bladder and shortened ductus choledochus) increased in the treated groups.

The first variation of dilated cerebral ventricle was only found in the BIBR1048 MS treated groups, increased with dose and was above the frequency in the historical controls. A search of the MARTA database (accessed in 2003) indicated 5 incidences dilated cerebral ventricle out of 14291 rabbit fetuses evaluated. However, the sponsor maintains that this finding is often seen in this strain and cites a spontaneous incidence of 1.35% in the control group of another study (U96-2578). This spontaneous incidence is above the incidence in the low and mid dose groups, but below the incidence of 3.57% in the high dose group. Furthermore, since the finding of dilated cerebral

ventricle occurred only in one litter (F421), the sponsor argues that the finding should be considered as a single occurrence because the litter is regarded as the biological and experimental unit. However, a compound-related effect cannot be unequivocally excluded at the high dose because it occurred in five fetuses of this litter.

Although the incidence of the second variation of additional vessels at the aortic arch in all groups was elevated over the incidence of the historical control (20.7%), the incidence in the high dose group (45.7%) was above that in the concurrent control group (36.4%). However, an incidence of 29.8% was observed in a control evaluation study (02B189) and an incidence of 30.7% was found in the control group of another study (U02-1648). These more recent control incidences are above the historical control incidence, suggesting more variation in the incidence of this finding than observed in the historical control data set. Therefore, a relationship to compound administration is equivocal.

The incidence of the third variation of hypoplasia of the gall bladder was also elevated in all groups over the value of the historical control (0.18%). Although the incidence of this variation was elevated in the BIBR 1048 MS treated groups (4.83 - 5.36%) over that in the concurrent control group (2.85%), a clear relationship to dose was lacking. Furthermore, in a control study (U05-1804), hypoplasia of the gall bladder occurred in three control groups at spontaneous incidences of 4.17%, 4.41%, and 2.94% with an overall mean of 3.85%. Therefore, the incidences of hypoplasia of the gall bladder in the present study were considered to be close to the current spontaneous incidence and not related to compound administration.

In addition, a shortened ductus choledochus was seen in one fetus each in the low and high dose groups (0.8% and 0.71%, respectively). However, in a control evaluation study (U05-1804), shortened ductus choledochus occurred in one control group at a spontaneous incidence of 1.47% with an overall mean of 0.48%. Therefore, a clear relationship to treatment is lacking in the present study.

The sponsor maintains that spontaneous deviations of extrahepatic biliary tract anatomy from normal are described in literature for rabbits including the Himalayan rabbit. The sponsor cites references indicating frequencies of 25.9% (Lee 1978), 0.91% (Palmer 1968), 0.62% (Lehmann et al. 1986), and 0.49% (Matsuo and Kast 1995).

The sponsor concluded that both the maternal and fetal NOAELs were 200 mg/kg. The reviewer concluded that the maternal NOAEL was 70 mg/kg, because of the clinical signs and reduced body weight gain at 200 mg/kg and that the fetal NOAEL was also 70 mg/kg because of the altered fetal sex ratio and increased resorptions at 200 mg/kg.

Table 142: Sponsor's Summary of Fetal Findings - Study U02-1648

Findings/Group n fetuses	Group 1 n =140	Group 2 n =124	Group 3 n =149	Group 4 n =140	Historical Data/%
		Dose mg/kg			
	Control n (%)	15 n (%)	70 n (%)	200 n (%)	
Runts	2 (1.42)	1 (0.80)	1 (0.67)	-	1.04 +
Flexures	5 (3.57)	4 (3.22)	4 (2.68)	1 (0.71)	0.95 +
Malformations:					
External:					
Microglossia	-	3 (2.41)	2 (1.34)	-	0.01
Less muscles of extremities	-	3 (2.41)	2 (1.34)	-	-
Joints of extremities stiffened	-	3 (2.41)	2 (1.34)	-	-
Skull hernia	1 (0.71)	1 (0.80)	-	-	-
Spina bifida (sacral)	-	1 (0.80)	-	-	
Visceral:					
Parts of brain not developed	-	1 (0.80)	-	-	-
Missing gallbladder	1 (0.71)	2 (1.61)	1 (0.67)	2 (1.42)	0.29
Diaphragm transparent	-	3 (2.41)	1 (0.67)	-	-
Doubled gallbladder with three drainages	1 (0.71)	-	-	-	-
Right A.subclavia at the dorsal side of aortic arch	1 (0.71)	-	-	-	-
Right A.subclavia at the A. pulmonalis	1 (0.71)	-	-	-	-
Skeletal:					
Fused sternebra	2 (1.42)	-	-	-	0.23
Missing (lumbar) vertebra	1 (0.71)	-	-	-	-
Variations:					
Visceral:					
Dilated cerebral ventricle	-	1 (0.80)	1 (0.67)	5 (3.57)	0.05+
Additional vessels at the aortic arch	51 (36.42)	44 (35.48)	50 (33.55)	64 (45.71)	20.66+
Additional vessels at the A. carotid	25 (17.85)	17 (13.70)	36 (24.16)	21 (14.99)	24.16+
Common trunk of right and left a. carotid	3 (2.14)	6 (4.83)	5 (3.35)	4 (2.85)	2.83+
4th artery at the aortic arch	3 (2.14)	1 (0.80)	-	-	-
Base of carotids separated	-	-	-	1 (0.71)	-
Common trunk of right and left pulmonary artery at the A. pulmonalis	2 (1.42)	-	2 (1.34)	-	-
Incompletely subdivided lung	1 (0.71)	-	-	-	0.21+
Hypoplasia of gallbladder	4 (2.85)	6 (4.83)	8 (5.36)	7 (4.99)	0.18+
Shortened ductus choledochus	-	1 (0.80)	-	1 (0.71)	-
Small testes	-	-	-	1 (0.71)	-
Skeletal:					
Cervical ribs	1 (0.71)	1 (0.80)	2 (1.34)	-	-
Lumbar ribs	1 (0.71)	1 (0.80)	-	-	0.52+
Ossif. delay of sternebra <5	1 (0.71)	-	-	-	0.04+
Split sternebra	1 (0.71)	-	4 (2.68)	-	-
Additional (thoracal) vertebra with ribs	-	1 (0.80)	-	-	0.10+
Ossification delay of talus	1 (0.71)	-	-	-	0.04+

Study title: Evaluation of the Rabbit Strain Chbb:HM in a Study for Effects on Embryo-Fetal Development by Oral Administration of Natrosol 250 HX, Gavage

Study no:	U05-1804 (02B189)
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharma KG, Biberach an der Riss, Germany
Date of study initiation:	09/26/02
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	Natrosol® 250 HX (hydroxyethylcellulose), Batch 101486, purity not indicated

Key Study Findings

Natrosol® 250 HX was administered orally from gestation day 6 to 18 to presumptive pregnant Himalayan rabbits (Chbb:HM) that were sacrificed on gestation day 29 to establish data on laboratory specific spontaneous deviations in this strain under the new breeding and rearing conditions. The sponsor concluded that intrauterine survival of embryos and fetuses fluctuated more widely in the rabbits bred by (b) (4) than in those breed by Boehringer Ingelheim Pharma GmbH & Co. KG. Also, deviations in fetal morphology occurring at low spontaneous incidences such as common trunk of right and left pulmonary artery at the A. pulmonalis, hypoplasia of gallbladder, missing gallbladder, persistent foramen interventriculare (VSD), lumbar ribs and split sternebrae were increased in the fetuses from rabbits bred by (b) (4)

Methods

Doses:	Only vehicle was administered
Frequency of dosing:	Daily from gestation day 6 to 18
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Himalayan rabbits (Chbb:HM)
Number/Sex/Group:	21 females
Satellite groups:	None

Study design: The females were dosed from day 6 up to and including day 18 of pregnancy. All animals were sacrificed on Day 29 of pregnancy. The following parameters and endpoints were evaluated: mortality, clinical signs, body weight (days 1, 21, 28 of pregnancy and daily during treatment), food consumption (weekly), and macroscopic pathology. The pregnancy performance parameters included fetal weight, sex, number of corpora lutea, number and position of implantation sites, live/dead fetuses and early or late resorptions. The fetuses were examined externally for malformations and variations.

Observations and Results

Mortality

No mortality occurred.

Clinical Signs

No significant clinical sign was observed.

Body Weight

Table 143: Sponsor's Summary of Body Weight – Study U05-1804

Groups	GD 1#	GD 7	GD 8	GD 10	GD 14	GD 18	GD 21	GD 28
t-test, Control Group I comparison with Control Groups II and III								
Control I (n=20)	-51.92	11.93	14.13	27.06	73.03	122.51	115.50	213.12
Control II (n=20)	-53.54	3.51	1.55	10.80	43.76*	99.79	96.30	189.36
Control III (n=20)	-67.33	-5.09*	-8.30*	0.19*	37.30*	80.61*	93.04	172.86
t-test, control II comparison with controls III and I								
Control II	-53.54	3.51	1.55	10.80	43.76	99.79	96.30	189.36
Control III	-67.33	-5.09	-8.30	0.19	37.30	80.61	93.04	172.86
Control I	-51.92	11.93	14.13	27.06	73.03*	122.51	115.50	213.12
t-test, control III comparison with controls I and II								
Control III	-67.33	-5.09	-8.30	0.19	37.30	80.61	93.04	172.86
Control I	-51.92	11.93*	14.13*	27.06*	73.03*	122.51*	115.50	213.12
Control II	-53.54	3.51	1.55	10.80	43.76	99.79	96.30	189.36
Overall mean +	-57.60	3.45	2.46	12.69	51.36	100.97	101.70	191.78

before administration of Natrosol , * significant difference ($p<0.05$) , GD Gestation Day
+ = calculated from all individual data

Food Consumption

Table 144: Sponsor's Summary of Food Consumption - Study U05-1804

Groups	GD 7/Week 1	GD 14/Week 2	GD 21/Week 3	GD 28/Week 4
Control I	744.46	828.72	644.21	602.96
Control II	718.85	752.38	620.71	573.12
Control III	780.35	780.66	656.93	608.94
Overall mean +	747.94	786.55	641.30	594.77

+ calculated from all individual data

Necropsy

Table 145: Sponsor's Summary of Most Frequent Findings - Study U05-1804

Finding	Frequency, data base: 58 cases in 38 does n (% cases)
calcification in the aortic arch	16 (27.6)
cysts on the liver (1-4)	10 (17.2)
growth on the adrenals	8 (13.8)
cysts on the ovaries (1-2, uni- or bilateral)	6 (10.3)
cysts on the adrenals	5 (8.6)
small gall bladder	4 (6.9)

Cesarean Section and Litter Parameters

Table 146: Sponsor's Summary of Litter Parameters - Study U05-1804

Groups	Control I	Control II	Control III	Overall mean++	Ranges of means/individual data
n litters +	20	20	20	60	
	Means				
corpora lutea	7.8	7.6	7.6	7.6	7.6-7.8/5-11
implantations	7.4	7.3	7.2	7.3	7.2-7.4/3-11
viable fetuses	7.2	6.8	6.8	6.9	6.8-7.2/2-10
dead fetuses	0	0.1	0.1	<0.1	0-0.1/0-1
sex (%)					
male	41.11	39.38	48.30	42.93	39.38-48.30/0[14.29]-83.33
female	58.89	60.62	51.70	57.07	51.70-60.62/16.67-100
total resorptions	0.15	0.45	0.35	0.32	0.15-0.45/0-2
early resorptions	0.15	0.40	0.30	0.28	0.15-0.40/0-2
late resorptions	0	0.05	0.05	0.03	0-0.05/0-1
fetal weight (g)	34.29	35.30	35.05	34.88	34.29-35.30/18.4-45.3 (44.9-47.9) #
pre-implantation loss (%)	5.94	3.48	3.96	4.46	3.48-5.94/0[11.11]-42.86 (66.67) #
resorption rate (%)	1.91	7.19	4.91	4.67	1.91-7.19/0[11.11]-25.0 (50.0) ##

+ non-pregnant animal No. 118 and animals with corpora lutea only Nos. 205, 304 excluded; pre-implantation loss for animals with corpora lutea only was calculated as 100%
++ calculated from all individual data
# outlier No. 305 in brackets
outlier No. 204 in brackets
[ ] the lowest number greater than 0

Breeder	(b) (4)		Boehringer Ingelheim Pharma GmbH & Co. KG		
Source of data	Data from the present study		Historical data 1968-1999 (Viertel and Trieb, 2003, R04-0190)		Historical data from 10 studies before 10 October, 1998
	Overall means	Ranges of means/individual data	Overall means and standard deviations	Ranges of means	Means/ranges of means
n litters +	60		1144		158
corpora lutea	7.6	7.6-7.8/5-11	8.4 ± 0.7	6.4-10	7.5/6.4-8.4
implantations	7.3	7.2-7.4/3-11	7.3 ± 0.7	5.1-8.5	7.0/6.1-7.8
viable fetuses	6.9	6.8-7.2/2-10	6.8 ± 0.7	4.8-8.0	6.5/5.3-7.4
dead fetuses	<0.1	0-0.1/0-1	-	-	0.01/0-0.08
sex (%)					
male	42.93	39.38-48.30/0[14.29]-80.00(83.33) #	49.9 ± 5.9	37.0-64.8	51/48-59
female	57.07	51.70-60.62/16.67-85.71(100) #	50.1-± 5.9	35.2-63.0	49/41-52
total resorptions	0.32	0.15-0.45/0-2	0.6± 0.3	0-1.25	0.5/0.2-1.2
early resorptions	0.28	0.15-0.40/0-2	-	-	0.4/0.1-1.1
late resorptions	0.03	0-0.05/0-1	-	-	0.1/0-0.1
fetal weight (g)	34.88	34.29-35.30/18.4-45.3 (44.9-47.9) #	37.7± 1.6	33.2-41.4	38.3/35.9-40.3
pre-implantation loss (%)	4.46	3.48-5.94/0[11.11]-42.86 (66.67) #	-	-	6.5/1.7-13.1
resorption rate (%)	4.67	1.91-7.19/0[11.11]-25.0 (50.0) #	7.5± 3.8	0-15.3	7.3/4.2-14.4

+ non pregnant animals excluded
# outlier in brackets
[ ] the lowest number greater than 0
- no data available

Offspring – Malformations and Variations

Table 147: Sponsor's Summary of Fetal Findings - Study U05-1804

Element	n fetuses (percentage [%])			
	Groups			
	Control I n=20 litters 144 fetuses	Control II n=20 136 fetuses	Control III n=20 136 fetuses	overall mean + n=60 416 fetuses
Forelimb				
flexure (right/left)	1(0.69)/0	1(0.74)/ 1(0.74)	4(2.94)/0	6(1.44)/1(0.24)
Head				
encephalocele	0	1(0.74)	0	1(0.24)

Bone	n fetuses (%)			
	Groups			
	Control I n=20 litters 144 fetuses	Control II n=20 136 fetuses	Control III n=20 136 fetuses	overall mean + n=60 416 fetuses
Cranium				
Calotte				
enlarged fontanelle	0	1(0.74)	0	1(0.24)
Upper jaws				
atrophy of tooth germ	0	1(0.74)	0	1(0.24)
Ribs				
lumbar ribs				
bilateral	1(0.69)	1(0.74)	0	2(0.48)
right	0	2(1.47)	0	2(0.48)
left	2(1.39)	4(2.94)	2(1.47)	8(1.92)
additional thoracal segment with ribs	0	1(0.74)	0	1(0.24)
Sternebrae				
split 1st sternebra in lateral axis (horizontally)	2(1.39)	1(0.74)	0	3(0.72)
fused sterenebrae				
2 and 3th	0	0	1(0.74)	1(0.24)
4 and 5th	0	1(0.74)	0	1(0.24)
2 – 4th	0	0	1(0.74)	1(0.24)
3 – 5th	0	0	1(0.74)	1(0.24)

Element	n fetuses (%)			
	Groups			
	Control I n=20 litters 144 fetuses	Control II n=20 litters 136 fetuses	Control III n=20 litters 136 fetuses	overall mean + n=60 litters 416 fetuses
dilated cerebral ventricle (1. and 2. = side ventricle)	0	1(0.74)	1(0.74)	2(0.48)
cauliflower-like increase of circumference in the region of the rhombencephalon	0	1(0.74)	0	1(0.24)
cyst in the caudal part of the brain cavity; mild dilatation of aqueductus mesencephali	0	0	1(0.74)	1(0.24)
two cysts in the caudal part of the brain cavity	0	0	1(0.74)	1(0.24)
missing cerebral parts/hydrocephalus internus (at histopathological examination)	0	1(0.74)	5(3.68)	6(1.44)
detailed information after histopathological examination				
moderate hydrocephalus internus: moderate dilatation of 3rd and side ventricles, aqueduct severely dilated, 4th ventricle severely dilated	0	0	1(0.74)	1(0.24)
moderate hydrocephalus internus: aqueduct moderately dilated	0	0	1(0.74)	1(0.24)
moderate hydrocephalus internus: moderate dilatation of 3rd ventricle, mild dilatation of side ventricles, aqueduct severely dilated	0	0	1(0.74)	1(0.24)
moderate hydrocephalus internus: aqueduct moderately dilated, 4th ventricle severely dilated	0	0	1(0.74)	1(0.24)
moderate hydrocephalus internus: 4th ventricle severely dilated	0	0	1(0.74)	1(0.24)
severe hydrocephalus internus	0	1(0.74)	0	1(0.24)

Element	n fetuses (%)			
	Groups			
	Control I n=20 litters 144 fetuses	Control II n=20 litters 136 fetuses	Control III n=20 litters 136 fetuses	Overall mean + n=60 litters 416 fetuses
Thorax				
Aortic arches				
additional vessel at the aortic arch				
one	48(33.33)	36(26.47)	35(25.74)	119(28.61)
two	2(1.39)	3(2.21)	0	5(1.20)
additional vessel at the A. carotis				
one right	42(29.17)	37(27.21)	37(27.21)	116(27.88)
one left	1(0.69)	0	1(0.74)	2(0.48)
one right and one left	0	1(0.74)	0	1(0.24)
two right	2(1.39)	5(3.68)	0	7(1.68)
two left	1(0.69)	0	0	1(0.24)
one right and two left	0	0	1(0.74)	1(0.24)
common trunk of carotids at the aortic arch	9(6.25)	8(5.88)	3(2.21)	20(4.81)
common origin of carotids at the aortic arch	7(4.86)	3(2.21)	6(4.41)	16(3.85)
distance between carotids	2(1.39)	1(0.74)	1(0.74)	4(0.96)
dilated truncus brachiocephalicus	0	1(0.74)	0	1(0.24)
origin of right A. subclavia from A. pulmonalis	2(1.39)	0	0	2(0.48)
ductus arteriosus Botalli dorsal of trachea	1(0.69)	0	0	1(0.24)
right A. carotis partially contracted	0	1(0.74)	0	1(0.24)
common trunk of pulmonary arteries at the A. pulmonalis	2(1.39)	6(4.41)	6(4.41)	14(3.37)
common origin of pulmonary arteries at the A. pulmonalis	1(0.69)	4(2.94)	12(8.82)	17(4.09)
venous system				
bifurcation of V. subclavia and V. jugularis caudally displaced / V subclavia and V. jugularis intertwisted	1(0.69)	0	0	1(0.24)

Element	n fetuses (%)			
	Groups			
	Control I n=20 litters 144 fetuses	Control II n=20 litters 136 fetuses	Control III n=20 litters 136 fetuses	Overall mean + n=60 litters 416 fetuses
Heart				
ventricular septal defect (VSD) persistent foramen interventriculare	0	2(1.47)	1(0.74)	3(0.72)
Lung				
missing lobus accessorius dexter of lung	15(10.42)	12(8.82)	12(8.82)	39(9.38)
missing left lobulation of lung	0	0	1(0.74)	1(0.24)
reduced cranial part of left lung	1(0.69)	0	0	1(0.24)
Diaphragm				
translucent centrum tendineum of diaphragm	2(1.39)	0	2(1.47)	4(0.96)
diaphragm fused with liver	1(0.69)	0	0	1(0.24)
Abdomen				
Gallbladder				
missing gall bladder	3(2.08)	2(1.47)	1(0.74)	6(1.44)
small gall bladder (hypoplasia of gall bladder)	6(4.17)	6(4.41)	4(2.94)	16(3.85)
ductus choledochus shortened	0	2(1.47)	0	2(0.48)
Urinary system				
missing kidney	0	1(0.74)	0	1(0.24)
missing ureter (bilateral)	0	1(0.74)	0	1(0.24)
bowed ureter				
bilateral	0	0	2(1.47)	2(0.48)
left	0	1(0.74)	2(1.47)	3(0.72)
wavy ureter (left)	2(1.39)	0	0	2(0.48)
dilated ureter (cranial)	1(0.69)	0	0	1(0.24)
double urinary bladder	0	1(0.74)	0	1(0.24)
persistent left mesonephros (missing left metanephros)	0	1(0.74)	0	1(0.24)
Reproductive organs				
missing ovaries	0	1(0.74)	0	1(0.24)
ovaries with hemorrhage	0	1(0.74)	0	1(0.24)
small ovary (right)	0	1(0.74)	0	1(0.24)
missing right uterine horn	0	1(0.74)	0	1(0.24)
shortened left uterine horn	1(0.69)	0	0	1(0.24)

Element	n fetuses (%)			
	Groups			
	Control I n=20 litters 144 fetuses	Control II n=20 136 fetuses	Control III n=20 136 fetuses	overall mean + n=60 416 fetuses
Liver				
yellowish focus on the lobus medialis sinister of the liver	1(0.69)	0	0	1(0.24)
whitish focus on the liver (at histopathology acute/subacute necrosis of liver parenchyma)	0	4(2.94)	3(2.21)	7(1.68)
indurate (pinhead-size) on the lobus medialis dexter of the liver (at histopathology moderate acute congestion)	0	1(0.74)	0	1(0.24)
Spleen				
edge of spleen whitish (at histopathology no changes observed)	2(1.39)	0	0	2(0.48)
Adrenals				
additional tissue on the cranial pole of the left adrenal (at histopathology no changes observed)	0	0	1(0.74)	1(0.24)
Eye				
lens (right) proximally displaced (at histopathology no changes observed)	1(0.69)	0	0	1(0.24)
cavity ventro-lateral of the left eye (at histopathology unilateral cystic dilatation)	1(0.69)	0	0	1(0.24)

+ calculated from all individual data

Table 148: Sponsor's Comparison to Other Control Data - Study U05-1804

Breeder	(b) (4)		
Source of data	Data from the present study	Control data from embryo-fetal study Viertel et al. 2002 (U02-1648) #	Control data from embryo-fetal study Viertel et al. 2005 (U05-1726) #
Findings	n=416 fetuses	n =140 fetuses	n=101 fetuses
	n (%)		
Malformations:			
External:			
skull hernia	1(0.24)##	1(0.71)	0
Visceral:			
ventricular septal defect (VSD) persistent foramen interventriculare	3(0.72)	0	1(0.99)
missing gallbladder	6(1.44)	1(0.71)	1(0.99)
doubled gallbladder with three drainages	0	1(0.71)	0
right A.subclavia at the dorsal side of aortic arch	0	1(0.71)	0
right A.subclavia at the A. pulmonalis	2(0.48)	1(0.71)	0
Skeletal:			
fused sternebra	4(0.96)	2(1.42)	0
missing (lumbar) vertebra	0	1(0.71)	0

% decimals truncated
encephalocele
0 no findings

Breeder	(b) (4)		
Source of data	**Data from the present study**	**Control data from embryo-fetal study Viertel et al. 2002 (U02-1648)**	**Control data from embryo-fetal study Viertel et al. 2005 (U05-1726)**
Findings	**n=416 fetuses**	**n=140 fetuses**	**n=101 fetuses**
	n (%)		
Runts	3(0.72)	2(1.42)	1(0.99)
Flexure	7(1.68)	5(3.57)	1(0.99)
Variations:			
Visceral:			
VSD minor	0	0	1(0.99)
additional vessels at the aortic arch	124(29.81)	51(36.42)	31(30.69)
additional vessels at the A. carotid	128(30.76)	25(17.85)	30(29.70)
common trunk of right and left a. carotid	20(4.81)	3(2.14)	17(16.83)
4th artery at the aortic arch	0	3(2.14)	0
base of carotids separated (distance between carotids)	4(0.96)	0	3(2.97)
common trunk of right and left pulmonary artery at the A. pulmonalis	14(3.37)	2(1.42)	2(1.97)
incompletely subdivided lung	0	1(0.71)	0
missing lobus accessorius of lung	39(9.38)	0	10(9.90)
hypoplasia of gallbladder	16(3.85)	4(2.85)	0
shortened ductus choledochus	2(0.48)	0	0
small spleen	0	0	2(1.98)
Skeletal:			
cervical ribs	0	1(0.71)	0
lumbar ribs	12(2.88)	1(0.71)	1(0.99)
ossif. delay of sternebra <5	0	1(0.71)	0
split sternebra	3(0.72)	1(0.71)	0
4th cervical vertebral body unilaterally ossified	0	0	1(0.99)
ossification delay of talus	0	1(0.71)	0

9.3 Prenatal and Postnatal Development

Study title: BIBR 1048 MS: Study for Effects on Pre- and Postnatal Development including Maternal Function in Rats by Oral Administration, Gavage

Study no:	04B182 (U06-1586)
Study report location:	EDR
Conducting laboratory and location:	Department of Non-Clinical Drug Safety Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
Date of study initiation:	March 14, 2005
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 8250250, 98.9%

Key Study Findings

In an acceptable , pre and postnatal development study, pregnant Han Wistar rats received 0, 15, 30, 70 mg/kg of BIBR 1048 MS daily from gestation day 6 to lactation day 21. Exposure to BIBR 951ZW was similar on gestation day 7 and lactation day 5 with a mean $AUC_{(0\text{-}24hr)}$ of 1450, 2760, and 6390 ng.hr/mL in the 15, 30 and 70 mg/kg dosage groups, respectively.

Treatment related F_0 mortalities were associated vaginal bleeding and occurred primarily, but not exclusively, during parturition with one and four deaths in the low and high dose groups, respectively. Vaginal bleeding also occurred in other high dose dams. The percentage of dams with delayed labor (GD 22 to GD 23) increased in the treated groups (14.3 to 33%) compared to the percentage in the control group (9.1%). F_0 body weight decreased in the high dose group during gestation and in both the mid and high dose groups during lactation. These decreases were associated with decreases in food consumption.

Although the number of implantation sites per F_0 dam was similar across all groups, the mean post-implantation loss and mean number of dead F1 offspring were increased in the high dose group resulting in a significantly decreased birth index. Survival of offspring after birth to LD 4 was lower in the mid and high dose groups compared to the low dose and control groups. Although four high dose F_0 litters had offspring with abnormalities and only one control litter had an offspring with abnormalities, these abnormalities were considered incidental.

Treatment of F_0 dams with BIBR 1048 MS during gestation and lactation did not significantly affect sex ratio, body weight at birth, body weight development, and postnatal survival after lactation day 4 or delay general physical development of the F_1 offspring. Neurological assessments showed no significant effect of BIBR 1048 MS treatment on F_1 reflexes, sensory functions, learning ability, memory and explorative behavior. Treatment of F_0 dams with BIBR 1048 MS during pregnancy and lactation did not impair the fertility of the F_1 animals or the intrauterine survival of the F_2 embryos pre- and post implantation.

The NOAEL for maternal (F_0) toxicity was 30 mg/kg, the NOAEL for pre/perinatal (to lactation day 4) toxicity in F_1 offspring was 30 mg/kg, the NOAEL for postnatal toxicity, development and fertility in F_1 offspring was 70 mg/kg. These NOAELs corresponded with BIBR 953 ZW AUC values of 2760, 2760, and 6390 ng.hr/mL, respectively.

Methods

Doses: 0, 15, 30, 70 mg/kg (as free base)

Group	Females per group	Weighed test article	Dose mg/kg	Free base equivalent	Dose mg/kg*
1	24	Natrosol 250 HX (0,5%)	0	Natrosol 250 HX (0.5%)	0
2	24	BIBR 1048 MS	17.3	BIBR 1048 BS	15
3	24	BIBR 1048 MS	34.6	BIBR 1048 BS	30
4	30	BIBR 1048 MS	80.7	BIBR 1048 BS	70

* final dose or concentration (free base)

Dose selection: Selection of 70 mg/kg as the high dose was based on a non-GLP PPD study (U05-2396-01, 05B020) in which rats were dosed at 0, 15, 50, 100 and 200 mg/kg from GD 6 to LD 4. One, four and four deaths occurred in the 50, 100 and the 200 mg/kg dose groups, respectively. Bloody vagina was observed in 6/20, 17/20, and 17/18 dams receiving 50, 100, and 200 mg/kg, respectively, generally in connection with increased post-implantation loss. At 200 mg/kg one dam had exclusively resorptions and another dam had only one fetus, one early resorption and no delivery. Significantly decreased body weight and body weight gain at 100 and 200 mg/kg correlated with decreased food consumption. The mean post-implantation loss increased and the gestation and birth indices decreased in the 100 and 200 mg/kg dose groups. The percentage of dams with live young decreased in the 50 and 100 mg/kg dose groups and significantly decreased in the 200 mg/kg dose group. A higher number of dams in the 100 and 200 mg/kg dose groups had longer gestation of 23 days. The mean number of dead offspring increased with dose in the 50, 100 and 200 mg/kg dose groups. On LD 4, the mean number of live offspring and the viability rate decreased in the 100 and 200 mg/kg dose groups. The sponsor's summary of litter parameters for Study U05-2396 is in Appendix 12.

Frequency of dosing: Daily from gestation day 6 to lactation day 21

Dose volume: 10 mL/kg

Administration route: Oral gavage

Formulation/Vehicle: 0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)

Species/Strain:	Rat (Crl:WI(Han)
Number/Sex/Group:	24 females/group
Satellite groups:	Groups 1-3: 6 females/group, Group 4: 12 females
Study design:	F_0 dams were mated and first day of gestation (GD1) determined. Dosing as indicated above on GD 6 and continued through lactation day (LD) 21. F_0 were evaluated for signs, weight, food, pregnancy duration/parturition. F_1 were not dosed, but were likely exposed in utero. Based on a PK study U06-1452, less than 0.13% of the dose to the dam is secreted into milk. F_1 pups were evaluated for survival, viability, body weight, physical development, sensory/motor functions, explorative behavior, learning and memory and fertility. F_1 pups were reared to reproductive maturity and the F_2 fetuses were evaluated to ensure fertility of the F_1 offspring. Toxicokinetics and hematology were evaluated in the F_0 satellite animals using blood samples collected on GD 7 and LD 5.
Deviation from study protocol:	None of the following deviations from the protocol impacted the validity of the study. 1. At study start the body weight range of the rats was 146-179 g instead of 165-185 g indicated in the protocol. 2. Body weight was recorded on GD 1 and 6-22 instead of GD 1 and 6-21. 3. Food consumption was recorded on GD 7, 14 and 21 instead of GD 6, 14 and 21.

Observations

F_0 Dams

Survival:	Animals were checked twice daily during the work week and once daily during weekends
Clinical signs:	Animals were checked twice daily during the work week and once daily during weekends
Body weight:	The bodyweight of each dam was determined during gestation on GD 1, 7, 12, 13, 16, 19, 22 and during lactation on LD 1, 2, 3, 11, 18, and 21.
Food consumption:	The quantity of food consumed by each dam was determined during gestation on GD 7, 14 and 21 and during lactation on LD 4, 7, 14 and 21.
Necropsy observations:	After weaning the offspring, all surviving females were sacrificed on LD 21 and subjected to necropsy and macroscopic examination. Those dams that did not give birth to offspring were sacrificed and necropsied on GD 25.
Uterine observations:	The uterus of each female was excised and the number of implantation sites was recorded.

Toxicokinetics:	On GD 7 and LD 5 blood samples were collected under isoflurane anesthesia at 0, 2, 8 and 24 hours after treatment via the retrobulbar venous plexus from six low and mid-dose satellite dams and from twelve high dose satellite dams (six on GD 7 and six on LD 5). Plasma was prepared and analyzed for BIBR 951ZW using a validated HPLC-MS-MS assay that incorporated an internal standard. The assay had a calibration range of 4 – 400 ng/mL, an inaccuracy of <5% and imprecision of < 6%.
Hematology:	After collection for TK at 24 hours after dosing on LD 5, blood samples for hematology were collected from 4, 5, 5, and 6 satellite dams of the control, low, mid and high dose groups, respectively. However, the sponsor did not provide these results because they were considered inconsistent and un-interpretable.
Formulation analysis:	The formulations were prepared daily. Samples were taken for analysis of homogeneity and concentration on one day in Week 1 and two days in Week 7.

F_1 Generation

Survival:	Mortality was determined on the day of delivery and on Day 4 when litter size was reduced to 5 males and 5 females.
Body weight:	The pups were weighed on Days 1, 4, 7, 14 and 21 post partum.
Physical development:	The following parameters were evaluated in 5 males and 5 females per litter: incisor eruption on Day 11, fur growth, ear opening and running ability on Day 13, and eye opening on Day 15. After determination of weaning on Day 21, each litter was reduced to 2 males and 2 females per litter. The following parameters were subsequently evaluated: descensus testis on Day 21, vaginal opening on Day 36, and preputial separation on Day 44.
Neurological assessment:	Reflexes and sensory functions of F_1 offspring were determined in week 4: papillary reflex, air-righting reflex, hearing and preyer reflex. Behavioral tests included spontaneous explorative activity during week 5, and a Biel water T-maze for learning and memory in weeks 6 and 7.

Reproduction: At 10-12 weeks of age, one F_1 male and one F_1 female per litter were mated within the same dose groups, but with mating of siblings avoided. The mating period was limited to 10 days and vaginal smears were taken after each mating day to detect sperm. Body weight of the F_1 females was measured on Days 1, 7, and 14 post coitum. After sacrifice of the F_1 females on Day 14 post coitum, the following litter parameters were determined: number of corpora lutea, implantations, live/dead fetuses and resorptions. The copulation index, fertility index, and gestation index were calculated. The F_1 males were killed after mating and subjected to an in situ examination. The reproductive organs (testes, epididymides, prostate, seminal vesicles) of those F_1 males whose F_1 female partners did not become pregnant were subjected to a histopathological examination after fixation. Additionally the pituitary glands, testes, epididymides, prostates, urinary bladders and seminal vesicles of 10 males per group were examined by histopathology.

Other: F_1 pups were examined for external abnormalities on the day of delivery. At culling, the killed F_1 pups were examined for visceral abnormalities by necropsy.

Sponsor's calculated indices

$$\text{Birth index} : 100x\left(\frac{\text{number of live offspring born}}{\text{number of implantation at necropsy}}\right)$$

$$\text{Copulation index} : 100x\left(\frac{\text{females successfully mated}}{\text{females paired for mating}}\right)$$

$$\text{Fertility index} : 100x\left(\frac{\text{sum females pregnant}}{\text{sum females mated}}\right)$$

$$\text{Fertility index during mating phase} = 100x\left(\frac{\text{females successfully mated and pregnant}}{\text{females successfully mated}}\right)$$

$$\text{Gestation index} : 100x\left(\frac{\text{number of females with live fetuses}}{\text{females pregnant}}\right)$$

$$\text{Viability rate} : 100x\left(\frac{\text{pups live on Day 4 of after delivery phase}}{\text{pups live born (first day of after delivery phase)}}\right)$$

$$\text{Weaning rate} : 100x\left(\frac{\text{pubs live on weaning Day} >= 21 \text{ after delivery phase}}{\text{pups live on Day 4 of lactation after litter reduction}}\right)$$

$$\text{Survival rate} : 100x\left(\frac{\text{pups live at the end of after delivery phase}}{\text{pups live on weaning Day} >= 21}\right)$$

$$\text{Resorption rate} : 100x\left(\frac{\text{early resorptions} + \text{late resorptions}}{\text{number of implantations at hysterectomy}}\right)$$

$$\text{Pre - Implantation loss} : 100x\left(1 - \left(\frac{\text{number of implantations at hysterectomy}}{\text{corpora lutea}}\right)\right)$$

$$\text{Post - Implantation loss} : 100x\left(1 - \left(\frac{\text{number of live offspring at delivery}}{\text{number of implantations at necropsy}}\right)\right)$$

F_2 Generation

Survival: The number of live and dead fetuses were determined at necropsy of the F_1 females on Day 14 post coitum

Results

Study acceptability:
Maternal toxicity was observed and at least 19 dams per group and their progeny were available for evaluation indicating the study was acceptable.

F_0 generation:

Survival and clinical signs:
Treatment related mortalities were associated with vaginal bleeding and occurred primarily, but not exclusively, during parturition (Table 149, Table 150). The number of F_0 deaths was highest in the high dose group. Three high dose dams (F429, 430 and 438) recovered from vaginal bleeding without further events. One high dose dam (F410) had vaginal bleeding on the day prior to labor, but survived to deliver offspring. However, of the dams that died, one high dose dam (F434) had vaginal bleeding and died on GD 20 prior to parturition, one high dose dam (F431) had vaginal bleeding and died during labor, and one low dose dam (F210) had vaginal bleeding and died during labor. The sponsor attributed the death of the dam in the low dose group to an individual increased sensitivity. Alternatively, since BIBR 951 ZW plasma concentrations varied more than two-fold, this dam may have had a higher BIBR 951 ZW plasma concentration. Vaginal bleeding was also observed in three high dose toxicokinetic animals, one of which died during gestation (GD17), again indicating that lethal consequences of the pharmacodynamic activity were not confined to parturition.

The perinatal maternal mortality in this study was attributed to prolongation of normal bleeding during parturition resulting from the pharmacodynamic action of BIBR 1048 MS, especially at the high dosage. Although the gestational maternal mortality was not expected, it was also related to the pharmacodynamic activity of BIBR 1048 MS. The sponsor maintains that no vaginal bleeding or no maternal mortality was observed after treatment with 70 mg/kg in the embryo-fetal study (U03-1284) and the fertility and early embryonic development study (U05-1550) where the treatment period excluded the perinatal phase. However, the reviewer notes that one of 24 dams treated at 70 mg/kg in that embryo-fetal study had blood in the uterine horn at necropsy.

Table 149: Reviewer's Summary of F_0 Mortality – Study U06-1586

	Number of F_0			Index (%)		Treatment-related death	
Group/ Dose	Mated	Pregnant	w Live pups	Fertility	Gestation	Number	Day
1 Control	24	23	22	95.8	95.7	0	
2 Low	23	22	21	95.7	95.5	1	GD24
3 Mid	24	21	21	87.5	100	0	
4 High	29	29	25	100	86.2	4	GD20, GD22, GD23, GD24

Table 150: Reviewer's Comments on Deaths and Clinical Signs - Study U06-1586

Group/ Dose	Total # pregnant.	Number Deaths	Comment on death	Comment on clinical sign
1 Control	23	0		F104: no delivery
2 Low	22	1	F 210: death on GD24	F210: Vaginal bleeding, no delivery F201: non-treatment related death
3 Mid	21	0		F327: unhealthy on LD5/6
4 High	29	4 (5)	F401, 402, 431 during labor; F434 on GD20 (F441 on LD 16 – dosing error)	F410, 429, 430, 431, 434, 438: Vaginal bleeding F410, F427, F431: Delivered dead pups F401, F402: Undelivered pups F441: non-treatment related death

Bodyweight:
During gestation only the dams in the high dose group showed a significant decrease (3%) in mean body weight and mean body weight gain (7.7%) relative to that for the control group from GD 16 until GD 20 (Table 151, Figure 35). During lactation, mean body weight was significantly decreased not only in the high dose group (5%), but also in the mid-dose group (3.9%) relative to that in the control group.

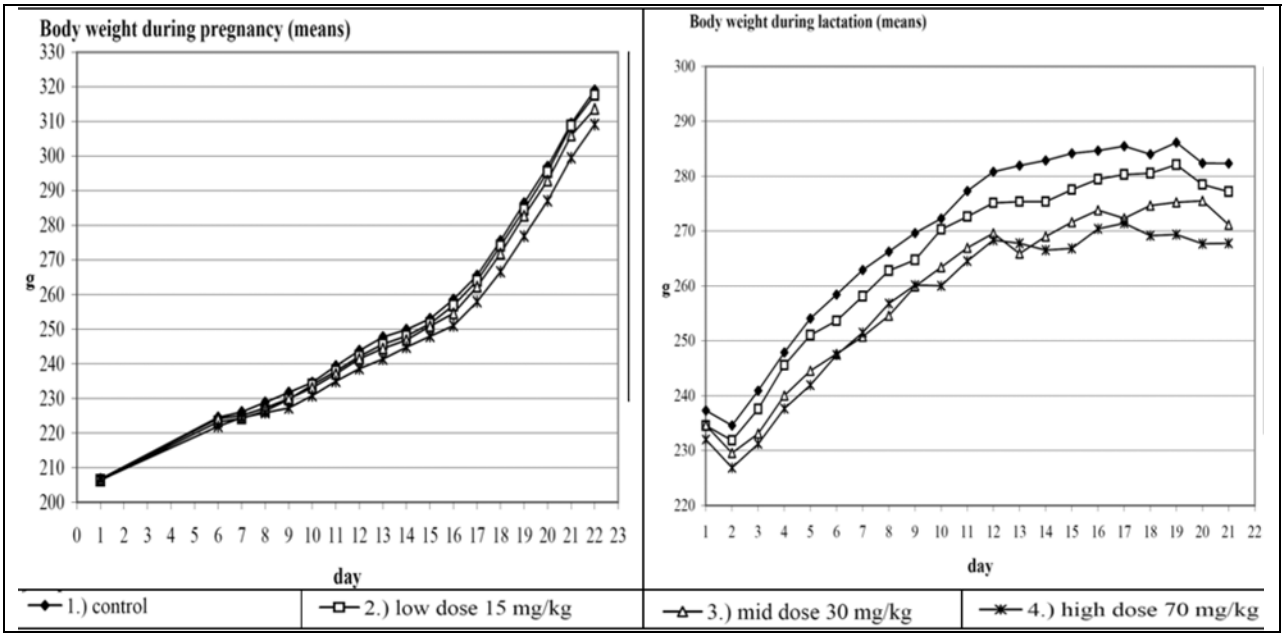
Table 151: Sponsor's Summary of F_0 Body Weight – Study U06-1586

Dose (mg/kg)	n dams	Mean of body weight (g) during gestation (postmating)					
		GD 7	GD 12	GD 13	GD 16	GD 19	GD 22
Control	22	226.10	243.85	247.75	258.60	286.47	319.04
15	21	224.28	242.16	245.72	256.91	284.59	317.65
30	21	225.14	241.47	244.50	254.64	282.84	313.66
70	25	224.49	238.61	241.44	251.06*↓	276.86*↓	309.17

Dose (mg/kg)	n dams	Mean of body weight (g) during lactation (after delivery)					
		LD 1	LD 2	LD 3	LD 11	LD 18	LD 21
Control	22	237.32	234.58	240.92	277.33	284.01	282.34
15	21	234.56	231.86	237.59	272.64	280.56	277.20
30	21	234.63	229.58	233.12*↓	266.97*↓	274.67	271.17*↓
70	#	232.03	226.90*↓	231.24*↓	264.57*↓	269.19*↓	267.78*↓

n= on LD 1 and 2 = 25, LD 3 = 24, LD 11, 18 and 21 = 23
* significant difference ($p<0.05$)
↓ decreased
GD = gestation day
LD = lactation day (equals postnatal day [PND])

Figure 35: Sponsor's Graphs of F_0 Body Weight - Study U06-1586



Food consumption:
During pregnancy, food consumption in the low and mid-dose groups was similar to that in the control group; however, food consumption in the high dose group decreased 4.4% during the last week of gestation. During lactation, food consumption in the mid and high dose groups decreased 3.5 to 7.9% during the last two weeks of lactation.

Table 152: Sponsor's Summary of Food Consumption - F_0 Dams - Study U06-1586

Dose (mg/kg)	n dams	Food consumption, mean (g) during pregnancy		
		GD 7	GD 14	GD 21
Control	22	112.53	144.63	155.65
15	21	112.19	146.34	157.34
30	21	114.35	146.15	157.03
70	25	111.39	141.15	148.89

Dose (mg/kg)	Food consumption, mean (g) during lactation							
	n dams	LD 4+	n dams	LD 7++	n dams	LD 14	n dams	LD 21
Control	20	80.30	21	195.36	22	396.63	22	480.04
15	19	87.01	19	215.64	21	393.35	21	481.28
30	21	77.72	21	193.07	21	375.62	21	463.68
70	24	75.55	24	195.24	23	373.27	23	442.16

+ registered manually, no analysis of variance performed
++ difference to LD 1 not to LD 4
GD = gestation day
LD = lactation day (equals postnatal day [PND])

Necropsy observations:
Of the five dams that died before delivery or during delivery, four (210, 401, 431, 434) had blood or clotted blood in the uterus or placenta and three (401, 402, 431) had undelivered offspring. Of the main study dams with scheduled necropsies, one, three and two dams in the low, mid and high dose groups, respectively, had enlarged

spleens. Of the 12 satellite high dose dams, one had an enlarged spleen and two had blood in the uterus.

Uterine content and litter parameters:
The litter parameters are summarized in Table 153. The mean post-implantation loss and mean number of dead offspring were increased in the high dose group resulting in a significantly decreased birth index. The mean post-implantation loss of 12.1% in the high dose group is twice the mean spontaneous post-implantation loss (5.7%) in the embryo-fetal evaluation study of Viertel and Nolte 2003 [U03-1549]. Furthermore, one dam (F410) in the current study had a post-implantation loss of 66.7% due to the delivery of 7 dead pups out of 11 total pups and 12 implantation sites

The sponsor attributes the increased post-implantation loss in the high dose group to uncertainty as to whether the dams 410 and 427 swallowed more of their offspring than was recorded. Excluding these animals, the sponsor maintains that the mean post-implantation loss is 8.33% (individual range of 0-46.15%), which is only slightly above the post-implantation loss in another study of Viertel [Internal No. 01B141 Natrosol 250 HX: Study for effects on embryo-fetal development in rats by oral administration (gavage), report in progress], (Control A mean 7.12%, range 0-50.0%). For comparison, a mean (range) post-implantation loss of 6.3% (3.7-8.6%) was reported in the MARTA database (accessed in 2005) for seven studies using Wistar rats.

No explanation was provided for the observation that only the treated groups had dams with post-implantation loss greater than 20%. The low, mid and high dose groups have 2, 2 and 4 dams, respectively, with post-implantation loss greater than 20%. Furthermore, 15 dams (60%) in the high dose group and only 8 dams (36.4%) in the control group had post-implantation loss.

In the high dose group, the mean number (0.4) and individual range of dead born offspring (0-7) were above the concurrent control values (mean: 0.090, range: 0-1). The values for the high dose group were above the value of zero in the embryo-fetal evaluation study of Viertel and Nolte 2003 [U03-1549] and the values (Control A mean 0.1, range: 0-2 and Control E mean 0.1, range: 0-1) from the study by Viertel [Internal No. 01B141]. Despite elimination of the four high dose dams that died prematurely or during labor, peri-natal survival of offspring in the high dose group decreased.

The percentage of dams with delayed labor from GD 22 to GD 23 increased in the treated groups (14.3 to 33%) compared to the percentage in the control group (9.1%). The one day delay in labor did not appear to be associated with vaginal bleeding, bleeding to death or poor health condition. The sponsor attributed the apparent delay in labor to the ambiguity in the method of defining the time of conception and the time of birth.

The perinatal deaths of three dams were attributed to the pharmacologic effect of BIBR 1048 MS. Parturition was delayed to GD 24 for a high dose dam (F431) who experienced vaginal bleeding during labor and died from uterine bleeding on GD 24 after delivery of one of twelve offspring. Parturition on GD23 for second high dose dam (F401) resulted in death from uterine bleeding after delivery of seven of eleven offspring. However, parturition of a third high dose dam (402) resulted in death on GD23

after delivery of three of nine offspring with no evidence of bleeding observed at necropsy. Parturition also did not occur in a low dose dam (F210) who experienced vaginal bleeding on GD 23 and was found dead on GD 24 with necropsy showing blood in the uterus, non-delivery of ten live fetuses, and one resorption.

Table 153: Reviewer's Summary of Litter Parameters – F_0 Dams - Study U06-1586

		Dose, mg/kg				Historical controls†	
Parameter		**0**	**15**	**30**	**70**	**Mean**	**Min/Max‡**
Total F_0 females		24	24	24	30		
Excluded		0	1	0	1		
Number F_0 females mated		24	23	24	29		
Number pregnant		23	22	21	29		
Dams non-pregnant		1 (F122)	1 (F203)	3 (F308, F309, F310)	0		
Pregnancy rate (%)		95.8	95.7	87.5	100		
Dams pregnant, no delivery		1 (F104)	0	0	0		
Dams prematurely dying		0	1	0	4		
Dams with death during labor		0	1 (F210)	0	3 (F401, F402, F431)		
Dams with viable pups		22	21	21	25	Total 85 litters	
Dams with only dead pups or no viable pups		0	0	0	0		
Dams with dead pups on PND 1 (Dam number)		2 (F119, F130)	2 (F217, F204)	1 (F303)	2 (F427, F410)		
Dams with dead pups discounted by sponsor		0	0	0	1 (F431: 1)		
Dams with all pups consumed		0	0	0	0		
Dams no viable offspring remaining after Day		1	0	0	2		
Dams with runts		0	1 (F217)	0	0		
Implantation sites/mated female (range)		11.4 (1-15)	11.7 (2-17)	11.3 (4-14)	11.4 (8-15)	11.1	5-15
Number viable offspring/ litter (range)		10.8 (1-15)	10.8 (2-16)	10.9 (4-14)	10.0 (4-14)	10.5	5-14
Birth index, % (range)		95.18 (81.8-100)	92.60 (70-100)	96.47 (72.7-100)	**87.89* (33.3-100)**	*94.6^{s}*	*33.3-100^{s}*
Number of dead offspring/litter		2/22 0.09	2/21 0.095	1/21 0.047	**10/25 0.40**	0	0
% Post-implantation (PI) loss (range)		4.82 (0-18.2)	7.40 (0-30)	3.53 (0-27.2)	**12.11* (0-66.7)**	5.7 [7.7]#	0-23.1 [0-54.6]#
# Dams with %PI loss >20 % (values)		0 -	3 (22.2, 23.1, 30)	2 (23.1, 27.3)	4 (33.3, 44.4, 46.2, 66.7)		
Dams with total born < # implant (%)		8 (36.4%)	9 (42.8%)	5 (23.8%)	**15 (60%)**		
Duration of gestation	<22 days	0	0	0	0		
	22 day	20	14	18	18		
	23 days (% total litters)	2 (9.1%)	7 (33.3%)	3 (14.3%)	7 (28%)		
	24 days	0	1 (F210)	0	1 (F431)		

† Viertel 2003, Study 00B203, ‡ Range of individual values, ^{s} Calculated from historical data using sponsor's equation,
* Statistically significant p<0.05, # Outlier of 54.6 included.

Toxicokinetics:
BIBR 953 ZW C_{max} and $AUC_{(0\text{-}24h)}$ values were similar on GD 7 and LD 5 (Table 154). Although the $AUC_{(0\text{-}24h)}$ values following the dose of 30 mg/kg in the present study are

similar to the $AUC_{(0\text{-}24h)}$ values obtained in Wistar female rats dosed at 30 mg/kg in a 13-week study [U05-1378], the Cmax values in the pregnant and lactating rats are approximately 30 % lower than the Cmax values in the non-pregnant females. However, the Cmax values at 15 and 70 mg/kg are lower than those obtained on GD 16 of the rat EFD study are also higher than those in the present study, although the AUC values are similar.

Table 154: Reviewer's Compilation of TK from Sponsor's Tables - Study U06-1586

			Cmax			$AUC_{(0\text{-}24h)}$		
	dose [mg/kg]	day	N	mean [ng/mL]	CV (%)	N	mean [(ng·h)/mL]	CV (%)
PPD Study – Study U06-1586	15	2	6	294	54.6	6	1520	45.0
	15	20	5	258	35.5	5	1380	42.2
	30	2	6	451	21.8	6	2470	26.3
	30	20	5	593	43.4	5	3110	38.1
	70	2	6	1220	27.3	6	6280	30.4
	70	20	6	1180	28.5	6	6500	16.4
13-week Tox – Study U05-1378	F 30 mg/kg	1		686	35.2		2310	
		91		754	20.4		2810	
EFD Study – Study U03-1284	F	GD 16						
	15 mg/kg			633	39.0		1470	40.0
	70 mg/kg			2560	9.1		7200	21.1
	200 mg/kg			4280	33.9		20800	47.0

Formulation analysis:
Analysis of the formulations on three days showed the formulations were homogeneous. The concentrations for all individual formulation samples were within the range of 89.8 – 105.5% of the nominal concentrations (Table 155).

Table 155: Reviewer's Summary of Formulation Analysis – Study U06-1586

Formulation date	**Group/Dose**	**Control**	**Low (15 mg/kg)**	**Mid (30 mg/kg)**	**High (70 mg/kg)**
	Nominal (mg/mL)	0	1.5	3.0	7.0
3/20/05	Mean, % nominal	0	104.3	97.6	93.9
	Range, %		102.3-105.5	96.3-99.0	93.3-94.9
5/2/05	Mean , % nominal	0	89.9	92.3	95.4
	Range, %		89.8-90.1	91.7-93.0	94.3-96.7
5/4/05	Mean, % nominal	0	96.9	98.2	99.4
	Range, %		95.6-98.0	97.3-98.7	96.4-104.7

F_1 generation:
F_1 Parameters at birth
As discussed above under litter parameters (Table 153), the mean number (0.4) and individual range of dead born offspring (0-7) in the high dose group were above the concurrent control values (mean: 0.090, range: 0-1). Although one runt of 3.3 gm was seen in the 15 mg/kg dose group (F217), the mean birth weight of the offspring was not affected by treatment and values for individual offspring from treated groups were within or above the historical control range (Table 156). Although the mean percentage of males in the high dose group (42.8%) was slightly lower than that in the control group (50.9%), it was within the historical control range and is partially attributable to the birth of only female offspring to F410.

Table 156: Reviewer's Summary of F_1 Parameters at Birth - Study U06-1586

					Historical controls[§]	
Parameter	**0 mg/kg**	**15 mg/kg**	**30 mg/kg**	**70 mg/kg**	**Mean**	**Min/Max**
# litters with viable offspring at delivery	22	21	21	25		
# viable offspring LD 1/ litter (individual range)	10.8 (1-15)	10.8 (2-16)	10.9 (4-14)	10.0 (4-14)	10.5	5-14
Birth Weight on LD 1: Offspring Mean/litter Range mean individual litters Range individual offspring value	 5.68 5.16-6.24 4.0-6.8	 5.77 4.68-7.50 4.2 (3.3[†])-8.0	 5.61 4.88-6.78 4.3-7.0	 5.74 4.58-6.54 4.4-7.2	 5.03	 2.3-6.3
% male of viable offspring (individual range)	50.85 (22.2-100)	46.92 (33.3-66.7)	47.63 (25-100)	42.77 (0-66.6)	49.4	18.2-80
# viable offspring on Day 1	238	227	229	251		
# viable offspring on Day 4	234	221	218	240		
% survival Day 1 – Day 4	98.3	97.3	95.2	95.6		
External examination						
# Litters with abnormal pups	1	0	0	4		
% litter with abnormal pups	4.54	0	0	16		
# Litters with malformations (offspring number)	0	0	0	2 (F433F1, F435F2)		
Malformation location				Head[º] Trunk[¹]		
% litters with malformations	0	0	0	8		
# litters with variations (offspring number)	1 (F120M1)	0	0	2 (F425M2, F426M1)		
Variation location (description)	Trunk^:			Head[²]: Trunk[³]:		
% litters with alterations	4.54	0	0	8.0		

[§] Viertel 2003, Study 00B203 * Statistically significant p<0.05; [†] Birth weight of the runt; ^ small testis;
º hydrocephalus; ¹ enlarged abdomen/ urogenital region; ² region of right eye; ³ right pelvis dilated; changed testis

In the high dose group, two F_1 offspring had malformations and two F_1 offspring had variations. This total number of malformations plus variations is higher than the one variation in F_1 offspring in the control group. This increase could be associated with the maternal toxicity (decreased body weight) in the high dose group.

F_1 Survival:
As indicated in Table 153, the birth index decreased significantly in the high dose group. Survival of offspring after birth to LD 4 was lower in the mid and high dose groups compared to that in the low dose and control groups (Table 157). From LD4 to weaning, survival in the treated groups was not statistically different from that in the control group, although survival was slightly decreased in the mid- and high dose groups. After weaning to LD 70, all males and females survived in all groups.

Table 157: Reviewer's Summary of F_1 Survival - Study U06-1586

Parameter	0 mg/kg	15 mg/kg	30 mg/kg	70 mg/kg
# litters with viable offspring at delivery	22	21	21	25
# litter with all offspring surviving to end	18	15	13	20
# litter not all offspring surviving to end	4	6	8	5
# viable offspring on Day 1	238	227	229	251
# viable offspring on Day 4	234	221	218	240
% survival Day 1 – Day 4	98.3	97.3	95.2	95.6
# viable offspring Day 4 post culling	198	185	182	199
# viable offspring on Day 21	197	184	180	196
% survival Day 4 – Day 21	99.5	99.5	98.9	98.4
# viable offspring Day 21 post culling	85	82	82	91
# viable offspring on Day 70	85	82	82	91
% survival Day 21 – Day 70	100	100	100	100

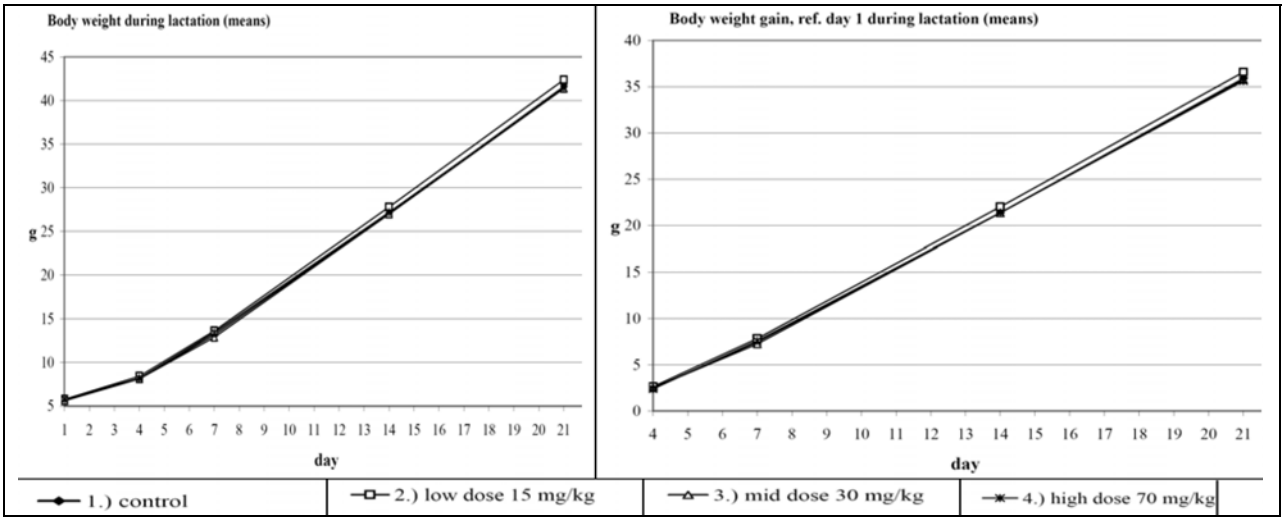
F_1 Body weight:
During lactation, the mean body weights and mean body weight gains of male and female F_1 offspring were similar among the three treated groups and the control group (Table 158, Figure 36).

Table 158: Sponsor's Summary of F_1 Body Weight - Lactation - Study U06-1586

Dose (mg/kg)	n litters	Mean of body weight (g) during lactation				
		LD 1+	LD 4	LD 7	LD 14	LD 21
Control	22	5.685	8.158	13.168	27.072	41.612
15	21	5.773	8.409	13.605	27.797	42.375
30	21	5.613	8.123	12.869	26.984	41.384
70	#	5.742	8.166	13.409	27.199	41.460

n= on LD 1 = 25, LD 4 = 24, LD 7, 14 and 21 = 23

Figure 36: Sponsor's Graphs - F_1 Body Weight - Lactation - Study U06-1586



After weaning, the mean body weights of the F_1 males in the mid- and high dose groups were slightly (<2%), but insignificantly lower than those in the low dose and control groups. In contrast, the mean body weights of the F_1 females in the mid- and high dose groups were slightly (≤3.5%) higher than those in the low dose and control groups.

Statistical significance was only achieved by the F_1 females of the high dose group on PND 63 (Table 159, Figure 37).

Table 159: Sponsor's Summary - F_1 Body Weight after Weaning – Study U06-1586

F1 Males

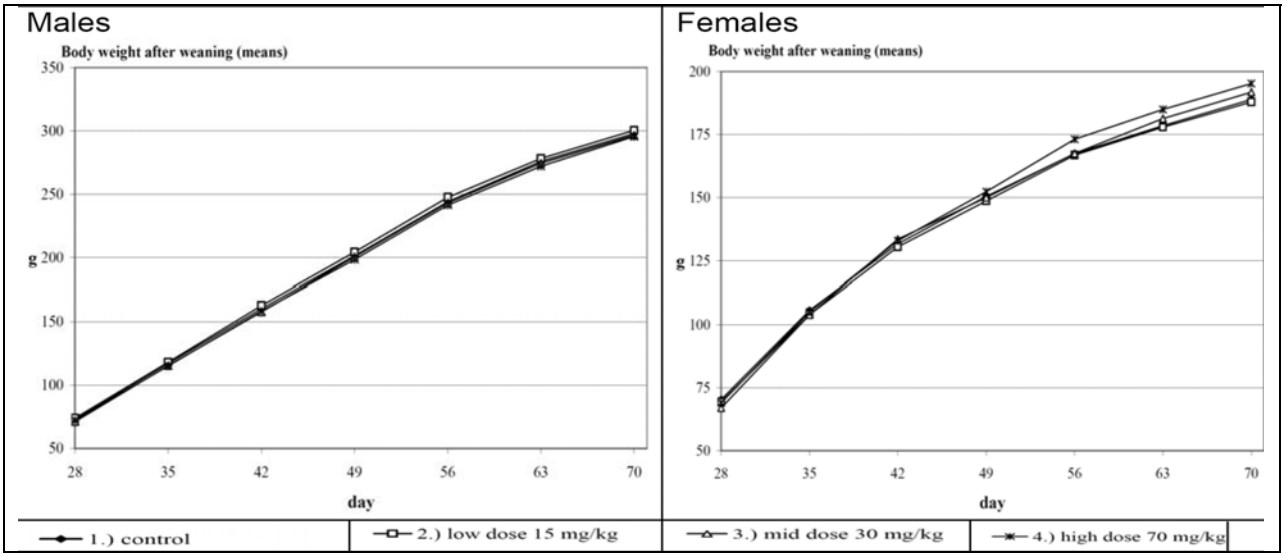
Dose (mg/kg)	n litters	Mean of body weight (g) of male offspring after weaning				
		PND 28	PND 35	PND 56	PND 63	PND 70
Control	22	73.014	117.189	244.159	276.107	298.073
15	21#	74.098	118.257	247.578	278.283	300.590
30	21	71.288	115.567	243.324	274.519	296.605
70	23##	72.222	115.350	241.511	272.230	295.609

F1 Females

Dose (mg/kg)	Mean of body weight (g) of female offspring after weaning					
	n litters	PND 28	PND 35	PND 56	PND 63	PND 70
Control	21	70.279	105.719	167.483	178.557	189.012
15	21#	69.402	104.026	166.915	177.945	187.993
30	20	67.018	103.913	167.548	181.365	191.888
70	23##	69.033	105.085	173.216	184.989*↑	195.267

* significant difference ($p<0.05$) # PND 56 n= 20 ## PND 56 n= 22
↑ increased
PND = postnatal day

Figure 37 : Sponsor's Graphs of F_1 Body Weight after Weaning - Study U06-1586



F_1 Physical Development:

Table 160 shows treatment with BIBR 1048 MS did not significantly affect the mean or individual range of time to appearance of physical landmarks of development compared to the concurrent control group. Table 161 shows the sponsor's analysis of the distribution of the time to appearance of physical landmarks of development for individual F_1 animals. The sponsor noted the delay in opening of the ears in the mid-dose group, but associated this delay with decreased body weight, and attributed this delay to inaccuracy in the conceptus time points. In addition, dose dependency was lacking. These data support the conclusion that BIBR 1048MS treatment did not delay general physical development of the F_1 animals. Although some parameters, such as testes descension, developed in more F_1 offspring in the treated groups at earlier timepoints, the earliest day of development was identical in all groups.

Table 160: Reviewer's Modification – F_1 Physical Parameters - Study U06-1586

Group		Incisors eruption	Fur growth	Ears opening	Eyes opening	Correct running	Descensus testis	Vagina opening	Preputial separation
control 0 [mg/kg]	n	22	22	22	22	22	22	21	22
	mv	11.90	13.89	13.73	15.96	13.68	22.27	36.43	44.41
	sd	0.58	0.63	0.72	0.55	0.50	0.51	0.71	0.97
Individual range		11-15	13-16	13-16	15-19	13-16	21-24	36-39	44-48
low dose 15 [mg/kg]	n	21	21	21	21	21	21	21	21
	mv	11.54	13.85	13.51	16.01	13.70	22.14	36.64	44.19
	sd	0.43	0.72	0.55	0.73	0.78	0.78	1.13	0.49
Individual range		11-13	13-17	13-15	15-18	13-17	21-24	36-42	44-46
mid dose 30 [mg/kg]	n	21	21	21	21	21	21	20	21
	mv	11.85	13.82	13.72	16.21	13.56	21.81	36.45	44.05
	sd	0.60	0.60	0.49	0.50	0.42	0.81	0.71	0.15
Individual range		11-14	13-17	13-15	15-18	13-18	21-23	36-38	44-45
high dose 70 [mg/kg]	n	23	23	23	23	23	23	23	23
	mv	11.63	13.80	13.56	16.15	13.57	21.89	36.28	44.09
	sd	0.47	0.67	0.57	0.64	0.37	0.93	0.58	0.25
Individual range		11-13	13-16	13-16	15-19	13-16	21-24	36-39	44-45

Table 161: Compilation of Sponsor's Distribution of the Time to Physical Landmark Development for Individual F_1 Animals - Study U06-1586

Group		Incisors eruption				Fur growth			
		nd	<12	12	>12	nd	<14	14	>14
control 0 [mg/kg]	f	0	62	97	38	0	72	86	39
	f%	0.0	31.5	49.2	19.3	0.0	36.5	43.7	19.8
low dose 15 [mg/kg]	f	0	92	76	16	0	74	68	42
	f%	0.0	50.0	41.3	8.7	0.0	40.2	37.0	22.8
	p		*0.0003				0.5270		
mid dose 30 [mg/kg]	f	0	70	68	43	0	58	96	27
	f%	0.0	38.7	37.6	23.8	0.0	32.0	53.0	14.9
	p		0.1606				0.3867		
high dose 70 [mg/kg]	f	0	91	87	19	0	78	70	48
	f%	0.0	46.2	44.2	9.6	0.0	39.8	35.7	24.5
	p		*0.0037				0.5342		

Group		Ears opening				Eyes opening			
		nd	<14	14	>14	nd	<16	16	>16
control 0 [mg/kg]	f	0	94	82	21	0	41	118	38
	f%	0.0	47.7	41.6	10.7	0.0	20.8	59.9	19.3
low dose 15 [mg/kg]	f	0	103	61	20	0	48	78	58
	f%	0.0	56.0	33.2	10.9	0.0	26.1	42.4	31.5
	p		0.1239				0.2287		
mid dose 30 [mg/kg]	f	0	64	100	17	0	23	94	63
	f%	0.0	35.4	55.2	9.4	0.0	12.8	52.2	35.0
	p		*0.0165				*0.0403		
high dose 70 [mg/kg]	f	0	102	75	20	0	32	111	53
	f%	0.0	51.8	38.1	10.2	0.0	16.3	56.6	27.0
	p		0.4807				0.2996		

Group		Correct running				Descensus testis			
		nd	<14	14	>14	nd	<22	22	>22
control 0 [mg/kg]	f	0	106	62	29	0	6	20	17
	f%	0.0	53.8	31.5	14.7	0.0	14.0	46.5	39.5
low dose 15 [mg/kg]	f	0	99	61	24	0	12	12	17
	f%	0.0	53.8	33.2	13.0	0.0	29.3	29.3	41.5
	p		1.0000				0.1131		
mid dose 30 [mg/kg]	f	0	96	70	15	0	21	8	13
	f%	0.0	53.0	38.7	8.3	0.0	50.0	19.0	31.0
	p		0.9179				*0.0004		
high dose 70 [mg/kg]	f	0	106	69	22	0	22	8	15
	f%	0.0	53.8	35.0	11.2	0.0	48.9	17.8	33.3
	p		1.0000				*0.0005		

Group		Vagina opening				Preputial separation			
		nd	<37	37	>37	nd	<45	45	>45
control 0 [mg/kg]	f	0	31	5	6	0	35	4	4
	f%	0.0	73.8	11.9	14.3	0.0	81.4	9.3	9.3
low dose 15 [mg/kg]	f	0	28	5	8	0	36	3	2
	f%	0.0	68.3	12.2	19.5	0.0	87.8	7.3	4.9
	p		0.6337				0.5495		
mid dose 30 [mg/kg]	f	0	29	5	6	0	40	2	0
	f%	0.0	72.5	12.5	15.0	0.0	95.2	4.8	0.0
	p		1.0000				0.0887		
high dose 70 [mg/kg]	f	0	36	8	2	0	41	4	0
	f%	0.0	78.3	17.4	4.3	0.0	91.1	8.9	0.0
	p		0.8028				0.2243		

F_1 Neurological Assessment:
All offspring had positive results in the papillary reflex and air-righting reflex tests.

In the hearing and Preyer reflex tests, the statistically significant effects noted in Table 162 were not found in the other sex and/or did not show dose dependence. Therefore, the effects were considered incidental and not toxicologically significant.

Table 162: From Report 0733: Hearing and Preyer Reflexes - Study U06-1586

	Dose [mg/kg]	Number of males with preyer reflexes at			Number of females with preyer reflexes at		
4 kHz		80dB	75dB	P value	80dB	75dB	P value
	control	8	35		10	32	
	15 mg/kg	7	34		6	35	
	30 mg/kg	6	36		7	33	
	70 mg/kg	7	38		8	38	
8 kHz		70dB	65dB		70dB	65dB	
	control	12	31		15	27	
	15 mg/kg	9	32		10	31	
	30 mg/kg	10	32		8	32	
	70 mg/kg	8	37		9	37	
12 kHz		70dB	65dB		70dB	65dB	
	control	1	42		0	42	
	15 mg/kg	0	41		0	41	
	30 mg/kg	3	39		0	40	
	70 mg/kg	0	45		0	46	
16 kHz		65dB	60dB		65dB	60dB	
	control	12	31		7	35	
	15 mg/kg	1	40	0.0016	10	31	
	30 mg/kg	10	32		10	30	
	70 mg/kg	14	31		9	37	
20 kHz		65dB	60dB		65dB	60dB	
	control	3	39	§	6	78	
	15 mg/kg	2	39		11	71	
	30 mg/kg	8	34		16	66	0.0218
	70 mg/kg	7	38		13	78	
	§ One male in control group could not hear the highest frequency						

No significant differences between the dose groups and the control group were detected in the Biel water T-maze test during Week 6, for the number of correct choices, for the correct choice or for memory. However, during Week 7 the significant better learning in the Biel water T-maze test for the F_1 males of the low and mid-dose groups and the worse learning for the F_1 females of the mid-dose group (Table 163) was considered incidental and not toxicologically relevant.

Table 163: From Report 0734: Biel Water T-maze Test – Week 7 - Study U06-1586

	Dose [mg/kg]	number of animals with 0-5 correct choices							p value
		0	1	2	3	4	5	total	
F1 Males	control	7	5	5	18	6	2	43	-
	15 mg/kg	2	2	7	13	14	3	41	**0.0244**
	30 mg/kg	0	2	8	13	13	6	42	**0.0037**
	70 mg/kg	3	4	8	16	9	5	45	0.1558
	Dose [mg/kg]	number of animals with 0-5 correct choices							p value
		0	1	2	3	4	5	total	
F1 Females	control	2	2	8	12	14	4	42	-
	15 mg/kg	2	2	3	19	11	4	41	0.8967
	30 mg/kg	4	8	10	6	7	5	40	**0.0497**
	70 mg/kg	5	7	8	13	10	3	46	0.0693

Last trial of Week 7

Males

Dose [mg/kg]	number of animals with correct choice			p value
	yes	no	total	
control	31	12	43	-
15 mg/kg	38	3	41	**0.0211**
30 mg/kg	36	6	42	0.1842
70 mg/kg	38	7	45	0.1991

Females

Dose [mg/kg]	number of animals with correct choice			p value
	yes	no	total	
control	38	4	42	-
15 mg/kg	34	7	41	0.3502
30 mg/kg	26	14	40	**0.0072**
70 mg/kg	35	11	46	0.0924

Evaluation of spontaneous activity showed a similar profile of the maximum interval within the first 15 minutes, intensity, and location of activity in the control and treated groups. Slight statistically significant changes in activity were generally present in one sex and not the other indicating the findings were incidental.

Table 164: From Report 0735: F_1 Spontaneous Activity - Study U06-1586

Combined F1 Males and Females

Lower frame		Total	P value	Center	P value	Border	P value
Time	Dose [mg/kg]	Mean		Mean		Mean	
15 min	control	488.1		62.7		425.3	
	15 mg/kg	547.8	0.0435	57.5		490.3	0.0167
	30 mg/kg	474.0		53.6		420.4	
	70 mg/kg	497.4		54.1		443.4	
30 min	control	192.7		25.0		167.6	
	15 mg/kg	202.3		22.6		179.7	
	30 mg/kg	215.2		22.5		192.7	0.0317
	70 mg/kg	217.3		22.9		194.5	0.0186
45 min	control	115.0		12.6		102.4	
	15 mg/kg	129.4		14.6		114.7	
	30 mg/kg	133.2		15.8		117.3	
	70 mg/kg	104.9		11.0		93.9	
60 min.	control	92.0		7.3		84.7	
	15 mg/kg	98.7		7.1		91.6	
	30 mg/kg	98.3		9.1		89.1	
	70 mg/kg	76.2		5.4		70.8	

Upper frame		Total	P value	Center	P value	Border	P value
Time	Dose [mg/kg]	Mean		Mean		Mean	
15 min	control	70.4		16.6		53.8	
	15 mg/kg	70.5		10.7		59.8	
	30 mg/kg	53.7	0.0228	8.6		45.1	
	70 mg/kg	60.6		12.5		48.1	
30 min	control	24.3		4.6		19.7	
	15 mg/kg	29.7		5.7		24.0	
	30 mg/kg	25.6		3.5		22.1	
	70 mg/kg	28.5		7.0		21.5	
45 min	control	9.5		1.7		7.8	
	15 mg/kg	15.2	0.0300	4.4	0.0124	10.8	
	30 mg/kg	12.3		2.6		9.7	
	70 mg/kg	9.8		2.6		7.1	
60 min.	control	7.8		1.6		6.2	
	15 mg/kg	9.6		2.5		7.1	
	30 mg/kg	9.7		3.6		6.1	
	70 mg/kg	4.4		1.2		3.2	

F_1 Reproduction:
Most of the F_1 offspring mated successfully within four nights of mating (Table 165). Histopathology revealed no changes in the reproductive tissues of the control F_1 male, who mated but whose female partner was not pregnant, and one of the low dose F_1 males, who did not successfully mate. Histopathology of the reproductive tissues the remaining males, who did not successfully mate, revealed acute dilatation of the prostate portion of the urethra and the urinary bladder in the low dose male and interstitial inflammatory infiltration of the prostate of the mid-dose male.

Table 165: Sponsor's Summary of Mating of F_1 Offspring - Study U06-1586

Group	Control	15 mg/kg	30 mg/kg	70 mg/kg
Sample size (n litters)	21	21	20	23
n/frequency (%) decimals truncated				
Day after start of mating				
1	7 (33.33)	4 (19.04)	4 (20.00)	6 (26.08)
2	4 (19.04)	5 (23.80)	1 (5.00)	4 (17.39)
3	6 (28.57)	4 (19.04)	10 (50.00)	4 (17.39)
4	4 (19.04)	6 (28.57)	4 (20.00)	9 (39.13)
Sperm found but not pregnant	1 (4.76)	0	0	0
Not successfully mated (no sperm found, non-pregnant)				
After 10 days of mating	0	2 (9.52)	1 (5.00)	0

Table 166 summarizes the reproductive parameters for the F_1 females. Slight increases in mean number of corpora lutea and implantations combined with slight decreases in resorption rate resulted in significant increase in the mean of viable fetuses in the F_1 high dose group. However, the values from both control and treated groups are within or close to range of values from historical or spontaneous evaluation studies. Likewise increases in means or individual ranges of total, early or late resorptions in the low dose group were all within or close to range of values from historical or spontaneous evaluation studies. Treatment of F_0 dams with BIBR 1048 MS during pregnancy and lactation did not impair the fertility of the F_1 animals or the intrauterine survival of their embryos pre- and post implantation.

Table 166: Reviewer's Summary of F_1 Reproductive Parameters - Study U06-1586

	Dose, mg/kg				Historical controls			
Parameter	**0**	**15**	**30**	**70**	**Study 1**	**Study 2**	**Study 3**	**CR**
Total F_1 females	21	21	20	23				22.89
F_1 1 females excluded	0	0	0	0				
F_1 Dams premature death	0	0	0	0				
Number F_1 females mated	21	21	20	23				
Number F_1 dams pregnant	20	19	19	23	23	90	92	21.1
F_1 Dams non-pregnant	1	2	1	0				
F_1 dams w sperm & non-pregnant	1	0	0	0				
F_1 dams wo sperm & non-pregnant	0	2	1	0				
F_1 Copulation index	100	90.4	95.0	100				
F_1 fertility index	95.2	100	100	100				
F_1 gestation index	100	100	100	100				
Pregnancy rate, mean %	95.2	90.4	95.0	100				92.79
Pregnancy rate, range								76-100
Dams with viable fetuses	20	19	19	23				
Dams with only dead fetuses	0	0	0	0				
Dams with abortions or resorptions only	0	0	0	0				
Dams with any resorption	13	14	10	13				7.89
% Dams with resorptions, mean	65	73.6	52.6	56.5				39.3
% Dams with resorptions, range								18.2-60
# Total Corpora lutea	246	249	241	303				
Corpora lutea/litter, mean (SD)	12.3 (1.7)	13.1 (1.9)	12.7 (1.6)	13.2 (1.5)	12.2	12.3	12.1	12.19
Corpora lutea/litter, range	10-16	10-18	9-16	11-16	10-16	11.9-12.5	11.6-12.5	10.6-14.3
# Total Implantations	228	232	222	288				
Implantations/litter, mean (SD)	11.4 (2.3)	12.2 (2.0)	11.7 (1.9)	12.5 (2.0)	11.8	11.3	11.3	10.39
Implantations/litter, range	6-16	8-16	9-15	8-16	9-16	11-11.6	10.9-11.5	8.2-13.2
# Total resorptions	19	25	16	16				
Total resorptions/litter, mean (SD)	0.95 (0.89)	1.32 (1.6)	0.84 (1.12)	0.70 (0.76)	1.43	0.83	0.96	0.58
Total resorptions/litter, range	0-3	0-7	0-4	0-3	0-4	0.6-1.15	0.8-1.24	0.2-1.3
Early resorptions/litter, mean (SD)	0.80 (0.83)	1.11 (1.49)	0.63 (1.01)	0.57 (0.73)	1.17	0.78	0.53	0.56
Early resorptions/litter, range	0-3	0-6	0-4	0-3	0-4	0.6-1.15	0.4-0.7	0.2-1.3
Late resorptions/litter, mean (SD)	0.15 (0.37)	0.21 (0.42)	0.21 (0.54)	0.13 (0.34)	0.26	0.06	0.42	0.02
Late resorptions/litter, range	0-1	0-1	0-2	0-1	0-3	0-0.2	0.38-0.52	0-0.10
% Resorption rate, mean (SD)	8.32 (8.0)	10.79 (13.37)	6.75 (8.42)	5.45 (5.73)	11.8	7.71	9.0	
% Resorption rate, range	0-30	0-58.33	0-30.8	0-20.0	0-27	4.9-11.3	7.4-10.7	
% Pre-implantation loss, mean (SD)	7.24 (13.7)	6.82 (8.0)	7.79 (10.07)	5.14 (8.20)	3.68	8.01	7.24	
% Pre-implantation loss , range	0-53.8	0-26.67	0-30.8	0-27.2	0-9.1	7.1-9.5	5.6-9.1	
Total viable fetuses	209	207	206	272				
Total dead fetuses	0	0	0	0				
Number viable fetuses/ litter, mean (SD)	10.5 (2.2)	10.9 (2.5)	10.8 (1.8)	**11.8* (1.9)**				9.8
Number viable fetuses/ litter (range)	6-14	5-15	9-15	8-16				7.8 – 12
Number of dead fetuses/ litter	0	0	0	0	0		0	

Study 1 = Control group from U05-1550, Study 2 = Control Study U04-1440, Study 3 = C (b) (4) from Reproductive Parameters and Fetal Data from Reproductive Toxicity Studies in the [Crl:WI(Han)] Rat, March 2009; , * statistically significant, p<0.05

Macroscopic findings:

The sponsor concluded that majority of the macroscopic and histopathologic findings found in offspring were considered incidental.

One of the offspring born to a high dose female (F401) that died during labor had a dilated left renal pelvis and bent left ureter. The incidence of this finding in the control EFD studies 04B226 and U03-1284 was 1.86% and 5.04% of fetuses, respectively. One offspring of the mid-dose female (F307) also had a bowed ureter. Dilated renal pelvis seen in the control EFD study 04B226 at 1.86% fetuses was observed in offspring from the low dose females (206, 207, and 221 (two offspring)), the mid-dose females (322, 322, 324, and 324),and the high dose females (424, and 426 (two offspring)). One offspring of a low dose female (223) had a marmorated kidney.

Subacute non-purulent interstitial inflammatory infiltration in the prostate of one offspring of a mid-dose female (305) was also described in three male offspring of control females (108, 119 and 123). Enlarged seminal vesicles were observed in one offspring of a mid-dose female (302) and offspring of a control female (106). One offspring of a low dose female (206) had a small left seminal vesicle. One male offspring of a control female (120) had a small testis that was considered a variation. One male offspring of the high dose female (426) had a normal right testis, but had a left testis that only contained an epididymis with no testicular tissue; however, the partner of this male became pregnant despite this variation.

One male offspring of a high dose female (425) had change in region of the right eye considered a variation that was described as small rosette formation of the retina close to the uveal tract upon histology.

A female offspring of high dose female (435) had a malformation described as an inflated external-abdomen. Necropsy showed an enlarged urinary bladder with retention of urine and an enlarged left kidney with dilated ureter. Although a definite cause of luminal obstruction was not observed in the histological sections, an obstructive narrowing of the urethral lumen was cited as the most probable cause of the dilatation of urinary bladder, ureter, and renal pelvis.

One female offspring of another high dose female (433) had a malformation described as a changed shape of head. Necropsy revealed this change was hydrocephalus internus with the dorsal cerebral parts missing. In the EFD study U03-1284, hydrocephalus internus was not observed at dosages (15, 70 and 200 mg/kg BIBR 1048 MS) that are equal to or higher than the high dose in the present study.

10 Special Toxicology Studies

Study title: Cytotoxicity Assay In Vitro with BALB/C 3T3 Cells: Neutral Red (NR) Test with BIBR 1048 MS During Simultaneous Irradiation with Artificial Sunlight

Study no.:	1110200 (U07-1799)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 11, 2007
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 1048 MS, Batch 1029452, 99.7%

Key Study Findings

In two valid neutral red phototoxicity assays using BALB/c 3T3 cells, BIBR 1048 MS at concentrations ≥ 15.6 µg/mL induced a slight phototoxic effect.

Methods

Cell line:	BALB/c 3T3 (mouse)
Concentrations in definitive study:	0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25 µg/mL
Basis of concentration selection:	The high dose was limited by solubility
Negative control:	Solvent control (final 1% DMSO)
Positive control:	Chlorpromazine (6.25 – 200 µg/mL)
Formulation/Vehicle:	Earle's Balanced Salt solution (EBSS)
Incubation & sampling time:	

Treatment: Cells were seeded in two 96-well plates. The cells were washed 24 hrs later and dilutions of the test articles in Earles's Balance Salt Solution were added to the cells (100 µL/well). Both plates were incubated for 1 hr in the dark, and then one plate was irradiated for 50 minutes through the lid at a dose of 1.7 mW/cm^2 (5 J/cm^2) of artificial sunlight and the other plate was kept in the dark. The cells were then washed, fresh culture medium added and the plates incubated overnight.
Neutral Red Uptake Determination of Cell Survival: The culture medium was removed and neutral red in serum free medium (500 µg/mL) was added and the plates incubated for 3 hours. The plates were washed and 0.15 mL of 50% ethanol, 1% acetic acid in water was added and the absorbance at 540 nm was measured.

Study Validity

Both of the assays met the following acceptance criteria.

1. Cell viability of the irradiated solvent control is at least 80% of that of the non-irradiated solvent control.

2. The photo-irritancy factor (PIF) for the positive control chlorpromazine is at least 6, where PIF = ED_{50} (-UV)/ED_{50}(+UV), and
3. The mean absorbance of the untreated controls is ≥0.4.

Results

The Mean Phototoxic Effect (MPE) is defined as the weighted average across a representative set of photo effect values. If the MPE value is ≥0.15, the chemical is classified as phototoxic. The results were evaluated bases on the following rules

If PIF ≤ 2 or MPE ≤ 0.1, no phototoxic potential is predicted.
If (2 < PIF < 5) or (0.1 < MPE < 0.15, a probable phototoxic potential is predicted.
If (PIF ≥ 5) or (MPE ≥ 0.15), a phototoxic potential is predicted.

The two experiments are summarized below. Both experiments were valid based on the PIF and MPE values for the positive control, chlorpromazine, that indicate a substantial phototoxic effect. The first experiment indicated a slight phototoxic effect for BIBR 1048 MS, since the MPE was 0.216 or a value >0.15. The second experiment indicated a non-phototoxic effect for BIBR 1048 MS, since the MPE was 0.097 or a value <0.15. However, the report indicated a slight cytotoxic effect for BIBR 1048 MS in the presence of irradiation in the second experiment. Therefore, the report concluded that BIBR 1048 MS induced a slight phototoxic effect.

Table 167: Reviewer's Summary of Phototoxicity Experiments – Study U07-1799

	Experiment 1		Experiment 2	
	-UV	+UV	-UV	+UV
BIBR 1048 MS, High dose, µg/mL	31.25	31.25	31.25	31.25
Solvent control, Abs 540 nm	1.004	0.848	1.126	1.156
High dose, Abs 540 nm	1.020	0.5381	1.016	0.402
ED_{50}, µg/mL	ND	ND	ND	11
PIF	ND		ND	
MPE*	0.216		0.097	
Chlorpromazine, High dose, µg/mL	200	4	200	4
Solvent control, Abs 540 nm	0.895	0.876	1.107	1.064
High dose, Abs 540 nm	0.076	0.099	0.072	0.113
ED_{50}, µg/mL	20.56	0.234	12.30	0.263
PIF	87.97		46.92	
MPE*	0.745		0.731	
* Mean Phototoxic Effect (MPE) is calculated from the following equations.				
$MPE = \frac{\sum_{i=1}^{n} w_i PE_{ci}}{\sum_{i=1}^{n} w_i}$	Pec = Rec X Dec Rec = Rc(-Irr) – Rc(+Irr)		$DE = \left\lvert \frac{C/C^* - 1}{C/C^* + 1} \right\rvert$	

Study title: BIBR 953 ZW: Local Tolerance after Single Intravenous Injection to Rabbits

Study no.:	99B152 (U00-1025)
Study report location:	EDR
Conducting laboratory and location:	Department of Non-Clinical Drug Safety Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
Date of study initiation:	September 30, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 951 ZW, Batch 8830251, 98.9%

Study Design

BIBR 953 ZW was formulated at 5 mg/ml in the vehicle ZB 1432. Solutions of the vehicle alone and BIBR 953 ZW in vehicle were administered intravenously in a volume of 0.5 ml into the V. auricularis rostralis of the left and right ears of albino rabbits in which the left ear hemoperfusion was transiently blocked with a soft intestinal clamp to the base of the ear. Each group consisted of two male and two female rabbits (NZW, body weight males: 3.2-3.4 kg, females: 3.7-4.0 kg). Observations made for 11 days post treatment included visual monitoring of clinical signs, food and water consumption and local macroscopic reactions.

Results

Reactions found only in the BIBR 953 ZW treated animals included swelling and bluish-red discolorations as summarized in Table 168. The discolorations were attributed to hematoma formation resulting from the pharmacodynamic effect of BIBR 953 ZW.

Table 168: Findings U00-1025

Macroscopic changes	Number of animals per group (n=4) which showed signs of intolerance			
	Vehicle (ZB 1433)		BIBR 953 ZW (5 mg/ml, ZB 1432)	
	left ear	right ear	left ear	right ear
Erythema	-	-		-
Swelling	-	-	1	-
Bluish-red discoloration	-	-	3	3
Scabbing	-	-	-	-
Induration	-	-	-	-
Necrosis	-	-	-	-
Result	Well tolerated		Tolerated	

Study title: BIBR 953 ZW: Local Tolerance after Single Intra-Arterial Injection to Rabbits

Study no.:	99B153 (U00-1026)
Study report location:	EDR
Conducting laboratory and location:	Department of Non-Clinical Drug Safety Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
Date of study initiation:	September 30, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch 8830251, 98.9%

Study Design

BIBR 953 ZW was formulated at 5 mg/ml in the vehicle ZB 1432. Solutions of the vehicle alone and BIBR 953 ZW in vehicle were administered intravenously in a volume of 0.5 ml into the A. auricularis of the left and right ears of albino rabbits in which the left ear hemoperfusion was transiently blocked with a soft intestinal clamp to the base of the ear. Each group consisted of two male and two female rabbits (NZW, body weight males: 3.4-3.6 kg, females: 3.9-4.2 kg). Observations made for 11 days post treatment included visual monitoring of clinical signs, food and water consumption and local macroscopic reactions.

Results

Reactions found in both the vehicle and the BIBR 953 ZW treated animals included swelling and bluish-red discolorations as summarized in Table 169. The discolorations were attributed to hematoma formation resulting from the pharmacodynamic effect of BIBR 953 ZW.

Table 169: Findings - Study U00-1026

Macroscopic changes	Number of animals per group (n=4) which showed signs of intolerance			
	Vehicle (ZB 1433)		BIBR 953 ZW (5 mg/ml, ZB 1432))	
	left ear	right ear	left ear	right ear
Erythema	-	-	-	-
Swelling	-	-	1	-
Bluish-red discoloration	1	2	4	4
Scabbing	-	-	-	-
Induration	-	-	-	-
Necrosis	-	-	-	-
Result	moderately tolerated		tolerated	

Study title: BIBR 953 ZW: Local Tolerance after Single Paravenous Injection in Rats

Study no.:	99B154 (U00-1027)
Study report location:	EDR
Conducting laboratory and location:	Department of Non-Clinical Drug Safety Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
Date of study initiation:	September 30, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch 8830251, 98.9%

Study Design

BIBR 953 ZW was formulated at 5 mg/ml in the vehicle ZB 1432. Solutions of the vehicle alone and BIBR 953 ZW in vehicle were administered were administered paravenously in a volume of 0.2 ml laterally and 0.2 ml medially to the jugular vein of rats (Chbb: THOM (SPF, 4/sex/group), 52 days of age; body weight of 258-268 g in males and 163-199 g in females). Observations made hourly for 6 hours and at 24 hours post treatment included visual monitoring of skin reactions and macroscopic findings at injection sites.

Results

Skin reactions found only in the BIBR 953 ZW treated animals included erythema and bluish-red discolorations as summarized in Table 170. The discolorations were attributed to hematoma formation resulting from the pharmacodynamic effect of BIBR 953 ZW.

Table 170: Findings - Study U00-1027

	Vehicle (ZB 1433)		BIBR 953 ZW (ZB 1432)	
	6 h	24 h	6 h	24 h
Skin reactions	0:6 0.00	0:7 0.00	12:6 2.00	21:7 3.00
macroscopic findings	0	0	0	0
Cumulative score	0.00	0.00	2.00	3.00
Evaluation	well tolerated		well tolerated	

Study title: Hemolysis Test with Injectable Solutions of BIBR 953 ZW (5 mg/ml, calculated as base) and Placebo

Study no.:	99B158 (U99-1743)
Study report location:	EDR
Conducting laboratory and location:	Department of Non-Clinical Drug Safety Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.
Date of study initiation:	September 15, 1999
GLP compliance:	Indicated
QA statement:	Indicated
Drug, lot #, and % purity:	BIBR 953 ZW, Batch ZB 1432, purity not indicated

Study Design

BIBR 953 ZW was formulated at 5 mg/ml in the vehicle ZB 1432. Solutions of the vehicle alone and BIBR 953 ZW in vehicle were mixed with saline in the following proportions 1:0, 1:1, 1:3, 1:6, 1:9 and 1:12. These mixtures were mixed with blood from three human donors according to a standard hemolysis protocol and evaluated for the percentage of hemolysis.

Results

The maximum hemolysis of the 100% BIBR 953 ZW solution was 2.6% (range 1.6-3.9%) and the maximum hemolysis of the vehicle was 1.0% (range 0.2-2.5%). BIBR 953 ZW at 5 mg/mL can be injected safely.

11 Integrated Summary and Safety Evaluation

Because of its central role in blood coagulation, thrombin (FIIa) is a major target for inhibition by therapeutic drugs for use in thromboembolic diseases. BIBR 1048 MS (dabigatran etexilate mesilate) is the orally active prodrug of BIBR 953 ZW, a non-peptide inhibitor of thrombin. BIBR 1048 MS is approvable for the proposed indication

from a nonclinical perspective because BIBR 1048 MS was shown to be efficacious in animal models of venous thrombosis and most of the toxicities observed are attributable to its pharmacodynamic or supra-pharmacodynamic (bleeding) effect.

In vitro studies indicated that the concentration of BIBR 953 ZW needed to double the aPTT clotting time (ED_{200} aPTT) was 2, 2.5, and 8-fold higher for rats, monkeys, and rabbits, respectively, than for humans. Intravenous administration of BIBR 953 ZW or oral administration of BIBR 1048 MS induced a dose- and time-dependent prolongation of ex-vivo clotting times in several species. In rat and rabbit models of venous thrombosis, intravenous BIBR 953 ZW induced a dose-dependent decrease in clot weight with IC_{50} values of 33 and 66 µg/kg, respectively, concomitant with prolongation of ex vivo aPTT values. The higher IC_{50} value in rabbits than rats is consistent with the higher ED_{200} value for rabbits than for rats.

Oral administration 5 mg/kg of BIBR 1048 MS to monkey produced 3-fold increase in ex vivo aPTT, whereas oral administration of 20 mg/kg BIBR 1048 MS to rats was needed to induce a 3-fold increase in ex vivo aPTT. This difference may reflect not only the difference in the ability of BIBR 953 ZW to inhibit monkey versus rat thrombin, but also a higher clearance in the rat than the monkey and the rapid glucuronidation of BIBR 953 ZE in the monkey. The BIBR 953 ZW glucuronides representing about 50% of total BIBR 953 ZW in monkey plasma are more active than BIBR 953 ZW in prolonging aPTT values.

Table 172 summarizes the major toxicities observed in the toxicology studies conducted with BIBR 1048 MS or BIBR 953 ZW and Table 173 summarizes NOAELs by toxicology study. Most of the toxicities are attributable to the pharmacodynamic effect of BIBR 953 ZW and include prolongation of coagulation and effects on red blood cell hematology associated with bleeding at suprapharmacodynamic exposures as evidenced by visible hemorrhages (macroscopic and microscopic) and/or hemosiderosis. Since blood for hematology was collected 24 hours after the previous dose in the toxicology studies, the prolongation of coagulation times at the highest dosages indicates that adequate doses of BIBR 1048 MS were administered to rats and monkeys to induce pharmacodynamic effects throughout most or all of the period between doses.

Ideally, one would like to project safety margins based on comparison of the NOAEL doses with doses that produce therapeutic anti-thrombotic activity. In venous thrombosis models in rats and rabbits, an oral dose of 10 mg/kg BIBR 1048 MS inhibited clot formation by 95% and 97% at 0.5 and 2 hours post dosing, respectively (See Figure 9 and Table 6). However, because the half-life of BIBR 953 ZW is approximately 1 and 7 hours in rats and rabbits inhibition of clot formation is approximately 50% at 2 and 7 hours and 0% at 5-7 and 24 hours, respectively, after an oral dose of 10 mg/kg BIBR 1048 MS.

In the human clinical trials, the mean aPTT ratio at trough was 1.4-1.48. Table 171 shows that a 200 mg/kg dose to monkeys induced prolongation of aPTT values similar to the prolongation observed in humans even 24 hours after dosing. Therefore, potential safety concerns were adequately evaluated in monkeys. In contrast, the aPTT ratios in rats were lower, indicating that a daily dose of 200 mg/kg was insufficient to maintain a pharmacodynamic effect similar to that in humans throughout the period between

doses. Lower doses in rats and monkeys induced even lower aPTT ratios. However, at doses of 200 mg/kg in rats and monkeys the Cmax values were 13-25 fold higher than the mean trough plasma concentration in humans. Thus, during the initial period following dosing in the general toxicology studies, the animals were subjected to supratherapeutic doses of BIBR 953 ZW.

Table 171: Reviewer's Comparison of aPTT Ratios and BIBR 953 ZW Plasma Concentrations

			Trough† (Cmax)		BIBR 953 ZW concentration, ng/mL			
			aPTT ratio§		Trough		Cmax	
Species	Study	Dose	M	F	M	F	M	F
Rat	13 week (W)	200 mg/kg	1.14	1.22	112	196	2155	2470
	26 week (T)	200 mg/kg	1.26	1.35	28	23	1959	2550
	2 year (W)	200 mg/kg	1.17	1.34	142	107	2560	2530
Monkey	26 week	200 mg/kg	1.44	1.76	201*	472*	1380*	2200*
	52 week	200 mg/kg	1.36	1.52	112*	133*	2158*	2388*
Human	PETRO[1]	150 mg bid	1.48		91.6*			
	RE-LY[2]	150 mg bid	1.40 (1.66)		114*		213*	

W = Wistar strain, T = Chbb: THOM strain
† Trough in animals 24 hours after dosing and in humans 12 hours after dosing, * Total BIBR 953 ZW plus glucuronides
§ Ratio relative to control group in animal studies and relative to pre-dose in human studies.
[1] PETRO Phase 2 Clinical Trial (Source: Study 1160.20, Tables 11.5.3: 1 and 11.5.2: 1)
[2] RE-LY Phase 3 Clinical Trial (Source: Study 1160.26, Tables 11.5.3: 1 and 11.5.2.1: 1).

Bleeding resulting in death occurred in some rat studies in which 300 mg/kg was the highest dosage. Therefore, the Executive CAC recommended a maximum dose of 200 mg/kg to be used in the two year carcinogenicity studies. In contrast, two deaths of high dose monkeys in the 52-week study were attributed to aspiration of the highly acidic formulation. The deaths of these monkeys could have been prevented based on experience in shorter duration studies with the same or higher doses of BIBR 1048 MS.

The maternal deaths in the rat embryo-fetal development study occurred at 200 mg/kg, a dose only slightly lower than the dose that caused deaths in the general toxicology studies. However, the maternal deaths in the pre-postnatal development study occurred at even lower doses (70 mg/kg) generally during parturition. The projected safety margin for use of BIBR 1048 MS during the peri-natal period in humans, calculated by comparing the exposure at the NOAEL in the rat versus human exposure is low (1.2 fold) and appropriate warnings need to given in the label.

After essentially a lifetime of treatment of mice and rats with BIBR 1048 MS in the two year carcinogenicity studies, an increased incidence of liver necrosis was observed in both species at the highest dosage of 200 mg/kg. However, these findings occurred without increases in liver function parameters (ALT, AST) in the rat. No finding of liver necrosis was observed at 200 mg/kg in the 26- or 52 week monkey studies. The relevance of the liver necrosis in rats and mice to humans is unknown.

The toxicology studies using intravenous administration produced toxicities related to the pharmacodynamic effect as observed in studies using oral administration. One exception was the increased serum potassium observed in both species. The increase was limited to females in rats, but was present in both sexes in monkeys.

Table 172: Reviewer's Summary of Safety Margins for Relevant Toxicities

Toxicity	Species (Duration of toxicology study)	NOAEL (mg/kg)	AUC M/F at NOAEL ng.hr/mL	Safety Margin Based on AUC*
Oral administration BIBR 1048 MS				
Deaths attributed to treatment (bleeding or acidic formulation)	Rat (13 weeks)	100	6260, 10500	3.4, 4.3
	Rat (4 weeks)	70	5557, 4692	3.0, 1.9
	Rat (EFD) (maternal)	70	7200	2.9
	Rat (PPND) (maternal)	**30**	2770	**1.2**
	Monkey (52 weeks)	36	6438, 3710	3.5, 1.5
Decreases in Hb, Hct, and/or RBC	Monkey (26 weeks)	36	5910, 8130	3.2, 3.3
	Monkey (52 weeks)	36	6438, 3710	3.5, 1.5
Increased reticulocytes	Rat (4 weeks)	15	985, 1064	0.5, 0.5
	Monkey (26 weeks)	36	5910, 8130	3.2, 3.3
Prolonged coagulation times (aPTT)	Rat (26 weeks, THOM)	10	739, 545	0.4, 0.2
	Rat (13 weeks, Wistar)	15	1260, 1050	0.7, 0.5
	Monkey (26 weeks)	36	5910, 8130	3.2, 3.3
	Monkey (52 weeks)	36	6438, 3710	3.5, 1.5
Hemorrhage, multiple tissues	Rat (13 weeks, Wistar)	100	6260, 10500	3.4, 4.3
	Rat (13 weeks, Wistar)	55	3150, 3445	1.7, 1.4
	Monkey (26 weeks)	36	5910, 8130	3.2, 3.3
Hemosiderosis and fibrosis – thymus and pancreas	Rat (26 weeks. THOM)	40	2640, 1420	1.7, 0.6
	Rat (13 weeks, Wistar))	55	3150, 3445	1.7, 1.4
	Rat (4 weeks, THOM)	15	985, 1064	0.5, 0.5
Increased incidence of liver necrosis	Rat (104 weeks)	100	7810, 6633	4.2, 2.7
	Mouse (104 weeks)	100	3830, 5400	2.1, 2.2
Decreased implantations	Rat (FEED)	**15**	**1560**	**0.6**
Increased embryo-fetal resorption rate, decreased number of viable fetuses/offspring	Rat (EFD)	**15**	**1560**	**0.6**
	Rabbit (EFD)	70	2770	1.1
	Rat (PPND)	**30**	**2760**	**1.1**
Intravenous administration BIBR 953 ZW				
Decreases in Hb, Hct, RBC (accompanied by Increases in reticulocytes)	Rat (4 weeks)	0.5	593	0.5, 0.3
	Monkey (4 weeks)	4	13050, 14400	7.2, 5.9
Prolonged coagulation times (aPTT)	Rat (4 weeks)	0.5	527, 593	0.3, 0.3
	Monkey (4 weeks)	4	13050, 14400	7.2, 5.9
Increased serum potassium	Rat (4 weeks)	5, 0.5	527, 593	0.3, 0.3
	Monkey (4 weeks)	4	13050, 14400	7.2, 5.9

*Human mean $AUC_{(0\text{-}12,\ ss)}$ for M: 911 ng*hr/mL, F: 1233 ng*hr/mL at 150 mg bid for healthy young subjects. Since the animal exposures are $AUC_{(0\text{-}24hr)}$ values, the human AUC values must be doubled for a 24 hour period to 1822 and 2466 ng*hr/mL in males and females, respectively. These values for humans are based on Table 10.2:9 in the Pharmacokinetic Metaanalysis Report (U09-1363-01, Descriptive statistics of AUCtau,ss grouped by gender and age (young <65 and elderly ≥65 after oral administration of 150 mg dabigatran capsules etexilate bid in healthy white subjects (see Appendix 13). Note the AUC values in elderly subjects are 50-88% higher than the AUC values in young subjects.

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Table 173: Toxicology Studies - NOAEL and Toxicity Levels

Species	Study	Sex	NOAEL (mg/kg)	AUC at NOAEL (ng*hr/mL)	Fold relative to human		Toxic dose, mg/kg	AUC at toxic dose	Fold relative to human	
					mg/m^2 basis	mean AUC†			mg/m^2 basis	mean AUC†
General toxicology										
Monkey	52 weeks	M	36	6438	2.3	3.5	200	15475	13	8.5
		F	36	3710	2.3	1.5	200	15300	13	6.2
Monkey	26 weeks	M	36	5910	2.3	3.2	200	14500	13	8.0
		F	36	8130	2.3	3.3	200	19000	13	7.7
Rat (THOM)	26 weeks	M	40	2640	1.3	1.4	200	8780	6.5	4.8
		F	40	1420	1.3	0.6	200	6640	6.5	2.7
Rat (HW)	13 weeks	M	55	3150	1.8	1.7	200	10900	6.5	6.0
		F	55	3545	1.8	1.5	200	11400	6.5	4.6
Reproductive and Developmental Toxicology										
Rat - FEED	Paternal	M	70	7320	2.3	4.0	200	22500	6.5	12.3
	Maternal	F	70	7320	2.3	3.0	200	22500	6.5	9.1
	Embryo		**15**	**1560**	0.5	**0.6**	70	7320	2.3	3.0
	Fertility		200	22500	6.5	8.5	-	-	>6.5	>9.1
Rat- EFD	Maternal	F	**15**	**1560**	**0.5**	**0.6**	70	7320	2.3	3.0
	F_1		**15**	**1560**	**0.5**	**0.6**	70	7320	2.3	3.0
Rabbit - EFD	Maternal	F	70	2770	4.5	1.1	200	8210	13	3.3
	F_1		70	2770	4.5	1.1	200	8210	13	3.3
Rat - PPD	Maternal	F	30	2760	1.0	1.1	70	6390	2.3	2.6
	F_1 (pre-)		30	2760	1.0	1.1	70	6390	2.3	2.6
	F_1 (peri)		30	2760	1.0	1.1	70	6390	2.3	2.6
	F_1 (post)		70	6390	2.3	2.6	-	-	>2.3	>2.6
	F_1 (fertility)		70	6390	2.3	2.6	-	-	>2.3	>2.6

† Human mean $AUC_{(0\text{-}12,\ ss)}$ for M: 911 ng*hr/mL, F: 1233 ng*hr/mL at 150 mg bid for healthy young subjects. Since the animal exposures are $AUC_{(0\text{-}24hr)}$ values, the human AUC values must be doubled for a 24 hour period to 1822 and 2466 ng*hr/mL in males and females, respectively. These values for humans are based on Table 10.2:9 in the Pharmacokinetic Metaanalysis Report (U09-1363-01, Descriptive statistics of AUCtau,ss grouped by gender and age (young <65 and elderly ≥65 after oral administration of 150 mg dabigatran capsules etexilate bid in healthy white subjects (see Appendix 13). Note the AUC values in elderly subjects are 50-88% higher than the AUC values in young subjects.

In the rat fertility and early embryo development study, BIBR 1048 MS treatment at doses of 200 mg/kg induced maternal toxicity based on decreased body weight gain accompanied by decreased food consumption (and lethality in a prior FEED dose range finding study with dosing to GD 16). Although BIBR 1048 MS treatment did not significantly affect the copulation, fertility and gestation indices, BIBR 1048 MS treatment produced embryo toxicity. Doses of 70 and 200 mg/kg significantly reduced the mean number of implantations, increased pre-implantation loss, and decreased the number of viable fetuses. The NOAEL for paternal and maternal toxicity was 70 mg/kg; however, the NOAEL for embryo toxicity was 15 mg/kg. These results are a concern because the embryo toxicity occurred at a lower dose (70 mg/kg) than the maternal toxicity (200 mg/kg). Since the maternal rat exposure ($AUC_{(0\text{-}24h)}$) at the embryo NOAEL (1560 ng•h/mL) is less than the exposure for a non-pregnant female human (2466 ng.hr/mL), no projected safety margin, calculated on the basis of exposure comparison, exists for early embryo toxicity based on this rat study.

In the rat embryo-fetal development study, BIBR 1048 MS treatment at 70 and 200 mg/kg induced maternal toxicity based on a significant decrease in body weight gain

and food consumption, and lethality at the high dose. The high dose increased the total, early and late resorptions, increased the resorption rate and decreased the mean number of viable fetuses. The mid dose increased total and early resorptions, increased resorption rate, decreased the percentage of males, and decreased number of viable fetuses. Excessive toxicity in the high dose group was indicated by two deaths, one of which accompanied an abortion and resorptions only in two dams. Furthermore, fetal body weight, a marker of excessive toxicity in embryo-fetal toxicity studies (Fleeman et al 2005) was significantly decreased at this dosage. Embryo findings included a rare malformation (cleft thoracal vertebral body) observed only in the treated groups at an incidence above the historical control range and with a dose-relationship. The incidences of skeletal variations (ossification delay of the supraoccipital bone, the occipital bone, cervical vertebral bodies, and calcaneum) were increased in the BIBR 1048 MS treated groups, particularly the high dose group. Although BIBR 1048 MS is not an overt teratogen, it is embryo-toxic in rats. Based on the findings of decreased number of viable fetuses, increased number of resorptions, and resorption rate in the mid dose group, NOAEL for embryo/fetal toxicity was 15 mg/kg, the same as that for maternal toxicity. Although the NOAELs for embryo/fetal and maternal toxicity were the same, the results are still a concern, since the maternal rat exposure at the embryo NOAEL (1560 ng•h/mL) is less than the exposure for a non-pregnant female human (2466 ng.hr/mL). Thus, no projected safety margin, calculated on the basis of exposure comparison, exists for embryo/fetal toxicity based on this rat study.

In the rat pre and postnatal development (PPND) study, BIBR 1048 MS treatment with the high dose of 70 mg/kg induced maternal toxicity that included not only decreases in body weight and food consumption, but also F_0 mortalities associated with vaginal bleeding primarily during parturition. This high dose increased post-implantation loss, number of dead F_1 offspring and resulted in a significantly decreased birth index. Survival of F_1 offspring after birth to LD 4 was decreased in the mid and high dose. Treatment of F_0 dams with BIBR 1048 MS during gestation and lactation did not significantly affect sex ratio, body weight at birth, body weight development, postnatal survival (after lactation day 4), general physical development, reflexes, sensory functions, learning ability, memory, explorative behavior, and fertility of the F_1 animals or the intrauterine survival of the F_2 embryos pre- and post implantation. Although the NOAEL for postnatal toxicity (after lactation day 4), development and fertility in F_1 offspring was 70 mg/kg, the NOAEL both for maternal (F_0) toxicity and for pre/perinatal (to lactation day 4) toxicity in F_1 offspring was 30 mg/kg. The maternal exposure at the NOAEL for maternal and pre/perinatal offspring toxicity (2760 ng.hr/mL) is similar to the exposure for a non-pregnant female human (2466 ng.hr/mL). Thus, little safety margin calculated on the basis of exposure comparison, exists for maternal and pre/perinatal offspring toxicity based on this rat study.

In a rabbit embryo fetal development study, BIBR 1048 MS treatment at 200 mg/kg induced maternal toxicity based on decreased body weight gain. Although treatment with BIBR 1048 MS did not significantly affect most of the litter parameters, the percentage of males in the high dose group was below the minimum of the historical range. Also, the number of total resorptions, number of early resorptions, and the resorption rate increased in the high dose group compared to the concurrent control group. Some apparent findings of missing gall bladder, dilated cerebral ventricle,

additional vessels at the aortic arch and hypoplasia of gall bladder in the treated fetuses were attributed to normal spontaneous variation in rabbits. The NOAEL for maternal toxicity is 70 mg/kg based on decreased body weight gain; however, the NOAEL for embryo fetal toxicity is also 70 mg/kg based on alteration of the sex ratio and increased resorption. The maternal exposure at the NOAEL for maternal and embryo fetal toxicity (2770 ng.hr/mL) is similar to the exposure for a non-pregnant female human (2466 ng.hr/mL). Thus, little safety margin, calculated on the basis of exposure comparison, exists for maternal and embryo fetal based on this rabbit study. The higher NOAEL for embryo-fetal toxicity in the rabbit compared to the rat is consistent with the lower anticoagulant activity of BIBR 953 ZW in rabbit plasma compared to rat plasma.

Administration of BIBR 1048 MS resulted in significant embryo/fetal/offspring toxicity in all three developmental toxicity studies in rats. The NOAELs for embryo/fetal/offspring toxicity in the FEED study, the EFD study, and the PPND study in rats were 15 mg/kg, 15 mg/kg and 30 mg/kg (or 0.5, 0.5 and 1 times the MRHD of 300 mg/day on a mg/m2 basis), respectively. The safety margins for embryo/fetal/offspring toxicity based on exposure comparisons are 0.6, 0.6 and 1.2 fold the a steady state AUC of about 2466 ng.hr/mL for the 150 mg BID dose in non-pregnant human females.

However, comparisons based solely on body surface area or exposure do not fully describe the treatment of the animals in the reproductive toxicology studies. Table 174 compares the plasma concentrations of BIBR 953 ZW in reproductive toxicology studies relative to the mean Cmax and trough plasma concentrations in humans. In the rat FEED, EFD and PPND studies, the plasma concentrations of BIBR 953 ZW at the toxic dose of 70 mg/kg in the dams were many multiples (5-23) of the mean human Cmax and trough plasma concentrations only during the first few hours after dosing. However, by 4 hours and certainly 8 hours after dosing, the plasma concentrations in the dams decreased and were similar to or less than the plasma concentrations in humans. In the rabbit EFD study, the plasma concentrations of BIBR 953 ZW at the toxic dose of 200 mg/kg in the dams were many multiples (5-23) of the mean human Cmax and trough plasma concentrations for up to 8 hours after dosing. In the rat and rabbit reproductive toxicology studies, the animals were subjected to supra therapeutic doses of BIBR 953 ZW for at least 12% and 33%, respectively, of each day following dosing.

Table 174: Reviewer's Comparison - Plasma Concentrations of BIBR 953 ZW in Reproductive Toxicology Studies Relative to Those in Humans

Time Post dose, hr	Rat, EFD – toxic at 70 mg/kg			Rat PPND – toxic at 70 mg/kg			Rabbit, EFD – toxic at 200 mg/kg		
	Plasma conc., ng/mL	Relative to human at		Plasma conc., ng/mL	Relative to human at		Plasma conc., ng/mL	Relative to human at	
		Cmax*	trough†		Cmax*	trough†		Cmax*	trough†
0	8						106	0.5	1
1	2350	11	23						
2	1090	5	10	1200	6	12	1750	8	17
4	322	1.5	2				1480	7	14
8	196	1	2	133	0.6	1	657	3	6
24	62	0.3	0.6	15	0.1	0.2	80	0.4	0.8

* Mean plasma concentration at Cmax was 213 ng/mL in the RE-LY Phase 3 Clinical Trial (Source: Study 1160.26, Tables 11.5.3: 1 and 11.5.2.1: 1).
† Mean plasma concentration at trough was 91.6 and 114 ng/mL in the PETRO Phase 2 and RE-LY Phase 3 Clinical Trial s, respectively (Source: Study 1160.20, Tables 11.5.3: 1 and 11.5.2: 1 and Study 1160.26, Tables 11.5.3: 1 and 11.5.2.1: 1).

The decreased implantation in the mid and high dose groups in the rat study FEED occurred in the presence of only a 2.8 and 3.7% decrease in absolute body weight and a 3.5 and 7.8% decrease in food consumption compared to the values in the control group. Terry et al (2005) demonstrated that feed restriction to about 90% of ad lib values resulted in approximately a 15% decrease in absolute body weight of pregnant Sprague Dawley rats, but had no effect on the number of implants and the number of viable embryos. Therefore, the 9.3 and 11% decrease in the number of implantations in the Han Wistar female rats treated with 70 and 200 mg/kg BIBR 1048 MS, respectively, is not likely attributable to maternal toxicity unless the two rat strains differ considerably in their response to decreased food consumption.

In the rat EFD study BIBR 1048 MS treatment at 70 and 200 mg/kg resulted in 3% and 8% decreases in absolute body weight gain accompanied by 5.6% and 17.6% decreases in food consumption, respectively, compared to values in the control group. Fleeman et al (2005) demonstrated that feed restriction to 50% of ad lib values resulted in 21% decrease in absolute maternal body weight and 7% decrease in fetal body weight, but had no effect on the number of viable fetuses, the number of resorptions, incidence of malformations or delayed ossification. Therefore, the 6.5% and 18% decrease in the number of viable fetuses, the 44% and 320% increase in resorptions, presence of a rare skeletal malformation only in treated groups, and increased incidence of delayed ossification in the Han Wistar rats treated with 70 and 200 mg/kg BIBR 1048 MS are not likely attributable to maternal toxicity unless the two rat strains differ considerably in their response to decreased food consumption.

The expression of both prothrombin (FII) and the thrombin receptor (PAR-1) during organogenesis in the mouse is consistent with a role of FIIa in development (Soifer et al 1994, Griffen et al 2001). During mouse development, prothrombin is expressed as early as 9.5 days post coitum in the visceral endoderm of the yolk sac (Sun et al 1998) and later in the liver (Sun et al 1998, Soifer et al 1994) using in situ hybridization. However, using a more sensitive RT-PCR assay, prothrombin gene expression was already at a relatively high level at 7.5 days post coitum (the earliest time point evaluated) (Ong et al 2000).

The importance of thrombin in embryo/fetal development is further supported by lethality in prothrombin- and PAR1-deficient mice. Transgenic mouse embryos made genetically deficient in prothrombin die either mid-gestation (9.5 days post coitum) or immediately after birth (Sun et al 1998; Xue 1998). In addition, approximately 50% of PAR1-deficient mouse embryos die at midgestation days (9-10 post coitum) (Connolly et al 1996). A similar early lethality may be associated with human FII deficiency, because no individual human has ever been identified that completely lack FII (Degen 1995).

The sponsor maintains that only a small amount of [^{14}C]-BIBR 953 ZW is transferred through the placenta to the fetus based on whole body autoradiography in pregnant albino rats on day 15-16 after oral administration of BIBR1 1048 MS or day 18-19 after subcutaneous administration of [^{14}C] BIBR 953 ZW. However, only two time points were examined with the later time point at 2 hours after dosing. In both instances, the level of radioactivity increased in the fetus at the second timepoint, whereas the levels in almost all other tissues decreased compared to the first time-point. The level of radioactivity in

the fetus after either administration was similar to that in caudal muscle. Therefore, some BIBR 953 ZW crosses the placenta. However, we do not know whether the levels of BIBR 953 ZW in the fetus continue to increase with time.

In the human fetus at 19-23 weeks of gestation, levels of both procoagulant factors and coagulation inhibitors range from only 10-25% of their corresponding adult values (Andrew and Paes 1990; Andrew et al 1987). At the end of gestation, all the serine protease zymogens are approximately 50% of the adult level (Andrew et al 1987). Similar to the human neonate, the rabbit neonate has plasma prothrombin levels in that are 50% of the adult mean (Hathaway et al 1964; Karpatkin et al 1991). On gestation day 25 and 29, the rabbit fetus has 10% and 49% of adult prothrombin protein levels, respectively, even though it has >60 and 99% of adult prothrombin mRNA levels (Karpatkin et al 2000).

The levels of FII protein earlier in development may be even lower than that indicated above. Sun et al (2002) generated additional transgenic mice in attempts to rescue the thrombin deficient mice from either the embryonic lethal phenotype or both the embryonic and neonatal lethal phenotype of prothrombin deficiency. One transgenic line was able to rescue both the embryonic and the neonatal lethality. In blood collected from adults of this transgenic line, the thrombin activity was found to be at 5-10% of wild-type adult levels. Assuming that the levels of prothrombin in the rat fetus are similar to that in the rabbit (10% of adult levels), the level prothrombin needed to rescue the embryonic and the neonatal lethality may be only 0.5-1% of adult levels. Therefore, a low level of placental transfer of BIBR 1048 MS to the fetus may still be sufficient to inhibit fetal thrombin and induce an adverse effect in the embryo/fetus. Some of the adverse effects seen in the reproductive and developmental toxicity studies in rats and rabbits with BIBR 1048 MS, especially loss occurring during parturition, may be the result of supra-pharmacodynamic effect of the drug. i.e., occult or overt bleeding. However, the animal studies do not clearly reveal the extent to which pharmacodynamic (therapeutic) as well as suprapharmacodynamic activity contribute to the fetal and embryonic loss.

The skeletal variations observed in the rat embryo-fetal study may also be a pharmacodynamic effect of BIBR 1048 MS on thrombin, which is important for osteoblast function and proliferation. Thrombin stimulates PAR-1-mediated proliferative responses in osteoblasts and chondrocytes (Mackie et al 2008). Although many of the effects of thrombin on bone cells are mediated by PAR-1, studies in PAR-1 null mice suggest that thrombin also is important in bone healing (Pagel et al 2006).

BIBR 1048 MS appears not to be an overt teratogen, but is an embryo/fetal toxicant, especially at suprapharmacodynamic dosages causing bleeding. However, the use of BIBR 1048 MS in treating thromboembolic disorders may be acceptable, despite the potential risks to the offspring as long as the label clearly indicates the risk.

12 Appendix/Attachments

Appendix 1: Lots of BIBR used in Toxicology Studies

Sponsor's table | **Reviewer's addition**

Study	Species	Dose(s)§ (mg/kg)	Remarks	Reference	Lot used	Maximum dose	NOAEL
13-week MTD study	Mouse	30, 100 and 300, p.o.	NOAEL 100 mg/kg	U05-1377	8250250	300 mg/kg	100 mg/kg
4-week toxicity study	Rat	15, 70 & 300 (unstressed versus stressed), 28 d, p.o.	Hemorrhages in heart (high dose only), thymus and pancreas	U98-2729	RAL-98	300 mg/kg	70 mg/kg
13-week MTD study	Rat	30, 100 and 300, p.o.	Hemorrhages in various organs	U05-1378	8250250	300 mg/kg	100 mg/kg
13-week toxicity study	Rat	15, 55 and 200 mg/kg	No toxicologically meaningful changes observed, NOAEL 200 mg/kg	U07-1693	1024879SPK	200 mg/kg	55 mg/kg *PD effect*
26-week toxicity study	Rat	10, 40 & 200, 183 d, p.o.	Hemosiderosis in pancreas and thymus, decreased liver enzymes	U03-1310	8050461	200 mg/kg	40 mg/kg
4-week toxicity study	Rhesus monkey	30, 100 & 300, 28 d, p.o.	No toxicologically meaningful changes observed, NOAEL 30 mg/kg	U98-2723	RAL-98	300 mg/kg	30 mg/kg
26-week toxicity study	Rhesus monkey	12, 36 & 200, 183 d, p.o.	Hemorrhages in various organs (high dose only), NOAEL 36 mg/kg	U03-1208	8050461	200 mg/kg	36 mg/kg
52-week toxicity study	Rhesus monkey	12, 36 & 200, 365 d, p.o.	No toxicologically meaningful changes observed, NOAEL 36 mg/kg	U05-1557	8250250	200 mg/kg	36 mg/kg
Carcinogenicity study	Mice	30, 100 & 200	No evidence for carcinogenic potential	U07-2181	8250250	200 mg/kg	*100 mg/kg PD effect*
Carcinogenicity study	Rat	30, 100 & 200	No evidence for carcinogenic potential	U07-2084	8250250	200 mg/kg	*100 mg/kg PD effect*

PD effect = Pharmacodynamic effect limited NOAEL to less than maximum dose

Appendix 2: Complete ICSAS Reports for BIBR 1048 and Impurity (b) (4)

To: Patricia Harlow
cc: Albert Defelice
From: CDER/OPS/SRS/ICSAS
Re: NDA 22512
Date: June 18, 2010

Dabigatran etexilate was evaluated by CDER/OPS/SRS/ICSAS for rodent carcinogenicity and genetic toxicity using four (quantitative) structure-activity relationship [(Q)SAR] computational toxicology software programs[1], and for reproductive and developmental toxicity and hepatobiliary adverse effects using Leadscope Model Applier and MC4PC. The results of the predictions from all software programs used were weighted equally and the analysis was optimized for sensitivity (minimizing false negatives) to reach the overall conclusions.

Rodent Carcinogenicity[2]

Software	Rat	Mouse
Derek for Windows	NSA	NSA
Leadscope	NC	NC
MC4PC	-	-
SciQSAR	-	-
Overall ICSAS Prediction	-	-

In considering the entire weight of evidence, ICSAS concludes that dabigatran etexilate is predicted to be negative for both rat and mouse carcinogenicity.

Genetic Toxicity for Predicting ICH S2 Battery[2]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity	Mouse Lymphoma	*In Vitro* Chromosome Aberrations	*In Vivo* Micronucleus
Derek for Windows	NSA	NSA	NSA	NSA	NSA
Leadscope	NC	NC	NC	NC	NC
MC4PC	-	NC	NC	+	-
SciQSAR	-	N/A	-	-	-
Overall ICSAS Prediction	-	**NC**	**NC**	**+**	-

In considering the entire weight of evidence, ICSAS concludes that dabigatran etexilate is predicted to be negative for genetic toxicity in the ICH S2 battery.

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[1] Derek for Windows 12, Leadscope Model Applier 1.3.3-3, MC4PC 2.2.0.22, and SciQSAR 2.2

[2] + = positive; − = negative; Eqv = equivocal; NSA = no structural alerts are identified by DfW; NC = test chemical features are not adequately represented in the model training data set, leading to a no call; N/A = no available model.

Genetic Toxicity for Predicting Carcinogenicity[2]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity	Fungal Mutagenicity	*Drosophila* Mutagenicity	*In Vivo* Rodent Mutation	hgprt	*In Vivo* Chromosome Aberrations	*In Vivo* Micronucleus	UDS
Derek for Windows	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA
Leadscope	NC	NC	NC	NC	NC	NC	NC	NC	NC
MC4PC	-	NC	NC	NC	NC	NC	N/A	-	NC
SciQSAR	-	N/A	+	N/A	+	-	-	-	-
Overall ICSAS Prediction	**-**	**NC**	**+**	**NC**	**+**	**NC**	**NC**	**-**	**NC**

In considering the entire weight of evidence, ICSAS concludes that dabigatran etexilate is predicted to be positive for genetic toxicity in the carcinogenicity prediction battery.

Reproductive Toxicity[2]

Software	Female Rat	Female Mouse	Male Rat	Male Mouse	Sperm Effects Male Rat	Sperm Effects Male Mouse
Leadscope	NC	NC	NC	NC	NC	NC
MC4PC	-	NC	NC	NC	NC	+
Overall ICSAS Prediction	**NC**	**NC**	**NC**	**NC**	**NC**	**+**

In considering the entire weight of evidence, ICSAS concludes that dabigatran etexilate is predicted to be positive for reproductive toxicity in rodents.

Behavioral Toxicity[2]

Software	Rat	Mouse
Leadscope	NC	NC
MC4PC	NC	NC
Overall ICSAS Prediction	**NC**	**NC**

In considering the entire weight of evidence, ICSAS concludes that no prediction can be made for dabigatran etexilate for behavioral toxicity in newborn rodents.

Fetal Dysmorphogenesis[2]

Software	Rat	Mouse	Rabbit
Leadscope	NC	NC	NC
MC4PC	-	NC	NC
Overall ICSAS Prediction	**NC**	**NC**	**NC**

In considering the entire weight of evidence, ICSAS concludes that no prediction can be made for dabigatran etexilate for fetal dysmorphogenesis in rodents.

Adverse Human Hepatobiliary Effects[2]

Software	Liver Damage	Liver Enzymes	Jaundice	Gall Bladder Disorders	Bile Duct Disorders
Leadscope	NC	NC	NC	NC	NC
MC4PC	-	-	Eqv	-	+
Overall ICSAS Prediction	**NC**	**NC**	**NC**	**NC**	**+**

In considering the entire weight of evidence, ICSAS concludes that dabigatran etexilate is predicted to be positive for adverse hepatobiliary effects in humans.

(b) (4) was evaluated by CDER/OPS/SRS/ICSAS for rodent carcinogenicity and genetic toxicity using four (quantitative) structure-activity relationship [(Q)SAR] computational toxicology software programs, and for reproductive and developmental toxicity and hepatobiliary adverse effects using Leadscope Model Applier and MC4PC[1]. The results of the predictions from all software programs used were weighted equally and the analysis was optimized for sensitivity (minimizing false negatives) to reach the overall conclusions.

Rodent Carcinogenicity[2]

Software	Rat	Mouse
Derek for Windows	NSA	NSA
Leadscope	NC	NC
MC4PC	-	-
SciQSAR	-	-
Overall ICSAS Prediction	**-**	**-**

In considering the entire weight of evidence, ICSAS concludes tha (b) (4) is predicted to be negative for both rat and mouse carcinogenicity.

Genetic Toxicity for Predicting ICH S2 Battery[2]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity	Mouse Lymphoma	*In Vitro* Chromosome Aberrations	*In Vivo* Micronucleus
Derek for Windows	NSA	NSA	NSA	NSA	NSA
Leadscope	NC	NC	NC	NC	NC
MC4PC	-	NC	NC	+	-
SciQSAR	-	N/A	-	-	+
Overall ICSAS Prediction	**-**	**NC**	**NC**	**+**	**+**

In considering the entire weight of evidence, ICSAS concludes that (b) (4) is predicted to be positive for genetic toxicity in the ICH S2 battery.

Genetic Toxicity for Predicting Carcinogenicity[2]

Software	*Salmonella* Mutagenicity	*E. coli* Mutagenicity	Fungal Mutagenicity	*Drosophila* Mutagenicity	*In Vivo* Rodent Mutation	hgprt	*In Vivo* Chromosome Aberrations	*In Vivo* Micronucleus	UDS
Derek for Windows	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA
Leadscope	NC	NC	NC	NC	NC	NC	NC	NC	NC
MC4PC	-	NC	NC	NC	NC	NC	N/A	-	NC
SciQSAR	-	N/A	+	N/A	+	-	-	+	-
Overall ICSAS Prediction	**-**	**NC**	**+**	**NC**	**+**	**NC**	**NC**	**+**	**NC**

In considering the entire weight of evidence, ICSAS concludes that (b) (4) s predicted to be positive for genetic toxicity in the carcinogenicity prediction battery.

Reproductive Toxicity[2]

Software	Female Rat	Female Mouse	Male Rat	Male Mouse	Sperm Effects Male Rat	Sperm Effects Male Mouse
Leadscope	NC	NC	NC	NC	NC	NC
MC4PC	-	NC	NC	NC	NC	+
Overall ICSAS Prediction	**NC**	**NC**	**NC**	**NC**	**NC**	**+**

In considering the entire weight of evidence, ICSAS concludes that (b) (4) is predicted to be positive for reproductive toxicity in rodents.

Behavioral Toxicity[2]

Software	Rat	Mouse
Leadscope	NC	NC
MC4PC	NC	NC
Overall ICSAS Prediction	**NC**	**NC**

In considering the entire weight of evidence, ICSAS concludes that no prediction can be made for (b) (4) (b) (4) for behavioral toxicity in rodents.

Fetal Dysmorphogenesis[2]

Software	Rat	Mouse	Rabbit
Leadscope	NC	NC	NC
MC4PC	-	NC	NC
Overall ICSAS Prediction	**NC**	**NC**	**NC**

In considering the entire weight of evidence, ICSAS concludes that no prediction can be made for (b) (4) (b) (4) for fetal dysmorphogenesis in rodents.

Adverse Human Hepatobiliary Effects[2]

Software	Liver Damage	Liver Enzymes	Jaundice	Gall Bladder Disorders	Bile Duct Disorders
Leadscope	NC	NC	NC	NC	NC
MC4PC	-	-	-	-	+
Overall ICSAS Prediction	**NC**	**NC**	**NC**	**NC**	**+**

In considering the entire weight of evidence, ICSAS concludes that (b) (4) s predicted to be positive for adverse hepatobiliary effects in humans.

This report has been reviewed and finalized by the Informatics and Computational Safety Analysis Staff.

Appendix 3: Executive CAC - Protocol Meeting Minutes

Executive CAC
Date of Meeting: March 16, 2004

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Josie Yang, Ph.D., HFD-550, Alternate Member
Albert Defelice, Ph.D., HFD-110, Team Leader

Presenting Reviewer and Author of Draft Minutes: Patricia Harlow, Ph.D., HFD-110

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

The Committee met to consider the adequacy of the protocols for a two-year carcinogenicity bioassays in rats and mice. The Committee did not address the sponsor's proposed statistical evaluation for the two-year bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND/NDA # 65, 813
Drug Name: BIBR 1048 MS
Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.

Background

BIBR 1048 MS is the methanesulfonate salt of a double pro-drug. The active form, BIBR 953 ZW, is a direct inhibitor of thrombin (Factor IIa).

The human dose for BIBR 1048 MS has not yet been finalized. In the Phase 3 trials, maximum doses of 150 mg BID and 300 mg BID are being used for prevention of deep-vein thrombosis in orthopedic patients and prevention of stroke in patients with atrial fibrillation, respectively.

Rat Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed a 104-week study using 55 Wistar rats/sex in the control, low and mid dose groups and 65 Wistar rats/sex in the high dose group. Based on the potential mortality due to hemorrhagic events and limitations of viscosity and acidity of the formulation, the sponsor proposed daily doses of 0, 30, 100 and 300 mg/kg/day administered by oral gavage.

In a 13-week toxicology study in Wistar rats, mortality, potentially due to hemorrhagic events, was 1.25 % at the dose of 300 mg/kg/day.

The sponsor also proposed separate satellite groups (10 animals/sex/dose group) for monitoring toxicokinetics on Day 1 and during weeks 26 and 52. Blood samples for hematology and clinical chemistry will be collected at scheduled necropsy from 20 main study animals/sex/group.

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

Mouse Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed a 104-week study using 54 CD-1 mice/sex in the control, low and mid dose groups and 63 CD-1 mice/sex in the high dose group. Based on the potential mortality due to hemorrhagic events and limitations of viscosity and acidity of the formulation, the sponsor proposed daily doses of 0, 30, 100 and 300 mg/kg/day administered by oral gavage.

In a 13-week toxicology study in CD-1 mice, mortality, potentially due to hemorrhagic events, was 0.9 % at the dose of 300 mg/kg/day.

The sponsor also proposed separate satellite groups (24 animals/sex/dose group) for monitoring toxicokinetics on Day 1 and during week 26. Blood samples for hematology will be collected at scheduled necropsy from 20 main study animals/sex/group.

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

Executive CAC Recommendations and Conclusions:

Rat:

***** The Committee recommended doses of 0, 30, 100 and 200 mg/kg/day in the rat bioassay, based on an MTD determined by the mortality due to hemorrhage, an expected consequence of the pharmacodynamic action of the drug at 300 mg/kg.

Mouse:

***** The Committee recommended doses of 0, 30, 100 and 200 mg/kg/day in the mouse bioassay, based on an MTD determined by the mortality due to hemorrhage, an expected consequence of the pharmacodynamic action of the drug at 300 mg/kg.

Overall comments:

* If the survival of any dose group approaches 25 animals during the study, the sponsor should contact the Division prior to termination of dose groups or stoppage/reduction of dosing. The Committee noted that the sponsor should NOT terminate any dose groups prior to the scheduled study termination or change any dosing without first contacting the Agency.

* The Committee noted that the toxicokinetic animals could be eliminated, since additional toxicokinetic data are not needed. Blood could be taken at terminal sacrifice for toxicokinetic data, if desired.

* The Committee noted that clinical pathology (hematology, blood chemistry and urinalysis) and organ weights are not needed for the evaluation of the carcinogenicity studies. However, measurements of coagulation parameters (aPTT and PT) would be valuable.

* The Committee was concerned about burns to the animals from the low pH of the formulation, and wondered if buffering with a citrate buffer would help. The ulceration and burning of the esophagus also contributes to an MTD. However, the sponsor would need to verify that exposure did not change with the change in formulation.

* The Committee recommended that at least the males be housed separately to prevent potential hemorrhage as the result of fighting.

The sponsor plans histological evaluation of tissues on the protocol list from all treatment groups and any macroscopic abnormalities. The sponsor will also need to conduct histopathologic examination of all dose groups for any macroscopic findings in any dose group for a given tissue not on the protocol list.

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

cc:\
/Division File, HFD 110
/A. Defelice, Team leader, HFD-110
/P. Harlow, Reviewer, HFD-110
/M. Pease-Fye, CSO/PM, HFD-110
/A. Seifried, HFD-024

Appendix 4: Executive CAC - Meeting Minutes - Study Reports

Executive CAC
Date of Meeting: February 16, 2010

Committee: David Jacobson-Kram, Ph.D., ONDIO, Chair
Abby Jacobs, Ph.D., ONDIO, Member
Paul Brown, Ph.D., ONDIO, Member
David Joseph, Ph.D., DGP, Alternate Member
Albert Defelice, Ph.D., DCRP, Team Leader

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

The following information reflects a brief summary of the Committee discussion and its recommendations. The Committee met to consider the results of two-year carcinogenicity bioassays in rats and mice.

IND/NDA: IND 65,813/NDA 22-512
Drug Name: BIBR 1048 MS (dabigatran etexilate)
Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.

Background

BIBR 1048 MS is the methanesulfonate salt of a double pro-drug. The active form, BIBR 953 ZW, is a direct inhibitor of thrombin (Factor IIa). In the Phase 3 trial for prevention of stroke in patients with atrial fibrillation, the maximum dose was 150 mg dabigatran etexilate bid.

Rat Carcinogenicity Study

In a 104-week study using 55 Han Wistar rats/sex in the control, low and mid dose groups and 65 Wistar rats/sex in the high dose group, daily doses of 0, 30, 100 and 200 mg/kg/day of BIBR 1048 MS were administered by oral gavage. The exposure in the high dose males and females was 12.4 and 9.6 fold, respectively, the mean human exposure in subjects receiving 150 mg dabigatran etexilate bid.

Although no treatment related effects were observed on body weight or food consumption, a dose-related increase in mortality was observed in both sexes compared to control groups and was attributed to the pharmacodynamic effect of BIBR 1048 MS. Likewise, hematology findings (decreased hemoglobin concentration and red blood cells along with increased reticulocyte counts and coagulation times), macroscopic findings (abnormal dark contents in multiple tissues) and microscopic findings of hemorrhage were dose-related and consistent with the pharmacodynamic action of BIBR 1048 MS.

Increased incidences of neoplasms were observed in the testes and the ovaries. The incidence of testicular Leydig cell adenomas was within the laboratory historical range,

and the attained p values for the pairwise test and trend test do not reach the thresholds to classify these tumors as positive by the criteria used by the Exec-CAC. In addition, there was lack of a clear dose-relationship for the incidence of Leydig cell adenomas and the absence of either Leydig cell hyperplasia or Leydig cell carcinoma in the high dose males.

Although the incidence of ovarian granulosa cell tumors was within the laboratory historical control range, the sponsor's statistical analysis showed that the trend test was statistically significant, but the pairwise test for the high dose group was not. Neither statistical test attained the threshold significance needed to classify ovarian granulosa cell tumors as a positive finding according to the draft FDA Guidance (2001). Furthermore, incidences of granulosa cell hyperplasia were found in the control group as well as the low dose and high dose groups. In addition, a Sertoli cell adenoma, another stromal tumor, was found only in the control group. The incidence of stromal cell tumors (granulosa plus Sertoli cell) does not attain the critical p values needed to classify the stromal cell tumors as positive. There was no anterior pituitary hyperplasia to suggest the possibility that increased LH and FSH might underlie the Leydig and granulosa cell neoplasia, respectively.

In an adequate carcinogenicity study, BIBR 1048 MS did not induce drug related statistically significant neoplasms in either male or female rats.

Mouse Carcinogenicity Study

Using 54 CD-1 mice/sex in the control, low and mid dose groups and 63 CD-1 mice/sex in the high dose group, daily doses of 0, 30, 100 and 200 mg/kg/day BIBR 1048 MS were administered by oral gavage. Males and females were dosed for up to 104 weeks and 102 weeks, respectively. The exposure in the high dose males and females was 5.9 and 7.7 fold, respectively, the mean human exposure in subjects receiving 150 mg dabigatran etexilate bid.

Although no treatment-related effect was observed on bodyweight gain or food consumption, many of the non-neoplastic macroscopic (abnormal dark contents in multiple tissues) and microscopic findings (hemorrhage) were related to the pharmacodynamic action of BIBR 1048 MS. No statistically significant difference in mortality between control and treated groups was observed for either sex; however, a slightly higher mortality in females at 100 mg/kg/d resulted in termination of all female groups during week 102. One factor identified as contributing to the death of female animals treated with BIBR 1048 MS at 100 or 200 mg/kg/day was the presence of large, hemorrhagic ovarian cysts, which are consistent with the pharmacodynamic effect of BIBR 1048 MS.

Increased incidences were observed of some tumors, including bronchioalveolar adenocarcinoma in mid-dose females, pleomorphic lymphoma in mid-dose males, and the combination of benign fibroma, malignant fibrosarcoma and malignant sarcoma in males. However, the incidences of these tumors were within the laboratory historical range and the attained p values do not reach the thresholds to classify these tumors as positive.

In an adequate carcinogenicity study, BIBR 1048 MS did not induce drug related

statistically significant neoplasms in either male or female mice.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee concluded that the rat bioassay was adequate and noted that the sponsor used the doses recommended by the prior Exec CAC protocol agreement.

- The Committee found that the rat carcinogenicity study was negative for any drug related statistically significant neoplasms.

Mouse:

- The Committee concluded that the mouse bioassay was adequate and noted that the sponsor used the doses recommended by the prior Exec CAC protocol agreement.

- The Committee found that the mouse carcinogenicity study was negative for any drug related statistically significant neoplasms.

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

cc:\
/Division File, DCRP
/A. Defelice, Team leader, DCRP
/P. Harlow, Reviewer, DCRP
/A. Blaus, CSO/PM, DCRP
/A. Seifried, ONDIO

Appendix 5: Sponsor's Summary - Neoplasms - Mouse Carcinogenicity Study

		--- NUMBER-OF-ANIMALS -							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	54	54	54	63	54	54	54	63
** TOP OF LIST **									
ADRENALS	NUMBER EXAMINED:	54	53	54	62	54	54	54	62
--B-CORTICAL ADENOMA		2	0	1	3	0	0	0	0
--B-PHAEOCHROMOCYTOMA		0	1	2	0	1	0	1	0
--B-SUBCAPSULAR CELL ADENOMA		5	6	2	5	1	0	2	1
--M-SUBCAPSULAR CELL CARCINOMA		0	1	0	0	0	0	0	0
BRAIN	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--M-ASTROCYTOMA		0	0	0	0	0	0	0	1
--M-SCHWANNOMA		0	1	0	0	0	0	0	0
CAECUM	NUMBER EXAMINED:	52	52	52	62	52	54	53	63
--M-ADENOCARCINOMA		0	0	0	0	2	0	0	0
COLON	NUMBER EXAMINED:	53	54	53	61	54	54	53	63
--M-ADENOCARCINOMA		0	0	0	0	1	0	0	0
DUODENUM	NUMBER EXAMINED:	45	52	46	60	52	50	53	63
--B-ADENOMA		0	0	0	0	1	1	0	2
FEMUR	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-HAEMANGIOMA		0	0	0	0	0	0	1	0
GALL BLADDER	NUMBER EXAMINED:	42	49	44	53	45	44	48	55
--B-PAPILLOMA		1	1	2	1	0	1	0	0
HARDERIAN GLANDS	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-ADENOMA		6	4	4	6	3	2	0	2
--M-ADENOCARCINOMA		0	1	0	2	0	0	1	0
JEJUNUM	NUMBER EXAMINED:	51	54	52	61	52	51	51	62
--M-ADENOCARCINOMA		0	0	1	0	0	0	0	0

		--- NUMBER-OF-ANIMALS-							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	54	54	54	63	54	54	54	63
KIDNEYS	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-TUBULAR ADENOMA		0	2	0	3	0	0	0	0
--M-HAEMANGIOSARCOMA		0	0	0	1	0	0	0	0
--M-TUBULAR CARCINOMA		1	0	0	0	0	0	0	0
LIVER	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-HEPATOCELLULAR ADENOMA		7	10	8	8	1	1	1	1
--M-HEPATOCELLULAR CARCINOMA		4	3	3	2	0	0	0	0
--B-HAEMANGIOMA		1	1	0	1	1	0	1	0
--M-HAEMANGIOSARCOMA		1	0	0	0	0	0	0	0
LN MESENTERIC	NUMBER EXAMINED:	54	54	54	61	54	54	53	62
--B-HAEMANGIOMA		1	0	0	0	0	0	0	1
LUNGS & BRONCHI	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-BRONCHIOLOALVEOLAR ADENOMA		8	9	6	10	8	13	14	11
--M-BRONCHIOLOALVEOLAR ADENOCARCINOMA		2	3	3	7	4	1	2	3
MAMMARY PROTOCOL	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--M-MAMMARY ADENOCARCINOMA		0	0	0	0	2	5	4	5
--M-ADENOACANTHOMA		0	0	0	0	2	1	1	1
--M-MAMMARY CARCINOSARCOMA		0	0	0	0	0	1	0	1
OVARIES+OVIDUCTS	NUMBER EXAMINED:	0	0	0	0	54	54	54	63
--B-CYSTADENOMA		0	0	0	0	2	2	0	1
--B-GRANULOSA CELL TUMOUR		0	0	0	0	0	0	2	0
--B-LUTEOMA		0	0	0	0	0	1	0	1
--B-SERTOLIFORM TUBULAR ADENOMA		0	0	0	0	0	1	0	0
--M-YOLK SAC CELL TUMOUR		0	0	0	0	1	0	0	0
PANCREAS	NUMBER EXAMINED:	54	54	53	63	54	54	54	63
--B-ISLET CELL ADENOMA		0	2	0	0	1	0	0	1

		--- NUMBER-OF-ANIMALS-							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	54	54	54	63	54	54	54	63
** FROM PREVIOUS PAGE **									
PANCREAS	NUMBER EXAMINED:	54	54	53	63	54	54	54	63
--M-SARCOMA		0	1	0	0	0	0	0	0
PITUITARY	NUMBER EXAMINED:	53	54	53	63	52	53	53	61
--B-ADENOMA, PARS DISTALIS		1	0	1	0	3	5	6	3
--M-SCHWANNOMA		0	0	0	0	1	0	0	0
SEMINAL VESICLES	NUMBER EXAMINED:	54	54	54	63	0	0	0	0
--B-ADENOMA		0	0	0	1	0	0	0	0
SKELETAL MUSCLE	NUMBER EXAMINED:	54	54	54	63	54	54	54	62
--B-HAEMANGIOMA		0	0	0	0	0	0	1	0
--M-FIBROSARCOMA		0	0	0	0	0	0	1	0
--M-OSTEOSARCOMA		0	0	1	0	0	1	0	0
--M-SARCOMA, NOS		0	0	0	1	0	0	0	0
SKIN (PROTOCOL)	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-FIBROMA		0	0	0	1	0	0	0	0
--B-SQUAMOUS CELL PAPILLOMA		1	0	1	0	0	0	0	0
--B-TRICHOEPITHELIOMA		0	0	0	1	0	0	0	0
--M-FIBROSARCOMA		0	4	3	4	2	0	0	0
--M-HAEMANGIOSARCOMA		0	0	0	0	0	1	0	0
--M-MALIGNANT FIBROUS HISTIOCYTOMA		1	0	0	0	0	0	0	0
--M-MALIGNANT HAIR FOLLICLE TUMOUR		0	0	0	0	1	0	0	0
--M-MALIGNANT SCHWANNOMA		0	0	0	0	0	1	0	0
--M-SARCOMA		0	0	0	1	0	0	1	0
SPLEEN	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-HAEMANGIOMA		0	0	0	1	0	0	0	0
--M-HAEMANGIOSARCOMA		1	0	1	0	0	0	0	1

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS -							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	54	54	54	63	54	54	54	63
STOMACH	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-SQUAMOUS CELL PAPILLOMA		0	0	1	0	0	1	0	0
TESTES	NUMBER EXAMINED:	54	54	54	63	0	0	0	0
--B-INTERSTITIAL (LEYDIG) CELL ADENOMA		4	1	2	2	0	0	0	0
THYMUS	NUMBER EXAMINED:	49	52	50	60	53	53	52	61
--B-THYMOMA		0	0	0	0	0	1	0	0
THYROIDS	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--B-FOLLICULAR CELL ADENOMA		0	0	0	0	0	1	0	0
UTERINE CERVIX	NUMBER EXAMINED:	0	0	0	0	54	54	54	63
--B-ENDOMETRIAL POLYP		0	0	0	0	0	1	2	0
--B-FIBROMA		0	0	0	0	0	1	0	0
--B-LEIOMYOMA		0	0	0	0	1	1	0	1
--M-ENDOMETRIAL STROMAL CELL SARCOMA		0	0	0	0	1	0	0	0
--M-LEIOMYOSARCOMA		0	0	0	0	0	2	0	1
--M-SQUAMOUS CELL CARCINOMA		0	0	0	0	1	0	0	0
UTERUS	NUMBER EXAMINED:	0	0	0	0	54	54	54	63
--B-ENDOMETRIAL POLYP		0	0	0	0	9	8	15	9
--B-HAEMANGIOMA		0	0	0	0	0	1	3	1
--B-LEIOMYOMA		0	0	0	0	1	2	3	2
--B-MIXED MUELLERIAN TUMOUR		0	0	0	0	0	1	0	0
--B-ENDOMETRIAL ADENOMA		0	0	0	0	0	0	1	0
--M-ENDOMETRIAL ADENOCARCINOMA		0	0	0	0	1	0	0	0
--M-HAEMANGIOSARCOMA		0	0	0	0	0	1	0	0
VAGINA	NUMBER EXAMINED:	0	0	0	0	52	52	51	59
--B-LEIOMYOMA		0	0	0	0	1	0	0	0

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS -							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	54	54	54	63	54	54	54	63
ABDOMEN	NUMBER EXAMINED:	1	0	0	1	2	2	0	1
--M-SARCOMA		1	0	0	0	0	0	0	0
ADIPOSE TISSUE	NUMBER EXAMINED:	2	1	0	0	3	3	3	2
--B-HAEMANGIOMA		0	0	0	0	1	0	0	0
BONE	NUMBER EXAMINED:	10	7	9	8	12	7	6	5
--B-OSTEOMA		0	1	0	0	0	2	0	0
--M-OSTEOSARCOMA		0	0	1	0	0	0	1	0
H'POIETIC TUMOUR	NUMBER EXAMINED:	54	54	54	63	54	54	54	63
--M-LYMPHOCYTIC/LYMPHOBLASTIC LYMPHOMA		3	2	2	3	7	8	7	8
--M-PLEOMORPHIC LYMPHOMA		0	3	5 a	2	4	7	2	5
--M-HISTIOCYTIC SARCOMA		0	0	0	0	2	2	1	2
--M-PLASMA CELL LYMPHOMA		0	1	0	0	0	0	0	0
--M-MYELOID LEUKAEMIA		0	0	0	0	0	1	0	0
--M-IMMUNOBLASTIC LYMPHOMA		0	0	0	0	0	1	0	0
THORAX	NUMBER EXAMINED:	4	1	0	1	2	1	1	2
--M-MESOTHELIOMA		1	0	0	0	0	0	0	0

** END OF LIST **

Significant when compared with Group 1: a - p<0.05; b - p<0.01

Appendix 6: Historical Control Data for CD-1 Mice from Conducting Laboratory

Historical Histopathology Data
Incidence of selected neoplastic findings in control CD-1 Mice from recent studies performed at H (b) (4)

MALES

code number	cdm109a	cdm109b	cdm111	Cdm112	cdm117	cdm119a	cdm119b	cdm120	cdm122			
start date	May-99	May-99	Sep-99	Apr-01	Aug-02	Mar-03	Mar-03	Mar-03	Jan-04			
route of admin.	og	og	og	og	dt	ih	ih	og	og			
study type	car	car	car	car	car	car	car	car	car			
supplier	cruk	cruk	cruk	cruk	cruk	cruk	cruk	cruk				
no/cage	2	2	1	1	2	2	2	1				
study duration (weeks)	104	104	104	104	104	104	104	104	104			
										totals	**range of percentages* min**	**max**
number of animals	56	56	70	54	64	60	60	54	51	525		
Lungs												
No examined	56	56	70	54	64	60	60	54	51	525		
B-Bronchioloalveolar Adenoma												
incidence	12	17	22	9	20	12	8	11	11	122		
percentage*	21.4%	30.4%	31.4%	16.7%	31.3%	20.0%	13.3%	20.4%	21.6%	23.24%	13.3%	31.4%
M-Bronchioloalveolar Adenocarcinoma												
incidence	4	5	6	2	11	2	2	7	1	40		
percentage*	7.1%	8.9%	8.6%	3.7%	17.2%	3.3%	3.3%	13.0%	2.0%	7.62%	2.0%	17.2%
Skin												
No examined	56	56	70	54	64	60	60	54	51	525		
M-Fibrosarcoma												
incidence	0	1	0	2	1	0	1	1	2	8		
percentage*	0.0%	1.8%	0.0%	3.7%	1.6%	0.0%	1.7%	1.9%	3.9%	1.52%	0.0%	3.9%
M-Sarcoma, NOS												
incidence	0	0	1	0	0	1	1	0	1	4		
percentage*	0.0%	0.0%	1.4%	0.0%	0.0%	1.7%	1.7%	0.0%	2.0%	0.76%	0.0%	2.0%

Historical Histopathology Data
Incidence of selected neoplastic findings in control CD-1 Mice from recent studies performed at (b) (4)

MALES

code number	cdm109a	cdm109b	cdm111	Cdm112	cdm117	cdm119a	cdm119b	cdm120	cdm122			
start date	May-99	May-99	Sep-99	Apr-01	Aug-02	Mar-03	Mar-03	Mar-03	Jan-04			
route of admin.	og	og	og	og	dt	ih	ih	og	og			
study type	car	car	car	car	car	car	car	car	car			
supplier	cruk	cruk	cruk	cruk	cruk	cruk	cruk	cruk				
no/cage	2	2	1	1	2	2	2	1				
study duration (weeks)	104	104	104	104	104	104	104	104	104			
										totals	**range of percentages* min**	**max**
number of animals	56	56	70	54	64	60	60	54	51	525		
Harderian Glands												
No examined	56	56	70	54	1	60	59	54	51	461		
B-Adenoma												
incidence	5	7	7	4	1	6	3	4	5	42		
percentage#	8.9%	12.5%	10.0%	7.4%	1.6%	10.0%	5.0%	7.4%	9.8%	8.00%	1.6%	12.5%
M-Carcinoma												
incidence	0	0	3	1	0	0	0	1	0	5		
percentage#	0.0%	0.0%	4.3%	1.9%	0.0%	0.0%	0.0%	1.9%	0.0%	0.95%	0.0%	4.3%

Historical Histopathology Data
Incidence of selected neoplastic findings in control CD-1 Mice from recent studies performed at (b) (4)

MALES

code number	cdm109a	cdm109b	cdm111	Cdm112	cdm117	cdm119a	cdm119b	cdm120	cdm122			
start date	May-99	May-99	Sep-99	Apr-01	Aug-02	Mar-03	Mar-03	Mar-03	Jan-04			
route of admin.	og	og	og	og	dt	ih	ih	og	og			
study type	car	car	car	car	car	car	car	car	car			
supplier	cruk	cruk	cruk	cruk	cruk	cruk	cruk	cruk				
no/cage	2	2	1	1	2	2	2	1				
study duration (weeks)	104	104	104	104	104	104	104	104	104			
										totals	**range of percentages* min**	**max**
number of animals	56	56	70	54	64	60	60	54	51	525		
Haemopoietic Tumour												
No examined	56	56	70	54	64	60	60	54	51	525		
M-Malignant Lymphoma (NOS)												
incidence	3	5	13	0	3	0	0	0	0	24		
percentage*	5.4%	8.9%	18.6%	0.0%	4.7%	0.0%	0.0%	0.0%	0.0%	4.57%	0.0%	18.6%
M-Pleomorphic Lymphoma												
incidence	4	0	0	3	4	3	3	1	2	20		
percentage*	7.1%	0.0%	0.0%	5.6%	6.3%	5.0%	5.0%	1.9%	3.9%	3.81%	0.0%	7.1%
M-Lymphocytic/Lymphoblastic Lymphoma												
incidence	0	0	0	8	5	6	2	0	1	22		
percentage*	0.0%	0.0%	0.0%	14.8%	7.8%	10.0%	3.3%	0.0%	2.0%	4.19%	0.0%	14.8%
M-Malignant Lymphoma (All combined)												
incidence	7	5	13	11	12	9	5	1	3	66		
percentage*	12.5%	8.9%	18.6%	20.4%	18.8%	15.0%	8.3%	1.9%	5.9%	12.57%	1.9%	20.4%

Historical Histopathology Data
Incidence of selected neoplastic findings in control CD-1 Mice from recent studies performed at (b) (4)

FEMAclock21LES

code number	cdm109a	cdm109b	cdm111	cdm112	cdm117	cdm119a	cdm119b	cdm120	cdm122			
start date	May-99	May-99	Sep-99	Apr-01	Aug-02	Mar-03	Mar-03	Mar-03	Jan-04			
route of admin.	og	og	og	og	dt	ih	ih	og	og			
study type	car	car	car	car	car	car	car	car	car			
supplier	cruk	cruk	cruk	cruk	cruk	cruk	cruk	cruk				
no/cage	2	2	1	2	2	2	2	2				
study duration (weeks)	104	104	104	104	104	104	104	104	104			
										totals	**range of percentages* min**	**max**
number of animals	56	56	70	54	64	60	60	54	51	525		
Mammary Area												
No examined	56	56	70	54	64	60	60	54	51	525		
M-Mammary Adenocarcinoma												
incidence	2	3	1	1	3	3	2	8	1	24		
percentage*	3.6%	5.4%	1.4%	1.9%	4.7%	5.0%	3.3%	14.8%	2.0%	4.57%	1.4%	14.8%
M-Adenoacanthoma												
incidence	0	0	0	0	0	2	1	0	1	4		
percentage*	0.0%	0.0%	0.0%	0.0%	0.0%	3.3%	1.7%	0.0%	2.0%	0.76%	0.0%	3.3%

Historical Histopathology Data
Incidence of selected neoplastic findings in control CD-1 Mice killed or dying during the treatment period from recent studies performed at (b) (4)

MALES												
code number		cdm109a	cdm109b	cdm111	cdm112	cdm117	cdm119a	cdm119b	cdm120	cdm122		
start date		May-99	May-99	Sep-99	Apr-01	Aug-02	Mar-03	Mar-03	Mar-03	Jan-04		
route of admin.		og	og	og	og	dt	ih	ih	og	og		
study type		car	car	car	car	car	car	car	car	car		
supplier		cruk	cruk	cruk	cruk	cruk	cruk	cruk	cruk			
no/cage		2	2	1	1	2	2	2	1			
study duration (weeks)		104	104	104	104	104	104	104	104	104		
											totals	**range of percentages* min max**
	number of animals	31	27	32	22	45	30	35	25	27	274	
Skin												
	No examined	31	27	32	22	45	30	35	25	27	274	
M-Fibrosarcoma												
	incidence	0	0	0	2	1	0	1	0	2	6	
	percentage*	0.0%	0.0%	0.0%	9.1%	2.2%	0.0%	2.9%	0.0%	7.4%	2.19%	0.0% 9.1%

BOI287 – Incidence of fibrosarcoma in animals killed or dying during the treatment period

Group/Sex		1M	2M	3M	4M
Dosage (mg/kg/day)		0	30	100	200
Skin (protocol)					
Number Examined		34	33	34	41
	M-Fibrosarcoma	0	4	2	3
	percentage	0.0%	12.1%	5.9%	7.3%

Appendix 7: Neoplastic Findings in Rat Carcinogenicity Study - All Animals

Histopathology - group distribution of neoplastic findings for all animals

Group	:	1	2	3	4
Compound	:	Control	BIBR 1048 MS	BIBR 1048 MS	BIBR 1048 MS
Dosage (mg/kg/day)	:	0	30	100	200

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Aorta Thoracic	Number Examined:	55	54	55	65	55	55	55	64
N-Lymphoma		1	0	0	0	0	0	0	0
Trachea	Number Examined:	55	55	55	65	55	55	52	65
N-Lymphoma		1	0	1	1	0	1	0	0
Lungs + Bronchi	Number Examined:	55	55	55	65	55	55	52	65
B-Bronchioloalveolar Adenoma		1	0	0	0	0	0	0	0
M-Bronchioloalveolar Adenocarcinoma		0	0	0	1	0	0	0	0
N-C Cell Carcinoma		0	1	0	0	0	0	0	0
N-Lymphoma		2	0	2	2	0	1	0	0
Liver	Number Examined:	55	55	55	65	55	55	55	65
B-Hepatocellular Adenoma		0	0	1	0	0	0	1	1
M-Hepatocellular Carcinoma		0	0	0	0	0	1	0	0
N-Lymphoma		2	0	2	2	0	1	0	0
Kidneys	Number Examined:	55	55	55	64	55	55	55	65
B-Lipoma		1	0	0	0	0	0	0	0
B-Tubular Adenoma		1	0	0	0	0	0	1	0
N-Lymphoma		1	0	2	2	0	0	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Urinary Bladder	Number Examined:	54	55	54	64	55	55	55	64
N-Lymphoma		1	0	0	0	0	0	0	0
Heart	Number Examined:	55	55	55	65	55	55	52	65
B-Endocardial Schwannoma		0	0	0	0	0	0	1	0
N-Lymphoma		0	0	2	1	0	1	0	0
N-Schwannoma		0	0	0	0	0	1	0	0
Spleen	Number Examined:	55	55	55	64	55	55	55	65
N-Adenocarcinoma		0	0	0	0	0	0	1	0
N-Lymphoma		2	0	2	2	0	1	0	0
Pancreas	Number Examined:	54	55	55	64	55	55	55	65
B-Acinar Cell Adenoma		0	1	0	0	0	0	0	0
B-Islet Cell Adenoma		2	1	0	3	0	0	1	0
B-Mixed Cell Adenoma		0	0	0	1	0	0	0	0
M-Acinar Cell Adenocarcinoma		0	0	0	0	0	0	0	1
M-Schwannoma		0	0	0	1	0	0	0	0
N-Lymphoma		1	0	1	1	0	0	0	0
N-Schwannoma		0	0	0	0	0	1	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
LN Mesenteric	Number Examined:	55	55	55	64	55	55	54	65
B-Haemangioma		5	8	3	6	3	3	1	1
N-Lymphoma		2	0	2	2	0	0	0	0
Thymus	Number Examined:	54	54	54	63	54	53	50	64
B-Thymoma (Epithelial)		0	0	1	0	0	1	0	0
B-Thymoma (Lymphoid)		2	0	1	1	5	4	3	3
M-Malignant Thymoma(Epithelial)		0	0	0	1	0	0	0	0
N-Lymphoma		1	0	2	2	0	1	0	0
Thyroids	Number Examined:	55	55	55	65	55	54	50	63
B-C-Cell Adenoma		1	4	3	4	4	3	1	0
B-Follicular Cell Adenoma		1	5	4	4	0	0	1	1
M-C-Cell Carcinoma		1	1	1	1	0	2	0	0
M-Follicular Cell Carcinoma		1	0	0	0	1	0	0	0
N-Lymphoma		1	0	2	1	0	1	0	0
Parathyroids	Number Examined:	50	48	50	62	52	47	46	56
B-Adenoma		0	0	1	0	0	0	0	0
N-Lymphoma		1	0	1	1	0	0	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Adrenals	Number Examined:	55	55	55	64	55	55	54	65
B-Cortical Adenoma		2	2	1	1	2	1	0	1
B-Phaeochromocytoma		2	2	1	2	1	0	1	1
M-Cortical Carcinoma		0	1	0	0	0	1	0	0
N-Adenocarcinoma		0	0	0	0	0	0	1	0
N-Lymphoma		0	0	1	1	0	0	0	0
Pituitary	Number Examined:	54	55	54	64	54	55	54	65
B-Adenoma, Pars Distalis		19	17	17	19	35	33	25	27
B-Adenoma, Pars Intermedia		1	2	2	2	0	0	0	0
M-Carcinoma, Pars Distalis		1	1	0	0	1	1	0	1
N-Lymphoma		1	0	1	1	0	0	0	0
LN Mandibular	Number Examined:	54	55	55	64	55	54	48	61
N-Lymphoma		2	0	2	2	0	0	0	0
Parotid S.G.	Number Examined:	54	55	55	65	55	54	50	62
B-Adenoma		0	0	0	0	0	0	1	0
M-Schwannoma		1	0	0	0	0	0	0	0
N-Lymphoma		1	0	1	1	0	1	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Salivary Glands	Number Examined:	55	55	54	65	55	54	48	62
N-Lymphoma		0	0	1	1	0	0	0	0
Skeletal Muscle	Number Examined:	55	55	55	64	55	55	55	65
B-Fibroma		0	0	1	0	0	0	0	0
N-Lymphoma		0	0	1	1	0	1	0	0
Stomach	Number Examined:	55	55	55	65	55	55	55	65
N-Adenocarcinoma		0	0	0	0	1	0	0	0
N-Lymphoma		1	0	0	1	0	0	0	0
Duodenum	Number Examined:	55	55	55	65	55	53	54	65
N-Lymphoma		1	0	0	0	0	0	0	0
Ileum/Peyers	Number Examined:	55	54	54	63	55	54	53	65
N-Lymphoma		1	0	1	1	0	0	0	0
Colon	Number Examined:	55	55	55	63	55	55	55	65
N-Lymphoma		0	0	0	1	0	0	0	0
Oesophagus	Number Examined:	55	55	55	65	55	55	54	65
N-Lymphoma		0	0	0	0	0	1	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Jejunum	Number Examined:	54	54	52	60	55	53	50	59
B-Leiomyoma		0	0	0	0	0	1	0	0
M-Adenocarcinoma		1	0	0	0	0	0	0	0
M-Leiomyosarcoma		0	0	0	1	1	0	0	0
Caecum	Number Examined:	55	55	55	62	55	55	54	63
N-Lymphoma		1	0	0	1	0	1	0	0
Rectum	Number Examined:	55	55	55	64	55	55	54	64
B-Fibroma		0	0	1	0	0	0	0	0
Mammary	Number Examined:	55	55	55	64	55	55	55	65
B-Lipoma		0	0	0	1	0	0	0	0
B-Mammary Adenoma		0	0	0	0	3	1	3	3
B-Mammary Fibroadenoma		0	1	0	0	16	8	10	7
M-Mammary Adenocarcinoma		0	0	0	0	3	5	1	2
N-Lymphoma		1	0	1	1	0	0	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Skin	Number Examined:	55	55	55	65	55	55	55	64
B-Basal Cell Tumour		0	0	2	1	0	1	0	0
B-Fibroma		1	2	1	2	0	0	0	0
B-Keratoacanthoma		3	2	1	1	2	0	0	0
B-Sebaceous Cell Adenoma		1	0	0	0	0	0	0	0
B-Squamous Cell Papilloma		1	0	1	1	0	0	1	0
M-Fibrosarcoma		0	1	0	1	0	0	0	0
M-Rhabdomyosarcoma		0	0	0	0	0	0	1	0
M-Sarcoma NOS		0	1	0	0	0	0	0	0
M-Squamous Cell Carcinoma		0	0	1	1	0	0	0	0
N-Lymphoma		0	0	1	0	0	1	0	0
Lachrymal Glds	Number Examined:	55	55	55	65	55	54	50	63
N-Lymphoma		1	0	2	1	0	0	0	0
N-Schwannoma		1	0	0	0	0	0	0	0
Eyes	Number Examined:	55	55	55	65	55	54	53	65
N-Lymphoma		1	0	1	1	0	0	0	0
Harderian Glands	Number Examined:	55	55	55	65	55	54	53	65
B-Adenoma		0	0	0	2	0	0	0	0
N-Lymphoma		1	0	1	1	0	0	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Sternum + Marrow	Number Examined:	55	55	55	65	55	55	55	65
N-Adenocarcinoma		0	0	0	0	0	0	1	0
N-Lymphoma		2	0	2	2	0	1	0	0
Femur	Number Examined:	55	55	55	65	55	55	55	65
N-Lymphoma		2	0	1	2	0	1	0	0
Brain	Number Examined:	55	55	55	65	55	55	55	65
B-Granular Cell Tumour		0	0	1	1	0	1	1	0
M-Astrocytoma		1	0	1	0	0	0	0	0
M-Mixed Glioma		1	0	0	0	0	0	0	0
M-Oligodendroglioma		0	1	0	1	0	1	0	0
N-Carcinoma		1	1	0	0	1	1	0	1
N-Lymphoma		1	0	1	0	0	0	0	0
Spinal C. Thor.	Number Examined:	55	55	55	65	55	55	55	65
N-Astrocytoma		0	0	1	0	0	0	0	0
Prostate	Number Examined:	55	54	55	64	-	-	-	-
B-Adenoma		1	0	0	1	-	-	-	-
N-Lymphoma		0	0	1	1	-	-	-	-

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Seminal Vesicles	Number Examined:	55	54	55	64	-	-	-	-
N-Lymphoma		1	0	1	1	-	-	-	-
Epididymides	Number Examined:	55	55	55	64	-	-	-	-
B-Mesothelioma		1	0	0	0	-	-	-	-
N-Lymphoma		0	0	1	1	-	-	-	-
Testes	Number Examined:	55	55	55	64	-	-	-	-
B-Interstitial (Leydig) Cell Adenoma		0	2	1	4	-	-	-	-
Ovaries	Number Examined:	-	-	-	-	55	55	54	65
B-Granulosa Cell Tumour		-	-	-	-	0	0	2	4
B-Sertoli Cell Adenoma		-	-	-	-	1	0	0	0
M-Schwannoma		-	-	-	-	0	1	0	0
Uterus	Number Examined:	-	-	-	-	55	55	55	65
B-Deciduoma		-	-	-	-	0	0	0	1
B-Endometrial Adenoma		-	-	-	-	0	1	0	0
B-Polyp (Stromal)		-	-	-	-	9	6	2	2
M-Adenocarcinoma		-	-	-	-	1	4	2	2
M-Schwannoma		-	-	-	-	0	1	0	2
N-Schwannoma		-	-	-	-	0	0	1	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
Uterine cervix	Number Examined:	-	-	-	-	55	55	55	65
M-Schwannoma		-	-	-	-	0	0	1	0
M-Squamous Cell Carcinoma		-	-	-	-	0	1	0	0
M-Stromal Sarcoma		-	-	-	-	1	0	0	0
N-Adenocarcinoma		-	-	-	-	1	0	0	0
Larynx	Number Examined:	55	55	55	65	55	54	50	63
N-C Cell Carcinoma		0	1	0	0	0	0	0	0
N-Follicular Cell Carcinoma		1	0	0	0	0	0	0	0
N-Lymphoma		1	0	1	1	0	0	0	0
Preputial glands	Number Examined:	54	55	54	63	-	-	-	-
M-Squamous Cell Carcinoma		1	1	1	1	-	-	-	-
N-Carcinoma		0	1	0	0	-	-	-	-
N-Lymphoma		1	0	1	1	-	-	-	-
Clitoral Glands	Number Examined:	-	-	-	-	55	55	54	65
M-Squamous Cell Carcinoma		-	-	-	-	0	0	1	0
Ureters	Number Examined:	54	54	52	61	55	54	53	63
N-Lymphoma		1	0	0	0	0	0	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
H-Poietic Tumour	Number Examined:	55	55	55	65	55	55	55	65
M-Lymphocitic / Lymphoblastic Lymphoma		1	0	1	2	0	0	0	0
M-Pleomorphic Lymphoma		1	0	1	0	0	1	0	0
LN Inguinal	Number Examined:	2	0	2	0	0	1	0	0
N-Lymphoma		1	0	2	0	0	0	0	0
LN Popliteal	Number Examined:	1	0	1	1	0	0	0	0
N-Lymphoma		1	0	1	0	0	0	0	0
LN Deep Cervical	Number Examined:	2	4	4	1	0	2	0	2
N-C Cell Carcinoma		1	1	1	1	0	1	0	0
N-Lymphoma		1	0	1	0	0	0	0	0
LN Axillary	Number Examined:	1	1	2	2	0	1	0	0
N-Lymphoma		1	0	1	1	0	0	0	0
LN Renal	Number Examined:	1	0	1	2	0	1	1	1
N-Adenocarcinoma		0	0	0	0	0	0	1	0
N-Lymphoma		1	0	1	1	0	0	0	0

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
LN Lumbar	Number Examined:	2	0	2	3	0	0	1	2
N-Adenocarcinoma		0	0	0	0	0	0	1	0
N-Lymphoma		1	0	2	1	0	0	0	0
Thorax	Number Examined:	1	0	1	1	0	2	0	0
M-Schwannoma		0	0	0	0	0	1	0	0
N-lymphoma		1	0	0	0	0	1	0	0
LN Pancreatic	Number Examined:	2	1	2	2	0	0	2	5
N-Lymphoma		2	0	2	1	0	0	0	0
LN Thymic	Number Examined:	0	1	2	2	0	0	1	1
N-Lymphoma		0	0	2	1	0	0	0	0
Adipose tissue	Number Examined:	2	4	6	13	11	7	7	16
B-Lipoma		0	0	0	0	0	0	0	1
Tail	Number Examined:	1	1	1	1	3	2	0	1
M-Histiocytic Sarcoma		0	0	0	0	1	0	0	0
Penis/Prepuce	Number Examined:	0	2	0	0	-	-	-	-
M-Squamous Cell Carcinoma		0	1	0	0	-	-	-	-

Tissue and Finding	Group/Sex: / Number:	1M 55	2M 55	3M 55	4M 65	1F 55	2F 55	3F 55	4F 65
LN Bronchial	Number Examined:	0	0	2	0	0	0	0	0
N-Lymphoma		0	0	2	0	0	0	0	0
Head	Number Examined:	0	3	8	3	1	0	4	5
M-Squamous Cell Carcinoma		0	0	0	0	0	0	0	1
Abdomen	Number Examined:	0	0	0	0	1	1	0	0
N-Adenocarcinoma		0	0	0	0	1	1	0	0

Appendix 8: Historical data - Wistar rats - (b) (4)

Historical Histopathology Data

Selected Testicular Findings
HAN Wistar Rats

Issued 9 March 2007

Historical Histopathology Data
Incidence of selected Testicular findings in control HAN Wistar Rats from recent studies performed at (b) (4)

MALES continued>>

code number	wsrh00a	wsrh00b	wsr027	wsr028	wsr029	wsr030	wsr031	wsr032
start date	Jul-00	Oct-00	Apr-01	Jul-01	Aug-01	Sep-01	Sep-01	Jan-02
route of admin.	ih	og	og	ih	ih	ih	dt	dt
study type	car	car	car	ct	ct	ct	ct	ct
supplier	HUK	BRL	HUK	HUK	HUK	HUK	HUK	HUK
no/cage	5	4	5	5	5	1	5	5
study duration (weeks)	104	104	104	104	104	104	104	104
number of animals	50	52	55	50	50	60	50	50
Testes								
No examined	50	52	55	50	50	60	50	50
B-Interstitial (LEYDIG) Cell Adenoma								
incidence	4	1	1	0	0	0	0	2
percentage*	8.0%	1.9%	1.8%	0.0%	0.0%	0.0%	0.0%	4.0%

Historical Histopathology Data
Incidence of selected Testicular findings in control HAN Wistar Rats from recent studies performed at (b) (4)

MALES <<from previous page

code number	wsr033	wsr034	wsr035	wsrh03a	wsr036	wsr037	wsr038	wsr039			
start date	Mar-02	Jul-02	Jul-02	Jan-03	Mar-03	Jun-03	Jan-04	Feb-04			
route of admin.	dt	dt	dt	ih	og	dt	dt	og			
study type	ct	ct	ct	car	car	ct	ct	car			
supplier	HUK	HUK	HUK	CRUK	HUK	HUK	HUK	HUK			
no/cage	5	1	5	4	4	5	5	3			
study duration (weeks)	104	104	104	104	104	104	104	104			
									totals	**range of percentages* min**	**max**
number of animals	50	55	50	60	52	50	50	50	834		
Testes											
No examined	50	55	50	60	52	50	50	50	834		
B-Interstitial (LEYDIG) Cell Adenoma											
incidence	0	1	0	1	0	2	1	2	15		
percentage*	0.0%	1.8%	0.0%	1.7%	0.0%	4.0%	2.0%	4.0%	1.80%	0.00%	8.00%

Historical Histopathology Data

Selected Ovarian Findings
HAN Wistar Rats

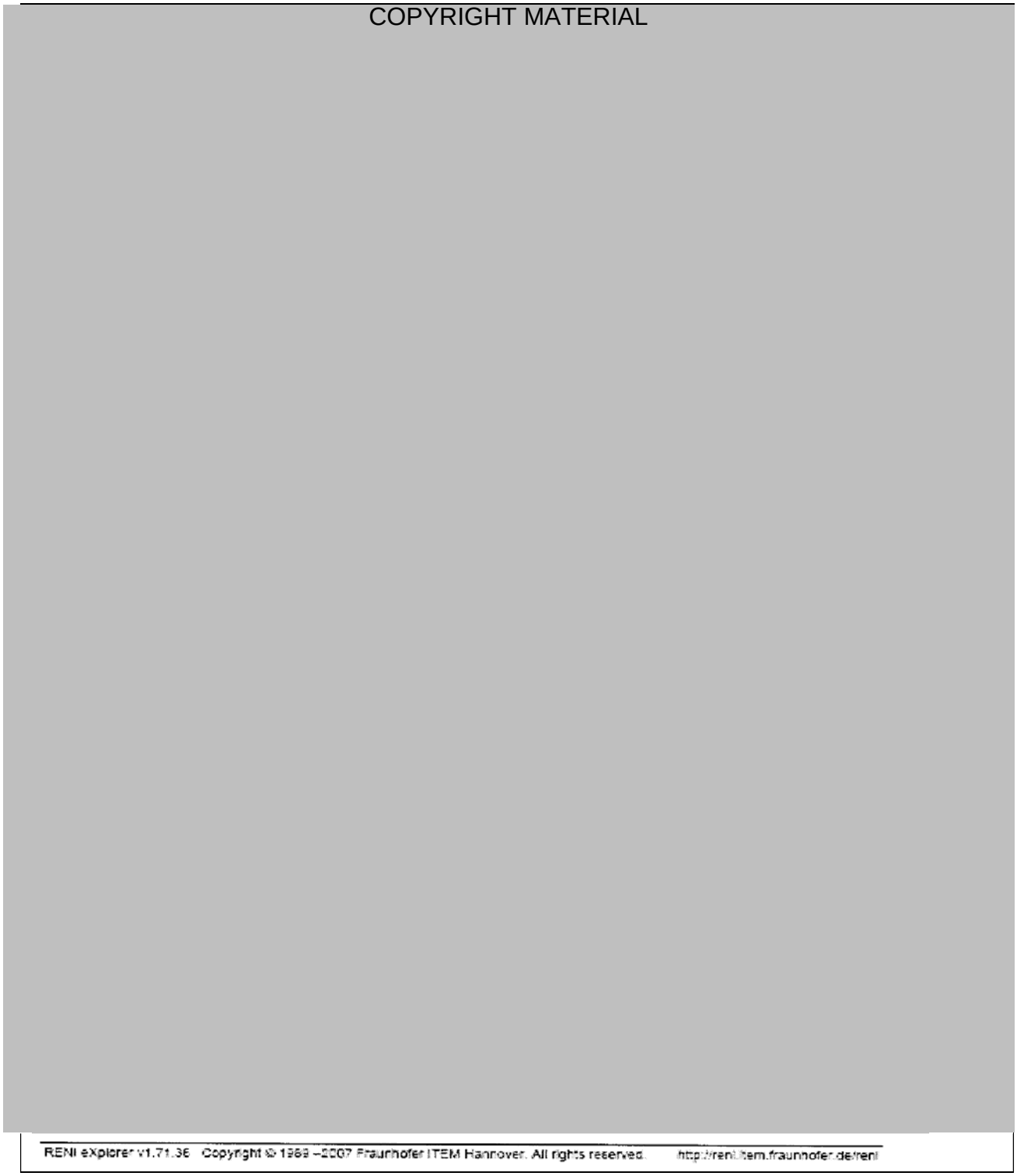
Issued 27 February 2007

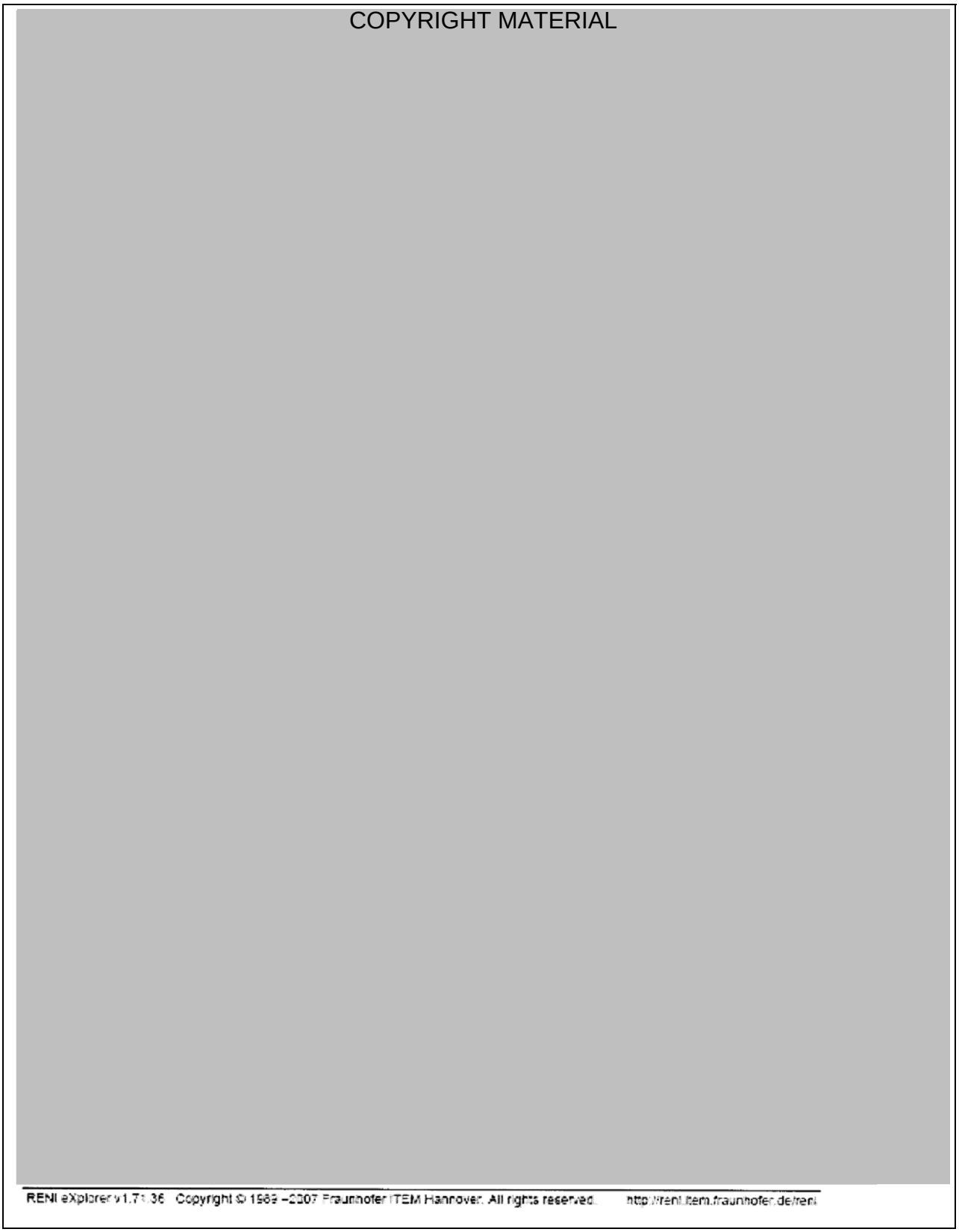
Historical Histopathology Data
Incidence of selected Ovary findings in control HAN Wistar Rats from recent studies performed a (b) (4) 27/

FEMALES

code number	wsr031	wsr032	wsr033	wsr034	wsr035	wsrh03a	wsrh03b	wsr036	wsr037			
start date	Sep-01	Jan-02	Mar-02	Jul-02	Jul-02	Jan-03	Jan-03	Mar-03	Jun-03			
route of admin.	dt	dt	dt	dt	dt	ih	ih	og	dt			
study type	ct	ct	ct	ct	ct	car	car	car	ct			
supplier	huk	huk	huk	huk	huk	cruk	cruk	huk	huk			
no/cage	5	5	5	1	5	4	4	4	5			
study duration (weeks)	104	104	104	104	104	104	104	104	104			
										totals	range of percentages* min	max
number of animals	50	50	50	55	50	60	60	52	50	477		
Ovaries												
No examined	50	50	50	55	49	60	60	52	50	476		
B-Granulosa Cell Tumour												
incidence	0	0	0	2	4	1	4	2	0	13		
percentage*	0.0%	0.0%	0.0%	3.6%	8.2%	1.7%	6.7%	3.8%	0.0%	2.73%	0.00%	8.16%
B-Granulosa-Thecal Cell Tumour												
incidence	0	0	0	0	0	0	0	0	1	1		
percentage*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.0%	0.21%	0.00%	2.00%
B-Luteinising Granulosa Cell Tumour												
incidence	0	0	0	0	0	0	0	0	1	1		
percentage*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.0%	0.21%	0.00%	2.00%
M-Malignant Granulosa Cell Tumour												
incidence	0	0	0	0	0	0	0	0	0	0		
percentage*	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.00%	0.00%	0.00%

Appendix 9: Historical Control Data - Wistar rats - RITA

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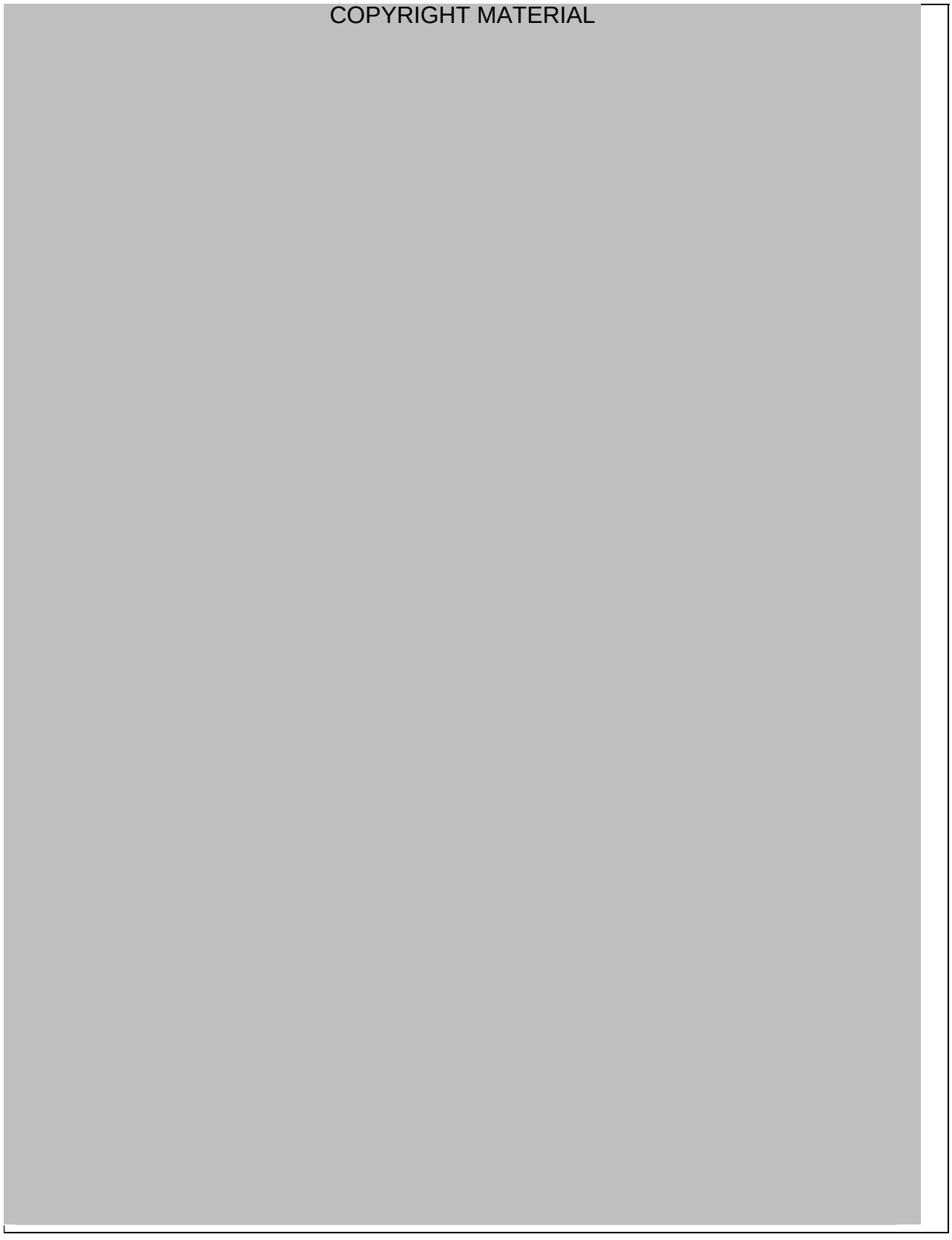
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Appendix 10: PTCC Reproductive and Developmental Toxicology Subcommittee Comments on Study U03-1284

I agree with Dr. Harlow's conclusion that the NOAEL for both maternal and embryo-fetal toxicity is 15 mg/kg (the low dose used in the study). In my opinion, the justification for setting the embryo-fetal NOAEL at 15 mg/kg is the evidence of embryofetal toxicity at the mid dose of 70 mg/kg/d, as demonstrated by: (1) increased embryofetal lethality (early resorptions mean of 1.2 vs. 0.8 in control), resulting in decreased number of viable fetuses at birth (mean 8.7 vs. 9.3 in control at similar numbers of implantations) - note that although these differences did not reach statistical significance at MD, they were clearly manifested in a dose-dependent manner, i.e., the early resorption mean values were 0.6, 1.2, and 2 at LD, MD and HD vs. 0.8 in control.; (2) embryofetal developmental retardation, as demonstrated by delayed skeletal ossification (skull, vertebral column) in 2 to 3 times larger proportion of fetuses at MD in comparison to control, again clearly dose-dependent, as these differences increased at the HD. In my opinion, there is no drug-related increase in skeletal malformations at any of the tested dose levels (as one of the 2 listed malformations, namely "flat and thickened ribs" was present in all groups, including control without dose-dependence (in 12, 9, 15, and 5 fetuses in control, LD, MD and HD, respectively) and is most likely a random spontaneous effect; the other one - "cleft thoracal vertebral body" was seen in a minimal number of fetuses (i.e.., 2 of 122 at HD, 1 of 173 at MD, and 1 of 220 at LD) and it could hardly be of toxicological significance; in addition it might be a sign of delayed vertebral ossification.

Embryofetal death/resorption is a teratogenic response at the extreme end of the continuum of effect. As such this drug is teratogenic at a dose shown to be maternally toxic (200mg/kg). It may also be teratogenic at non maternally toxic doses (≥70mg/kg) based on data trend in resorption rate, evidence of developmental delay (delayed ossification) and potential rib malformation incidence. As currently presented in the attached summary, it appears that the fetus was used as the unit of analysis rather than the litter, and interpretation of these data is difficult. A 'per litter analysis' of effect is needed to fully interpret these data.
Pending the appropriate analyses, my guess is that the NOAEL for both maternal and embryo-fetal toxicity will be 15 mg/kg, as concluded by Dr. Harlow.

I agree with Pat's assessment for the NOAEL based on the maternal and the dose-related fetal toxicities. The label should state that there are findings at the specified MD 7 HD doses and their equivalent AUCs/Cmax.
I agree with Pat's conclusions expressed in her review and that the maternal and fetal NOAELs are both the low dose, 15 mg/kg.
I also agree that the lower % total malformations in the high dose group relative to the mid-dose may be due to the high incidence of resorptions in that group, and that there was a dose and treatment relationship to the incidence of cleft thoracic vertebral body (note the higher incidence of split sternebrae in the HD group as well). While the latter could possibly argue for there being no NOAEL demonstrated for fetal toxicity, I believe it is reasonable to consider the low dose a NOAEL.

I agree that the NOAEL should be called at the low dose, 15 mg/kg free base. Hard to rationalize a higher NOAEL for the embryofetal effects.
The mid and high doses appear to cause maternal toxicity.
There is a note that one animal in the high dose group aborted. Is that correct? I thought abortions were not seen in rats.
I am not familiar with the M:F ratios on the Han Wistar rat. The Control and low dose ratios seem as skewed for males as the mid dose is skewed for females.

Is the cleft thoracal [thoracic?] vertebrae the same vertebrae in each fetus? Is this really malformation or just another manifestation of ossification delay in these fetuses?
It is just a clarification of language on page 5 of the summary but relative to the ossification delay, this is likely a result of developmental delay in the fetuses possibly brought on by the maternal toxicity in the dams.

Appendix 11: Individual Malformations in EFD Study U03-1284

Malformations in fetuses

Individual values

Dam no.	Uterus pos. (Fetus no.)	Sex	Weight(g)	Findings
107	L07	f	5.3	proximal double ureter,left
108	L02	m	4.9	flat and thickened ribs, left 6.-9.,11.12., right 5.-12. flat and thickened ribs, Z-shaped ribs: left 11., 12., right 10.-12.
	R01	f	4.7	flat and thickened ribs, left 11., 12., right 9.-12.
	R03	f	4.5	flat and thickened ribs, right 8.-12.
112	L02	f	4.4	flat and thickened ribs 5.-12.
	L03	m	4.2	flat and thickened ribs, Z-shaped ribs: 4.-12.
	L04	m	5.0	flat and thickened ribs 5.-12.
116	R03	m	6.0	flat and thickened ribs, right 5.-10., left 8.-11.
118	L03	m	5.3	flat and thickened ribs 4.-12.
	R01	m	5.2	flat and thickened ribs, right 8.-12.
130	L01	m	5.5	flat and thickened ribs, left1., 2.,5.-12., right 1., 2., 4-13.
	L02	f	4.7	flat and thickened ribs, left 1.-12., right 1., 2., 4.-13.
	L06	m	4.9	flat and thickened ribs, left 1., 2., 4.-12. right 1., 2., 4.-13.
201	L06	m	4.8	flat and thickened ribs, right 5.-12.
	R02	f	4.5	2nd thoracal vertebral body asymmetrical
202	L02	f	5.1	cleft thoracal vertebral body (12.)
210	L01	f	5.0	flat and thickened ribs, right 9.-12. wavy ribs, 10., 11. left
	R03	f	5.0	flat and thickened ribs, right 10.-12.
212	R01	m	5.1	flat and thickened ribs, left 10.-12., right 9.-12.
213	L04	m	5.3	flat and thickened ribs 5.-12.
	R01	m	5.4	flat and thickened ribs 5.-12.
	R03	f	5.2	flat and thickened ribs 6.-12.
219	R02	f	4.9	flat and thickened ribs, right 5.-12.

Dam no.	Uterus pos. (Fetus no.)	Sex	Weight(g)	Findings
303	L05	m	5.0	flat and thickened ribs 6.-12.
304	L02	m	5.7	flat and thickened ribs 7.-12.
	L03	m	5.3	flat and thickened ribs 6.-13.
	R02	f	5.1	flat and thickened ribs, left 6.-13., right 5.-13.
309	L02	m	5.6	flat and thickened ribs 6.-12.
	R02	f	5.2	flat and thickened ribs 5.-12.
311	R04	f	5.3	flat and thickened ribs 6.-12.
313	R02	m	5.7	flat and thickened ribs 7.-12.
	R05	f	5.1	flat and thickened ribs 7.-12.
314	R07	m	5.3	cleft thoracal vertebral body (12.)
318	R02	f	4.2	brachygnathia inferior cleft in the soft palate
321	R02	m	5.1	flat and thickened ribs 5.-13.
322	R01	f	5.4	flat and thickened ribs, right 6.-10.
324	R01	f	4.8	flat and thickened ribs, left 5.-12, right 7.-12.
	R02	f	4.7	flat and thickened ribs, left 10-12., right 5.-13.
	R04	f	5.2	flat and thickened ribs, left 8.-10. right 8.-12.
	R05	f	4.5	flat and thickened ribs 5.-13.
402	L03	f	4.9	flat and thickened ribs 5.-11.
406	L01	m	4.6	flat and thickened ribs 6.-11.
	L02	m	4.8	flat and thickened ribs 5.-12.
	R06	m	4.1	flat and thickened ribs 5.-11.
413	R04	f	4.7	flat and thickened ribs 6.-12.
419	L01	f	4.6	cleft thoracal vertebral body (13.)
424	L03	f	5.0	cleft thoracal vertebral body (13.)

Appendix 12: Sponsor's Summary - Litter Parameters - PPND Study U05-2396-01

	Control	Dose groups BIBR 1048 BS [mg/kg]				Spontaneous incidences from Evaluation Study [U03-1549, Viertel, 2003] ++	
		15	50	100	200		
n litters +	21	24	19	16	12	85	
Litter parameters (means/individual range per dam)						overall means	ranges of means/ individual data
Corpora lutea $, $$	10.71 /9-13	10.58 /6-13	10.31 /9-12	10.25 /8-13	10.83 /8-14	12.0	11.8-12.3/ 9.0-15.0
Implantations	9.6 /7-13	9.3 /5-13	9.5 /4-12	9.6 /8-13	9.8 /6-12	11.1	10.9-11.4/ 5.0-15.0
Dams with viable offspring (%)	87.50	100	83.33	79.17	58.33*↓	-	-
Pre-implantation loss (%) $, $$	10.01 /0.00-36.36	12.70 /0.00-50.00	7.97 /0.00-60.00 (n=20)	10.24 /0.00-80.00 (n=20)	12.25 /0.00-77.77 (n=18)	-	-
Post-implantation loss (%)	8.39 /0.00-70.00	7.05 /0.00-40.00	5.19 /0.00-18.18	15.51 /0.00-55.56	34.32 /9.09-66.67	5.70	2.30-7.29/ 0[7.69]-23.08# (54.55)
Gestation index (%)	100	100	100	95	77.78	-	-
Birth index (%)	91.61 /30.00-100	92.95 /60.00-100	94.81 /81.82-100	84.49 /44.44-100	65.68 /33.33-90.91	-	-
Duration of gestation (days)							
<22/22/23/>23	0/19/2/0	0/21/3/0	0/17/2/0	0/11/5/0	0/2/10/0	-	-
Sex of offspring (%)							
male	47.82 /20.00-75.00	44.76 /10.00-72.73	56.03 /33.33-100	48.94 /0-75	45.19 /25-77.78	49.41	46.92-52.18/ 18.18-80.0
female	52.18 /25-75	55.24 /27.27-90	43.97 /0-66.67	51.36 /25-100	54.81 /22.22-75	50.59	47.82-53.08/ 20.0-81.82
Newborn viable offspring (LD 1)	8.81 /3-11	8.67 /4-13	9.00 /4-12	8.13 /4-11	6.42*↓ /3-10	viable fetuses	
						10.5	10.1-10.7/ 5.0-14.0
Dead newborn offspring $	0	0	0.05 /0-1	0.18 /0-3	0.33 /0-2	dead fetuses	
						0	0
Birth weight [g] of offspring (LD 1)	5.966 /5.34-6.90	6.010 /5.30-7.73	5.871 /5.02-7.70	5.801 /4.40-6.66	5.852 /4.74-6.68	fetal weight	
						5.03	4.90-5.13/ 2.3-6.3
Offspring weight on LD 4	8.774 /7.52-10.30	8.974 /7.46-12.30	8.574 /6.35-12.33	8.212 /5.89-10.92	8.729 /7.11-10.46	-	-
Live offspring (LD 4)	8.81 /3-11	8.63 /4-13	8.84 /3-12	7.63 /4-10	6.18*↓ /0-10	-	-
Viability rate	100	99.52	98.25	93.85	88.31	-	-

+ non-pregnant and prematurely died animals excluded
++ Evaluation of the rat strain CrlGlxBrlHan:WI (=Crl:WI(HAN)) in a study for effects on embryofetal development by oral administration of Natrosol 250 HX, gavage (comment see chapter 2.8 Spontaneous Data)
* significantly different to control ($p<0.05$)
↓ decreased
one outlayer (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlyer included
[ ] lowest number greater than 0
$ decimals truncated
$$ animals prematurely died or animals with no delivery included , no analysis of variance performed

Appendix 13: Sponsor's Table of Human AUC Values

Descriptive statistics of $AUC_{0\text{-}12,ss}$ ($AUC_{tau,ss}$) grouped by gender and age (young <65 and elderly ≥65 years) after oral administration of 150 mg dabigatran etexilate capsule b.i.d. in healthy white subjects

$AUC_{0\text{-}12,ss}$ [ng·h/mL] 150 mg b.i.d.	**Total Dabigatran**			
	Young		**Elderly**	
	Female	**Male**	**Female**	**Male**
No of studies	**4**	**4**	**1**	**1**
N	41	39	16	18
Mean	1233	911	1902	1720
SD	485	434	578	279
CV [%]	39.4	47.6	30.4	16.2
Min	293	241	1096	959
Max	2317	2068	3134	2259
5th percentile	595	322	1223	1382
10th percentile	635	420	1358	1475
25th percentile	880	596	1510	1606
Median	1169	865	1828	1718
75th percentile	1604	1108	2107	1880
90th percentile	1784	1391	2686	2006
95th percentile	2118	1837	3126	2072
gMean	1131	812	1828	1695
gCV [%]	46.3	53.9	29.4	18.3

Source U09-1363; Table 10.2: 9

Appendix 14: References

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22512	GI-1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	PRADAXA (DABIGATRAN ETEXILATE MESYLATE)
NDA-22512	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	PRADAXA (DABIGATRAN ETEXILATE MESYLATE)

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/s/

PATRICIA P HARLOW
09/13/2010

ALBERT F DEFELICE
09/13/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 22-512 **Applicant: Boehringer Ingelheim** **Stamp Date: 4/19/2010**

Drug Name: dabigatran etexilate **NDA/BLA Type: Commercial**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Oral dosing of BIBR 1048 MS was generally conducted using 0.5% Natrosol 250 HX (hydroxyethylcellulose) solution in water. Some studies used parenteral dosing of the pharmacologically active form, BIBR 953 ZW.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Statements are included with individual study reports
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable – Dabigatran has little distribution to the brain and the central nervous system.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Patricia Harlow, Ph.D.	May 17, 2010
Reviewing Pharmacologist	Date
Albert DeFelice, Ph.D.	May 17, 2010
Team Leader/Supervisor	Date

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

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22512	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	PRADAXA (DABIGATRAN ETEXILATE MESYLATE)

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PATRICIA P HARLOW
05/17/2010

ALBERT F DEFELICE
05/17/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 22-512 **Applicant: Boehringer Ingelheim** **Stamp Date: 12/15/2009**

Drug Name: dabigatran etexilate (**NDA/BLA Type: Commercial**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Oral dosing of BIBR 1048 MS was generally conducted using 0.5% Natrosol 250 HX (hydroxyethylcellulose) solution in water. Some studies used parenteral dosing of the pharmacologically active form, BIBR 953 ZW.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Statements are included with individual study reports
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable – Dabigatran has little distribution to the brain and the central nervous system.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Patricia Harlow, Ph.D.	January 14, 2010
Reviewing Pharmacologist	Date
Albert DeFelice, Ph.D.	January 14, 2010
Team Leader/Supervisor	Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22512	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	PRADAXA (DABIGATRAN ETEXILATE MESYLATE)

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01/14/2010

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