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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Product:	Pradaxa™ (Dabigatran etexilate mesylate)
Indication:	Reduction in Risk or Treatment of Pediatric VTE
Applicant:	Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division:	DPT-CHEN
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1 Executive Summary

1.1 Introduction

Dabigatran etexilate mesylate (BIBR 1048 MS) is a prodrug that is hydrolyzed to dabigatran (BIBR 953 ZW), a direct thrombin inhibitor previously approved for the treatment, prevention, or reduction of risk of deep vein thrombosis and pulmonary embolism in adults. In support of a pediatric indication, the sponsor submitted two nonclinical juvenile toxicity studies under NDA 022512 (dabigatran etexilate mesylate capsules). These studies are (b) (4) intended to support (b) (4) NDA 0214358 (dabigatran etexilate mesylate pellets).

1.2 Brief Discussion of Nonclinical Findings

All excipients and impurities (b) (4) are considered qualified and acceptable for use. In both the preliminary and definitive juvenile toxicity studies, bleeding-related mortality was observed. The bleeding is an extension of the pharmacodynamic effect of BIBR 953 ZW. In the case of the definitive study, bleeding-related mortality occurred at the lowest dose, and therefore a NOAEL could not be determined from the study.

1.3 Recommendations

1.3.1 Approvability

NDAs 022512, (b) (4) and 0214358 are approvable from a nonclinical perspective for the proposed indications. Most of the toxicities identified in the juvenile toxicity are attributable to the pharmacodynamic effect of dabigatran (BIBR 953 ZW).

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

For all (b) (4) dosage forms, the risk of bleeding has already been stated in the Warnings and Precautions (5.2) and includes the need for prompt evaluation of signs and symptoms of blood loss. No additional labeling is required under Nonclinical Toxicology (Section 13). No nonclinical labeling update is needed for Section 8 (no new animal data submitted).

2 Drug Information

2.1 Drug

CAS Registry Number

211915-06-0

Generic Name

Dabigatran etexilate mesylate (Pradaxa™)

Code Name

BIBR 1048 MS

Chemical Name

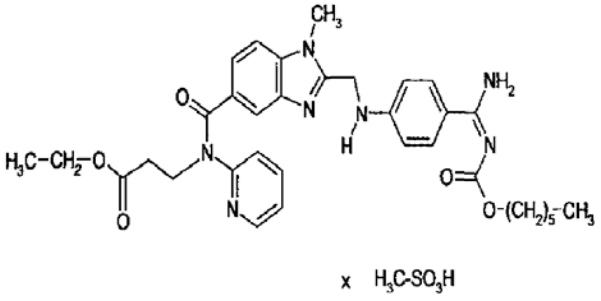
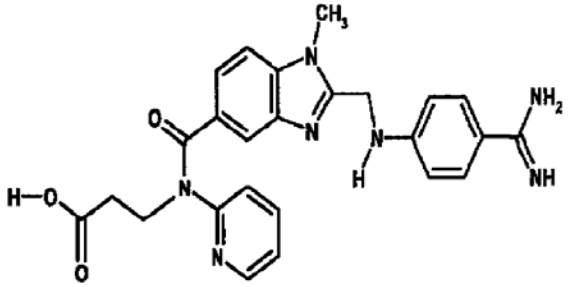
IUPAC name:
Ethyl N-{[2-({[4-((E)-amino{[(hexyloxy)carbonyl]imino}methyl)phenyl]amino}methyl)-1-methyl-1H-benzimidazol-5-yl]carbonyl}-N-pyridin-2-yl-β-alaninate methanesulfonate

Molecular Formula/Molecular Weight

Molecular formula: $C_{34}H_{41}N_7O_5 \times CH_3O_3S$
Molecular weight: free base: 627.7 g/mole; mesylate salt: 723.8 g/mole

Structure or Biochemical Description

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)
	

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 INDs, NDAs, BLAs and DMFs

(b) (4)

NDA 214358 (dabigatran etexilate mesylate granules, Boehringer Ingelheim)
IND 063267

2.3 Drug Formulation

PRADAXA™ capsules are supplied in 75 mg, 110 mg, and 150 mg strengths for oral administration. Each capsule contains dabigatran etexilate mesylate as the active ingredient: 150 mg dabigatran etexilate (equivalent to 172.95 mg dabigatran etexilate mesylate), 110 mg dabigatran etexilate (equivalent to 126.83 mg dabigatran etexilate mesylate), or 75 mg dabigatran etexilate (equivalent to 86.48 mg dabigatran etexilate mesylate) along with the following inactive ingredients: acacia, dimethicone, hypromellose, hydroxypropyl cellulose, talc, and tartaric acid. The capsule shell is composed of carrageenan, hypromellose, potassium chloride, titanium dioxide, black edible ink, and FD&C Blue No. 2 (150 mg and 110 mg capsules only).

(b) (4)

Each packet of PRADAXA™ pellets (NDA 214358) contains 20 mg, 30 mg, 40 mg, 50 mg, 110 mg, or 150 mg dabigatran etexilate (equivalent to 23.06 mg, 34.59 mg, 46.12 mg, 57.65 mg, 126.83 mg, or 172.95 mg dabigatran etexilate mesylate) along with the following inactive ingredients: acacia, dimethicone, hypromellose, hydroxypropyl cellulose, talc, and tartaric acid.

2.4 Comments on Novel Excipients

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

2.5 Comments on Impurities/Degradants of Concern

Dabigatran etexilate mesylate impurities were previously assessed for genotoxicity, anti-thrombin activity, and % in batches used in tox studies under NDA 022512 (adult oral capsule) in the pharm/tox review by Patricia Harlow (dated April 9, 2010 in DARRTS). None of the impurities were genotoxic. For NDA 022512 (pediatric oral capsule), the only impurities exceeding the threshold set forth in ICH Q3A/Q3B wer (b) (4) he other two impurities are considered qualified based on the use of an adult rat NOAEL (appropriate for intended population of 8 years and older, assuming a k_m of 37 for an adult), as illustrated in the Table 1 below:

Table 1. Reviewer's Summary of Specified Impurities for Dabigatran Etexilate Mesylate Capsules (b) (4)

Impurity	Specification (≤%)		Batches of BIBR 1048 MS used in tox studies				Max Exposure DP (mg/m^2)	
	DS	DP	(b) (4) 98	8050461	8250250	1024879 (b) (4)	Human†	Animal&
(b) (4)								

For (b) (4) 214358, the only impurities exceeding the threshold set forth in ICH Q3A/Q3B wer (b) (4) has no anti-thrombin activity and the structure of (b) (4) is similar to (b) (4) The proposed limits of this impurity are NMT (b) (4) % in the drug substance (b) (4) (b) (4) % and (b) (4) % for the drug product in the (b) (4) (b) (4) pellets, respectively. These limits exceed the thresholds in drug substance and drug product set forth in ICH Q3A/Q3B.

Due to the youngest age of the intended population (b) (4) for the (b) (4) (b) (4) pellets, the juvenile tox study is appropriate for calculating a margin of safety. However, a NOAEL could not be determined from this tox study as the lowest dose in the study (15 mg/kg/day) resulted in bleeding-related mortality (see Section 5.2).

Conservatively applying 10X uncertainty factor to this LOAEL and performing a margin of safety calculation resulted in a number (b) (4)

As an alternative approach to qualify the impurity, we asked Dr. Fadi Nossair (Clinical) if he could look at the adverse events from the Phase 3 trial to determine if the younger population was more sensitive to bleeding than adolescents/adults. He presented us with this table below:

Table 2. Clinical Adverse Reactions by Dosing form in Phase III Trial.

Bleeding Adverse Reactions (AR) in Dabigatran arm (b) (4)	Pellet Granules (n=41)	Capsules (n=121)
Any bleeds from AE STDM – n (%)	6 (15)	29 (24)
Mucosal Bleeds – n (%)	3 (7)	8 (7)
Cutaneous Bleeds – n (%)	1 (2)	11 (9)
GI Bleeds – n (%)	2 (5)	6 (5)
Other Bleeds[1]	0 (0)	6 (5)
Serious bleeding AEs	0 (0)	5 (4)
Major bleeding – Total – n (%)	0 (0)	3 (3)
CRNM bleeding – Total – n (%)	0 (0)	2 (2)
Minor bleeding – Total – n (%)	6 (15)	23 (19)

Using a contingency table with a p value computed by Fisher's exact test, there was not a significant difference in any bleeds from AE STDM between the (b) (4) (b) (4) capsules (patient> (b) (4) p = (b) (4).

Given (b) (4) is not genotoxic, has no anti-thrombin activity, (b) (4) (b) (4) and there is no significant difference between frequencies of bleeding between these young vs old population in the clinical studies, we conclude that for the purpose of impurity qualification for (b) (4) we can use the adult rat NOAEL for our margin of safety calculation. This results in an adequate margin of safety (b) (4) for (b) (4) NDA 214358. All impurities are therefore determined to be qualified (assuming a k_m of (b) (4) for a 6-month-old) as shown below in Table 3 and Table 4.

(b) (4)

(b) (4)

Table 4. Reviewer's Summary of Specified Impurities for Dabigatran Etexilate Mesylate Pellets

Impurity	Specification (≤%)		Batches of BIBR 1048 MS used in tox studies				Max Exposure DP (mg/m^2)	
	DS	DP	(b) (4) 98	8050461	8250250	1024879 (b) (4)	Human†	Animal&
(b) (4)								

(b) (4)

(b) (4) is observed in the drug substance and drug product of all (b) (4) dosage forms. According to ICH (M7), this is a class 2 impurity with an allowable daily intake of 1.5 ug/day per lifetime. (b) (4) does not increase during product manufacture or stability for either the capsule or pellet formulations at the maximum dosages. The sponsor set a (b) (4) specification of (b) (4) ppm ((b) (4) mg/kg) for (b) (4) in these two dosage forms assuming a maximum daily dose of (b) (4) mg/day, resulting in an allowable intake of 1.5 ug/day. (b) (4)

The sponsor proposed a shelf-life acceptance criteria of ≤ (b) (4) ppm ((b) mg/kg) for (b) (4) assuming a maximum daily dose of (b) (4) mg/day, resulting in an allowable intake of 1.5 ug/day. (b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

The proposed dosing regimen of each dabigatran etexilate mesylate formulation for the treatment and prophylaxis of venous thromboembolism events (VTE) is twice daily according to age- and weight-based nomograms provided in the product label. Dabigatran etexilate mesylate capsules are intended for pediatric patients aged 8 years or older, up to a maximum dose of (b) (4) mg/day. (b) (4)

Dabigatran etexilate mesylate pellets are intended for children less than 12 years old as soon as the child can swallow soft food, up to a maximum dose of (b) (4) mg/day.

2.7 Regulatory Background

The sponsor notified the sponsor of its intention to perform a single animal toxicology study to support chronic administration of dabigatran etexilate mesylate in children on June 10, 2014. The sponsor submitted the protocol of the definitive juvenile toxicity study under IND 063267 for review/feedback on August 5, 2014. The study design was determined to be acceptable on August 25, 2014. The final study was submitted under NDA 022512 (Supplement 41) on September 21, 2020 and will also cover the indications in (b) (4) NDA 0214358.

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
n0024900	BIBR 1048 MS: Preliminary Neonatal Toxicity Study in the Han Wistar Rat by Oral (Gavage) Administration	DDB0435
n00251085	BIBR 1048 MS: Neonatal Toxicity Study in the Han Wistar Rat by Oral (Gavage) Administration for 8 Weeks followed by a 4-Week Recovery Period	DDB0467

4 Pharmacology

4.1 Primary Pharmacology

The target and mechanism of action of dabigatran etexilate mesylate (BIBR 1048 MS) was previously reviewed by Patricia Harlow (see PharmTox review in DARRTS dated April 9, 2010). Briefly, BIBR 1048 MS is an oral prodrug of the active pharmaceutical ingredient, dabigatran (BIBR 953 ZW). BIBR 953 ZW is a synthetic, direct thrombin (Factor IIa) inhibitor, which interacts with the active site in the catalytic domain of the thrombin enzyme. Thrombin is a serine protease that cleaves fibrinogen to fibrin monomers, is central to both the intrinsic and extrinsic pathways of blood coagulation.

5 Special Toxicology Studies

5.1 Preliminary Juvenile Toxicity Study

Study title: BIBR 1048 MS: Preliminary Neonatal Toxicity Study in the Han Wistar Rat by Oral (Gavage) Administration

Study no.:	n0024900
Study report location:	Study n00249900
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11 September 2014
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BIBR 1048 MS, Lot #'s 1075414/1076019/1077470, Purity > 99% for all lots

Key Study Findings

1- Increased mortality/termination due to animal welfare in 100 and 200 mg/kg/day groups
2- An increased incidence of hemorrhage in 100 and 200 mg/kg/day groups
3- A dose dependent increase in the severity of hemorrhage
4- Toxicological findings were predictable due to mechanism of action of test article (direct thrombin inhibitor)

Methods

Doses:	0, 30, 100, and 200 mg/kg/day (Phase 1) 0, 20, 45, and 70 mg/kg/day (Phase 2)
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	0.5% Natrosol 250 HX solution
Species/Strain:	Han Wistar rats
Number/Sex/Group:	8/sex/group (main phase)[1]
Satellite groups:	Day 1 Toxicokinetic (TK) phase (12/sex/group)[2]

Study design: Han Wistar rat pups were treated with either vehicle or test article from post natal day (PND) 7 to PND 28 at the specified doses. Study groups were divided into 2 phases based on dose levels and then further subdivided into a main phase and TK group. TK blood samples were collected at 1, 3, 8, and 24 hours after single (Day 1 TK phase) or repeated (Day 21 main phase) dosing. Blood samples for hematology were collected for Phase 2 animals only. Main phase animals were evaluated for viability, clinical observations, and body weights during the treatment period. Macroscopic pathology and histopathology were evaluated for select organs/tissues in all main phase animals at the end of the treatment period.

Deviation from study protocol: none

Observations and Results

Mortality:

Methods: A viability check was performed twice daily on all animals.

Results: In the 200 mg/kg/day dosing group, three animals (2 males, 1 female) were found dead at first room check, and the remainder of the group was terminated early due to deteriorating clinical condition. In the 100 mg/kg/day dosing group, two female rats were terminated early due to deteriorating clinical condition. There were no premature deaths reported in the remainder of the Phase 1 animals or in any of the dosing groups in Phase 2.

Clinical Signs:

Methods: Animals were inspected visually twice daily for clinical condition. Except on days of blood sampling, detailed observations were recorded pre-dose, on completion of dosing, one to two hours after dosing, and at the end of the working day. Animals were subjected to a full physical examination on PND 21, 24, and 28.

Results: In the 200 mg/kg/day dosing group, dull eyes, pale extremities, underactive behavior, and significant weight loss were common clinical signs that necessitated termination of the entire group on PND 22. In the 100 mg/kg/day dosing group pallor, cold to touch, piloerection, and partially closed eyes were clinical signs among the two animals that were terminated early. Two male rats in the 70 mg/kg/day group were noted to have bruising on the tail with one of these animals exhibiting underactive

behavior along with an enlarged, dark red eye. One male rat dosed at 45 mg/kg/day had a dark right eye. There were no clinical signs noted in the remainder of the dosing groups in Phase 1 or Phase 2 animals.

Body Weight:

Methods: Body weights were individually recorded for juvenile animals on PND 6 through 28 and on the day of necropsy.

Results: In surviving male rats from PND 7 through PND 21, there were no clear test article related changes in body weight in 200 (0.98X), 100 (0.98X), 70 (0.92X), 45 (1.04X), 30 (0.88X), or 20 (0.96X) mg/kg/day groups compared to their corresponding controls. In surviving male rats from PND 7 through PND 28 (end of treatment), there were no clear test-article related changes in body weight in the 100 (0.96X), 70 (0.92X), 45 (1.05X), 30 (0.92X) or 20 (1.01X) mg/kg/day groups compared to their corresponding controls.

In surviving female rats from PND 7 through PND 21, there were no clear test article related changes in body weight in 200 (0.98X), 100 (0.98X), 70 (0.94X), 45 (1.01X), 30 (0.89X), or 20 (0.96X) mg/kg/day groups compared to their corresponding controls. In surviving female rats from PND 7 through PND 28, there were no clear test-article related changes in body weight in the 100 (0.95X), 70 (0.97X), 45 (1.01X), 30 (0.91X), 20 (1.00X), compared to their corresponding controls.

Hematology:

Methods: In Phase 2 animals only, blood was collected via the sublingual vein under terminal anesthesia at the end of treatment. Animals were not fasted.

Results: There was a statistically significant increase in Mean cell volume (MCV) in 70 mg/kg/day male rats compared to their respective male controls (64.1± 1.94 vs 61.6 ± 2.25 fL). There were no other apparent treatment-related changes in hematology parameters.

Toxicokinetics:

Methods**:** Blood samples were obtained from Day 1 TK phase animals on PND 7 following a single dose administration and main phase groups on PND 21 following repeated dose administration. Samples were collected at 1, 3, 8, and 24 hours postdose.

Table 5. Reviewer's Summary – Toxicokinetic Parameters

Dosage (mg/kg/day)	Sex	Postnatal Day 1 C_{max} (ng/mL)	Postnatal Day 1 T_{max} (h)	Postnatal Day 1 $AUC_{(0-24hr)}$ (ng*h/mL)	Postnatal Day 21 C_{max} (ng/mL)	Postnatal Day 21 T_{max} (h)	Postnatal Day 21 $AUC_{(0-24hr)}$ (ng*h/mL)
20	M	740	8	10300	1020	1	2290
	F	540	8	8210	618	1	1470
30	M	1610	3	17500	2220	1	4380
	F	1340	8	18500	754	1	1660
45	M	1190	8	17400	1610	1	4240
	F	1070	8	16600	1480	1	3550
70	M	1390	8	21300	2690	1	6610
	F	1020	3	14600	1820	1	5410
100	M	1350	8	19400	1920	1	5380
	F	1630	8	24300	2020	1	4690
200	M	1170	8	17000	-	-	-
	F	1010	8	16000	-	-	-

Results: Data was not collected for the 200 mg/kg/day dosing group on PND 21. Exposure levels were generally similar between males and females on PND 1, however, at lower dosages (20 and 30 mg/kg/day) lower exposure levels were seen in females compared to males after repeated administration. Plasma levels were lower after repeated administration (PND 21) compared to single dose (PND 1) in both males and females. There was not a dose-dependent increase in exposure parameters after single or repeated dose administration in males or females.

Necropsy:

Methods: All main phase animals that were found dead or euthanized after the last day of dosage (PND 28) were subjected to gross necropsy of the cranial, thoracic, and abdominal cavities.

Organs and tissues preserved at necropsy were processed, embedded in paraffin, sectioned at 3-4 microns and stained with H&E

The following tissues were retained: trachea, lungs, esophagus, pharynx, and head

Results:

There were no treatment-related macroscopic abnormalities among the 3 animals found dead in the 200 mg/kg/day group. In the remainder of the animals in the group (terminated on PND 22), pale liver and kidney were a common macroscopic finding in both sexes. Macroscopic findings in 200 mg/kg day males included dark contents rectum (n=1) and dark inner ears (n=1). Macroscopic findings in 200 mg/kg/day females

included pale skeletal muscle (n=2), dark contents in cecum (n=1), slightly enlarged spleen (n=1), clotted blood in rectum (n=1), stained and wet fur around anus (n=1), milk present in stomach (n=1), firm pellets in rectum and colon (n=1), and pale internal organs (n=1). Macroscopic findings in the 100 mg/kg/day group were limited to two the females terminated early and included blood on muzzle (n=1), dark material in stomach and jejunum (n=1), reduced cecum contents (n=1), milk present in stomach (n=1), small thymus (n=1), pale pituitary (n=1), dark and enlarged left eye (n=1), blood in left eye (n=1), pale liver (n=1), and trauma to right pinna O(n=1). One male rat in the 70 mg/kg/day dosing group had an opaque right eye. There were no treatment-related macroscopic findings in 45, 30, or 20 mg/kg/day dosing groups.

In the histopathology report, the high dose was reported as 300 mg/kg/day, not 200 mg/kg/day as stated above. The number of animals with histopathologic findings of hemorrhage was similar between 0, 20, 30, 45, and 70 mg/kg/day groups (n= 0-4 animals/group), but higher in the 100 (n=8) and 300 (n=6) mg/kg/day groups. The severity of hemorrhage was dose-dependent with a slight-to-minimal range of grading in 0 to 30 mg/kg/day, minimal-to-mild in 45 to 70 mg/kg/day, slight-to-moderate in 100 mg/kg/day, and slight-to-severe in 300 mg/kg/day. The sites of hemorrhage were most common in the lungs and thymus, but hemorrhages in the nose (70, 100, and 300 mg/kg.day), trachea (100 mg/kg/day) and skin (100 mg/kg/day) were also observed. Blood was also observed in the stomach (100 mg/kg/day, n=1), esophagus (100 mg/kg/day, n=1), and rectum (300 mg/kg/day, n=1). Extramedullary hematopoiesis was observed in the liver (n=4) and spleen (n=1) in the 300 mg/kg/day dosing group. Mononuclear infiltrates were observed in the lung in the 100 mg/kg/day (n=3) and 300 mg/kg/day (n=4) dosing group. Inflammation and debris were also present in the nasolacrimal duct of the 100 mg/day and 300 mg/kg/day dosing group within 3 and 4 animals, respectively.

Study Summary

Juvenile Wistar Han rats (8/sex/group) were divided into two main phases (Phase 1 and 2) and treated with 0, 20, 30, 45, 70, 100, or 200 mg/kg/day of the test article from PND 7 to 28. Main phase animals were evaluated for viability, clinical observations, and body weights during the treatment period. Blood samples for toxicokinetics were obtained from Day 1 TK phase animals on PND 7 following a single dose administration and main phase groups on PND 21 following repeated dose administration. Macroscopic pathology and histopathology evaluation were limited to the larynx, trachea, esophagus, lungs (with bifurcation). Phase 2 animals (0, 20, 45, and 70 mg/kg/day) had blood drawn for hematology. Three rats in the 200 mg/kg/day were found dead and due to deteriorating clinical condition (dull eyes, pale extremities, underactive behavior, and significant weight loss) the remaining animals in the group were terminated early. Two rats in the 100 mg/kg/day were terminated early due to deteriorating clinical condition (pallor, cold to touch, piloerection, and partially closed eyes). Two male rats in the 70 mg/kg/day group were noted to have bruising on the tail with one of these animals exhibiting underactive behavior along with an enlarged, dark red eye. Histopathological findings demonstrated hemorrhage in all treatment groups, including control, with

increased incidence of hemorrhage (lungs, nose, thymus) in the 100 and 200 mg/kg/day groups along with a dose-dependent increase in the graded severity of hemorrhage. Extramedullary hematopoiesis, mononuclear infiltrates, and nasolacrimal debris/inflammation were also among histopathological findings in 100 and 200 mg/kg/day dosing groups. There was a statistically significant increase in MCV in 70 mg/kg/day male rats compared to their respective male controls. Toxicological findings were related to the mechanism of action of the test article (direct thrombin inhibitor). There was no clear effect of the test article on body weight at any dose. There were no clinical, macroscopic, or histopathologic treatment-related findings in the 20 or 30 mg/kg/day group. Based on the results of this study, 70 mg/kg/day is acceptable for the high dose in the definitive study.

5.2 Definitive Juvenile Toxicity Study

Study title: BIBR 1048 MS: Neonatal Toxicity Study in the Han Wistar Rat by Oral (Gavage) Administration for 8 weeks followed by a 4-Week Recovery Period

Study no.:	n00251085-1
Study report location:	Study n00251085-01
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 July 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BIBR 1048 MS, Lot #1079776, Purity > 99%

Key Study Findings

1- Increased mortality/termination due to animal welfare in all dosage groups
2- Common findings among premature deaths included evidence of bleeding/hemorrhage (e.g. eye lesions, blood in abdomen, intraocular hemorrhage)
3- Other toxicological findings in surviving animals (eye abnormalities, cataracts, failure of pupil dilation reflex) were considered secondary to bleeding.
4- There was no clear effect of BIBR 1048 MS on growth or development.
5- Toxicological findings were predictable due to mechanism of action of test article (direct thrombin inhibitor)
6- Due to mortality in the lowest dosing group (15 mg/kg/day) a NOAEL could not be established for the study.

Methods

Doses:	0, 15, 32.5, and 70 mg/kg/day)
Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	0.5% Natrosol 250 HX solution
Species/Strain:	Han Wistar rats
Number/Sex/Group:	12/sex/group (main phase) 20 /sex/group (recovery phase)
Satellite groups:	Day 1 Toxicokinetic (TK) phase (12/sex/group) Clinical Pathology phase (20/sex/group)
Study design:	Han Wistar rat pups were treated with either vehicle or test article and divided into four phases. Main phase and recovery phase animals were dosed from post-natal day (PND) 7 to PND 62 at the specified doses. Clinical pathology phase animals were dosed from PND 7 to PND 20. Single dose TK phase animals were dosed once on PND 7. Recovery phase animals were observed for a four-week period following treatment. All animals were evaluated for viability and clinical observations. Main phase and recovery phase animals were evaluated for body weight, food consumption, air righting, auditory function, visual function, limb measurements, ophthalmic examination, sexual maturation, and urinalysis. Recovery phase animals underwent neurobehavioral examination. Blood samples for hematology and clinical chemistry were collected at PND 21 for clinical pathology phase animals and at necropsy for main phase and recovery phase animals. TK blood samples were collected at 1, 3, 8, and 24 hours after single (Day 1 TK phase) or repeated (Week 8, recovery phase) dosing. Macroscopic examination was performed at the end of the treatment period on all main and recovery phase animals, as well on any premature deaths from any study phase. Histopathology was evaluated in main phase animals at the end of the treatment period, as well as in any premature deaths from any study phase.

Results were presented as mean ± standard deviation (SD). For parametric data, inter group comparisons were made using William's test (monotonic dose-response) or Dunnett's test (non-monotonic dose-response). For non-parametric data inter group comparisons were made using Shirley's test (monotonic dose-response) or Steel's test (non-monotonic dose-response). For ulna length, neurobehavioral examination, sexual maturation, and clinical pathology, Fisher's Exact test was used to compare against the control (if 75% of the data across all groups were the same). Significant differences between control and treated groups were expressed at the $p<0.05$ or $p<0.01$ level.

Deviations from study protocol: The protocol had stated TK samples were to be collected from animals during Week 8 on PND 62, however due to an oversight TK samples for each time point were collected on different days during Week 8 (e.g. 1-hour post dose collected on PND 57, 8-hour post dose collected on PND 59.

Unselected neonates were weighed in the time between PND 7 and PND 10 instead of on PND 7 and PND 10.

For three males in the 70 mg/kg/day (recovery phase) balano pre-putial separation may have started on Day 38 instead of Day 39 as stated in the protocol.

Observations and Results

Mortality:

Methods: A viability check was performed twice daily on all animals.

Results: There were 28 premature deaths in the study broken down per dosing group as follows: 15 mg/kg/day (1 female), 32.5 mg/kg/day (9 males, 4 females), 70 mg/kg/day (10 males, 3 females). There were no deaths in control animals. Many of these animals (n=8) were cannibalized by the dam, making it impossible for macroscopic/microscopic evaluation. Among animals killed for welfare reasons in the 15 mg/kg/day (n=1 female), 32.5 mg/kg/day (3 males, 2 females), and 70 mg/kg/day (4 males, 1 female) groups,

common finding was dark and/or enlarged eye which correlated to hemorrhage in all but one death.

Clinical Observations:

Methods: Animals were inspected visually at least twice daily for clinical condition. Detailed observations were daily during the ore-weaning period and twice weekly during the post-weaning period at the following times: pre-dose, on completion of dosing, one to two hours after dosing, and at the end of the working day. All treated animals were subjected to a full physical examination weekly starting from PND 21 until termination.

Results: Eye abnormalities were the most common clinical observation. A large right/left eye was observed in the 15 (2 females), 32.5 (2 females, 5 males) and 70 (5 males, 1 female) mg/kg/day dosing groups. A dark right/left eye was observed in the 32.5 (3 males) and 70 (5 males) mg/kg/day dosing groups. These eye changes persisted until the end of recovery in one male and two males in the 32.5 and 70 mg/kg/day dosing groups, respectively. Underactive behavior was also observed in the 15 (1 female), 32.5 (1 male, 1 female), and 70 (1 male, 1 female) mg/kg/day dosing groups. Pale skin color was observed in the15 (2 males, 3 females), 32.5 (1 male, 4 females), and 70 (2 males, 1 female) dosing groups.

Body Weight:

Methods: Body weights were individually recorded for juvenile animals on PND 6 through 28 and then twice daily thereafter until termination. In addition, all main and recovery phase animals were weighed on the day of sexual maturation and on the day of necropsy.

Results: Female rats dosed at 70 mg/kg/day had significantly reduced (approximately 5%) group mean bodyweights compared to their respective female controls on days PND 23 through PND 35, however there was no significant differences between these groups at the end of treatment, between PND 21 through 63, or between PND 7 through 63. There were no other effects of BIBR 1048 MS on body weight gain of males or females throughout treatment or during the recovery period.

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Food Consumption:

Methods: Food consumption was recorded twice weekly for main and recovery phase animals from PND 21 until termination.

Results: There were no effects of BIBR 1048 MS on food consumption of males or females throughout treatment or during the recovery period

Pre-weaning Development

Methods: For main and recovery phase animals, air righting was measured from PND 16 to PND 21 and both auditory function (startle reflex) and visual function (pupil reflex) were measured on PND 20.

Results: There were no effects of BIBR 1048 MS on mean day of age of air righting or startle reflex. Failure of the pupil reflex was observed in the 15 (1 male/64 total animals), 32.5 (4 males + 1 female/61 total animals), and 70 (5 males + 1 female/63 total animals) mg/kg/day dosing groups.

Limb measurements

Methods: For main phase animals, the length of the left ulna was recorded on PND 14, 28, 42, 56, and at necropsy. For recovery phase animals, the length of the left ulna was recorded on PND 14, 28, 42, 56, 70, 84 and at necropsy.

Results: There were no effects of BIBR 1048 MS on ulna length and growth in males throughout the treatment or during the recovery period. In female rats dosed at 70 mg/kg/day, there was a statistically significant reduction in ulna length (24.3±1.9 mm) versus respective female controls (25.2±0.55 mm) measured on PND 28. This reduction was not seen versus controls in any other measurement on specified days or in the change in ulna length from PND 14 to PND 56. Also, by the end of treatment and recovery periods, there was no difference in ulna length between treated females and controls.

Neurobehavioral examinations:

Methods: Recovery animals were assessed for effects of dosing on behavior during Weeks 7 and 8 of dosing. On PND 50/51, individual animals were monitored for motor activity over a 1-hour period using an automated infra-red system within a plastic cage. The system employed high beam and low beam detectors to measure rearing and ambulatory activity, respectively, in 6-minute intervals. From PND 52-55, learning ability was assessed in a series of three trials run on for consecutive days using a Morris water maze.

Results: At the 30 to 36-minute interval, there was a statistically significant reduction in mean high beam (rearing activity) in male mice dosed at 32.5 (6.1±11.8 min) and 70 (10.8±13.9) mg/kg/day compared to the respective male controls (29±27.8 min). There was a non -significant decrease in total mean rearing activity in all treated animals versus control, but this effect was not dose-dependent. In females, an opposite effect was observed, where at the 18 to 24 minute-interval, there was a statistically significant increase in 32.5 (63.4±36.4) and 70 (52.3±34.0) mg/kg/day dosing groups compared to respective female controls (34.1 ± 28.1 min). A non-significant increase in mean rearing time was observed at the 6, 12, 18, and 30-minute intervals as well as the total mean rearing activity.

There was no effect of BIBR 1048 MS on the ambulatory activity of male rats. Female rats dosed at 32.5 and 70 mg/kg/day had significantly increased mean ambulatory activity at both the 6 to 12-minute and 18 to 24-minute time interval versus their respective controls. At the 6 to 12-minute interval, mean ambulatory activity time was 124.7±43.1 min in female controls versus 176±45.7 and 159.9±56.0 min in the 32.5 and 70 mg/kg/day dosing group, respectively. At the 18 to 24-minute interval, mean ambulatory activity time was 60.5±37.9 min in female controls versus 90.2±40.7 and 85.0±30.4 in the 32.5 and 70 mg/kg/day dosing group, respectively. At 15 mg/kg/day dosing in females, there was a non-significant decrease in mean ambulatory time at all intervals. There was no clear effect of BIBR 1048 MS on total mean ambulatory time across all female dosing groups.

There was no effect of BIBR 1048 MS on Morris maze performance in male rats. In female rats dosed at 70 mg/kg/day, there was a statistically significant increase in the number of pool quadrants (sectors) crossed (14.2 ± 5.9) on the first day of maze testing versus their respective female controls (10.7±4.3). There was also a statistically significant increase observed on the fourth day of maze testing where 70 mg/kg/day dosed females crossed a mean of 5.5 ± 3.0 sectors versus their respective controls, which crossed 3.9±2.3 sectors. In all female BIBR 1048 MS dosing groups, mean trial times on day 1 and day 2 of maze testing were increased nonsignificantly and without dose dependence by approximately 20% and 40%, respectively, when compared to their respective female controls. On Day 1, 75% of 15 mg/kg/day females, 84.2% of 32.5 mg/kg/day females, and 75.0% of 70 mg/kg/day females failed at least 1 maze trial compared to 45% of female controls ($p>0.05$). On Day 2, 25.0% of 15 mg/kg/day females, 15.8% of 32.5 mg/kg/day females, and 20% of 70 mg/kg/day females failed at least 1 maze trial compared to 10% of female control ($p>0.05$), There were no clear BIBR 1048 MS effects on mean trial time or percentage of animals failing trials on day 3 and 4 of maze testing. For all female groups, trial time, number of failed trials, and number of sector crossings progressively decreased with each day of maze testing.

Ophthalmic examination:

Methods: All main phase and recovery phase animals were examined using an indirect ophthalmoscope during either Week 8 of treatment or Week 4 of recovery, respectively.

Results: In the 70 mg/kg/day dosing group, 2 males were noted to have slight cornea opacities and 2 males were observed to have cataracts. One male with cataracts also presented with a slight cornea opacity that was associated with vascularization. Among females dosed at 70 mg/kg/day, 2 were observed to have a linear superficial opacity and 1 presented with persistent pupillary membrane. There were no other clear effects of treatment on any of the dosing groups.

Sexual maturation:

Methods: For males, sexual maturation was assessed by daily examination from PND 38 for completion of balano-preputial separation. For females, it was assessed by daily

examination from PND 25 until vaginal opening. Body weights were recorded on the day of balano-preputial separation and vaginal opening.

Results: There were no effects of BIBR 1048 MS on the age or body weight at completion of sexual maturation in both male and female rats.

Hematology:

Methods: Blood samples were drawn on PND 21 for clinical pathology phase animals at necropsy for main phase (PND 63) and recovery phase (PND 91) animals. The following parameters were measured: hematocrit (Hct), hemoglobin concentration (Hb), erythrocyte count (RBC), absolute reticulocyte count (Retic), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), red cell distribution width (RDW), total leucocyte count (WBC), differential leucocyte count (neutrophils, lymphocytes, eosinophils, basophils, monocytes, large unstained cells), platelet count (Plt), and morphology (anisocytosis, microcytosis, hypochromasia, hyperchromasia), prothrombin time (PT), and activated partial thrombolastin. A blood film using Romanowsky stain was prepared for all samples to examine abnormalities.

Results: In male rats at PND 21, MCHC was significantly reduced in the 32.5 (28.0 ± 0.97 g/dL) and 70 (28.0 ± 1.30 g/dL) mg/kg/day dosing groups compared to their respective male controls (28.8 ± 0.91 g/dL). The following parameters were significantly increased in male rats dosed at 70 mg/kg/day (compared to controls): Retic (0.951 ±.0.19 x 10^{12}/L vs 0.805 ± 0.19 x 10^{12}/L), WBC (3.47 ± 1.5 x 10^{12}/L vs 2.67 ± 0.43 x 10^{12}/L), lymphocytes (2.75 ± 1.14 x 10^{12}/L vs. 2.13 ± 0.36 x 10^{12}/L), monocytes (0.12 ± 0.07 x 10^{12}/L vs. 0.07 ± 0.03 x 10^{12}/L), and large unstained cells (0.02 ± 0.01 x 10^{12}/L vs 0.01 ± 0.01 x 10^{12}/L). There were no treatment related findings in female rats at PND 21.

In main phase rats at PND 63, there were no treatment related findings in male rats. However, in main phase female rats at 70 mg/kg/day, the following parameters were significantly reduced (compared to control animals): Hct (0.409 ± 0.01 L/L vs. 0.428 ± 0.02 L/L), Hb (13.9 ± 0.51 g/dL vs. 14.5 ± 0.38 g/dL), and RBC (7.28 ± 0.39 g/dL vs. 7.65 ± 0.27 g/dL).

In recovery phase rats at PND 91 there were no treatment related findings in either sex.

Clinical Chemistry:

Methods: Methods: Blood samples were drawn on PND 21 for clinical pathology phase animals at necropsy for main phase (PND 63) and recovery phase (PND 91) animals. The following blood chemistry parameters were measured: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (gGT), glutamate dehydrogenase (GLDH), total bilirubin (Bili), urea, creatinine (Creat), glucose (Gluc), Total cholesterol (Chol), Triglycerides (Trig), Sodium (Na), Potassium (K), Chloride (Cl), Calcium (Ca), Inorganic phosphorus (Phos),

Magnesium (Mg), Total protein (Total Prot), Albumin (Alb), α1 globulin (a1), α2 globulin (a2), β globulin (Beta), and γ globulin (Gamma).

Results:

In male rats, on PND 21, AST was significantly reduced in the 15, 32.5, and 70 mg/kg/day dosing groups compared to male controls. ALT and γ-globulin were also both significantly reduced in 32.5 and 70 mg/kg/day dosing groups compared to male controls. Magnesium was significantly increased in the 15, 32.5 and 70 mg/kg/day dosing groups compared to male controls, while bilirubin was significantly increased in 70 mg/kg/day only. In female rats, on PND 21, creatinine was significantly increased in the 32.5 and 70 mg/kg/day dosing groups compared to respective female controls. Glucose was significantly increased in the 70 mg/kg/day dosing group only. ALT and albumin-to-globulin ratio were significantly decreased in 70 mg/kg/day dosing group compare to their respective female controls. These findings (with values) are summarized in the table below:

Table 6. Reviewer's Table of Select Mean Clinical Chemistry Parameters – Clinical Pathology Phase (Day 21)

	0 (control)	15 mg/kg/day	32.5 mg/kg/day	70 mg/kg/day
MALE				
ALT (U/L)	45 ± 8.4	42 ± 9.2	38 ± 8.0*	33 ± 6.8**
AST (U/L)	110 ± 24.8	89 ± 11.0**	88 ± 12.2**	93 ± 15.1**
BIL (µmol/L)	2 ± 0.5	2 ± 0.6	2 ± 0.6	2 ± 0.7*
Mg (mmol/L)	0.82 ± 0.03	0.86 ± 0.05*	0.88 ± 0.06**	0.89 ± 0.1**
Gamma (g/L)	2 ± 0.6	2 ± 0.7	2.0 ±0.5**	1 ± 0.5**
FEMALE				
ALT (U/L)	43 ± 6.7	39 ± 6.5	43 ± 10.0	36 ± 7.2**
Cr (µmol/L)	9 ± 2.0	10 ± 3.2	11 ± 2.6*	12 ± 3.9*
Glu (mmol/L)	11.2 ± 0.98	11.2 ± 0.96	11.9 ± 1.0	12.3 ± 1.7*
A/G Ratio	1.40 ± 0.1	1.41 ± 0.2	1.45 ± 0.1	1.32 ± 0.1*

*p<0.05 vs control, **p<0.01 vs control

In male rats, on PND 63, α1-globulin was significantly reduced in the 32.5 and 70 mg/kg/dosing group compared to their respective male controls. α1-globulin, however, was significantly increased in the 70 mg/kg/day dosing group compared to their respective male controls. In female rats, on PND 63, potassium was significantly increased in 15, 32.5, and 70 mg/kg/day dosing groups compare to female controls

while γ-globulin was significantly increased in 32.5 and 70 mg/kg/day dosing groups only. These findings (with values) are summarized in the table below:

Table 7. Reviewer's Table of Select Mean Clinical Chemistry Parameters – Main Phase (Day 63)

	0 (control)	15 mg/kg/day	32.5 mg/kg/day	70 mg/kg/day
MALE				
a1 (g/L)	12 ± 0.9	12 ± 0.7	11 ± 1.3*	11 ± 0.6*
a2 (g/L)	4 ± 0.5	4 ± 0.3	4 ± 0.3	5 ± 0.6**
FEMALE				
K (mmol/L)	5.1 ± 0.34	4.9 ± 0.26*	4.8 ± 0.19*	4.9 ± 0.29*
Gamma (g/L)	1 ± 0.5	1 ± 0.5	2.0 ±0.4*	2 ± 0.5*

*p<0.05 vs control

In female rats, on PND 91, glucose, cholesterol and albumin-to-globulin ratio were significantly decreased in the 15, 32.5, and 70 mg/kg/day dosing groups compared to their respective female controls. Sodium was significantly increased in the 70 mg/kg/day dosing group compared to respective female controls. These findings (with values) are summarized in the table below:

Table 8. Reviewer's Table of Select Mean Clinical Chemistry Parameters – Recovery Phase (Day 91)

	0 (control)	15 mg/kg/day	32.5 mg/kg/day	70 mg/kg/day
MALE				
No apparent treatment-related findings				
FEMALE				
Glu (mmol/L)	5.1 ± 0.34	4.9 ± 0.26*	4.8 ± 0.19*	4.9 ± 0.29*
Chol (mmol/L)	1.91 ± 0.3	1.69 ± 0.3*	1.63 ±0.4**	1.64 ± 0.4**
Na (mmol/L)	140 ± 1.3	140 ± 1.7	141 ± 1.5	141 ± 1.2*
A/G Ratio	1.06 ± 0.06	0.99 ± 0.06*	1.04 ± 0.06*	1.01 ± 0.06**

*p<0.05 vs control, **p<0.01 vs control

Urinalysis:

Methods: Urine was collected overnight for all main phase animals during week 8 of treatment and for all recovery phase animals during week of recovery. The following urine parameters were assessed or analyzed: clarity/color, volume, pH, specific gravity, ketones, bile pigments, blood pigments, protein, creatinine, glucose, sodium, potassium, chloride. Urine sediment was analyzed for epithelial cells, leucocytes, erythrocytes, casts, or other abnormal components.

Results: In main phase female rats, during week 8 of treatment, urine glucose and sodium were significantly increased in all rats receiving test article compared to their

respective female controls. Glucose was 2.47 ± 0.6, 2.39 ± 0.8, and 2.51 ± 0.8 µmol in 15, 32.5, and 70 mg/kg/day dosing groups compared to 1.82 ± 0.5 µmol in female controls. Sodium was 0.35 ± 0.1, 0.37 ± 0.1, and 0.31 ± 0.1 mmol in 15, 32.5. and 70 mg/kg/day dosing groups compared to 0.25 ± 0.1 mmol in female controls. Urine specific gravity was significantly increased in female rats dosed at 70 mg/kg/day (1036 ± 5.0 g/L) compared to female controls (1032 ± 4.9 g/L).

In recovery phase male rats, during week 4 of recovery, urine pH was significantly decreased in the 70 mg/kg/day dosing group (6.9 ± 0.7) compared to male controls (7.2 ± 0.5). In recovery phase female rats, urine pH was significantly increased in the 70 mg/kg/day dosing group (6.6 ± 0.70) compared to female controls (6.1 ± 0.40).

Toxicokinetics:

Methods**:** Blood samples for BIBR 953 ZW measurement were obtained from Day 1 TK phase animals on PND 7 following a single dose administration and from select animals in the recovery phase group after week 8 following repeated dose administration. Samples were collected at 1, 3, 8, and 24 hours postdose.

Results:

Table 9. Reviewer's Table of Toxicokinetic Parameters

		Postnatal Day 1			Week 8		
Dosage (mg/kg/day)	**Sex**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$AUC_{(0-24hr)}$ (ng*h/mL)**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$AUC_{(0-24hr)}$ (ng*h/mL)**
15	M	876	3	9740	543	1	2110
	F	643	3	7050	538	1	1480
32.5	M	971	3	12700	1250	1	3870
	F	889	8	11500	1720	1	4440
70	M	909	8	13600	2170	1	6200
	F	1370	8	18300	2710	1	7760

C_{max} and AUC of BIBR 953 ZW were generally similar between males and female rats after single (PND 1) and repeated dosing (Week 8). For each sex, both parameters increased dose-dependently during Week 8, but less than dose proportionally on PND 1. After repeated administration, AUC was much lower compared to PND 1 in both males and females across all doses.

Necropsy:

Methods**:** All main phase and recovery animals that were found dead or euthanized after the last day of dosage (PND 63) or recovery (PND 91) were subjected to full necropsy (with organ weights and tissue retentions recorded) and macroscopic examination. Organs and tissues preserved at necropsy were processed, embedded in paraffin, sectioned at 4-5 microns and stained with H&E. Macroscopic evaluation for

both phases included examination of cranial, thoracic, and abdominal cavities. Microscopic examination was performed on all premature deaths in the study and main phase (control and 70 mg/kg/day). If abnormalities were present in the former, main phase animals dosed at 15 mg/kg/day and 32.5 mg/kg/day were also examined.

The following tissues were microscopically examined histology and light microscopy: adrenals, aorta, bone marrow, brain (cerebellum, cerebrum, midbrain), caecum, colon, duodenum, epididymides, eyes, femur (femorotibial joint), harderian glands, heart (including auricular and ventricular regions), ileum, jejunum, kidneys, lachrymal glands, larynx, liver, lungs, lymph node (mandibular, mesenteric; left axillary), esophagus, optic nerves, ovaries, pancreas, Peyer's patches, pituitary, prostate, rectum, salivary glands (submandibular, parotid, sublingual), sciatic nerves, seminal vesicles, skeletal muscle, skin with mammary glands, spinal cord (cervical, thoracic, and lumbar levels), spleen, sternum, stomach, testes, thymus, thyroid (with parathyroids), tongue, trachea, ulna (left), ureters, urinary bladder, uterus with cervix, and vagina.

Results: In recovery phase male rats surviving until scheduled termination, there was a significant increase in brain weight relative to body weight in the 15 (0.559 ± 0.05), 32.5 (0.558 ± 0.04), and 70 (0.562 ± 0.05) mg/kg/day dosing groups compared to their respective male controls (0.529 ± 0.04). Statistically significant decreases in heart, liver, and spleen-relative to brain weight were also observed in recovery phase male rats. These findings are summarized in the table below:

Table 10. Reviewer's Table of Organ relative to brain weight ratio in recovery phase male rats

	0 mg/kg/day	15 mg/kg/day	32.5 mg/kg/day	70 mg/kg/day
Organ				
Heart	52.1 ± 4.6	49.5 ± 4.8	49.8 ± 4.1	48.0 ± 4.3**
Liver	696 ± 82	641 ± 81*	642 ± 82*	632 ± 61*
Spleen	34.3 ± 4.9	30.5 ± 4.4*	30.3 ± 3.9*	32.4 ± 4.4*

*p<0.05 vs controls, **p<0.01 vs controls

There were no changes noted in absolute or relative (to brain or weight) in female recovery phase animals or in main phase animals of either sex.

In recovery phase male rats surviving until scheduled termination, at 70 mg/kg/day macroscopic changes not observed in control animals included opaque eyes (n=1) and thymus mass (n=2). In recovery phase female rats surviving until schedule termination, at 70 mg/kg/day, macroscopic changes not observed in control animals included kidney depression (n=1). There were no apparent treatment-related macroscopic changes observed in main phase animals of either sex.

In main phase male rats surviving until scheduled termination, slight pelvic dilatation was seen in 15 (n=1), 32.5 (n=1), and 70 (n=1) mg/kg/day dosing groups but not in

corresponding male controls. In main phase male rats at 70 mg/kg/day, other microscopic changes not observed in male controls included kidney tubular cast (n=1), epithelial hyperplasia of larynx (n=1), and larynx inflammation (n=1). In main phase female rats at 70mg/kg/day, microscopic changes not observed in female controls included minimal pelvic dilatation (n=1) and axillary sinus erythrocytosis (n=1).

Macroscopic observations in animals that died prematurely included evidence of hemorrhage/bleeding. In the female rat killed prematurely in the 15 mg/kg/day dosing group, there was blood found in abdomen. Of the 5 animals killed for welfare reasons in the 32.5 mg/kg/day dosing group, 4 animals (3 male, 1 female), had dark, enlarged, right or left eyes. The other animal killed prematurely (female) in the 32.5 mg/kg dosing group had dark stomach contents. Of the 5 animals killed for welfare reasons in the 70 mg/kg/day dosing group, 4 animals (3 male, 1 female), had dark, enlarged right or left eyes. The other animal killed prematurely (male) in the 70 mg/kg/day group had abdominal adhesions with a distended, thickened, and dark ileum.

Microscopic observations were only recorded for animals in the 32.5 and 70 mg/kg/day dosing groups which died prematurely. All 4 animals with recorded microscopic observations in the 32.5 mg/kg/day dosing group that died prematurely had eye lesions, with 3 of these animals (2 males, 1 female) having findings of intraocular hemorrhage and corneal vacuolation. Of the 5 animals with recorded microscopic evaluations in the 70 mg/kg/day dosing group that died prematurely, 4 (3 males, 1 female) had eye lesions and intraocular hemorrhage. The other animal prematurely (male) in the 70 mg/kg/day group had microscopic intestinal ulceration.

Study Summary

Juvenile Wistar Han pups were divided into four treatment groups: main phase (12/sex/group), recovery phase (20/sex/group), Day 1 TK phase (12/sex/group), and clinical pathology phase (20/sex/group). Animals were treated with 0, 15, 32.5, 45, or 70 mg/kg/day of the test article from PND 7 to 28 (main phase, recovery phase), PND 7 to 20 (clinical pathology phase), or PND 7 alone (Day 1 TK phase). All animals were evaluated for viability and clinical observations, while main phase and recovery animals only were evaluated for body weight, food consumption, air righting, auditory function, visual function, limb measurements, ophthalmic examination, neurobehavioral examination (recovery phase only). sexual maturation, and urinalysis during the treatment period (and four weeks after for recovery phase animals). Blood samples for hematology and clinical chemistry were collected at PND 21 for clinical pathology phase animals and at necropsy for main phase and recovery phase animals. Blood for TK samples were drawn on PND 7 (Day 1 TK Phase, single dose) and during Week 8 (repeated dose, recovery phase). Macroscopic pathology and histopathology (main phase only) were performed at the end of the treatment or recovery period. There were 28 premature deaths in the study broken down per dosing group as follows: 15 mg/kg/day (1 female), 32.5 mg/kg/day (9 males, 4 females), 70 mg/kg/day (10 males, 3 females) with no premature deaths in control animals. These animals were killed for welfare reasons with the common observation of enlarged, dark eye(s) associated with

histopathology findings of hemorrhage. In animals surviving until the end of the study, eye abnormalities (enlarged, dark eyes), underactive behavior, and pale skin color were observed in all treatment groups, but not controls. In three male animals receiving either 32.5 or 70 mg/kg/day BIBR 1048 MS, the eye abnormalities lasted until the end of the recovery period. Failure of the pupil closure reflex was observed in all dosing groups but corresponded in almost all cases to the presence of an enlarged, dark eye. Cataracts were observed in two males in the 70 mg/kg/day group, both animals of which had observed dark/large eye at PND 17. suggesting they were secondary to eye hemorrhage. At the end of the treatment period, main phase female rats dosed at 70 mg/kg/day had reduced hematocrit, hemoglobin, and RBC count compared to their respective female controls, consistent with blood loss via hemorrhage. There were statistically significant changes in clinical chemistry parameters (ALT, AST, BIL, Mg, Cr, Glu, K, γ-globulin, α1 globulin, α2 globulin) and urinalysis (pH, Glu, Na) of both sexes at PND 21 and/or PND 63, but these changes overall were mild and not likely to have clinical implications. In all three male dosing groups after recovery, there was a statistically significant increase in brain-to-body weight and a statistically significant decrease in liver-to-brain weight ratio. Also, in male rats dosed at 70 mg/kg/day, after recovery, there was a significant decrease in spleen- and heart- to brain ratio compared to control. As these changes were not reflected in either the absolute or relative (to body weight) weight changes in the same recovery animals, and there were no organ weight changes (relative or absolute) noted at the end of treatment period in main phase animals, these findings are not likely clinically significant. C_{max} and AUC of BIBR 953 ZW were generally similar between males and female rats after single (PND 1) and repeated dosing (Week 8). For each sex, both parameters increased dose-dependently during Week 8, but less than dose proportionally on PND 1. After repeated administration, AUC was much lower compared to PND 1 in both males and females across all doses. A similar observation of AUC was made after single and repeat-dosing of BIBR 1048 MS in adult male rats and adult male monkeys in the previous Pharm/Tox review by Patricia Harlow (see PharmTox review in DARRTS dated April 9, 2010). There was no clear effect of the test article on body weight, food consumption, neurobehavior, or growth and development at any dose. Toxicological findings were related to the exaggerated pharmacology of the test article (direct thrombin inhibitor) and due to bleeding-related mortality at the lowest dose, a NOAEL could not be determined for this study.

6 Integrated Summary and Safety Evaluation

All excipients and impurities for all (b) (4) dosage forms (capsule, (b) (4) and pellet) are considered qualified and acceptable for use in their intended population. One impurity ((b) (4) is a class 2 mutagen but does not exceed the specific threshold set forth in ICH M7 for any of the (b) (4) dosage forms. In both the preliminary and definitive juvenile toxicity studies, bleeding-related mortality was observed. The bleeding is due to an exaggeration of the pharmacology of BIBR 953 ZW. In the case of the definitive study, bleeding-related mortality occurred at the lowest dose, and therefore a NOAEL could not be determined from the study. Other toxicological findings (eye abnormalities, cataracts, failure of pupil closure reflex) were considered secondary to

bleeding/hemorrhage. There was no impact of BIBR 1048 MS on growth or development. Furthermore, in Phase 3 clinical trial (which was used to assist in the qualification of impurities), BIBR 1048 MS appeared to be well tolerated among all age groups. The labeling for all (b) (4) dosage forms/ages indicates there is an increased risk of bleeding with this drug and need for prompt evaluation of signs and symptoms of blood loss. Therefore, the use of BIBR 1048 MS in the treatment and prophylaxis of thromboembolic disorders in pediatric patients is approvable from a nonclinical perspective.

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