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APPLICATION NUMBER:

206111Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: NDA 206,111
Supporting document/s: SN/SDN: 0000/0001
Applicant's letter date: August 4th 2014
CDER stamp date: August 4th 2014
Product: Empagliflozin/Metformin FDC, Synjardy™
Indication: T2DM
Applicant: Boehringer Ingelheim Pharmaceuticals Inc.
Review Division: DMEP
Reviewer: Mukesh Summan, PhD, DABT
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1 Executive Summary

1.1 Introduction

The proposed empagliflozin plus metformin immediate release (IR) fixed dose combination (FDC), film-coated tablet was submitted in accordance with 21 USC 505(b)2 for the treatment of type 2 diabetes mellitus as an adjunct to diet and exercise. Both drugs are approved for use as monotherapies for the same indication. Boehringer Ingelheim Pharmaceuticals Inc. owns empagliflozin and submitted a letter of authorization for DMF # (b) (4) for metformin hydrochloride drug substance, owned by (b) (4)

Nonclinical studies were conducted using co-administration of empagliflozin and metformin rather than the FDC, and were reviewed in this NDA or under NDA 204629 (for empagliflozin, AP 08.01.2014) or under NDA 201281 (for linagliptin + metformin hydrochloride FDC, AP 01.30.2012). This pharmacology/toxicology NDA review summarizes the pertinent previously reviewed studies (as needed). The nonclinical recommendation will rely in part on the FDA's prior determination of safety and efficacy for the individual drugs and the focus of this review is to evaluate interactions between empagliflozin and metformin in a FDC tablet based on the nonclinical data submitted in support of the FDC drug product.

Safety margins to expected human exposure were estimated for empagliflozin using $AUC_{0-24h, ss} = 4740 \text{ nM} \cdot \text{h}$ plasma exposure at the proposed maximum recommended human dose (MRHD) of 25mg empagliflozin. Safety margins to expected human exposure were estimated for metformin IR using $AUC_{0-24h, ss} = 159,000 \text{ nM} \cdot \text{h}$ at the MRHD of 2000mg metformin IR.

1.2 Brief Discussion of Nonclinical Findings

The sponsor is seeking the approval of empagliflozin (Jardiance[®]) as a fixed dose combination (FDC) with metformin immediate release (Glucophage[®]). Empagliflozin is a selective inhibitor of sodium-glucose co-transporter 2 (SGLT2), approved by the FDA August 1st 2014. Metformin immediate release (IR) is a biguanide class insulin sensitizer antihyperglycemic agent approved by the FDA March 3rd 1995.

Pivotal toxicology studies with co-administered drugs were conducted to bridge potential toxicity of the combination treatment of empagliflozin and metformin. Empagliflozin pharmacological activity and nonclinical toxicology studies were reviewed under NDA 204629. The safety of the empagliflozin/metformin FDC as co-administered drugs was supported by 2 week rat, 3-month rat and rat dose range finding and definitive embryo-fetal development (EFD) studies. The pharmacological activity of the co-administered empagliflozin and metformin was evaluated in a single dose and multiple dose Zucker Diabetic Fatty (ZDF) rat model studies. Standalone toxicology studies with metformin (2 week rat and rat EFD study) were also submitted in support of the FDC application.

Nonclinical exposure margins are based on the current proposed maximum recommended human dose (MRHD) of 25 mg empagliflozin ($AUC_{0-24h,ss} = 4740 \text{ nM}\cdot\text{h}$) and 2000 mg metformin ($AUC_{0-24 \text{ h, ss}} = 159 \text{ }\mu\text{M}\cdot\text{h}$).

Impurities and Degradants

[REDACTED] (b) (4)

These degradants/impurities were non-mutagenic by their evaluation in a bacterial reverse mutation test by (Q)SAR analysis. [REDACTED] (b) (4) was also qualified by its use in 4- and 13-week pivotal nonclinical studies in the rat and also by evaluation in an Ames assay and an in vitro micronucleus study in CHO cells, which were negative.

Pharmacology Studies With Co-Administered Empagliflozin and Metformin

In the single dose ZDF rat model, the co-administration of empagliflozin and metformin as a single dose, augmented the reduction of blood glucose relative to the individual components alone, following an oral glucose tolerance test.

In the 28-day multiple dose ZDF study, many diabetes parameters were evaluated for both the co-administration of empagliflozin and metformin and the single drug entities alone. Of note, plasma glucose and HbA1c were significantly reduced and plasma insulin and leptin were significantly increased in all treatment groups. However, the co-administration of empagliflozin and metformin further reduced plasma glucose and HbA1c and also augmented the increase in plasma insulin and leptin.

Toxicology Studies With Metformin Alone

Pivotal repeat dose studies with metformin were conducted in the Wistar (Han) rat for 2 weeks at 1-18x MRHD (2000 mg). Minimal ventricular myocardium hypertrophy was observed at 1000 mg/kg with correlative increases in absolute weight, heart to body weight and heart to brain weight ratios. Absolute and organ to body weight/brain weights were significantly increased in the pituitary gland and adrenal glands at 1000 mg/kg and correlative pituitary hyperplasia and adrenal gland vacuolation were observed. Depletion of pancreatic zymogen granules, atrophy of the seminal vesicles and inflammatory infiltration of the parotid salivary gland were also noted microscopically at 1000 mg/kg. The cardiovascular pathology at the high dose was considered adverse, and the NOAEL is 200 mg/kg (2x MRHD).

Toxicology Studies With Co-Administered Empagliflozin and Metformin

Pivotal repeat dose studies with the co-administration of empagliflozin + metformin were conducted in the Wistar (Han) rat for 2 weeks and 90-days in duration, respectively. In these studies the overall empagliflozin exposure was 4-69x MRHD (25 mg empagliflozin) and the overall metformin exposure was 2-7x MRHD (2000 mg metformin).

In the 2-week study rat study empagliflozin and metformin were administered alone or empagliflozin was co-administered with metformin. The high dose of 500/1000 mg/kg empagliflozin + metformin was not tolerated and resulted in both mortality and moribundity in both sexes at days 8-11 post-dose. These animals failed to gain weight and showed kidney tubular degeneration, mineralization of pelvic calculi and hydronephrosis. Hydronephrosis and mineralization of pelvic calculi was also observed at the mid dose (300/600 mg/kg). For the co-administration of empagliflozin + metformin in the 2-week rat study, the NOAEL was the low dose 100/200 mg/kg (13x MRHD 25 mg empagliflozin and 3x MRHD 2000 mg metformin).

In the 90-day rat study empagliflozin and metformin were administered alone or empagliflozin was co-administered with metformin. The high dose of 200/400 mg/kg (23x/7x MRHD) empagliflozin + metformin resulted in mortality in a single female at recovery day 30 and the cause of mortality was not identified. The co-administration of empagliflozin + metformin at 200/400 mg/kg exacerbated both reduced body weight and body weight gain observed in the high dose empagliflozin alone group (200/0 mg/kg) and the changes were irreversible in recovery animals.

Empagliflozin alone or in combination with metformin also resulted in dose-dependent hypochloremia and elevations of hepatic transaminases and alkaline phosphatase. Pharmacodynamic effects as a result of exposure to empagliflozin alone or co-administered with metformin were also evident with glucosuria and polyuria present. There were no toxicologically adverse or toxicokinetic drug-drug interactions, with the exception of a slight increase in the exposure of empagliflozin or metformin when co-administered relative to the individual drugs alone.

Treatment with co-administered empagliflozin and metformin resulted in minimal mineralization of the kidney pelvic epithelium at the high dose (200/400 mg/kg), particularly in the females. For the co-administration of empagliflozin + metformin in the 90-day rat study, the NOAEL was the low dose 50/100 mg/kg (4-6x MRHD 25 mg empagliflozin and 2x MRHD 2000 mg metformin).

Reproductive Toxicology Studies With Metformin Alone

Reproductive toxicity was assessed in early embryonic development studies in the rat with metformin at 4-23x MRHD (2000 mg). In the embryo-fetal developmental study, metformin at 1000 mg/kg was teratogenic at high exposures (23x MRHD), resulting in anophthalmia and polydactylia. In addition, dose-related increases in skeletal malformations in the rib (flat, thickened or z-shaped), scapula (bent inward) were seen in several litters and fetuses at ≥ 500 mg/kg. The fetal NOAEL was 200 mg/kg (4x MRHD). A maternal NOAEL was not identified due to a dose-dependent 20-153% decrease in BW gain in all treatment groups.

Reproductive Toxicology Studies With Co-Administered Empagliflozin and Metformin

Reproductive toxicity was assessed in early embryonic development studies in the rat with co-administered empagliflozin + metformin at up to 27x MRHD for empagliflozin and 8x MRHD for metformin, respectively.

The co-administration of empagliflozin and metformin was not teratogenic at 100/200 mg/kg. Higher exposure resulted in a dose-dependent increase in the skeletal malformation of flat and thickened rib in the empagliflozin and metformin treated groups, particularly at 300/600 mg/kg. This malformation was also present at a lower incidence in the empagliflozin alone (300/0 mg/kg) and the metformin alone (0/600 mg/kg) groups. Other skeletal malformations were only present in one to three fetuses of the same litter in the 300/600 mg/kg empagliflozin + metformin-treated group and treatment at this dose caused a reduction in fetal weight. Microglossia and micrognathia were present in one empagliflozin alone (300/0 mg/kg) fetus. Consequently the fetal NOAEL is 100/200 mg/kg (14x/4x MRHD). A NOAEL for maternal toxicity was empagliflozin and metformin at 30/60 mg/kg (3-4x/1x MRHD) due to reduced body weight, body weight gain and food consumption at \geq 100/200 mg/kg.

Overall, the co-administration of empagliflozin with metformin was consistent with toxicological findings observed in pivotal nonclinical toxicology studies with the empagliflozin and metformin monotherapy and did not yield new adverse findings. The nonclinical co-administration studies support the safety and approval of the fixed dose combination.

1.3 Recommendations

1.3.1 Approvability

AP (Approval)

Pharmacology/Toxicology recommends approval of NDA 206,111.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

The nonclinical sections of the proposed Synjardy™ label have incorporated the information present in the labels for the approved individual products empagliflozin and the linagliptin and metformin FDC (Jentaducto®), respectively.

Established Pharmacological Class (Highlights/Indications & Usage):

Synjardy™ is a combination of empagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, and metformin, a biguanide, indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

Empagliflozin: 864070-44-0

Metformin: 1115-70-4

Generic Name

Empagliflozin: Jardiance[®]

Metformin: Glucophage[®]

Code Name

Empagliflozin/BI 10773 ^{(b) (4)}

Metformin IR

Synjardy[™]

Chemical Name

Empagliflozin:

(1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl)-D-glucitol

Metformin:

N,N-dimethylimidodicarbonimidic diamide monohydrochloride

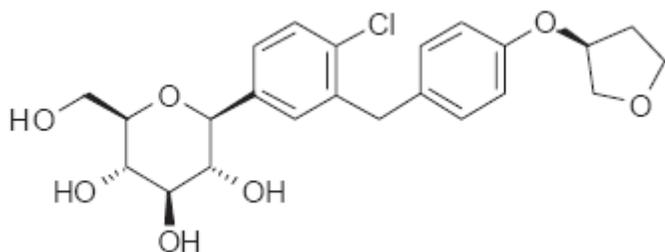
Molecular Formula/Molecular Weight

Empagliflozin: C₂₃ H₂₇ Cl O₇ / MW = 450.91 g/mol

Metformin: C₄H₁₂N₅ / MW = 165.62 g/mol

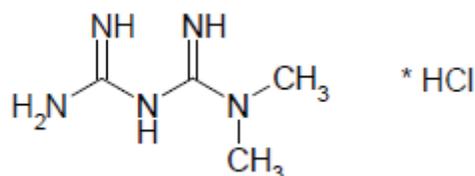
Structure or Biochemical Description

Empagliflozin:



Structure or Biochemical Description

Metformin:



Pharmacologic Class

Empagliflozin: Sodium Glucose co-Transporter 2 (SGLT2) Inhibitor

Metformin: Biguanide antihyperglycemic

2.2 Relevant INDs, NDAs, BLAs and DMFsEmpagliflozin: IND 102,145 and NDA 204,629 (Jardiance[®], AP August 1st 2014).Metformin IR: NDA 203,357 (Glucophage[®], AP March 3rd 1995), from DMF (b) (4)**2.3 Drug Formulation**

Empagliflozin/Metformin immediate release (IR) fixed dose combination (FDC) film coated tablets will be manufactured as empagliflozin/metformin strengths as follows: 5/500, (b) (4) 5/1000, 12.5/500, (b) (4) and 12.5/1000 mg/mg.

Both drug substances are approved for use in the United States and only manufacturing of the new empagliflozin/metformin FDC drug products in the strengths described above is unique to this application. There are no novel excipients in the FDC drug product formulation and all excipients are compendial.

Active ingredients: empagliflozin and metformin hydrochloride

Inactive ingredients: corn starch, copovidone, colloidal silicon dioxide, magnesium stearate, (b) (4) and a film coating layer: (b) (4) (hypromellose (b) (4), polyethylene glycol 400, titanium dioxide, yellow ferric oxide and talc) (see sponsor's tables below).

Table 1. Composition of Empagliflozin/Metformin Tablets -5 mg Series (sponsor’s table)

Ingredient	Reference to Standards	Function	Empagliflozin / metformin hydrochloride	
			5 mg / 500 mg [mg/tablet]	5 mg / 1000 mg [mg/tablet]
Empagliflozin	Company standard	Drug substance	5.000	5.000
Metformin hydrochloride	USP	Drug substance	500.000	1000.000
Corn starch	NF			(b) (4)
Copovidone	NF			
Colloidal silicon dioxide	NF			
Magnesium stearate	NF			(b) (4)
Total weight (film-coated tablet)				(b) (4)

(b) (4)

Table 2. Composition of Empagliflozin/Metformin Tablets - Film Coat Layer 5 mg Series (sponsor’s table)

Ingredient	Reference to standards	Function	Empagliflozin / metformin hydrochloride film-coated tablets	
			5 mg / 500 mg	5 mg / 1000 mg
Quantity [mg / tablet]				
Hypromellose	USP			(b) (4)
Polyethylene glycol 400	NF			
Titanium dioxide	USP			
Ferrous oxide, yellow	NF			
Talc	USP			
Total				

2.5 Comments on Impurities/Degradants of Concern

Metformin: None. No new degradants or impurities were identified.

Empagliflozin and empagliflozin/metformin FDC product:

(b) (4)

(b) (4) the sponsor determined that the degradation products/impurity were non-mutagenic in the bacterial reverse mutation test by (Q)SAR analysis. The sponsor has set a specification limit of (b) (4)% for the (b) (4) degradation product. Drug substance impurity (b) (4) was also qualified by its use in 4- and 13-week pivotal nonclinical studies in the rat and also by evaluation in an Ames assay and an in vitro micronucleus study in CHO cells, which were negative.

2.6 Proposed Clinical Population and Dosing Regimen

The clinical population of type 2 diabetes mellitus (T2DM) patients indicated for use with the individual drug components are proposed for the FDC product. T2DM subjects will be treated BID with empagliflozin/metformin at 5/500, (b) (4) 5/1000, 12.5/500, (b) (4) and 12.5/1000 mg/mg. The maximum recommended daily dose is 25 mg empagliflozin/2000 mg metformin and the treatment regimen is BID.

2.7 Regulatory Background

NDA's for empagliflozin and metformin were approved by the FDA August 1st 2014 and March 3rd 1995, respectively. The sponsor is seeking approval of BID orally administered fixed dose combination (FDC) of empagliflozin and metformin hydrochloride immediate release (IR) (b) (4) (e/m: 5/500 (b) (4) 5/1000, 12.5/500, (b) (4) 12.5/1000 mg/mg). This NDA for the FDC was submitted to the Agency August 4 2014, and relies primarily on the sponsor's approved NDA for empagliflozin and letters of authorization for the DMF relating to metformin hydrochloride.

3 Studies Submitted

3.1 Studies Reviewed

Combination of BI 10773 and Metformin in a Diabetic Rat Model (ZDF): Effect on Glucose Tolerance (non-GLP, Study# MD2010-004-lab8, U10-1604-01)

Effect of Chronic Administration of BI 10773, Metformin and The Combination of BI 10773 With Metformin On Glucose Homeostasis and On Body Weight, Food and Water Intake, And Various Plasma Parameters in Male Diabetic ZDF Rats (GLP, Study# RS618)

Metformin: 2-Week Oral (Gavage) Toxicity Study in Rats (GLP, Study# 09B040, U09-2246-01)

BI 10773 ^{(b) (4)} and Metformin: A 90-Day Oral (Gavage) Toxicity Study in Rats With a 1-Month Recovery Period (GLP, study#10r045, U11-3632-01)

Metformin: Study for Effects on Embryo-fetal Development in rats by Oral (gavage) Administration (GLP, study# 09B099, U10-2386-01)

BI 10773 and Metformin: Dose-Range Finding Study for effects on Embryo-fetal Development in Rats (Oral Administration by Gavage) (non-GLP, study# 11b2252, U13-2538-01)

BI 10773 and Metformin: Study for effects on Embryo-fetal Development in Rats (Oral Administration by Gavage) (GLP, study# 12b056, U13-2227-01)

3.2 Studies Not Reviewed

BI 10773: Determination of the Potential Effects of BI10773 (Empagliflozin) On the Activity of Human SLC2A9 (GLUT9A & GLUT9B) (non-GLP, Study# n00231735-01)

BI ^{(b) (4)} and BI10773: Study for effects on Embryo-fetal Development in Rats (Oral Administration by Gavage) (GLP, study# 12B124, U13-2287-01) [**Reviewer note:** this is an linagliptin + empagliflozin FDC product study report; which was reviewed under NDA 206073, AP 01.20.2015].

3.3 Previous Reviews Referenced

NDA 204629 (for empagliflozin, AP 08.01.2014)

NDA 201281 (for linagliptin + metformin hydrochloride FDC, AP 01.30.2012).

All other pharmacology/toxicology studies relevant to evaluation of Synjardy™ have been reviewed previously under NDA 204629 for Jardiance® (empagliflozin) or NDA 201281 for the linagliptin /metformin hydrochloride FDC and thus only a brief summary is provided for reference and labeling purposes (if applicable).

4 Pharmacology

4.2 Secondary Pharmacology

Combination of BI 10773 and Metformin in a Diabetic Rat Model (ZDF): Effect on Glucose Tolerance (non-GLP, Study# MD2010-004-lab8, U10-1604-01)

Method

Male ZDF rats (n = 5) received a single dose of vehicle (Natrosol 250 HX), empagliflozin (BI 10773) (3 mg/kg), metformin (300 mg/kg) or a combination of empagliflozin and metformin (3 + 300 mg/kg). An oral glucose tolerance test (OGTT) was performed 30 minutes later. Blood was collected pre-dose (fasted 12 hr), 15, 30, 60, 90, 120 and 180 minutes post-glucose load via a tail bleed. Blood glucose was measured with a glucometer.

Results

Following a single dose, empagliflozin alone and metformin alone, significantly reduced the oral blood glucose $AUC_{0-180 \text{ min}}$ (see sponsor's figure below) by 37% and 39%, respectively. The co-administration of empagliflozin and metformin exacerbated the oral blood glucose $AUC_{0-180 \text{ min}}$ reduction by 63% compared to the single entities alone (see sponsor's figure below). The blood glucose reduction with empagliflozin, metformin or the co-administration of empagliflozin and metformin occurs as early as 15 minutes post-glucose load until the last measured time point of 180 minutes (see sponsor's figure below)

Figure 1. Blood Glucose AUC Following A Single Dose in the ZDF Rat (sponsor's table)

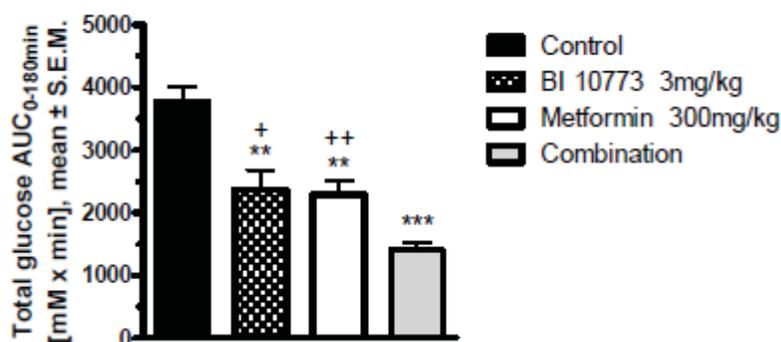
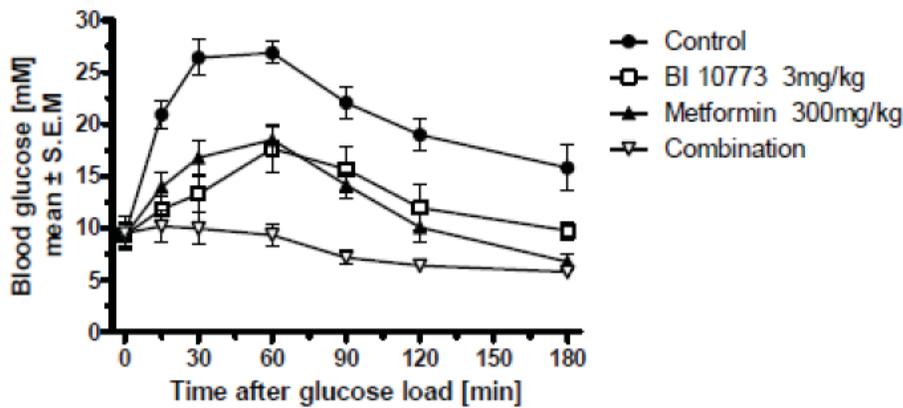


Table 5. Blood Glucose AUC in the ZDF Rat

BLOOD GLUCOSE AUC _{0-180 MIN} ± SEM (mM.min)		
Doses: empagliflozin/metformin mg/kg	NA	% Difference from Vehicle, p value
0/0	3775.95 ± 240.03	NA
3/0	2377.05 ± 308.97	-37, p=0.0072
0/300	2293.80 ± 225.32	-39, p=0.0020
3/300	1403.55 ± 126.17	-63, p=0.0000

NA = not applicable

Figure 2. Blood Glucose in the ZDF Rat (sponsor's table)



Effect of Chronic Administration of BI 10773, Metformin and The Combination of BI 10773 With Metformin, On Glucose Homeostasis and On Body Weight, Food and Water Intake, And Various Plasma Parameters in Male Diabetic ZDF Rats (GLP, Study# RS618) — Summary

**SPECIES
DOSES AND
ADMINISTRATION
ANIMALS
FOLLOW-UP**

Male ZDF rat
28 days

0/0 (lean control), 0/0, 3/0, 0/300, 3/300 mg/kg empagliflozin/metformin (see opposite)

Oral gavage, 3 mL/kg (vehicle 0.5% Natrosol) for 32 days

Main: 8-9/males/group (see opposite)

Recovery: None.

All groups: 7-day “run-in” with vehicle.

Treatment was daily at the beginning of the 12 h dark period.

OGTT at day 31.

Treatment (dosing to commence at 08.45 h)	N
Vehicle ZDF lean control 3 ml/kg po	9
Vehicle ZDF control 3 ml/kg po	9
BI 10773 3 mg/kg po	8
Metformin 300 mg/kg po	8
Metformin 300 mg/kg po plus BI 10773 3 mg/kg po	8

All drug doses are for the free base.

Mortality: None.

Clinical Signs: Unremarkable.

Body Weight: At day 28, mean body weight (BW) was increased 7% in the 3 mg/kg empagliflozin (nss), 300 mg/kg metformin alone (nss) male ZDF rats. In contrast, treatment with 3/300 mg/kg empagliflozin + metformin combination significantly increased mean BW 16% ($p < 0.001$) (see sponsor’s table below). From weeks 1-4, BW gain was increased compared to controls in males treated with 3 mg/kg empagliflozin (28%, nss), 300 mg/kg metformin alone (29%, nss) or 3/300 mg/kg empagliflozin + metformin combination (64% $p < 0.001$) (see sponsor’s table below). Increased BW and BW gain did not correlate with increased food consumption. **Reviewer note: group A, the lean vehicle was excluded**

from the statistical analysis.**Mean Body Weight (sponsor's table)**

Day 28	Vehicle ZDF control 3 ml/kg po	9	392.9	5.2			
	BI 10773 3 mg/kg po	8	420.7	6.0	27.8	7.1	0.065
	Metformin 300 mg/kg po	8	421.8	8.4	28.8	7.3	0.056
	Metformin 300 mg/kg + BI 10773 3 mg/kg	8	454.9	6.9	61.9	15.8	<0.001***

Body Weight Gain (sponsor's table)

Weeks 1-4	Vehicle ZDF control 3 ml/kg po	9	38.3	4.0			
(Day 1-29)	BI 10773 3 mg/kg po	8	66.3	5.5	28.0		0.065
	Metformin 300 mg/kg po	8	67.1	8.1	28.9		0.058
	Metformin 300 mg/kg + BI 10773 3 mg/kg	8	102.7	6.6	64.4		<0.001***

nss = not statistically significant

Food Consumption: Unremarkable.

Water Consumption: Water consumption in the 3 mg/kg empagliflozin alone (3/0 mg/kg) was unremarkable (data not shown). Water consumption in the 300 mg/kg metformin alone (0/300 mg/kg) was reduced 28% ($p < 0.05$) in week 2 (see sponsor's table below). The combination of empagliflozin and metformin at 3/300 mg/kg reduced water consumption 24% ($p < 0.05$) at week 3 (see sponsor's table below). This is likely due to the increasing uncontrolled diabetes and subsequent increased water consumption in the vehicle control male ZDF rats.

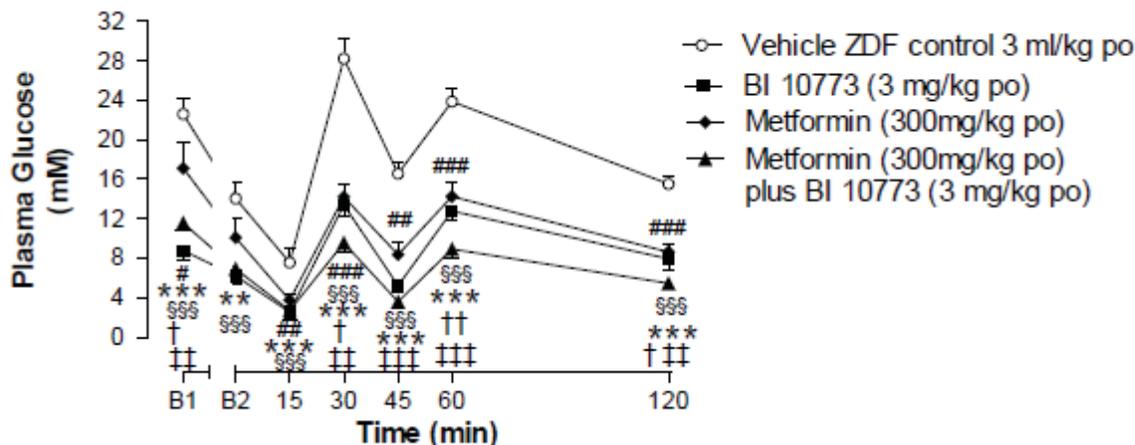
Water Consumption (sponsor's table)

Week 2	Vehicle ZDF control 3 ml/kg po	9	91.7	7.8			
(Day 8-14)	BI 10773 3 mg/kg po	8	90.6	10.3	-1.1	-1.2	0.914
	Metformin 300 mg/kg po	8	66.5	6.6	-25.2	-27.5	0.013*
	Metformin 300 mg/kg + BI 10773 3 mg/kg	8	74.2	3.8	-17.5	-19.0	0.080
Week 3	Vehicle ZDF control 3 ml/kg po	9	97.4	7.5			
(Day 15-21)	BI 10773 3 mg/kg po	8	96.0	12.3	-1.4	-1.4	0.899
	Metformin 300 mg/kg po	8	77.6	7.4	-19.8	-20.3	0.074
	Metformin 300 mg/kg + BI 10773 3 mg/kg	8	74.0	2.1	-23.4	-24.0	0.037*

Overall, water consumption at weeks 1-4 was unremarkable (data not shown).

OGTT and Plasma Glucose: Following the OGTT, plasma glucose was reduced from 15-120 minutes post dose in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the empagliflozin/metformin combination group (3/300 mg/kg), respectively (sponsor's figure below).

Plasma Glucose (sponsor's figure)



Following the OGTT, the plasma glucose AUC (AUCB) was significantly reduced 45% in the metformin alone (0/300 mg/kg) group and 34% in the empagliflozin/metformin combination group (3/300 mg/kg) (see sponsor's table below).

Plasma Glucose AUC (sponsor's truncated table)

Treatment	AUC		Mean	AUCB	
	SEM	p		SEM	p
Vehicle ZDF control 3 ml/kg po	1.88		8.69	2.62	
BI 10773 3 mg/kg po	1.26	<0.001***	4.41	1.36	0.070
Metformin 300 mg/kg po	1.91	<0.001***	0.79	1.44	0.001**
Metformin 300 mg/kg + BI 10773 3 mg/kg	1.49	<0.001***	-1.14	1.72	<0.001***

OGTT and Plasma Insulin: Following the OGTT, plasma insulin AUC was unchanged in the empagliflozin alone (3/0 mg/kg) group. Plasma insulin was significantly increased 81% in the metformin alone (0/300 mg/kg) group and 113% in the in the empagliflozin/metformin combination group (3/300 mg/kg) (see sponsor's table below).

Plasma Insulin AUC (sponsor's truncated table)

Treatment	AUC			AUCB		
	Mean	SEM	p	Mean	SEM	p
Vehicle ZDF control 3 ml/kg po	2.59	0.21		0.17	0.40	
BI 10773 3 mg/kg po	3.30	0.44	0.189	-0.29	0.28	0.481
Metformin 300 mg/kg po	4.70	0.75	0.002**	0.18	0.57	0.978
Metformin 300 mg/kg + BI 10773 3 mg/kg	5.51	0.62	<0.001***	-0.11	0.62	0.885

Clinical Chemistry: Plasma AST and ALT were unremarkable in the empagliflozin alone (3/0 mg/kg) group. On day 28 metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) increased plasma AST by 77% and 72%, respectively, and plasma ALT by 57% and 64%, respectively.

Day 28 Plasma AST and ALT (sponsor's table)

Treatment	n	Mean	SEM	% of vehicle	p
<u>Day 28 AST (U/L)</u>					
Vehicle ZDF control 3 ml/kg po	9	46.2	7.2		
BI 10773 3 mg/kg po	8	51.0	9.5	110.3	0.717
Metformin 300 mg/kg po	8	82.0	19.4	177.4	0.038*
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	79.7	17.6	172.4	0.050*
<u>Day 28 ALT (U/L)</u>					
Vehicle ZDF control 3 ml/kg po	9	59.5	6.4		
BI 10773 3 mg/kg po	8	69.0	7.5	115.9	0.468
Metformin 300 mg/kg po	8	93.2	22.3	156.6	0.034*
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	97.6	17.1	163.9	0.019*

At day 14 plasma glucose was significantly reduced 47%, 33% and 54% in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) groups, respectively (see sponsor's table below). Thus showing a slight exacerbation of the plasma glucose reduction with the combination treatment. At day 28 plasma glucose was significantly reduced 46%, 26% and 50% in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) groups, respectively. Again showing a slight exacerbation with the combination treatment that appears to be maintained up to day 28 (see sponsor's table below).

Day 14 Plasma Glucose (sponsor's table)

Treatment	n	Mean	SEM	% of vehicle	p
<u>Day 14 Glucose (mM)</u>					
Vehicle ZDF control 3 ml/kg po	9	21.96	0.77		
BI 10773 3 mg/kg po	8	11.65	0.58	53.1	<0.001***
Metformin 300 mg/kg po	8	14.80	1.59	67.4	<0.001***
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	10.16	0.59	46.3	<0.001***

Day 28 Plasma Glucose (sponsor's table)

Treatment	n	Mean	SEM	% of vehicle	p
<u>Day 28 Glucose (mM)</u>					
Vehicle ZDF control 3 ml/kg po	9	26.11	1.30		
BI 10773 3 mg/kg po	8	14.10	0.68	54.0	<0.001***
Metformin 300 mg/kg po	8	19.40	1.39	74.3	0.009**
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	13.06	0.66	50.0	<0.001***

Plasma non-esterified fatty acids (NEFA) were increased 14% at day 14 in the empagliflozin alone (3/0 mg/kg) group. NEFA were decreased 11% and 28% at days 14 and 28, respectively, in the metformin alone (0/300 mg/kg) group (see sponsor's table below)

Day 14 NEFA (sponsor's table)

Treatment	n	Mean	SEM	Difference from vehicle	p
<u>Day 14 NEFA (µM)</u>					
Vehicle ZDF control 3 ml/kg po	9	1349	57		
BI 10773 3 mg/kg po	8	1533	88	184	0.028*
Metformin 300 mg/kg po	8	1178	62	-172	0.035*
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	1314	60	-35	0.666

Day 28 NEFA (sponsor's table)

Day 28 NEFA (μ M)					
Vehicle ZDF control 3 ml/kg po	8	1331	77		
BI 10773 3 mg/kg po	8	1308	86	-23	0.831
Metformin 300 mg/kg po	8	958	109	-373	<0.001***
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	1277	89	-54	0.608

Plasma triacylglycerol (TAG) was increased 45% at day 14, in the metformin alone (3/0 mg/kg) group (see sponsor's table below). Plasma triacylglycerol (TAG) was increased 61% and 69% at day 28, in the metformin alone (3/0 mg/kg) group and the combination of empagliflozin and metformin (3/300 mg/kg) group, respectively (see sponsor's table below).

Day 14 and 28 Plasma TAG

Treatment	n	Mean	SEM	Difference from vehicle	p
Day 14 TAG (mM)					
Vehicle ZDF control 3 ml/kg po	9	3.912	0.292		
BI 10773 3 mg/kg po	8	3.084	0.309	-0.828	0.151
Metformin 300 mg/kg po	8	5.680	0.649	1.767	0.002**
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	4.746	0.395	0.833	0.155
Day 28 TAG (mM)					
Vehicle ZDF control 3 ml/kg po	8	3.829	0.273		
BI 10773 3 mg/kg po	8	3.292	0.412	-0.537	0.454
Metformin 300 mg/kg po	8	6.228	0.673	2.399	0.001***
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	6.461	0.675	2.632	<0.001***

Plasma lactate was decreased 22% at day 14 in the empagliflozin alone (3/0 mg/kg) group. In contrast, plasma lactate was increased 43% and 30% in the metformin alone (3/0 mg/kg) group and the combination of empagliflozin and metformin (3/300 mg/kg) group, respectively (see sponsor's table below). At day 28, plasma lactate was increased 80% and 60% in the metformin alone (3/0 mg/kg) group and the combination of empagliflozin and metformin (3/300 mg/kg) group, respectively (see sponsor's table below).

Day 14 and 28 Plasma Lactate

Treatment	n	Mean	SEM	Difference from vehicle	p
Day 14 Lactate (mM)					
Vehicle ZDF control 3 ml/kg po	9	5.41	0.37		
BI 10773 3 mg/kg po	8	4.21	0.35	-1.20	0.025*
Metformin 300 mg/kg po	8	7.76	0.24	2.35	<0.001***
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	7.06	0.47	1.65	0.003**
Day 28 Lactate (mM)					
Vehicle ZDF control 3 ml/kg po	8	4.53	0.78		
BI 10773 3 mg/kg po	8	4.31	0.65	-0.23	0.786
Metformin 300 mg/kg po	8	8.48	0.48	3.95	<0.001***
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	7.26	0.52	2.73	0.003**

Day 14 plasma glycerol was unremarkable (data not shown). At day 28, plasma glycerol was increased 80% in the combination of empagliflozin and metformin (3/300 mg/kg) group (see sponsor's table below).

Day 28 Plasma Glycerol (sponsor's table)

Treatment	n	Mean	SEM	% of vehicle	p
Day 28 Glycerol (mM)					
Vehicle ZDF control 3 ml/kg po	8	1.154	0.154		
BI 10773 3 mg/kg po	8	1.048	0.153	90.7	0.655
Metformin 300 mg/kg po	8	1.488	0.278	128.9	0.246
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	2.084	0.369	180.5	0.014*

At day 14, plasma leptin was increased 44% in the combination of empagliflozin and metformin (3/300 mg/kg) group (see sponsor's table below). At day 28 plasma leptin was increased 26%, 45% and 100% in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) groups, respectively (see sponsor's table below). The increase in plasma leptin correlated with the increase in plasma insulin at day 28 and this is expected due to the obese and insulin resistant phenotype of the ZDF rat.

Day 14 and 28 Plasma Leptin (sponsor's table)

Treatment	n	Mean	SEM	% of vehicle	p
Day 14 Leptin (ng/ml)					
Vehicle ZDF control 3 ml/kg po	9	36.3	2.7		
BI 10773 3 mg/kg po	8	43.2	3.2	119.0	0.075
Metformin 300 mg/kg po	8	43.2	3.3	119.0	0.075
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	52.3	2.4	144.4	<0.001***
Day 28 Leptin (ng/ml)					
Vehicle ZDF control 3 ml/kg po	8	25.7	2.2		
BI 10773 3 mg/kg po	8	35.0	3.1	136.0	0.020*
Metformin 300 mg/kg po	8	37.3	2.6	144.7	0.006**
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	51.5	3.4	199.9	<0.001***

Day 14 and day 28 plasma c-reactive protein (CRP), adiponectin and ketone bodies were unremarkable (data not shown).

Plasma Insulin: At day 14 plasma insulin was significantly increased 60% ($p < 0.05$) in the combination of empagliflozin and metformin (3/300 mg/kg) group (see sponsor's table below). At day 28 plasma insulin was significantly increased 65%, 74% and 223% in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) group, respectively. Thus showing a synergistic effect on the plasma insulin increase with the combination treatment (see sponsor's table below).

Day 14 and 28 Plasma Insulin (sponsor's table)

Treatment	n	Mean	SEM	% of vehicle	p
Day 14 Insulin (ng/ml)					
Vehicle ZDF control 3 ml/kg po	9	4.59	0.81		
BI 10773 3 mg/kg po	8	5.29	0.84	115.2	0.501
Metformin 300 mg/kg po	8	6.20	0.82	134.8	0.156
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	7.35	0.60	160.0	0.029*
Day 28 Insulin (ng/ml)					
Vehicle ZDF control 3 ml/kg po	9	3.15	0.52		
BI 10773 3 mg/kg po	8	5.19	1.01	164.8	0.027*
Metformin 300 mg/kg po	8	5.49	1.05	174.2	0.015*
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	10.16	0.71	322.6	<0.001***

Plasma HbA1c: At day 14 plasma HbA1c was significantly reduced 15%, 11% and 26% in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) groups, respectively (see sponsor's table below). Thus showing an exacerbation of the plasma HbA1c reduction with the combination treatment. At day 28 plasma HbA1c was significantly reduced 23%, 15% and 33% in the empagliflozin alone (3/0 mg/kg), metformin alone (0/300 mg/kg) and the combination of empagliflozin and metformin (3/300 mg/kg) groups, respectively, (see sponsor's table below).

Day 14 and 28 Plasma HbA1c

Treatment	n	Mean	SEM	Difference from vehicle	p
Day 14 HbA1c (%)					
Vehicle ZDF control 3 ml/kg po	9	11.34	0.28		
BI 10773 3 mg/kg po	8	9.83	0.28	-1.71	<0.001***
Metformin 300 mg/kg po	8	10.06	0.20	-1.27	0.002**
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	8.42	0.21	-2.92	<0.001***
Day 28 HbA1c (%)					
Vehicle ZDF control 3 ml/kg po	9	14.40	0.32		
BI 10773 3 mg/kg po	8	11.08	0.42	-3.32	<0.001***
Metformin 300 mg/kg po	8	12.27	0.47	-2.12	0.001**
Metformin 300 mg/kg + BI 10773 3 mg/kg	8	9.59	0.10	-4.80	<0.001***

Urinalysis: Urine volume was significantly increased 51% in the empagliflozin alone (3/0 mg/kg) group at day 15 (see sponsor's table below). The diuresis is an expected pharmacodynamics response of empagliflozin. However, urine volume was significantly decreased 46% and 43% at day 15 and 29, respectively, in the metformin alone (0/300 mg/kg) group (see sponsor's table below).

Day 15 and 29 Urine Volume (sponsor's table)

Group	n	Day 15				Day 29				
		Mean	SEM	Difference from vehicle	p	n	Mean	SEM	Difference from vehicle	p
Vehicle ZDF control (3 ml/kg po)	9	5.2	0.8			9	9.9	1.1		
BI 10773 (3 mg/kg po)	8	7.9	1.2	2.7	0.012*	8	11.3	1.9	1.4	0.35
Metformin (300 mg/kg po)	8	2.8	0.4	-2.4	0.022*	8	5.6	0.7	-4.3	0.006**
Metformin (300 mg/kg po) plus BI 10773 (3 mg/kg po)	8	5.4	0.5	0.2	0.863	8	7.2	0.6	-2.7	0.076

Urine glucose excretion (UGE) was significantly increased 127% in the empagliflozin alone (3/0 mg/kg) group at day 15 (see sponsor's table below). The UGE is an expected pharmacological response of empagliflozin. However, UGE was significantly decreased 62% and 44% at day 15 and 29, respectively, in the metformin alone (0/300 mg/kg) group (see sponsor's table below).

Day 15 and 29 UGE

Group	n	Day 15				Day 29				
		Mean	SEM	Difference from vehicle	p	n	Mean	SEM	Difference from vehicle	p
Vehicle ZDF control (3 ml/kg po)	9	204.6	90.8			9	636.9	42.2		
BI 10773 (3 mg/kg po)	8	465.3	43.9	227.5	0.038*	8	673.7	55.4	105.8	0.812
Metformin (300 mg/kg po)	8	77.1	56.7	37.7	0.015*	8	357.6	179.6	56.1	0.018*
Metformin (300 mg/kg po) plus BI 10773 (3 mg/kg po)	8	290.6	34.6	142.1	0.364	8	426.7	57.5	67.0	0.094

Summary: Mean BW and BW gain was increased 16-64% in the combination of empagliflozin and metformin (3/300 mg/kg) group. Following the OGTT, the plasma glucose AUC was significantly reduced 34-45% with metformin alone and the combination of empagliflozin and metformin (3/300 mg/kg) group and the plasma insulin AUC was increased 81-113% in the same groups. Plasma glucose was significantly reduced in all treatment groups with a slight exacerbation of treatment with the combination of empagliflozin and metformin (3/300 mg/kg) group. Plasma insulin was significantly increased in all treatment groups with a significant exacerbation (223%) of treatment with the combination of

empagliflozin and metformin (3/300 mg/kg) group. This in turn reduced the plasma HbA1C in all treatment groups. These results demonstrate that empagliflozin and metformin in combination was effective in the management of some diabetes parameters in the male ZDF rat.

6 General Toxicology

Pivotal toxicology studies were designed to identify unexpected interactions from empagliflozin and metformin combination (co-administration) treatment that may exacerbate toxicity. Nonclinical toxicity of empagliflozin and metformin monotherapy were previously reviewed and included in the labels of the listed drugs.

6.1 Single-Dose Toxicity

No single dose studies were submitted

6.2 Repeat-Dose Toxicity

BI 10773 ^{(b) (4)} and Metformin: 2-Week Oral (Gavage) Combination Toxicity and Toxicokinetics Study in the Wistar Han IGS Rat

GLP study, signed 01/18/2012 (Study# 10r021, U12-3045-01)

Doses: 0, 100/200, 300/600, 500/1000 mg/kg empagliflozin/metformin
500/0 mg/kg empagliflozin alone
0/1000 mg/kg metformin alone
n = 10/sex/group (main) and *n* = 9/sex/group TK

Exposure: 0, 61900^μ/429,000^μ; 242,000^μ/1,240,000^μ; not done^τ/1,110,000^μ
(day 14) nM*h empagliflozin/metformin
329,000^μ/0 nM*h (day 14) empagliflozin alone
0/1,580,000^μ (day 14) metformin alone
[^μ male and female mean exposure],
[^τ not done due to excess mortality in the group]

NOAEL 100/200 empagliflozin + metformin (13x empagliflozin/ 3x metformin MRHD)

Key Study Findings:

- Fatalities were seen in males treated with 500/1000 mg/kg empagliflozin + metformin, with one male found dead on day 9 and another on day 11. The remainder of the empagliflozin + metformin group (including all females), were sacrificed as moribund on days 8-11.
- In the 500/1000 mg/kg empagliflozin + metformin group, mean body weight failed to increase at day 3, 7 or 10 in males and was minimally increased 4% in the females at day 10. This correlated with reduced food consumption (28%) at day 3 in the same male and female groups. Mean body weight was reduced 10% at day 14 in the 300/600 mg/kg empagliflozin + metformin group males.
- In males absolute lymphocytes were reduced 26-28% in the 300/600 mg/kg and 500/0 empagliflozin + metformin groups which correlated with lymphocyte depletion in the spleen, thymus and lymph nodes.
- Diminished body fat of similar incidence was found in the 300/600 mg/kg and 500/1000 mg/kg empagliflozin + metformin groups. This likely due to emaciation.
- Hepatocellular rarefaction with glycogen accumulation was observed in the 300/600 mg/kg and 500/1000 mg/kg empagliflozin + metformin groups. Periportal hepatocellular hypertrophy was also noted in the 500/1000 mg/kg and 500/0 empagliflozin + metformin groups.

- Kidney cortical tubular degeneration was observed in the 500/1000 mg/kg. empagliflozin + metformin group.
- Increased incidence of mineralization of the pelvic calices (calculi) was observed in the 300/600 mg/kg and 500/1000 mg/kg empagliflozin + metformin groups, that was also associated with hydronephrosis in most of the same animals.
- The NOAEL is 100/200 mg/kg (13x/3x MRHD) due to the kidney lesions, emaciation and fatalities at \geq 300/600 mg/kg.

Metformin: 2-Week Oral (Gavage) Toxicity Study in Rats

Study no.: 09B040, U09-2246-01
Study report location: EDR
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co. KG
Birkendorfer Str. 65
88397 Biberach an der Riss
Germany
Date of study initiation: February 19th 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Metformin hydrochloride (free base),
801941 and 100.1%

Key Study Findings

- Increased heart weight, heart to body weight and heart to brain weight (20-30%) with correlative minimal ventricular myocardium hypertrophy and muscle fiber degeneration was noted in both sexes at 1000 mg/kg and is considered adverse.
- Minimal to slight hyperplasia of pituitary gland (par distalis) with correlative increases in organ weight/organ to body/brain weight ratios (14-30%) was observed in the 1000 mg/kg females.
- Minimal to slight reduction of the thymus cortical area with correlative decreases in WBC (19%), in thymus weight and thymus organ to body/brain weight ratios (16-22%) was noted in the 1000 mg/kg animals.
- Minimal to slight adrenal vacuolation with correlative increases in adrenal weight and adrenal to body/brain weight ratios (15-24%) was observed in the 1000 mg/kg males.

- Minimal to slight depletion of pancreatic zymogen granules, atrophy of the seminal vesicles (males only) and parotid salivary gland infiltration/inflammation was noted in the 1000 mg/kg animals.
- Red cell mass was reduced in the 1000 mg/kg animals and modest changes in clinical chemistry were observed in the high dose animals.
- The NOAEL for metformin in this study is 200 mg/kg which is equivalent to 2x MRHD (2000 mg metformin).

Methods

Doses:	0, 100, 200 and 1000 mg/kg
Frequency of dosing:	Daily (14 days)
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% aqueous hydroxyethylcellulose (Natrosol® 250HX)
Species/Strain:	Wistar (Han): Crl:WI(Han)
Number/Sex/Group:	10/sex/group (main) and 3/sex/group (TK)
Age:	7-8 weeks old
Weight:	M: 194.3 to 231.2g and F: 143.8 to 176.2g
Satellite groups:	3/sex/group (TK)
Unique study design:	None
Deviation from study protocol:	None that affected the study outcome.

Observations and Results

Mortality

None.

Clinical Signs

Clinical signs in general were unremarkable, with the exception of a soiled anogenital region that was observed from day 4 to day 15 in all 1000 mg/kg-treated males (results not shown).

Body Weights

Absolute mean body weight was significantly reduced 4% at days 11 and 14, respectively, in the 200 mg/kg females. At days 4, 8, 11 and 14, body weight gain was significantly reduced 4-5% in the 1000 mg/kg males (see sponsor's tables below). The reduced absolute body weight and body weight gain did not correlate with reduced food consumption.

Table 6. Metformin 2 Week Rat Absolute Mean Body Weight - Females (sponsor's table)

Body wt. [g]		female					
[day]		-7	1	4	8	11	14
Group							
Group 1 0	n	10	10	10	10	10	10
	mv	145.47	164.08	163.57	174.66	184.09	189.93
	[mg/kg] sd	3.41	6.57	8.16	9.44	7.74	7.63
Group 2 100	n	10	10	10	10	10	10
	mv	144.34	164.58	164.71	175.55	180.59	188.20
	[mg/kg] sd	4.51	4.31	3.10	4.91	6.86	5.42
	p	0.5792	0.8691	0.6992	0.7890	0.2858	0.6113
Group 3 200	n	10	10	10	10	10	10
	mv	144.05	160.31	160.57	170.21	177.26	182.89
	[mg/kg] sd	4.19	8.18	5.42	6.02	5.75	7.82
	p	0.4864	0.2190	0.3122	0.1860	* 0.0415	* 0.0441
Group 4 1000	n	10	10	10	10	10	10
	mv	145.15	165.00	160.49	171.11	179.74	186.98
	[mg/kg] sd	5.66	7.28	8.11	8.27	8.30	8.89
	p	0.8750	0.7619	0.2996	0.2892	0.1866	0.3878

Table 7. Metformin 2 Week Rat Body Weight Gain – Males (sponsor's table)

Body wt. [%]		male					
[day]		-7	1	4	8	11	14
Group							
Group 1 0	n	10	10	10	10	10	10
	mv	83.71	100.00	100.36	110.92	116.78	122.75
	[mg/kg] sd	1.80	0.00	2.03	1.94	2.74	3.12
Group 2 100	n	10	10	10	10	10	10
	mv	82.93	100.00	100.35	110.25	117.58	123.33
	[mg/kg] sd	2.91	0.00	1.49	1.99	2.84	3.95
	p	0.4194	1.0000	0.9948	0.4609	0.4943	0.7047
Group 3 200	n	10	10	10	10	10	10
	mv	83.22	100.00	100.62	111.61	117.33	123.89
	[mg/kg] sd	2.04	0.00	1.59	1.96	2.14	3.86
	p	0.6091	1.0000	0.7284	0.4502	0.6386	0.4618
Group 4 1000	n	10	10	10	10	10	10
	mv	82.63	100.00	95.56	106.28	111.64	117.10
	[mg/kg] sd	1.49	0.00	1.68	2.15	2.57	2.53
	p	0.2655	1.0000	* < 0.0001	* < 0.0001	* < 0.0001	* 0.0007

Feed and Water Consumption

Food consumption was unremarkable. Water consumption was increased in the 1000 mg/kg females (11-17%) and males (23-30%) at day 11 and 14, respectively (results not shown). The toxicological significance of the increased water consumption is unknown.

Ophthalmoscopy

The sponsor states that there were no adverse ophthalmology findings. However, animal ophthalmology data and/or study report was not provided/submitted by the sponsor.

ECG

Not Applicable.

Hematology

Hemoglobin (HGB), red blood cells (RBC) and hematocrit (HCT) were significantly reduced in the 1000 mg/kg males (4-5%) and females (3-6%), respectively (see sponsor's tables below) but were without a histopathology correlate. MCHC was also minimally reduced in the 1000 mg/kg females. White blood cells (WBC) were also dose-dependently decreased and significantly decreased 19% in the 1000 mg/kg females and this correlated histopathologically with reduced thymic cortical areas and reduced organ weight. Although available, bone marrow smears were not assessed in this study. Prothrombin time (PT) was also significantly increased 5% in the 1000 mg/kg males and females, respectively.

Table 8. Metformin 2 Week Rat Hematology – Males Day 15 (sponsor's table)

male		15 [day]					
Parameter		HGB	RBC	HCT	PLT	PT	
		[g/dL]	[10 ⁶ /uL]	[vol%]	[10 ³ /uL]	[s]	
Group 1	n	10	10	10	n	10	10
0	mv	15.28	8.569	45.37	mv	1065.6	16.26
[mg/kg]	sd	0.56	0.274	1.52	sd	89.6	0.50
Group 2	n	10	10	10	n	10	10
100	mv	15.44	8.488	45.84	mv	994.0	16.15
[mg/kg]	sd	0.42	0.295	1.06	sd	146.8	0.46
	p	0.4655	0.4576	0.4343	p	0.1463	0.6537
Group 3	n	10	10	10	n	10	10
200	mv	15.41	8.415	45.25	mv	998.2	16.36
[mg/kg]	sd	0.41	0.132	1.37	sd	97.9	0.48
	p	0.5527	0.1622	0.8411	p	0.1707	0.6833
Group 4	n	8	8	8	n	8	10
1000	mv	14.60	8.154	43.74	mv	962.4	17.01
[mg/kg]	sd	0.55	0.227	1.32	sd	77.3	0.70
	p	* 0.0056	* 0.0009	* 0.0140	p	0.0512	* 0.0039

Table 9. Metformin 2 Week Rat Hematology – Females Day 15 (sponsor's table)

female		16 [day]						
Parameter		HGB	RBC	HCT	MCHC	PLT	PT	WBC
		[g/dL]	[10 ⁶ /uL]	[vol%]	[g/dL]	[10 ³ /uL]	[s]	[10 ³ /uL]
Group								
Group 1	n	10	10	10	10	n	10	10
0	mv	14.34	7.998	42.27	33.93	mv	1060.1	16.23
[mg/kg]	sd	0.69	0.299	1.84	0.55	sd	131.9	0.67
								1.31
Group 2	n	10	10	10	10	n	10	10
100	mv	14.72	8.164	43.24	34.05	mv	1082.7	16.68
[mg/kg]	sd	0.49	0.271	1.15	0.67	sd	89.0	0.65
	p	0.0973	0.2288	0.1088	0.6678	p	0.6505	0.1252
								0.0760
Group 3	n	10	10	10	10	n	10	10
200	mv	14.63	8.096	43.23	33.84	mv	1059.2	16.10
[mg/kg]	sd	0.42	0.379	1.15	0.83	sd	132.0	0.61
	p	0.2022	0.4745	0.1123	0.7474	p	0.9856	0.6527
								0.0670
Group 4	n	10	10	10	10	n	10	9
1000	mv	13.61	7.555	40.88	33.32	mv	1000.5	16.97
[mg/kg]	sd	0.31	0.246	0.96	0.32	sd	78.6	0.63
	p	* 0.0024	* 0.0024	* 0.0240	* 0.0344	p	0.2361	* 0.0171
								* 0.0359

Clinical Chemistry

Liver transaminases (ALT and AST) and alkaline phosphatase were in general decreased in the treated animals at day 3 or at the end of treatment in both metformin-treated males and females (see sponsor's table below). ALP was also decreased in the 1000 mg/kg males and females at the end of treatment. There were no histopathology correlates and the significance of the decrease in these liver function tests is unknown (see sponsor's table below).

Table 10. Metformin 2 week Rat Clinical Chemistry (sponsor's table)

Parameter [unit]	Day	Group	Daily dose of metformin BS [mg/kg]							
			0		100		200		1000	
			1	2	3	4	mean	Δ%	mean	Δ%
ALT [U/L]	3	M	30.4	27.6	-9.1	32.3	6.3	27.2	-10.4	
	3	F	24.6	24.6	0.2	↓21.6	-12.1	↓14.4	-41.3	
	15	M	40.7	↓35.5	-12.8	↓34.9	-14.3	↓28.3	-30.5	
	16	F	24.4	23.4	-4.1	23.5	-3.7	↓19.6	-19.7	
AST [U/L]	3	M	63.4	↓43.8	-31.0	70.1	10.5	49.4	-22.1	
	3	F	70.6	↑87.3	23.7	68.9	-2.4	↓60.1	-14.9	
	15	M	73.6	68.5	-6.9	↓59.4	-19.2	72.3	-1.8	
	16	F	63.2	63.6	0.7	58.4	-7.6	↓56.1	-11.3	
ALP [U/L]	3	M	154	181	17.5	169	10.2	168	9.2	
	3	F	105	112	6.8	91.5	-13.1	↓79.2	-24.8	
	15	M	143	170	18.5	125	-12.7	↓109	-24.0	
	16	F	68.7	71.8	4.5	68.1	-0.8	↓53.8	-21.7	
GLDH [U/L]	3	M	5.99	5.41	-9.7	7.22	20.5	6.16	2.8	
	3	F	6.57	6.86	4.4	5.92	-9.9	↓4.86	-26.0	
	15	M	5.62	5.94	5.7	5.04	-10.3	↓4.78	-15.0	
	16	F	5.30	7.52	41.9	4.91	-7.4	3.31	-37.6	

M: males; F: females

Δ% percent deviation from Control (calculated from original raw data/rounded)

↑ statistically significant increase compared with Control; p<=0.05, many to one t-test, two sided

↓ statistically significant decrease compared with Control; p<=0.05, many to one t-test, two sided

Urinalysis

At day 9 post-dose, specific gravity was increased in the 1000 mg/kg males (2%) and females (1.6%) (data not shown).

Gross Pathology

Four 1000 mg/kg males were found with a soiled perianal region at necropsy. Liquid feces were found in the cecum in two 100 mg/kg females, one 200 mg/kg male and one animal of each gender at 1000 mg/kg.

Organ Weights

Absolute heart weight, heart to body weight and heart to brain weight ratios were significantly increased 21%, 30% and 22% in the 1000 mg/kg males and 20%, 24% and 20% in the 1000 mg/kg females, respectively, (see sponsor's table below). The increased heart weight correlated with a minimal hypertrophy of the myocardium in the 1000 mg/kg metformin treated animals.

Absolute liver weight was dose-dependently and significantly increased (males only) in the 200 mg/kg males (7%) and the 1000 mg/kg males (17%) and also in the 1000 mg/kg females (20%). Liver to body weight ratios were dose dependently significantly

increased in the 200 mg/kg (6-7%) and 1000 mg/kg (24-26%) animals of both genders (see sponsor's table below). This was without a histopathology correlate.

Absolute adrenal weight, adrenal to body weight ratio and the adrenal to brain weight ratio were significantly increased 15%, 24% and 16% in the 1000 mg/kg males (see sponsor's table below). This correlated histopathologically with minimal to slight cytoplasmic vacuolation in zona fasciculata.

Table 11. Metformin 2 Week Rat – Organ Weights (sponsor's table)

Parameter	Group	1	2	3	4
	Daily dose of Metformin BS [mg/kg]	0	100	200	1000
End of treatment period					
Males	Body weight [g]	233.44	234.06	235.31	217.90↓
Heart (n=10)	absolute [g]	0.8112	0.8303	0.8644	0.9850↑
	relative to BW §	0.3476	0.3545	0.3678	0.4514↑
	relative to BrW &	42.78	43.54	45.28	52.14↑
Liver (n=10)	absolute [g]	6.4524	6.5522	6.9257↑	7.5674↑
	relative to BW §	2.762	2.797	2.945↑	3.476↑
	relative to BrW &	340.6	343.6	362.7	400.2↑
Adrenals (n=10)	absolute [mg]	56.94	55.26	60.70	65.75↑
	relative to BW §	0.02446	0.02363	0.02585	0.03022↑
	relative to BrW &	3.003	2.899	3.183	3.479↑
Thymus (n=10)	absolute [g]	0.4167	0.4094	0.4192	0.3266↓
	relative to BW §	0.1778	0.1730	0.1776	0.1495
	relative to BrW &	21.94	21.50	21.86	17.21↓
Thyroid (n=10)	absolute [mg]	16.59	15.95	16.18	11.84↓
	relative to BW §	0.00705	0.00683	0.00688	0.00543↓
	relative to BrW &	0.871	0.836	0.846	0.625↓
Females	Body weight [g]	171.95	170.83	166.66	166.54
Heart (n=10)	absolute [g]	0.6509	0.6785	0.6579	0.7802↑
	relative to BW	0.3790	0.3970	0.3950	0.4691↑
	relative to BrW	36.18	37.40	36.79	43.33↑
Liver (n=10)	absolute [g]	4.9224	5.0555	5.0551	5.9110↑
	relative to BW §	2.862	2.960	3.035↑	3.551↑
	relative to BrW &	273.5	278.7	282.9	328.3↑
Thymus (n=10)	absolute [g]	0.4206	0.3973	0.4036	0.3507↓
	relative to BW	0.2434	0.2322	0.2415	0.2113
	relative to BrW	23.30	21.87	22.61	19.51↓
Pituitary (n=10)	absolute [mg]	9.92	10.38	10.83	11.32↑
	relative to BW	0.00578	0.00609	0.00649↑	0.00679↑
	relative to BrW	0.5514	0.5704	0.6053	0.6299↑

§, &: BW = body weight; BrW = brain weight

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

Absolute thymus weight and the thymus to brain weight ratio were significantly reduced in the 1000 mg/kg males (22%) and females (16-17%) (see sponsor's table above). This

correlated histopathologically with a minimal to slight reduction of the thymic cortical areas and a reduction of WBC (but not lymphocytes), in the 1000 mg/kg females.

Absolute thyroid weight and thyroid to body and brain weight ratios were significantly reduced (23% - 28%) in the 1000 mg/kg males (see sponsor's table above). These changes were without histopathology correlates.

Absolute pituitary and pituitary to brain weight ratio were dose-dependently and significantly increased 14% in the 1000 mg/kg females. Pituitary to body weight ratios were also dose-dependently and significantly increased in the 200 mg/kg (12%) and the 1000 mg/kg females (30%) (see sponsor's table above). This correlated histopathology with minimal to slight pituitary hyperplasia in the 1000 mg/kg females.

Histopathology

Adequate Battery Yes

Peer Review Yes

Histological Findings

Three males and five females at 1000 mg/kg showed a minimal concentric hypertrophy of the ventricular myocardium which was consistent with the increased absolute and organ to body weight/brain weight ratios. Additionally, minimal degeneration of single heart muscle fibers (in some cases with resorptive inflammation) was present in three male and one female at 1000 mg/kg, one of each gender at 200 mg/kg, but also in one control male (see sponsor's table below).

Table 12. Rat Two Week Metformin – Heart Histopathology (sponsor's table)

Group	1		2		3		4	
Group name	Group 1		Group 2		Group 3		Group 4	
Dose level [mg/kg]	0		100		200		1000	
	m	f	m	f	m	f	m	f
Animals in group :	10	10	10	10	10	10	10	10
Animals examined :	10	10	10	10	10	10	10	10
<u>heart</u>	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
-degeneration	1	0	0	0	1	1	3	1
-hypertrophy	0	0	0	0	0	0	3	5
-infiltration	0	3	0	2	0	0	1	0

Minimal to slight adrenal cytoplasmic vacuolation was observed predominantly in males with 7, 4 and 8 occurrences noted in the 100, 200 and 1000 mg/kg groups but also in 3 control males (see sponsor's table below). This correlated with the increased adrenal

weight and adrenal to body weight/brain weight ratios observed in the 1000 mg/kg males.

Table 13. Rat Two Week Metformin – Adrenal Histopathology (sponsor’s table)

Group	1		2		3		4	
Group name	Group 1		Group 2		Group 3		Group 4	
Dose level [mg/kg]	0		100		200		1000	
	m	f	m	f	m	f	m	f
Animals in group :	10	10	10	10	10	10	10	10
Animals examined :	10	10	10	10	10	10	10	10
adrenal gland	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
-accessory cortical nodule	0	1	0	0	0	0	0	0
-vacuolation, cytoplasmic	3	0	7	1	4	0	8	2

Minimal to slight hyperplasia of the pituitary gland (pars distalis) was noted in four 1000 mg/kg females and correlated with the increased absolute pituitary and body/brain weight ratios in the 1000 mg/kg females (see sponsor’s table below).

Table 14. Rat Two Week Metformin – Pituitary Histopathology (sponsor’s table)

Group	1		2		3		4	
Group name	Group 1		Group 2		Group 3		Group 4	
Dose level [mg/kg]	0		100		200		1000	
	m	f	m	f	m	f	m	f
Animals in group :	10	10	10	10	10	10	10	10
Animals examined :	10	10	10	10	10	10	10	10
pituitary gland	(10)	(9)	(10)	(10)	(10)	(10)	(9)	(10)
-cyst(s)	1	0	0	0	0	0	0	0
-hyperpl., pars distalis (H)	0	0	0	0	0	0	0	4
(not examined)	0	1	0	0	0	0	1	0

Minimal to slight reduction in thymus cortical area was observed in seven males and eight females at 1000 mg/kg, one of each gender at 200 mg/kg and three males and one female at 100 mg/kg (see sponsor’s table below). For the 1000 mg/kg animals this was correlated with a decrease in absolute and thymus to brain weight ratios. Thymic atrophy also correlated with a general reduction of WBC in the 1000 mg/kg females (but not lymphocytes) and was not related to reduced food consumption.

Minimal to slight depletion of pancreatic zymogen granules was noted in two male and four 1000 mg/kg animals (see sponsor’s table below). This is suggestive of malnutrition but was without corroborative reduced food consumption. In addition, minimal to slight infiltration and/or inflammation of the parotid salivary gland was found in 5 male and 6 female 1000 mg/kg animals (see sponsor’s table below).

Minimal to slight atrophy of the seminal vesicles was observed in 3 males at 1000 mg/kg and this together with the reduction in thymic weight and absolute body weight is likely to be a stress related response.

Table 15. Rat Two Week Metformin – Histopathology (sponsor’s table)

Group	1		2		3		4	
Group name	Group 1		Group 2		Group 3		Group 4	
Dose level [mg/kg]	0		100		200		1000	
	m	f	m	f	m	f	m	f
Animals in group :	10	10	10	10	10	10	10	10
Animals examined :	10	10	10	10	10	10	10	10
thymus	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
-apoptosis	8	8	8	3	6	6	7	9
-hemorrhage	2	0	1	1	0	0	3	0
-hyperpl., epith.tub.&cords (H)	0	0	0	0	1	0	0	0
-reduced in size	0	0	3	1	1	1	7	8
pancreas	(10)	(10)	(0)	(0)	(0)	(0)	(10)	(10)
-apoptosis	0	0	-	-	-	-	0	1
-atrophy, lobular	0	1	-	-	-	-	0	0
-depletion	0	0	-	-	-	-	2	4
parotid salivary gland	(10)	(10)	(0)	(0)	(0)	(0)	(10)	(10)
-atrophy	0	0	-	-	-	-	1	1
-degeneration	0	0	-	-	-	-	1	0
-infiltration	0	1	-	-	-	-	3	1
-infiltration, inflammatory	0	0	-	-	-	-	1	5
-inflammation	0	0	-	-	-	-	1	0
-mineralisation	0	0	-	-	-	-	2	1
-vacuolation, cytoplasmic	2	0	-	-	-	-	1	0
seminal vesicle	(10)	(0)	(0)	(0)	(0)	(0)	(10)	(0)
-atrophy	0	-	-	-	-	-	3	-

Special Evaluation

NA

Toxicokinetics

At day 14 the AUC 0-24h increased dose proportionately from 100 to 200 mg/kg and was greater than dose proportional from 200 to 1000 mg/kg (see sponsor’s table below). There were no gender differences for the exposure.

Table 16. Rat 2 Week Metformin Toxicokinetics (sponsor's table)

Parameter	Day	Gender	100 mg/kg	200 mg/kg	1000 mg/kg
C(max) [nmol/L]	1	m	40,800	79,200	129,000
	1	f	43,500	72,000	157,000
	14	m	40,500	66,800	203,000
	14	f	35,900	73,400	204,000
AUC(0-24h) [nmol-h/L]	1	m	218,000	377,000	1,920,000
	1	f	188,000	345,000	2,080,000
	14	m	196,000	387,000	2,710,000
	14	f	159,000	360,000	2,880,000

n= 3 per group per gender
m, f = males, females

Dosing Solution Analysis

Metformin was within the acceptable range of the nominal concentrations.

BI 10773^{(b)(4)} and Metformin: A 90-Day Oral (Gavage) Toxicity Study in Rats With a 1-Month Recovery Period

Study no.: 10r045, U11-3632-01
 Study report location: eCTD
 Conducting laboratory and location: (b)(4)
 Date of study initiation: June 16 2010
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BI 10773^{(b)(4)} (empagliflozin),
 BI10773^{(b)(4)} and 99.6 -100.1
 Metformin, 80194 and 100.1%

Key Study Findings

- Mortality in one high dose empagliflozin+metformin (200/400 mg/kg) recovery female with unknown cause.
- Reduced absolute body weight (10-13%) and body weight gain (17-19%) in the empagliflozin only-treated (200/0 mg/kg) males that was irreversible in recovery animals.
- Exacerbation of reduced body weight (7-15%) and body weight gain (26-36%) in the high dose empagliflozin and metformin (200/400 mg/kg) males that was irreversible in recovery animals.

- Empagliflozin alone, metformin alone or empagliflozin in combination with metformin resulted in dose-dependent hypoglycemia in both males and females.
- Empagliflozin alone or in combination with metformin resulted in dose-dependent hypochloremia in both males and females and elevations in transaminases and alkaline phosphatase.
- Empagliflozin alone or in combination with metformin resulted in glucosuria and aciduria.
- Adrenal weight was increased in all treated males.
- Liver and kidney organ weights, organ to body weight/brain weight ratios were increased in all treatment groups, but exacerbated in the mid and high dose empagliflozin and metformin co-administration groups.
- Kidney pelvic mineralization was noted in the high dose empagliflozin and metformin co-administration group (mostly females).
- The NOAEL is 50/100 mg/kg based on the findings of hypochloremia and pronounced reduced mean body weight/gain at higher doses. Empagliflozin/metformin at 50/100 mg/kg is equivalent to 4-6x MRHD in both males and females for empagliflozin and 2x MRHD in both males and females for metformin.

Methods

Doses:	Empagliflozin (e)/Metformin (m): 0/0, 200/0, 0/400, 50/100, 100/200 and 200/400 mg/kg
Frequency of dosing:	Daily
Route of administration:	PO (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Rat/Crl:WI(Han)
Number/Sex/Group:	25/sex/group (e+m): 0/0, 200/0, 0/400 and 200/400 mg/kg 15/sex/group (e+m): 50/100 and 100/200 mg/kg
Age:	Approx. 7 weeks old
Weight:	M: 221-295g; F: 170-200g
Satellite groups:	3/sex/control TK and 9/sex/group TK
Unique study design:	None
Deviation from study protocol:	None that affected the study outcome

Observations and Results

Mortality

One 200/400 (empagliflozin (e)/metformin (m)) mg/kg recovery female was found dead at recovery day 30. This female was without clinical signs and the cause of death was not determined.

Clinical Signs

Ano-genital staining was observed in 1-3 animals in the 0/400 (e+m) mg/kg males and females from weeks 2-13 for the males and weeks 7-13 for the females. Ano-genital staining was also observed in one male during week 1 in the 200/400 (e+m) mg/kg group (see sponsor’s table below). Unformed stool and watery stool was observed in 10 and 8 males and 5 and 3 females, respectively, in the 200/400 (e+m) mg/kg during week one of treatment (see sponsor’s tables below). The GI effects were reversed in recovery animals.

GI effects are known to occur with metformin and can also occur due to off-target SGLT1 inhibition for SGLT2 inhibitors. Due to the short duration of occurrence, these results suggest that the GI effects became tolerable.

Table 17. Male Clinical Signs (sponsor’s table)

Males

Observations	Phase		Week													
	PRE	RND	DOS	1	2	3	4	5	6	7	8	9	10	11	12	13
Dermal General, Ano-Genital Staining	3						2	2	2	2	2	2	2	2	1	1
Dermal General, Stains on Fur (Head/Neck)	6		1													
Dermal General, Stains on Fur (Limbs)	1			1	1	1	1	1	1	1	1	1	1	1		
Gastrointestinal, Unformed Stool	6		10													
Gastrointestinal, Watery Stool	6		8													
Ocular, Lacrimation (Unilateral)	1								1	1						
Oral/Buccal, Incisor(s) Broken/Missing	2														1	

Table 18. Female Clinical Signs (sponsor's table)

Females

Observations	Phase	DOS															
		Day 2	5	5	6	6	6	7	8	9	10	10	11	11	45	54	
Group	Session	PM	AM	PM	AM	PDO	PM	AM	AM	AM	AM	PM	AM	PM	US	AM	
Daily Observations, Decreased Fecal Volume	2																1
Daily Observations, Red Exudate Source Unknown	2																1
Daily Observations, Red Nasal Discharge	2																1
Daily Observations, Unformed Stool	6			1	1	1		1	1								
Gastrointestinal, Unformed Stool	6																5
Gastrointestinal, Watery Stool	6																3

Body Weights

Mean absolute body weight was significantly reduced 5% relative to the vehicle control from weeks 10-13 in the e+m 200/0 mg/kg-treated males. Mean body weight remained significantly reduced 8% in these males throughout the recovery period, thus showing irreversibility (see sponsor's figure below). Body weight gain was also significantly reduced 9-15% from weeks 7-13 of the dosing phase in the 200/0 mg/kg treated males (see table below). Body weight gain was also significantly reduced 17-19% in the 200/0 mg/kg recovery males, thus showing irreversibility (see sponsor's figure below).

Treatment with metformin alone (0/400 mg/kg) had no effect on absolute body weight in both the males and females. Body weight gain was significantly reduced 11% in week 2 of recovery in the 0/400 mg/kg males. Body weight gain was also significantly reduced 8-10% in weeks 7-13 in the 0/400 mg/kg metformin-treated females; but was unaffected in the recovery females (see tables below).

Treatment with low and mid dose of e+m at 50/100 mg/kg or 100/200 mg/kg did not result in significantly reduced absolute body weight or body weight gain when compared to the vehicle control (0/0 mg/kg) animals (see tables below).

The co-administration of empagliflozin and metformin at 200/400 mg/kg exacerbated the reduced mean body weight in males when compared to the mean body weight reduction with empagliflozin alone at 200/0 mg/kg. During the dose administration, mean body weight was significantly reduced 7-15% in males with e+m at 200/400 mg/kg with the maximum reduction occurring at week 13. Mean body weight remained significantly reduced 12-16% in the e+m 200/400 mg/kg recovery males, but showed a trend of recovery as the duration of the recovery progressed (see sponsor's figure below). In contrast, mean body weight was only significantly reduced 4% at weeks 11 and 13 in the 200/400 mg/kg females.

Body weight gain was significantly reduced 81% at week one and reduced 26-42% from weeks 2-13 in the 200/400 mg/kg males. Body weight gain remained reduced 26-36% in the 200/400 mg/kg males during the recovery period, thus showing irreversibility (see table below). Similarly, body weight gain was significantly reduced 49% at week one and reduced 11-19% at weeks 3, 5, 7 and 10-13 in the 200/400 mg/kg females (see table below). Body weight gain remained reduced 14-17% in the 200/400 mg/kg females during the recovery period (see sponsor's figures below).

The reduced body weight/body weight gain did not correlate with reduced food consumption.

Figure 3. Male Body Weight (sponsor's figure)

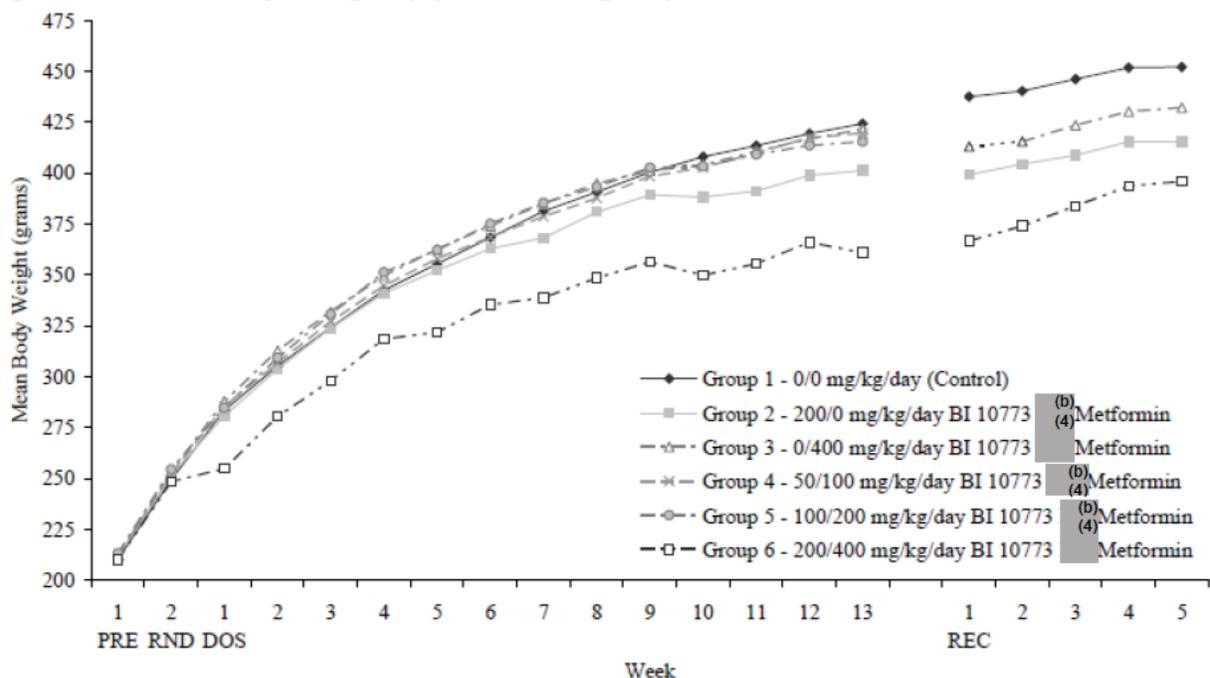
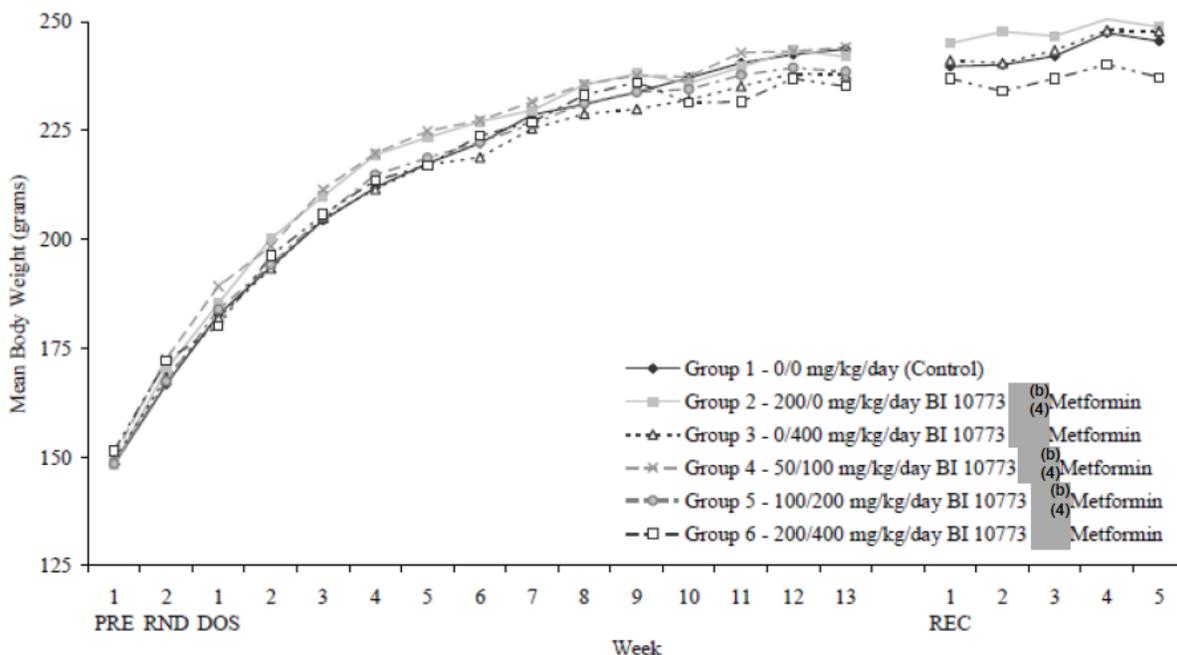


Figure 4. Female Body Weight (sponsor's figure)



MALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrease in BW	BW % control
Dosing Phase Week 1 – 13	0/0	175	-	-
	200/0	149	↓15	86
	0/400	168	↓4	96
	50/100	167	↓5	96
	100/200	161	↓7	92
	200/400	113	↓35	64
Recovery Phase Week 1 – 5	0	14.5	-	-
	200/0	16.1	↓8	92
	0/400	19.1	↓4	96
	200/400	29.2	↓12	88

FEMALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Change in BW	BW % control
Dosing Phase Week 1 - 13	0/0	77	-	-
	200/0	72	↓1	99
	0/400	69	↓2	98
	50/100	72	0	100
	100/200	71	↓2	98
	200/400	63	↓4	96
Recovery Phase Week 1 - 5	0	5.8	-	-
	200/0	4	↑1	101
	0/400	7	↑1	100
	200/125	0.3	↓3	97

Feed Consumption

Food consumption (FC) was increased in all male and female treatment groups during the dosing phase of the study (see sponsor's figures and table below). FC was exacerbated in e+m treated animals (50/100, 100/200 or 200/400 mg/kg) compared to metformin alone (0/400 mg/kg) animals) (see sponsor's table and figures below). FC for the co-administration of high dose e+m (200/400 mg/kg) was in the same range (males) or increased (females) relative to the low (50/100 mg/kg) or mid dose (100/200 mg/kg) e+m combination (see sponsor's table below). During recovery week one, FC continued to increase in the empagliflozin alone (200/0 mg/kg) and the high dose e+m combination (200/400 mg/kg) treated animals. FC thereafter appeared to normalize to control animal FC (see sponsor's figures below).

The increased FC did not correlate with an increased body weight. Increased FC is an expected pharmacodynamics outcome observed with treatment with empagliflozin and SGLTs inhibitors, in general.

Figure 5. Food Consumption – Males (sponsor’s figure)

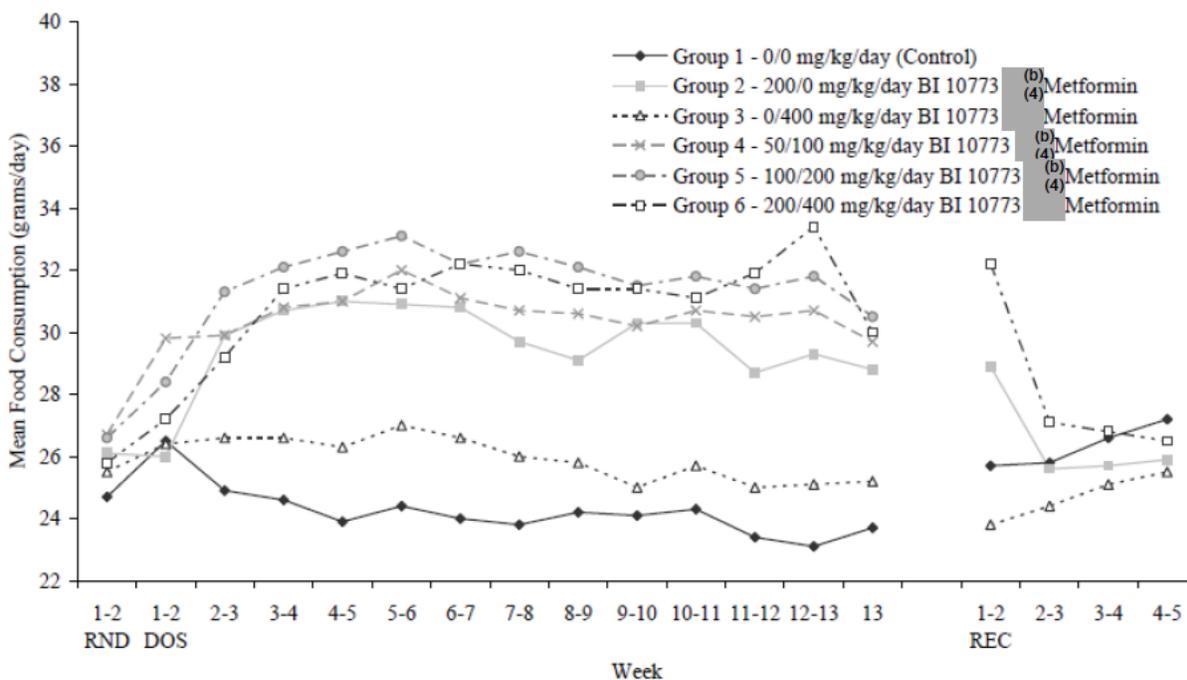


Figure 6. Food Consumption – Females (sponsor’s figure)

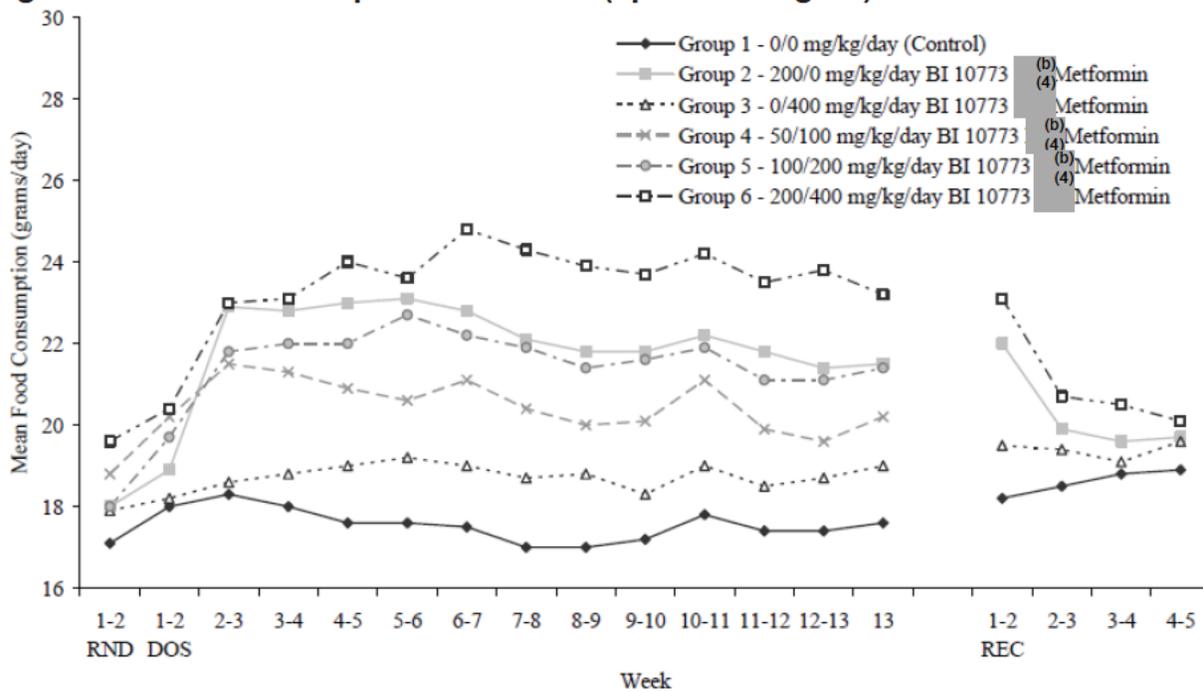


Table 19. 90-Day Food Consumption (sponsor's table)

Group	BI 10773 ^{(b) (4)} Metformin (mg/kg/day)	Males	Females
2	200/0	20 - 30%	22 - 31%
3	0/400	4 - 11%	6 - 11%
4	50/100	20 - 33%	13 - 21%
5	100/200	26 - 38%	19 - 29%
6	200/400	17 - 45%	26 - 43%

Ophthalmoscopy

N/A. The sponsor did not conduct eye exams.

ECG

N/A. The sponsor did not conduct ECG exams.

Hematology

Hematocrit (HCT) and mean corpuscular volume (MCV) were significantly increased approximately 3-5% in the 200/400 mg/kg males and females (see sponsor's table below). In addition, mean corpuscular hemoglobin concentration (MCHC) was significantly increased 4.4% in the 200/400 mg/kg males and females (see sponsor's table below). These hematological parameters were without correlation to red blood cell, reticulocyte or histopathological changes. Hematological parameters were unremarkable in recovery animals.

Table 20. Hematology (sponsor's table)

Group	Males					Females				
	2	3	4	5	6	2	3	4	5	6
BI 10773 ^{(b) (4)}	200	0	50	100	200	200	0	50	100	200
Metformin	0	400	100	200	400	0	400	100	200	400
HCT	-	-	-	-	+3.7 ^a	-	+4.6 ^a	-	-	+5.1 ^a
MCV	-	-	-	-	+4.4 ^a	-	-	-	-	+4.0 ^{a,b}
MCHC	-	-	-	-	-4.4 ^{a,b}	-	-	-	-	-4.4 ^a

^aStatistically significant versus controls.

Clinical Chemistry

Treatment with empagliflozin (BI 10773 ^{(b) (4)}) alone (200/0 mg/kg) resulted in a significant reduction of serum glucose (up to 51%) and chloride (up to 1-2%) in both males and females. The reduction of serum glucose is a known pharmacodynamics action of

empagliflozin and correlated with the observed glucosuria. Serum calcium, total protein (TP), albumin (ALB) and globulin (Glob) were also significantly reduced (2-5%) in the 200/0 mg/kg females (see sponsor's table below). Triglycerides (TRIG) (males only), BUN (males only), AST (males only), ALT, alkaline phosphatase (ALKP) and phosphorus (PHOS) were significantly increased (12-31%) in the 200/0 mg/kg rats (see sponsor's table below). The slightly elevated transaminases and ALKP suggest hepatic and/or cholestatic injury, but were without histopathology correlates, but correlated with increased liver weight.

Treatment with metformin alone (0/400 mg/kg) also resulted in a significant reduction of serum glucose (13%) (females only). Serum TP, ALB and GLOB were also reduced (4-5%) in 0/400 mg/kg females. Cholesterol (CHOL) (males only), PHOS, calcium (Ca^{++}) were significantly increased (6-32%) in the metformin only (0/400 mg/kg) animals (see sponsor's table below).

The co-administration of e+m resulted in a dose-dependent decrease in serum glucose (14-51%) and chloride (1-8%) in both males and females and exacerbated the significant increase in serum TRIG (200%) and ALKP (29-139%) particularly in the high dose e+m (200/400 mg/kg) treated animals (see sponsor's table below). The elevation of serum PHOS and reduction of serum Ca^{++} , TP, ALB and Glob observed with empagliflozin or metformin alone were also present in the combination treated animals (see sponsor's table below). Serum transaminases were significantly elevated in the mid dose 100/200 mg/kg (18-33%) and high dose 200/400 mg/kg (31-83%) e+m combination animals of both genders. The elevation of liver enzymes correlated with increased liver weights but not histopathology.

Table 21. Clinical Chemistry Percent Difference Relative to Vehicle (sponsor's table)

Males					
Group	2	3	4	5	6
BI 10773 ^{(b) (4)}	200	0	50	100	200
Metformin	0	400	100	200	400
GLU	-50.4 ^{a,d}	-	-28.7 ^a	-44.3 ^{a,d}	-51.3 ^{a,d}
Cl ⁻	-1.9 ^{a,d}	-	-1.9 ^{a,d}	-2.9 ^{a,d}	-6.8 ^{a,b,d}
Na ⁺	-	-	-	-	-1.4 ^a
TRIG	31.0	-	-	69.0 ^{a,d}	212.1 ^{a,b,d}
BUN	25.0 ^{a,d}	-12.5 ^a	-	12.5	18.8 ^c
AST	12.0 ^a	-	-	18.5 ^a	40.2 ^{a,b,d}
ALT	-	-	-	22.2 ^a	38.9 ^a
ALKP	19.2 ^a	-	-	28.8 ^{a,d}	51.9 ^{a,b,d}
CHOL	-	17.7 ^a	-	11.3	8.1
PHOS	21.1 ^a	31.6 ^a	-	21.1 ^a	35.1 ^{a,b}
Ca ⁺⁺	-	6.0 ^a	-	2.0	4.0 ^{a,c}
TP	-	-	-	-	-
ALB	-	-	-	-	-
Glob	-	-	-	-	-
Females					
Group	2	3	4	5	6
BI 10773 ^{(b) (4)}	200	0	50	100	200
Metformin	0	400	100	200	400
GLU	-36.6 ^{a,d}	-12.9 ^a	-14.0 ^a	-17.2 ^a	-31.2 ^{a,c,d}
Cl ⁻	-1.0 ^a	-	-1.0 ^a	-2.9 ^{a,d}	-7.7 ^{a,b,d}
Na ⁺	-	-	-	-	-3.5 ^a
TRIG	-	-	-	-	281.0 ^{a,d}
BUN	-	-22.2 ^a	-	11.1	11.1 ^c
AST	23.4 ^{a,d}	-	10.4	22.1 ^{a,d}	31.2 ^{a,d}
ALT	33.3 ^a	-	-	33.3 ^a	83.3 ^{a,b}
ALKP	34.8	30.4 ^{a,d}	-	47.8 ^a	139.1 ^{a,b,c,d}
CHOL	-	-	-	-	-
PHOS	16.4 ^a	18.2 ^a	-	14.5 ^a	23.6 ^a
Ca ⁺⁺	-1.9 ^a	1.9	-	-	-2.9 ^{a,c}
TP	-3.2 ^a	-4.8 ^a	-	-3.2	-7.9 ^{a,b,c,d}
ALB	-5.1 ^a	-5.1 ^a	-2.6 ^a	-2.6 ^a	-7.7 ^{a,b,c,d}
Glob	-4.2	-4.2 ^a	-	-	-8.3 ^{a,b}

Urinalysis

Treatment with empagliflozin (BI 10773 ^{(b) (4)}) alone (200/0 mg/l/kg) resulted in significant glucosuria and an increase in urinary specific gravity in both genders. In addition, the empagliflozin + metformin males exhibited dose-dependent polyuria (46-155%) (see sponsor's table below). Polyuria was also evident in the metformin alone 0/400 mg/kg animals, particularly in males (155%). The co-administration of e+m resulted in glucosuria, polyuria, aciduria and an increase in urinary specific gravity, particularly in the males (see sponsor's table below). Urinalysis was unremarkable in recovery animals. Glucosuria and polyuria are known pharmacodynamic effects from treatment with empagliflozin.

Table 22. Day 90 Urinalysis Percent Difference Relative to Vehicle (sponsor's table)

Males					
Group	2	3	4	5	6
BI 10773 ^{(b)(4)}	200	0	50	100	200
Metformin	0	400	100	200	400
Glucose (mg/dL)	≥1000	0	≥1000	≥1000	≥1000
Sp.G.	0.8 ^a	-0.9 ^a	1.7 ^{a,c}	1.4 ^a	1.3 ^{a,b}
pH	-10.9 ^{a,c}	-	-6.3 ^a	-9.4 ^a	-14.1 ^{a,b,c}
VOL	127.3 ^{a,c}	154.5 ^{a,c}	45.5 ^a	109.1 ^{a,c}	154.5 ^{a,c}
Females					
Group	2	3	4	5	6
BI 10773 ^{(b)(4)}	200	0	50	100	200
Metformin	0	400	100	200	400
Glucose (mg/dL)	≥1000	0	≥1000	≥1000	≥1000
Sp.G.	1.7 ^{a,c}	-0.2	1.6 ^{a,c}	1.8 ^{a,c}	1.3 ^{a,b,c}
pH	-	-	-	-	-6.9 ^{a,b}
VOL	-	18.8	-	-	25.0 ^a

Gross Pathology

Five male and two female 200/400 mg/kg animals were found with a distended cecum (results not shown). This was without a histopathology correlate and was not present in recovery animals. **Reviewer note:** this may represent off-target SGLT1 inhibition.

Organ Weights

Absolute adrenal weight was increased 14-28% in the treated males with the organ weight being slightly increased due to metformin alone or to the empagliflozin/metformin combination, relative to empagliflozin alone (see sponsor's table below). Adrenal to body weight ratios were increased in all male treatment groups and exacerbated dose-dependently 25-47% in the male empagliflozin and metformin combination groups (see table below). Adrenal to brain weight ratios were also increased in all male treatment groups and slightly exacerbated 22-30% in the male empagliflozin and metformin combination groups (see table below). Adrenal weights were without histopathology correlates and unremarkable in recovery animals. No effect on adrenal weight was observed in females.

Absolute kidney weight was increased 13% and 21% in the 200/0 mg/kg males and females, respectively. Kidney weight was also increased 16% in the metformin alone (0/400 mg/kg) males (see sponsor's table below). Kidney weight was however, further increased in the empagliflozin/metformin combination treated males (23-34%) and females (14-45%) (see tables below). Kidney to body weight ratios were increased in all male and female treatment groups and increased dose-dependently 25-61% in the male and 18-55% in the female, empagliflozin and metformin combination groups, respectively (see table below). Kidney to brain weight ratios were also increased in all male and female treatment groups and increased dose-dependently 24-38% in the male

and 15-49% in the female empagliflozin and metformin combination groups, respectively (see table below). Increased kidney weight was not correlated to histopathology except for tissue mineralization at 200/400 mg/kg, but remained elevated 9-12% in the 200/400 mg/kg recovery animals (see sponsor's table below).

Table 23. Organ Weights – Main Study Animals (sponsor's table)

Group	Males					Females				
	2	3	4	5	6	2	3	4	5	6
BI 10773 ^(b) (4)	200	0	50	100	200	200	0	50	100	200
Metformin	0	400	100	200	400	0	400	100	200	400
Kidney	+13 ^a	+16 ^a	+23 ^a	+31 ^a	+34 ^{a,b,c}	+21 ^a	-	+14 ^a	+19 ^a	+45 ^{a,b,c}
Liver	-	+15 ^a	+12 ^a	+16 ^a	+18 ^a	+7 ^a	-	-	+12 ^a	+42 ^{a,b,c}
Thymus	-20 ^a	-	-	-20 ^a	-46 ^{a,b,c}	-19 ^a	-	-	-20 ^a	-23 ^a
Adrenal	+14 ^a	+21 ^a	+22 ^a	+28 ^a	+21 ^a	-	-	-	-	-

^aStatistically significant versus controls.

^bStatistically significant versus BI 10773 ^(b)(4) alone (included when relevant).

^cStatistically significant versus Metformin alone (included when relevant).

-: no change

For males absolute liver weight was increased 12-18% in all treatment groups except the empagliflozin alone treatment group (200/0 mg/kg). Absolute liver weight was also increased 7% in the empagliflozin alone females (200/0 mg/kg) and the mid dose 100/200 mg/kg e+m combination females (12%). Absolute liver weight was further increased in the high dose 200/400 e+m combination females (42%) (see sponsor's table above).

Liver to body weight ratios were increased in all male treatment groups and dose-dependently increased 13-43% in the male empagliflozin and metformin combination groups (see table below). Liver to brain ratios were also increased in all male treatment groups, except in the metformin alone group, and increased dose-dependently 13-23% in the male empagliflozin and metformin combination groups (see table below). For females, the liver to body weight ratios were increased 10-13% in the empagliflozin alone (200/0 mg/kg) and the metformin alone (0/400 mg/kg) groups and dose-dependently increased 10-52% in the empagliflozin and metformin combination groups (see table below). Similarly, body weight to brain weight ratios were increased 7% in the female empagliflozin alone group (200/0 mg/kg) and the mid (13%) and high (46%) dose empagliflozin and metformin combination groups (see table below).

The increased liver weight was without a histopathology correlate. The absolute liver weight, liver to body weight/brain weight ratio remained increased 9-15% in the 200/400 mg/kg recovery females.

The absolute thymus weight was decreased 14% in the empagliflozin only-treated males and dose-dependently decreased 20% and 46% in the mid (100/200 mg/kg) and high (200/400 mg/kg) empagliflozin/metformin combination group males, respectively (see sponsor's table above). Corresponding thymus to body weight (reduced 15-35%) and thymus to brain weight (21-45%) ratios were reduced in the same males (see table below). Absolute thymus weight was also decreased 19% in the empagliflozin only-

females dose-dependently decreased 20% and 23% in the mid (100/200 mg/kg) and high (200/400 mg/kg) empagliflozin/metformin combination group females, respectively (see sponsor's table above). Corresponding thymus to body weight (reduced 14-18%) and thymus to brain weight (18-21%) ratios were reduced in the same females (see table below). These findings were without a histopathology correlate and thymus weight was unremarkable in recovery animals.

Absolute ovary weight was increased 19% and 30% in the metformin alone (0/400 mg/kg) and high dose empagliflozin (200/400 mg/kg) females, respectively. There was also a corresponding increase 19-40% in the ovary to body weight/brain ratio in the same animals (see table below). These observations were without a histopathology correlate and ovary weight was unremarkable in recovery females.

Table 24. Organ Weights – Recovery Animals (sponsor's table)

	Males	Females
Group	6	6
BI 10773 ^{(b) (4)}	200	200
Metformin	400	400
Kidney	+12	+9
Liver	-	+9

Organ Weights							
Dose, mg/kg		Males					
Empagliflozin		0	200	0	50	100	200
Metformin		0	0	400	100	200	400
Terminal BW	g	400	373 *	409	395	386	332 ***
Adrenal	g	0.0546	0.0621 *	0.0661 **	0.0664 **	0.0701 ***	0.0663 ***
	% BW	0.0136	0.0167 **	0.0161 **	0.017 ***	0.0182 ***	0.02 ***
	% Br. W	2.702	3.068 *	3.225 **	3.309 **	3.523 ***	3.38 **
Kidney	g	2.06	2.33 *	2.39 **	2.54 ***	2.70 ***	2.76 ***
	% BW	0.51	0.62 ***	0.58 **	0.64 ***	0.69 ***	0.83 ***
	%	102					

	Br. W		115 *	117 *	127 ***	136 ***	141 ***
Liver	g	8.47	8.44	9.74 ***	9.5 ***	9.86 ***	10.04 ***
	% BW	2.12	2.26 **	2.38 **	2.40 ***	2.55 ***	3.02 ***
	% Br. W	419.08	417.37	475.78 **	473.5 **	497.43 **	512.45 ***
Heart	g	1.01	0.96	1.12 **	1.06	1.08	1.06
	% BW	0.25	0.25	0.27 **	0.27 **	0.28 **	0.32 ***
	% Br. W	50.16	47.6	54.92 *	53.25	54.61 *	54.31 *
Thymus	g	0.33	0.26 **	0.31 **	0.30	0.26 ***	0.25 ***
	% BW	0.14	0.12 *	0.14	0.13	0.12 **	0.11 ***
	% Br. W	17.71	14.46 **	17.17	16.62	14.26 **	14.01 **

* $p \leq 0.05$, ** $p \leq 0.01$ $p \leq 0.001$ (from sponsor's assessment).

Organ Weights							
Dose, mg/kg		Females					
Empagliflozin		0	200	0	50	100	200
Metformin		0	0	400	100	220 *	400
Terminal BW	g	234	221 *	223 *	226	220	218 **
Kidney	g	1.38	1.67 ***	1.42	1.57 ***	1.64 ***	2 ***
	%	0.59	0.75	0.64	0.69	0.74	0.91

	BW		***	*	***	***	***
	% Br. W	74	90 ***	76	85 ***	88 ***	110 ***
Liver	g	5.31	5.67 *	5.56	5.61	5.94 ***	7.52 ***
	% BW	2.26	2.56 ***	2.49 ***	2.47 ***	2.69 ***	3.44 ***
	% Br. W	284.77	305.88 *	299.49	305.46	321.52 ***	415.35 ***
Heart	g	0.72	0.69	0.76 *	0.71	0.73	0.82 **
	% BW	0.30	0.31	0.34 ***	0.31	0.33 ***	0.37 ***
	% Br. W	38.67	37.48	41.13	39.06	39.88	45.50 ***
Ovaries	g	0.094	0.105	0.111 *	0.111	0.102	0.122 **
	% BW	0.040	0.047 *	0.050 *	0.049 *	0.046 *	0.056 ***
	% Br. W	5.02	5.72	5.99 *	6.06	5.50	6.73 **
Thymus	G	0.33	0.26 ***	0.31	0.30	0.26 **	0.25 ***
	% BW	0.14	0.12 *	0.14	0.13	0.11 **	0.11 **
	% Br. W	17.71	14.46 **	17.17	16.62	14.26 **	14.00 **

* $p \leq 0.05$, ** $p \leq 0.01$ $p \leq 0.001$ (from sponsor's assessment).

Histopathology

Adequate Battery

Yes

Peer Review Yes

Histological Findings

Minimal pelvic epithelium mineralization was observed in one male and 6 female 200/400 mg/kg animals at termination (see sponsor’s table below). Minimal cortical tubular basement membrane thickening was observed primarily in the 200/400 mg/kg group and the 0/400 mg/kg metformin alone females (see sponsor’s table below). This observation was reversed in recovery animals.

Table 25. Histopathology at the Terminal Sacrifice (sponsor’s table)

	M				F			
	0	0	200	200	0	0	200	200
BI 10733 (b) (4) mg/kg/day	0	0	200	200	0	0	200	200
Metformin mg/kg/day	0	400	0	400	0	400	0	400
No. Examined	15	15	15	15	15	15	15	15
Kidney								
Mineralization, pelvic epithelium								
Minimal	0	0	0	1	0	0	0	6

Table 26. Kidney Histopathology at the Terminal Sacrifice (sponsor’s table)

```

-----
Controls from group(s): 1
Animal sex: -- Animals --
              -- Males --
Dosage group: Ctls  2  3  4  5  6
Tissues With Diagnoses No. in group: 15 15 15 15 15 15
-----
Kidneys .....Number examined: 15 15 15 15 15 15 | 15 15 15 15 15 15
  BASEMENT MEMBRANE THICKENING, TUBULAR, CORTICAL,
  -FOCAL/MULTIFOCAL
  -> 15 15 15 15 15 12 | 15 15 14 15 15 14
  1> 0 0 0 0 0 3 | 0 0 1 0 0 1
  .....Total Incidence of Finding Observed: 0 0 0 0 0 3 | 0 0 1 0 0 1
-----
    
```

Minimal to marked sperm granuloma in the epididymides was noted with increased incidence in the empagliflozin alone (200/0 mg/kg), metformin alone (0/400 mg/kg) and the high dose e+m combination (200/400 mg/kg) males (see sponsor’s table below). The high incidence of granuloma was reversed in the recovery males (see sponsor’s table below).

Table 27. Male Epididymal Sperm Granuloma at Termination (sponsor’s table)

```

-----
Controls from group(s): 1
Animal sex: -- Animals --
              -- Males --
Dosage group: Ctls  2  3  4  5  6
Tissues With Diagnoses No. in group: 15 15 15 15 15 15
-----
Epididymides .....Number examined: 15 15 14 0 0 15
  GRANULOMA, SPERM
  -> 14 9 12 0 0 13
  1> 0 1 0 0 0 0
  2> 1 2 2 0 0 0
  3> 0 2 0 0 0 2
  4> 0 1 0 0 0 0
  .....Total Incidence of Finding Observed: 1 6 2 0 0 2
-----
    
```

Table 28. Male Epididymal Sperm Granuloma at Recovery (sponsor's table)

Controls from group(s): 1		Animal sex:	-- Animals			
		Dosage group:	-- Males --			
Tissues With Diagnoses		No. in group:	Ctl's	2	3	6
			10	10	10	10
Epididymides	Number examined:		10	10	10	10
GRANULOMA, SPERM						
	->		8	8	10	9
	1>		0	0	0	1
	3>		0	1	0	0
	4>		2	1	0	0
.....Total Incidence of Finding Observed:			2	2	0	1

Special Evaluation

None.

Toxicokinetics

Ninety days of empagliflozin combined with metformin resulted in empagliflozin exposure that was dose proportional or slightly greater than dose proportional. The T_{max} ranged from 1-2 hr. At day 90 in males, the high dose empagliflozin/metformin combination (200/400 mg/kg) increased both the C_{max} and exposure (AUC) of empagliflozin 2-3-fold compared to the high dose of empagliflozin alone. At day 90, the exposure to empagliflozin alone (200/0 mg/kg) was 2-fold higher in females than in the males (see sponsor's table below).

Table 29. Empagliflozin Toxicokinetics in Rats (sponsor's table)

Parameter	Sex	Day	BI 10773/Metformin HCl (mg/kg/day)			
			50/100	100/200	200/400	200/0
C_{max} (nM)	Male	1	5,650	5,940	6,870	7,880
		90	3,470	8,390	18,500	6,530
	Female	1	4,690	12,400	22,200	15,300
		90	7,850	12,300	22,200	26,600
AUC_{0-24} (nM·h)	Male	1	21,400	35,100	67,400	58,200
		90	18,000	44,200	110,000	46,100
	Female	1	24,600	49,000	79,500	55,900
		90	28,300	57,700	115,000	101,000
t_{max} (h)	Male	1	1	2	1	1
		90	2	2	1	1
	Female	1	1	2	2	1
		90	1	2	1	1

Empagliflozin combined with metformin resulted in metformin exposure that was slightly less than dose proportional. There were no sex differences in exposure to the combination of empagliflozin and metformin. The exposure to high dose metformin alone at 0/400 mg/kg was slightly lower (1.35 -1.49 –fold) compared to the high dose e+m combination at 200/400 mg/kg. This suggests a slight enhancement of exposure

to metformin when combined with empagliflozin (see sponsor's table below). T_{max} was generally 2 hr.

Table 30. Metformin Toxicokinetics in Rats (sponsor's table)

Parameter	Sex	Day	BI 10773/ Metformin HCl (mg/kg/day)			
			50/100	100/200	200/400	0/400
C_{max} (nM)	Male	1	34,000	46,400	70,500	116,000
		90	38,200	66,900	146,000	117,000
	Female	1	25,300	30,000	56,800	104,000
		90	42,000	93,200	166,000	140,000
AUC_{0-24} (nM•h)	Male	1	198,000	436,000	632,000	658,000
		90	388,000	549,000	1,130,000	758,000
	Female	1	206,000	312,000	508,000	610,000
		90	278,000	575,000	1,030,000	762,000
t_{max} (h)	Male	1	1	2	2	2
		90	2	2	2	2
	Female	1	2	2	2	2
		90	1	2	2	2

Overall when dosed in combination, metformin slightly increases empagliflozin exposure in male rats. However, metformin exposure is also slightly increased when dosed in combination with empagliflozin.

Dosing Solution Analysis

All dosing solutions were within nominal concentrations.

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Metformin: Study for Effects on Embryo-fetal Development in rats by Oral (gavage) Administration

Study no.:	09B099, U10-2386-01
Study report location:	eCTD
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Birkendorfer Str. 65 88397 Biberach an der Riss Germany
Date of study initiation:	05.28.2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Metformin HCl, Batch 801941, 100.1%

Reviewer note: Study# 09B099, U10-2386-01 was reviewed under NDA 201281 for the linagliptin/metformin FDC (submitted to DARRTs 10.04.2011 and AP 01.30.2015). This reviewer concurs with the conclusions of the primary reviewer for this study; and the study key findings and NOAEL are presented are from NDA 201281.

Key Study Findings

- Treatment generally did not affect pregnancy success but metformin was teratogenic at ≥ 500 mg/kg dosing during embryo-fetal development. Dose-related increases in skeletal malformations, predominantly in rib (flat, thickened, and/or z-shaped). Scapula (bent inward) was seen in several litters and fetuses at ≥ 500 mg/kg along with isolated incidences (1 or 2 fetuses/litters) of other skeletal malformations.
- Anophthalmia and polydactylia malformations were seen externally in two fetuses from one HD litter, providing further support for teratogenic potential of metformin.
- Decreased blood glucose was observed at some time points but results were variable and there was no evidence of sustained hypoglycemia (which is not expected with metformin treatment). Teratogenic findings cannot be explained by maternal hypoglycemia alone but may be related to changes in peripheral glucose metabolism and insulin sensitivity that affect fetal development in unknown ways.

NOAEL determination –

A maternal NOAEL could not be determined based on decreased BW gain at the start of treatment in all groups, which resulted in dose-related, significantly

reduced (49-123%) BW gain at 200 and 500 mg/kg and approximately 120-150% decreased BW gain at 1000 mg/kg. Skeletal variations increased slightly in the LD but there was no evidence of drug-related teratogenesis in the 200 mg/kg group. Metformin treatment caused fetal skeletal malformations at 500 and 1000 mg/kg which could not be attributed to reduced maternal BW gain.

Doses (mg/kg/d) 0, 200, 500, 1000 metformin (free base)
638, 1730, 3690 $\mu\text{M}\cdot\text{h}$ (4X, 11X, 23X MRHD)

NOAEL (maternal) <200 mg/kg/d (<4X MRHD)

NOAEL (fetal) = 200 mg/kg/d (4X MRHD)

BI 10773 and Metformin: Dose-Range Finding Study For Effects on Embryo-Fetal Development in Rats (Oral Administration by Gavage)

Study no.:	U13-2538-01, 11b225
Study report location:	eCTD
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Birkendorfer Str. 65 88397 Biberach an der Riss, Germany
Date of study initiation:	January 5 th 2012 (animal arrival)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BI 10773 (b) (4) 1045396 and 99.6% Metformin hydrochloride, 000902 and 99.7%

Key Study Findings

- The co-administration of empagliflozin and metformin at up to 300/600 mg/kg did not affect rat maternal pregnancy or result in variations or malformations in the fetuses.
- Mean gestational body weight and body weight gain were reduced 4-7% with empagliflozin and metformin in the 200/400 mg/kg and 300/600 mg/kg-treated dams.
- The reduced mean body weight and body weight gain correlated with reduced food consumption in the 200/400 mg/kg and 300/600 mg/kg empagliflozin and metformin-treated dams at GD 14.
- The maternal NOAEL is empagliflozin and metformin at 50/100 mg/kg (7x/2x MRHD). The fetal NOAEL is empagliflozin and metformin at 300/600 mg/kg (54x/16x MRHD).

Methods

Doses: Empagliflozin (e)/Metformin (m): 0/0, 50/100, 200/400 and 300/600 mg/kg
 Frequency of dosing: Daily GD 7-16
 Dose volume: 10 mL/kg
 Route of administration: Per os (gavage)
 Formulation/Vehicle: 0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
 Species/Strain: Rat/Crl:WI (Han) (Wistar)
 Number/Sex/Group: 16 females/group (10 females in main study and 6 females in TK and blood glucose group)
 Satellite groups: 6 females in TK and blood glucose group
 Study design: Pregnant females treated QD via oral gavage from GD 7 -16. At each of GD 7 and 16, 3 satellite pregnant females per group, were used to measure TK and 3 additional satellite pregnant females per group were used to measure blood glucose. Laparohysterectomy and necropsy were at GD 22. Fetuses were examined for external, visceral and skeletal variations and malformations.

Deviation from study protocol: None that affected the study outcome.

Observations and Results

Reviewer note: the sponsor's study design and animal number table is below. Of note females #112, 202, 209, 304, 311, 403, 404 and 407 were non-gravid.

Table 31. Sponsor's Study Design and Animal Number Designation Table

Group	Females/group	Daily dose [mg/kg]			Animal numbers [†]	
		(b) (4) BI 10773	Metformin HCl	Metformin BS	Main study	Toxicokinetics/ Clinical chemistry
1	16	0	0	0	107-116	101-106
2	16	50	100	78	207-216	201-206
3	16	200	400	312	307-316	301-306
4	16	300	600	468	407-416	401-406

[†] non-pregnant main study animals: 112, 209, 311, 407; non-pregnant satellite animals: 202, 304, 403, 404

Mortality

None.

Clinical Signs

Soft feces was noted in two 200/400 mg/kg empagliflozin and metformin (e+m) dams and also six 300/600 mg/kg e+m dams either on a single day or over two days at GD 8,9 or 12 (see sponsor's table below). Female #407 was observed with diarrhea on GD 9 (see sponsor's table below)

Table 32. Clinical Signs (sponsor's table)

Animal No.	Finding	GD
G 1, 0 mg/kg		
107-116	no finding	
G 2, 50:100 mg/kg BI 10773^{(b) (4)}:Metformin HCl		
207-216	no finding	
G 3, 200:400 mg/kg BI 10773^{(b) (4)}:Metformin HCl		
307, 310-316	no finding	
308	soft faeces	12
309	soft faeces	9
G 4, 300:600 mg/kg BI 10773^{(b) (4)}:Metformin HCl		
412-414	no finding	
407	diarrhoea	9
408, 409, 410, 411	soft faeces	9
415, 416	soft faeces	8-9

Body Weight

During the treatment period mean gestational body weight and body weight gain was dose-dependently reduced, in particular, for the 200/400 mg/kg and 300/600 mg/kg empagliflozin and metformin treated-dams (see sponsor's figures below). Mean body weight was significantly reduced 4% in the 200/400 mg/kg dams at GD 9, and 5-7% in the 300/600 mg/kg empagliflozin and metformin dams at GD 9-14, respectively (see sponsor's table below). The reduced body weight and body weight gain in the 200/400 mg/kg and 300/600 mg/kg females, correlated with reduced food consumption at GD 14 in these groups.

Figure 7. Body Weight (sponsor's figure)

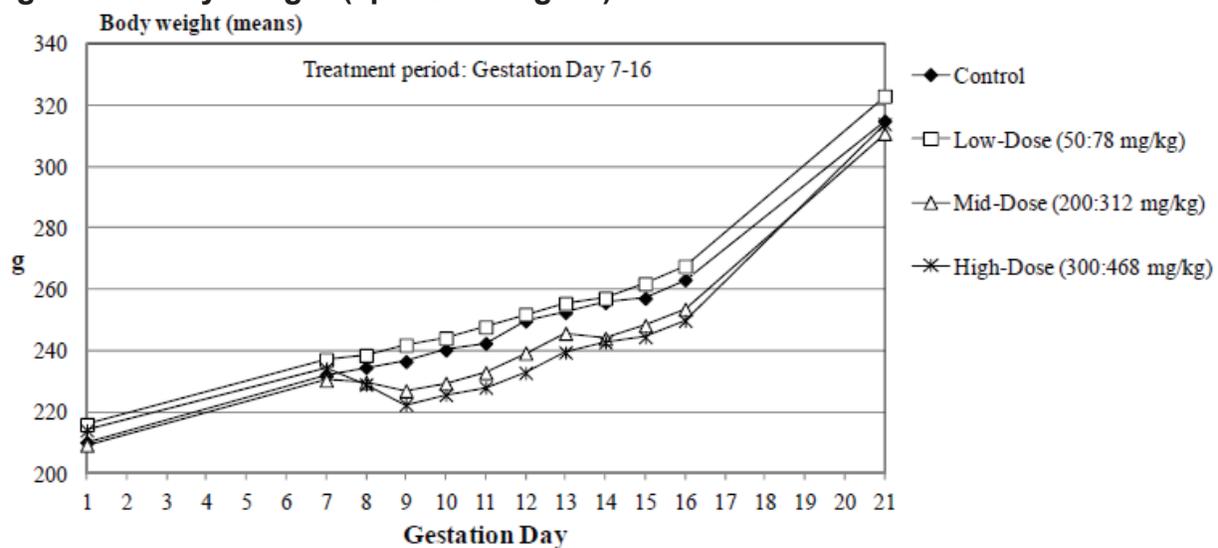


Figure 8. Body Weight Gain (sponsor's figure)

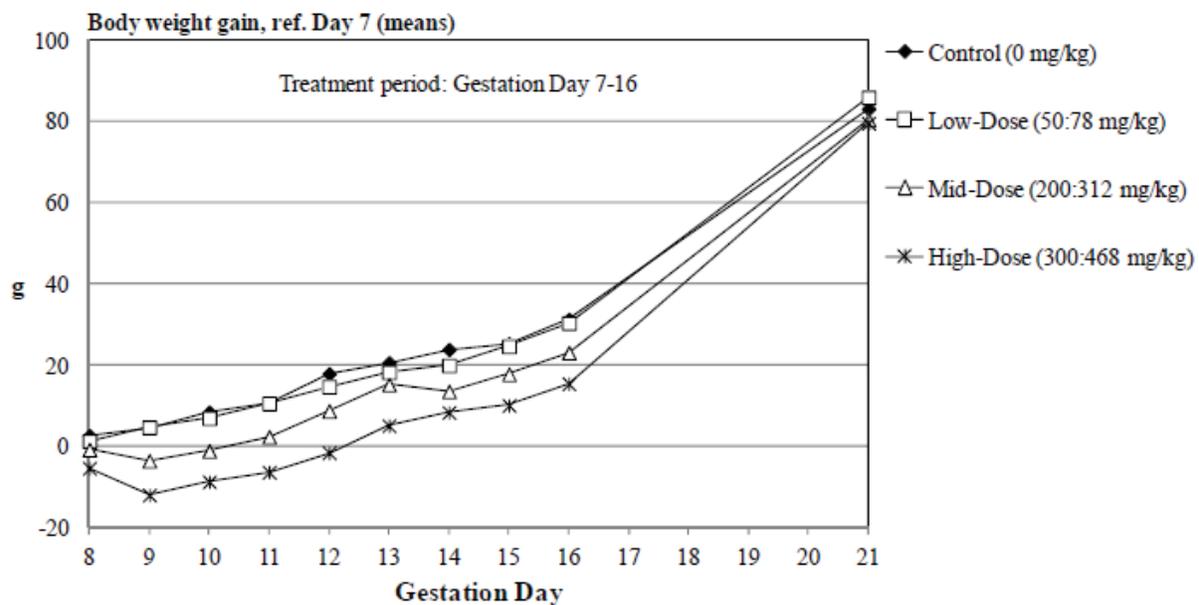


Table 33. Mean Gestational Body Weight (sponsor's table)

Body wt. [g]							
<u>female</u>							
Obs. time	[day]	1	7	8	9	10	11
Group							
Control	n	9	9	9	9	9	9
0 [mg/kg]	mv	210.08	231.80	234.53	236.47	240.26	242.41
	sd	6.56	6.73	8.30	6.24	7.38	7.30
50:78	n	9	9	9	9	9	9
<multi comp.>	mv	215.92	237.11	238.48	241.86	244.10	247.76
	sd	10.62	11.02	9.46	11.46	10.49	10.52
	p	0.2174	0.2899	0.4324	0.2928	0.4752	0.3156
200:312	n	9	9	9	9	9	9
<multi comp.>	mv	209.27	230.43	229.70	226.97	229.38	232.86
	sd	10.28	10.13	11.68	10.85	12.42	11.35
	p	0.8625	0.7836	0.3372	0.0685	*0.0492	0.0777
300:468	n	9	9	9	9	9	9
<multi comp.>	mv	214.09	234.30	228.98	222.40	225.63	228.00
	sd	11.26	13.00	12.17	12.99	13.81	14.21
	p	0.3942	0.6159	0.2711	*0.0088	*0.0098	*0.0097

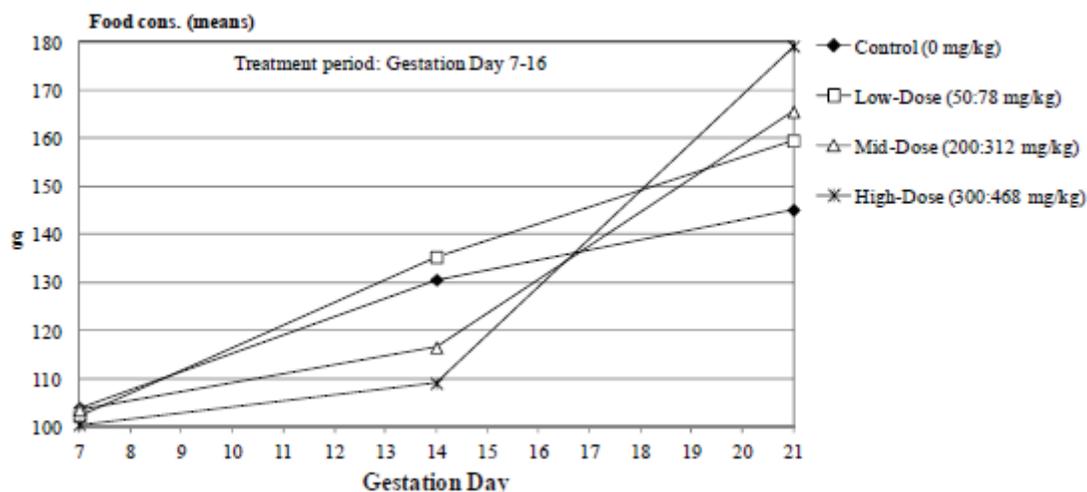
Table 34. Mean Gestational Body Weight (sponsor's table) continued

Body wt. [g]							
<u>female</u>							
Obs. time	[day]	12	13	14	15	16	21
Group							
Control	n	9	9	9	9	9	9
0 [mg/kg]	mv	249.69	252.44	255.69	257.10	263.11	314.88
	sd	7.88	7.64	7.84	8.44	6.70	16.36
50:78	n	9	9	9	9	9	9
<multi comp.>	mv	251.84	255.40	257.07	261.80	267.52	322.98
	sd	10.53	11.20	12.23	12.85	12.78	18.05
	p	0.7127	0.6386	0.8288	0.4583	0.5090	0.3625
200:312	n	9	9	9	9	9	9
<multi comp.>	mv	239.24	245.69	244.03	248.26	253.54	310.83
	sd	14.05	13.83	12.35	13.02	13.00	18.04
	p	0.0812	0.2865	0.0744	0.1674	0.1572	0.6477
300:468	n	9	9	9	9	9	9
<multi comp.>	mv	232.78	239.46	242.71	244.43	249.76	313.86
	sd	15.35	18.00	18.84	17.31	20.20	21.56
	p	*0.0064	*0.0452	*0.0483	0.0515	0.0516	0.9079

Feed Consumption

Food consumption was dose-dependently reduced 11-16% at GD 14 in the 200/400 and 300/600 mg/kg empagliflozin and metformin-treated dams and correlated with the significant reduced body weight and body weight gain observed in these groups (see sponsor's figure below). Food consumption recovered in the post-treatment period.

Figure 9. Mean Food Consumption



Clinical Chemistry: Glucose

At GD 7 treatment with the low dose empagliflozin and metformin at 50/100 mg/kg significantly reduced plasma glucose at 8 hr post-dose. At higher doses at GD 7, plasma glucose was significantly reduced from 1-24 hr post-dose at $\geq 200/400$ mg/kg (see sponsor's table below). At GD 16, plasma glucose was significantly reduced in treatment groups at most sampling time points (see sponsor's table below).

Table 35. Plasma Glucose During Gestation

Daily dose of BI 10773:Metformin HCl [mg/kg]	Group	Gestation Day	Sample time point					
			Pre-Dose	1 h	2 h	4 h	8 h	24 h
			Pre-Treatment					
0:0	1	7	9.72	13.20	13.02	13.13	12.11	12.62
		16	9.70	10.93	10.78	10.47	10.15	8.30
50:100	2	7	10.42	10.47	10.49	7.68	9.52 ↓	9.92
		16	9.54	8.20 ↓	8.81	8.55 ↓	7.50 ↓	7.97
200:400	3	7	11.78	10.23 ↓	7.66 ↓	3.81 ↓	6.33 ↓	7.83 ↓
		16	7.35 ↓	7.49 ↓	8.41 ↓	7.25	5.07 ↓	7.08
300:600	4	7	9.15	10.40 ↓	8.35 ↓	4.90 ↓	3.93 ↓	6.18 ↓
		16	7.89	6.82 ↓	6.25	10.61	6.14 ↓	7.83

↓: significantly decreased compared with Control; $p < 0.05$

Clinical Chemistry: Fructosamine

Fructosamine was used as a marker of average blood sugar concentration over the preceding 2-3 weeks. Fructosamine was unremarkable in the treated animals at GD 22 (necropsy) (data not shown).

Toxicokinetics

For the empagliflozin and metformin co-administered females, empagliflozin C_{max} was less than dose proportional at either GD 7 (Drug day 1) or 16 (Drug day 10). The empagliflozin exposure (AUC_{0-24}) increased approximately dose-proportionately from 50 mg/kg to 300 mg/kg at either GD 7 or 16 (see sponsor's table below). The T_{max} ranged from 1-2 hr.

Table 36. Empagliflozin TK Parameters When Co-Administered With Metformin (sponsor's table)

Mean BI 10773 toxicokinetic parameters after oral administration of BI 10773 ^{(b) (4)} in combination with metformin HCl on Drug Days 1 and 10 of a range-finding embryo-fetal developmental study in pregnant Wistar Han rats					
Parameter	Drug Day	Statistic	BI 10773 (mg/kg/day)		
			50 ^a	200 ^b	300 ^c
C_{max} (nM)	1	Mean	8,830	21,800	24,000
		SD	1,940	2,970	12,000
	10	Mean	8,570	29,300	37,000
		SD	2,350	2,190	13,300
AUC_{0-24} (nM·h)	1	Mean	33,800	128,000	108,000
		SD	8,770	46,500	21,900
	10	Mean	32,600	139,000	254,000
		SD	8,480	23,300	83,400
t_{max} (h)	1	Median	1.5	2	2
		Range	1-2	1-2	1-2
	10	Median	1	1.5	1.5
		Range	1-2	1-2	1-2

^a Administered with 100 mg/kg/day metformin HCl.

^b Administered with 400 mg/kg/day metformin HCl.

^c Administered with 600 mg/kg/day metformin HCl.

For the empagliflozin and metformin co-administered females, metformin C_{max} was less than dose proportional at either GD 7 (Drug day 1) or 16 (Drug day 10). The metformin exposure (AUC_{0-24}) was less than dose-proportional in the 50/100 mg/kg group to the 200/400 mg/kg empagliflozin and metformin group, at either GD 7 (Drug day 1) or 16 (Drug day 10) (see sponsor's table below). Metformin exposure (AUC_{0-24}) was approximately dose-proportional from 200/400 mg/kg to the 300/600 mg/kg empagliflozin and metformin groups. For the 200:400 mg/kg and 300/600 mg/kg empagliflozin and metformin groups, the exposure to metformin increased as the duration of exposure increased, suggesting accumulation of metformin (see sponsor's table below).

Table 37. Metformin TK Parameters When Co-Administered With Empagliflozin (sponsor's table)

Mean metformin toxicokinetic parameters after oral administration of metformin HCl in combination with BI 10773 ^{(b)(4)} on Drug Days 1 and 10 of a range-finding embryo-fetal developmental study in pregnant Wistar Han rats					
Parameter	Drug Day	Statistic	Metformin HCl (mg/kg/day)		
			100 ^a	400 ^b	600 ^c
C_{max} (nM)	1	Mean	25,800	77,000	103,000
		SD	3,680	18,800	56,500
	10	Mean	30,000	140,000	220,000
		SD	1,880	9,190	36,800
AUC_{0-24} (nM•h)	1	Mean	242,000	675,000	1,000,000
		SD	28,300	114,000	27,600
	10	Mean	245,000	1,430,000	2,490,000
		SD	56,900	226,000	339,000
t_{max} (h)	1	Median	3	2	5
		Range	2-4	1-4	2-8
	10	Median	2	2	3
		Range	2-2	2-2	2-4

^a Administered with 50 mg/kg/day BI 10773 ^{(b)(4)}

^b Administered with 200 mg/kg/day BI 10773 ^{(b)(4)}

^c Administered with 300 mg/kg/day BI 10773 ^{(b)(4)}

Dosing Solution Analysis

Within nominal concentrations.

Necropsy

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

At the terminal necropsy non-gravid animals numbered 1/16, 2/16, 2/16 and 3/16 in each group, respectively. The maternal necropsy was unremarkable for dams treated with empagliflozin and metformin at up to 200/400 mg/kg. One 300/600 mg/kg empagliflozin and metformin-treated female was found with a urinary bladder filled with turbid urine (data not shown).

One late resorption was noted in a 300/600 mg/kg dam, but this was not outside of the historical control range. As expected from the reduced maternal body weight/weight gain, fetal weight was slightly lower in the 200/400 and 300/600 mg/kg empagliflozin and metformin groups, but was also not statistically significant (see sponsor's table below).

Table 38. Selected C-Section Parameters (sponsor's table)

	Daily dose of BI 10773/Metformin HCl [mg/kg]				Spontaneous Incidences from Evaluation Study [U03-1549] mean/range	
	Control	50:100	200:400	300:600		
n litters +	9	9	9	9	85	
Litter parameters (means/individual range per dam)					overall means	ranges of means/ individual data
Corpora lutea	13.1/10-15	13.1/12-15	12.9/11-15	13.6/11-15	12.0	11.8-12.3/ 9.0-15.0
Implantations	11.6/5-14	11.2/6-13	12.4/10-14	12.3/7-15	11.1	10.9-11.4/ 5.0-15.0
Viable fetuses	10.7/4-14	10.9/6-13	11.7/9-14	11.4/7-15	10.5	10.1-10.7/ 5.0-14.0
Dead fetuses	0.1/0-1	0	0	0	0	0
Fetal sex (%)						
Male	44.01/ 14.29-54.55	50.54/ 18.18-80.00	46.29/ 16.67-76.92	50.39/ 27.27-71.43	49.41	46.92-52.18/ 18.18-80.0
Female	55.99/ 45.45-85.71	49.46/ 20.00-81.82	53.71/ 23.08-83.33	49.61/ 28.57-72.73	50.59	47.82-53.08/ 20.0-81.82
Total resorptions	0.78/0[1]-2	0.33/0-1	0.78/0[1]-2	0.89/0[1]-4	0.65	0.3-0.8/ 0[1.0]-3.0 [#] (6.0)
Early resorptions	0.78/0[1]-2	0.33/0-1	0.78/0[1]-2	0.78/0[1]-3	0.60	0.3-0.7/ 0[1.0]-3.0 [#] (6.0)
Late resorptions	0	0	0	0.11/0-1	0.05	0-0.1/ 0-1.0
Fetal weight [g]	5.03/ 4.45-5.58	5.06/ 4.87-5.61	4.88/ 4.57-5.14	4.86/ 4.62-5.16	5.03	4.90-5.13/ 2.3-6.3
Pre-implantation loss (%)	11.57/ 0[6.67]- 61.54	13.72/ 0[8.33]- 57.14	3.53/ 0[6.67]-9.09	8.31/ 0[7.14]- 53.33	7.57	6.13-10.06/ 0[6.67]-46.15 ^{##} (61.54)
Resorption rate [%]	7.30/ 0[7.69]- 20.00	2.86/ 0[8.33]- 9.09	6.29/ 0[7.69]- 18.18	7.64/ 0[6.67]- 36.36	5.70	2.30-7.29/ 0[7.69] -23.08 [#] (54.55)

+ non-pregnant animals, animals with resorptions only and prematurely died animals excluded

one outlier (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlier included

one outlier (No. 222) excluded (in brackets: No. 222 included); mean calculated with the outlier included

[] the lowest number greater than 0

Offspring (Malformations, Variations, etc.)

No fetal variations or malformations were observed in the study.

BI 10773 and Metformin: Study for effects on Embryo-Fetal Development in Rats (Oral Administration by Gavage)

Study no.: 12b056, U13-2227-01
 Study report location: eCTD
 Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co. KG
 Birkendorfer Str. 65
 88397 Biberach an der Riss, Germany
 Date of study initiation: May 10th 2012 (animal arrival)
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: BI 10773 (b) (4) 1045396 and 99.6%
 Metformin HCL, 000902 and 99.7%

Key Study Findings

- Maternal toxicity was manifest as reduced body weight, body weight gain and reduced food consumption at $\geq 100/200$ mg/kg empagliflozin/metformin.
- Co-administration of 300/600 mg/kg empagliflozin/metformin exacerbated reduced body weight, body weight gain and food consumption.
- Early, late and total resorptions were increased in the empagliflozin alone (300/0 mg/kg) dams.
- The malformations microglossia and micrognathia was present in one 300/0 mg/kg (empagliflozin alone) fetus.
- Bilateral flat and thickened rib was dose-dependently increased and exacerbated in the empagliflozin and metformin co-administered groups. Other skeletal malformations were present only in the high dose 300/600 mg/kg empagliflozin/metformin group (e.g. scapula bent inwardly) usually only in 1-3 fetuses and one litter.
- The embryo-fetal NOAEL is 100/200 mg/kg (14x/4x MRHD) due to the increase in skeletal variations and malformations at 300/600 mg/kg empagliflozin/metformin.
- The maternal NOAEL is 30/60 mg/kg empagliflozin/metformin due to reduced body weight, body weight gain and food consumption at higher doses. This is equivalent to 3-4x MRHD for empagliflozin and 1x MRHD for metformin.

Methods

Doses: Empagliflozin (e)/Metformin (m): 0/0, 30/60, 100/200, 300/600, 300/0 and 0/600 mg/kg

Frequency of dosing: Daily GD 7-16
Dose volume: 10 mL/kg
Route of administration: Per os (gavage)
Formulation/Vehicle: 0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain: Rat/Crl:WI (Han) (Wistar)
Number/Sex/Group: 36 females/group (24 females in main study and 12 females in TK and blood glucose group)
Satellite groups: 12 females in TK and blood glucose group
Study design: Pregnant females treated QD via oral gavage from GD 7 -16. At each of GD 7 and 16, 6 satellite pregnant females per group, were used to measure TK and 6 additional satellite pregnant females per group were used to measure blood glucose. Laparohysterectomy and necropsy were at GD 22. Fetuses were examined for external, visceral and skeletal variations and malformations. Selected fetal tissues examined histologically.

Deviation from study protocol: Not provided by the sponsor

Observations and Results

Reviewer note: the sponsor's study design and animal number table is below. Of note females #101, 111, 116, 211, 213, 220, 234, 235, 308, 411, 412, 511, 522, 525, 610, 615, 629 and 634 were non-gravid.

Table 39. Sponsor's Study Design and Animal Number Designation Table

Group	Females/group	Daily dose [mg/kg]			Animal numbers ^a	
		BI 10773 (b) (4)	Metformin HCl	Metformin BS	Main study	Toxicokinetics/ Clinical chemistry
1	36	0	0	0	101-106, 113, 114, 118-120, 124-136	107-112, 115-117, 121-123
2	36	30	60	46.8	201-206, 213, 214, 218-220, 224-236	207-212, 215-217, 221-223
3	36	100	200	156	301-306, 313, 314, 318-320, 324-336	307-312, 315-317, 321-323
4	36	300	600	468	401-406, 413, 414, 418-420, 424-436	407-412, 415-417, 421-423
5	36	300	0	0	501-506, 513, 514, 518-520, 524-536	507-512, 515-517, 521-523
6	36	0	600	468	601-606, 613, 614, 618-620, 624-636	607-612, 615-617, 621-623

^a non-pregnant main study animals: 101, 213, 220, 234, 235, 525, 629, 634; non-pregnant satellite animals: 111, 116, 211, 308, 411, 412, 511, 522, 610, 615

Mortality

None.

Clinical Signs

Clinical signs were unremarkable.

Body Weight

Body weight and body weight gain were unremarkable in the 30/60 mg/kg and 100/200 mg/kg empagliflozin and metformin groups except for a minimal decrease in body weight gain in the 100/200 mg/kg empagliflozin and metformin group at GD 8 (see sponsor's figure below).

Mean body weight was unremarkable in the metformin alone (0/ 600 mg/kg) group at GD 7-11. However, mean body weight was significantly reduced 3-4% at GD 13-16 in this group and correlated with reduced food consumption at GD 14. In addition, body weight gain in the metformin alone (0/ 600 mg/kg) females was significantly reduced 55-62% from GD 8-10 and became more tolerable as the duration of the study progressed as the body weight gain was significantly reduced 27-33% from GD 11-16. The body weight gain remained reduced at 9% at GD 21 (see sponsor's figure and table below).

Table 40. Metformin Body Weight (sponsor's table)

Body wt. [g]							
female							
Obs. time	[day]	1	7	8	9	10	11
Metformin	n	21	21	21	21	21	21
468 [mg/kg]	mv	218.57	235.53	236.74	237.50	239.30	244.29
	sd	9.02	7.69	7.90	9.00	8.92	8.62
	p	0.6386	0.7729	0.3995	0.2094	0.1060	0.2307
Body wt. [g]							
female							
Obs. time	[day]	12	13	14	15	16	21
Metformin	n	21	21	21	21	21	21
468 [mg/kg]	mv	247.51	249.28	251.75	254.32	260.03	313.36
	sd	9.31	9.44	10.04	10.23	10.03	15.15
	p	0.0885	*0.0347	*0.0223	*0.0099	*0.0137	0.1229

Table 41. Metformin Body Weight Gain (sponsor's table)

Body wt. [g]							
female							
Obs. time	[day]	1	8	9	10	11	12
Metformin	n	21	21	21	21	21	21
468 [mg/kg]	mv	-16.97	1.21	1.97	3.77	8.76	11.98
	sd	2.92	2.98	3.36	3.16	3.83	4.32
	p	0.0817	*0.0465	*0.0016	*<0.0001	*0.0091	*0.0001
Body wt. [g]							
female							
Obs. time	[day]	13	14	15	16	21	
Metformin	n	21	21	21	21	21	
468 [mg/kg]	mv	13.74	16.21	18.79	24.50	77.82	
	sd	4.59	5.08	5.84	5.73	12.81	
	p	*<0.0001	*<0.0001	*<0.0001	*<0.0001	*0.0421	

Mean body weight was unremarkable in the empagliflozin alone (300/0 mg/kg) group, however the body weight gain was significantly reduced at GD 8 (79%) and 9 (47%) (see sponsor's tables below). The co-administration of empagliflozin and metformin (300/600 mg/kg) exacerbated the reduced body weight and body weight gain. Mean body weight was significantly reduced 7-8% from GD 9-16 and remained reduced 4% at GD 21 (see sponsor's table below). Body weight gain was also significantly reduced 79% and 47% at GD 8 and 9, respectively, in the 300/600 mg/kg females (see sponsor's table and figures below). The body weight gain remained reduced (59-96%) from GD 13-16 and became more tolerable as the duration of the study progressed and the body weight gain was significantly reduced 17% at GD 21 (see sponsor's tables below). The reduced body weight/body weight gain in the 300/600 mg/kg group correlated with reduced food consumption at GD 14.

Table 42. Empagliflozin Alone (300 mg/kg) and Empagliflozin/Metformin 300/600 mg/kg Body Weight (sponsor's table)

Body wt. [g]

female

Obs. time	[day]		1	7	8	9	10	11
300:468	n		24	24	24	24	24	24
<multi comp.>	mv		233.58	237.56	240.94	246.22	250.78	307.92
	sd		10.30	11.80	11.23	11.39	13.77	17.37
	p		*<0.0001	*<0.0001	*<0.0001	*<0.0001	*<0.0001	*0.0092
BI 10773 (b) (4)	n		23	23	23	23	23	23
300 [mg/kg]	mv		249.15	251.87	255.81	259.29	264.47	316.38
	sd		14.86	13.99	16.35	15.99	15.84	20.59
	p		0.1988	0.1476	0.2086	0.1715	0.1537	0.3105

Body wt. [g]

female

Obs. time	[day]		12	13	14	15	16	21
300:468	n		24	24	24	24	24	24
<multi comp.>	mv		233.58	237.56	240.94	246.22	250.78	307.92
	sd		10.30	11.80	11.23	11.39	13.77	17.37
	p		*<0.0001	*<0.0001	*<0.0001	*<0.0001	*<0.0001	*0.0092
BI 10773 (b) (4)	n		23	23	23	23	23	23
300 [mg/kg]	mv		249.15	251.87	255.81	259.29	264.47	316.38
	sd		14.86	13.99	16.35	15.99	15.84	20.59
	p		0.1988	0.1476	0.2086	0.1715	0.1537	0.3105

Table 43. Empagliflozin Alone (300 mg/kg) and Empagliflozin/Metformin 300/600 mg/kg Body Weight Gain (sponsor's table)

Body wt. [g]

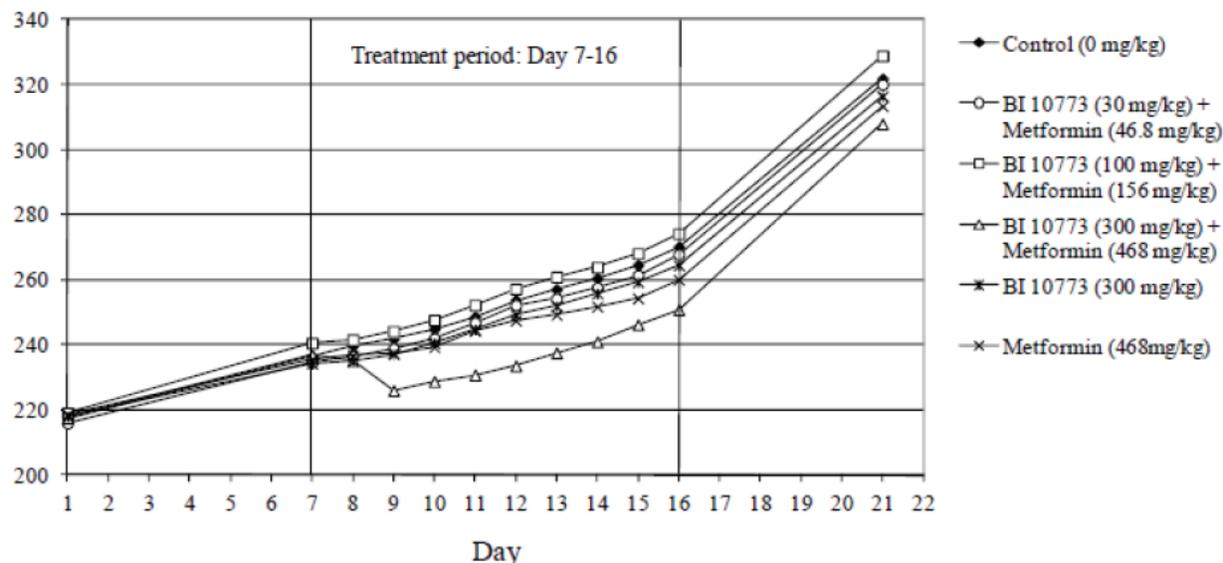
female

Obs. time	[day]		1	8	9	10	11	12
300:468	n		24	24	24	24	24	24
<multi comp.>	mv		-19.44	-1.75	-10.93	-8.17	-6.18	-3.35
	sd		6.26	4.75	4.85	4.58	5.26	6.04
	p		0.9805	*<0.0001	*<0.0001	*<0.0001	*<0.0001	*<0.0001
BI 10773 (b) (4)	n		23	23	23	23	23	23
300 [mg/kg]	mv		-16.36	0.68	2.83	6.30	10.43	14.85
	sd		4.16	2.71	2.85	3.11	3.77	3.24
	p		*0.0265	*0.0106	*0.0149	0.0548	0.1854	0.0701

Table 43. Empagliflozin Alone (300 mg/kg) and Empagliflozin/Metformin 300/600 mg/kg Body Weight Gain (sponsor’s table) - continued

Body wt. [g]						
female						
Obs. time	[day]	13	14	15	16	21
300:468	n	24	24	24	24	24
<multi comp.>	mv	0.63	4.01	9.29	13.85	70.99
	sd	7.84	6.95	6.00	7.52	11.32
	p	*<0.0001	*<0.0001	*<0.0001	*<0.0001	*<0.0001
BI 10773 (b) (4)	n	23	23	23	23	23
300 [mg/kg]	mv	17.57	21.52	24.99	30.18	82.08
	sd	3.27	4.20	4.58	4.58	9.91
	p	0.0501	0.1011	0.0530	*0.0392	0.3708

Figure 10. Post-mating Mean Body Weight (sponsor’s figure)



Feed Consumption

Food consumption was unremarkable in the 30/60 mg/kg group. Food consumption was significantly increased 6-15% at GD 7-21 in the 100/200 mg/kg group. Food consumption was also significantly increased 9% at GD 21 in the empagliflozin alone (300/0 mg/kg) group. Conversely, treatment with metformin alone (0/600 mg/kg) resulted in a significant 10% reduction of food consumption at GD 14. The co-administration of empagliflozin and metformin at 300/600 mg/kg exacerbated the reduction of food consumption to 24% at GD 14 (see sponsor’s table below).

Table 44. Food Consumption (sponsor's table)

Food cons. [g]

female

Obs. time	[day]		7	14	21
Group					
Control	n		23	23	23
0 [mg/kg]	mv		113.40	142.08	154.53
	sd		9.63	11.54	10.21
30:46.8	n		20	20	20
<multi comp.>	mv		111.28	143.47	158.59
	sd		9.45	13.17	11.31
	p		0.4439	0.6992	0.3395
100:156	n		24	24	24
<multi comp.>	mv		119.81	151.00	178.13
	sd		9.26	13.63	14.11
	p		*0.0163	*0.0099	*<0.0001
300:468	n		24	24	24
<multi comp.>	mv		116.57	108.65	176.76
	sd		8.71	11.42	20.11
	p		0.2315	*<0.0001	*<0.0001
BI 10773 ^{(b)(4)}	n		23	23	23
300 [mg/kg]	mv		111.09	140.17	167.96
	sd		9.31	10.16	14.97
	p		0.3874	0.5802	*0.0013
Metformin	n		21	21	21
468 [mg/kg]	mv		114.49	128.09	149.57
	sd		7.58	9.51	7.79
	p		0.6897	*0.0001	0.2377

Clinical Chemistry: Glucose

There was a dose-dependent trend of reduced plasma glucose in the GD 7-treated dams that was not present at GD 16 (see sponsor's table below). The sponsor did not carry out a statistical analysis.

Table 45. Embryo-Fetal Development Plasma Glucose in Dams

Daily dose of BI 10773 (b)(4) Metformin HCl [mg/kg]	Group	Gestation Day	Sample time point					
			Pre-Dose	1 h	2 h	4 h	8 h	24 h
			Pre- Treatment					
0:0	1	7	9.397	10.323	11.703	12.397	12.463	12.443
		16	6.817	7.883	9.147	9.060	8.503	6.753
30:60	2	7	10.457	10.663	11.090	10.913	9.590	10.520
		16	8.110	8.557	8.067	8.937	8.557	6.950
100:200	3	7	10.623	9.477	9.590	8.550	7.090	9.087
		16	8.943	8.180	9.445	9.640	8.340	8.630
300:600	4	7	9.587	8.827	8.807	2.913	7.793	7.470
		16	7.147	8.040	8.117	9.940	7.120	5.493
300:0	5	7	10.287	9.630	9.637	8.027	8.163	9.593
		16	8.840	9.177	8.710	8.135	8.680	7.923
0:600	6	7	10.263	12.483	12.950	11.550	8.750	11.237
		16	7.650	8.973	9.807	7.697	6.777	8.620

Toxicokinetics

For the empagliflozin: metformin co-administered pregnant females, the metformin exposure (AUC_{0-24}) increased dose proportionally from low to mid dose (30/60 to 100/200 mg/kg), but was less than dose proportional in the mid to high dose exposed (100/200 to 300/600 mg/kg) females when evaluated at drug day 1 (GD 7) and drug day 10 (GD 16), respectively (see sponsor's table below). The co-administration of empagliflozin and metformin at the high dose (300/600 mg/kg) or metformin alone (0/600 mg/kg) did not interfere with the metformin exposure (AUC_{0-24}) (see sponsor's table below). C_{max} was less than dose-proportional in all co-administered empagliflozin and metformin groups and T_{max} ranged from 1-2 hours.

Table 46. Metformin TK Parameters in Embryo-Fetal Development Rats (sponsor's table)

Mean Metformin toxicokinetic parameters after oral administration of Metformin HCl in combination with BI 10773 ^{(b)(4)} on Drug Days 1 and 10 of an embryo-fetal developmental study in pregnant Wistar Han rats (n = 3 rats/group ^a)						
Parameter	Drug Day	Statistic	BI 10773 ^{(b)(4)} :Metformin HCl [mg/kg]			
			30:60	100:200	300:600	0:600
C_{max} (nM)	1	Mean	20,200	30,500 ^b	77,400	147,000
		SD	2,150	12,700	26,100	16,800
	10	Mean	19,600 ^b	80,900	156,000 ^c	133,000 ^b
		SD	1,060	5,600	NC	13,400
AUC_{0-24} (nM·h)	1	Mean	106,000	355,000 ^b	771,000	782,000
		SD	16,900	195,000	88,600	88,200
	10	Mean	149,000 ^b	568,000	1,330,000 ^c	1,330,000 ^b
		SD	9,980	67,800	NC	11,700
t_{max} (h)	1	Median	2	1 ^b	2	2
		Range	1-2	1-1	2-2	1-2
	10	Median	1.5 ^b	1	2 ^c	2 ^b
		Range	1-2	1-2	NC	2-2

^a Nonpregnant rats excluded from means.

^b N=2 pregnant rats.

^c N=1 pregnant rat.

NC= not calculated.

For empagliflozin, the sponsor states that an interfering peak in the empagliflozin chromatogram, prevented the analysis of nearly all of the 0 and 24 h plasma drug day 10 (GD 16) samples. These samples were designated a concentration of zero for the AUC_{0-24} assessment. For the empagliflozin: metformin co-administered pregnant females, the empagliflozin exposure increased less than dose proportionally when evaluated at drug day 1 (GD 7) and drug day 10 (GD 16), respectively (see sponsor's table below).

The exposure to co-administered empagliflozin: metformin at 300/600 mg/kg empagliflozin and metformin appears to increase the exposure to empagliflozin approximately 1-2-fold at drug day 10 (GD 16) when compared to the empagliflozin alone (300/0 mg/kg)-treated dams. There is also some minimal empagliflozin accumulation particularly at the high dose (300/600 mg/kg and 300/0 mg/kg) (see sponsor's table below). However, the lack of 0 and 24h empagliflozin plasma samples confounds these findings. C_{max} was less than dose-proportional in all co-administered empagliflozin and metformin groups and T_{max} ranged from 1-2 hours.

Table 47. Empagliflozin TK Parameters in Embryo-Fetal Development Rats (sponsor's table)

Mean BI 10773 toxicokinetic parameters after oral administration of BI 10773 ^{(b)(4)} in combination with Metformin HCl on Drug Days 1 and 10 of an embryo-fetal developmental study in pregnant Wistar Han rats (n = 3 rats/group ^a).						
Parameter	Drug Day	Statistic	BI 10773 ^{(b)(4)} Metformin HCl [mg/kg]			
			30:60	100:200	300:600	300:0
C _{max} (nM)	1	Mean SD	5,220 874	17,700 ^b 2,400	23,100 6,740	14,200 854
	10	Mean SD	3,620 ^b 658	15,800 3,560	66,000 ^c NC	19,900 ^b 2,970
AUC _{all} ^d (nM•h)	1	Mean SD	15,900 1,300	44,500 ^b 250	109,000 23,600	85,400 8,270
	10	Mean SD	12,500 ^b 64.0	41,900 7460	163,000 ^c NC	79,800 ^b 2,480
AUC ₀₋₂₄ ^e (nM•h)	1	Mean SD	15,900 1,300	44,500 ^b 250	109,000 23,600	85,400 8,270
	10	Mean SD	16,700 ^b 6,000	64,600 9,470	231,000 ^c NC	126,000 ^b 16,100
t _{max} (h)	1	Median Range	2 2-2	1 ^b 1-1	1 1-2	2 1-2
	10	Median Range	2 ^b 2-2	2 2-2	2 ^c NC	1.5 ^b 1-2

^a The toxicokinetic parameters of non-pregnant rats were excluded from the means.

^b N=2 pregnant rats.

^c N=1 pregnant rat.

^d AUC_{all} was calculated using only reportable values from bioanalysis.

^e AUC₀₋₂₄ was calculated using 0- and 24-h values of 0 nM for rats without reportable 0- and 24-h concentrations.

NC= not calculated.

Dosing Solution Analysis

Within nominal concentrations.

Necropsy

One 0:600 mg/kg empagliflozin and metformin female dam was found with an enlarged placenta with four fetuses (see sponsor's table below).

Table 48. Necropsy Findings

Animal No.	Finding
G 1, 0 mg/kg	
All females	no finding
G 2, 50:100 mg/kg BI 10773 ^{(b)(4)}	Metformin HCl
All females	no finding
G 3, 200:400 mg/kg BI 10773 ^{(b)(4)}	Metformin HCl
All females	no finding
G 4, 300:600 mg/kg BI 10773 ^{(b)(4)}	Metformin HCl
All females	no finding
G 5, 300:0 mg/kg BI 10773 ^{(b)(4)}	Metformin HCl
All females	no finding
G 6, 0:600 mg/kg BI 10773 ^{(b)(4)}	Metformin HCl
631	Enlarged placenta in 4 fetuses (uterus pos. L04, R01, R02, R04)

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Early, late and total resorptions were significantly increased, greater than the concurrent control and historical control range in the 300/0 mg/kg empagliflozin alone group (see sponsor's table below). This in turn, significantly increased the resorption rate in the same group. Fetal body weight was significantly reduced in the 300:600 mg/kg empagliflozin and metformin group and correlated with reduced body weight/body weight gain observed in the dams.

Table 49. Selected Litter/Necropsy Parameters (sponsor's table)

	Daily dose of BI 10773:Metformin HCl [mg/kg]						Spontaneous Incidences from Evaluation Study [U03-1549] mean/range	
	Control	30:60	100:200	300:600	300:0	0:600		
n litters +	23	20	24	24	23	21	85	
Litter parameters (means/individual range per dam)							overall means	ranges of means/individual data
Corpora lutea	12.0/ 5-16	12.0/ 8-15	12.4/ 3-16	12.3/ 8-15	11.7/ 9-15	12.1/ 5-14	12.0	11.8-12.3/ 9.0-15.0
Implantations	11.3/ 5-14	11.3/ 8-15	11.5/ 3-16	11.5/ 6-15	11.1/ 9-15	11.2/ 5-14	11.1	10.9-11.4/ 5.0-15.0
Viable fetuses	10.8/ 5-14	10.7/ 7-15	11.1/ 2-16	10.7/ 5-14	9.9/ 5-14	10.5/ 4-14	10.5	10.1-10.7/ 5.0-14.0
Dead fetuses	0.0/ 0-1	0.1/ 0-1	0	0	0	0	0	0
Fetal sex (%)								
Male	47.77/ 20.00- 80.00	47.88/ 25.00- 85.71	50.19/ 25.00- 100.00	48.10/ 30.77- 70.00	44.68/ 10.00- 70.00	52.50/ 20.00- 77.78	49.41	46.92-52.18/ 18.18-80.0
Female	52.23/ 20.00- 80.00	52.12/ 14.29- 75.00	49.81/ 0.00- 75.00	51.90/ 30.00- 69.23	55.32/ 30.00- 90.00	47.50/ 22.22- 80.00	50.59	47.82-53.08/ 20.0-81.82
Total resorptions	0.48/ 0[1]-2	0.55/ 0[1]-2	0.33/ 0[1]-2	0.79/ 0[1]-2	1.22* †/ 0[1]-6	0.71/ 0[1]-3	0.65	0.3-0.8/ 0[1.0]-3.0# (6.0)
Early resorptions	0.48/ 0[1]-2	0.55/ 0[1]-2	0.33/ 0[1]-2	0.75/ 0[1]-2	1.09* †/ 0[1]-4	0.71/ 0[1]-3	0.60	0.3-0.7/ 0[1.0]-3.0# (6.0)
Late resorptions	0	0	0	0.04/ 0-1	0.13* †/ 0[1]-2	0	0.05	0-0.1/ 0-1.0
Fetal weight [g]	5.01@/ 4.65-5.65	5.08/ 4.74-5.58	5.13/ 4.73-5.77	4.85* †/ 4.06-5.48	5.09/ 4.41-5.62	5.03/ 4.31-5.58	5.03	4.90-5.13/ 2.3-6.3
Pre-implantation loss (%)	4.64/ 0[7.14]- 21.43	5.70/ 0[7.69]- 33.33	7.56/ 0[7.14]- 38.46	6.22/ 0[7.69]- 25.00	4.35/ 0[7.14]- 18.18	7.33/ 0[7.69]- 33.33	7.57	6.13-10.06/ 0[6.67]-46.15## (61.54)
Resorption rate [%]	3.90/ 0[8.33]- 18.18	5.19/ 0[7.69]- 22.22	4.16/ 0[7.14]- 33.33	7.21/ 0[6.67]- 18.18	10.39* †/ 0[6.67]- 54.55	7.06/ 0[7.69]- 37.50	5.70	2.30-7.29/ 0[7.69]-23.08# (54.55)

* significant difference (p<0.05)

† increased

‡ decreased

+ non-pregnant animals and animals with resorptions only excluded

one outlier (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlier included

one outlier (No. 222) excluded (in brackets: No. 222 included); mean calculated with the outlier included

[] the lowest number greater than 0

@ Body weight of fetus 103R04 was excluded from the calculation because it was accidentally injected during anesthesia of its mother.

Offspring (Malformations, Variations, etc.)

No external variations were observed.

Visceral Variations

The sponsor reports visceral variations that were present in all groups including the control group as per the following sponsor's text:

- one additional vessel at the right pulmonary artery
- shortened truncus brachiocephalicus
- no distance between the carotids
- lobus accessorius dexter of lung small
- lobus accessorius dexter of lung missing
- additional hepatic lobe on lobus hepatis medialis dexter
- dilated ureter, unilateral
- bowed ureter, unilateral
- kinked ureter, unilateral

In the 0/600 mg/kg metformin alone group, the following visceral variations were greater than the concurrent control (and on occasion the historical control range): enlarged distance between carotids; distance between the left A. carotis and left A. subclavian enlarged and bowed ureter (see sponsor's table below).

Table 50. Visceral Variations (sponsor's table)

Findings	Daily dose of BI 10773 ^{(b)(4)} Metformin HCl [mg/kg]						Spontaneous incidences from evaluation study [U03-1549]
	G 1	G 2	G3	G4	G5	G6	
	Control	30:60	100:200	300:600	300:0	0:600	
n fetuses / incidences (%)							Decimals rounded
Decimals rounded							
Total number of litters	23	20	24	24	23	21	85
Total number of fetuses	248	213	267	257	228	221	889
Runts	0	2/0.94	0	0	1/0.44	0	2/0.22
Variations							
Visceral Variations	Decimals truncated						Decimals rounded
Number of litters	23	20	24	24	23	21	85
Number of fetuses	116	100	127	123	108	106	424
Small distance between the carotids	0	0	3/2.36	1/0.81	3/2.77	1/0.94	5/1.18 6/5.50 ⁺
Enlarged distance between the carotids	0	0	0	1/0.81	0	<u>2/1.88</u>	1/0.24 1/1.17 ⁺⁺⁺
Distance between left A. carotis and left A. subclavia small	1/0.86	1/1.00	2/1.57	3/2.43	1/0.92	3/2.83	7/1.65 12/11.00 ⁺
Distance between left A. carotis and left A. subclavia enlarged	0	0	1/0.78	0	0	<u>1/0.94</u>	3/0.71 1/0.91 ⁺
No distance between left A. carotis and left A. subclavia	2/1.72	6/6.00	2/1.57	2/1.62	4/3.70	1/0.94	5/1.18 7/6.42 ⁺
Bowed ureter	bil	0	0	1/0.78	0	1/0.92	<u>1/0.94</u> 1/0.24 1/0.91 ⁺ 1/0.92 ⁺⁺

Findings at incidences above the actual Control group and above spontaneous incidences from evaluation study [U03-1549] or Control groups from other embryo-fetal development studies underlined and in bold letters

uni = unilateral

bil = bilateral

⁺ = data from Control group of [U10-2386-01](#), 21 litters, 109 fetuses

⁺⁺ = data from Control group of [U12-2018-01](#), 20 litters, 108 fetuses

⁺⁺⁺ = data from Control group of [U10-2448-01](#), 19 litters, 85 fetuses

Skeletal Variations

The sponsor reports skeletal variations that were present in all groups including the control group as per the following sponsor's text:

- frontal bone partly not ossified, bilateral
- fontanelle enlarged
- parietal bone partly not ossified, bilateral
- interparietal bone partly not ossified
- squamosal bone partly not ossified, unilateral
- processus zygomaticus of maxillar bone partly not ossified, uni- and bilateral
- processus zygomaticus of squamosal bone partly not ossified, bilateral
- processus zygomaticus of maxillar bone and jugal bone fused, bilateral
- orbitosphenoidal bone not ossified, unilateral
- ossification centres of bulla ossea <3, unilateral
- basisphenoidal bone partly not ossified
- hyoid bone partly not ossified
- early onset of ossification of processus coracoideus, unilateral
- cervical vertebral arch partly not ossified, bilateral
- cervical rib, bilateral
- thoracal vertebral arch partly not ossified
- rib nodulated proximal end, unilateral
- rib nodulated distal, unilateral
- sternebra unilaterally ossified
- additional sternebra
- lumbar vertebral body flat
- lumbar rib, bilateral
- caudal vertebral body partly not ossified

Other skeletal variations above the concurrent control are shown in the following sponsor's table (see below). Of note was the high number of fetuses/incidence with skeletal variations in either the 300/600 mg/kg empagliflozin and metformin group or the metformin alone (0:600 mg/kg) group or both groups. The maternal toxicity of reduced body weight/body weight gain and reduced food consumption in these two groups, likely led to an intrauterine growth delay with a likely increase in skeletal variations.

Table 51. Skeletal Variations (sponsor's table)

Findings	Daily dose of BI 10773 ^{(b) (4)} Metformin HCl [mg/kg]						Spontaneous incidences from evaluation study [U03-1549]	
	G 1	G 2	G3	G4	G5	G6		
	Control	30:60	100:200	300:600	300:0	0:600		
n fetuses / incidences (%)								
Variations								
Skeletal Variations		Decimals truncated						Decimals rounded
Number of litters		23	16 [§]	23 [§]	21 [§]	21 [§]	19 [§]	85
Number of fetuses		133	89 [§]	135 [§]	117 [§]	111 [§]	106 [§]	465
Frontal bone partly not ossified	uni	0	2/2.24	1/0.74	0	0	0	9/1.94 (bil + uni) 2/1.63 [†] 5/4.80 [‡]
Parietal bone partly not ossified	uni	2/1.50	2/2.24	<u>5/3.70</u>	3/2.56	2/1.80	<u>4/3.77</u>	2/0.44 3/2.50 ^{††} 4/3.30 ^{‡‡}
Supraoccipital bone partly not ossified		4/3.00	5/5.61	2/1.48	7/5.98	1/0.90	0	18/3.87 7/5.83 ^{††} 9/7.89 [*]
Mandibular bone partly not ossified	bil	36/ 27.06	28/ 31.46	34/ 25.18	41/ 35.04	23/ 20.72	33/ 31.13	110/23.66 (bil + uni) 39/37.49 [‡]
	uni	15/ 11.27	10/ 11.23	18/ 13.33	11/ 9.40	7/ 6.30	7/ 6.60	110/23.66 (bil + uni) 15/14.42 [‡]
Jugal bone partly not ossified	bil	1/0.75	0	3/2.22	1/0.85	0	0	8/1.72 3/2.50 ^{††}
	uni	0	<u>2/2.24</u>	2/1.48	1/0.85	1/0.90	0	5/1.08 2/1.68 ^{**}
Squamosal bone partly not ossified	bil	1/0.75	6/6.74	2/1.48	3/2.56	0	0	19/4.09 5/9.25 [§]
Processus zygomaticus of squamosal bone partly not ossified	uni	1/0.75	4/4.49	2/1.48	1/0.85	2/1.80	0	3/0.65 4/4.49 ^{†††}

Findings at incidences above the actual Control group and above the spontaneous incidences from evaluation study U03-1549 or Control groups from other embryo-fetal development studies underlined and in bold letters

uni = unilateral

bil = bilateral

[§] differing number of litters and fetuses for the skeletal observation due to a relocation accident (b) (4) (affected litters 201, 203, 205, 206, 304, 401, 405, 406, 501, 503, 603, 604; the findings are listed in Tables 11.2.2: 4 and 11.2.2: 5)

[†] = data from Control group of U10-2386-01, 21 litters, 122 fetuses

^{††} = data from Control group of U12-2018-01, 20 litters, 120 fetuses

^{†††} = data from Control group of U10-2448-01, 19 litters, 89 fetuses

[‡] = data from Control group of U07-1325, 20 litters, 104 fetuses

^{‡‡} = data from Control group of U11-1920-01, 21 litters, 121 fetuses

^{*} = data from Control group of U06-1637, 21 litters, 114 fetuses

^{**} = data from Control group of U08-1715-01, 22 litters, 119 fetuses

[§] = data from Control group 2 (Natrosol) of U11-1221-01, 9 litter, 54 fetuses

continued

Table 51. Skeletal Variations (sponsor's table) - continued

Findings		Daily dose of BI 10773 ^{(b) (4)} Metformin HCl [mg/kg]					Spontaneous incidences from evaluation study [U03-1549]	
		G1	G2	G3	G4	G5		G6
		Control	30:60	100:200	300:600	300:0		0:600
n fetuses / incidences (%)								
Variations								
Skeletal Variations		Decimals truncated					Decimals rounded	
Number of litters		23	16 [§]	23 [§]	21 [§]	21 [§]	19 [§]	85
Number of fetuses		133	89 [§]	135 [§]	117 [§]	111 [§]	106 [§]	465
Processus zygomaticus of maxillar bone and jugal bone fused	uni	6/4.51	2/2.24	1/0.74	<u>6/5.12</u>	5/4.50	2/1.88	0
Orbitosphenoidal bone not ossified	bil	4/3.00	2/2.24	0	<u>7/5.98</u>	2/1.80	3/2.83	0
Ossification centres of bulla ossea <3	uni	2/1.50	0	0	<u>6/5.12</u>	1/0.90	1/0.94	28/6.02 (bil + uni)
Occipital bone not ossified		6/ 4.51	3/ 3.37	1/ 0.74	<u>24/ 20.51</u>	0	6/ 5.66	11/2.37 11/9.16 ^{††}
Cervical vertebral bodies <7		15/ 11.27	7/ 7.86	9/ 6.66	<u>22/ 18.80</u>	12/ 10.81	8/ 7.54	76/16.34
Cervical vertebral body asymmetrically ossified		10/ 7.51	3/ 3.37	17/ 12.59	16/ 13.67	8/ 7.20	11/ 10.37	0 27/22.68 ^{**}
Cervical vertebral body dumbbell-shaped		0	2/2.24	5/3.70	4/3.41	0	1/0.94	10/2.15 17/14.28 ^{**}
Cervical rib	uni	7/5.26	5/5.61	5/3.70	11/9.40	4/3.60	3/2.83	31/6.67 14/11.66 ^{††}
Thoracal vertebral body flat		1/0.75	0	0	2/1.70	3/2.70	2/1.88	0 7/5.88 ^{**}
Thoracal vertebral body asymmetrically ossified		1/0.75	0	0	1/0.85	0	1/0.94	0 3/2.52 ^{**}
Sternebrae <6		0	<u>1/1.12</u>	0	<u>1/0.85</u>	0	0	2/0.43
Sternebra partly not ossified		20/ 15.03	<u>18/ 20.22</u>	12/ 8.88	<u>21/ 17.94</u>	17/ 15.31	<u>22/ 20.75</u>	0 9/15.78 ^{***}
Sternebra asymmetrically ossified		2/1.50	1/1.12	5/3.70	2/1.70	2/1.80	3/2.83	11/2.37 6/4.76 ^{***}
Dorsal-ventrally split sternebra		0	0	1/0.74	<u>2/1.70</u>	1/0.90	0	1/0.22 2/1.63 [†]

Findings at incidences above the actual Control group and above the spontaneous incidences from evaluation study U03-1549 or Control groups from other embryo-fetal development studies underlined and in bold letters

uni = unilateral

bil = bilateral

[§] differing number of litters and fetuses for the skeletal observation due to a relocation accident; ^{(b) (4)} (affected litters 201, 203, 205, 206, 304, 401, 405, 406, 501, 503, 603, 604; the findings are listed in Tables 11.2.2: 4 and 11.2.2: 5)

[†] = data from Control group of U10-2386-01, 21 litters, 122 fetuses

^{††} = data from Control group of U12-2018-01, 20 litters, 120 fetuses

^{***} = data from Control group of U09-1990-01, 22 litters, 126 fetuses

^{**} = data from Control group of U08-1715-01, 22 litters, 119 fetuses

^{***} = data from Control group of U07-1814, 10 litters, 57 fetuses

continued

Table 51. Skeletal Variations (sponsor's table) - continued

Findings	Daily dose of BI 10773 ^{(b)(4)} Metformin HCl [mg/kg]						Spontaneous incidences from evaluation study [U03-1549]	
	G 1	G 2	G3	G4	G5	G6		
	Control	30:60	100:200	300:600	300:0	0:600		
n fetuses / incidences (%)								
Variations								
Skeletal Variations		Decimals truncated					Decimals rounded	
Number of litters		23	16 [§]	23 [§]	21 [§]	21 [§]	19 [§]	85
Number of fetuses		133	89 [§]	135 [§]	117 [§]	111 [§]	106 [§]	465
Calcaneus not ossified	bil	60/ 45.11	35/ 39.32	52/ 38.51	<u>72/ 61.53</u>	44/ 39.63	55/ 51.88	205/44.08 63/52.94**
	uni	6/ 4.51	10/ 11.23	15/ 11.11	7/ 5.98	4/ 3.60	12/ 11.32	20/4.30 13/12.49 [†]
Metatarsalia <5	bil	2/1.50	<u>3/3.37</u>	0	<u>7/5.98</u>	<u>2/1.80</u>	<u>3/2.83</u>	R: 7/1.51 L: 6/1.29
	uni	1/0.75	<u>1/1.12</u>	0	<u>1/0.85</u>	0	<u>2/1.88</u>	
Proximal posterior phalanges <3	bil	14/ 10.52	9/ 10.11	7/ 5.18	22/ 18.80	7/ 6.30	15/ 14.15	R: 72/15.48 L: 70/15.05 25/21.92*
	uni	11/8.27	4/4.49	12/8.88	11/9.40	4/3.60	8/7.54	R: 72/15.48 L: 70/15.05 17/14.16 ^{††}
Proximal posterior phalanges not ossified	bil	20/ 15.03	8/ 8.98	6/ 4.44	<u>27/ 23.07</u>	10/ 9.00	7/ 6.60	R: 65/13.98 L: 63/13.55
	uni	3/2.25	2/2.24	5/3.70	3/2.56	2/1.80	2/1.88	R: 65/13.98 L: 63/13.55 7/5.88**

Findings at incidences above the actual Control group and above the spontaneous incidences from evaluation study U03-1549 or Control groups from other embryo-fetal development studies underlined and in bold letters

uni = unilateral

bil = bilateral

R = right

L = left

[§] differing number of litters and fetuses for the skeletal observation due to a relocation accident ^{(b)(4)} (affected litters 201, 203, 205, 206, 304, 401, 405, 406, 501, 503, 603, 604; the findings are listed in Tables 11.2.2: 4 and 11.2.2: 5)

^{††} = data from Control group of U12-2018-01, 20 litters, 120 fetuses

[†] = data from Control group of U07-1325, 20 litters, 104 fetuses

* = data from Control group of U06-1637, 21 litters, 114 fetuses

** = data from Control group of U08-1715-01, 22 litters, 119 fetuses

Malformations

Microglossia and micrognathia were noted in one fetus in the 300/0 mg/kg empagliflozin alone group. Flat and thickened rib incidence, although present in the concurrent control group fetuses, was also dose-dependently increased in the empagliflozin and metformin treated groups and also present in the empagliflozin alone (300/0 mg/kg) and metformin alone (0/600 mg/kg) groups (see sponsor's table below). The co-administration of empagliflozin and metformin appears to increase the incidence of this malformation. Cleft cervical vertebral body occurs at higher incidence in the 300/600 mg/kg empagliflozin and metformin group and 0/600 mg/kg metformin alone group. Other skeletal malformations also occurred in the 300:600 mg/kg empagliflozin and metformin group and 0:600 mg/kg metformin alone groups, but were present in a single fetus and litter (see sponsor's table below).

Table 52. Malformations (sponsor's table)

Findings	Daily dose of BI 10773 ^{(b)(4)} Metformin HCl [mg/kg]						Spontaneous incidences from evaluation study [U03-1549]	
	G 1	G 2	G3	G4	G5	G6		
	Control	30:60	100:200	300:600	300:0	0:600		
n fetuses / incidences (%)								
Malformations								
External Malformations	Decimals truncated						Decimals rounded	
Number of litters	23	20	24	24	23	21	85	
Number of fetuses	248	213	267	257	228	221	889	
Microglossia	0	0	0	0	<u>1/0.43</u> 531R06	0	0	
Micrognathia	0	0	0	0	<u>1/0.43</u> 531R06	0	0	
Skeletal Malformations	Decimals truncated						Decimals rounded	
Number of litters	23	16 [§]	23 [§]	21 [§]	21 [§]	19 [§]	85	
Number of fetuses	133	89 [§]	135 [§]	117 [§]	111 [§]	106 [§]	465	
Cleft cervical vertebral body	1/0.75	0	1/0.74	<u>5/4.27</u>	0	<u>2/1.88</u>	5/1.08	
Cervical vertebral body unilaterally ossified	2/1.50	0	1/0.74	3/2.56	0	2/1.88	1/0.22 3/2.88 [#] 4/3.27 [*]	
Cleft thoracal vertebral body	0	1/1.12	0	0	0	1/0.94	1/0.22 1/1.12 ^{***}	
Flat and thickened rib	bil	10/ 7.51	<u>7/ 7.86</u>	<u>14/ 10.37</u>	<u>23/ 19.65</u>	<u>11/ 9.90</u>	<u>11/ 10.37</u>	45/9.68 (bil + uni)
	uni	3/2.25	2/2.24	10/7.40	7/5.98	1/0.90	5/4.71	
Costal cartilage misaligned	0	0	0	<u>1/0.85</u> 427R05	0	0	0	
Sternebrae branched dorsad	0	0	0	<u>1/0.85</u>	0	<u>1/0.94</u>	0	
Sternebrae branched caudad	0	0	0	<u>1/0.85</u>	0	<u>1/0.94</u>	0	
Sternebrae fused	0	0	0	<u>1/0.85</u> 427R05	0	0	0	
Sternebra misshapen	0	0	0	<u>1/0.85</u> 427R05	0	0	0	
Femur misshapen proximal	0	0	0	0	0	<u>1/0.94</u>	0	

Findings at incidences above the actual Control group and above the spontaneous incidences from evaluation study U03-1549 or Control groups from other embryo-fetal development studies underlined and in bold letters

uni = unilateral

bil = bilateral

[§] differing number of litters and fetuses for the skeletal observation due to a relocation accident ^{(b)(4)} (affected litters 201, 203, 205, 206, 304, 401, 405, 406, 501, 503, 603, 604; the findings are listed in Tables 11.2.2: 4 and 11.2.2: 5)

^{*} = data from Control group of U10-2386-01, 21 litters, 122 fetuses

^{***} = data from Control group of U10-2448-01, 19 litters, 89 fetuses

[#] = data from Control group of U07-1325, 20 litters, 104 fetuses

Other skeletal malformations (e.g. radius bent, scapula bent inwardly) were only found in the 300:600 mg/kg empagliflozin and metformin group (see sponsor's table below).

These skeletal malformations were only found in individual fetuses in one litter with the exception of three fetuses from one litter and also lacked a correlation to dose.

Table 53. Unclassified Skeletal Malformations

Findings	Daily dose of BI 10773 ^{(b) (4)} Metformin HCl [mg/kg]						Spontaneous incidences from evaluation study [U03-1549]	
	G 1	G 2	G3	G4	G5	G6		
	Control	30:60	100:200	300:600	300:0	0:600		
n fetuses / incidences (%)								
Fetal findings without classification								
Skeletal		Decimals truncated					Decimals rounded	
Number of litters		23	16 ^s	23 ^s	21 ^s	21 ^s	19 ^s	85
Number of fetuses		133	89 ^s	135 ^s	117 ^s	111 ^s	106 ^s	465
Radius bent	uni	0	0	0	<u>1/0.85</u>	0	0	0
Scapula bent inwardly	uni	0	0	0	<u>2/1.70</u>	0	0	0 1/0.87*
Scapula and acromion process (proximal) bent inwardly	bil	0	0	0	<u>3/2.56</u>	0	0	0
	uni	0	0	0	<u>2/1.70</u>	0	0	0
Longitudinal axis of humerus distally bent to the dorsal side	uni	0	0	0	<u>1/0.85</u>	0	0	0

Findings at incidences above the concurrent Control group and above the spontaneous incidences from evaluation study U03-1549 or Control groups from other embryo-fetal development studies underlined and in bold letters

uni = unilateral

bil = bilateral

^s differing number of litters and fetuses for the skeletal observation due to a relocation accident ^{(b) (4)} (affected litters 201, 203, 205, 206, 304, 401, 405, 406, 501, 503, 603, 604; the findings are listed in Tables [11.2.2: 4](#) and [11.2.2: 5](#))

* = data from Control group of [U06-1637](#), 21 litters, 114 fetuses

11 Integrated Summary and Safety Evaluation

The sponsor is seeking the approval of empagliflozin (Jardiance[®]) as a fixed dose combination (FDC) with metformin immediate release (Glucophage[®]). Empagliflozin is a selective inhibitor of sodium-glucose co-transporter 2 (SGLT2), approved by the FDA August 1st 2014, under NDA 204629. Empagliflozin exerts its pharmacological activity by inhibiting SGLT2, the major transporter responsible for renal glucose absorption. By inhibiting renal glucose reabsorption, the urinary excretion of glucose is enhanced, thereby reducing fasting and postprandial glucose levels, in a mechanism that is independent of insulin. Thus, there is extensive nonclinical experience with empagliflozin that has been reviewed under NDA 204629.

Metformin immediate release (IR) is a biguanide class insulin sensitizer antihyperglycemic agent approved by the FDA March 3rd 1995. Metformin lowers both basal and postprandial glucose by decreasing hepatic glucose production and intestinal glucose absorption. Metformin also improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Thus, there is extensive clinical experience for the use of metformin in T2DM in adults and children.

Nonclinical studies with the fixed-dose combination (FDC) were not performed. Toxicity unique to the combination of empagliflozin and metformin was evaluated in rats co-administered each drug separately and in combination in pivotal 2 week rat, 3 month rat and dose-range finding and definitive rat embryo-fetal development (EFD) studies. The sponsor also submitted a metformin only 2-week rat study and a rat embryo-fetal development study in support of their application. All nonclinical studies were conducted using the oral administration of the drug, which is the clinical route of exposure.

Nonclinical exposure margins are based on the current proposed maximum recommended human dose (MRHD) of 25 mg empagliflozin ($AUC_{0-24h,ss} = 4740 \text{ nM}\cdot\text{h}$) and 2000 mg metformin ($AUC_{0-24 \text{ h, ss}} = 159 \text{ }\mu\text{M}\cdot\text{h}$).

Impurities and Degradants

(b) (4)
These degradants/impurities are of little toxicological concern as they were qualified and found to be negative by (Q)SAR analysis for a bacterial mutation test or were qualified by their use in nonclinical toxicology studies of 4- and 13-weeks as described in the review of the empagliflozin monotherapy NDA.

Pharmacology Studies With Co-Administered Empagliflozin and Metformin

Proof of concept studies in single dose and multiple dose diabetic mice (ZDF rat model) for up to 28-days, showed the improvement in glucose excursion and reduction of HbA1c following an oral glucose tolerance test. The co-administration of empagliflozin and metformin caused a greater reduction of blood glucose and HbA1C compared to the individual components alone.

Toxicology Studies With Metformin Alone

Pivotal repeat dose studies with metformin were conducted in the Wistar (Han) rat for 2 weeks at 1-18x MRHD (2000 mg). Cardiovascular toxicity at the high dose (1000 mg/kg) with correlative increases in heart weight and heart to body/brain weight ratios was identified and considered adverse. The NOAEL is 200 mg/kg (2x MRHD).

Toxicology Studies With Co-Administered Empagliflozin and Metformin

Pivotal toxicology studies were conducted to bridge the potential toxicity of the combination treatment of empagliflozin and metformin. No unexpected or synergistic toxicity was identified in the co-administration studies at clinically relevant exposures to empagliflozin and metformin. However, at high exposure multiples of empagliflozin (>51x MRHD) co-administration with metformin in the rat (7x MRHD), resulted in moribundity and mortality at doses of empagliflozin that were not associated with these toxicities when administered alone.

In the 2-week study rat, the high dose of 500/1000 mg/kg empagliflozin and metformin was not tolerated and resulted in both mortality and moribundity in both sexes. However, the exposure at this dose is unlikely to be seen clinically and corresponds to >51x MRHD for empagliflozin and 7x MRHD for metformin, respectively. A NOAEL in the 2-week rat study was identified and corresponds to the low dose 100/200 mg/kg; which is equivalent to 13x MRHD for empagliflozin and 3x MRHD for metformin, thus providing an adequate safety margin.

Similarly, in the 90-day rat study with empagliflozin and metformin, the high dose of 200/400 mg/kg empagliflozin + metformin resulted in mortality in a single female at recovery day 30 and the cause of mortality was not identified. Although the cause the death was not identified the mortality occurred at high exposure multiples of 23x MRHD for empagliflozin and 7x MRHD for metformin, respectively.

Pharmacodynamic effects as a result of exposure to empagliflozin alone or co-administered with metformin were also evident with glucosuria and polyuria (primarily males) present. The latter may have contributed to the dose-dependent hypochloremia observed with empagliflozin alone or in combination with metformin. Treatment with co-administered empagliflozin and metformin resulted in minimal mineralization of the kidney pelvic epithelium at the high dose (200/400 mg/kg), but this was likely to be driven by empagliflozin, as this finding is also observed with the empagliflozin monotherapy in the rat, at high exposures.

There were no toxicologically adverse or toxicokinetic drug-drug interactions, with the exception of a slight increase in the exposure of empagliflozin (2-3 fold) or metformin (1-fold) when co-administered relative to the individual drugs alone. Clinically, empagliflozin has been used in T2DM subjects on a background of metformin and a pharmacokinetic drug-drug interaction has not been found. Thus, the current finding may be unique to this study in the rat.

For the co-administration of empagliflozin + metformin in the 90-day rat study, the NOAEL was the low dose 50/100 mg/kg (4-6x MRHD 25 mg empagliflozin and 2x MRHD 2000 mg metformin), thus providing an adequate safety margin for the proposed clinical use of the FDC product.

Reproductive Toxicology Studies With Metformin Alone

Reproductive toxicity was assessed in the embryo-fetal developmental study in the rat with metformin at 4-23x MRHD. Metformin at 1000 mg/kg was teratogenic at high exposures (23x MRHD) resulting in anophthalmia and polydactylia. In addition, dose-related increases in skeletal malformations in the rib (flat, thickened or z-shaped), scapula (bent inward) were seen in several litters and fetuses at ≥ 500 mg/kg. The fetal NOAEL was the low dose of 200 mg/kg (4x MRHD). A maternal NOAEL was not identified due to a dose-dependent 20-153% decrease in BW gain in all treatment groups.

Metformin is not listed as teratogenic at approximate clinical exposures based on the current label; and the co-administration with empagliflozin did not result in metformin-related malformations. However, the rat strain used in the embryo-fetal development (EFD) studies in the metformin (Glucophage[®]) label appears to be the Sprague-Dawley (SD) rat and this differs from the Wistar (Han) rat strain used in the present metformin alone and empagliflozin and metformin co-administration EFD studies. Lastly, the contribution of the maternal toxicity to malformation formation in the metformin EFD study cannot be excluded.

Reproductive Toxicology Studies With Co-Administered Empagliflozin and Metformin

Reproductive toxicity was assessed in the embryo-fetal developmental study in the rat with co-administered empagliflozin + metformin at up to 27x MRHD for empagliflozin and 8x MRHD for metformin, respectively.

The co-administration of empagliflozin and metformin was not teratogenic at 100/200 mg/kg. Higher exposure at 300/600 mg/kg (high dose) resulted in a dose-dependent increase in the skeletal malformations (flat and thickened rib). Other skeletal malformations were also present at this dose, but only in one to three fetuses of the same litter. Consequently the fetal NOAEL is 100/200 mg/kg which is equivalent to 9-14x MRHD of empagliflozin and 3-4x MRHD of metformin.

Maternal toxicity was evident due to reduced body weight, body weight gain and food consumption at $\geq 100/200$ mg/kg. Thus the maternal NOAEL is 30/60 mg/kg which is equivalent to 3-4x MRHD of empagliflozin and 1x MRHD of metformin. The co-administration of empagliflozin and metformin did not result in an exacerbation or identification of unique malformations.

In summary, no unexpected or synergistic interactions were uncovered in nonclinical studies when empagliflozin was co-administered with metformin. Toxicity in animals occurred at high clinical exposures and NOAELs at or above approximate clinical

exposures in pivotal rat 2-week, 90-day and embryo-fetal development studies were found.

Table 54. Summary of Empagliflozin and Metformin Co-Administration Toxicology Studies.

RAT TOXICOLOGY STUDIES			
SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD Empagliflozin 25 mg: 4740 nM.hr Metformin 2000 mg: 159,000 nM.hr AUC basis*	BASIS
Rat 2 weeks: 0/0 100/200 300/600 500/100 500/0 0/1000 mg/kg/day	100/200 mg/kg/day	Empagliflozin: 13x Metformin: 3x	-Mortality and moribundity at 500/100 mg/kg. -No BW/BW gain at 500/1000 mg/kg. - Renal hydronephrosis at 300/600 mg/kg and 500/1000 mg/kg.
Rat 90 days: 0/0 200/0 0/400 50/100 100/200 200/400 mg/kg/day	50/100 mg/kg/day	Empagliflozin: 4-6x Metformin: 2x	-Mortality at 200/400 mg/kg -Irreversible BW/BW gain at 200/0 mg/kg and 200/400 mg/kg -Hypochloremia at \geq 100/200 mg/kg.
Rat EFD: 0/0 30/60 100/200 300/600 300/0 0/600 mg/kg/day	Dams: 30/60 mg/kg/day Fetal: 100/200 mg/kg/day	Empagliflozin: 3x Metformin: 1x Empagliflozin: 14x Metformin: 4x	-Reduced BW, BWG and food consumption in dams at \geq 100/200 mg/kg - Skeletal malformations at \geq 300/600 mg/kg

*AUC in human: 4740 nM.hr at 25 mg/day for empagliflozin and 159,000 nM.hr at 2000 mg/day for metformin.

Table 55. Summary of Metformin Toxicology Studies.

RAT TOXICOLOGY STUDIES			
SPECIES/ STUDY	NOAEL	MULTIPLE OF MRHD* Metformin 2000 mg: 159, 000 nM.hr AUC basis*	BASIS
Rat 2 weeks: 0 100 200 1000 mg/kg/day	200 mg/kg/day	2x	-Ventricular myocardial hypertrophy and degeneration at 1000 mg/kg. -Increased heart weight, and heart to body/brain weight ratios at 1000 mg/kg.
Rat EFD: 0 200 500 1000 mg/kg/day	Dams: ND mg/kg/day Fetal: <200 mg/kg/day	<4x <4x	-Dose-dependent decreases in BW gain (20-153%) during treatment. - Anophthalmia and polydactylia at 1000 mg/kg. -Skeletal malformations at ≥ 500 mg/kg.

*AUC in human: 159,000 nM.hr at 2000 mg/day for metformin. ND – not determined

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

/s/

MUKESH SUMMAN
04/15/2015

RONALD L WANGE
04/15/2015
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206111

**Applicant: Boehringer Ingelheim Stamp Date: August 4th 2014
Pharmaceuticals Inc.**

**Drug Name:
Empagliflozin/Metformin
FDC**

NDA Type: 505(b)2

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		Co-administration of the individual components of the FDC was used in the nonclinical studies, rather than the FDC itself.
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 <u>submitted</u> a rationale to justify the alternative route?	X		Oral dose administration was used in the nonclinical studies and this is the intended route of human exposure.
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² 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		<div style="background-color: #cccccc; padding: 5px; margin-bottom: 5px;">(b) (4)</div> Thus, the structure (u) (4) was determined and the sponsor independently conducted a SAR analysis and this was negative (per the sponsor).
11	Has the applicant addressed any abuse potential issues in the submission?	n/a		Abuse of either component is not expected.
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?	n/a		Not applicable. Empagliflozin/Metformin FDC will not be marketed OTC.

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.

Reviewing Pharmacologist

Date

Team Leader/Supervisor

Date

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

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

/s/

MUKESH SUMMAN
09/18/2014

RONALD L WANGE
09/18/2014
I concur that the NDA is fileable.