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APPLICATION NUMBER:

201281Orig1s000

PHARMACOLOGY REVIEW(S)

REV-NONCLINICAL-05 (Review Noted (NAI))
NDA-201281
ORIG-1
Supporting Document 23
Resubmission/Class 1
Submit Date: 11/30/2011 - FDA Received Date: 11/30/2011

Class 1 resubmission of NDA 201281, linagliptin plus metformin HCl FDC tablets. The resubmission addresses CMC issues in the original NDA review. No nonclinical data were included in the resubmission. The pharmacology/toxicology approval recommendation and labeling recommendations from the original NDA review remain unchanged.

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/s/

DAVID B CARLSON
01/26/2012
Pharmtox approval recommendation (unchanged from original NDA review recommendation)

TODD M BOURCIER
01/26/2012

Memorandum

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Date:	15 November, 2011
NDA #	201280 201281
Sponsor:	Boehringer Ingelheim
Drug:	Tradjenta® (Linagliptin) Jentadueto® (Linagliptin + Metformin FDC)
Reviewer:	David B. Carlson, Ph.D.

Boehringer Ingelheim's drug linagliptin, a DPP4 inhibitor, was recently reviewed for treatment of type 2 diabetes mellitus as both a monotherapy and a fixed dose combination with metformin. The initial pharmacology/toxicology reviews of rabbit embryofetal development studies were completed during the IND phase and results were further reviewed and summarized in the NDA reviews. During the course of labeling discussions it became apparent that the pharmacology/toxicology reviews for NDA 201280 and NDA 201281 inadvertently listed an incorrect rabbit strain. This memo serves as an amendment to the original pharmacology/toxicology reviews to clarify that Himalayan rabbits, not New Zealand White rabbits, were used in the pivotal embryofetal development studies with linagliptin.

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/s/

DAVID B CARLSON
11/15/2011
Correction regarding rabbit strain -- no change in pharmtox conclusions or recommendations

TODD M BOURCIER
11/16/2011
Correction memo

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:	201281
Supporting document/s:	Original submission (and updates)
Applicant's letter date (CDER Stamp Date):	19 January, 2011 (1/19/11)
Product:	Linagliptin + metformin HCl FDC tablet (b) (4)
Indication:	Type 2 Diabetes Mellitus treatment
Applicant:	Boehringer Ingelheim Pharmaceuticals, Inc.
Review Division:	Metabolism and Endocrinology Products
Reviewer:	David B. Carlson, Ph.D.
Supervisor/Team Leader:	Todd Bourcier, Ph.D.
Division Director / Deputy Director:	Mary Parks, M.D. Eric Colman, M.D.
Project Manager:	Mehreen Hai, Ph.D.
Review Completion Date:	30 September, 2011

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 201281 are owned by Boehringer Ingelheim Pharmaceuticals Inc. (BIPI) or are data for which BIPI has obtained a written right of reference. Any information or data necessary for approval of NDA 201281 that BIPI does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 201281.

Review Notes and Abbreviations/Key

Some of the sponsor's tables and figures from the electronic NDA submission have been included and cited in this review. All drug-related trends are discussed in relation to concurrent vehicle control groups in each study unless otherwise noted. Drugs were administered by oral gavage suspension in 0.5% hydroxyethylcellulose (Natrosol® 250 HX) unless otherwise noted. Common animal strains were used and abbreviated by common animal name, unless noted, as follows: Wistar Han rat, CD-1 mouse, Beagle dog, Cynomolgus monkey, New Zealand White rabbit. Results and conclusions of some studies previously reviewed by this reviewer under NDA 201280 are cited and summarized in this NDA review.

Key: Linagliptin (lina., BI 1356); metformin HCl (metformin, met.); Dipeptidyl peptidases – DPP4 (aka DPP-4), DPP2, DPP8, DPP9; type 2 diabetes mellitus (T2DM); USP (United States Pharmacopeia); NF (National Formulary); Dosing groups – LD (low dose), MD (mid dose), HD (high dose); mg/kg (mg/kg/day); once daily dosing (QD), twice daily dosing (BID); MRHD (maximum recommended human dose); NOAEL (no observed adverse effect level); LOAEL (lowest observed adverse effect level); statistically significant (ss); not statistically significant (nss); PD (pharmacodynamic), PK (pharmacokinetic), TK (toxicokinetic); BW (body weight); AUC (integrated 'area under the curve'); GD (gestation day); LD (lactation day); central nervous system (CNS), peripheral nervous system (PNS); OGTT (oral glucose tolerance test); LFT (liver function test).

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

The proposed linagliptin plus metformin fixed-dose combination, film-coated tablet was submitted in accordance with 21 USC 505(b)(2) for treatment of type 2 diabetes mellitus as an adjunct to diet and exercise. Both drugs are approved for use as monotherapy agents and linagliptin monotherapy is expected to be used in patients with inadequate blood sugar control on a background of metformin treatment. Boehringer Ingelheim owns linagliptin and submitted a written 'right of reference' for metformin manufactured by (b) (4) pertaining to the listed Glucophage® tablets. Several new nonclinical studies were conducted with linagliptin and metformin coadministration to support development of FDC clinical use. Consistent with standard practice, nonclinical studies assessed combination treatment of the drug substances but the exact FDC drug product was not assessed in animals. All pivotal studies with linagliptin were conducted in compliance with current GLP standards.

1.2 Brief Discussion of Nonclinical Findings

All pivotal nonclinical studies were conducted using oral administration of drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by Sponsor and confirmed by this reviewer[1]. Toxicity studies in healthy, non-diabetic animals were sufficient to identify NOAEL exposures for comparison to clinical exposure.

Safety margins to expected human exposure were estimated using linagliptin AUC_{0-24} = 158 nM*h and metformin AUC_{0-24} = 159 µM*h plasma exposure in diabetic subjects at the proposed maximum recommended human doses (MRHD) of 2.5 mg linagliptin plus 1000 mg metformin BID. The proposed clinical FDC doses range from 1:200 to 1:400 linagliptin:metformin. Doses in the nonclinical studies used similar ratios to estimate minimum and maximum clinical ratios. Nonclinical dose ratios were considered acceptable based on proposed clinical uses but it is important to note that animal dosing was not expected to achieve maximum tolerated doses (MTD) of each drug. No toxicity was expected from linagliptin alone because doses were well below NOAELs identified in previously reviewed toxicity studies (under NDA 201280). Metformin-mediated toxicity was expected to limit the maximum nonclinical doses and study designs were expected to allow assessment of potential supra-additive or synergistic effects of combination linagliptin and metformin treatment.

Linagliptin and metformin have distinct, complementary mechanisms of action which lead to improved glucose metabolism compared to monotherapy. Linagliptin inhibits the

[1] Pivotal studies were conducted in accordance with OECD and/or member states GLP principles, which are acceptable under US agreements

DPP4 enzyme, which prevents DPP4-mediated cleavage of endocrine active substrates including gut incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). The incretins, in combination but with GLP-1 playing a key role, increase insulin secretion, enhance insulin receptor sensitivity, and decrease hepatic glucose, gastric emptying, and food intake. DPP4 inhibition prevents the natural rapid breakdown of GLP-1 and GIP after their postprandial expression. Metformin pharmacology is still not entirely understood but it seems to improve insulin sensitivity at peripheral target tissues including liver, muscle, and adipose. Metformin does not seem to directly affect insulin production or metabolism, but treatment increases glucose uptake and utilization in peripheral tissues, decreases hepatic glucose production, and decreases intestinal glucose absorption.

A single, 'proof of concept' study in diabetic mice showed a slight improvement in baseline fasting glucose and improved glucose excursion after oral glucose tolerance test (OGTT) with combined linagliptin and metformin treatment compared to either drug alone. The study was limited to one week duration and the Sponsor did not conduct a more robust *in vivo* assessment of glucose metabolism such as HbA_{1c} after 13-week treatment. Nevertheless, the diabetic mouse study supports the clinical finding of improved blood glucose control with combined linagliptin plus metformin treatment over monotherapy treatments.

Linagliptin monotherapy is listed for once daily dosing while metformin is given twice daily. The proposed FDC tablet is designed for BID dosing, thus linagliptin dosing will differ from the current monotherapy dosing. Total linagliptin exposure measured as $AUC_{0\text{-}24\ h}$ has been shown to be similar while maximum plasma exposure, C_{max}, will be lower clinically. Linagliptin plasma levels after 2.5 mg linagliptin BID dosing have been shown to be sufficient for clinical DPP4 inhibition and no effect on efficacy is expected with the FDC tablet BID dosing. Nonclinical studies did not identify any efficacy or safety endpoints that may be affected by a change from QD to BID dosing of linagliptin.

Pivotal toxicity studies were conducted to bridge potential toxicity of combination treatment of linagliptin and metformin. No unexpected toxicity or significant supra-additive or synergistic interactions attributed to combination treatment were identified that would alter previous pharmacology and toxicology conclusions about the safe use of linagliptin and metformin in the treatment of type 2 diabetes. Toxicity in nonclinical studies was driven by metformin, as expected based on dosing ratios and large safety margins with linagliptin. Major target organs of metformin were heart and liver, as evidenced by heart hypertrophy with immune cell infiltration/inflammation and liver hypertrophy with concomitant hepatic injury and elevated LFT biomarkers, starting at approximately 10-times the expected clinical AUC exposures. Linagliptin coadministration did not have any apparent effect on heart, liver or other metformin-related toxicity on target organs including stomach and GI tract, salivary glands, lymphoreticular tissues, or reproductive tissues.

Metformin clinical toxicity hallmarks include lactic acidosis and gastrointestinal side effects (e.g., diarrhea, bloating, discomfort). Based on nonclinical toxicity findings,

linagliptin is not expected to exacerbate potential metformin-induced metabolic acidosis or GI-related toxicity. No evidence of metabolic acidosis or GI distress were seen nonclinically with linagliptin alone at exposures exceeding 50-times human exposures.

No carcinogenicity studies were conducted with the linagliptin and metformin combination. Neither linagliptin nor metformin are genotoxic and neither are considered to pose a significant carcinogenic risk at clinical exposures. Several linagliptin impurities, including potential or theoretical impurities and degradants, were identified and adequately qualified to show no significant toxicologic or carcinogenic risk. No further carcinogenicity testing with the combined drugs is necessary.

A notable new toxicity issue was identified in the nonclinical program suggesting potential metformin-induced teratogenicity. Metformin is not listed as teratogenic at approximate clinical exposures (body surface area estimates) on current labels. Studies conducted by BI clearly demonstrated that metformin at 10- to 20-times human exposure caused skeletal malformations in Wistar Han rats. The studies confirmed that metformin is not teratogenic at approximate clinical exposures (clear NOAELs were established). Linagliptin combination treatment did not have any remarkable effect on the metformin-related malformations. The use of Wistar Han rats seems significant because most embryofetal development studies are conducted in Sprague Dawley (SD) rats. The reference Glucophage® label does not note the rat strain used but it seems clear from the original pharmacology/toxicology review(s) that SD rats were used. Wistar rats are reported to be more sensitive to heart malformations than SD rats[2] and they seem to be more susceptible to the rib and scapula malformations seen with metformin treatment[3]. More importantly, metformin was clearly toxic to pregnant rats at the teratogenic doses, causing reduced body weight gain, modestly reduced plasma glucose (albeit not to marked hypoglycemic levels), and signs of metabolic acidosis. Body weight decrements typically cause developmental delays (e.g., delayed skeletal ossification), but even maternal body weight loss does not seem to cause fetal malformations in rats. On the other hand, both hypoglycemia and metabolic acidosis are known to cause fetal malformations. Importantly, data from other DPP4 inhibitor development programs do not confirm metformin-induced skeletal malformations. Several important trends were apparent from those recent studies: all were conducted in SD rats; all confirmed the absence of metformin teratogenicity at clinical exposures; all confirmed the absence of a significant effect of DPP4 inhibitor plus metformin combination treatment on embryofetal development; and, some of the studies confirmed metformin was not teratogenic at exposures up to approximately 10X MRHD.

The mechanism of metformin-induced teratogenicity was not investigated. Clearly there are differences in the embryofetal study results for this NDA compared to those described on the existing metformin labels and in other DPP4 inhibitor nonclinical programs. It is likely the maternal toxicity at teratogenic doses contributed to fetal findings, since there were no fetal malformations in the absence of metformin-induced

[2] Fujino H et al. 2005. Congenital Anomalies. **45:**52-58
[3] Wilby OK et al. 2007. Reproductive Toxicology **24:**57(abstract)

maternal toxicity. It is equally important to emphasize that both metformin alone and the combined linagliptin plus metformin treatment were not teratogenic at approximate clinical exposures (by AUC), consistent with the current metformin label. Nevertheless, the teratogenicity findings at maternally toxic metformin doses in Wistar Han rats should be noted in the label for the proposed linagliptin plus metformin FDC tablets.

Summary of nonclinical issues relevant to clinical use of linagliptin or the combination with metformin:

1. Metformin caused fetal skeletal malformations in Wistar Han rats at maternally toxic doses, at approximately 10-times and higher the expected clinical exposure. Neither metformin alone nor combined with linagliptin was teratogenic at approximate clinical exposures. Linagliptin and metformin cross the placenta and are secreted in milk in rats. Exposure to linagliptin and metformin in nursing infants may present a risk of hypoglycemia.

2. Hypersensitivity / pseudoallergy may occur in susceptible individuals in the clinical population based on linagliptin findings in dogs and minipigs. The evidence suggests that this reflects a histamine-related response rather than an immunoglobulin-mediated allergic response. This reaction is distinct from the ulcerative necrotic skin lesions associated with some members of the DPP4 inhibitor class. Linagliptin did not cause necrotic skin lesions in any species in the non-clinical program. There is no indication that the addition of metformin changes this adverse response to linagliptin.

3. Since DPP4 cleaves substrates other than the targeted incretin hormones, inhibition of DPP4 may have unintended consequences with prolonged dosing that were not evident in the nonclinical program. As noted in the Januvia review “Effects on human immunity, specifically recall responses to antigens and immune cell trafficking, may be adversely affected by DPP4 inhibition. This risk is an unavoidable characteristic of…the drug class.” Addition of metformin would not change the primary pharmacology of linagliptin.

4. Pancreatitis has arisen as a safety concern for GLP1 targeted therapeutics, including the DPP4 inhibitors. Linagliptin has not caused histological changes in the pancreas of animals indicative of pancreatitis or pancreatic injury after long-term exposure to very high doses of drug. A limitation to extrapolating these studies to the intended diabetic clinical population is that toxicity studies are conducted in normoglycemic healthy animals. The combination toxicity study did not uncover evidence of pancreatic injury with either drug separately or combined.

1.3 Recommendations

1.3.1 Approvability

Nonclinical data support the safe use of linagliptin plus metformin FDC tablets under the proposed uses. The pharmacology/toxicology reviewer recommends approval.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are needed.

1.3.3 Labeling

Specific labeling recommendations are shown below, based on the updated proposed label submitted by BI. Recommended reviewer changes are shown in **bold** type and double strikethrough (i.e., ~~strikethrough~~) and consistent with recommended updates to the Glucophage® label.

8.1 Pregnancy

Pregnancy Category C is recommended for the combination. While neither metformin nor the linagliptin plus metformin combination were teratogenic at clinical exposures, the current Pregnancy Category B label is not supported by the teratogenic findings in Wistar Han rats. It is possible a Category B label can be supported by existing "adequate and well-controlled studies in pregnant women" that failed to demonstrate a risk to the fetus, but CFR language does not support a Category B label recommendation by the pharmtox reviewer in light of the skeletal malformations in Wistar Han rats.

Linagliptin

BI's language is generally acceptable, with minor modifications recommended as shown: (b) (4)

Metformin Hydrochloride

(b) (4)

(b) (4)

Metformin administered to female Sprague Dawley rats from gestation day 6 to lactation day 21 up to 600 mg/kg/day (about 2 times the maximum clinical dose based on body surface area comparisons) had no effect on prenatal or postnatal development of offspring.

Metformin crosses the placenta into the fetus in rats and humans[4].

13 NONCLINICAL TOXICOLOGY

BI's language for the FDC combination is acceptable and other language accurately represents the approved linagliptin and Glucophage® labels. A minor change is recommended by pharmtox, in order to separate fertility findings from genetic toxicity.

Metformin Hydrochloride

"There was no evidence of a mutagenic potential of metformin in the following *in vitro* tests: Ames test (*S. typhimurium*), gene mutation test (mouse lymphoma cells), or chromosomal aberrations test (human lymphocytes). Results in the *in vivo* mouse micronucleus test were also negative. ***[note – paragraph break inserted]***

Fertility of male or female rats was unaffected by metformin when administered at doses as high as 600 mg/kg/day, which is approximately (b) **2** times to MRHD based on body surface area."

[4] Vanky E et al. 2005. Fertil Steril **83**:1575-1578.

2 Drug Information

2.1 Drug

(b) (4)

2.1.1 CAS Registry Number

Linagliptin – 668270-12-0
Metformin HCl – 115-70-4; metformin (free base) 657-24-9

2.1.2 Generic Name

Linagliptin and metformin hydrochloride.

2.1.3 Code Name

Linagliptin – BI 1356 BS; BI 1356

2.1.4 Chemical Name

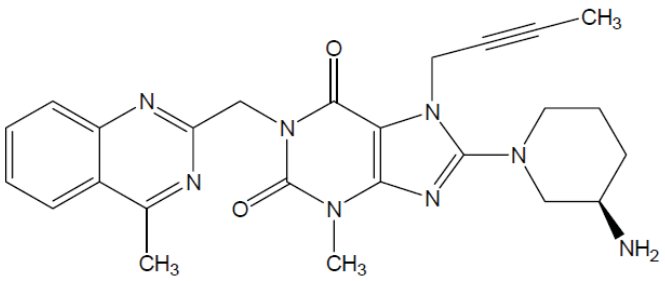
Linagliptin – 1H-purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butynyl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl)methyl]-
Metformin HCl – 1,1-dimethylbiguanide hydrochloride;
N,N-Dimethylimidodicarbonimidic diamide hydrochloride

2.1.5 Molecular Formula/Molecular Weight

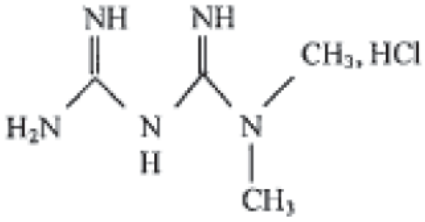
Linagliptin – $C_{25}H_{28}N_8O_2$ / 472.54 g/mol
Metformin HCl – $C_4H_{12}ClN_5$ / 165.62 g/mol

2.1.6 Structure (or Biochemical Description)

Linagliptin –



Metformin HCl –



2.1.7 Pharmacologic class

Dipeptidyl peptidase IV (DPP4) inhibitor plus biguanide.

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

Linagliptin – IND 105,055 (linagliptin + metformin FDC); NDA 201280 (Tradjenta™, linagliptin monotherapy); IND 70,963 (linagliptin); IND 108,288 (linagliptin + pioglitazone); IND 108,388 (linagliptin + BI10773)

Metformin – NDA 20-357 (metformin monotherapy); DMF (b) (4) (metformin hydrochloride; (b) (4) Draft labeling references Glucophage® (metformin hydrochloride) tablets

DPP4 Inhibitor NDAs – NDA 21-995 (sitagliptin, Januvia®) and NDA 22-044 (sitagliptin + metformin, Janumet®); (b) (4) NDA 22-350 (saxagliptin, Onglyza®) and NDA 200678 (saxagliptin + metformin, Kombiglyze XR ®); NDA 22-271 (alogliptin) and NDA 22-426 (alogliptin + pioglitazone)

2.2 Drug Formulation

Linagliptin and metformin hydrochloride FDC tablets are proposed in three dosage strengths of 2.5/500, 2.5/850, 2.5/1000 mg linagliptin/mg metformin.

Individual drug substances linagliptin and metformin are listed for use under the same manufacturing conditions for the proposed FDC formulation. No novel interactions between drug substances or unacceptable levels of combined impurities or residual solvents were identified. The current pharmacology/toxicology review is limited to the new FDC tablet drug product formulation.

All excipients in the drug product are compendial or previously reviewed for the individual listed drugs (listed in Table 1). All of the individual inactive ingredients except arginine, including food-grade pigments, have been previously included at higher concentrations in oral drugs. Arginine is a common amino acid synthesized in humans and also consumed in food. Arginine is utilized in protein production and routinely metabolized and recycled through normal cellular processes. Arginine has been approved for *iv* injection directly into blood at up to 88% in solution, further supporting the absence of toxicity expected from systemic exposure from a drug. Thus, humans are normally exposed to arginine orally and systemically and drug-related oral exposure poses no known or unique risks at levels proposed in the FDC tablet.

No residual solvents are listed in the drug product specification (b) (4)

Table 1 – Drug product composition

		linagliptin / metformin hydrochloride		
		2.5 mg / 500 mg	**2.5 mg / 850 mg**	**2.5 mg / 1000 mg**
Ingredient	**Function**	**[mg/ tablet]**	**[mg/ tablet]**	**[mg/ tablet]**
Linagliptin	Active ingredient	2.500	2.500	2.500
Metformin hydrochloride	Active ingredient	500.000	850.000	1000.000
Arginine	(b) (4)			
Corn starch				
Copovidone				
Colloidal silicon dioxide				
Magnesium stearate				
(b) (4)				
Titanium dioxide				
Yellow ferric oxide				
Red ferric oxide				
Propylene glycol				
Hypromellose (b) (4)				
Talc				
(b) (4)				
	Total weight (film coated tablet)	**602.0**	**1016.0**	**1198.0**

(b) (4)

2.4 Comments on Novel Excipients

None. All excipients are compendial or have been previously approved in listed drug products.

2.5 Comments on Impurities/Degradants of Concern

Several actual and potential impurities and degradants were identified in the linagliptin drug substance and drug product. All of the compounds of concern were qualified in accordance with current guidance and none were considered to pose a significant toxicologic risk in the original linagliptin review[5]. The Sponsor identified three impurities of linagliptin (b) (4) Two of the impurities, (b) (4) (potential degradant) and (b) (4) were identified, qualified, and reviewed during the original linagliptin review. The (b) (4) potential impurity was not previously identified (b) (4) did not have any structural alerts for genotoxicity (b) (4) No specification is listed for (b) (4) because it is only considered a potential impurity and it was not identified in the absence of stress storage conditions. No further qualification of (b) (4) was considered necessary based on the absence of any genotoxic structural alert or other predicted toxicity, consistent with ICH guidelines[6]. (b) (4) specified at (b) (4) as a degradant in the drug product, was tested for potential genetic toxicity *in vitro* and found to be negative for mutagenic and clastogenic genotoxic potential. (b) (4) was also qualified in the 13-week rat combination study at approximately (b) (4) total linagliptin dose (or about (b) (4) MRHD at the NOAEL)[7]. Individual genetic toxicity studies are reviewed in Section 7.4, below.

[5] NDA 201280, Pharmacology/Toxicology Review (D. Carlson, 3/4/11)
[6] ICH Q3B(R), Guidance for Industry Impurities in New Drug Products, 2003
[7] *Reviewer's note* – the Sponsor estimated (b) (4) MRHD based on mg/kg comparison, but the proper estimate is actual exposure (estimate using actual AUC linagliptin exposures in rats and humans provides (b) (4) MRHD assuming specification limit of (b) (4) (b) (4) in drug product) or alternatively body surface area comparison (b) (4)

Table 2 – Impurities

(b) (4)

[redacted]

2.6 Proposed Clinical Population and Dosing Regimen

The proposed linagliptin and metformin FDC tablets are indicated for treatment of adults with type 2 diabetes mellitus to improve glycemic control as an adjunct to diet and exercise. Proposed dosage strengths are 2.5 mg/500 mg, 2.5 mg/850 mg, and 2.5 mg/1000 mg immediate release tablets of linagliptin/metformin HCl for twice daily dosing (BID). Clinical efficacy and safety were primarily investigated with combination treatment with the individual monotherapies (i.e., co-administration) across the proposed dosing range. Additional Phase III trials investigated treatment with 5 mg linagliptin as add-on therapy to patients with inadequate glycemic control with metformin. Bioequivalence of the FDC tablet was assessed in a separate trial to bridge results from combination treatments of the individual drugs with the proposed FDC tablet. Clinical trial results confirmed bioequivalence of the different linagliptin plus metformin treatments and determined superiority of combined treatment compared to either monotherapy alone. In the pivotal clinical efficacy trial (1218.46), 24-weeks of treatment resulted in statistically significant HbA_{1c} decreases of 0.5-0.6% and 0.8-1.1%

in the LD and HD combination treatments compared to metformin and linagliptin alone, respectively. The proposed clinical doses represent a range of 1:200 to 1:400 linagliptin:metformin and similar drug ratios were investigated in the pivotal nonclinical studies.

Pediatric clinical trial (Trial 1218.56)

The Sponsor submitted information on the pediatric program for linagliptin + metformin, including reference to a proposed pediatric trial for linagliptin monotherapy (Trial 1218.56). They formally requested a waiver for pediatric trials in children ages 0-9 and deferral for ages 10-16, based on the limited diabetic population in children under aged 10. The proposed doses include the listed adult 5 mg/day dose (b) (4) There are no unique risks predicted from the nonclinical development program at the anticipated exposures in the proposed pediatric population. The major potential risks to children in pediatric trials are toxicity to tissues and organ systems that continue to develop until adulthood, such as brain, bone, muscle, and the immune system. DPP4 is synonymous with CD26, which is present on various T-cells and is an active immune system component as a receptor/co-stimulatory molecule, adhesion molecule, and enzymatic activity on various chemokine substrates. DPP4 is also present in developing brain and skeletal muscle in low levels but evidence suggests it may lack enzymatic activity developmentally. It is not clear whether linagliptin or other DPP4 inhibitors alter the immune system or brain very early in development. In animals linagliptin readily crosses the placenta and it is excreted in breast milk, which suggests it may cross the blood:brain barrier. Whatever functions DPP4/CD26 may have in the developing nervous and immune systems, data from $DPP4^-$ knockout mice lacking DPP4 show it is not essential for normal development and there is apparently redundancy for DPP4-mediated developmental activity. Importantly, the target clinical trial population is limited to children older than 10, thus immune system and brain development should be advanced past critical early life stages.

Juvenile animal studies are not considered necessary prior to pediatric clinical trials for linagliptin because neither neurotoxicity nor immune system perturbation are expected at therapeutic exposures and the therapeutic index is high for adults and children. Juvenile animal studies have not been required prior to pediatric clinical trials for any listed DPP4 inhibitor drug.

2.7 Regulatory Background

This is the original submission for a linagliptin and metformin FDC oral therapy. Linagliptin and metformin are approved for treatment of T2DM as individual monotherapies as adjuncts to diet and exercise modification.

3 Studies Submitted

3.1 Studies Reviewed

Pivotal nonclinical studies included 13-week combination toxicity and combination embryofetal development in Wistar rats. Individual studies reviewed are shown in the Table of Contents.

3.2 Studies Not Reviewed

None.

3.3 Previous Reviews Referenced

Pivotal nonclinical studies were previously reviewed to support the safe use of linagliptin and metformin monotherapies. Pharmacology/toxicology reviews were referenced as appropriate.

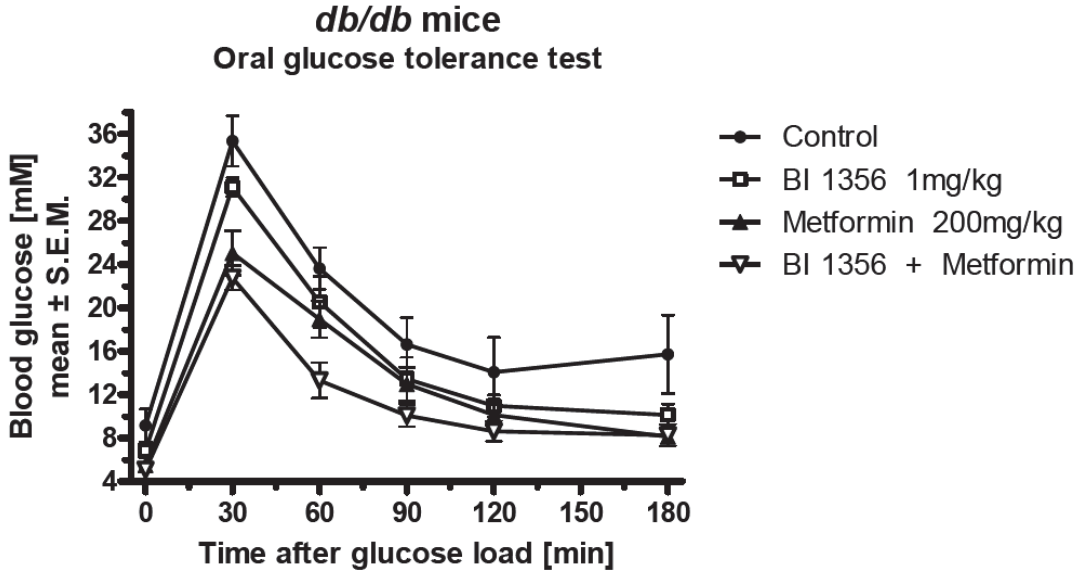
4 Pharmacology

4.1 Primary Pharmacology

Combination linagliptin + metformin in diabetic db/db mouse – Glucose tolerance
Non-GLP, 08/24/09 (Report No. U09-1799-01; Doc. MD2009-006-Lab8)

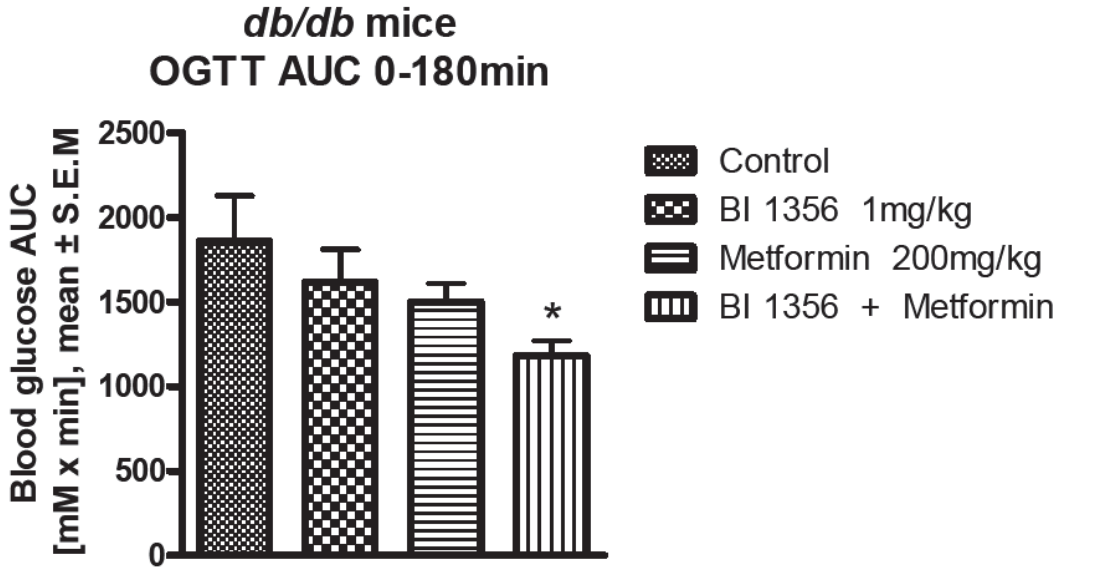
Summary: Male obese diabetic *db/db* mice (C57BL/KSJ@Rj-db) were co-treated with linagliptin and metformin by oral gavage for 1 week. The anti-diabetic effects of drug treatment were assessed by OGTT measured 16 h after the last dose. Mice were treated with 0, 1/0, 0/200, or 1/200 mg/kg linagliptin/metformin, 10 ml/kg gavage volume, in 0.5% hydroxyethylcellulose (Natrosol® 250 HX). Results showed improved baseline fasting glucose and improved glucose excursion after OGTT in all treatments, with superior improvement in the combination linagliptin plus metformin group compared to either drug alone. After OGTT, glucose excursion was decreased by 13% (nss), 19% (nss), 37% (ss) and fasting glucose was decreased 25% (nss), 40% (nss), and 44% (ss) with linagliptin, metformin, and combination treatment, respectively. Sponsor's summary data are shown below in Figure 1 and Figure 2.

Figure 1 – Oral glucose tolerance test in diabetic mice



Effect of multiple once daily oral dosing for one week of BI 1356, metformin, or a combination thereof, on oral glucose tolerance in overnight fasted db/db mice. For combination, the same doses as for monotherapy were used. The last compound administration was 16 h before the test.

Figure 2 – Glucose excursion after OGTT in diabetic mice



Baseline-corrected area under the blood glucose-time curves in an oral glucose tolerance test in db/db mice after treatment with BI 1356, metformin, or a combination thereof. (*, $p < 0.05$)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical Methods and Validation

Bioanalytical methods were previously validated for extraction, quantification, and stability of linagliptin and metabolite CD 1750 XX in plasma of all experimental animal species under the original NDA for linagliptin. Validation of metformin in rat plasma was confirmed as described below.

Metformin in rat plasma (LC-MS/MS detection and validation)
Signed GLP statement, 01/18/10 (Report No. U09-2204-01;Study CP085227)

Summary: Metformin extraction, quantification, and stability were validated in rat plasma collected on EDTA. Quantification was confirmed for 50 to 25,000 nmol/l metformin from 25 µl of plasma. Metformin stability in rat plasma was confirmed for 24 h at room temperature, for 132 h at 10°C, and for 294 h at -24°C.

Metformin in human plasma (LC-MS/MS detection and validation)
Signed GLP statement, 12/13/05 (Report No. U10-1482-01;Study CP055376)

Summary: Metformin extraction, quantification, and stability were validated in human plasma collected on EDTA and cross-validated for plasma collected on heparin. Quantification was confirmed for 5 to 2500 ng/ml metformin from 25 µl of plasma. Metformin stability in human plasma was confirmed for 4 h at room temperature, for 164 h at 10°C, and for 22 d at 5°C.

Metabolism

Metformin *in vitro* CYP P450 inhibition
Non-GLP, no date (Report No. U08-2174-01;Study A244/08/LU (B3511))

Summary: Inhibitory potential of metformin was assessed in human liver microsome incubations with various substrates for specific cytochrome P450s (CYPs) at concentrations up to 100 µM metformin. Competitive inhibition was assessed for the most common CYPs involved in drug metabolism and mechanism-based inhibition (e.g. heme interactions and/or irreversible binding) for CYP 3A4. **Metformin did not show any inhibitory potential for any individual CYPs (IC_{50} > 100 µM), including assays for mechanism-based inhibition of CYP3A4.**

Sponsor's CYP inhibition summary

Location in Module: 4.2.2.4
Report No. U08-2174
Study No. A244/08LU (B3511)

Type of study: **In vitro inhibition of cytochrome P450 enzymes, in vitro drug-drug interaction study**
Test item: Metformin hydrochloride (DI 618105 CL)

Method:
Experiments with pooled human liver microsomes in the presence of NADPH using test substrates for 10 human cytochrome P450 enzymes:

phenacetin O-dealkylation	test for CYP 1A1/1A2;	coumarin 7-hydroxylation	test for CYP 2A6
bupropion hydroxylation	test for CYP 2B6;	amodiaquine N-deethylation	test for CYP 2C8
diclofenac 4'-hydroxylation	test for CYP 2C9;	flurbiprofen 4'-hydroxylation	test for CYP 2C9
S-mephenytoin 4'-hydroxylation	test for CYP 2C19	dextromethorphan O-deethylation	test for CYP 2D6;
lauric acid 11-hydroxylation	test for CYP 2E1	nifedipine oxidation	test for CYP 3A;
testosterone 6β-hydroxylation	test for CYP 3A	midazolam 1'-hydroxylation	test for CYP 3A
lauric acid 12-hydroxylation	test for CYP 4A11		

The test substrates were incubated in the presence of 0.1 µM, 1 µM, 10 µM and 100 µM of metformin. The potential in vitro inhibition of the selective test reactions was used to assess the potential for drug-drug interactions on comedications that may be caused by metformin.
In addition, the potential for mechanism-based inhibition of CYP 3A4 by metformin was investigated. CYP 3A4 dependent nifedipine oxidation, testosterone 6β-hydroxylation and midazolam 1'-hydroxylation were used as test assays.

Tabulated results:
No pronounced inhibition of the various test reactions by metformin hydrochloride was observed. There was no indication of a mechanism-based inhibition of CYP 3A4 by metformin hydrochloride.

Tabulated results:

test reaction	CYP enzyme	IC_{50} [µM]	K_i [µM]
phenacetin O-dealkylation	1A1/1A2	> 100	n.d.
coumarin 7-hydroxylation	2A6	> 100	n.d.
bupropion hydroxylation	2B6	> 100	n.d.
amodiaquine N-deethylation	2C8	> 100	n.d.
diclofenac 4'-hydroxylation	2C9	> 100	n.d.
flurbiprofen 4'-hydroxylation	2C9	> 100	n.d.
S-mephenytoin 4'-hydroxylation	2C19	> 100	n.d.
dextromethorphan O-deethylation	2D6	> 100	n.d.
lauric acid 11-hydroxylation	2E1	> 100	n.d.
nifedipine oxidation	3A4	> 100	n.d.
testosterone 6ß-hydroxylation	3A4	> 100	n.d.
midazolam 1'-hydroxylation	3A4	> 100	n.d.
lauric acid 12-hydroxylation	4A11	> 100	n.d.

n.d.: not determined

Additional information:
no

6 General Toxicology

Pivotal toxicology studies were designed to identify unexpected interactions from linagliptin and metformin combination treatment that may exacerbate toxicity. Nonclinical toxicity of linagliptin 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

Dose-ranging metformin – 2-Week rat
Signed GLP statement, 10/12/10 (Report No. U09-2246-01)

Doses: 0, 100, 200, 1000 mg/kg
*Exposure: 178, 374, 2790 µM*h*

NOAEL = 200 mg/kg (2X MRHD)

Key Study Findings:

- NOAEL = 200 mg/kg; the 1000 mg/kg high dose was tolerable in the 2-week study and there was no remarkable toxicity at lower doses.
- Increased heart weight (+20-30%) with correlative minimal myocardium hypertrophy in both sexes at 1000 mg/kg was considered the major adverse finding.
- Soiled anogenital region and liquid feces in caecum were seen at the HD.
- Modest changes in hematology, clinical chemistry, and urinalysis values were observed in the HD. Various serum electrolytes differed from controls but variability compared to historical controls limited confidence in any apparent trends.
- Additional HD organ weight findings included increased liver weight (+17-26%; +6-7% in MD), which may be due to exaggerated pharmacology, and modest changes (generally 15-20% differences) in immune-related organs including increased male adrenal glands and female pituitary glands, and decreased thymus and thyroid (male only).
- Modest correlative lesions were observed histologically with the exception of liver. Minimal to slight parotid salivary gland infiltration/inflammation, pancreatic zymogen granules, and seminal vesicle atrophy were also seen in the HD.

Dose-ranging linagliptin + metformin – 2-Week rat (high dose)
Non-GLP, signed 08-04-09 (Report No. U09-1632-01; Study 08B080)

Doses: *0, 2.5/500, 5/1000, 10/2000 mg/kg linagliptin/metformin (1:200 = expected clinical ratio)*
Exposure: *0.459/1795, 0.562/3625, 0.500/3135 (HD day 1) µM*h linagliptin/metformin*

NOAEL < 2.5/500 linagliptin/metformin (< 3X linagliptin / < 11X metformin MRHD)

Key Study Findings:

- NOAEL < 2.5/500 mg/kg linagliptin/metformin in non-GLP preliminary dose-ranging study (n-5/group), with dose-related toxicity. Modest toxicity attributable to metformin in LD combination included electrolyte changes and gross and histological findings in target organs of heart (resorptive infiltration/inflammation, cytoplasmic eosinophilia), thymus (↑ apoptosis), adrenals (vacuolation), and parotid salivary gland (vacuolation, degeneration, infiltration, apoptosis).

- Wistar Han rats were more sensitive to metformin-induced toxicity than reported for other rat strains. Combination doses were based on the minimum expected 1:200 ratio of linagliptin:metformin; the maximum 10 mg/kg linagliptin was below the NOAEL for linagliptin alone and toxicity was driven by metformin.

- The HD combination of 10/2000 mg/kg clearly exceeded the MTD, leading to mortality and humane sacrifice within the first week of dosing.

- Target organs included heart, lymphoreticular system, pancreas, stomach and GI tract, bone marrow, liver, adrenal glands, salivary glands (parotid, tongue), and reproductive tissues (male and female)

- Additional toxicity evident as reduced BW, increased serum creatinine, P, and Mg, clinical signs (piloerection, hair loss, abnormal activity, soiled anus), and gross pathology in target organs

Dose-ranging linagliptin + metformin – 2-Week rat (low dose)
Signed GLP statement, 06-23-10 (Report No. U09-2243-01; Study 09B029)

Doses: 0, 0.5/100, 1/200 mg/kg linagliptin/metformin
*Exposure: 0.138/236, 0.303/458 µM*h linagliptin/metformin*

NOAEL = 1/200 linagliptin/metformin (2X linagliptin / 3X metformin MRHD)

Key Study Findings:

- NOAEL = 1/200 mg/kg linagliptin/metformin; HD combination was well tolerated, no mortality or abnormal clinical signs at any dose and no remarkable histopathology findings.

- Minor dose-related changes in serum electrolytes (K +7-12%; Na +1-4%, Cl +2-4%) were notable and consistent with more pronounced, albeit variable, findings in the separate high dose range finding studies with metformin alone and combination treatments

- Absolute and relative thyroid weights increased slightly at both doses, generally +15-17% and maximum +31% (LD ♀), but the modest changes and absence of a dose-response suggest limited biological significance

- Dose-related changes in reticulocytes (↑ 13-24%) and absolute (but not percent) lymphocytes (maximum ↓ 17%) were modest and not likely biologically significant

Subchronic 13-Week linagliptin + metformin in rat
Signed GLP statement, 10/08/10

BI 1356 BS (linagliptin) and metformin: 13-week oral (gavage) combination toxicity study in rats (Study 09B104, Doc. No. U10-1492-01)

Doses (mg/kg/d) *0/0 (vehicle control)*
0.5/100, 2/400, 4/800, 2/800 (linagliptin/metformin)
4/0 (linagliptin control)
0/800 (metformin control)

*Exposures: (µM*h)* *0.158 / 224, 0.301 / 1180, 0.327 / 3620, 0.235 / 3710 (linagliptin / met)*
(1X/1X, 2X/7X, 2X/23X, 1X/23X MRHD)
0.361 / 0 (2X MRHD linagliptin)
0 / 3320 (21X MRHD metformin)

NOAEL = *0.5 / 100 linagliptin/metformin (1X linagliptin / 1X metformin MRHD)*
4 mg/kg linagliptin control (2X MRHD)

NOAEL determination – Findings in the 0.5/100 mg/kg linagliptin/metformin low dose combination were limited to low incidence and severity of kidney hyaline droplets, which were not considered adverse in the absence of functional kidney changes. Metformin-related toxicity was evident at ≥ 400 mg/kg alone or in combination.

Key Study Findings:

- Heart hypertrophy with immune cell infiltration and inflammation and liver hypertrophy coupled with modest functional deficit suggested by increased LFTs defined the major target organ toxicity.

- The high dose of 800 mg/kg metformin alone or in combination exceeded the MTD based on unexplained deaths and moribundity.

- Linagliptin exposures were well below expected adverse exposures. Combination doses were based on expected clinical ratios (1/200 or 1/400 linagliptin:metformin) and toxicity was driven by metformin, as expected.

- There was no synergism and no remarkable additive effect of linagliptin on metformin-related toxicity. A slight additive effect (~10%) on decreased BW gain was evident with high dose linagliptin + metformin co-treatment.

- Clear, dose-related trend of decreased urine pH at ≥400 mg/kg metformin (±linagliptin), which was consistent with metformin-induced lactic acidosis. No other markers of lactic acidosis were assessed, but altered serum electrolytes provided further support to metabolic changes and possible anion gap perturbation.

Study no.:	*Study 09B104, Doc. No. U10-1492-01*
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Birkendorfer Str. 65 88397 Biberach an der Riss Germany
Date of study initiation:	7/9/09 (animal arrival)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linagliptin, Batch PR4AZU00546A1, 95.8% purity (spiked with (b) (4) degradant for qualification); Metformin HCl, Batch 801941, 100.1% purity

13-Week Repeat Dose Combination Toxicity in Rats — Summary

SPECIES DOSES AND ADMINISTRATION # ANIMALS FOLLOW-UP	**NOAEL = 0.5 / 100 MG/KG/DAY LINAGLIPTIN / METFORMIN; 1X LINAGLIPTIN / 1X METFORMIN MRHD**
Wistar Han rat 13-wk + 6-wk recovery 0/0, 0.5/100, 2/400, 4/800, 2/800, 4/0, 0/800 mg/kg linagliptin/metformin Oral gavage, 10 ml/kg (0.5% HEC vehicle) Main: 10/sex/group Recovery: 10/sex/group (control, HD combination, HD metformin)	

Linagliptin AUC_{0-24h} (µg*h/ml)

Dose (mg/kg)	Males		Females	
	Day 1	Day 88	Day 1	Day 88
0.5 / (100 met)	0.123	0.166	0.100	0.149
2 / (400 met)	0.251	0.308	0.313	0.297
4 / (800 met)	0.289	0.344	0.407	0.309
2 / (800 met)	0.201	0.253	0.222	0.218
4 / (0 met)	0.203	0.265	0.208	0.457

Metformin AUC_{0-24h} (µg*h/ml)

(0.5 lina) / 100	272	221	147	226
(2 lina) / 400	790	1160	613	1200
(4 lina) / 800	1690	2330	1480	4900
(2 lina) / 800	1500	3220	1360	4200
(0 lina) / 800	1610	2770	1300	3870

Mortality: HD metformin group deaths or moribund sacrifice, in combination (1/10 ♂, 2/10 ♀, 2 TK ♂) and metformin control (1/10 ♀). One female death was attributed to gavage error. Cause of death/moribundity were not consistent, but heart and liver toxicity were apparent in some cases. Signs preceding death included stool abnormalities, increased defecation, soiled anogenital region, reduced activity/prostration and 'preferred isolation' post-dose, reduced BW gain or weight loss/emaciation. Gross pathology in individual early decedents enlarged adrenal glands, visceral fibrin and adhesions (heart, liver, duodenum), hardened heart, discolored tissues/organs (liver, duodenum, gastric mucosa), small spleen and thymus.
Clinical Signs: Signs attributed to metformin included 'preferred isolation' and prostrate position post-dose at ≥ 400 mg/kg, and additional signs at ≥ 800 mg/kg of increased defecation, soft stool, and soiled anogenital region. Transient increased salivation at dosing was seen in combination groups (≥ 2/400), possibly due to taste of the combined drugs.
Body Weight: BW was reduced compared to controls in males (↓ 11-19%) treated with ≥ 400 mg/kg metformin, alone or in combination, and for females (↓ 6%) only in the 4/800 mg/kg group. The HD combination group male BW was also ↓ 9% compared to HD metformin alone, suggesting a slight additive interaction with linagliptin. BW gain trends were even more pronounced (↓ 15-34% ♂ and ↓ 6-9% ♀), including the 2/800 mg/kg female group. BW gain increased in metformin groups during recovery but remained ↓ 5-10% compared to controls at the end of recovery. BW effects were clearly more pronounced in males.
Hematology: Lymphocytes were increased up to approximately 30% in groups treated with ≥ 400 mg/kg metformin. There was no effect of linagliptin alone or linagliptin in combination. Findings were within the labs' historical control range and they were reversed in recovery animals. Neither lymphocyte elevations or other sporadic changes were considered toxicologically meaningful.
Clinical Chemistry: ALT and AST increased approximately +50% in metformin alone and +60-84% in 2/800 and 4/800 combination groups. Linagliptin alone did not increase ALT or AST on day 92. Mean changes were of marginal biological significance (< 2-fold) and reversible, although ALT, AST, GLDH were elevated 2- to 3-fold indicating toxicity in some individual animals. Metformin ≥ 400 mg/kg, alone or in combination, altered plasma electrolytes. K increased 7-29%, Cl decreased 1-3%, Mg increased 5-39%, and P increased 7-29%, with changes slight more pronounced in females. Electrolyte changes were consistent with metformin-related findings from range-finding studies and likely toxicologically significant due to normally tight regulation within narrow physiological ranges.
Urinalysis: Effects of ≥ 400 mg/kg metformin were observed, with no apparent interaction of linagliptin co-treatment and no consistent dose-response to [metformin]. Specific gravity increased 2-3%, urine volume decreased slightly approximately 20-50%, and trends of elevated ketone bodies and decreased pH. Decreased urine pH was consistent with lactic acidosis associated with metformin toxicity.
Organ Weights: Several organ weights (absolute and/or relative to BW and brain weight) were affected by ≥ 400 mg/kg metformin treatment (± linagliptin), including marked increased **heart (+50-75%)** and **liver (+30-65%) weights**, and moderate increased **kidney (+30-40%)** and **adrenal gland weights (~ ≥35%)** and decreased

thymus (↓ 25-40%) weights in both sexes. In **females spleen (~+20%)** and **ovary (~+35%)** also increased and **pituitary glands (↓ 15%)** decreased. Correlative histologic lesions included heart myocardial hypertrophy, liver hepatocyte hypertrophy, kidney tubular dilatation, adrenal cortex hypertrophy and cytoplasmic vacuolation, ovary increased number of corpora lutea, and thymus involution and apoptotic rates. Organ weight changes were reversible, although small increases in heart weights (~+12%, both sexes) and female liver (~+10%) and ovary (~+20%) weights showed incomplete recovery after 6-weeks.
Gross Pathology: NOEL = 100 mg/kg metformin (+ 0.5 mg/kg linagliptin); findings at 400 mg/kg met limited to discolored salivary glands (5/20) and stomach (4/20). HD met major target organs included heart (enlarged, hard), liver (enlarged, discolored), salivary glands (discolored), thymus (small), and stomach/GI (discolored, abnormal contents). Findings observed or exacerbated in HD met + linagliptin included small thymus, small uterus, and emaciation (2/20 females). Gross findings were generally absent in recovery animals with the exception of sporadic discolored organs.
Histopathology: LOAEL = 2/400 mg/kg linagliptin/metformin; minimal kidney hyaline drops (2/10 ♂) in the LD combination were not considered adverse in the absence of other evidence of renal toxicity. Dose-related lesions were evident in several target organs, with incidence and severity increasing with metformin dose and no apparent contribution of linagliptin. Major target organs were heart (myocardium hypertrophy, infiltration/inflammation), liver (hypertrophy), kidneys (tubule dilatation, vacuolation, hyaline droplets, pigmentation), and salivary glands (infiltration, hypertrophy). Additional target organs were female reproductive organs, thymus, adrenal glands, and stomach/GI. Correlates with gross pathology included myocardial hypertrophy (gross enlarged heart) and hepatocyte hypertrophy (gross enlarged liver). Lesions were largely reversed in recovery animals.
Toxicokinetics: Both drugs were readily absorbed and increased with increasing dose. Linagliptin increased less than dose proportionally. At steady state metformin increased greater than dose-proportionally and exposure was up to 2-fold higher than single dosing at ≥ 400 mg/kg. Combination treatment did not affect individual drug exposures.
Summary: **NOAEL = 0.5/100 mg/kg linagliptin/metformin.** Toxicity was evident at ≥ 400 mg/kg metformin alone or in combination, with 800 mg/kg metformin exceeding the MTD based on mortality/moribundity. Major target organ toxicity was evident as heart hypertrophy with infiltration and inflammation and liver hypertrophy with modest functional deficit suggested by increased LFTs (marked in individual animals). Additional toxicity was evident based on plasma electrolytes altered up to 30%, GI distress, kidney hypertrophy and histologic changes, and immune tissue and stress related changes. BW was also decreased, consistent with altered glucose metabolism (a PD effect) at low doses but excessive at higher doses. There was no synergism and no remarkable additive effect of linagliptin on metformin-related toxicity. Degradant (b) (4) was qualified at a final concentration of (b) (4) of linagliptin, or about (b) (4) potential exposure at the drug product specification limit based on (b) (4) MRHD linagliptin NOAEL exposure in this study.

Sponsor's study design summary

Group	Daily dose		Animal numbers					
	BI 1356 BS	Metformin BS	Males			Females		
	[mg/kg]	[mg/kg]	Main Study animals	Recovery animals	Kinetic animals	Main Study animals	Recovery animals	Kinetic animals
1	0	0	101-110	111-120	121-124	151-160	161-170	171-174
2	0.5	100	201-210	-	211-214	251-260	-	261-264
3	2	400	301-310	-	311-314	351-360	-	361-364
4	4	800	401-410	411-420	421-424	451-460	461-470	471-474
5	2	800	501-510	-	511-514	551-560	-	561-564
6	4	0	601-610	-	621-624	651-660	-	671-674
7	0	800	701-710	721-730	711-714	751-760	771-780	761-764

Methods

Doses:	*See study design (above)*
Frequency of dosing:	QD
Route of administration:	Oral
Dose volume:	10 ml/kg
Formulation/Vehicle:	Suspension in 0.5% hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Wistar Han rat (Crl:WI(Han), SPF confirmed)
Number/Sex/Group:	10
Age:	7-8 weeks
Weight:	Males: 204-257 g / Females: 144-190 g
Satellite groups:	10/sex recovery (6-week – control, HD combination, HD metformin) 4/sex/group TK
Unique study design:	Group housed up to 4/sex/cage; food consumption and water consumption (visual only) were monitored per cage, confounding precise individual values. Urinalysis performed for 5 h after 20 ml/kg water gavage (~0.5-1 h post-treatment) in individual metabolism cages. Linagliptin drug substance spiked with (b) (4) (b) (4), to qualify potential degradant exposure.
Deviation from study protocol:	HD linagliptin and metformin control dosing solutions were reportedly interchanged on dosing day 1, however, it appears groups were switched for the entire study because the data do not indicate HD control groups were dosed with multiple compounds.

Observations and Results (additional details)

Mortality – HD metformin group deaths or moribund sacrifice, in combination (1/10 ♂, 1/10 ♀, 2 TK ♂) and metformin control (1/10 ♀). Signs preceding death included stool abnormalities, increased defecation, soiled anogenital region, reduced activity/prostration and 'preferred isolation' post-dose, reduced BW gain or weight loss.

Cause of death was unclear for most (TK animals were not examined); one female had moderate to severe thymus, spleen, lymph node, and hematopoiesis atrophy/depletion and one male had peritonitis and liver necrosis. Gross pathology in individuals included emaciation, enlarged adrenal glands, visceral fibrin and adhesions (heart, liver, duodenum), hardened heart, discolored tissues/organs (liver, duodenum, gastric mucosa), small spleen and thymus.

An additional HD combination female death (day 79) was considered a gavage error based on bloody, coagulated contents in thoracic cavity and esophagus perforation and hemorrhage.

Body Weights (twice weekly through week 4, then weekly) –

Sponsor's body weight summary

Body weight on	Group/ gender	Group 1 Control	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
	Dose BI 1356 BS [mg/kg]	0	0.5	2	4	2	4	0
	Dose metformin [mg/kg]	0	100	400	800	800	0	800
Day 1[a]	Males	228.22	231.64	224.60	229.39	228.83	227.98	223.48
	Females	161.61	162.73	157.36	158.58	167.31	157.92	160.69
Day 91	Males	392.53	389.77	**↓343.08**	**↓318.04**	**↓328.72**	401.01	**↓351.11**
	Females	231.09	222.70	221.34	**↓217.00**[b]	225.02	225.79	226.51[c]
Day 133	Males	417.09	-	-	**↓376.38**[d]	-	-	397.80
	Females	240.14	-	-	232.89	-	-	232.89[e]

↓: significantly decreased compared to Control (Group 1); p< = 0.05, many-to-one t-test, two-sided
a: n = 20 per gender in Groups 1, 4 and 7, n = 10 in Groups 2, 3, 5 and 6
b: n = 18
c: n = 19
d, e: n = 9

Feed Consumption (food consumption measured per cage with body weights, water consumption daily visual qualitative inspection) **–**

Minimal, transient decreased in food consumption were observed at the beginning of treatment. Male consumption in ≥ 400 mg/kg metformin groups decreased 9-14% weeks 1-3 only and female consumption in ≥ 800 mg/kg decreased 10-21% in week 1 only. There was a slight additive effect of linagliptin plus metformin treatment on decreased food consumption. Food consumption increased at the beginning of the

recovery period. The biological significance of food consumption trends was considered minimal based on small, transient changes from controls and because of difficulty interpreting food consumption measurements for housed in groups.

Ophthalmoscopy (pre-test, week 12, and recovery) – Unremarkable. The ophthalmologist noted a "more excited behavior" in HD combination animals during examinations, suggesting an increased sensitivity to light. No correlative lesions were found on histological examination.

Clinical Chemistry (lithium heparin or sodium citrate (clotting markers) anticoagulant) –

Transaminase activity increased modestly in HD metformin groups (alone or in combination), with ALT and AST increased approximately +50% in metformin alone and +60-84% in 2/800 and 4/800 combination groups. Linagliptin alone did not increase ALT or AST on day 92. Increases were of marginal biological significance (< 2-fold) and reversible.

Sponsor's clinical chemistry summary (enzymes)

Parameter [unit]	Day		Daily dose of BI 1356 BS/metformin [mg/kg]												
			0	0.5/100		2/400		4/800		2/800		4/0		0/800	
		Group	1	2		3		4		5		6		7	
		Gender	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
ALT [U/L]	3	M	36.4	33.5	-8.0	42.4	16.3	35.4	-3.0	34.3	-6.0	33.8	-7.2	↓28.8	-20.9
		F	28.2	26.1	-7.7	29.3	3.8	29.3	3.8	26.9	-4.6	↑37.4	32.7	**↑36.3**	28.7
	92	M	30.9	26.2	-15.3	37.7	22.0	**↑57.0**	84.3	**↑49.4**	59.8	29.8	-3.7	**↑46.8**	51.3
		F	26.0	25.1	-3.3	30.6	17.5	**↑45.4**	74.6	**↑42.8**	64.7	32.2	23.6	**34.7**	33.6
	134	M	31.1	n.a.	n.a.	n.a.	n.a.	30.4	-2.3	n.a.	n.a.	n.a.	n.a.	26.7	-14.3
		F	23.8	n.a.	n.a.	n.a.	n.a.	↓16.7	-29.9	n.a.	n.a.	n.a.	n.a.	↓20.5	-13.9
AST [U/L]	3	M	86.5	87.8	1.5	84.0	-2.9	95.4	10.3	88.8	2.7	78.1	-9.7	83.5	-3.5
		F	88.2	82.7	-6.2	80.1	-9.2	97.3	10.4	94.8	7.5	94.5	7.1	87.6	-0.7
	92	M	63.4	57.6	-9.2	69.4	9.5	**↑78.6**	24.0	74.7	17.9	58.5	-7.7	66.0	4.1
		F	76.7	61.1	-20.3	71.5	-6.8	75.7	-1.2	67.9	-11.4	68.2	-11.0	63.4	-17.3
	134	M	53.0	n.a.	n.a.	n.a.	n.a.	56.4	6.6	n.a.	n.a.	n.a.	n.a.	61.7	16.5
		F	61.5	n.a.	n.a.	n.a.	n.a.	↓53.4	-13.1	n.a.	n.a.	n.a.	n.a.	57.3	-6.7

M: males; F: females
n.a.: not applicable
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

In HD metformin groups plasma glucose decreased slightly (max. ↓ 27%) in males and total glycogen increased in both sexes (approximately 2- to 3-fold), consistent with the expected PD effects of lower blood glucose and increased insulin sensitivity. Creatinine (↓ 30%) and albumin (↓6-18%) decreased slightly at ≥ 400 mg/kg metformin alone or in combination. Biological significance of decreased creatinine is unclear and decreased albumin may be indicative of modest changes associated with BW decreases.

Sponsor's clinical chemistry summary (metabolism markers)

Parameter [unit]	Day		Dose of BI 1356 BS/metformin [mg/kg]												
			0	0.5/100		2/400		4/800		2/800		4/0		0/800	
		Group	1	2		3		4		5		6		7	
		Gender	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
Glucose [mmol/L]	92	M	7.79	7.94	1.9	7.21	-7.5	↓5.66	-27.4	↓5.86	-24.8	8.13	4.3	↓6.11	-21.7
		F	6.39	6.95	8.7	7.55	18.2	7.25	13.5	5.28	-17.4	7.14	11.7	6.88	7.7
	134	M	8.61	n.a.	n.a.	n.a.	n.a.	8.47	-1.6	n.a.	n.a.	n.a.	n.a.	8.34	-3.1
		F	6.08	n.a.	n.a.	n.a.	n.a.	6.28	3.3	n.a.	n.a.	n.a.	n.a.	6.54	7.5
Creat [mmol/L]	92	M	33.7	35.5	5.5	↓30.6	-9.0	↓27.1	-19.5	↓29.3	-13.1	32.3	-4.1	↓28.4	-15.7
		F	39.6	39.3	-0.9	↓31.2	-21.2	↓29.0	-26.8	↓29.6	-25.3	37.5	-5.3	↓30.9	-22.0
	134	M	33.9	n.a.	n.a.	n.a.	n.a.	30.4	-10.3	n.a.	n.a.	n.a.	n.a.	35.3	4.1
		F	46.2	n.a.	n.a.	n.a.	n.a.	46.5	0.5	n.a.	n.a.	n.a.	n.a.	40.9	-11.6
Tot-Chol [mmol/L]	92	M	2.02	2.08	3.3	1.91	-5.0	↓1.76	-12.7	↓1.54	-23.8	2.20	9.0	↓1.78	-11.5
		F	1.78	↑2.29	28.3	1.97	10.6	1.84	3.3	1.54	-13.9	2.00	11.9	1.75	-2.1
	134	M	1.99	n.a.	n.a.	n.a.	n.a.	1.74	-12.6	n.a.	n.a.	n.a.	n.a.	↓1.66	-16.6
		F	1.86	n.a.	n.a.	n.a.	n.a.	1.80	-3.1	n.a.	n.a.	n.a.	n.a.	1.69	-9.0
Tot-Glyc [mmol/L]	92	M	1.29	1.46	13.4	1.49	15.7	↑2.21	71.3	↑2.43	88.6	1.58	22.7	↑2.49	93.4
		F	0.56	0.80	44.1	0.97	74.7	↑1.24	122.5	↑1.91	242.7	0.67	20.2	↑1.00	79.7
	134	M	1.03	n.a.	n.a.	n.a.	n.a.	1.2	17.1	n.a.	n.a.	n.a.	n.a.	1.30	26.3
		F	0.47	n.a.	n.a.	n.a.	n.a.	0.52	10.6	n.a.	n.a.	n.a.	n.a.	↓0.39	-16.4

M: males; F: females
n.a.: not applicable
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

Electrolyte levels were affected by ≥ 400 mg/kg metformin alone or in combination, with little or no additive interactions from linagliptin co-treatment. Plasma K increased 7-10% in males and 14-29% in females, Cl decreased 1-3% in both sexes, Mg increased 5-19% in males and 8-39% in females, and P increased 7-21% in males and 9-29% in females. Electrolyte changes were moderate to high, consistent with metformin-related findings from range-finding studies, and likely toxicologically significant because electrolytes levels are usually tightly regulated within narrow physiological ranges that were exceeded in this study (compared to historical levels).

Sponsor's clinical chemistry summary (electrolytes)

Parameter [unit]	Day		Daily dose of BI 1356 BS / metformin [mg/kg]												
			0	0.5/100		2/400		4/800		2/800		4/0		0/800	
		Group	1	2		3		4		5		6		7	
		Gender	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
K [mmol/L]	92	M	3.78	3.95	4.6	↑**4.05**	7.3	↑**4.17**	10.5	↑**4.05**	7.34	3.68	-2.5	↑**4.04**	7.0
		F	3.29	3.42	3.8	↑**3.74**	13.6	↑**4.26**	29.3	↑**4.24**	28.8	3.49	6.1	↑**4.02**	22.2
	134	M	3.68	n.a.	n.a.	n.a.	n.a.	3.84	4.4	n.a.	n.a.	n.a.	n.a.	3.67	-0.3
		F	3.34	n.a.	n.a.	n.a.	n.a.	3.19	-4.4	n.a.	n.a.	n.a.	n.a.	↓**3.11**	-6.9
Cl [mmol/L]	92	M	104	104	0.5	104	0.3	↓**103**	-1.3	↓**102**	-1.40	104.0	0.1	103	-0.6
		F	105	105	-0.4	105	-0.1	↓**102**	-2.7	↓**103**	-2.50	106	0.2	↓**103**	-2.2
	134	M	104	n.a.	n.a.	n.a.	n.a.	104	0.5	n.a.	n.a.	n.a.	n.a.	104	-0.4
		F	106	n.a.	n.a.	n.a.	n.a.	107	0.8	n.a.	n.a.	n.a.	n.a.	↑**108**	1.5
Mg [mmol/L]	92	M	0.81	0.80	-1.6	0.85	5.2	↑**0.92**	13.3	↑**0.97**	19.2	0.81	-0.2	↑**0.89**	9.5
		F	0.82	0.89	8.1	↑0.93	13.2	↑**0.93**	13.3	↑**1.15**	39.4	0.86	4.7	↑**0.92**	11.9
	134	M	0.78	n.a.	n.a.	n.a.	n.a.	0.78	-0.2	n.a.	n.a.	n.a.	n.a.	0.78	0.1
		F	0.82	n.a.	n.a.	n.a.	n.a.	0.85	3.5	n.a.	n.a.	n.a.	n.a.	↑**0.86**	4.2
Inorg-P [mmol/L]	92	M	1.48	1.50	1.6	1.59	7.4	↑**1.72**	16.3	↑**1.69**	14.2	1.36	-8.1	↑**1.78**	20.7
		F	1.22	1.27	3.7	1.33	8.8	↑**1.58**	29.0	↑**1.53**	25.3	1.22	0.0	↑**1.51**	23.0
	134	M	1.29	n.a.	n.a.	n.a.	n.a.	1.37	5.6	n.a.	n.a.	n.a.	n.a.	↑**1.43**	10.7
		F	1.17	n.a.	n.a.	n.a.	n.a.	1.09	-6.6	n.a.	n.a.	n.a.	n.a.	1.07	-8.4

M: males; F: females
n.a.: not applicable
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

Historical range for
[17] potassium in male rats (age 155 - 196 days): 3.4 - 4.2 [mmol/L]
[18] potassium in female rats (age 155 - 196 days): 3.1 - 3.9 [mmol/L]
[19] magnesium in male rats (age 155 - 196 days): 0.7 - 0.9 [mmol/L]
[20] magnesium in female rats (age 155 - 196 days): 0.8 - 0.9 [mmol/L]

Urinalysis – There was a clear trend toward lower pH with increased metformin dose (± linagliptin). No statistical analysis was done on pH trends but data are summarized in the reviewer's summary table, below. Recovery animals showed reversibility, as there was no trend of treatment-related decreased pH during recovery week 6 and pH values in individual rats confirmed reversibility. Decreased urine pH was consistent with lactic acidosis, a known side effect of metformin exposure.

Urine pH†					
Linagliptin/metformin (mg/kg)	pH				
	≤6.5 (n)	7.0 (n)	7.5 (n)	8.0 (n)	≥8.5 (n)
0/0 [a] (n = 40)	1	10	17	9	3
0.5/100 (n = 20)	0	0	5	5	10
2/400 (n = 20)	10	4	4	0	2
4/800 [a] (n = 40)	39	1	0	0	0
2/800 (n = 19)	19	0	0	0	0
4/0 (n = 20)	3	6	5	3	3
0/800 [a] (n = 39)	39	0	0	0	0

† Distribution of urine pH in treatment groups, shown as number of rats with various measured pH (sexes combined)
[a] Includes recovery animal data from urinalysis during the treatment period

Sponsor's urinalysis summary

Parameter [unit]	Day		Daily dose of BI 1356 BS / metformin [mg/kg]											
			0	0.5/100		2/400		4/800		2/800		4/0		0/800	
		Group	1	2		3		4		5		6		7	
		Gender	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
U-Vol [mL]	85	M	7.43	6.30	-15.2	↓4.90	-34.0	↓5.50	-25.9	↓3.30	-55.6	6.25	-15.8	↓4.38	-41.1
		F	4.48	3.50	-21.8	5.10	14.0	4.58	2.4	4.35	-2.8	4.05	-9.5	3.34	-25.3
	130	M	7.25	n.a.	n.a.	n.a.	n.a.	6.28	-13.4	n.a.	n.a.	n.a.	n.a.	6.70	-7.6
		F	4.25	n.a.	n.a.	n.a.	n.a.	3.85	-9.4	n.a.	n.a.	n.a.	n.a.	3.89	-8.5
U-s.gr. [g/mL]	85	M	1.012	1.016	0.4	↑1.032	2.0	↑1.034	2.2	↑1.047	3.4	1.015	0.3	↑1.038	2.6
		F	1.013	1.014	0.1	1.020	0.7	↑1.032	1.9	↑1.034	2.1	1.013	0.0	↑1.035	2.1
	130	M	1.011	n.a.	n.a.	n.a.	n.a.	1.011	0.0	n.a.	n.a.	n.a.	n.a.	1.011	0.0
		F	1.009	n.a.	n.a.	n.a.	n.a.	1.012	0.2	n.a.	n.a.	n.a.	n.a.	↑1.013	0.4

M: males; F: females
n.a.: not applicable
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

Organ Weights

Sponsor's organ weight summary tables

Males

Parameter	Group	1	2	3	4	5	6	7
	Dose BI 1356 BS [mg/kg]	0	0.5	2	4	2	4	0
	Dose metformin BS [mg/kg]	0	100	400	800	800	0	800
Males	Body weight [g]	366.65	368.34	**317.78↓**	**298.77↓**	**297.65↓**	378.50	**325.34↓**
Heart (n=10)	absolute [g]	1.0544	1.0168	1.0916	**1.3314↑**	**1.4046↑**	1.0377	**1.3613↑**
	relative to BW[1)]	0.2886	0.2773	**0.3442↑**	**0.4586↑**	**0.4705↑**	0.2745	**0.4185↑**
	relative to BrW[2)]	49.1	48.1	52.2	**67.1↑**	**72.0↑**	50.0	**67.1↑**
Liver (n=10)	absolute [g]	8.5034	8.8782	9.0751	**9.6908↑**	**10.0140↑**	8.9685	**10.1207↑**
	relative to BW[1)]	2.326	2.409	**2.863↑**	**3.275↑**	**3.357↑**	2.374	**3.112↑**
	relative to BrW[2)]	395.6	420.5	434.6	486.0↑	**512.6↑**	432.3	**499.4↑**
Kidneys (n=10)	absolute [g]	2.1418	2.1330	2.1579	2.2518	2.2699	2.1217	2.2515
	relative to BW[1)]	0.586	0.580	**0.679↑**	**0.758↑**	**0.762↑**	0.562	**0.692↑**
	relative to BrW[2)]	99.6	101.0	103.1	**112.8↑**	**116.1↑**	102.2	**110.8↑**
Thymus (n=10)	absolute [g]	0.2531	0.2617	**0.1779↓**	**0.1608↓**	**0.1670↓**	0.2574	**0.2048↓**
	relative to BW[1)]	0.0695	0.0717	**0.0558↓**	**0.0523↓**	**0.0563↓**	0.0678	0.0633
	relative to BrW[2)]	11.76	12.39	**8.46↓**	**8.00↓**	**8.55↓**	12.44	10.13
Adrenals (n=10)	absolute [mg]	52.29	54.28	57.88	**65.43↑**	**61.91↑**	48.92	**62.29↑**
	relative to BW[1)]	0.01428	0.01486	**0.01821↑**	**0.02252↑**	**0.02081↑**	0.01291	**0.01911↑**
	relative to BrW[2)]	2.433	2.568	2.761	**3.292↑**	**3.170↑**	2.353	**3.054↑**

1) BW = body weight
2) BrW = brain weight
↑, ↓: significantly increased, decreased compared with Control; p<=0.05, many-to-one t-test, two-sided

Females

Parameter	Group	1	2	3	4	5	6	7
	Dose BI 1356 BS [mg/kg]	**0**	**0.5**	**2**	**4**	**2**	**4**	**0**
	Dose metformin BS [mg/kg]	**0**	**100**	**400**	**800**	**800**	**0**	**800**
Females	Body weight [g]	210.69	211.04	201.72	**190.84↓**	199.56	213.02	205.90
Heart (n=10; Group 4: n=8)	absolute [g]	0.7162	0.7193	0.8015	**1.1516↑**	**1.1801↑**	0.7608	**1.0577↑**
	relative to BW	0.3396	0.3407	0.3975	**0.6087↑**	**0.5998↑**	0.3576	**0.5146↑**
	relative to BrW	37.0	36.5	42.2	**63.0↑**	**64.5↑**	39.5	**56.1↑**
Liver (n=10; Group 4: n=8)	absolute [g]	5.3458	5.4932	**6.3136↑**	**7.7156↑**	**8.2530↑**	5.3077	**7.6003↑**
	relative to BW[1)]	2.549	2.607	**3.136↑**	**4.056↑**	**4.181↑**	2.493	**3.700↑**
	relative to BrW[2)]	276.3	279.4	**332.7↑**	**421.5↑**	**450.1↑**	275.7	**402.9↑**
Kidneys (n=10; Group 4: n=8)	absolute [g]	1.3503	1.3741	1.4383	**1.6520↑**	**1.7701↑**	1.4100	**1.6847↑**
	relative to BW[1)]	0.642	0.652	0.714	**0.871↑**	**0.899↑**	0.663	**0.818↑**
	relative to BrW[2)]	69.7	69.8	75.7	**90.3↑**	**96.4↑**	73.2	**89.3↑**
Thymus (n=10; Group 4: n=8)	absolute [g]	0.2312	0.2229	**0.1928↓**	**0.1248↓**	**0.1417↓**	0.2257	**0.1580↓**
	relative to BW[1)]	0.1093	0.1057	0.0955	**0.0655↓**	**0.0705↓**	0.1064	**0.0764↓**
	relative to BrW[2)]	11.95	11.34	10.17	**6.83↓**	**7.70↓**	11.73	**8.36↓**
Spleen (n=10; Group 4: n=8)	absolute [g]	0.4641	0.5097	0.4531	0.4853	**0.5379↑**	0.5077	**0.5435↑**
	relative to BW	0.2200	0.2424	0.2251	**0.2543↑**	**0.2709↑**	0.2390	**0.2641↑**
	relative to BrW	23.97	25.91	23.88	26.49	**29.33↑**	26.38	**28.85↑**
Ovaries (n=10; Group 4: n=8)	absolute [mg]	86.84	88.04	94.51	104.39	**113.88↑**	80.47	**113.03↑**
	relative to BW	0.04149	0.04174	0.04701	**0.05455↑**	**0.05740↑**	0.03798	**0.05492↑**
	relative to BrW	4.486	4.480	4.967	**5.693↑**	**6.202↑**	4.183	**5.994↑**
Adrenals (n=10; Group 4: n=8)	absolute [mg]	59.99	61.37	60.60	68.26	**76.78↑**	60.55	**74.81↑**
	relative to BW	0.02865	0.02904	0.03003	**0.03569↑**	**0.03909↑**	0.02840	**0.03640↑**
	relative to BrW	3.086	3.119	3.185	**3.721↑**	**4.187↑**	3.139	**3.962↑**
Pituitary (n=10; Group 4: n=8)	absolute [mg]	12.11	13.16	10.73	10.51	10.58	12.34	**10.21↓**
	relative to BW	0.00579	0.00627	0.00532	0.00552	0.00532	0.00578	**0.00498↓**
	relative to BrW	0.6236	0.6708	0.5651	0.5745	0.5767	0.6407	**0.5404↓**

1) BW = body weight
2) BrW = brain weight
↑, ↓: significantly increased, decreased compared with Control; p<=0.05, many-to-one t-test, two-sided

Recovery (Males and Females)

Parameter	Group	1	2	3	4	5	6	7
	Dose BI 1356 BS [mg/kg]	**0**	**0.5**	**2**	**4**	**2**	**4**	**0**
	Dose metformin BS [mg/kg]	**0**	**100**	**400**	**800**	**800**	**0**	**800**
Males	Body weight [g]	395.42	-	-	**353.50↓**	-	-	376.82
Heart (n=10; Group 4: n=9)	absolute [g]	1.0454	-	-	1.1125	-	-	1.1360
	relative to BW	0.2651	-	-	**0.3145↑**	-	-	**0.3025↑**
	relative to BrW	49.27	-	-	**54.34↑**	-	-	**55.28↑**
Females	Body weight [g]	223.67	-	-	216.52	-	-	216.44
Heart (n=10; Group 7: n=9)	absolute [g]	0.7578	-	-	**0.8402↑**	-	-	0.8247
	relative to BW	0.3384	-	-	**0.3879↑**	-	-	**0.3807↑**
	relative to BrW	38.32	-	-	**43.32↑**	-	-	**42.93↑**
Liver (n=10; Group 7: n=9)	absolute [g]	5.3276	-	-	5.6863	-	-	5.7527
	relative to BW	2.387	-	-	**2.623↑**	-	-	**2.664↑**
	relative to BrW	269.0	-	-	293.0	-	-	**299.8↑**
Ovaries (n=10; Group 7: n=9)	absolute [mg]	84.50	-	-	91.78	-	-	**99.80↑**
	relative to BW	0.03782	-	-	0.04237	-	-	**0.04639↑**
	relative to BrW	4.261	-	-	4.728	-	-	**5.195↑**

1) BW = body weight
2) BrW = brain weight
↑, ↓: significantly increased, decreased compared with Control; p<=0.05, many-to-one t-test, two-sided

Histopathology

Adequate Battery - Yes (all tissues from control and HD combination fixed and examined; all target organs examined from all animals of all other groups)

Peer Review - Yes

Histological Findings

Major histology findings were consistent with metformin-related toxicity and were summarized above with additional details described here. Findings in the linagliptin HD control group were considered unremarkable which was consistent with low linagliptin exposures compared to metformin. Linagliptin findings were limited to low incidence and severity of immune cell infiltration and/or inflammation in heart and kidneys. Findings in the LD combination, limited to minimal kidney hyaline droplets in 2/10 males, were consistent with dose-related findings at higher combination doses (increased incidence and severity) but were not considered adverse in the absence of any functional kidney changes.

Histological lesions in MD and HD combinations were consistent with metformin HD controls and most signs of toxicity were reversible after the drug-free recovery period. In the major target organs there were a low number of recovery animals with minimal to slight lesions in heart and kidney, although kidney basophilic tubules remained in up to 50% of HD metformin males. Minimal salivary gland hypertrophy and degeneration and increased numbers of corpora lutea (minimal severity) also remained in up to 60% of females. Recovery lesions are summarized in the Sponsor's table, below.

Histopathology summary (recovery)

	Recovery					
Group	1		4		7	
Dose BI 1356 BS [mg/kg]	0		4		0	
Dose metformin BS [mg/kg]	0		800		800	
Gender	M	F	M	F	M	F
No. of animals examined	10	10	9[1)]	10	10	9[2)]
Heart	n=10	n=10	n=9	n=10	n=10	n=9
fibrosis/fibroplasia myocard	0	0	1	0	0	2
infiltration	1	0	0	1	2	0
inflammation	5	0	1	2	1	2
vacuolation, cytoplasm.	0	0	0	0	0	1
Kidneys	n=10	n=10	n=9	n=10	n=10	n=9
tubules, basophilic	1	0	1	3	5	3
infiltration, cellular	0	1	1	3	2	2
inflammation	0	1	2	3	1	1
vacuolation, tubuloep.	0	0	0	0	0	1
Sublingual salivary glands	n=8	n=9	n=7	n=10	n=9	n=9
hypertrophy, ductular epithelium	0	0	0	4	0	0
Parotid salivary glands	n=10	n=10	n=9	n=10	n=10	n=9
hypertrophy, ductular epithelium	0	0	0	3	0	0
degeneration	0	0	0	6	3	4
Submandibular salivary glands	n=10	n=10	n=9	n=10	n=10	n=9
hypertrophy, ductular epithelium	0	0	0	3	0	0
Ovaries		n=10		n=10		n=9
Corpora lutea, increased in number	-	0	-	2	-	5

1) recovery animal No. 414 excluded
2) recovery animal No. 774 excluded

Special Evaluation – Additional staining was performed for all kidney samples (chromotrope-aniline-blue (CAB)) and on heart (fat staining) for selected animals.

Stability and Homogeneity – Stock assay solutions and dosing solutions were tested appropriately for stability, homogeneity, and drug concentration. Mean concentrations ranged from 88.5% to 105.7% of linagliptin and 94.9% to 106.6% of metformin nominal concentrations.

Toxicokinetics – Both drugs were readily absorbed and increased with increasing dose. Linagliptin increased less than dose proportionally from 0.5 to 4 mg/kg. Linagliptin exposure was slightly higher, approximately 20-25%, after repeated dosing. Co-treatment with metformin did not consistently affect linagliptin exposure and there was no consistent sex difference in exposure.

Metformin exposure increased greater than dose-proportionally on day 88 and exposure was up to 2-fold higher after repeated dosing at ≥ 400 mg/kg. There was no apparent effect of linagliptin co-treatment on metformin exposure.

Metformin was detected in only one control (out of 80 samples), at an insignificant concentration, 69.4 nmol/l, slightly above the detection limit (50 nmol/l). However, metformin was detected in most linagliptin only animals (64 of 80 samples), ranging from 53 to 2340 nmol/l. Metformin concentrations were approximately 10- to 1000-fold lower than mean C_{max} in the lowest metformin treated group (100 mg/kg + 0.5 mg/kg linagliptin). Based on Sponsor calculations, approximately 0.5 mg/ml dosing solution would be needed to account for plasma metformin levels, which was not excluded by the 1 mg/ml quantification limit in the administered dosing formulations. Data showed variability was very high within and between individual rats and time course data were not consistent with absorption and elimination curves in metformin-treated rats (see Table, below). The TK report concluded plasma sample contamination *ex vivo* was likely responsible for detectable metformin. However, the presence of metformin in nearly all samples on days 1 and 88 suggest possible cross-contamination exposure *in vivo*, albeit to very low levels of metformin. The absence of metformin exposure in the vehicle control group is puzzling compared to the consistent presence of metformin in the linagliptin only group. Nevertheless, even assuming low-level metformin exposure to the linagliptin only animals, toxicity trends confirm any metformin exposure was below the NOAEL.

Sponsor's table of plasma metformin in linagliptin-only group

Group 6 gender: males

day	time [h]	621 [nmol/L]	622 [nmol/L]	623 [nmol/L]	624 [nmol/L]	N	mean [nmol/L]	CV %	SD [nmol/L]
1	1	<50.0	<50.0	<50.0	53.2	4	13.3	200.0	26.6
1	2	2340	167	82.3	<50.0	4	647	174.6	1130
1	4	91.7	244	113	102	4	138	51.9	71.4
1	8	88.5	365	109	74.2	4	159	86.7	138
1	24	<50.0	<50.0	<50.0	<50.0	4	0	NC	0
88	1	1030	140	796	245	4	553	77.6	429
88	2	433	677	128	<50.0	4	310	98.5	305
88	4	130	679	208	73.1	4	273	101.5	277
88	8	839	753	346	176	4	529	60.3	319
88	24	139	131	75.5	55.5	4	100	41.0	41.1

Group 6 gender: females

day	time [h]	671 [nmol/L]	672 [nmol/L]	673 [nmol/L]	674 [nmol/L]	N	mean [nmol/L]	CV %	SD [nmol/L]
1	1	64.9	<50.0	<50.0	<50.0	4	16.2	200.0	32.5
1	2	<50.0	57.3	104	<50.0	4	40.3	124.8	50.3
1	4	191	133	75.6	83.1	4	121	44.2	53.4
1	8	68.3	1450	179	109	4	452	147.7	667
1	24	<50.0	<50.0	<50.0	58.0	4	14.5	200.0	29.0
88	1	488	88.7	465	114	4	289	75.1	217
88	2	421	163	1770	112	4	617	126.7	781
88	4	198	1160	1030	832	4	805	53.0	427
88	8	497	368	1590	561	4	754	74.7	563
88	24	155	204	131	NOR	3	163	22.8	37.2

NOR no result
<x.x below the quantification limit
NC not calculated

Table 3 – 13-Week rat linagliptin + metformin TK summary

BI 1356 BS (linagliptin) and metformin: 13-week oral (gavage) combination toxicity study in rats. Mean toxicokinetic parameters of BI 1356 BS

Parameter	Day	Gender/ Dose	Group 2	Group 3	Group 4	Group 5	Group 6
			0.5 mg/kg +100 mg/kg metformin	2 mg/kg +400 mg/kg metformin	4 mg/kg +800 mg/kg metformin	2 mg/kg +800 mg/kg metformin	4 mg/kg without metformin
C(max) [nmol/L]	Day 1	m	9.18	39.0	37.2	18.8	17.7
		f	13.9	64.3	63.0	25.2	25.4
		m&f	11.5	51.6	50.1	22.0	21.5
	Day 88	m	16.1	37.1	25.3	15.6	38.3
		f	20.1	50.5	21.8	16.2	99.4
		m&f	18.1	43.8	23.5	15.9	68.9
AUC(0-24h) [nmol·h/L]	Day 1	m	123	251	289	201	203
		f	99.7	313	407	222	208
		m&f	112	282	348	211	205
	Day 88	m	166	308	344	253	265
		f	149	297	309	218	457
		m&f	158	302	327	235	361

m&f: male & female

Mean toxicokinetic parameters of metformin

Parameter	Day	Gender/ Dose	Group 2	Group 3	Group 4	Group 5	Group 7
			100 mg/kg +0.5 mg/kg BI 1356 BS	400 mg/kg +2 mg/kg BI 1356 BS	800 mg/kg +4 mg/kg BI 1356 BS	800 mg/kg +2 mg/kg BI 1356 BS	800 mg/kg without BI 1356 BS
C(max) [nmol/L]	Day 1	m	44,900	103,000	143,000	144,000	170,000
		f	37,200	112,000	182,000	183,000	178,000
		m&f	41,000	108,000	162,000	163,000	174,000
	Day 88	m	51,400	123,000	198,000	227,000	219,000
		f	57,400	198,000	361,000	324,000	305,000
		m&f	54,400	161,000	279,000	275,000	262,000
AUC(0-24h) [nmol·h/L]	Day 1	m	272,000	790,000	1,690,000	1,500,000	1,610,000
		f	147,000	613,000	1,480,000	1,360,000	1,300,000
		m&f	210,000	702,000	1,580,000	1,430,000	1,450,000
	Day 88	m	221,000	1,160,000	2,330,000	3,220,000	2,770,000
		f	226,000	1,200,000	4,900,000	4,200,000	3,870,000
		m&f	224,000	1,180,000	3,620,000	3,710,000	3,320,000

m&f: male & female

7 Genetic Toxicology

Genetic toxicity of linagliptin and metformin were assessed individually to support the safe use of each monotherapy. Neither linagliptin nor metformin were found to have genotoxic potential. Several known and potential impurities and degradation products were identified for linagliptin. Most compounds were qualified in the development program and discussed in the original linagliptin review.[8] Genetic toxicity studies on an additional potential linagliptin impurity, (b) (4) are reviewed below.

7.4 Other Genetic Toxicity Studies

The Sponsor identified impurities of the FDC tablet drug product which required identification and potentially qualification. Assays conducted to qualify potential genetic toxicity of impurities are reviewed below. Unless otherwise noted, assays were conducted at the Sponsor's laboratory under the following conditions.

Study report location:	eCTD, 4.2.3.7.6
Boehringer laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Dept. Non-Clinical Drug Safety (NDS) Birkendorfer Str. 65 88397 Biberach an der Riss Germany

Ames Assay Methods (Mutagenesis)

Primary Conducting Lab:	Boehringer Ingelheim (Germany)
Strains:	Standard *S. typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102. See Sponsor's strain summary table below.
Basis of concentration selection:	Exploratory plate incorporation assay and tested up to the limit dose or solubility limit (precipitate that prevented plate reading) or excessive toxicity based on reduced background lawn.
Negative control:	Vehicle (water or DMSO unless otherwise noted)
Positive control:	See Sponsor's summary table below.
Formulation/Vehicle:	Water or DMSO (unless otherwise noted)
Incubation & sampling time:	Standard Ames plate incorporation and pre-incubation (20 min at 37°C) assays ± Aroclor 1254-induced rat liver S9 (b) (4) Bacteria plated for 2 d (3 d for TA102) at 37°C and counted with an automatic colony counter (or manually if necessary based on amount of precipitation). Assays run in triplicate.

[8] NDA 201280, Pharmacology/Toxicology Review (D. Carlson, 3/4/11)

Criteria for a valid study: Negative control revertants within the laboratory's historical range (approx. 120 assays) and positive control revertants clearly increased. Criteria for a positive result were based simply on concentration-dependent increase in revertants (rather than any multiple over background)

Criteria for a positive result: Reproducible, concentration-dependent increase in revertants over vehicle control and/or outside the historical range in at least one tester strain.

Typical sponsor's bacterial tester strain summary

(Ames test) – Bacterial tester strains

Bacterial strain	His- gene	Additional markers LPS	Repair	R-factor	Detected mutation
S. typhimurium					
TA 1537	*hisC3076*	*rfa*	*uvrB*	-	Frameshift
TA 98	*hisD3052*	*rfa*	*uvrB*	pKM101	Frameshift
TA 100	*hisG46*	*rfa*	*uvrB*	pKM101	Base substitution
TA 1535	*hisG46*	*rfa*	*uvrB*	-	Base substitution
TA 102	*hisG428*	*rfa*	+	pKM101, pAQ1	Base substitution

Boehringer Ingelheim's bacterial tester strain summary

Reference Substances	Abbreviation	CAS No.	Test concentration (µg/plate)
Without activation:			
2-Nitrofluorene	2-NF	607-57-8	10
Sodium azide	NaN_3	26628-22-8	5
Mitomycin C	MMC	50-07-7	0.5
9-Aminoacridine	9-AA	90-45-9	50
With Activation:			
2-Aminoanthracene	2-AA	613-13-8	4 or 10 (TA 102)

In vitro HPBL Chromosome Aberration Assay (Clastogenesis)

Strains:	Primary human lymphocyte cultures from peripheral blood of a single or pooled donor(s) per experiment
Basis of concentration selection:	Pre-test for solubility up to the limit dose of 5000 µg/ml or 10 mmol/L and/or assessment of mitotic index (proportion of mitotic cells/1000 cells) to identify cytotoxic concentrations
Negative control:	Vehicle (water or DMSO unless otherwise noted)
Positive control:	See Sponsor's summary table, below.
Formulation/Vehicle:	Water or DMSO (unless otherwise noted)
Incubation & sampling time:	Primary human lymphocytes in culture (stimulated to divide with phytohaemagglutinin (PHA)) exposed to test article for: (1) 4 h ± Aroclor 1254-induced rat liver S9 (b) (4) 20 h recovery growth; (2) 24 h -S9 continuous treatment. Cell division arrested with Colcemid® 2 h prior to end of incubation, cells harvested, slides prepared for examination of chromosome damage during metaphase. Duplicate cultures per treatment.
Criteria for a valid study:	Assays acceptable if negative and positive control values were within the conducting labs historical control range. Evaluation of ≥ 2000 polyploid cells, mitotic index evaluated, ≥ 100 metaphase cells per culture scored in duplicate for each concentration.
Criteria for a positive result:	Reproducible, concentration-dependent statistically significant increase in chromosomal aberrations (excluding gaps), with consideration of increases compared to the historical control range.

Boehringer Ingelheim's HPBL chrom. ab. assay positive control summary

Adriamycin (ADR) 0.05 and 0.075 µg/mL	Batch: 070646 CAS-No. 25316-40-9 (b) (4)	
Endoxan® Cyclophosphamide (CP) 7 µg/mL	Batch: 8B590E CAS-No. 6055-19-2 (b) (4)	Factor: 1.069

7 µg/mL CP is equivalent to 7.483 µg/mL Endoxan®.

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8 Carcinogenicity

Carcinogenic potential was previously assessed in lifetime rat and mouse bioassays for linagliptin and metformin. Results are described in the labels of the individual drugs and no potential interactions were uncovered in combination toxicity studies to suggest altered or additional carcinogenic potential.

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity were previously assessed in rats and rabbits for linagliptin and metformin, with results described in the labels for the individual drugs. Additional pivotal embryofetal development studies were conducted in Wistar rats, which are reviewed below.

9.2 Embryonic Fetal Development

Metformin embryofetal development in rat (Seg 2 Rat)
GLP statement, signed 10/21/10

Metformin: Study for effects on embryo-fetal development in rats by oral (gavage) administration (Study 09B099, Doc. No. U10-2386-01)

Doses (mg/kg/d) *0, 200, 500, 1000 metformin (free base)*
*638, 1730, 3690 µM*h* ***(4X, 11X, 23X MRHD)***

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

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, slightly reduced (<10%) BW gain at 200 and 500 mg/kg and approximately 15% 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.

Key study findings:

- Treatment generally did not affect pregnancy success but metformin was teratogenic at ≥ 500 mg/kg dosing during embryofetal 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.

Study no:	09B099 (Doc. No. U10-2386-01)
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Birkendorfer Str. 65 88397 Biberach an der Riss Germany
Date of study initiation:	5/28/09 (animal arrival)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Metformin HCl, Batch 801941, 100.1% purity

Methods

Doses:	0, 200, 500, 1000 mg/kg metformin
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	Suspension in 0.5% hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Wistar Han rat (Crl:WI(Han) (SPF confirmed)
Number/Sex/Group:	24 pregnant females
Satellite groups:	6 pregnant females/gr. TK (3 controls) 6 pregnant females/gr. blood glucose (6 controls)
Study design:	Pregnant females treated QD by oral gavage GD 7-16, necropsy and C-section GD 22. Pregnancy parameters determined macroscopically, fetuses examined for external, visceral, and skeletal variations and malformations. Selected fetal tissues examined histologically. Historical control data for comparison were from 2 comprehensive studies with Natrosol 250 HX vehicle and from vehicle control groups in 6 other studies in the conducting lab between 2003 and 2009.
Deviation from study protocol:	Unremarkable.

Observations and Results:

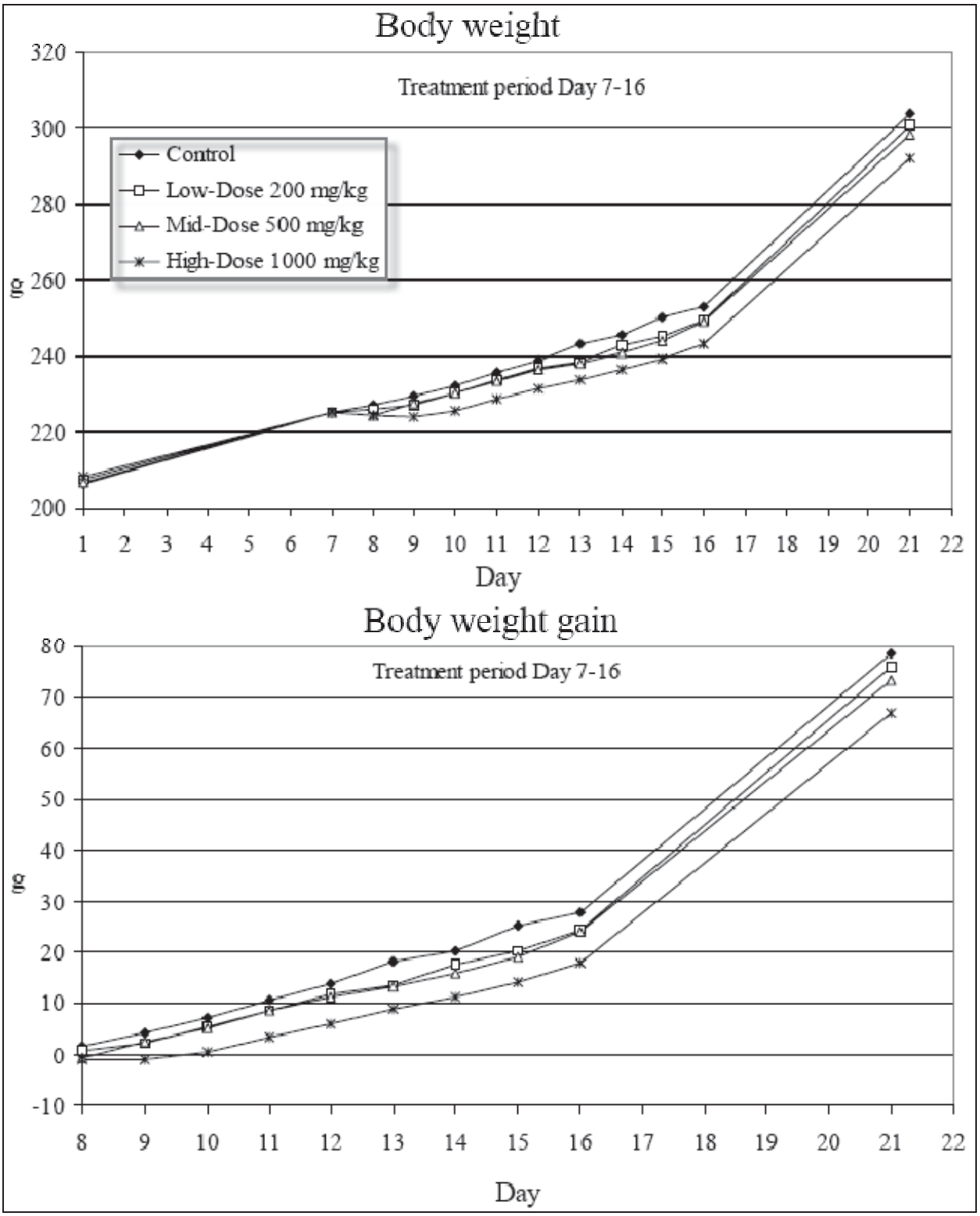
Mortality and Clinical Signs – Pregnant rats seemed to tolerate all metformin doses. There were no deaths and no consistent clinical signs

Body Weight (GD 1, 7-16, and 21) – Maternal BW gain was reduced with increasing metformin dose. BW gain was minimal in the LD and MD for one day and in the HD for three days after the beginning of dosing, after which BW gain increased at a similar rate as controls. BW and BW gain were significantly reduced at various time points in treatment groups. Mean BW data are shown in the Sponsor's table and weight gain trends are apparent in the Sponsor's figures, below.

Sponsor's BW gain summary table

Dose [mg/kg]	n dams	Mean of body weight gain [g] relative to GD 7							
		GD 8	GD 9	GD 10	GD 11	GD 14	GD 15	GD 16	GD 21
Control	21	1.76	4.22	7.19	10.56	20.41	25.20	27.78	78.60
200	22	0.72	2.13*↓	5.30	8.70	17.54	20.16*↓	24.44	75.93
500	22	-0.55*↓	2.54	5.14	8.52	16.00*↓	19.06*↓	24.06	73.27
1000	18	-0.94*↓	-1.10*↓	0.48*↓	3.40*↓	11.22*↓	14.17*↓	17.96*↓	67.03*↓

* significant difference ($p<0.05$)
↓ decreased
GD gestation day


Body weight
Treatment period Day 7-16
Control
Low-Dose 200 mg/kg
Mid-Dose 500 mg/kg
High-Dose 1000 mg/kg
g
320
300
280
260
240
220
200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Day
Body weight gain
Treatment period Day 7-16
g
80
70
60
50
40
30
20
10
0
-10
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Day

Feed Consumption (GD 7, 14, 21) – Food consumption was measured in weekly intervals, so analyses are limited to weekly periods. Food consumption was significantly decreased in MD (↓9%) and HD (↓22%) groups during the first week of dosing (Week 2, GD 14 time point), corresponding to decreased BW gain at the beginning of dosing. Food consumption likely increased stabilized after the first few days of dosing, similar to BW trends, but it was not possible to decipher daily trends. Food consumption was unchanged in the LD and similar in all groups between GD 14 and GD 21.

Toxicokinetics and Blood Glucose (GDs 7 & 16, 0, 1, 2, 4, 8, 24 h post-dose, K-EDTA (TK) and lithium heparin (glucose) anticoagulants) **–**

Metformin exposure was confirmed in satellite pregnant females. Exposure increased with increasing dose, slightly less than dose-proportional for C_{max} (3.5X increase) and approximately dose proportional for AUC_{0-24} over the 5-fold dose range. Total exposure (AUC) but not C_{max} was slightly increased after repeated dosing on GD 16 compared to initial dosing on GD 7. Metformin was detected in 3 of 21 control samples (and 2 samples did not have a valid assessment), all of which were "far below the trough values (C(24h)) at the LD. Slight contamination of samples ex vivo was suggested but not considered toxicologically significant.

Sponsor's metformin TK summary

parameter	Gestation day*	200 mg/kg	500 mg/kg	1000 mg/kg
C(max)	7	68200	181000	294000
[nmol/L]	16	79200	135000	275000
AUC(0-24h)	7	377000	1360000	2130000
[nmol·h/L]	16	638000	1730000	3690000

*: GD 7 = day 1 of dosing; GD 16 = day 10 of dosing

Blood glucose data on GD 7 and GD 16 were variable. Several blood glucose time points were not analyzed statistically because of non-pregnant females and a MD death during blood sampling. Glucose was decreased in some animals postdose, but there were no clear dose-related trends for decreased blood glucose. Metformin is thought to decreased hyperglycemia predominantly by increasing peripheral glucose uptake and insulin sensitivity, which is consistent with the absence of a hypoglycemic effect in healthy animals.

Sponsor's blood glucose summary

Parameter [unit]	Day		Daily dose of [mg/kg]						
			0	200		500		1000	
		Group	1	2		3		4	
		Time point#	mean	mean	Δ%	mean	Δ%	mean	Δ%
Glucose [mmol/L]	1+	0	11.1	11.7	n.a.	11.5	n.a.	10.9	n.a.
		1	9.75	10.0	n.a.	8.93	n.a.	11.4	n.a.
		2	11.4	11.1	n.a.	11.0	n.a.	12.3	n.a.
		4	11.0	9.20	n.a.	8.30	n.a.	6.78	n.a.
		8	9.90	9.32	n.a.	5.95	n.a.	5.43	n.a.
		24	12.4	12.0	n.a.	6.88	n.a.	6.16	n.a.
	10++	0	7.08	6.29	-11.2	5.86	-17.3	7.23	2.2
		1	7.08	4.82	-31.9	7.53	6.4	**9.89**↑	39.6
		2	7.07	6.33	10.4	8.67	22.7	**11.4**↑	60.4
		4	7.44	6.93	-6.86	8.25	10.8	9.19	23.6
		8	7.35	5.01	-31.2	4.99	-32.1	7.67	4.4
		24	6.97	5.33	-23.4	6.55	-6.0	6.78	-2.7

hours after treatment
+ gestation day 7
++ gestation day 16
non-pregnant females Nos. 108, 110, 215 and 311 excluded
animal No. 315 died during blood sampling on time point 0, day 10
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
n.a.: not applicable

Necropsy

Cesarean Section Data (Implantation sites, pre- / post-Implantation loss, etc.) –

Maternal gross necropsy was unremarkable for all dams, suggesting short term dosing during pregnancy did not cause metformin-induced toxicity seen in standard toxicity studies.

Several animals were not pregnant at terminal necropsy (3/24, 1/24, 2/24, 5/24, respectively) and 1/24 LD and 1/24 HD had complete resorption of embryos. Animals were mated prior to treatment so fertility and pregnancy success were not assessed in this study. The absence of a dose-response in dams with complete resorptions does not support a treatment-related effect of metformin on embryonic loss.

Total mean implantations were slightly lower in the HD dams ($\bar{x}$ = 10.3 HD vs. 11.7 controls, ss). Small numbers of implantation and viable fetuses in individual dams of MD (1/22) and HD (1/18) groups were also outside the range of a dedicated vehicle control study. Mean pre-implantation loss was also slightly higher in MD and HD (nss). Similar to fertility parameters, implantation and pre-implantation loss occurred prior to beginning of treatment and thus cannot be attributed to metformin. There was a trend of decreased HD mean viable fetuses ($\bar{x}$ = 11.0, 11.0, 10.6, 9.8, respectively, nss), which is consistent with slightly lower total implantations in the HD (which were occurred prior to treatment). Selected data are shown in the table below.

Other pregnancy parameters were not different in metformin groups, including: dead fetuses (none in any group); fetal sex; resorptions (total, late, or early); or, fetal weight.

Selected pregnancy parameters

		Metformin			Spontaneous Incidences from Evaluation Study [U03-1549] mean/range	
	Control	200 mg/kg	500 mg/kg	1000 mg/kg		
n litters +	21	22	22	18	85	
Litter parameters (means/individual range per dam)					overall means	ranges of means/ individual data
Corpora lutea	12.7/10-15	12.8/10-15	13.0/10-15	12.3/10-15	12.0	11.8-12.3/ 9.0-15.0
Implantations	11.7/9-13	11.5/9-14	11.3/4-15	10.3*↓/2-14	11.1	10.9-11.4/ 5.0-15.0
Viable fetuses	11.0/9-13	11.0/8-14	10.6/3-15	9.8/2-14	10.5	10.1-10.7/ 5.0-14.0
Pre-implantation loss (%)	7.66/ 0[7.14]-23.08	9.62/ 0[7.14]-26.67	13.44/ 0[7.14]-69.23	16.31/ 0[7.69]-81.82	7.57	6.13-10.06/ 0[6.67]-46.15## (61.54)
Resorption rate [%]	6.06/ 0[7.69]-25.00	4.44/ 0[7.69]-18.18	6.79/ 0[7.14]-25.00	5.34/ 0[9.09]-20.00	5.70	2.30-7.29/ 0[7.69] -23.08# (54.55)

\+ non-pregnant animals and animals with resoptions only excluded
\# one outlayer (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlayer included
\# # one outlayer (No. 222) excluded (in brackets: No. 222 included); mean calculated with the outlayer included
[ ] the lowest number greater than 0

Reviewer's note – selected data from Sponsor's summary table.

Offspring (Malformations, Variations, etc.) –

Variations

Total variations were similar across treatment groups, with no apparent total increase in variations with metformin treatment. The Sponsor did not analyze variations on a per litter basis so litter comparisons across treatment groups were not clear.

Several **visceral variations** were seen in control and treatment groups at rates outside the range seen in reference vehicle studies. Shortened truncus brachiocephalicus and small kidney caudal (unilateral) were increased in the HD and outside the reference vehicle control studies, suggesting an effect of metformin treatment.

Bowed or dilated ureter (unilateral or bilateral) were increased in LD and MD groups (nss) compared to controls, but the absence in the HD and occurrence in concomitant controls outside the historical comparator group suggest the finding was not likely caused by metformin treatment. Selected data are shown in the table below.

Notable visceral variations

Findings			Metformin			Spontaneous incidences
		G 1	G 2	G 3	G 4	
			Dose [mg/kg]			
		Control	200	500	1000	
		n fetuses / incidences (%)				
		decimals rounded				decimals rounded
Variations						
Visceral variations		decimals truncated				decimals rounded
Number of litters		**21**	**22**	**22**	**18**	**85**
Number of fetuses		**109**	**118**	**111**	**83**	**424**
Bowed ureter	bil	1/0.91	3/2.54	0	0	1/0.24−
	uni	2/1.83	5/4.23	4/3.60	1/1.20	3/0.71−
Dilated ureter	bil	2/1.83	4/3.38	0	0	1/0.24−
	uni	3/2.75	5/4.23	7/6.30	0	13/3.06+
Common trunk of pulmonary arteries at the Truncus pulmonalis		0	0	0	**1/1.20**	1/0.87§§
Truncus brachiocephalicus shortened		3/2.75	3/2.54	3/2.70	**7/8.43**	1/1.08$$
Small kidney caudal	uni	0	0	0	**3/3.61**	uni: 2/0.48+
Dilated renal pelvis	uni	0	0	0	1/1.20	2/1.86$

Findings at incidences above the actual Control group and above the spontaneous incidences from other studies underlined and in bold letters.

Reviewer's note – selected data from Sponsor's summary table

Skeletal variations were generally higher in concomitant controls and metformin treatment groups compared to reference vehicle studies. Compared to concomitant controls (and reference controls), skeletal variations were clearly increased in HD metformin fetuses and modestly increased in MD fetuses for a few variation types. Skeletal variations consisted almost entirely of absent, decreased, or incomplete ossification. Additional increased HD variations included flat thoracal vertebral body (11 fetuses (12% incidence) vs. 2 fetuses (1.6% incidence) in controls) and changes in anterior or posterior phalanges.

Findings in the MD that were consistent with a dose-related trend, whether statistically significant or not, included incomplete ossifications, flat thoracal vertebral body, and changes in phalanges. Trends that included MD findings are shown in the data table below.

Notable trends in skeletal variations

Findings			Metformin			Spontaneous incidences	
		G 1	G 2	G 3	G 4		
			Dose [mg/kg]				
		Control	200	500	1000		
		n fetuses / incidences (%)					
Variations							
Skeletal variations		decimals truncated				decimals rounded	
Number of litters		21	22	22	18	85	
Number of fetuses		122	125	122	93	465	
Frontal bone partly not ossified	bil	4/3.27	1/0.80	3/2.45	**14/15.05**	9/4.04++	9/1.94 +
	uni	2/1.63	0	1/0.81	**3/3.22**	-	
Parietal bone partly not ossified	bil	6/4.91	8/6.40	5/4.09	**28/30.10**	72/15.48+; 20/17.54$	
	uni	4/3.27	2/1.60	**6/4.91**	**9/9.67**	2/0.44+	
Interparietal bone partly not ossified		21/17.21	26/20.80	32/26.22	**51/54.83**	177/38.06+; 50/43.85$	
Orbitosphenoidal bone not ossified	bil	3/2.45	2/1.60	**4/3.27**	2/2.15	-	
	uni	7/5.73	6/4.80	5/4.09	**11/11.82**	-	
Thoracal vertebral body flat		2/1.63	2/1.60	7/5.73	**11/11.82**	small 1/0.22+; small: 4/1.79++; 3/2.88$$; 7/5.88##	
Proximal anterior phalanges <4	bil	15/12.29	17/13.60	23/18.85	**22/23.65**	19/ 16.66$	R: 86/ 18.50+
	uni	5/4.09	3/2.40	3/2.45	12/12.90	6/5.26$; 7/ 6.73$$;	L: 87/ 18.71+
Calcaneus not ossified	bil	82/67.21	77/61.6	**88/72.13**	**70/75.26**	225/48.39+; 42/40.38$$	
	uni	11/9.01	10/8.00	8/6.55	8/8.60	20/4.30+; 13/12.49$$;	

R = right side; L = left side; uni = unilateral; bil = bilateral
- = no finding
Findings at incidences above the actual Control group and above the spontaneous incidences from other studies underlined and in bold letters.

Reviewer's note – selected data from Sponsor's summary table, highlighting trends that were evident at both MD and HD (not inclusive of all findings at the HD)

Malformations **– Skeletal malformations** were clearly increased above concomitant and reference (historical) controls in the MD and HD. Dose-related increases in skeletal malformations were observed predominantly in rib (flat, thickened, and/or z-shaped). Scapula (bent inward) was seen in several litters at ≥500 mg/kg in fetuses with flat and thickened ribs. There were other isolated incidences (1 or 2 fetuses/litters) of other skeletal malformations. Two fetuses from a single HD fetus also had external

malformations of anophthalmia and polydactylia, providing further support for metformin-induced teratogenesis. Fetal malformations are shown in the Sponsor's summary table, below.

Fetal malformation summary

Findings			Metformin			Spontaneous incidences	
		G 1	G 2	G 3	G 4		
			Dose [mg/kg]				
		Control	200	500	1000		
		n fetuses / incidences (%)					
Malformations							
External malformations		decimals truncated				decimals rounded	
Number of litters		**21**	**22**	**22**	**18**	**85**	
Number of fetuses		**231**	**243**	**233**	**176**	**889**	
Encephalocele		1/0.43	0	0	0	-	
Anophthalmia	uni	0	0	0	**1/0.56**	-	
Polydactylia (right hind limb with six digits)	uni	0	0	0	**1/0.56**	-	
Skeletal malformations		decimals truncated				decimals rounded	
Number of litters		**21**	**22**	**22**	**18**	**85**	
Number of fetuses		**122**	**125**	**122**	**93**	**465**	
Cleft cervical vertebral body		2/1.63	0	0	2/2.15	5/1.08+; 6/2.69++	
Cervical vertebral body unilaterally ossified		4/3.27	1/0.80	1/0.81	0	1/0.22+	
Cleft thoracal vertebral body		0	0	**2/1.63**	**1/1.07**	1/0.22+; 1/0.84##	
Thoracal vertebral bodies fused unilateral		0	0	1/0.81	0		
Split sternebra lateral axis		0	0	0	**1/1.07**	1/0.25++ [day 23]	
Missing rib	uni	0	0	1/0.81	0		
Bifurcated rib	uni	0	0	1/0.81	0		
Flat and thickened rib	bil	5/4.09	17/13.60	**27/22.13**	**37/39.78**	45/9.68+; 33/14.80++; 5/13.51#	
	uni	7/5.73	5/4.00	7/5.73	**15/16.12**		
Rib z-shaped	bil	0	0	0	**13/13.97**	0##	2/0.43+;
	uni	0	0	1/0.81	4/4.30	2/1.68 ##	24/10.77 ++
Findings without classification							
Scapula bent inwardly	bil	0	0	**2/1.63**	**7/7.52**	-	
	uni	0	0	**2/1.63**	**5/5.37**	1/0.87$	
Scapula and acromion process (proximal) bent inwardly	bil	0	0	1/0.81	0	-	

R = right side; L = left side; uni = unilateral; bil = bilateral

- = no finding

Findings at incidences above the actual Control group and above the spontaneous incidences from other studies underlined and in bold letters.

Linagliptin + metformin embryofetal development in rat (Seg 2 Rat)
GLP statement, signed 10/26/10

BI 1356 (Linagliptin) and metformin: Study for effects on embryo-fetal development in rats by oral (gavage) administration (Study 09B138, Doc. No. U10-2448-01)

Doses	*0/0 (vehicle control)*
(mg/kg/d)	*1/200, 2.5/500, 5/1000, 2.5/1000*
lina./met.	*5/0 (linagliptin control)*
	0/1000 (metformin control)
	30/0 (MD linagliptin control for placental transfer)
	240/0 (HD linagliptin control for placental transfer)

Exposures:	*0.238 / 528, 0.347 / 1510, 0.618 / 4810, 0.347 / 3830 (linagliptin / met)*
*(µM*h)*	***(1.5X/3X, 2X/9X, 4X/30X, 2X/24X MRHD)***
	*0.470 / 0 (**3X MRHD** linagliptin)*
	*0 / 3670 (**23X MRHD** metformin)*

NOAEL (maternal)** = 5 mg/kg linagliptin **(3X MRHD)**; < 200 mg/kg met. **(<3X MRHD)
NOAEL (fetal)** = 1/200 mg/kg (linagliptin / metformin) **(1.5X/3X MRHD)

NOAEL determination – Linagliptin alone was not considered teratogenic (multiple malformations in a single fetus were considered incidental). Metformin ± linagliptin ≥500 mg/kg/d (≥9X MRHD) caused skeletal malformations at maternally toxic doses which were slightly exacerbated in combination with linagliptin. At the lowest metformin dose of 200 mg/kg, maternal BW effects were modest but significant and fetal findings were considered within the background range.

Key study findings:

- Metformin caused a dose-related increase in skeletal variations and malformations at maternally toxic doses, slightly exacerbated with linagliptin coadministration.
- Metformin caused dose-related decreased BW (consistent with decreased food consumption) during the first few days of treatment, exacerbated by linagliptin coadministration, with BW gain reduced throughout treatment and remaining slightly reduced in combination HD metformin groups until termination.
- No remarkable treatment-related effects on pregnancy parameters. Consistent with reduced maternal BW gain, mean fetal body weights were slightly reduced in 1000 mg/kg metformin (± linagliptin) to maximum -4% (ss only in HD combination).

- Linagliptin:metformin ratios of 1:200 and 1:400 bracketed the expected clinical ratios of 1:200, 1:340, and 1:400.

- Satellite linagliptin 30 and 240 mg/kg control groups were used to determine maternal transfer of linagliptin and confirmed fetal exposure of approximately 40-50% maternal exposure.

Study no:	09B138, Doc. No. U10-2448-01
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	Boehringer Ingelheim Pharma GmbH & Co. KG Birkendorfer Str. 65 88397 Biberach an der Riss Germany
Date of study initiation:	10/1/09 (animal arrival)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linagliptin, Batch 5060160, 98.0% purity; Metformin HCl, Batch 801941, 100.1% purity

Methods

Doses:	See study design summary, below
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	Suspension in 0.5% hydroxyethylcellulose (Natrosol® 250 HX)
Species/Strain:	Wistar Han rat (Crl:WI(Han) (SPF confirmed)
Number/Sex/Group:	42 pregnant females (48 vehicle controls) [12/group (16 vehicle controls) used for TK] [6/group for blood glucose analysis]
Satellite groups:	36 pregnant females/gr. linagliptin controls for TK determination of maternal transfer
Study design:	Pregnant females treated QD by oral gavage GD 7-16, necropsy and C-section GD 22. Pregnancy parameters determined macroscopically, fetuses examined for external, visceral, and skeletal variations and malformations. Selected fetal tissues examined histologically. Historical control data for comparison were from 2 comprehensive studies with Natrosol 250 HX vehicle and from vehicle controls from “other studies” between 2003 and 2010 in the conducting lab (cited in the study report when referenced).
Deviation from study protocol:	Unremarkable.

Study Design Summary

Group	Females/ group	Ratio of BI 1356 BS: Metformin	Daily dose			Animal No.
			BI 1356 BS [mg/kg/day]	Metformin HCl [mg/kg/day]	Metformin BS [mg/kg/day]	
1	48	0	0	0	0	101-148
2	42	1:200	1.0	256.4	200	201-242
3	42		2.5	641.0	500	301-342
4	42		5	1282.0	1000	401-442
5	42	1:400	2.5	1282.0	1000	501-542
6	42	-	5	0	0	601-642
7	42	-	0	1282.0	1000	701-742
8#	36	-	30.0	0	0	801-836
9#	36	-	240.0	0	0	901-936

Conversion factor metformin free base to salt 1.282 , # without evaluation of maternal, embryo-fetal or litter parameters, evaluation of placental transfer of BI 1356 BS

Observations and Results:

Mortality and Clinical Signs – There was no mortality in main study dams. Treatment-related clinical signs were limited to soft feces in HD metformin groups (± linagliptin). Technicians also observed increased digging activity and chewing on bedding for 5-10 min post-dose in HD metformin groups (± linagliptin).

One linagliptin HD TK dam from the satellite maternal transfer study was killed *in extremis* with clinical signs and macroscopic and histologic signs of gavage error. Two TK dams in the HD metformin only group died, one during anesthesia and one after blood sampling.

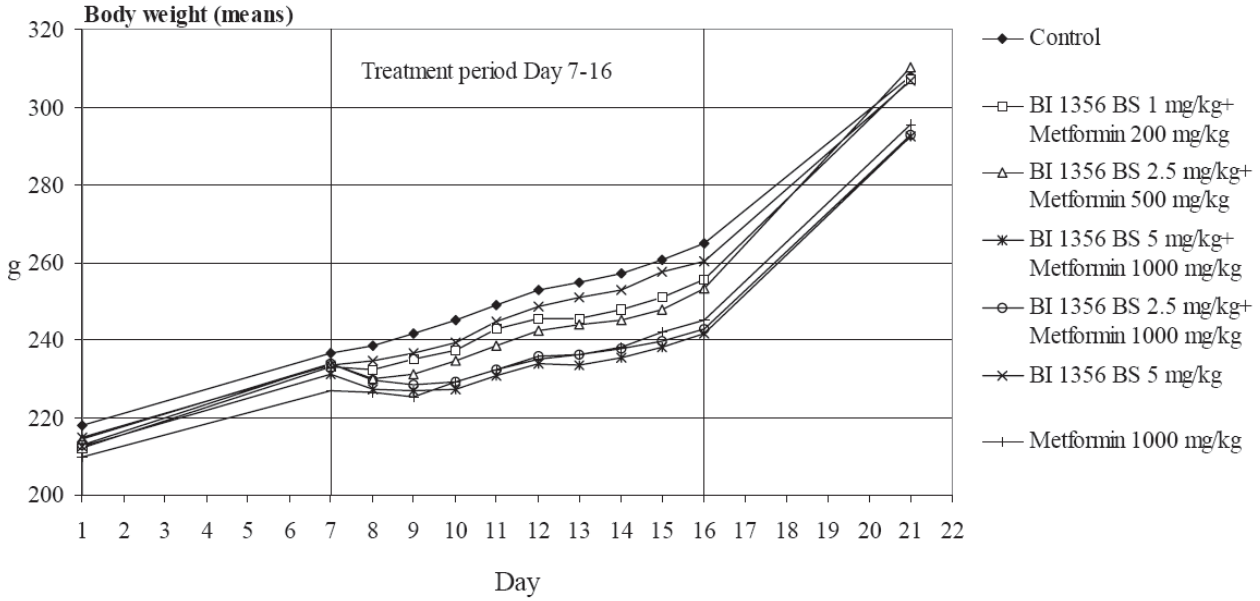
Body Weight (GDs 1, 7-16, and 21) –Metformin treatment caused decreased BW and reduced BW gain at all doses with severity increasing with dose. Dams lost weight during the first few days of metformin treatment, with the dose-related effect attenuated by about dosing day 4 and positive BW gain throughout the rest of treatment and pregnancy. Linagliptin alone had no apparent effect on BW but a slight trend (nss) to reduce BW gain during the first few days of treatment. BW trends were similar with combination treatment, as linagliptin coadministration with 1000 mg/kg metformin had no apparent effect on overall BW but exacerbated reduced BW gain. BW data are shown in the Sponsor's table and figures, below.

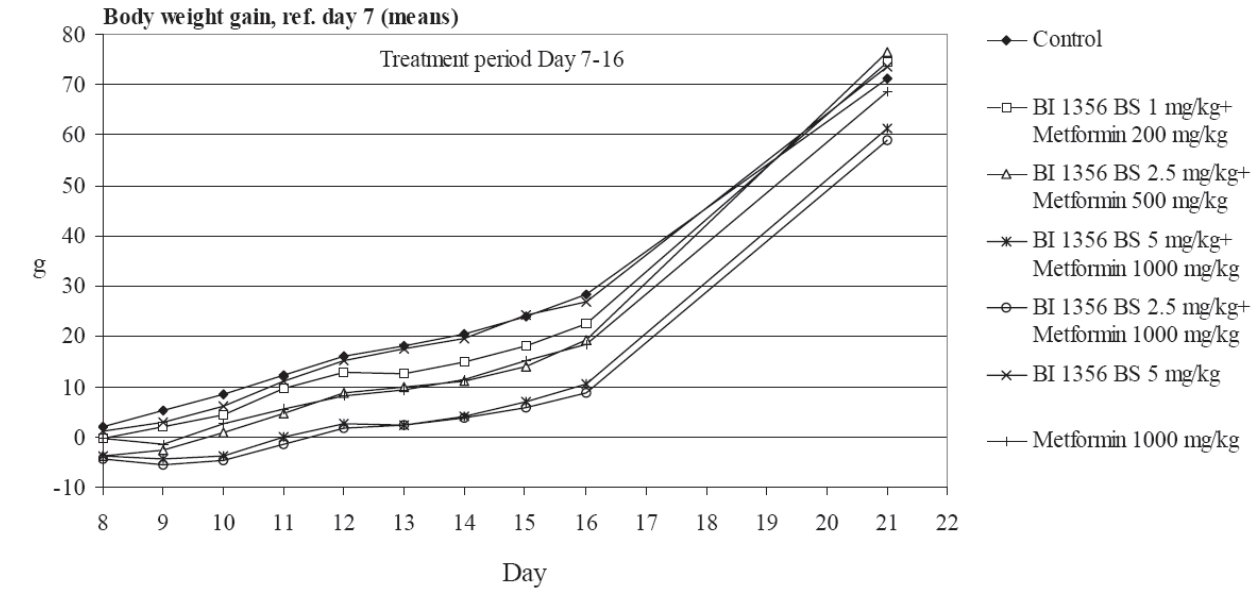
Sponsor's BW gain summary table

Dose# [mg/kg]	n dams	Mean of body weight gain [g] relative to GD 7							
		GD 8	GD 9	GD 10	GD 11	GD 14	GD 15	GD 16	GD 21
Control	19	1.93	5.05	8.45	12.18	20.48	23.79	28.19	71.29
1/200	21	-0.47*↓	1.92*↓	4.30*↓	9.71	14.95*↓	18.01*↓	22.52*↓	74.48
2.5/500	19	-3.91*↓	-2.69*↓	0.89*↓	4.73*↓	11.18*↓	14.09*↓	19.18*↓	76.43
5/1000	22	-3.75*↓	-4.42*↓	-3.90*↓	-0.20*↓	4.13*↓	6.98*↓	10.42*↓	61.30*↓
2.5/1000	18	-4.47*↓	-5.71*↓	-4.79*↓	-1.62*↓	3.79*↓	5.91*↓	8.82*↓	58.83*↓
5/0	18	1.17	2.99	5.98	11.12	19.44	24.13	26.86	73.57
0/1000	20	-0.28	-1.58*↓	2.42*↓	5.58*↓	11.27*↓	15.27*↓	18.31*↓	68.68

* significant difference ($p<0.05$) , ↓ decreased
\# BI 1356 BS/metformin
GD gestation day

Sponsor's body weight and body weight gain





Feed Consumption (GDs 7, 14, 21) – Dose-related decreased food consumption, up to -30%, was evident during treatment (week 2, GD 14) in metformin groups. Linagliptin did not affect food consumption alone or in combination with metformin. Analysis of food consumption trends was limited due to limited observations during treatment. Food consumption remained slightly lower (-9%) in the HD metformin (± linagliptin) group during week 3.

Toxicokinetics and Blood Glucose (GDs 7 & 16, at 1, 2, 3, 4, 8, 24 h post-dose, K-EDTA (TK) and lithium heparin (glucose) anticoagulants) **–**

Sponsor's blood sampling summary

Group	Females/ group	Blood samples				Daily dose			Animal No.
		n mothers			n mothers & embryos				
		TK GD 7	BG GD 7	TK GD 16	TK GD 16	BI 1356 BS [mg/kg/ day]	Metformin HCl [mg/kg/day]	Metformin B S [mg/kg/day]	
1	48	6	6	6	6	0	0	0	101-148
2	42	6	6	6	-	1.0	256.4	200	201-242
3	42	6	6	6	-	2.5	641.0	500	301-342
4	42	6	6	6	-	5.0	1282.0	1000	401-442
5	42	6	6	6	-	2.5	1282.0	1000	501-542
6	42	6	6	6	-	5.0	0	0	601-642
7	42	6	6	6	-	0	1282.0	1000	701-742
8	36	**6**	**6**	-	24 +**12**	30.0	0	0	801-836
9	36	**6**	**6**	-	24 +**12**	240.0	0	0	901-936

Conversion factor metformin base to salt 1.282
TK toxicokinetics (in-life, complete profile), GD gestation day; BG blood glucose

Linagliptin exposure increased less than dose-proportional between 1 and 5 mg/kg in combination with metformin (2X C_{max} and 2.7X AUC) over the 5-fold linagliptin dose range. Linagliptin exposure was slightly lower when given alone (5 mg/kg) compared to combination treatment with metformin, although differences were within the range of inter-individual variability and only a single dose was compared so calculated differences were likely biologically meaningless. There was no appreciable linagliptin accumulation after repeat dosing (up to 20-30% increased AUC in most dose groups).

Metformin total exposure increased greater than dose-proportional between 200 and 1000 mg/kg in combination with linagliptin (AUC increased >5-fold). Maximum (C_{max}) plasma exposure increased less than dose-proportional from 200 to 1000 mg/kg, although C_{max} increase from 500 to 1000 mg/kg was dose-proportional. There was a slight increase in metformin exposure in combination with linagliptin (+20-30% C_{max} and AUC). Metformin total exposure (AUC) increased approximately 2-fold after repeated dosing, although there was no consistent difference in C_{max} after repeated dosing.

Exposure data are shown in the Sponsor's summary tables, below.

Exposure summary

Linagliptin

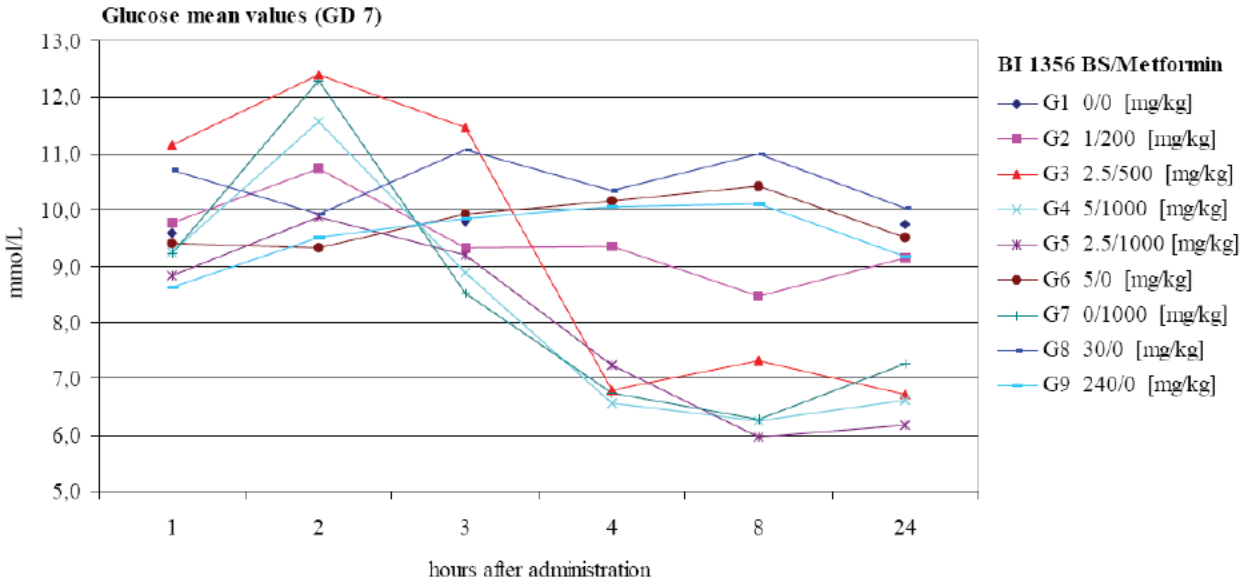
Parameter	Dosing Day	1 mg/kg +200 mg/kg metformin (Group 2)	2.5 mg/kg +500 mg/kg metformin (Group 3)	5 mg/kg +1000 mg/kg metformin (Group 4)	2.5 mg/kg +1000 mg/kg metformin (Group 5)	5 mg/kg without metformin (Group 6)
C(max) [(nmol/L)]	1	38.9	67.4	76.6	33.5	65.6
	10	62.7	50.4	39.7	35.9	87.1
AUC(0-24h) [(nmol·h/L)]	1	184	372	509	268	361
	10	238	347	618	347	470

Metformin

Parameter	Dosing Day	200 mg/kg +1 mg/kg BI 1356 BS (Group 2)	500 mg/kg +2.5 mg/kg BI 1356 BS (Group 3)	1000 mg/kg +5 mg/kg BI 1356 BS (Group 4)	1000 mg/kg +2.5 mg/kg BI 1356 BS (Group 5)	1000 mg/kg without BI 1356 BS (Group 7)
C(max) [(nmol/L)]	1	81000	141000	251000	233000	211000
	10	94000	141000	306000	278000	255000
AUC(0-24h) [(nmol·h/L)]	1	329000	902000	2010000	1810000	1760000
	10	528000	1510000	4810000	3830000	3670000

Blood glucose – Linagliptin alone and low dose metformin (200 mg/kg) had no effect on blood glucose. Higher doses of metformin (≥500 mg/kg), with or without linagliptin, caused slightly reduced blood glucose from 4 to 24 h postdose. While glucose levels were reduced, they remained above hypoglycemic levels throughout the 24 h period post-dose (minimum blood glucose > 6 mmol/l). Glucose trends are shown in the Sponsor's figure, below.

Mean blood glucose (GD 7)



Maternal Transfer (GD 16 at 1, 3, 8, 24 h post-dose in sub-groups for maternal TK and embryo blood from A. carotis (pooled for analysis) immediately after maternal sampling)

Two satellite groups of dams were treated with 30 or 240 mg/kg/d linagliptin to assess maternal transfer during the period of organogenesis (GD 7 to GD 16). Embryo exposure was confirmed on GD 16, with maximum exposure (C_{max}) of 27-29% and total exposure (AUC_{0-24}) of 42-54%) that of mothers. In dams, linagliptin maximum plasma exposure (C_{max}) increased approximately dose-proportionally and total exposure (AUC) was greater than dose-proportional across the 8-fold dose range. Exposure trends in embryos were similar to dams across the dose range. Data are summarized in the Reviewer's table, below.

Linagliptin exposure (maternal and embryonal)[†]				
Dose (mg/kg)		Dams		Embryos
		GD 7	GD 16	GD 16
30	C_{max}	2420	1960	526
	AUC_{0-24}	8900	10,600	4560
240	C_{max}	17,500	13,000	5050
	AUC_{0-24}	178,000	146,000	79,000

[†] Linagliptin mean exposure measured in dams after single exposure (GD 7) and in dams and embryos after exposure during the period of organogenesis (GD 16)
C_{max} = ng/ml, $AUC_{0-24\ h}$ = ng*h/ml

Necropsy

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

Several animals were not pregnant (5, 3, 5, 2, 6, 6, 4 in respective groups 1-7), which cannot be attributed to treatment because females were mated prior to the study. Mean implantations and corresponding viable fetuses in the control group were below historical comparator groups. Pre-implantation loss was also very high in the vehicle control group. A single fetus was found dead at c-section in the HD metformin control. Single runts were found in various groups including the control, with no correlation to treatment.

There were no remarkable treatment-related effects on pregnancy parameters. Mean total resorptions, attributable to early resorptions, were slightly higher in 2.5/1000, 5/0 (linagliptin control), and 0/1000 (metformin control) groups, which resulted in higher mean resorption rates. Resorption differences were not statistically significant and means were skewed by 1 dam in each group that had high numbers of resorptions. Mean fetal body weights were slightly reduced in 1000 mg/kg metformin groups (± linagliptin), which was consistent with reduced mean BW gain in dams and reached statistical significance only in the HD combination group (-4% fetal BW). Notable pregnancy data are highlighted in the Sponsor's summary table (abbreviated), below.

		BI 1356 BS/metformin						Spontaneous Incidences from Evaluation Study [U03-1549, Viertel and Nolte, 2003] mean/range	
	G1	G2	G3	G4	G5	G6	G7		
	Control	1/200	2.5/500	5/1000	2.5/1000	5/0	0/1000		
n litters +	19	21	19	22	18	18	20	85	
Litter parameters (means/individual range per dam)								overall means	ranges of means/ individual data
Corpora lutea	13.6/11-20	13.1/9-15	13.5/10-16	13.0/10-16	13.4/6-17	13.3/10-21	12.8/10-15	12.0	11.8-12.3/ 9.0-15.0
Implantations	9.8/2-14	11.6/7-14	12.3*↑/6-16	11.4/3-16	11.4/2-17	11.3/5-14	11.8*↑/7-15	11.1	10.9-11.4/ 5.0-15.0
Viable fetuses	9.2/2-14	11.0*↑/7-14	11.7*↑/6-16	10.8/3-16	10.4/1-17	10.2/4-14	10.8/5-14	10.5	10.1-10.7/ 5.0-14.0
Total resorptions	0.68/0[1]-2	0.57/0[1]-2	0.53/0[1]-3	0.59/0[1]-3	1.00/0[1]-6	1.06/0[1]-8	0.90/0[1]-4	0.65	0.3-0.8/ 0[1.0]-3.0# (6.0)
early resorptions	0.68/0[1]-2	0.52/0[1]-2	0.53/0[1]-3	0.55/0[1]-3	1.00/0[1]-6	1.00/0[1]-8	0.80/0[1]-3	0.60	0.3-0.7/ 0[1.0]-3.0# (6.0)
late resorptions	0	0.05/0-1	0	0.05/0-1	0	0.06/0-1	0.10/0-1	0.05	0-0.1/ 0-1.0
Fetal weight [g]	4.99/4.45-5.73	4.99/4.61-5.53	4.99/4.47-5.47	4.78*↓/4.38-5.17	4.86/4.47-5.67	4.85/4.19-5.38	4.81/3.94-5.41	5.03	4.90-5.13/ 2.3-6.3
Pre-implantation loss (%)	26.91/ 0[7.14]-86.67	10.82*↓/ 0[6.67]-53.33	9.16*↓/ 0[6.25]-57.14	11.83*↓/ 0[7.14]-78.57	17.44/ 0[6.25]-69.23	14.36*↓/ 0[7.14]-61.54	7.94*↓/ 0[7.14]-30.77	7.57	6.13-10.06/ 0[6.67]-46.15## (61.54)
Resorption rate [%]	5.70/0[7.69]-20.00	4.65/0[7.14]-16.67	4.02/0[7.14]-27.27	4.97/0[6.67]-23.08	9.74/0[7.69]-50.00	9.12/0[7.69]-61.54	7.96/0[7.69]-44.44	5.70	2.30-7.29/ 0[7.69] -23.08# (54.55)

* = significant difference ($p<0.05$) , ↓ decreased , ↑ increased , + = non-pregnant animals excluded
# = one outlayer (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlayer included , ## = one outlayer (No. 222) excluded (in brackets: No. 222 included); mean calculated with the outlayer included , [ ] the lowest number greater than 0

Reviewer's note – selected data shown and data described in text are highlighted.

Offspring (Malformations, Variations, etc.)

Variations

No trends could be discerned from total variations in different treatment groups. Numerical visceral variations were increased slightly in treatment groups (nss) and specific visceral variations seemed increased in various treatment groups. Skeletal variations were seen in every fetus but, similar to visceral variations, specific skeletal variations were increased in treatment groups.

Visceral variations above concomitant and historical comparator control groups included shortened truncus brachiocephalicus (5/1000 and 2.5/1000 groups), altered distance between carotids (various groups, no dose-response), small or no distance between left A. carotis and left A. subclavia (≥200 mg/kg metformin ± linagliptin), and bowed or dilated ureter (≥500 mg/kg metformin ± linagliptin). Increased shortened truncus brachiocephalicus and ureter variations at higher metformin doses were consistent with results from a study with metformin only (Study 09B099/U10-2386-01, reviewed above). Unlike the separate metformin alone study, there was no apparent affect of metformin treatment on kidney variations.

Skeletal variations were prevalent in all groups including controls. Delayed, incomplete, or absent ossification was the most prominent finding increased in treatment groups, consistent with developmental delays associated with reduced maternal BW gain. Ossification changes were most prominent at ≥ 500 mg/kg metformin (± linagliptin). In addition to ossification changes, there were treatment-related variations in cervical, thoracal, and caudal vertebral bodies (altered number or shape) and in number of proximal posterior phalanges. Notably, in several bones there was evidence of a greater effect in combination metformin and linagliptin groups compared to HD metformin only controls.

Findings		Natrosol	BI 1356 BS / Metformin BS				BI 1356 BS	Metformin BS	Spontaneous incidences from evaluation study Viertel 2003 U03-1549
		G1	G2	G3	G4	G5	G6	G7	
			Dose [mg/kg]						
		Control	1.0 / 200	2.5 / 500	5.0 / 1000	2.5 / 1000	5.0	1000	
		n fetuses / incidences (%) decimals rounded							
Total number of litters		**19**	**21**	**19**	**22**	**18**	**18**	**20**	**85**
Total number of fetuses		**174**	**231**	**223**	**238**	**187**	**184**	**216**	**889**
Runts		1/0.57	1/0.43	0	0	0	1/0.54	1/0.46	2/0.22
Variations									
External variations		decimals truncated							decimals rounded
Number of litters		**19**	**21**	**19**	**22**	**18**	**18**	**20**	**85**
Number of fetuses		**174**	**231**	**223**	**238**	**187**	**184**	**216**	**889**
Thin skin in region of parietal/interparietal bone		1/0.57	0	0	0	0	0	0	0
Visceral variations		decimals truncated							decimals rounded
Number of litters		**19**	**21**	**19**	**22**	**17**	**18**	**20**	**85**
Number of fetuses		**85**	**110**	**108**	**114**	**88**	**88**	**103**	**424**
Shortened truncus brachiocephalicus		2/2.35	2/1.81	2/1.85	7/6.14	8/9.09	3/3.40	2/1.94	1/0.24
Small distance between the carotids		2/2.35	3/2.72	4/3.70	1/0.87	2/2.27	1/1.13	3/2.91	5/1.18
Enlarged distance between the carotids		1/1.17	0	0	0	0	2/2.27	0	1/0.24
No distance between the carotids		2/2.35	0	3/2.77	1/0.87	1/1.13	2/2.27	1/0.97	4/0.94
Distance between left A. carotis and left A. subclavia small		3/3.52	15/13.63	13/12.03	11/9.64	3/3.40	7/7.95	8/7.76	7/1.65
Distance between left A. carotis and left A. subclavia enlarged		0	0	0	0	1/1.13	0	1/0.97	3/0.71
No distance between left A. carotis and left A. subclavia		4/4.70	8/7.27	3/2.77	2/1.75	11/12.50	0	6/5.82	5/1.18
Common trunk of pulmonary arteries at the Truncus pulmonalis		0	0	1/0.92	0	0	0	0	0
Stomach focal reddish discoloured		0	0	1/0.92	0	0	0	0	0
Dilated ureter	uni	1/1.17	3/2.72	5/4.62	5/4.38	3/3.40	1/1.13	2/1.94	13/3.06
	bil	1/1.17	1/0.90	1/0.92	4/3.50	0	0	2/1.94	1/0.24
Bowed ureter	uni	4/4.70	4/3.63	6/5.55	4/3.50	5/5.68	1/1.13	1/0.97	3/0.71
	bil	1/1.17	1/0.90	0	0	1/1.13	0	4/3.88	1/0.24
Kinked ureter	uni	0	1/0.90	2/1.85	1/0.87	1/1.13	0	0	0
	bil	1/1.17	0	0	0	0	0	1/0.97	0
Dilated renal pelvis	bil	1/1.17	0	0	0	0	1/1.13	0	0

Findings		Natrosol	BI 1356 BS / Metformin BS				BI 1356 BS	Metformin BS	Spontaneous incidences	
		G1	G2	G3	G4	G5	G6	G7		
			Dose [mg/kg/day]							
Skeletal variations		Control	1/200	2.5/500	5/1000	2.5/1000	5	1000		
		n fetuses / incidences (%)								
Number of litters		**19**	**21**	**19**	**22**	**18**	**18**	**20**		
Number of fetuses		**89**	**121**	**115**	**124**	**99**	**96**	**113**		
Parietal bone partly not ossified	bil	3/3.37	3/2.47	10/8.69	20/16.12	**34/34.34**	1/1.04	11/9.73	72/15.48+; 20/17.54$	
	uni	3/3.37	3/2.47	4/3.47	3/2.41	1/1.01	1/1.04	3/2.65	4/3.27*	
Interparietal bone partly not ossified		14/15.73	11/9.09	34/29.56	53/42.74	**48/48.48**	9/9.37	33/29.20	177/38.06+; 50/43.85$	
Supraoccipital bone partly not ossified		1/1.12	3/2.47	5/4.34	8/6.45	**19/19.19**	5/5.20	3/2.65	18/3.87+; 18/8.07++	
Maxillar bone partly not ossified	bil	0	0	0	0	**3/3.03**	1/1.04	1/0.88	6/1.29+	
Jugal bone partly not ossified	bil	1/1.12	0	**5/4.34**	**4/3.22**	**15/15.15**	0	**3/2.65**	8/1.72+; 1/1.85**	
	uni	1/1.12	2/1.65	**7/6.08**	**7/5.64**	**8/8.08**	1/1.04	0	5/1.08+; 2/3.70**	
Squamosal bone partly not ossified	bil	0	2/1.65	7/6.08	**21/16.93**	**24/24.24**	4/4.16	10/8.84	19/4.09+; 2/1.63*; 34/15.25++	
	uni	1/1.12	2/1.65	4/3.47	7/5.64	3/3.03	2/2.08	5/4.42	8/1.73+; 8/6.55*	
Processus zygomaticus of squamosal bone partly not ossified	bil	0	0	**5/4.34**	**7/5.64**	**17/17.17**	0	2/1.76	15/3.23+; 1/2.70#; 1/1.85**	
	uni	4/4.49	1/0.82	5/4.34	5/4.03	**5/5.05**	1/1.04	**6/5.30**	3/0.65+; 1/0.84##; 1/1.85**	
Orbitosphenoidal bone not ossified	bil	2/2.24	1/0.82	5/4.34	5/4.03	**8/8.08**	2/2.08	3/2.65	3/2.45*; 3/5.55**	
	uni	4/4.49	4/3.30	3/2.60	6/4.83	**8/8.08**	3/3.12	5/4.42	7/5.73*; 4/7.40**	
Occipital bone not ossified		4/4.49	6/4.95	7/6.08	**22/17.74**	**17/17.17**	**10/10.41**	**24/21.23**	11/2.37+; 8/6.55*	
Early onset of ossification of processus coracoideus	bil	0	0	1/0.86	0	0	0	0	3/2.63$	
	uni	1/1.12	1/0.82	0	0	1/1.01	**5/5.20**	0	3/2.63$	
Cervical vertebral bodies <7		7/7.86	16/13.22	8/6.95	**23/18.54**	**27/27.27**	12/12.50	19/16.81	76/16.34+	
Thoracal vertebral body dumbbell-shaped		0	2/1.65	1/0.86	**6/4.83**	**6/6.06**	2/2.08	2/1.76	10/4.48++	
Thoracal vertebral body flat		0	2/1.65	1/0.86	6/4.83	5/5.05	1/1.04	**12/10.61**	7/5.88##	
Thoracal vertebral body asymmetrically ossified		0	0	1/0.86	**6/4.83**	2/2.02	0	1/0.88	1/0.96$$; 3/2.52##	
Sternebrae <6		0	**1/0.82**	0	**1/0.80**	0	**1/1.04**	0	2/0.43+	
Sternebra partly not ossified		7/7.86	**19/15.70**	10/8.69	14/11.29	11/11.11	6/6.25	**18/15.92**	14/13.46$$	
Caudal vertebral bodies <6		10/11.23	9/7.43	9/7.82	14/11.29	**23/23.23**	**17/17.70**	10/8.84	66/14.19+	
Proximal posterior phalanges <3	bil	13/14.60	26/21.48	16/13.91	**28/22.58**	17/17.17	**16/16.66**	**27/23.89**	L: 70/15.05+ R: 72/15.48+	25/21.92$
	uni	9/10.11	11/9.09	8/6.95	**18/14.51**	9/9.09	13/13.54	12/10.61		5/4.38$
Proximal posterior phalanges not ossified	bil	11/12.35	10/8.26	12/10.43	26/20.96	**32/32.32**	16/16.66	19/16.81	L: 63/13.55+ R: 65/13.98+	8/6.72##; 13/24.07**
	uni	4/4.49	4/3.30	2/1.73	**9/7.25**	3/3.03	5/5.20	2/1.76		7/5.88##; 2/3.70**

Malformations

Trends of increased skeletal malformations were seen in rib (flat, thickened, z-shaped) and scapula (bent inward, with or without acromion process bent inward) in ≥ 500 mg/kg metformin (± linagliptin) treatment groups. With the exception of bilateral flat and thickened or z-shaped ribs, there was no difference between metformin alone groups and metformin plus linagliptin combination groups. The metformin-related increased rib and scapula malformations were consistent with a separate metformin only embryofetal development study, although scapula malformations were increased in the HD combination group compared to lower dose combinations or metformin alone. In the low dose (1/200) combination group the only malformation increased above concomitant and historical controls was cleft cervical vertebral body, which was also increased in the 1000 mg/kg metformin group but no other combination groups.

There were low incidences of additional malformations that were not clearly associated with metformin treatment. Hydronephrosis was seen in a single fetus in the 5/1000 HD combination, which as not seen concomitant or historical controls. A single 5 mg/kg linagliptin fetus had multiple thoracal vertebral malformations which were distinct from malformations in other fetuses, which was considered likely incidental and unlikely to be related to linagliptin treatment. Similarly, two 5/1000 HD combination fetuses had multiple vertebral malformations that were different from malformations in other metformin groups.

Findings		Natrosol	**BI 1356 BS/Metformin BS**				**BI 1356 BS**	**Metformin BS**	**Spontaneous incidences**	
		G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**		
			Dose [mg/kg/day]							
		Control	**1/200**	**2.5/500**	**5/1000**	**2.5/1000**	**5**	**1000**		
Malformations		**n fetuses / incidences (%)**								
Skeletal malformations		decimals truncated							decimals rounded	
Number of litters		**19**	**21**	**19**	**22**	**18**	**18**	**20**		
Number of fetuses		**89**	**121**	**115**	**124**	**99**	**96**	**113**		
Cleft cervical vertebral body		0	**4/3.30**	0	2/1.61	1/1.01	2/2.08	**4/3.53**	5/1.08+; 6/2.69++	
Cervical vertebral body unilaterally ossified		0	1/0.82	0	0	0	0	3/2.65	1/0.22+; 4/3.27*	
Cleft thoracal vertebral body		1/1.12	0	1/0.86	**4/3.22** (No. 428R04)	0	1/1.04 (No. 626L03)	1/0.88	1/0.22+; 1/0.84##; 1/1.85**	
Flat and thickened rib	bil	3/3.37	10/8.26	**18/15.65**	**46/37.09**	**25/25.25**	3/3.12	**31/27.43**	45/9.68+;	9/8.65$$
	uni	3/3.37	5/4.13	**12/10.43**	**11/8.87**	**8/8.08**	5/5.20	**13/11.50**	33/14.80++	4/3.84$$
Rib z-shaped	bil	0	0	1/0.86	**4/3.22**	**11/11.11**	0	**5/4.42**	2/0.43+;	1/0.96$$
	uni	0	0	0	**3/2.41**	**2/2.02**	0	1/0.88	24/10.77++	2/1.68##
Z-shaped ribs in two dimensions, longitudinal axis (body axis) and dorso-ventral axis	bil	0	0	0	0	1/1.01	0	0	0+	
Skeletal findings without classification										
1st sternebra medially partly not ossified and 3rd-6th sternebrae split in the longitudinal axis		0	0	0	**1/0.80** (No. 441L04)	0	0	0	0+	
Scapula bent inwardly	bil	0	0	**1/0.86**	**9/7.25**	**2/2.02**	0	**3/2.65**	0+	
	uni	0	1/0.82	**2/1.73**	**16/12.90**	**4/4.04**	0	**4/3.53**	0+; 1/0.87$	
Scapula and acromion process (proximal) bent inwardly	bil	0	0	0	**1/0.80**	**2/2.02**	0	0	0+	
	uni	0	0	0	**1/0.80**	0	0	0	0+	
Longitudinal axis of humerus distally bent to the dorsal side	bil	0	0	0	**1/0.80**	0	0	0	0+	

Findings at incidences above the actual Control group and above the spontaneous incidences from other studies underlined and in bold letters.

11 Integrated Summary and Safety Evaluation

The proposed linagliptin and metformin FDC tablet was submitted in accordance with 21 USC 505(b)(2) for treatment of type 2 diabetes mellitus. Both drugs have previously been approved for chronic use as monotherapies in conjunction with diet and exercise. Nonclinical studies to support chronic use of the FDC tablet primarily addressed the potential for drug interactions that may increase toxicity when administered together. All pivotal studies were conducted in compliance with current GLP standards. The Sponsor also discussed peer-reviewed literature and public data pertaining to linagliptin and metformin nonclinical pharmacology and toxicology. All supporting nonclinical information was reviewed for this NDA, including public literature and NDA and drug master file information for which the Sponsor owns or has a written 'right-of-reference'.

Pharmacology

Linagliptin and metformin have distinct mechanisms of action which lead to improved glucose metabolism in diabetic patients. Linagliptin inhibits the DPP4 enzyme, which prevents DPP4-mediated cleavage of endocrine active substrates including gut incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). The incretins, in combination but with GLP-1 playing a key role, increase insulin secretion, enhance insulin receptor sensitivity, and decrease hepatic glucose, gastric emptying, and food intake. DPP4 inhibition prevents the natural rapid breakdown of GLP-1 and GIP after their postprandial expression.

Metformin seems to indirectly improve insulin sensitivity, primarily related to peripheral glucose metabolism at liver, muscle, and adipose target tissues rather than acting directly on insulin production or metabolism. As noted in the label for the reference drug, Glucophage®:

> "Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization…With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease."

The complementary mechanisms for linagliptin- and metformin-induced glucose lowering are expected to result in an overall additive effect when given together to diabetic patients. Clinical trials with metformin, with and without a DPP-4 inhibitor, showed metformin treatment increases plasma GLP-1, likely by stimulating GLP-1 secretion rather than inhibiting GLP-1 degradation. Indeed, clinical data show the proposed FDC tablet resulted in improved glucose metabolism in diabetics as

evidenced by an additional 0.3% to 0.5% decrease in HbA_{1c} after 24-weeks of treatment.

A single pharmacology study in male obese diabetic mice supported the additive effect of linagliptin and metformin on glucose metabolism. After a week of treatment, the mice showed improved baseline fasting glucose and improved glucose excursion after OGTT with monotherapies and superior improvement in the combination treatment compared to either drug alone. As expected, the effect was more pronounced on glucose excursion compared to the less sensitive measure of fasting plasma glucose. Only the lowest proposed clinical ratio of 1:200 linagliptin:metformin was used, so the effect at the higher 1:300 and 1:400 clinical ratios may be expected to provide a more pronounced effect.

PK/ADME

No dedicated PK or ADME studies were conducted with linagliptin and metformin coadministration. TK trends from combination toxicity studies suggest limited to no interaction between drugs. Coadministration did not affect individual drug levels in the 13-week bridging toxicity study but there was a trend of slightly increased metformin exposure with coadministration in pregnant rats. The slight increases in metformin, up to approximately 30%, were consistent across combination doses in the embryofetal development study but trends were generally within the range of inter-individual variability. It was not clear if the trend seen in the pregnant rats was a distinct, biologically significant effect compared to males and non-pregnant females but the finding was notable because of slightly exacerbated teratogenic effects of the linagliptin and metformin high dose combination.

Neither linagliptin nor metformin are extensively metabolized in animals or humans. A single major, inactive metabolite, CD 1790, was identified for linagliptin and accounted for 13% of administered human dose in plasma. The majority of administered linagliptin is excreted unchanged in feces. No major metformin metabolites have been described. Approximately 90% of absorbed metformin is excreted renally within the first 24 h in humans.

No potential drug-drug interactions related to CYP 450 inhibition were identified. Linagliptin binds with high affinity to plasma DPP4 and non-specifically to plasma proteins after saturation of DPP4 binding sites. Metformin has negligible protein binding in plasma and it does not inhibit or interact directly with DPP4 proteins. Thus, no drug-drug interactions related to protein binding are predicted. No other potential drug-drug interactions, for example drug transporters related to intestinal absorption of tissue-specific uptake and elimination, have been identified at expected clinical exposures.

Linagliptin monotherapy is listed for once daily dosing while metformin is given twice daily. The proposed FDC tablet is designed for BID dosing, thus linagliptin dosing will differ from the current monotherapy dosing. Total linagliptin exposure measured as $AUC_{0\text{-}24\ h}$ has been shown to be similar while maximum plasma exposure, C_{max}, will be

lower clinically. Linagliptin plasma levels after 2.5 mg linagliptin BID dosing have been shown to be sufficient for clinical DPP4 inhibition and no effect on efficacy is expected with the FDC tablet BID dosing. Nonclinical studies did not identify any efficacy or safety endpoints that may be affected by a change from QD to BID dosing of linagliptin.

Toxicology

No remarkable additive or synergistic toxicity was identified in the pivotal linagliptin and metformin combination toxicity study in rats. Dose-limiting toxicity was due to metformin which was expected based on doses of linagliptin and metformin. Maximum linagliptin doses were chosen based on expected 1:200 to 1:400 clinical ratios, which were limited to 1- to 2-times expected human exposures and more than 100-fold lower than toxic doses in prior rat studies. Linagliptin and metformin lower blood glucose by different mechanisms but there is limited predicted overlap in target organ toxicity. The most sensitive targets of metformin toxicity were heart hypertrophy with immune cell infiltration/inflammation and liver hypertrophy with concomitant hepatic injury and elevated LFT biomarkers, starting at approximately 10-times the expected clinical AUC exposures. Linagliptin coadministration did not have any apparent effect on heart, liver or other metformin-related toxicity on target organs including stomach and GI tract, salivary glands, lymphoreticular tissues, or reproductive tissues.

Metformin clinical toxicity hallmarks include lactic acidosis and gastrointestinal side effects (e.g., diarrhea, bloating, discomfort). Based on nonclinical toxicity findings, linagliptin is not expected to exacerbate potential metformin-induced metabolic acidosis or GI-related toxicity. No evidence of metabolic acidosis or GI distress were seen nonclinically with linagliptin at exposures exceeding 50-times human exposures. In dedicated safety pharmacology studies linagliptin caused a slight reduction in gastric juice volume and acid output which had no effect on gastric emptying but coincided with a very modest effect on intestinal transit (≤ 10% reduced transit). The slight effects on GI motility were seen in rats at doses well in excess of expected human exposures (> 50-times MRHD). Linagliptin at clinical exposures is not expected to have any effect on metabolic acidosis or GI distress and thus no interactions with any potential metformin-induced metabolic or GI toxicity.

Pivotal embryofetal development studies were conducted in Wistar Han rats. Neither linagliptin nor metformin are listed as teratogenic at clinical exposures in animals on current labels. Published reports of metformin nonclinical reproductive toxicity studies are limited but both metabolic acidosis and hypoglycemia have been implicated in animal teratogenicity. The Sponsor conducted a GLP-compliant embryofetal development study with metformin alone to determine an MTD and appropriate doses for the combination embryofetal rat study. Data from both the range finding study and linagliptin plus metformin combination showed metformin causes skeletal malformations at maternally toxic doses (reduced BW gain and decreased plasma glucose) with exposures approximately 10- to 20-times clinical exposures. Teratogenicity was attributed to metformin and there was no apparent synergistic effect, although incidence of certain malformations was slightly increased in linagliptin plus high dose metformin

groups. Metformin concentrations were slightly (+20-30%) higher in the combination linagliptin groups, which may provide a simple explanation for the slight increase in fetuses with malformations.

Several important factors distinguish the current studies from the existing database. The reference metformin label does not list the rat strain used and exposures were limited to approximate clinical exposures based on body surface area estimates. Sprague Dawley (SD) rats are the most common strain for toxicity studies whereas an acceptable alternative, Wistar Han rats, were used in the Sponsor's nonclinical program. Different strains may be more susceptible to certain malformations. For example, Wistar rats were reported to be more sensitive to heart malformations than SD rats[9]. The Sponsor also noted a potential Wistar-specific condition, Chondrodystrophy Syndrome[10], which may result in increased rib malformations (flat and thickened, z-shaped) and scapula bent inward. Evidence supporting the syndrome is limited but the findings are consistent with an apparent recent increase in skeletal malformations in the conducting lab. The absence of reported skeletal malformations in SD rats treated with metformin also lends support to increased sensitivity in the Wistar Han strain. Nevertheless, malformations were more prominent in metformin treatment groups compared to controls, supporting a drug-induced effect in the Wistar Han rat study.

Importantly, there was no evidence that the combination of linagliptin and metformin was teratogenic because malformations were attributable to metformin. Evidence of maternal toxicity at the teratogenic metformin doses included reduced body weight gain and reduced plasma glucose. Decreased urine pH and altered serum electrolytes in the 13-week combination toxicity study also suggest potential for lactic acidosis and/or metabolic acidosis in pregnant rats. Reduced maternal BW gain is often indicative of general maternal toxicity and can cause delayed fetal growth. Reduced maternal BW gain in the combination rat study was consistent with slight decreases in fetal weights and observed ossification delays. However, reduced maternal BW gain and even maternal weight loss is not associated with skeletal malformations in the absence of other maternal toxicity. On the contrary, both metabolic acidosis and hypoglycemia are associated with teratogenicity. Plasma glucose was slightly reduced to approximately 6 mM minimum starting at 4 h post-dose in the combination embryofetal development study. Rat plasma glucose of 6 mM is typically tolerable and may not cause sufficient fetal hypoglycemia to account for the skeletal malformations. However, maternal toxicity from combined effects on BW and blood glucose, potentially coupled with metabolic acidosis, may be stressful enough to cause fetal malformations.

No carcinogenicity studies were conducted with the linagliptin and metformin combination. Neither linagliptin nor metformin are genotoxic and neither are considered to pose a significant carcinogenic risk at clinical exposures. Several linagliptin impurities, including potential or theoretical impurities and degradants, were identified

[9] Fujino H et al. 2005. Congenital Anomalies. **45:**52-58
[10] Wilby OK et al. 2007. Reproductive Toxicology **24:**57(abstract)

and adequately qualified to show no significant toxicologic or carcinogenic risk. No further carcinogenicity testing with the combined drugs is necessary.

In summary, no unexpected or synergistic interactions were uncovered in nonclinical studies when linagliptin was coadministered with metformin. Linagliptin is well tolerated in animals and target organ toxicity occurs only at very high multiples of human exposure (approximately 50-times or greater). Toxicity in animals is attributable to metformin at exposures approximately 10-times or greater than clinical exposures when coadministered with linagliptin at expected clinical ratios of 1:200 to 1:400 linagliptin:metformin. NOAELs were established at or slightly above approximate clinical exposures in pivotal 13-week toxicity and embryofetal development combination toxicity studies in rats.

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/s/

DAVID B CARLSON
10/04/2011
Pharmtox review -- recommend approval

TODD M BOURCIER
10/04/2011
I concur with Dr Carlson's recommendations. The preclinical data supports AP.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 201281 **Applicant:** Boehringer Ingelheim **Stamp Date:** 1/19/11

Drug Name: Linagliptin + metformin FDC **NDA/BLA Type:** 505(b)(2)

On **initial** overview of the NDA/BLA application for RTF:

	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		NDA 201280 (linagliptin) is cross-referenced for studies with linagliptin drug substance
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		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Statement included in NDA 201280 for cross-referenced linagliptin studies; statement not found for current NDA 201281 but bridging toxicology studies are GLP-compliant.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Label will need to be updated to be consistent with changes required for linagliptin monotherapy label.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Drug product impurities identified and qualified. Drug substance impurities addressed in linagliptin NDA.
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

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

/s/

DAVID B CARLSON
03/02/2011
Sufficient nonclinical submission to permit filing

TODD M BOURCIER
03/02/2011
Fileable for pharm/tox