CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209803Orig1s000
209805Orig1s000
209806Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	209805
Supporting document/s:	SDN 1
Applicant's letter date:	12/19/2016
CDER stamp date:	12/19/2016
Product:	Ertugliflozin and Sitagliptin
Indication:	Type 2 Diabetes Mellitus
Applicant:	Merck Sharp and Dohme Corp
Review Division:	DMEP
Reviewer:	Jessica J. Hawes, Ph.D.
Supervisor/Team Leader:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Elizabeth Godwin

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209805 are owned by Merck Sharp and Dohme Corp are data for which Merck Sharpe and Dohme Corp has obtained a written right of reference.
Any information or data necessary for approval of NDA 209805 that Merck Sharp and Dohme Corp 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 reflected 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 209805.

TABLE OF CONTENTS

Table of Tables

Table of Sponsor's Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Merck Sharp and Dohme Corp. has submitted NDA application packages for the new molecular entity (NME) Ertugliflozin (PF-04971729, MK-8835) alone (NDA #209803, IND #106447) and as fixed dose combination (FDC) products with the marketed drugs Metformin (MK-8835B, NDA #209806, IND #122329) and Sitagliptin (MK-8835A, NDA #209805, IND #122330) for the treatment of type 2 diabetes mellitus (T2DM).

The nonclinical profile for the NME, ertugliflozin, was fully evaluated in the Pharm/Tox review under NDA #209803. This review focuses on evaluation of additional information pertinent to the ertugliflozin + sitagliptin hydrochloride FDC product.

1.2 Brief Discussion of Nonclinical Findings

Coadministration of ertugliflozin and sitagliptin in rats for 13-weeks was generally well-tolerated with sufficient margins of safety and was not associated with significant adverse systemic toxicities. Furthermore, no new clinically relevant or synergistic adverse toxicities due to coadministration of PF-04971729 and sitagliptin were observed. Thus, the rat combination toxicology study adequately bridges the proposed FDC product to the ertugliflozin nonclinical safety profile under NDA #209803, with sufficient safety margins based on AUC exposures at the maximum recommended high doses ($MRHD_{AUC}$) of 15 mg/day ertugliflozin (89x $MRHD_{AUC}$) and 100 mg/day sitagliptin (9x $MRHD_{AUC}$). Overall, the nonclinical data were considered to be sufficient and support clinical dosing of the FDC product at ertugliflozin doses up 15 mg/day ertugliflozin and sitagliptin doses up to 100 mg/day.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of the ertugliflozin/sitagliptin FDC

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Nonclinical labeling recommendations are below. See Section 11 Labeling Review for a full discussion of proposed changes. Only labeling specific to the FDC or the sitagliptin component are captured in this review. Please see the NDA review under #209803 for labeling recommendations regarding the ertugliflozin component.

Only one minor edit specific for sitagliptin was recommended (Section 8.1).

Section: 8 USE IN SPECIFIC POPULATIONS

Section 8.1 Pregnancy

Sitagliptin

Sitagliptin administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) did not adversely affect developmental outcomes at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100 mg/day based on AUC comparisons. Higher doses increased the incidence of rib malformations in offspring at 1,000 mg/kg, or approximately 100 times human exposure at the MRHD. (b) (4)

Sitagliptin administered to female rats from gestation day 6 to lactation day 21 decreased body weight in male and female offspring at 1,000 mg/kg. No functional or behavioral toxicity was observed in offspring of rats.

Placental transfer of sitagliptin administered to pregnant rats was approximately 45% at 2 hours and 80% at 24 hours postdose. Placental transfer of sitagliptin administered to pregnant rabbits was approximately 66% at 2 hours and 30% at 24 hours.

2 Drug Information

2.1 Drug

CAS Registry Number

Ertugliflozin: 1210344-57-2
Sitagliptin: 654671-77-9

Generic Name

Ertugliflozin + sitagliptin

Code Name

Ertugliflozin + sitagliptin FDC: MK-8835A

Ertugliflozin: PF-04971729, MK-8835
Ertugliflozin L-pyroglutamic acid (L-PGA) co-crystal form: PF-04971729 (b) (4)

It is noted that the neutral amorphous form was used for most exploratory toxicology studies, whereas the L-PGA co-crystalline form intended for marketing was used in pivotal toxicology and safety pharmacology studies.

Sitagliptin: MK-0431, L-000224715-010X, sitagliptin phosphate

Chemical Name

PF-04971729:	(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol
PF-04971729 (b) (4)	(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol L-pyroglutamic acid
Sitagliptin:	7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-α]pyrazine phosphate (1:1) monohydrate

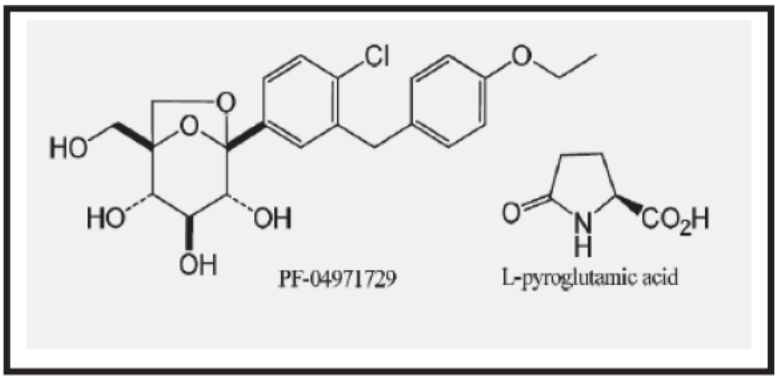
Molecular Formula/Molecular Weight

PF-04971729:	C22H25ClO7 / 436.88 g/mol
PF-04971729 (b) (4)	C27H32ClNO10 / 566.00 g/mol
Sitagliptin:	C16H15F6N5O / 407.32 g/mol

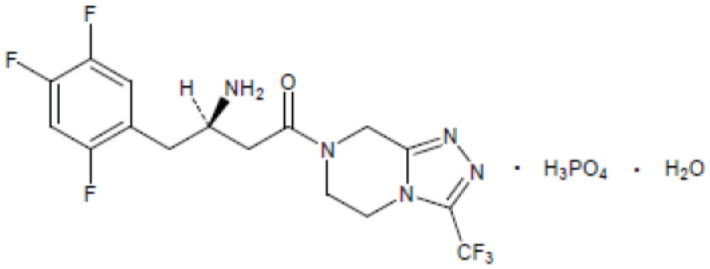
Sitagliptin phosphate monohydrate salt: C16H15F6N5O ● H3PO4 ● H2O / 523.32 g/mol

Structure or Biochemical Description

Ertugliflozin L-PGA



Sitagliptin



Pharmacologic Class

Ertugliflozin: Sodium glucose co-transporter 2 (SGLT2) Inhibitor
Sitagliptin: Dipeptidyl peptidase-4 (DPP-4) inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA #209803 (IND #106447):	MK-8836 Ertugliflozin, Merck Sharp and Dohme Corp
NDA #209806 (IND #122329):	MK-8835**A** (Ertugliflozin + metformin FDC), Merck Sharp and Dohme Corp
NDA #21995:	Januvia (Sitagliptin), Merck Sharp and Dohme Corp

2.3 Drug Formulation

The ertugliflozin/sitagliptin FDC will be formulated as film coated tablet in 4 strengths: (b) (4) (b) (4) 5 mg ertugliflozin + 100 mg sitagliptin, (b) (4) and 15 mg ertugliflozin + 100 mg sitagliptin. The following two tables are representative of the formulations for all 4 strengths.

Sponsor's Table 1: Ertugliflozin/Sitagliptin FDC Tablet Formulation Composition

Table 1 Ertugliflozin (b) (4) Tablet Composition

Component	Quality Reference	Function	Amount per tablet (mg)
Core Tablet			
Ertugliflozin L-PGA*	In-house	Active	(b) (4)
Sitagliptin Phosphate Monohydrate†	In-house	Active	
Cellulose, Microcrystalline	USP-NF	(b) (4)	
Dibasic Calcium Phosphate, Anhydrous	USP-NF		
Croscarmellose Sodium	USP-NF		
Sodium Stearyl Fumarate	USP-NF		
Magnesium Stearate	USP-NF		
		Core Tablet Weight	
Film Coat			
(b) (4)			
Carnauba Wax	USP-NF		(b) (4)
(b) (4)			
Hypromellose	USP-NF		(b) (4)
Hydroxypropyl Cellulose	USP-NF		
Titanium Dioxide	USP-NF		
Iron Oxide Red	USP-NF		
Iron Oxide Yellow	USP-NF		
Ferrosoferric Oxide/Black Iron Oxide	USP-NF		

(Tables excerpted from sponsor's package)

2.4 Comments on Novel Excipients

All excipients are compendial and controlled at acceptable levels.

2.5 Comments on Impurities/Degradants of Concern

Ertugliflozin-related impurities and degradants were qualified under NDA #209803.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adults with T2DM.

The sponsor's recommended starting dose is 5 mg ertugliflozin/100 mg sitagliptin once daily with or without food. The sponsor recommends that the dose may be increased to a maximum dose of 15 mg ertugliflozin/100 mg sitagliptin once daily if additional glycemic control is needed.

Sponsor's Maximum Recommended Human Dose:

FDC: Once daily dose of 15 mg ertugliflozin and 100 mg sitagliptin

- **Total: 15 mg/day ertugliflozin + 100 mg/day sitagliptin**
  - Ertugliflozin: * **AUC = 1.38 μg·h/mL**, C_{max} = 266 ng/mL ≈ 0.6 μM
    *Based on the clinical population pharmacokinetic (PK) analysis
  - Sitagliptin: ****AUC_{0-24} = 6.9 μM·h = 2.81 μg·h/mL**, C_{max} = 805 nM
    ** Based on study #PB022/1033 (CSR, Table 12)

Ertugliflozin: The proposed MRHD under NDA #209803 for the NME alone is also 15 mg/day.

Sitagliptin: Approved maximum daily dose of Sitagliptin is 100 mg once daily

2.7 Regulatory Background

- An IND for Ertugliflozin was originally submitted as PF04971729 in September 2009.
- On 4/14/2014, the sponsor submitted a Type B meeting request and a pre-IND package for the FDC Ertugliflozin + Sitagliptin product. On 4/22/2014, a Pre-IND/Type B meeting was granted with written responses sent to the sponsor on 6/12/2014. Within the pre-IND package, the sponsor submitted 3 clinical questions, but no non-clinical questions.
- On 7/30/2014, the sponsor submitted and cross-referenced the new IND #122330 for the FDC product MK-8835A containing ertugliflozin and sitagliptin for the treatment of T2DM.
- On 3/25/2015, the sponsor submitted a meeting request to discuss a revised clinical pharmacology and biopharmaceutics plan and written responses were sent on 6/8/2015
- Pediatric study plan (PSP) written responses were provided on 7/2/2015 and a PSP initial agreement was provided on 8/20/2015.
- On 7/6/2016, the sponsor submitted a Type-B Pre-NDA meeting request. A pre-NDA meeting was held on 9/6/2016. Two nonclinical questions were submitted,

but not discussed at the meeting. The sponsors was informed via written responses that the nonclinical safety package was adequate to support filing of the NDA, but that conclusions regarding the carcinogenicity findings were a matter of review. A total of 13 additional questions were discussed and/or addressed, but were not nonclinical.

Ertugliflozin

- Ertugliflozin was originally submitted as PF04971729 under IND #106447 in September 2009.
- And NDA package for ertugliflozin as an NME alone (non-FDC) drug formulation was submitted at the same time as the ertugliflozin/metformin FDC NDA on 12/19/2016.

Sitagliptin

Sitagliptin was approved under NDA #21995 as Januvia® (Merck & Co., Inc.) on 10/16/2006 as an adjunct to diet and exercise to improve glycemic control in adults with T2DM with a maximum approved adult dose set at 100 mg/day. Sitagliptin has been prescribed for treatment of type II diabetes in patients worldwide for 11 years. The label for sitagliptin was updated in January 2017.

3 Studies Submitted

3.1 Studies Reviewed

All nonclinical studies for ertugliflozin were submitted and reviewed under NDA#209803 and IND #106447. All nonclinical coadministration studies have been previously reviewed under IND #106447 and #122330.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

A preliminary 2-week combination toxicology study studies was reviewed in Pharmacology and Toxicology (Pharm/Tox) review #1 under IND #122330 by Dr. David Carlson. A pivotal 13-week combination toxicology studies was reviewed in detail in Pharm/Tox review #7 under IND #106447 by Dr. Jessica Hawes. Summaries of these studies are included in this review.

Table 1: Summaries of Pivotal Previously-Reviewed Nonclinical Studies

Combination Toxicology

Study #	Brief Title	Primary Review
TT147809 ((b) (4) #8294467, Pfizer #13GR342)	2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats	IND #122330 Pharm/Tox review #1, Dr. Carlson, 8/28/2014
TT147808 ((b) (4) #8300338, Pfizer #14GR162)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats **(GLP)**	IND #106447 Pharm/Tox review #7, Dr. Hawes, 12/3/2015

Brief summaries of nonclinical studies for sitagliptin were based on the Pharm/Tox review by Dr. Bourcier for Januvia under NDA #21995.

4 Pharmacology

4.1 Primary Pharmacology

Ertugliflozin is an inhibitor of SGLT2, thereby blocking the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells and resulting in significant glucosuria. Ertugliflozin is highly selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

Sitagliptin is a competitive inhibitor of the DPP-4 enzyme that functions to digest the gastrointestinal hormone incretins GLP-1 and GIP, which are released in response to a meal. Thus, sitagliptin inhibits inactivation of GLP-1 and GIP, thereby resulting in increases the secretion of insulin and suppressed glucagon release by pancreatic alpha cells.

Drug activity related to proposed indication:
Ertugliflozin administration in rats results in concentration-dependent glucosuria, which is directly related to the pharmacodynamic (PD) activity of SGLT2 inhibition. Ertugliflozin administration in rats is associated with concomitant decreases in plasma glucose levels despite compensatory increases in food consumption. Glucosuria has also been reported in humans.

Sitagliptin suppresses glucagon release and increases insulin secretion; leading to normalization of blood glucose levels. Sitagliptin has also been shown to lower HbA1c levels in human.

4.2 Secondary Pharmacology

Ertugliflozin

Nonclinical secondary pharmacology studies for ertugliflozin were fully evaluated in the Pharm/Tox review by Dr. Hawes under NDA #209803.

Briefly, significant drug-drug interactions (DDI) with ertugliflozin administration and drugs metabolized by cytochrome P450 (CYP) enzymes or transported by organic anion transporters (OATs), organic cation transporters (OCTs) or organic anion transporting polypeptides (OATPs) are not likely at clinical exposures. Significant DDI with

diphosphate-glucuronosyltransferase (UGT) enzyme inhibition is also unlikely at clinical concentrations.

Sitagliptin

Sitagliptin is metabolized by CYP3A4 and CYP2C8 enzymes; thus, concomitant use of sitagliptin with drugs that interfere with CYP3A4 and/or CYP2C8 may lead to increases in systemic sitagliptin exposures.

Ertugliflozin/Sitagliptin FDC

Drug-drug interactions between ertugliflozin and sitagliptin are not anticipated.

Ertugliflozin and sitagliptin are predominantly eliminated by different mechanisms and are not expected to affect each other's elimination pathways. Ertugliflozin is predominantly metabolized by UGT1A9 and UGT2By, with minor contributions by CYP3A4, and even less by CYP3A5 and CYP2C8. Sitagliptin is predominantly eliminated via filtration at the glomerulus and excreted in the urine unchanged, accounting for 79% of the dose, with the remaining being eliminated via hepatic metabolism by CYP3A4 and CYP2C8. Although both drugs are partially metabolized by CYP2C8, the role of CYP2C8 metabolism for sitagliptin is minor and very minor for ertugliflozin; thus, competition for CYP2C8-mediated metabolism is unlikely to occur or lead to changes in PK parameters of either drug.

Ertugliflozin does not exhibit reversible or time-dependent inhibition of CYP3A4 or CYP2C8 in human liver microsomes (HLMs) in vitro, with IC_{50} values of >30 μM, which is at least 50-fold higher than clinical ertugliflozin C_{max} concentrations (0.6 μM). The ertugliflozin metabolites M5a and M5c also do not inhibit CYP3A4 or CYP2C8 enzymes. Thus, ertugliflozin and its disproportional metabolites are not likely to interfere with sitagliptin metabolism at clinical exposure levels.

Sitagliptin does not inhibit or induce metabolizing enzymes involved in ertugliflozin metabolism; thus sitagliptin is not anticipated to affect ertugliflozin exposures. Furthermore, since sitagliptin plasma protein binding is relatively low (38%), it is less likely to interact with highly protein-bound drugs, such as ertugliflozin.

4.3 Safety Pharmacology

Both ertugliflozin and sitagliptin may be associated with some concern for cardiovascular (CV) effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. Given that the margins of safety for cardiovascular effects are sufficient for each drug alone and a DDI is not likely, the margins of safety for the FDC product is considered to be sufficient as well.

Ertugliflozin

Standard CV, neurological and pulmonary safety pharmacology studies were completed for ertugliflozin under IND #106447 and NDA #209803.

Central Nervous System (CNS): At 500 mg/kg ertugliflozin in male rats, drug-related decreases in average body temperature of 0.4°C, and 30-40% decreases in locomotor activity, were observed at C_{max} exposures approximately 339-fold higher than clinical C_{max} exposure at the maximum recommended high dose ($MRHD_{Cmax}$) of 15 mg/day. The no observed adverse effect level (NOAEL) for CNS effects in rats was set at 25 mg/kg, which is associated with a safety margin of ~36x $MRHD_{Cmax}$.

Cardiovascular System: Ertugliflozin weakly inhibited the human ether-a-go-go-related gene (hERG) potassium channel in vitro with an IC_{50} of 59 μM and an IC_{20} of 8.11 μM in CHO cells, but was a poor inhibitor in human embryonic kidney (HEK293) cells with an IC_{50} value of >300 μM. Ertugliflozin also weakly inhibited Nav1.5 currents with an IC_{50} of 188 μM. Although significant inhibition of hERG and Nav1.5 currents were reported at concentrations ≥30 μM (50x $MRHD_{Cmax}$), significant hERG or Nav1.5 inhibition is not anticipated at biologically relevant exposure levels. In dogs, single doses of 50 mg/kg ertugliflozin (163x $MRHD_{Cmax}$) were associated with moderate decreases in the QTc interval, cardiac contractility, and heart rate corresponding with T_{max}, as well as increases in systolic blood pressure (sBP) and lengthening of the PR interval, with a NOAEL of 5 mg/kg and a safety margin of ~13x $MRHD_{Cmax}$. In the 27-day pair-fed study #PD001 in spontaneous hypertensive rats (SHR), ertugliflozin-related decreases in blood pressures and heart rate were associated with treatment-related diuresis and activation of the renin-angiotensin-aldosterone-system (RAAS) at 36 mg/kg/day (11x $MRHD_{Cmax}$). Furthermore, based on similar effects observed with a diuretic positive control anti-hypertensive, it's likely that ertugliflozin-related CV effects in the SHR model are, at least in part, secondary to PD-related diuresis.

Respiratory System: In rats, dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed for up to 120 minutes post-dose and correlated with C_{max} at doses of ≥25 mg/kg (~36x $MRHD_{Cmax}$), with a NOAEL of 5 mg/kg (9x $MRHD_{Cmax}$).

Supplemental

Renal/Urinary System: No specific renal safety pharmacology studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes increased urinary glucose excretion and kidney alterations in rats and dogs at clinical exposure levels.

Gastrointestinal System: No GI-specific safety studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs.

Immunotoxicity: There were no indications of immunotoxicity or antigenicity in repeat-dose toxicology studies.

Sitagliptin

Standard CV, neurological and pulmonary safety pharmacology studies for sitagliptin were reviewed under NDA #21995, and are summarized below.

Neurological: No drug-related CNS effects were identified in rat or mouse CNS safety pharmacology studies with functional observational battery (FOB) assessments of CNS activity at doses up to 180 mg/kg in rats and 100 mg/kg in mice. The no observed effect level (NOEL) for neurological effects is >180 mg/kg in rats and >100 mg/kg in mice.

Cardiovascular: Sitagliptin inhibits hERG activity with an IC_{50} of 147 μM, an IC_{20} of ~50 μM, and complete inhibition at 1000 μM, with 80% reversibility. In anesthetized dogs, decreases in blood pressure (↓56 mm Hg) and heart rate (↓40 bpm) were observed with IV infusions of 30 mg/kg and plasma concentrations of 202 μM (253x $MRHD_{Cmax}$), which is associated with a NOAEL of 10 mg/kg (plasma levels ≤59 μM = 74x $MRHD_{Cmax}$). In conscious telemetered dogs, an oral dose of 50 mg/kg (1-hour postdose plasma level of 34 μM = 43x $MRHD_{Cmax}$) was associated with an increase in heart rate (↑30 bpm) and shortening in PR interval in 75% of animals, with a NOAEL of 10 mg/kg (1-hour postdose plasma levels of 7 μM = 9x $MRHD_{Cmax}$). No drug-related changes in QT or other ECG interval were reported. Sitagliptin-related CV risks were evaluated in the CV outcome trial TECOS, wherein the sponsor reported that there was not a drug-related increase in risk of major adverse CV events or the risk of hospitalization for heart failure.

Pulmonary: No meaningful effects on pulmonary parameters were reported in rats at oral doses up to 180 mg/kg. In anesthetized dogs, no changes in respiratory parameters were observed at an IV dose of 10 mg/kg; however, decreases in blood pressure and increases in heart rate of ≤15 minutes were observed.

Renal: No consistent changes in renal function parameters including glomerular filtration rate, effective renal plasma flow, electrolyte excretion, plasma electrolyte concentrations, and filtration fraction were reported in dogs at oral doses up to 10 mg/kg.

Gastrointestinal: No significant effects on GI motility, basal gastric acid secretion, or gastrin-stimulated gastric acid output were reported in dogs at 10 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Ertugliflozin

Ertugliflozin PK parameters were characterized in human, dog, rat, and mouse species. Ertugliflozin protein binding is high in all 4 species, ranging from 92 to 97%. Significant species differences in absorption were associated with oral bioavailability ranging from moderate to high across species and oral absorption ranges of 75-87% in mice, 56-88% in rats, 94-97% in dogs, and up to 100% in humans. T_{max} is achieved within 30 minutes in mice, 0.7 to 2.3 hours in rats, and 0.8 to 1.5 hours in dogs. In humans, T_{max} is

achieved after 1 hour in humans (fasted), but after 2 hours in humans in the fed state, indicating absorption delays in the presence of food. Systemic exposures follow linear pharmacokinetics with a trend for slight increases in female exposures over time at high doses in rodents, indicating a potential gender effect which is likely related to gender differences in metabolism in rodents. Ertugliflozin has a moderate half-life ($t_{1/2}$) of 3 to 4 hours in rodents and 8 hours in dogs, but is 1.5 to 4 times longer in humans ranging from 12 to 18 hours.

Ertugliflozin may be a substrate for the efflux transporter permeability glycoprotein 1/multidrug resistance protein 1 (P-gp/MDR1), but is not affected by P-gp/MDR1 inhibitors; thus, P-gp/MDR1 is unlikely to be a limiting factor in Ertugliflozin absorption. Ertugliflozin has a moderate volume of distribution in rats with preferential distribution into plasma relative to red blood cells. The highest distribution is primarily to organs responsible for drug metabolism and elimination, such as the bladder, liver, and kidney. Ertugliflozin is also highly distributed to rat adrenal gland, Harderian gland, and pancreas. Ertugliflozin crosses the adult blood:brain barrier, but only reaches concentrations 3 to 63-fold lower than that of blood; whereas distribution to the choroid plexus and pituitary gland is 2-fold greater than blood. In fetal rats, ertugliflozin more readily crosses the blood:brain barrier, resulting in significantly more drug exposure to fetal CNS tissues and eyes than in corresponding adult tissues relative to plasma levels. Ertugliflozin is excreted in rat milk at exposures comparable to maternal plasma levels. Ertugliflozin also readily crosses the rat placental barrier, but with fetal exposures remaining lower than maternal plasma levels.

In rats, elimination of radiolabeled drug and metabolites was virtually complete by 168 hours (7 days) postdose. Ertugliflozin is primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans.

The predominant route of elimination of ertugliflozin is via metabolism, wherein glucuronidation is the major metabolic pathway in all species, with minor contributions from oxidative metabolism involving hydroxylation, oxidation, and oxidative desethylation. There are no unique human metabolites; however, the 2-O-β glucuronide M5a (PF-06685948) and the 3-O- β glucuronide M5c (PF-06481944) are disproportional human metabolites, making up 12.2% and 24.1% of total drug in human plasma, respectively.

Sitagliptin

Sitagliptin is absorbed rapidly with an oral bioavailability of 60-90% in rats and dogs, with AUC and C_{max} exposures generally increasing dose-proportionally. Sitagliptin is widely distributed to tissues, with tissue exposures generally higher than plasma, with the exceptions of brain, eyes and bone. Sitagliptin binding to plasma proteins is similar across species, ranging from 32% to 38%. Clearance is moderate in dogs and high in rats, with half-lives of 2 to 5 hours. In Humans, the plasma half-life is 13 hours, which is 3 to 6-fold longer due to slower clearance than both dogs and rats. Sitagliptin is primarily eliminated via urine, possibly by organic anion transporters in the renal tubules and is a substrate for OAT3 transport. Sitagliptin crosses the placenta in pregnant rats and is excreted in the mild of lactating rats. Placental transfer reaches 45% at 2 hours and 80% at 24 hours postdose in rats, and 66% at 2 hours and 30% at 24 hours postdose in rabbits. Sitagliptin is excreted in the milk of lactating rats.

5.2 Toxicokinetics

In rats, PK parameters of ertugliflozin and sitagliptin were not significantly affected by coadministration. Similarly, in the Phase 1 clinical PK study (#P022/1033) in healthy subjects, there were no meaningful differences in ertugliflozin or sitagliptin PK parameters when co-administered together compared to administration of each drug alone. Thus, concomitant administration of ertugliflozin and sitagliptin are not associated with significant changes in drug exposures in humans.

Sponsor's Table 2: Summary of Ertugliflozin PK Parameters with Coadministration in Humans – Study #P022/1033

Parameter (unit)	Parameter Summary Statistics[a] for Ertugliflozin by Treatment	
	Ertugliflozin 15 mg SD	Ertugliflozin 15 mg SD + sitagliptin 100 mg SD
N, n	12, 12	12, 12
AUC_{inf} (ng·hr/mL)	1413 (26)	1445 (25)
AUC_{last} (ng·hr/mL)	1385 (26)	1412 (24)
C_{max} (ng/mL)	262.9 (25)	258.1 (26)
T_{max} (hr)	1.00 (1.00 - 3.00)	1.00 (0.500 - 2.10)
CL/F (mL/min)	177.0 (26)	173.1 (25)
V_z/F (L)	181.4 (41)	203.3 (21)
$t_{½}$ (hr)	12.63 ± 5.15	14.17 ± 4.55

PK parameters are defined in Table S3.

Abbreviations: %CV = percent coefficient of variation; hr = hour(s); N = number of subjects in the treatment group; n = number of subjects contributing to the summary statistics; PK = pharmacokinetic(s); SD = single dose.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean ± standard deviation for $t_{½}$.

Sponsor's Table 3: Summary of Sitagliptin PK Parameters with Coadministration in Humans – Study #P022/1033

Parameter (unit)	Parameter Summary Statistics[a] for Sitagliptin by Treatment	
	Sitagliptin 100 mg SD	Ertugliflozin 15 mg SD +Sitagliptin 100 mg SD
N, n	12, 12	12, 12
AUC_{inf} (uM•hr)	6.882 (21)	6.997 (20)
AUC_{last} (uM•hr)	6.814 (21)	6.912 (21)
C_{max} (nM)	792.0 (24)	805.3 (24)
T_{max} (hr)	2.00 (1.00-4.00)	3.00 (1.00-6.00)
CL/F (mL/min)	594.4 (21)	584.4 (20)
V_z/F (L)	548.2 (28)	579.3 (23)
$t_{½}$ (hr)	11.00 ± 2.89	11.79 ± 2.98

PK parameters are defined in Table S3.
Abbreviations: %CV=percent coefficient of variation; hr = hour(s); N = Number of subjects in the treatment group; n = Number of subjects with reportable $t_{½}$ and AUC_{inf}; SD = single dose
a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean (± standard deviation) for $t_{½}$.

(Tables excerpted from sponsor's package)

6 General Toxicology

6.1 Ertugliflozin

Toxicology studies with administration of ertugliflozin alone were reviewed under NDA #209803 and include pivotal 6-month rat and 9-month dog studies.

Safety margins from the 6 month rat and 9 month dog studies support the proposed 15 mg/day dose of ertugliflozin with safety margins of at least 13x and 46x, respectively, based on AUC exposures at the nonclinical NOAELs. Most findings in the chronic nonclinical toxicology studies can be attributed to drug-related glucosuria and osmotic diuresis. Drug-related gastrointestinal findings in dogs (excessive vomiting, salivation and abnormal feces) and rats (stomach erosion/ulcers, pyloric crypt degeneration and foveolar hyperplasia) are consistent with off-target inhibition of SGLT1. Hyperostosis and changes in calcium regulation have also been observed in rats, and are also likely to be related to SGLT1 inhibition.

Table 2: Ertugliflozin Summary of Pivotal General Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
9-Month + 8-Week Recovery Beagle Dogs **Dose:** 1, 10 & 150 mg/kg ♂ AUC: 6, 63 & 1040 µg·h/mL ♀ AUC: 7, 78 & 767 µg·h/mL	**10 mg/kg** (♂ & ♀)	♂: **46x** ♀: **57x**	**≥1 mg/kg (♂4x/♀6x MRHD):** adrenal gland (↑organ weight & cortex vacuolation), glucosuria **≥10 mg/kg (♂46x/♀57x MRHD):** thyroid mineralization (♀, irreversible) **150 mg/kg (♂754x/♀556x MRHD):** Adverse: GI intolerance (excessive vomiting, diarrhea, salivation), possibly related mortalities, systemic inflammatory response Non-adverse: ↓BW & gain, ↑thymus weight, persistent ↑reticulocytes, ↑urine calcium (partially reversible), irreversible

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
			urine ↑volume
6-Month + 8-Week Recovery Sprague Dawley (SD) Rats **Dose:** 5, 25 & 100 mg/kg ♂ AUC: 18, 128 & 397 µg·h/mL ♀ AUC: 27, 167 & 814 µg·h/mL	**5 mg/kg** (♂ & ♀)	♂: **13x** ♀: **19x**	**≥5 mg/kg (♂13x/♀19x MRHD):** stomach erosion/ulcer, ↓pancreatic zymogen, ↑food consumption, ↓blood glucose, glucosuria, ↓serum electrolytes (minimal), ↑phosphates, possible dehydration, minimal ↑BUN **≥25 mg/kg (♂93x/♀121x MRHD):** Adverse: stomach (pyloric crypt degeneration, discoloration, ↑severity of erosion/ulcer) Non-adverse: minimal-slight kidney findings (pelvic & tubule dilatation, hyperplasia, mineral deposition) **100 mg/kg (♂288x/♀590x MRHD):** bone [severe hyperostosis (♂) & hyperplasia (♀)], digestive tract (stomach hyperplasia, ↑severity of erosions/ulcers & crypt degeneration), ↑severity of kidney findings, adrenal gland (↑organ weight, hypertrophy & cortex vacuolation), ↓BW & gain, ↓RBC parameters, ↓reticulocytes, ↑urine calcium, ↓PTH, significant ↓serum electrolytes (Ca, Na, K, & Cl), mild ↑BUN (1.5-fold)

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 µg·h/mL and C_{max} = 266 ng/mL
♂ = males only; ♀ = females only

6.2 Sitagliptin

Sitagliptin target organs of toxicity identified in rats include kidney (necrosis), liver (necrosis), heart (myocardial degeneration) and bone marrow (necrosis); however, these effects were only observed at high doses (~150x MRHD) and were likely due to inhibition of off-target enzymes DPP8/9. Administration of doses up to ~20x MRHD for 6 months in rats was not associated with any significant toxicity findings. In a 12-month dog toxicology study, the NOAEL at 5x MRHD was based on clinical signs of reduced activity, hunched posture, ataxia, tremor, and sporadic emesis at 50 mg/kg (20x MRHD). Respiratory distress was identified in some animals, but no consistent target organs were identified in dogs.

6.3 FDC Ertugliflozin/Sitagliptin

In accordance with ICH and FDA guidances, the sponsor submitted a GLP-compliant 3-month repeat dose toxicity study in rats with coadministration of ertugliflozin and sitagliptin under IND #106447. The sponsor also submitted a preliminary, non-GLP 2-week study under IND #122330.

Study: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats (Study #TT147808 / #8300338 / #14GR162)

Study #	TT147808 / 8300338 / 14GR162
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	7/14/2014
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729: Lot #E010014849, 76.0% purity Sitagliptin: Lot #010X054, 99.6% purity

Key Study Findings

- Ertugliflozin + Sitagliptin Coadministration-related Findings:
  - Potential exacerbation of stomach erosion and discoloration of glandular mucosa in males and incidences of hemorrhage in females
  - Adrenal Gland
    - ↑Adrenal organ weight
    - Exacerbation of zona glomerulosa hypertrophy of the adrenal cortex
  - Prostate mixed cell inflammation (low incidences)
  - Improvement of ertugliflozin-mediated ketonuria
- Ertugliflozin-related Findings:
  - Trends for ↓body weight and ↓weight gain
  - ↑Food consumption
  - Stomach:
    - Discoloration of glandular stomach mucosa
    - Erosion
    - Submucosal inflammation
    - Hemorrhage
  - ↓Pancreatic acinar cell zymogen granules (♂&♀)
  - Clinical chemistry: ↓glucose, ↓Cl, ↓Ca, and ↑BUN
  - Urine: ↑specific gravity, ↑volume, ↑glucose, ↑ketones, and ↓pH
  - Kidney:
    - ↑Kidney organ weight
    - Tubule and pelvic dilatation
  - Adrenal Gland
    - Adrenal cortex hypertrophy
- Sitagliptin-related Findings:
  - No biologically significant findings were attributed primarily to sitagliptin

SD Rat, 13 Weeks	NOAEL (AUC)	Multiple of MRHD*
No significant adverse systemic toxicities	25 mg/kg Ertugliflozin (123 μg ·h/mL) + 60 mg/kg Sitagliptin	Ertugliflozin: **89x** Sitagliptin: **9x**

	(24.4 μg ·h/mL)	

*Based on a maximum once daily dose of 15 mg ertugliflozin / 100 mg sitagliptin with an ertugliflozin exposure of AUC = 1.38 μg·h/mL and sitagliptin exposure of AUC = 2.81 μg·h/mL

METHODS

SD rats were co-administered doses of 0/0, 0/60, 25/0, 5/20, 5/60, 25/20, and 25/60 mg/kg PF-04971729/mg/kg sitagliptin (10/sex/group) via oral gavage daily for 91 days. The vehicle for PF-04971729 was 0.5% MC/10% PEG 400 and the vehicle for sitagliptin was 0.5% MC/5 mM HCl. Animals were evaluated for clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, macroscopic findings, and microscopic findings. Satellite TK groups were included for each dose (4/sex/group).

RESULTS

The NOAEL was set at the high combination dose of 25 mg/kg ertugliflozin and 60 mg/kg sitagliptin due to a lack of significant adverse systemic toxicities. The safety margins for ertugliflozin were 89x $MRHD_{AUC}$ for the proposed clinical high dose of 15 mg/day ertugliflozin and 9x $MRHD_{AUC}$ for a 100 mg/day sitagliptin clinical dose.

Reduced serum glucose, increased urinary volume and glucosuria underlie most of the findings in this study, which include reduced body weight and increased food consumption, as well as histopathology changes in the kidney, adrenal gland, and pancreas. Overall, the key findings associated with PF-04971729 in this study are consistent with administration of PF-04971729 alone for 1, 3, and 6 months in rats. Similarly, findings associated with coadministration of PF-04971729 and sitagliptin, as well as sitagliptin alone, are consistent with findings in the 2-week coadministration study in rats (study #13GR342). Overall, there were no new significant drug-related toxicities and the majority of the findings are considered to be secondary to the PD activity of PF-04971720. Thus, there were no toxicity interactions due to coadministration of PF-04971729 and sitagliptin.

Decreases in body weight gain with reciprocal increases in food consumption were observed in animals receiving ertugliflozin independent of sitagliptin, and are consistent with observations from previous studies with PF-04971729 administration in rats. Thus, these findings are attributable to ertugliflozin. It is noted that treatment-related decreases in body weights and weight gains, as well as increases in food consumption, were not exacerbated with coadministration of sitagliptin coadministration.

PF-04971729-related glucosuria was associated with reciprocal decreases in blood glucose levels, reflecting inhibition of SGLT2 and reduced renal tubular reabsorption of glucose from the glomerular filtrate. Observed reductions in Ca (↓4-5%) and Cl (↓3-5%) electrolyte concentrations in the blood are consistent with osmotic diuresis. Furthermore, increases in urine volume (↑2 to 3-fold in males, ↑18-37% in females) and urine specific gravity (↑2-4%), as well as decreases in urinary pH (↓3-13%), were also observed with PF-04971729 administration independent of sitagliptin and are also consistent with osmotic diuresis. Since increases in BUN levels correlate with increases in urine volume while creatinine levels were not increased, the observed increases in

BUN (↑29-93%) are likely to be secondary to dehydration resulting from glucosuria, rather than kidney toxicity. Ultimately, coadministration of sitagliptin did not exacerbate any of the clinical chemistry findings and there were no clear signs of kidney dysfunction. Overall, the clinical pathology changes observed with coadministration treatment were considered to be non-adverse findings attributable to PD-related effects of PF-04971729.

Drug-related ketonuria was attributed to ertugliflozin administration, but was considered to be non-adverse. The highest incidences and severity of ketonuria were observed at 25 mg/kg ertugliflozin in both males and females, which is consistent with findings in 6-month rat and other 13-week rat toxicology studies with ertugliflozin administration. Importantly, improvement of ketonuria was observed with coadministration of sitagliptin in both males and females. Thus, there was not a negative toxicological interaction regarding ketonuria with co-administration.

Increases in kidney weights were observed in all male (↑14-35%) and female (↑21-35%) PF-04971729 treatment groups, but not in animals treated with sitagliptin alone. Correlating histopathological findings of minimal to marked tubular dilatation in the kidney, consistent with osmotic diuresis, were also observed in animals receiving PF-04971729 but not with sitagliptin alone. Overall, the kidney findings were independent of sitagliptin and attributable to the PD activity of ertugliflozin. Since, there were no indications of kidney dysfunction or toxicity, these findings were considered to be non-adverse. Furthermore, there was no indication of exacerbation of increased kidney weight with coadministration of sitagliptin.

Findings of discolored stomach and/or erosion were observed in animals treated with PF-04971729 in the absence or presence of sitagliptin. Increases in the number of incidences of minimal to mild stomach erosion and discoloration findings were reported in males with sitagliptin coadministration, indicating potential exacerbation. Incidences of stomach hemorrhage were reported in males independent of sitagliptin, but were only observed in females with sitagliptin coadministration. These stomach findings are consistent with previous rat toxicology studies with PF-04971729 administration, and are likely related to off-target inhibition of SGLT1 in this species, which is not likely to occur at clinical exposure levels. It's noted that other members of the DPP4 inhibitor drug class have been associated with stomach findings of necrosis, ulceration and erosion; thus, exacerbation of the stomach findings in coadministration groups may be due to additive toxicities of both PF-04971729 and sitagliptin. Nevertheless, the stomach findings were of low severity and were not dose-limiting; thus, they were not considered to be a significant adverse systemic toxicity in this study.

Pancreatic zymogen depletion was observed in all groups treated with PF-04971729, independent of sitagliptin administration, with increased severity in males. Similar findings were also described in previous toxicology studies with ertugliflozin alone or in combination with sitagliptin, and are considered to be non-adverse findings secondary to PD-related increases in food consumption.

Decreases in blood calcium levels were reported in PF-04971729 treatment groups are attributable to PF-04971729-related glucosuria, and are also consistent with disruption of absorption and calcium homeostasis secondary to off-target inhibition of SGLT1. Although, there were no abnormal bone findings in this study, increased trabecular bone was observed at doses of 25 mg/kg PF-04971729 with longer exposures in males in the 6-month rat toxicology study. Thus, the decreased serum calcium levels observed in this study may be indicative of early drug-related effects on bone in this species. Nevertheless, since PF-04971729-mediated inhibition of SGLT1 is unlikely at clinical exposure levels, this finding is not likely to be clinically relevant.

Adrenal gland hypertrophy was reported in both sexes of PF-04971729 treatment groups and was associated with increased incidence and severity with coadministration of sitagliptin, and also correlated with increased adrenal gland weights. These findings are consistent with previous studies with PF-04971729 alone in rats and dogs, and are likely due to a compensatory response to fluid and electrolyte losses related to the PF-04971729-induced glucose excursion. However, it's also noted that the adrenals are a potential class-related target organ of sitagliptin. The coadministration data indicate that that sitagliptin and ertugliflozin likely work together to increase severity and incidence rates of adrenal gland organ weight increases and hypertrophy. Nevertheless, these effects are not considered to be adverse.

It is noted that there were no abnormal heart organ weight changes or histopathological findings.

Low incidence rates of minimal to moderate prostate mixed cell infiltration were reported in males with coadministration, but not with administration of either drug alone. The DPP4 inhibitor drug class is associated with cellular infiltration of multiple organs. Although this finding was not dose-related and occurred at low incidences of 1 to 2 animals per group, it may be treatment-related with coadministration of PF-04971729 and sitagliptin. Nevertheless, it was also considered to be non-adverse.

Coadministration of PF-04971729 did not affect sitagliptin exposures. PF-04971729 exposures were predominantly unaffected by sitagliptin coadministration. However, it is noted that PF-04971729 exposures were slightly higher on Day 91 by 12-28% with coadministration, indicating a possible trend for accumulation with high doses of both drugs. Nevertheless, given the small amount of increase, it is unclear if this is a significant finding.

Methods

Doses	PF-04971729 (mg/kg) + Sitagliptin (mg/kg): 0+0, 0+60, 25+0, 5+20, 5+60, 25+20, and 25+60
Frequency of dosing	Once daily for 91 days. Animals were dosed 1st with PF-04971729, then dosed with sitagliptin 2nd within 2 minutes.
Route of administration	Oral gavage
Dose volume	5 mL/kg PF-04971729 + 5 mL/kg sitagliptin = 10 mL/kg total
Formulation/Vehicle	Vehicle #1 (PF-04971729): 0.5% (w/v) methylcellulose, 10% (v/v) polyethylene glycol 400 (PEG 400) Vehicle #2 (sitagliptin): 0.5% (w/v) methylcellulose, 5 mM hydrochloric acid
Species/Strain	Crl:CD(SD) rats, (b) (4)
Number/Sex/Group	10/sex/group
Age	6-7 weeks
Weight	♂: 202-282 g ♀: 153-216 g
Satellite groups	TK animals including 4/sex/group
Unique study design	Co-administration of PF-04971729 and sitagliptin
Deviation from study protocol	On some occasions, some animals received only a partial dose; however, the identity of the animals was not reported. Day 17-87: various animals (11 total) across most groups received the sitagliptin dose more than 2 minutes after the PF-04971729 dose.

Study Design

		No. of Animals		PF-04971729		Sitagliptin	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Dose Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	10	10	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Control/ High)	1 (Toxicity)	10	10	0	0	60	12
	2 (Toxicokinetic)	4	4	0	0	60	12
3 (High/ Control)	1 (Toxicity)	10	10	25	5	0	0
	2 (Toxicokinetic)	4	4	25	5	0	0
4 (Low/ Low)	1 (Toxicity)	10	10	5	1	20	4
	2 (Toxicokinetic)	4	4	5	1	20	4
5 (Low/ High)	1 (Toxicity)	10	10	5	1	60	12
	2 (Toxicokinetic)	4	4	5	1	60	12
6 (High/ Low)	1 (Toxicity)	10	10	25	5	20	4
	2 (Toxicokinetic)	4	4	25	5	20	4
7 (High/ High)	1 (Toxicity)	10	10	25	5	60	12
	2 (Toxicokinetic)	4	4	25	5	60	12

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg sitagliptin (or Vehicle Control Article 2, as applicable).

b PF-04971729 dose concentrations were corrected for lot specific potency of 0.760 (76.0%). A correction factor of 1.316 was used for Lot No. E010014849.

c Sitagliptin dose concentrations were corrected for salt content and lot specific potency of 0.996 (99.6%). A correction factor of 1.285 was used for Lot No. 010X054.

d Group 1 received Vehicle Control Article 1 (0.5% [w/v] methylcellulose [4000 cps] with 10% [v/v] polyethylene glycol 400 prepared in reverse osmosis water) and Vehicle Control Article 2 (0.5% [w/v] methylcellulose [4000 cps] with 5 mM hydrochloric acid prepared in reverse osmosis water) only.

Parameters Measured

Clinical Findings	Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. Detailed observations were conducted on all animals prior to dosing on Day 1, weekly during the dosing phase, and on Day 91. Cageside observations were also conducted at 1 hour postdose.
Body weights	Animals were weighed once during the predose phase, prior to dosing of Day 1, weekly thereafter, and on Day 91.
Food consumption	Food consumption was quantified for each cage weekly, beginning on Day 1, for Weeks 1-13 and Days 85-91.
Ophthalmoscopy	Ophthalmic examinations were conducted by a veterinarian using an indirect ophthalmoscope and a mydriatic agent once during the predose phase and during Week 13 of the dosing phase.
EKG	Not evaluated
Hematology	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.2 Hematology Tests** red blood cell (erythrocyte) count hemoglobin hematocrit mean corpuscular volume mean corpuscular hemoglobin mean corpuscular hemoglobin concentration platelet count white blood cell (leukocyte) count differential blood cell count blood smear reticulocyte count mean platelet volume red blood cell distribution width **3.5.1.3 Coagulation Tests** prothrombin time activated partial thromboplastin time
Clinical chemistry	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.4 Clinical Chemistry Tests** glucose urea nitrogen creatinine total protein albumin globulin albumin:globulin ratio cholesterol total bilirubin alanine aminotransferase alkaline phosphatase gamma glutamyltransferase aspartate aminotransferase calcium inorganic phosphorus sodium potassium chloride
Urinalysis	Urine samples were collected at necropsy on Day 92 **3.5.1.5 Urinalysis Tests** appearance (clarity and color) bilirubin blood glucose ketones microscopic examination of sediment pH protein specific gravity urobilinogen volume
Gross pathology	Animals were fasted overnight and necropsied on Day 92. External features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues were examined.
Organ weights	Organ weights were measured (W) according the table below. Paired organs were weighed together.
Histopathology	Tissues were collected from all animals and prepared (P) by preserving in 10% NBF, embedding in paraffin, sectioning, and staining with H&E. All tissues in Groups 1 (0+0), 2 (0+60), 3 (25+0), and 7 (25+60) were examined microscopically. The kidneys, ureter, duodenum, pancreas, glandular stomach, adrenal cortex, and prostate from Groups 4 (5+20), 5 (5+60), and 6 (25+20) were also examined microscopically.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		P,E
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	right upper incisor tooth with root		P,E
epididymis (2)	W	P,E	salivary gland (mandibular [2])		P,E
esophagus		P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
eye (2)[b]		P,E	seminal vesicle		P,E
femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	skin/subcutis {skin and adnexa}		P,E
gross lesions		P,E	spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
gut-associated lymphoid tissue {GALT}		P,E	spleen	W	P,E
Harderian gland[b]		P,E	sternum with bone marrow {sternum}		P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[b]	W	P,E
jejunum		P,E	thymus	W	P,E
kidney (2)	W	P,E	thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
larynx			tongue		P,E
liver	W	P,E	trachea		P,E
lower mandible			ureter		P,E
lungs with large bronchi {lung}		P,E	urinary bladder		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	uterus		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	vagina		P,E
mammary gland (males and females)		P,E			

E = Examined microscopically; P = Processed; W = Weighed.

a Brain was sectioned according to published recommendations (Bolon et al., 2013).

b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Bone marrow smears were prepared from the femur, but were not examined.

Toxicokinetics	Non-fasted blood samples were collected from all groups (2 animals/time point/group) on Days 1 and 91 at 1, 4, 7, and 24 hours postdose.

Observations and Results

Mortality

There were no mortalities.

Clinical Signs

There were no drug-related findings.

Body Weights

Male body weight gains were generally lower (↓6-14%) with administration of ≥5 mg/kg PF-04971729 and were associated with lower final body weights (↓3-8%). However, there was not a clear dose-dependent response. Decreases in body weight gains (↓1-9%) and final body weights (↓1-4%) were less pronounced in females and were also independent of dose. Decreases in body weights and weight gains are consistent with PF-04971729-related findings in rats in multiple other studies and are considered to be drug-related. There were no indications of exacerbation by co-administration with sitagliptin.

Table 3: Body Weights - 13-week Rat Study #14GR162

MALES: Body Weight				
Study Time	Dose (mg/kg+mg/kg)	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	346	-	-
	0+600	347	100.3%	100%
	25+0	325	93.9% (↓6.1%)	96.6% (↓3.4%)
	5+200	302	87.3% (↓12.7%)	92.3% (↓7.7%)
	5+600	307	88.7% (↓11.3%)	93.2% (↓6.8%)
	25+200	298	86.1% (↓13.9%)	92.0% (↓8.0%)
	25+600	318	91.9% (↓8.1%)	95.1% (↓4.9%)
FEMALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	130	-	-
	0+600	133	102.3%	101.0% (↑1.0%)
	25+0	122	93.8% (↓%6.2)	96.8% (↓3.2%)
	5+200	129	99.2% (↓0.8%)	98.7% (↓1.3%)
	5+600	118	90.8% (↓9.2%)	95.9% (↓4.1%)
	25+200	118	90.8% (↓9.2%)	95.9% (↓4.1%)
	25+600	124	95.4% (↓4.6%)	97.8% (↓2.2%)

Sponsor's Figure 1: Body Weights - 13-week Rat Study #14GR162

Figure 7.1: Summary of Body Weight - Males

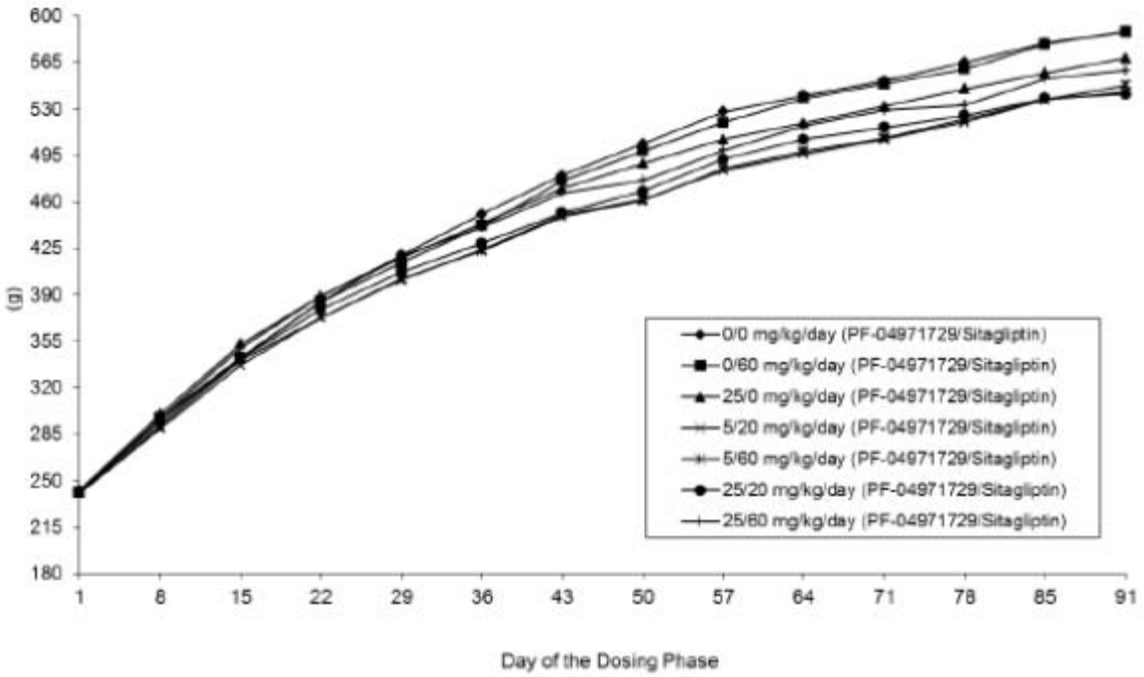
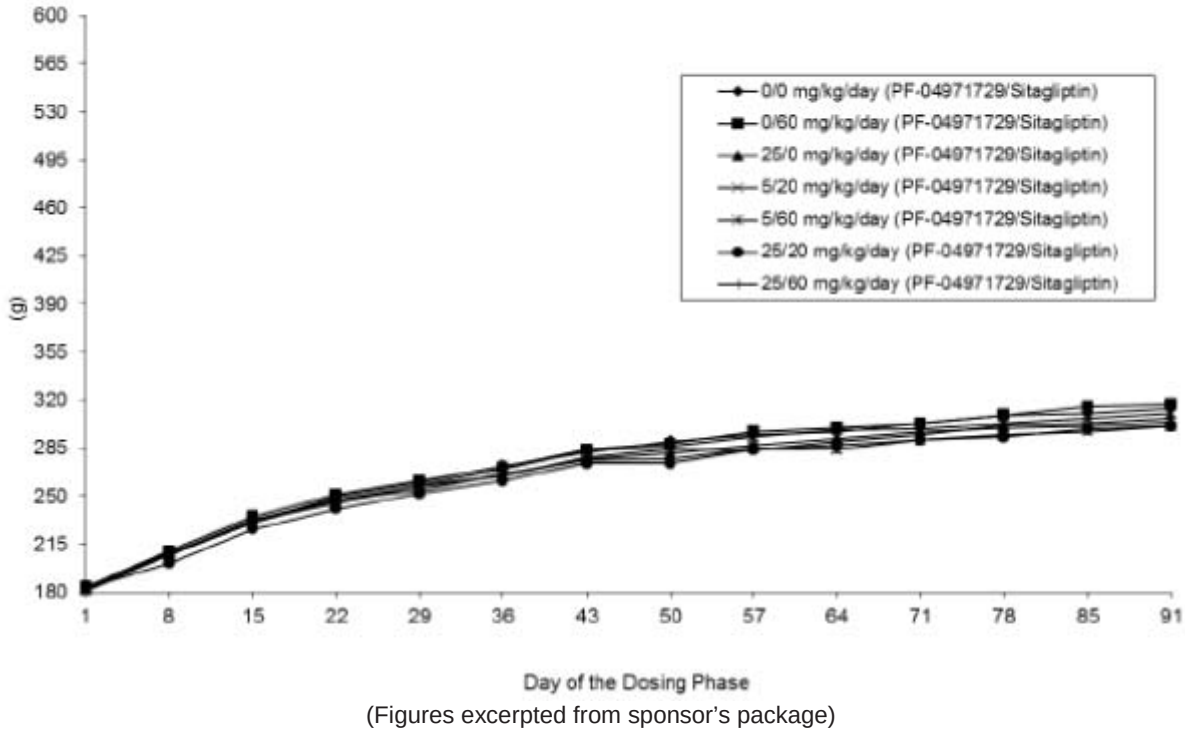


Figure 7.2: Summary of Body Weight - Females



(Figures excerpted from sponsor's package)

Feed Consumption

Food consumption was higher in females (↑11-22%) treated with PF-04971729, but was independent of dose and did not reach statistical significance in all groups due to variability. Similarly, food consumption was also increased in males (↑7-14%) treated with PF-04971729 independent of dose, but did not reach statistical significance in any dose group. Increases in food consumption are consistent with PF-04971729-related findings in rats in multiple other studies and are considered to be drug-related. There were no indications of exacerbation by co-administration with sitagliptin.

Table 4: Food Consumption - 13-week Rat Study #14GR162

Food Consumption				
Dose, mg/kg	Males		Females	
	Consumption (g/animal/day)	% Control	Consumption (g/animal/day)	% Control
0+0	29	-	18	-
0+60	28	96.6%	17	94.4%
25+0	32	110.3%	21*	116.7%
5+20	31	106.9%	20	111.1%
5+60	31	106.9%	21	116.7%
25+20	32	110.3%	22*	122.2%
25+60	33	113.8%	21	116.7%

* p value < 0.05

Ophthalmoscopy

There were no treatment-related findings.

Hematology

There were no biologically significant changes in hematology parameters with either drug treatment or co-administration.

Several statistically significant changes in hematocrit, neutrophil counts and prothrombin time were reported in various treatment groups; however, these findings were considered to be of small magnitude, within the normal biological range, and unlikely to be biologically significant. Small 5% decreases in hematocrit (Hct) were observed in females receiving 25 mg/kg PF-04971729 and sitagliptin, but were not observed in males. Decreases of 25-37% in neutrophils (NEUT) were observed in males treated with PF-04971729, but only reached statistical significance in males receiving 25 mg/kg PF-04971729 co-administered with sitagliptin. An 8% decrease in

pro-thrombin time (PT) was reported for males treated with 25 mg/kg PF-04971729 and 20 mg/kg sitagliptin, but was not dose-dependent nor observed in females.

Table 5: Hematology Parameters - 13-week Rat Study #14GR162

Hematology						
Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	Hct (%)		NEUT (10^3/uL)		PT (sex)	
	♂	♀	♂	♀	♂	♀
0+0	52.5	51.5	2.12	0.82	10.8	9.2
0+60	51.4	51.2	2.39	1.05	10.5	9.3
25+0	51.5	49.9	1.54 (↓27.4%)	0.79	10.4	9.2
5+20	52.2	50.7	2.78	0.74	10.2	9.5
5+60	51.4	49.6	1.60 (↓24.5%)	0.65	10.6	9.0
25+20	51.6	49.1* (↓4.7%)	1.32* (↓37.3%)	1.04	9.9* (↓8.3%)	9.0
25+60	51.9	48.8* (↓5.2%)	1.36* (↓35.8%)	1.02	10.6	9.3

* p value < 0.05

Clinical Chemistry

Drug-related decreases in steady-state fasting serum glucose levels were observed with PF-04971729 treatment in both males (↓17-33%) and females (↓7-28%), but were independent of sitagliptin treatment or co-administration. Thus, maintenance of reduced blood glucose levels was attributed to PF-04971729 administration alone. It is noted that the reduced steady-state blood glucose levels were harvested 24 hours postdose and were at or below the lower limit of normal (LLN), but were not within the hypoglycemic range (<50 mg/dL) for this species.

Drug-related increases in BUN levels were observed with PF-04971729 treatment in males (↑57-93%) and females (↑29-79%), reaching levels above the upper limit of normal (ULN) in males treated with 25 mg/kg PF-04971729. The increases were dose-dependent with regard to PF-04971729, but were independent of sitagliptin treatment or co-administration. Thus, the increases in BUN levels were attributed to Pf-04971729 administration alone.

Statistically significant decreases in total protein levels (↓6-7%) were observed in females treated with 25 mg/kg PF-04971729 alone or in combination with 60 mg/kg sitagliptin. Significant decreases in albumin levels (↓9%) were also observed in females receiving the highest dose combination (25+60). Nevertheless, the decreases in blood protein levels remained within the normal biological range for this species and are not considered to be biologically significant.

Table 6: Clinical Chemistry Parameters - 13-week Rat Study #14GR162

Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	Glucose (mg/dL)		BUN (mg/dL)		TP (g/dL)		ALB (g/dL)	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	102	101	14	14	7.2	8.1	4.4	5.4
0+60	108	111	14	14	7.6	8.1	4.5	5.4
25+0	76* (↓25.5%)	73* (↓27.7%)	27* (↑92.9%)	25* (↑78.6%)	7.1	7.6* (↓6.2%)	4.4	5.0
5+20	85* (↓16.7%)	90 (↓10.9%)	22* (↑57.1%)	20* (↑42.9%)	7.4	7.8 (↓3.7%)	4.4	5.2
5+60	84* (↓17.6%)	94 (↓6.9%)	22* (↑57.1%)	18* (↑28.6%)	7.0	8.1	4.3	5.4
25+20	68* (↓33.3%)	82* (↓18.8%)	27* (↑92.9%)	24* (↑71.4%)	7.1	7.8 (↓3.7%)	4.3	5.2
25+60	70* (↓31.4%)	73* (↓27.7%)	26* (↑85.7%)	24* (↑71.4%)	7.1	7.5* (↓7.4%)	4.3	4.9* (↓9.3%)

* p value < 0.05

Drug-related increases in ALT levels were observed in males (↑24-44%) at ≥5 mg/kg PF-04971729 and in females (↑35-48%) at 25 mg/kg in a dose-dependent manner with regard to PF-04971729, but independent of sitagliptin treatment or co-administration. Thus, the increases in ALT were attributed to PF-04971729 administration; however, the degrees of increase were considered to be minimal and unlikely to be biologically significant.

Statistically significant decreases in cholesterol were observed in females with PF-04971729 administration, but were not consistently dose-dependent and remained within the normal biological range for this species. Although attributed to PF-04971729 treatment, this finding is not considered to be biologically significant.

Drug-related decreases in electrolytes were observed in both sexes. Statistically significant decreases in chloride levels were observed in both males (↓4-5%) and females (↓3%) with PF-04971729 administration in a dose-dependent manner with regard to PF-04971729, but independent of sitagliptin. Statistically significant decreases in calcium levels were observed in both males (↓5%) and females (↓4%) with 25 mg/kg PF-04971729 administration alone or in combination with 20 mg/kg sitagliptin in males (↓4%). The observed decreases in electrolytes are consistent with findings from other studies with PF-04971729 administration in rats and are considered to be drug-related. Nevertheless, it is noted that the mean levels of both calcium and chloride remained within the normal biological range for this species.

Table 7: Clinical Chemistry Parameters Continued - 13-week Rat Study #14GR162

Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	ALT (U/L)		CHOL (mg/dL)		Calcium (mg/dl)		Cl (mmol/L)	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	34	29	79	113	11.4	11.5	103	102
0+60	32	30	84	105	11.4	11.6	102	102
25+0	49* (↑44.1%)	43* (↑48.3%)	76	89* (↓21.2%)	10.8* (↓5.3%)	11.0* (↓4.3%)	98* (↓4.9%)	99* (↓2.9%)
5+20	42* (↑23.5%)	31	72	92 (↓18.6%)	11.2	11.3	99* (↓3.9%)	100 (↓2.0%)
5+60	43* (↑26.5%)	36 (↑24.1%)	66	88* (↓22.1%)	11.0 (↓3.5%)	11.5	99* (↓3.9%)	101 (↓1.0%)
25+20	46* (↑35.3%)	39* (↑34.5%)	80	94 (↓16.8%)	11.0* (↓3.5%)	11.2 (↓2.6%)	98* (↓4.9%)	99* (↓2.9%)
25+60	46* (↑35.3%)	41* (↑41.4%)	70	88* (↓22.1%)	11.1 (↓2.6%)	11.1 (↓3.5%)	98* (↓4.9%)	99* (↓2.9%)

* p value < 0.05

Urinalysis

Moderate to marked glucosuria was reported in both sexes with PF-04971729 administration alone or in combination with sitagliptin.

Statistically significant drug-related increases in specific gravity were reported in males (↑2-3%) and females (↑3-4%) with PF-04971729 administration, but were independent of dose and sitagliptin administration.

Significant drug-related increases in urine volume reaching 2 to 3-fold above concurrent controls were observed in males with PF-04971729 administration and were dose-dependent with regard to PF-04971729, but were independent of sitagliptin administration and dose. A trend for minimal increases (↑18-37%) in urine volume was also observed with 25 mg/kg PF-04971729 administration in females independent of sitagliptin, but did not reach statistical significance.

Trends for decreased urine pH were observed in both males (↓3-8%) and females (↓10-13%), but were less severe with co-administration.

Table 8: Urine Parameters - 13-week Rat Study #14GR162

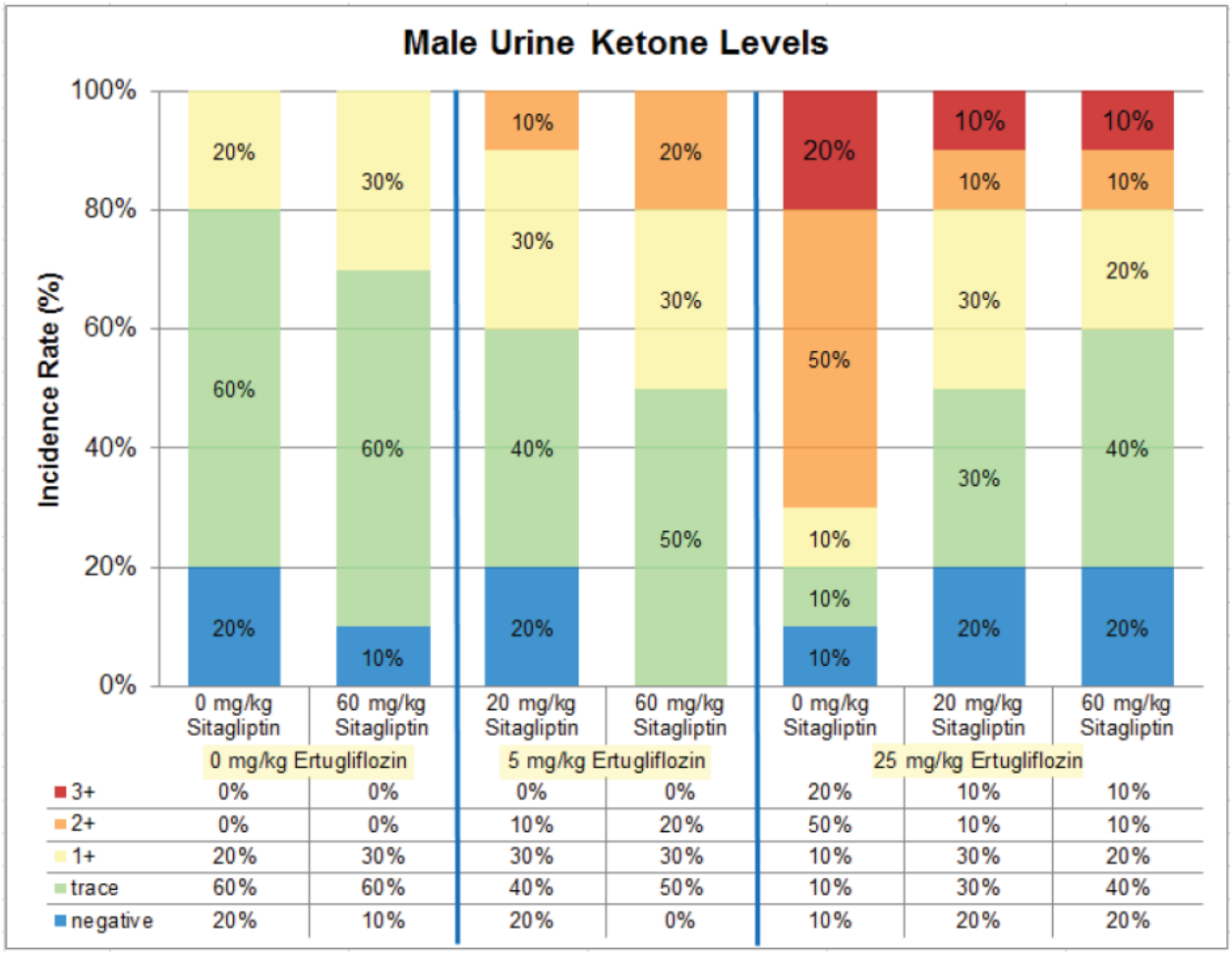
Urine Parameters						
Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	Specific Gravity		Volume (mL)		pH^	
	♂	♀	♂	♀	♂	♀
0+0	1.037	1.015	11.0	15.9	6.7	7.0
0+60	1.040	1.028	8.9	9.0	6.6	6.6
25+0	1.056*	1.053*	32.9*	19.0	6.2	6.1

	(↑1.8%)	(↑3.7%)	(↑3-fold)	(↑19.5%)	(↓7.5%)	(↓12.9%)
5+20	1.063* (↑2.5%)	1.055* (↑3.9%)	21.0* (↑2-fold)	13.9	6.7	6.4
5+60	1.056* (↑1.8%)	1.047* (↑3.2%)	24.1* (↑2-fold)	16.2	6.5	6.4
25+20	1.054* (↑1.6%)	1.056* (↑4.0%)	31.0* (↑3-fold)	18.7 (↑17.6%)	6.4 (↓4.5%)	6.2 (↓11.4%)
25+60	1.051* (↑1.4%)	1.048* (↑3.3%)	34.5* (↑3-fold)	21.8 (↑37.1%)	6.5 (↓3.0%)	6.3 (↓10.0%)

^ Statistical analysis not performed
* p value < 0.05

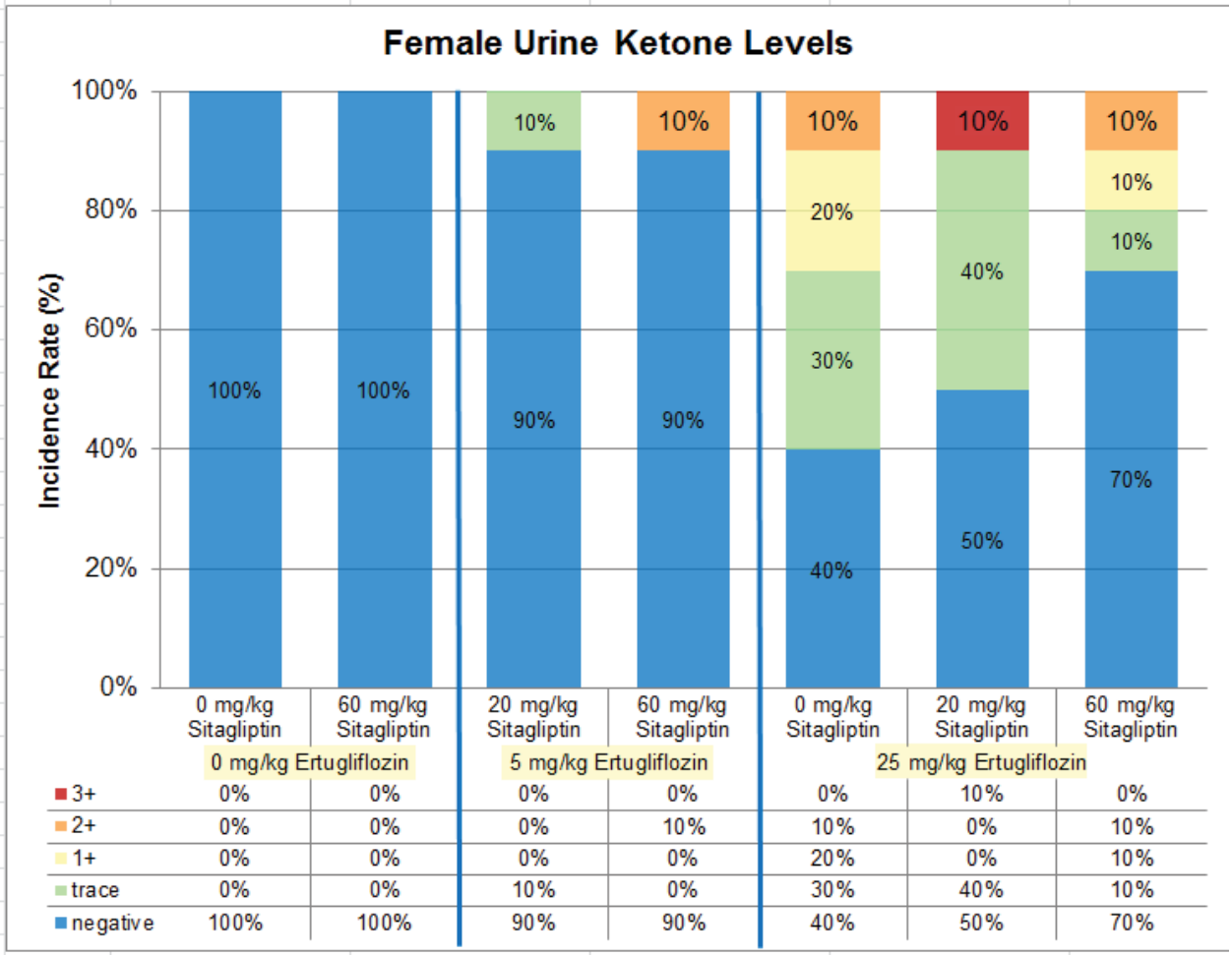
Moderate to marked urine ketone levels of ≥40 mg/dL (2+ and 3+) were observed in 90% of males receiving 25 mg/kg PF-04971729 alone and 20% of males receiving coadministration of 25 mg/kg PF-04971729 and sitagliptin. Decreases in incidence and severity of ketones were observed with co-administration of sitagliptin, which indicates improvement of ketone levels. Importantly, there was not a negative interaction regarding ketonuria with sitagliptin co-administration in male rats.

Figure 1: Male Ketone Urinalysis - 13-week Rat Study #14GR162



In general, urine ketone levels were lower in females, regardless of vehicle or drug administration. Increases in incidence and severity of ketones were observed in females with PF-04971729 administration, and were generally independent of sitagliptin coadministration. The highest incidence rates of females with ketone bodies were observed with 25 mg/kg PF-04971729, with mild (15 mg/dL, 1+) to marked (80 mg/dL, 3+) levels of ketonuria in 30% to 50% of females. It is noted that a decrease in incidence of ketones was observed with co-administration of 60 mg/kg, despite administration of 25 mg/kg PF-04971729, which indicates improvement of ketone levels. Importantly, there was not a negative interaction regarding ketonuria with sitagliptin co-administration in male rats.

Figure 2: Female Ketone Urinalysis - 13-week Rat Study #14GR162



Gross Pathology

Incidences of mucosal discoloration were observed in the glandular stomach were observed in both sexes treated with ≥25 mg/kg PF-04971729. Furthermore, there was an apparent dose-dependent increase in males with co-administration of sitagliptin, indicating exacerbation. In females, the largest incidence of mucosal discoloration was observed in animals treated with 25 mg/kg PF-04971729 alone. Mucosal discoloration

was most often dark red and sometimes black, grey, or white, corresponding with erosion, submucosal inflammation, and/or hemorrhage.

Pelvic enlargement was reported in the kidneys of 2 males treated with 25 mg/kg PF-04971729, which correlated with microscopic findings of renal dilatation.

Sponsor's Table 4: Macroscopic Findings - 13-week Rat Study #14GR162

Table
Incidence of Macroscopic Observations
Terminal Euthanasia (Dosing Phase)

Test Article	(dosage)	1	2	3	4	5	6	7
PF-04971729	mg/kg/day	0	0	25	5	5	25	25
Sitagliptin	mg/kg/day	0	60	0	20	60	20	60

Tissue/ Observation	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	4/1/M	5/1/M	6/1/M	7/1/M
	Number of Animals:	10	10	10	10	10	10	10
Stomach	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	9	10	9	8	6
Discolored		0	0	1	0	1	2	4
Kidney	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	9	10	10	9	10
Large		0	0	1	0	0	1	0

Table
Incidence of Macroscopic Observations
Terminal Euthanasia (Dosing Phase)

Test Article	(dosage)	1	2	3	4	5	6	7
PF-04971729	mg/kg/day	0	0	25	5	5	25	25
Sitagliptin	mg/kg/day	0	60	0	20	60	20	60

Tissue/ Observation	Group/Subgroup/Sex:	1/1/F	2/1/F	3/1/F	4/1/F	5/1/F	6/1/F	7/1/F
	Number of Animals:	10	10	10	10	10	10	10
Stomach	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	6	10	10	9	9
Discolored		0	0	4	0	0	1	1

(Tables excerpted from Sponsor's report and highlighted)

Organ Weights

PF-04971729-related increases in kidney weights (absolute, relative body and relative brain weights) were observed in all male (↑14-35%) and female (↑21-35%) PF-04971729 treatment groups, but not in animals treated with sitagliptin alone. There was no indication of exacerbation of increased kidney weight with co-administration of sitagliptin. The increased kidney weights were considered to be due to PF-04971729 treatment, but were independent of sitagliptin administration.

Increases in adrenal weights were observed in both sexes treated with co-administration of 25 mg/kg PF-04971729 and sitagliptin. However, statistical significance was only achieved in weights relative to body weight and only in males (↑32%) at 25+60 and in females (↑20%) at 25+20. Nevertheless, this finding is consistent with other studies involving PF-04971729 administration in rats and is considered likely to be a drug-related finding that is exacerbated by sitagliptin co-administration.

Sponsor's Table 5: Organ Weights - 13-week Rat Study #14GR162

Text Table 4.1: Test Article-Related Changes in Organ Weight Parameters

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/0	0/60	25/0	5/20	5/60	25/20	25/60	0/0	0/60	25/0	5/20	5/60	25/20	25/60
Kidney														
Absolute Weight (g)	3.0101	1.07	1.25*	1.19*	1.14	1.21*	1.23*	1.6836	.99	1.25*	1.21*	1.20*	1.25*	1.22*
Body Weight Ratio (%)	0.5445	1.07	1.33*	1.32*	1.25*	1.35*	1.33*	0.5745	.99	1.33*	1.25*	1.27*	1.35*	1.30*
Brain Weight Ratio (%)	135.2726	1.06	1.27*	1.21*	1.17*	1.22*	1.25*	80.7133	1.03	1.29*	1.25*	1.22*	1.27*	1.27*
Adrenal														
Absolute Weight (g)	0.0612	1.09	1.15	1.01	1.13	1.05	1.23	0.0669	.94	1.07	.94	.95	1.11	1.03
Body Weight Ratio (%)	0.0111	1.08	1.23	1.11	1.24	1.16	1.32*	0.0228	.94	1.14	.98	1.00	1.20*	1.09
Brain Weight Ratio (%)	2.7525	1.07	1.17	1.02	1.16	1.05	1.25	3.2085	.98	1.10	.97	.96	1.12	1.07

* = Statistically significant ($p \leq 0.05$) difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold of control mean value.

(Table excerpted from sponsor's package)

Histopathology

Battery Considered Adequate? Yes
Peer Review Performed? Yes

Increases in incidence and severity of kidney tubule dilatation was observed in males and females with increasing PF-04971729 administration, but were independent of sitagliptin treatment. Furthermore, all animals treated with 25 mg/kg PF-04971729 presented with minimal to mild tubule dilatation. Increased incidence or severity of pelvis dilatation was also observed in males in all PF-04971729 treatment groups except 5+20 and in females at 5+20 and 25+60. Overall, the kidney findings of tubule and pelvic dilatation are considered likely to be drug-related.

Increases of mixed cell inflammation in the prostate were observed in males co-administered both drugs, with an apparent dose-dependence of sitagliptin with 25 mg/kg PF-04971729 co-administration.

Stomach findings of erosion, hemorrhage and inflammation were reported in both sexes. In general, increases in incidence and/or severity of glandular stomach erosion were observed in both sexes. However, there was not a clear dose-dependence in females since this effect was not seen in the highest co-administration group 25+60. Findings of minimal acute submucosal inflammation were also noted in both sexes with PF-04971729 administration alone or in combination with sitagliptin, and with PF-04971729 dose-dependence in males, but not in females. Incidences of minimal hemorrhage were also reported with PF-04971729 administration alone in males and with sitagliptin co-administration with 25 mg/kg PF04971729 in both sexes. Overall, the stomach histopathological findings are consistent with previous findings in rats with PF-04971729 and are considered to be PF-04971729-related, but largely independent of sitagliptin administration.

Hypertrophy in the zona glomerulosa of the adrenal cortex was observed in both sexes with PF-04971729 administration. Furthermore, the increases in incidence and/or severity were increased with sitagliptin co-administration.

Pancreatic zymogen depletion was observed in all groups treated with PF-04971729, independent of sitagliptin administration, with increased severity in males.

Sponsor's Table 6: Histopathology - 13-week Rat Study #14GR162

Text Table 3.3: Incidence and Severity of Test Article-Related Microscopic Findings

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/ 0	0/ 60	25/ 0	5/ 20	5/ 60	25/ 20	25/ 60	0/ 0	0/ 60	25/ 0	5/ 20	5/ 60	25/ 20	25/ 60
Number Examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Kidney														
Dilatation, tubule(s)														
Not Present	6	6	0	0	1	0	0	6	8	0	3	1	0	0
Minimal	4	4	1	9	9	6	5	3	2	5	7	9	4	3
Mild	0	0	9	1	0	4	5	1	0	5	0	0	6	7
Dilatation, pelvis														
Not Present	9	9	7	10	8	4	7	10	10	10	9	10	10	8
Minimal	1	1	3	0	2	6	3	0	0	0	0	0	0	2
Mild	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Prostate														
Inflammation, mixed cell														
Not Present	10	10	10	9	10	9	8	NA	NA	NA	NA	NA	NA	NA
Minimal	0	0	0	0	0	1	2	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA
Stomach, Glandular														
Erosion														
Not Present	9	10	8	10	9	7	6	10	9	9	10	9	8	10
Minimal	1	0	1	0	0	0	4	0	1	0	0	0	2	0
Mild	0	0	1	0	1	3	0	0	0	1	0	1	0	0
Inflammation, acute, submucosa														
Not Present	10	10	8	9	9	6	8	10	10	9	10	9	9	10
Minimal	0	0	2	1	1	4	2	0	0	1	0	1	1	0
Hemorrhage														
Not Present	10	10	8	10	10	7	8	10	10	10	10	10	8	9
Minimal	0	0	2	0	0	3	2	0	0	0	0	0	2	1
Adrenal, Cortex														
Hypertrophy, zona glomerulosa														
Not Present	10	10	1	5	2	1	0	10	9	6	6	4	5	2
Minimal	0	0	8	5	4	6	7	0	1	4	3	3	4	5
Mild	0	0	1	0	4	3	3	0	0	0	1	3	1	3
Pancreas														
Zymogen depletion														
Not Present	10	10	1	0	1	0	0	10	9	2	7	6	2	4
Minimal	0	0	4	8	4	7	5	0	1	8	3	4	8	6
Mild	0	0	5	2	5	3	5	0	0	0	0	0	0	0

NA = Not applicable.

(Table excerpted from sponsor's package and highlighted)

Toxicokinetics

PF-04971729 exposures increased dose-proportionally. Exposures in females tended to be slightly higher, but were not considered to be significant. Exposures were also slightly higher on Day 91 by 12-28% with co-administration, indicating a possible trend for accumulation with high doses of both drugs. T_{max} ranged from 1 to 7 hours postdose, with a trend for delayed T_{max} with high dose co-administration of both drugs (25+60).

Sponsor's Table 7: PF-04971729 Toxicokinetics - 13-week Rat Study #14GR162

6.1. Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
25 / 0	1	Male	7060	4	99100
		Female	10700	4	125000
		Overall	8880	4	112000
	91	Male	8930	4	91200
		Female	9240	4	138000
		Overall	9080	4	114000
5 / 20	1	Male	1920	7	24900
		Female	2350	4	24100
		Overall	1700	4	24000
	91	Male	1400	1	14100
		Female	2970	1	27800
		Overall	2180	1	21000
5 / 60	1	Male	1720	7	23700
		Female	1940	4	25700
		Overall	1800	4	24700
	91	Male	2520	1	21900
		Female	3060	1	27700
		Overall	2790	1	24800
25/20	1	Male	8390	7	120000
		Female	9910	4	120000
		Overall	8930	4	120000
	91	Male	12500	4	157000
		Female	15100	4	150000
		Overall	13800	4	153000
25/60	1	Male	6380	7	89700
		Female	9370	7	131000
		Overall	7870	7	110000
	91	Male	9710	7	122000
		Female	11800	4	124000
		Overall	8240	7	123000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package)

Sitagliptin exposures increased proportionally with dose and were not significantly affected by co-administration of PF-04971729. There were no significant signs of gender effects or accumulation. T_{max} ranged between 1 and 7 hours post-dose.

Sponsor's Table 8: Sitagliptin Toxicokinetics - 13-week Rat Study #14GR162

6.2. Mean Toxicokinetic Parameters for Sitagliptin in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
0 / 60	1	Male	2670	4	27500
		Female	2120	4	19000
		Overall	2400	4	23300
	91	Male	3660	4	37800
		Female	2900	1	22500
		Overall	3140	1	30100
5 / 20	1	Male	778	4	8670
		Female	613	4	5640
		Overall	695	4	7140
	91	Male	853	4	7390
		Female	1000	4	6460
		Overall	929	4	6930
5 / 60	1	Male	3450	7	45300
		Female	2670	4	29300
		Overall	2900	4	37300
	91	Male	4450	1	32700
		Female	3740	1	24500
		Overall	4090	1	28600
25/20	1	Male	382	7	5260
		Female	455	4	5490
		Overall	417	4	5380
	91	Male	1170	4	9050
		Female	807	4	7720
		Overall	989	4	8400
25/60	1	Male	2370	7	28600
		Female	1670	7	22500
		Overall	2020	7	25500
	91	Male	2360	4	26200
		Female	2780	4	22500
		Overall	2570	4	24400

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package)

Dosing Formulation Analysis

PF-04971729 and sitagliptin dose formulations were analyzed using a validated HPLC method. Overall mean concentrations of PF-04971729 and sitagliptin formulations were within ±10% of the target concentrations.

7 Genetic Toxicology

Genetic toxicology studies with the FDC or coadministration of ertugliflozin and sitagliptin have not been conducted, but are not required. Since both ertugliflozin and sitagliptin are not genotoxic; there is not a genotoxic concern with the FDC product.

Ertugliflozin

Based on the weight of evidence, ertugliflozin is not considered to be genotoxic. Ertugliflozin was evaluated for genotoxic potential in a standard battery of valid genotoxicity assays, including in vitro microbial reverse mutation (Ames), in vitro human lymphocyte cytogenetic, and in vivo rat micronucleus assays.

Sitagliptin

There is no evidence of a mutagenic potential for sitagliptin based on in vitro Ames, hepatocyte alkaline elution, and chromosome aberration assays or an in vivo mouse micronucleus induction assay.

8 Carcinogenicity

Combination carcinogenicity studies with the FDC or coadministration of ertugliflozin and sitagliptin have not conducted, but are not required.

Ertugliflozin

Rat and mouse carcinogenicity studies with administration of ertugliflozin alone were reviewed under NDA #209803.

In the 2-year carcinogenicity study conducted in male and female Crl:CD1(ICR) mice, ertugliflozin was administered daily at doses of 5, 15 or 40 mg/kg/day, in accordance with ECAC dosing recommendations. All male groups were terminated during Week 97 and all female groups were terminated during Week 102 due to low survival that was not drug-related. There were no significant drug-related neoplastic findings in male or female mice at any of the doses examined, and the NOAEL for neoplasms was set at the high dose of 40 mg/kg/day (~50x $MRHD_{AUC}$). It is also noted that non-adverse PD-related kidney and bladder findings were considered to be comparable to similar findings observed in shorter toxicology studies.

In the 2-year carcinogenicity study conducted in male and female SD rats, ertugliflozin was administered daily at doses of 1.5, 5, or 15 mg/kg/day ertugliflozin, in accordance with ECAC dosing approval with the exception of exclusion of a saline/water control group. In female rats, there were no statistically significant increases in incidences of benign or malignant neoplasms in any tissues, with a neoplastic NOAEL of 15 mg/kg/day (74x $MRHD_{AUC}$). However, in male rats, drug-related increases in the incidences of adrenal medulla benign pheochromocytoma (PCC) and combined benign + malignant PCC neoplasms were reported at 15 mg/kg/day (66x $MRHD_{AUC}$), resulting in a NOAEL for neoplasms of 5 mg/kg/day (18x $MRHD_{AUC}$). The incidence rates and timing of PCC observations correlated with drug-related increases in adrenal medulla hyperplasia at ≥5 mg/kg/day in a manner that was considered to be consistent with a continuum of tumor development. Thus, the increased incidences of adrenal medulla hyperplasia and PCC observed at ≥5 mg/kg/day were considered possibly drug-related, but were not considered to be unequivocally drug-related.

Sitagliptin

Chronic carcinogenicity studies with sitagliptin were been performed in rats and mice. In rats, increases in the incidence of combined liver adenoma/carcinoma were observed in males and females at 500 mg/kg/day (~62x MRHD), which was considered possibly related to non-genotoxic chronic hepatotoxicity. No drug-related neoplasms were

observed in mice at doses up to 500 mg/kg/day (~72x MRHD). Overall, the carcinogenic risk to humans at clinical doses was considered to be minimal.

9 Reproductive and Developmental Toxicology

Combination reproductive and developmental toxicology studies with the FDC or coadministration of ertugliflozin and sitagliptin have not conducted, but are not required. Based on results from a juvenile toxicology study in rats, ertugliflozin exposure poses a potential risk to human renal development. Thus, the FDC product will also have a potential risk to human renal development and a combination embryonic fetal development (EFD) study is not required, in accordance ICH M3(R2), as a hazard has already been identified for the ertugliflozin component. Therefore labeling for reproductive and developmental hazards of the FDC will be based on each individual drug component. Please see original NDA reviews for ertugliflozin (NDA 209803) and sitagliptin (NDA 21995) for experimental detail.

10 Special Toxicology Studies

No special toxicology studies were conducted the combination.

11 Labeling Review

Only labeling specific to the FDC or the sitagliptin component are captured in this review. Please see the NDA review under #209803 for labeling recommendations regarding the ertugliflozin component.

Section 8 Use in Specific Populations

Section 8.1 Pregnancy

Excerpt 1: Sponsor's Proposed Section 8.1 Text

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to sitagliptin during pregnancy. Health care providers are encouraged to report any prenatal exposure to TRADEMARK by calling the Pregnancy Registry at 1-800-986-8999.

Risk Summary

(b) (4)

(b) (4) TRADEMARK is not recommended during the second and third trimesters of pregnancy.

(b) (4)

In rats and rabbits, sitagliptin doses of 250 and 125 mg/kg, respectively (approximately 30 and 20 times the human exposure at the maximum recommended human dose) did not adversely affect development outcomes of either species.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk

Poorly-controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, spontaneous abortions, preterm delivery, stillbirth, and delivery complications. It can also increase the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Animal Data

Ertugliflozin

In embryo-fetal development studies, ertugliflozin (50, 100 and 250 mg/kg/day) was administered orally to rats on gestation days 6 to 17 and to rabbits on gestation days 7 to 19. Ertugliflozin did not adversely affect developmental outcomes in rats and rabbits at maternal exposures that were (b) (4) (b) (4) the human exposure at the maximum clinical dose of 15 mg/day, based on AUC. At a maternally toxic dose in rats (250 mg/kg/day), lower fetal viability, (b) (4) a higher incidence of a visceral malformation (membranous ventricular septal defect) (b) (4) (b) (4) (b) (4) In the pre- and postnatal development study, decreased postnatal growth (b) (4) (b) (4) were observed in rats administered ertugliflozin gestation day 6 through lactation day 21 at ≥100 mg/kg/day ((b) (4) times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC).

When ertugliflozin was orally administered to juvenile rats from PND 21 to PND 90, increased kidney weight, renal tubule and renal pelvis dilatation, and renal mineralization occurred at doses greater than or equal to 5 mg/kg (13-fold human exposures). These effects did not fully reverse within the 1 month recovery period. (b) (4)

Sitagliptin

Sitagliptin administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) did not adversely affect developmental outcomes at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100 mg/day based on AUC comparisons. Higher doses increased the incidence of rib malformations in offspring at 1,000 mg/kg, or approximately 100 times human exposure at the MRHD. (b) (4) (b) (4)

Sitagliptin administered to female rats from gestation day 6 to lactation day 21 decreased body weight in male and female offspring at 1,000 mg/kg. No functional or behavioral toxicity was observed in offspring of rats.

Placental transfer of sitagliptin administered to pregnant rats was approximately 45% at 2 hours and 80% at 24 hours postdose. Placental transfer of sitagliptin administered to pregnant rabbits was approximately 66% at 2 hours and 30% at 24 hours.

(Excerpted from sponsor's package)

Reviewer's Comments

The following statement under the Animal Data section for sitagliptin was removed " (b) (4) ." Although not necessarily inaccurate, this statement is considered to be unnecessary and is not included in the current 01/2017 label for sitagliptin.

Reviewer's Proposed Section 8.1 Text

Sitagliptin

Sitagliptin administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) did not adversely affect developmental outcomes at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100 mg/day based on AUC comparisons. Higher doses increased the incidence of rib malformations in offspring at 1,000 mg/kg, or approximately 100 times human exposure at the MRHD. (b) (4)

Sitagliptin administered to female rats from gestation day 6 to lactation day 21 decreased body weight in male and female offspring at 1,000 mg/kg. No functional or behavioral toxicity was observed in offspring of rats.

Placental transfer of sitagliptin administered to pregnant rats was approximately 45% at 2 hours and 80% at 24 hours postdose. Placental transfer of sitagliptin administered to pregnant rabbits was approximately 66% at 2 hours and 30% at 24 hours.

Section 8.2 Lactation

Excerpt 2: Sponsor's Proposed Section 8.2 Text

8.2 Lactation

Risk Summary

There is no information regarding the presence of TRADEMARK, (b) (4) in human milk, the effects on the breastfed infant, or the effects on milk production. Ertugliflozin and sitagliptin are present in the milk of lactating rats *[see Data]*. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney, based on data with ertugliflozin.

(b) (4)

Data

Animal Data

Ertugliflozin

The lacteal excretion of radiolabeled ertugliflozin in lactating rats was evaluated 10 to 12 days after parturition. Ertugliflozin derived radioactivity exposure in milk and plasma were similar, with a milk/plasma ratio of 1.07, based on AUC.

Sitagliptin

Sitagliptin is secreted in the milk of lactating rats at a milk to plasma ratio of 4:1.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's animal data regarding sitagliptin are supported by the available data and are considered to be adequate.

Section 12 Clinical Pharmacology

Section 12.1 Mechanism of Action

Excerpt 3: Sponsor's Proposed Section 12.1 Text

12.1 Mechanism of Action

TRADEMARK

TRADEMARK combines two antihyperglycemic agents with complementary mechanisms of action to improve glycemic control in patients with type 2 diabetes: ertugliflozin, a SGLT2 inhibitor, and sitagliptin, a DPP-4 inhibitor.

Ertugliflozin

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Ertugliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, ertugliflozin reduces renal reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion.

Sitagliptin

Sitagliptin is a DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones. Concentrations of the active intact hormones are increased by sitagliptin, thereby increasing and prolonging the action of these hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal. These hormones are rapidly inactivated by the enzyme, DPP-4. The incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells by intracellular signaling pathways involving cyclic AMP. GLP-1 also lowers glucagon secretion from pancreatic alpha cells, leading to reduced hepatic glucose production. By increasing and prolonging active incretin levels, sitagliptin increases insulin release and decreases glucagon levels in the circulation in a glucose-dependent manner. Sitagliptin demonstrates selectivity for DPP-4 and does not inhibit DPP-8 or DPP-9 activity *in vitro* at concentrations approximating those from therapeutic doses.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's proposed text is supported by the nonclinical data and is considered to be acceptable.

Section 12.3 Pharmacokinetics

Excerpt 4: Sponsor's Proposed Section 12.3 Text

Metabolism

Ertugliflozin

Metabolism is the primary clearance mechanism for ertugliflozin. The major metabolic pathway for ertugliflozin is UGT1A9 and UGT2B7-mediated O-glucuronidation to two glucuronides that are pharmacologically inactive at clinically relevant concentrations. CYP-mediated (oxidative) metabolism of ertugliflozin is minimal (12%).

Sitagliptin

Approximately 79% of sitagliptin is excreted unchanged in the urine with metabolism being a minor pathway of elimination.

Following a [^{14}C]sitagliptin oral dose, approximately 16% of the radioactivity was excreted as metabolites of sitagliptin. Six metabolites were detected at trace levels and are not expected to contribute to the plasma DPP-4 inhibitory activity of sitagliptin. *In vitro* studies indicated that the primary enzyme responsible for the limited metabolism of sitagliptin was CYP3A4, with contribution from CYP2C8.

Elimination

Ertugliflozin

The mean systemic plasma clearance following an intravenous 100 µg dose was 11.2 L/hr. The mean elimination half-life in type 2 diabetic patients with normal renal function was estimated to be 16.6 hours based on the population pharmacokinetic analysis. Following administration of an oral [^{14}C]-ertugliflozin solution to healthy subjects, approximately 40.9% and 50.2% of the drug-related radioactivity was eliminated in feces and urine, respectively. Only 1.5% of the administered dose was excreted as unchanged ertugliflozin in urine and 33.8% as unchanged ertugliflozin in feces, which is likely due to biliary excretion of glucuronide metabolites and subsequent hydrolysis to parent.

Sitagliptin

Following administration of an oral [^{14}C]sitagliptin dose to healthy subjects, approximately 100% of the administered radioactivity was eliminated in feces (13%) or urine (87%) within one week of dosing. The apparent terminal $t_{1/2}$ following a 100-mg oral dose of sitagliptin was approximately 12.4 hours and renal clearance was approximately 350 mL/min.

Elimination of sitagliptin occurs primarily via renal excretion and involves active tubular secretion. Sitagliptin is a substrate for human organic anion transporter-3 (hOAT-3), which may be involved in the renal elimination of sitagliptin. The clinical relevance of hOAT-3 in sitagliptin transport has not been established. Sitagliptin is also a substrate of p-glycoprotein, which may also be involved in mediating the renal elimination of sitagliptin. However, cyclosporine, a p-glycoprotein inhibitor, did not reduce the renal clearance of sitagliptin.

Ertugliflozin

In Vitro Assessment of Drug Interactions

In *in vitro* studies, ertugliflozin and ertugliflozin glucuronides did not inhibit CYP450 isoenzymes (CYPs) 1A2, 2C9, 2C19, 2C8, 2B6, 2D6, or 3A4, and did not induce CYPs 1A2, 2B6, or 3A4. Ertugliflozin was not a time-dependent inhibitor of CYP3A *in vitro*. Ertugliflozin did not inhibit UGT1A6, 1A9, or 2B7 *in vitro* and was a weak inhibitor (IC_{50} >39 µM) of UGT1A1 and 1A4. Ertugliflozin glucuronides did not inhibit UGT1A1, 1A4, 1A6, 1A9, or 2B7 *in vitro*. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of drugs eliminated by these enzymes. Ertugliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and is not a substrate of organic anion transporters (OAT1, OAT3), organic cation transporters (OCT1, OCT2), or organic anion transporting polypeptides (OATP1B1, OATP1B3). Ertugliflozin or ertugliflozin glucuronides do not meaningfully inhibit P-gp, OCT2, OAT1, or OAT3 transporters at clinically relevant concentrations. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of concurrently administered medications that are substrates of these transporters.

Sitagliptin

In Vitro Assessment of Drug Interactions

Sitagliptin is not an inhibitor of CYP isozymes CYP3A4, 2C8, 2C9, 2D6, 1A2, 2C19 or 2B6, and is not an inducer of CYP3A4. Sitagliptin is a p-glycoprotein substrate, but does not inhibit p-glycoprotein mediated transport of digoxin. Based on these results, sitagliptin is considered unlikely to cause interactions with other drugs that utilize these pathways.

Sitagliptin is not extensively bound to plasma proteins. Therefore, the propensity of sitagliptin to be involved in clinically meaningful drug-drug interactions mediated by plasma protein binding displacement is very low.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's nonclinical data regarding sitagliptin are supported by the available data, are consistent with the current sitagliptin label (01/2017), and are considered to be adequate.

Section 13 Nonclinical Toxicology

Section 13.1 Carcinogenicity & Mutagenesis & Impairment of Fertility

Excerpt 5: Sponsor's Proposed Section 13.1 Text

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Ertugliflozin

In the (b) (4) mouse (b) (4) study, ertugliflozin was administered by oral gavage at doses of 5, 15, and 40 mg/kg/day. There were no ertugliflozin-related neoplastic findings at doses up to 40 mg/kg/day (approximately (b) (4) times human exposure at the maximum recommended human dose [MHRD] of 15 mg/day based on AUC). In the (b) (4) rat (b) (4) study, ertugliflozin was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg/day. Ertugliflozin-related neoplastic findings included an increased incidence of (b) (4) adrenal medullary pheochromocytoma in male rats at 15 mg/kg/day. This finding (b) (4) to carbohydrate malabsorption leading to altered calcium homeostasis (b) (4) to human risk. The no-observed-effect level (NOEL) for neoplasia was 5 mg/kg/day (approximately 16 times human exposure at the MRHD of 15 mg/day).

Sitagliptin

A two year carcinogenicity study was conducted in male and female rats given oral doses of sitagliptin of 50, 150, and 500 mg/kg/day. There was an increased incidence of combined liver adenoma/carcinoma in males and females and of liver carcinoma in females at 500 mg/kg. This dose results in exposures approximately 60 times the human exposure at the maximum recommended daily adult human dose (MRHD) of 100 mg/day based on AUC comparisons. Liver tumors were not observed at 150 mg/kg, approximately 20 times the human exposure at the MRHD.

A two year carcinogenicity study was conducted in male and female mice given oral doses of sitagliptin of 50, 125, 250, and 500 mg/kg/day. There was no increase in the incidence of tumors in any organ up to 500 mg/kg, approximately 70 times human exposure at the MRHD.

Mutagenesis

Ertugliflozin

Ertugliflozin was not mutagenic or clastogenic with or without metabolic activation in the microbial reverse mutation, *in vitro* cytogenetic (human lymphocytes), and *in vivo* rat micronucleus assays.

Sitagliptin

Sitagliptin was not mutagenic or clastogenic with or without metabolic activation in the Ames bacterial mutagenicity assay, a Chinese hamster ovary (CHO) chromosome aberration assay, an *in vitro* cytogenetics assay in CHO, an *in vitro* rat hepatocyte DNA alkaline elution assay, and an *in vivo* micronucleus assay.

Impairment of Fertility

Ertugliflozin

In the rat fertility and embryonic development study, male and female rats were administered ertugliflozin at 5, 25, and 250 mg/kg/day. No effects on fertility were observed at 250 mg/kg/day (approximately (b) (4) times human exposure at the MRHD of 15 mg/day based on AUC comparison).

Sitagliptin

In rat fertility studies with oral gavage doses of 125, 250, and 1000 mg/kg, males were treated for 4 weeks prior to mating, during mating, up to scheduled termination (approximately 8 weeks total) and females were treated 2 weeks prior to mating through gestation day 7. No adverse effect on fertility was observed at 125 mg/kg (approximately 12 times human exposure at the MRHD of 100 mg/day based on AUC comparisons). At higher doses, nondose-related increased resorptions in females were observed (approximately 25 and 100 times human exposure at the MRHD based on AUC comparison).

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's text regarding sitagliptin is reflected in the most recent sitagliptin label (01/2017) and is considered to be appropriate.

Section 13.2 Animal Pharmacology and/or Toxicology

This section was not included in the sponsor's proposed label.

Reviewer's Comments

This section was also not included in the label for ertugliflozin or sitagliptin. Thus, omission of this section is considered to be acceptable.

11 Integrated Summary and Safety Evaluation

This review evaluates the nonclinical safety profile of the NME ertugliflozin FDC with sitagliptin submitted by Merck Sharp and Dohme Corp for the treatment of T2DM. The non-clinical pharmacology, general toxicology, carcinogenicity, reproductive and developmental, and special toxicology studies establishing the safety profile of the NME alone were fully evaluated under NDA #209803, but were also submitted in support of the FDC product under NDA #209805. The approved label for Januvia® (sitagliptin) is also referenced.

Ertugliflozin inhibits SGLT2 resulting in significant glucosuria, which is associated with concomitant decreases in plasma glucose levels despite compensatory increases in food consumption. Sitagliptin suppresses glucagon release and increases insulin secretion; leading to normalization of blood glucose levels. Thus, although both ertugliflozin and sitagliptin reduce blood glucose levels, they act through different mechanisms.

Based on pathways of elimination, absorption and metabolization, significant DFI on PK parameters between ertugliflozin and sitagliptin are not anticipated. Furthermore, concomitant administration of ertugliflozin and sitagliptin were not associated with significant changes in drug exposures in humans.

Based on safety pharmacology studies, both ertugliflozin and sitagliptin may be associated with some concern for CV effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. However, no CV-related toxicities were observed in the combination toxicology studies. Thus, further evaluation of the FDC product in nonclinical safety pharmacology studies is not warranted.

In accordance with ICH guidelines, the sponsor submitted a GLP-compliant 3-month combination toxicology study with coadministration of ertugliflozin and sitagliptin in rats. The majority of the drug-related findings were attributed to PD-related inhibition of SGLT2 mediated by ertugliflozin. Ertugliflozin PD-related kidney tubule dilatations and increased organ weights were not associated with indications of kidney dysfunction or toxicity and were considered likely to be non-adverse. It is noted that similar ertugliflozin-related findings were observed with coadministration of metformin (reference NDA #209806); however, the degree of glucose excursion was lower in animals receiving coadministration of sitagliptin compared to those being co-administered metformin. The DPP4 inhibitor drug class has been associated with decreases in glucose excursion, which may partially counteract the PD activity of ertugliflozin to some extent.

Potential additive effects of ertugliflozin and sitagliptin coadministration were observed in the stomach and adrenal gland. Potential combination-related increases in the incidences of stomach discoloration and minimal to mild erosion/ulcer were observed in male rats. In female rats, potential combination-related increases in incidences of stomach hemorrhage were reported. Since both ertugliflozin and sitagliptin have been associated with stomach findings in previous nonclinical toxicology studies, it's likely that exacerbation of the stomach findings is due to additive toxicities of both PF-04971729 and sitagliptin. However, the stomach findings were of low severity and were not considered to be adverse. Furthermore, since off-target SGLT1 inhibition is not likely in humans, additive ertugliflozin-related effects on the stomach and digestive tract are not likely to be observed clinically. Increases in adrenal gland weights and hypertrophy were considered likely related to additive effects of ertugliflozin and sitagliptin, but were non-adverse. Overall, there were no toxicologically significant adverse synergistic toxicities due to coadministration of ertugliflozin and sitagliptin. Thus, the NOAEL was set at the high combination dose of 25 mg/kg ertugliflozin and 60 mg/kg sitagliptin, with safety margins of 89x $MRHD_{AUC}$ for a high dose of 15 mg/day ertugliflozin 9x $MRHD_{AUC}$ for a high dose of 100 mg/day sitagliptin.

Observations of ertugliflozin-related ketonuria in rats correlate with reductions in body weights and may be secondary to ertugliflozin PD-related inhibition of carbohydrate absorption and decreases in glucose levels, which may be consistent with non-adverse nutritional ketosis. Although ertugliflozin-mediated ketonuria is non-adverse in rats, given that diabetic ketoacidosis (DKA) has been observed clinically in diabetic patients treated with SGLT2 inhibitors, this finding is notable. In the 13-week rat combination toxicology study, improvement of ketonuria was observed with coadministration of sitagliptin in both males and females.

In summary, the 13-week coadministration toxicology study in rats adequately bridges the proposed FDC to the ertugliflozin nonclinical studies and did not identify and new clinically relevant, toxicologically significant toxicities or interactions. Since the safety margins for coadministration of ertugliflozin (111x MRHD) and sitagliptin (9x MRHD) in the 13-week rat study are sufficient, the nonclinical data support clinical dosing of the FDC product at ertugliflozin doses up 15 mg/day ertugliflozin and sitagliptin doses up to 100 mg/day.

FDC Toxicology Summary Table

Table 9: Ertugliflozin + Sitagliptin Coadministration Human Safety Margins

Study	NOAEL (mg/kg)	Human Safety Margin (Based on AUC*)	Findings
2 Week (Non-GLP) **Ertugliflozin/Sitagliptin:** 5/20, 5/60, 25/20, 25/60, 25/0, & 0/60 mg/kg Ertugliflozin AUC: 27.5, 24.2, 133, 132, 163, & - µg·h/mL Sitagliptin AUC: 7.61, 26.7, 7.65, 26.8, -, & 30.6 µg·h/mL	Ertugliflozin / Sitagliptin **25 / 60**	Ertugliflozin: **56x** Sitagliptin: **7x**	*No significant adverse effects.* **5 / 20 mg/kg (20x/3x MRHD):** ↑food consumption, Blood (↓glucose, ↑BUN, ↓Ca), urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight, **5 / 60 mg/kg (18x/10x MRHD):** ↑food consumption, Blood [↓glucose, ↑BUN, ↓protein, <2-fold ↑AST, <2-fold ↑ALT, ↓Ca], urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight, **25 / 20 mg/kg (96x/3x MRHD):** ↑food, Blood (↓glucose, ↑BUN, ↓Cl, ↓Ca), urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight), pancreas (↓zymogen) **25 / 60 mg/kg (96x/10x MRHD):** ↑food, Blood (↓glucose, ↑BUN, ↓Cl, <2-fold ↑AST, <2-fold ↑ALT, ↓Ca), urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight, tubule mineralization in 2 females), pancreas (↓zymogen) **25 / 0 mg/kg (118x/- MRHD):** ↑food consumption, Blood (↓glucose, ↑BUN, ↓protein, ↓Cl, ↓Ca) urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight), pancreas (↓zymogen) **0 / 60 mg/kg (-/11x MRHD):** Blood (↓protein (♀)

Study	NOAEL (mg/kg)	Human Safety Margin (Based on AUC*)	Findings
13 Week (GLP) **Ertugliflozin/Sitagliptin:** 5/20, 5/60, 25/20, 25/60, 25/0, & 0/60 mg/kg Ertugliflozin AUC: 21, 24.8, 153, 123, 114, & - µg·h/mL Sitagliptin AUC: 6.93, 28.6, 8.4, 24.4, -, & 30.1 µg·h/mL	Ertugliflozin / Sitagliptin **25 / 60**	Ertugliflozin: **66x** Sitagliptin: **5x**	*No significant adverse effects.* **5 / 20 mg/kg (15x/2x MRHD):** ↓Body weight, ↑Food consumption, Blood (↓Cl, ↓glucose, <2-fold ↑BUN, <2-fold ↑ALT), urine [glucosuria, ↑specific gravity (♀), ↑volume (♂)], kidney [↑weight, minimal-mild tubule dilatation, mild pelvis dilatation (1♀)], stomach minimal inflammation (1♂), pancreas (↓zymogen), adrenal hypertrophy **5 / 60 mg/kg (18x/10x MRHD):** ↓Body weight, ↑Food consumption, Blood [↓Cl, ↓glucose, ↓cholesterol (♀),<2-fold ↑BUN, <2-fold ↑ALT], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂)], kidney (↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation (♂)], stomach [mild erosion (1♂), minimal inflammation], pancreas (↓zymogen), adrenal hypertrophy **25 / 20 mg/kg (110x/3x MRHD):** ↓Body weight, ↑Food consumption, Blood (↓glucose, ↓Cl, ↓Ca, <2-fold ↑BUN, <2-fold ↑ALT), urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], stomach (discolored, minimal-mild erosion, minimal inflammation, minimal hemorrhage), kidney [↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation (♂)], pancreas (↓zymogen), adrenal hypertrophy **25 / 60 mg/kg (89x/9x MRHD):** ↓Body weight, ↑Food consumption, Blood [↓glucose, ↓Cl, ↓Ca, ↓protein (♀), ↓cholesterol (♀),<2-fold ↑BUN, <2-fold ↑ALT], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], stomach [discolored, minimal-mild erosion (♂), minimal inflammation, minimal hemorrhage], kidney (↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation), adrenal (↑weight, hypertrophy), pancreas (↓zymogen) **25 / 0 mg/kg (83x/0x MRHD):** ↑↓Body weight, ↑Food consumption, Blood [↓ Cl, ↓Ca (♀), ↓glucose, ↓protein (♀), ↓cholesterol (♀),<2-fold ↑BUN, <2-fold ↑ALT], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], kidney (↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation (♂)], pancreas (↓zymogen), stomach [discolored, minimal-mild erosion, minimal inflammation, minimal hemorrhage (♂)], adrenal hypertrophy **0 / 60 mg/kg (0x/11x MRHD):** Adrenal hypertrophy (1♀), pancreas ↓zymogen (1♀)

* Based on a maximum once daily dose of 15 mg ertugliflozin / 100 mg sitagliptin with a predicted ertugliflozin exposure of $AUC_{0\text{-}24}$ = 1.38 µg·h/mL and sitagliptin exposure of AUC = 2.81 µg·h/mL

♂ = Males only
♀ = Females only

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

/s/

JESSICA HAWES
12/14/2017

RONALD L WANGE
12/14/2017
I concur with Dr. Hawes' recommendation for approval.

Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 209803 (ertugliflozin); 209805 (ertugliflozin and sitagliptin); 209806 (ertugliflozin and metformin)
Agency receipt date: December 19, 2016
Drug: ertugliflozin alone and in fixed dose combinations with sitagliptin or metformin
Sponsor: Merck Sharpe and Dohme Corp.

Indication: Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of ertugliflozin alone or in a fixed dose combination product with either metformin, an antihyperglycemic agent, or sitagliptin, an approved dipeptidyl peptidase inhibitor, for the indication listed above.

The recommended pharmacologic class for ertugliflozin is a sodium glucose co-transporter 2 (SGLT2) inhibitor. Other approved members of this class include canagliflozin, empagliflozin, and dapagliflozin. These approved SGLT2 inhibitors are also approved in combination with metformin, or with saxagliptin (dapagliflozin) and linagliptin (empagliflozin).

A complete nonclinical program was conducted by the sponsor to support approval of ertugliflozin. Ertugliflozin elicited expected pharmacological responses in the species evaluated. The characterized toxicity profile relates to a great degree to the pharmacodynamic response related to glucosuria and osmotic diuresis secondary to SGLT2 inhibition. Key findings included effects on the renal system in all species evaluated, disrupted calcium homeostasis, and increased incidence of neoplasms (adrenal medulla benign pheochromocytoma (PCC) and combined benign and malignant PCC neoplasms). Gastrointestinal system and bone effects were also observed and were likely due to off-target inhibition of SGLT1, which is of low concern in humans. In all cases, the findings were either of relatively low severity, associated with a no-observed-adverse-effect-level providing acceptable exposure margins compared to the maximum recommended dose of 15 mg, or not identified during the clinical program. In all, they were not considered to represent significant clinical safety risks. The sponsor conducted additional studies to demonstrate that significant human metabolites and degradation products in the formulation were not associated with any additional clinically relevant findings. Ertugliflozin tested negatively in a battery of genetic toxicity studies.

In a battery of reproductive toxicity studies, no effects on fertility were observed and drug-related fetal effects were observed only at doses associated with maternal toxicity and with sufficient exposure margins associated with no effect levels. Ertugliflozin was observed in the milk of lactating rats following drug administration. Ertugliflozin may

present a potential clinical risk to renal development and maturation during the second and third trimester based on irreversible findings in a juvenile rat toxicology study, similar to other drugs in this class.

The sponsor conducted 13-week toxicology studies in rats to support the safety of the proposed fixed dose combinations with the previously approved drugs, metformin and sitagliptin. Co-administration studies demonstrated that dosing was well tolerated and not associated with significant adverse toxicities and with adequate margins of safety compared to the maximum recommended clinical doses of 15 mg ertugliflozin, 100 mg sitagliptin, and 2000 mg metformin. No new clinically relevant or synergistic toxicities were observed.

Conclusion:
I agree with the Division pharmacology/toxicology conclusions that ertugliflozin, as a single drug product or as a fixed dose combination with either metformin or sitagliptin, can be approved from the nonclinical perspective. I have reviewed the proposed wording for the nonclinical sections of the three product labels and agree with the Division recommendations.

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

/s/

TIMOTHY J MCGOVERN
12/11/2017

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	209805
Supporting document/s:	SDN 1
Applicant's letter date:	12/19/2016
CDER stamp date:	12/19/2016
Product:	Ertugliflozin and Sitagliptin
Indication:	Type 2 Diabetes Mellitus
Applicant:	Merck Sharpe and Dohme Corp
Review Division:	DMEP
Reviewer:	Jessica J. Hawes, Ph.D.
Supervisor/Team Leader:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Elizabeth Godwin

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209805 are owned by Merck Sharpe and Dohme Corp. are data for which Merck Sharpe and Dohme Corp. has obtained a written right of reference.
Any information or data necessary for approval of NDA 209805 that [name of applicant] 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 reflected 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 2098005.

TABLE OF CONTENTS

Table of Tables

Table of Sponsor's Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Merck Sharp and Dohme Corp. has submitted NDA application packages for the new molecular entity (NME) Ertugliflozin (PF-04971729, MK-8835) alone (NDA #209803, IND #106447) and as fixed dose combination (FDC) products with the marketed drugs Metformin (MK-8835B, NDA #209806, IND #122329) and Sitagliptin (MK-8835A, NDA #209805, IND #122330) for the treatment of type 2 diabetes mellitus (T2DM).

The nonclinical profile for the NME, ertugliflozin, was fully evaluated in the Pharm/Tox review under NDA #209803. This review focuses on evaluation of additional information pertinent to the ertugliflozin + sitagliptin hydrochloride FDC product.

1.2 Brief Discussion of Nonclinical Findings

Coadministration of ertugliflozin and sitagliptin in rats for 13-weeks was generally well-tolerated with sufficient margins of safety and was not associated with significant adverse systemic toxicities. Furthermore, no new clinically relevant or synergistic adverse toxicities due to coadministration of PF-04971729 and sitagliptin were observed. Thus, the rat combination toxicology study adequately bridges the proposed FDC product to the ertugliflozin nonclinical safety profile under NDA #209803, with sufficient safety margins based on AUC exposures at the maximum recommended high doses ($MRHD_{AUC}$) of 15 mg/day ertugliflozin (89x $MRHD_{AUC}$) and 100 mg/day sitagliptin (9x $MRHD_{AUC}$). Overall, the nonclinical data were considered to be sufficient and support clinical dosing of the FDC product at ertugliflozin doses up 15 mg/day ertugliflozin and sitagliptin doses up to 100 mg/day.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of the ertugliflozin/sitagliptin FDC

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Nonclinical labeling recommendations are below. See Section 11 Labeling Review for a full discussion of proposed changes. Only labeling specific to the FDC or the sitagliptin component are captured in this review. Please see the NDA review under #209803 for labeling recommendations regarding the ertugliflozin component.

Only one minor edit specific for sitagliptin was recommended (Section 8.1).

Section: 8 USE IN SPECIFIC POPULATIONS

Section 8.1 Pregnancy

Sitagliptin

Sitagliptin administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) did not adversely affect developmental outcomes at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100 mg/day based on AUC comparisons. Higher doses increased the incidence of rib malformations in offspring at 1,000 mg/kg, or approximately 100 times human exposure at the MRHD. (b) (4)

Sitagliptin administered to female rats from gestation day 6 to lactation day 21 decreased body weight in male and female offspring at 1,000 mg/kg. No functional or behavioral toxicity was observed in offspring of rats.

Placental transfer of sitagliptin administered to pregnant rats was approximately 45% at 2 hours and 80% at 24 hours postdose. Placental transfer of sitagliptin administered to pregnant rabbits was approximately 66% at 2 hours and 30% at 24 hours.

2 Drug Information

2.1 Drug

CAS Registry Number

Ertugliflozin: 1210344-57-2
Sitagliptin: 654671-77-9

Generic Name

Ertugliflozin + sitagliptin

Code Name

Ertugliflozin + sitagliptin FDC: MK-8835A

Ertugliflozin: PF-04971729, MK-8835
Ertugliflozin L-pyroglutamic acid (L-PGA) co-crystal form: PF-04971729 (b) (4)

It is noted that the neutral amorphous form was used for most exploratory toxicology studies, whereas the L-PGA co-crystalline form intended for marketing was used in pivotal toxicology and safety pharmacology studies.

Sitagliptin: MK-0431, L-000224715-010X, sitagliptin phosphate

Chemical Name

PF-04971729: (1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol

PF-04971729 (b) (4): (1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol L-pyroglutamic acid

Sitagliptin: 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-α]pyrazine phosphate (1:1) monohydrate

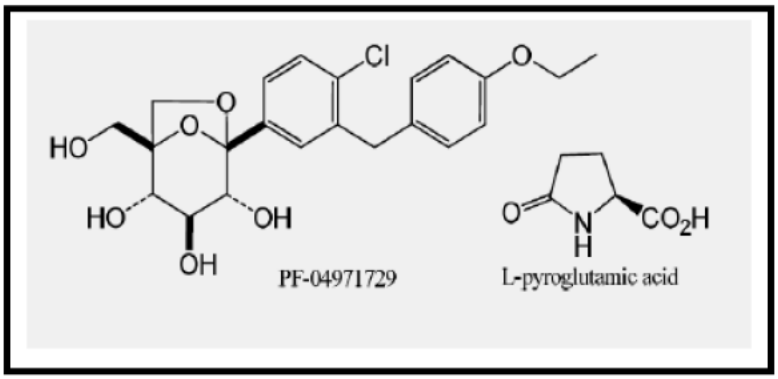
Molecular Formula/Molecular Weight

PF-04971729: C22H25ClO7 / 436.88 g/mol
PF-04971729 (b) (4): C27H32ClNO10 / 566.00 g/mol

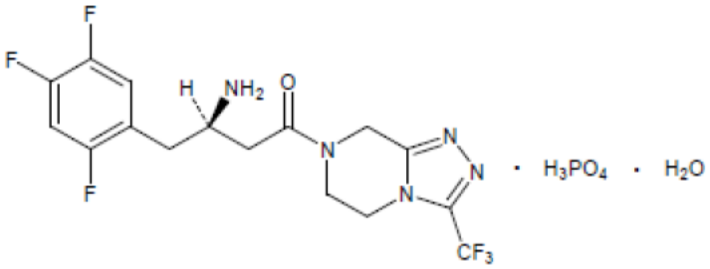
Sitagliptin: C16H15F6N5O / 407.32 g/mol
Sitagliptin phosphate monohydrate salt: C16H15F6N5O ● H3PO4 ● H2O / 523.32 g/mol

Structure or Biochemical Description

Ertugliflozin L-PGA



Sitagliptin



Pharmacologic Class

Ertugliflozin: Sodium glucose co-transporter 2 (SGLT2) Inhibitor
Sitagliptin: Dipeptidyl peptidase-4 (DPP-4) inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA #209803 (IND #106447):	MK-8836 Ertugliflozin, Merck Sharp and Dohme Corp
NDA #209806 (IND #122329):	MK-8835**A** (Ertugliflozin + metformin FDC), Merck Sharp and Dohme Corp
NDA #21995:	Januvia (Sitagliptin), Merck Sharp and Dohme Corp

2.3 Drug Formulation

The ertugliflozin/sitagliptin FDC will be formulated as film coated tablet in 4 strengths: (b) (4) (b) (4) 5 mg ertugliflozin + 100 mg sitagliptin, (b) (4) and 15 mg ertugliflozin + 100 mg sitagliptin. The following two tables are representative of the formulations for all 4 strengths.

Sponsor's Table 1: Ertugliflozin/Sitagliptin FDC Tablet Formulation Composition

Table 1 Ertugliflozin (b) (4) Tablet Composition

Component	Quality Reference	Function	Amount per tablet (mg)
Core Tablet			
Ertugliflozin L-PGA*	In-house	Active	(b) (4)
Sitagliptin Phosphate Monohydrate†	In-house	Active	
Cellulose, Microcrystalline	USP-NF	(b) (4)	
Dibasic Calcium Phosphate, Anhydrous	USP-NF		
Croscarmellose Sodium	USP-NF		
Sodium Stearyl Fumarate	USP-NF		
Magnesium Stearate	USP-NF		
		Core Tablet Weight	
Film Coat			
			(b) (4)
Carnauba Wax	USP-NF		(b) (4)
			(b) (4)
Hypromellose	USP-NF		(b) (4)
Hydroxypropyl Cellulose	USP-NF		
Titanium Dioxide	USP-NF		
Iron Oxide Red	USP-NF		
Iron Oxide Yellow	USP-NF		
Ferrosoferric Oxide/Black Iron Oxide	USP-NF		

(Tables excerpted from sponsor's package)

2.4 Comments on Novel Excipients

All excipients are compendial and controlled at acceptable levels.

2.5 Comments on Impurities/Degradants of Concern

Ertugliflozin-related impurities and degradants were qualified under NDA #209803.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adults with T2DM.

The sponsor's recommended starting dose is 5 mg ertugliflozin/100 mg sitagliptin once daily with or without food. The sponsor recommends that the dose may be increased to a maximum dose of 15 mg ertugliflozin/100 mg sitagliptin once daily if additional glycemic control is needed.

Sponsor's Maximum Recommended Human Dose:

FDC: Once daily dose of 15 mg ertugliflozin and 100 mg sitagliptin

- **Total: 15 mg/day ertugliflozin + 100 mg/day sitagliptin**
  - Ertugliflozin: * **AUC = 1.38 μg·h/mL**, C_{max} = 266 ng/mL ≈ 0.6 μM
    *Based on the clinical population pharmacokinetic (PK) analysis
  - Sitagliptin: ****AUC_{0-24} = 6.9 μM·h = 2.81 μg·h/mL**, C_{max} = 805 nM
    ** Based on study #PB022/1033 (CSR, Table 12)

Ertugliflozin: The proposed MRHD under NDA #209803 for the NME alone is also 15 mg/day.

Sitagliptin: Approved maximum daily dose of Sitagliptin is 100 mg once daily

2.7 Regulatory Background

- An IND for Ertugliflozin was originally submitted as PF04971729 in September 2009.
- On 4/14/2014, the sponsor submitted a Type B meeting request and a pre-IND package for the FDC Ertugliflozin + Sitagliptin product. On 4/22/2014, a Pre-IND/Type B meeting was granted with written responses sent to the sponsor on 6/12/2014. Within the pre-IND package, the sponsor submitted 3 clinical questions, but no non-clinical questions.
- On 7/30/2014, the sponsor submitted and cross-referenced the new IND #122330 for the FDC product MK-8835A containing ertugliflozin and sitagliptin for the treatment of T2DM.
- On 3/25/2015, the sponsor submitted a meeting request to discuss a revised clinical pharmacology and biopharmaceutics plan and written responses were sent on 6/8/2015
- Pediatric study plan (PSP) written responses were provided on 7/2/2015 and a PSP initial agreement was provided on 8/20/2015.
- On 7/6/2016, the sponsor submitted a Type-B Pre-NDA meeting request. A pre-NDA meeting was held on 9/6/2016. Two nonclinical questions were submitted,

but not discussed at the meeting. The sponsors was informed via written responses that the nonclinical safety package was adequate to support filing of the NDA, but that conclusions regarding the carcinogenicity findings were a matter of review. A total of 13 additional questions were discussed and/or addressed, but were not nonclinical.

Ertugliflozin

- Ertugliflozin was originally submitted as PF04971729 under IND #106447 in September 2009.
- And NDA package for ertugliflozin as an NME alone (non-FDC) drug formulation was submitted at the same time as the ertugliflozin/metformin FDC NDA on 12/19/2016.

Sitagliptin

Sitagliptin was approved under NDA #21995 as Januvia® (Merck & Co., Inc.) on 10/16/2006 as an adjunct to diet and exercise to improve glycemic control in adults with T2DM with a maximum approved adult dose set at 100 mg/day. Sitagliptin has been prescribed for treatment of type II diabetes in patients worldwide for 11 years. The label for sitagliptin was updated in January 2017.

3 Studies Submitted

3.1 Studies Reviewed

All nonclinical studies for ertugliflozin were submitted and reviewed under NDA#209803 and IND #106447. All nonclinical coadministration studies have been previously reviewed under IND #106447 and #122330.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

A preliminary 2-week combination toxicology study studies was reviewed in Pharmacology and Toxicology (Pharm/Tox) review #1 under IND #122330 by Dr. David Carlson. A pivotal 13-week combination toxicology studies was reviewed in detail in Pharm/Tox review #7 under IND #106447 by Dr. Jessica Hawes. Summaries of these studies are included in this review.

Table 1: Summaries of Pivotal Previously-Reviewed Nonclinical Studies

Combination Toxicology		
Study #	**Brief Title**	**Primary Review**
TT147809 ([(b) (4)] #8294467, Pfizer #13GR342)	2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats	IND #122330 Pharm/Tox review #1, Dr. Carlson, 8/28/2014
TT147808 ([(b) (4)] #8300338, Pfizer #14GR162)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats **(GLP)**	IND #106447 Pharm/Tox review #7, Dr. Hawes, 12/3/2015

Brief summaries of nonclinical studies for sitagliptin were based on the Pharm/Tox review by Dr. Bourcier for Januvia under NDA #21995.

4 Pharmacology

4.1 Primary Pharmacology

Ertugliflozin is an inhibitor of SGLT2, thereby blocking the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells and resulting in significant glucosuria. Ertugliflozin is highly selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

Sitagliptin is a competitive inhibitor of the DPP-4 enzyme that functions to digest the gastrointestinal hormone incretins GLP-1 and GIP, which are released in response to a meal. Thus, sitagliptin inhibits inactivation of GLP-1 and GIP, thereby resulting in increases the secretion of insulin and suppressed glucagon release by pancreatic alpha cells.

Drug activity related to proposed indication:
Ertugliflozin administration in rats results in concentration-dependent glucosuria, which is directly related to the pharmacodynamic (PD) activity of SGLT2 inhibition. Ertugliflozin administration in rats is associated with concomitant decreases in plasma glucose levels despite compensatory increases in food consumption. Glucosuria has also been reported in humans.

Sitagliptin suppresses glucagon release and increases insulin secretion; leading to normalization of blood glucose levels. Sitagliptin has also been shown to lower HbA1c levels in human.

4.2 Secondary Pharmacology

Ertugliflozin

Nonclinical secondary pharmacology studies for ertugliflozin were fully evaluated in the Pharm/Tox review by Dr. Hawes under NDA #209803.

Briefly, significant drug-drug interactions (DDI) with ertugliflozin administration and drugs metabolized by cytochrome P450 (CYP) enzymes or transported by organic anion

transporters (OATs), organic cation transporters (OCTs) or organic anion transporting polypeptides (OATPs) are not likely at clinical exposures. Significant DDI with diphosphate-glucuronosyltransferase (UGT) enzyme inhibition is also unlikely at clinical concentrations.

Sitagliptin

Sitagliptin is metabolized by CYP3A4 and CYP2C8 enzymes; thus, concomitant use of sitagliptin with drugs that interfere with CYP3A4 and/or CYP2C8 may lead to increases in systemic sitagliptin exposures.

Ertugliflozin/Sitagliptin FDC

Drug-drug interactions between ertugliflozin and sitagliptin are not anticipated.

Ertugliflozin and sitagliptin are predominantly eliminated by different mechanisms and are not expected to affect each other's elimination pathways. Ertugliflozin is predominantly metabolized by UGT1A9 and UGT2By, with minor contributions by CYP3A4, and even less by CYP3A5 and CYP2C8. Sitagliptin is predominantly eliminated via filtration at the glomerulus and excreted in the urine unchanged, accounting for 79% of the dose, with the remaining being eliminated via hepatic metabolism by CYP3A4 and CYP2C8. Although both drugs are partially metabolized by CYP2C8, the role of CYP2C8 metabolism for sitagliptin is minor and very minor for ertugliflozin; thus, competition for CYP2C8-mediated metabolism is unlikely to occur or lead to changes in PK parameters of either drug.

Ertugliflozin does not exhibit reversible or time-dependent inhibition of CYP3A4 or CYP2C8 in human liver microsomes (HLMs) in vitro, with IC_{50} values of >30 μM, which is at least 50-fold higher than clinical ertugliflozin C_{max} concentrations (0.6 μM). The ertugliflozin metabolites M5a and M5c also do not inhibit CYP3A4 or CYP2C8 enzymes. Thus, ertugliflozin and its disproportional metabolites are not likely to interfere with sitagliptin metabolism at clinical exposure levels.

Sitagliptin does not inhibit or induce metabolizing enzymes involved in ertugliflozin metabolism; thus sitagliptin is not anticipated to affect ertugliflozin exposures. Furthermore, since sitagliptin plasma protein binding is relatively low (38%), it is less likely to interact with highly protein-bound drugs, such as ertugliflozin.

4.3 Safety Pharmacology

Both ertugliflozin and sitagliptin may be associated with some concern for cardiovascular (CV) effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. Given that the margins of safety for cardiovascular effects are sufficient for each drug alone and a DDI is not likely, the margins of safety for the FDC product is considered to be sufficient as well.

Ertugliflozin

Standard CV, neurological and pulmonary safety pharmacology studies were completed for ertugliflozin under IND #106447 and NDA #209803.

Central Nervous System (CNS): At 500 mg/kg ertugliflozin in male rats, drug-related decreases in average body temperature of 0.4°C, and 30-40% decreases in locomotor activity, were observed at C_{max} exposures approximately 339-fold higher than clinical C_{max} exposure at the maximum recommended high dose ($MRHD_{Cmax}$) of 15 mg/day. The no observed adverse effect level (NOAEL) for CNS effects in rats was set at 25 mg/kg, which is associated with a safety margin of ~36x $MRHD_{Cmax}$.

Cardiovascular System: Ertugliflozin weakly inhibited the human ether-a-go-go-related gene (hERG) potassium channel in vitro with an IC_{50} of 59 μM and an IC_{20} of 8.11 μM in CHO cells, but was a poor inhibitor in human embryonic kidney (HEK293) cells with an IC_{50} value of >300 μM. Ertugliflozin also weakly inhibited Nav1.5 currents with an IC_{50} of 188 μM. Although significant inhibition of hERG and Nav1.5 currents were reported at concentrations ≥30 μM (50x $MRHD_{Cmax}$), significant hERG or Nav1.5 inhibition is not anticipated at biologically relevant exposure levels. In dogs, single doses of 50 mg/kg ertugliflozin (163x $MRHD_{Cmax}$) were associated with moderate decreases in the QTc interval, cardiac contractility, and heart rate corresponding with T_{max}, as well as increases in systolic blood pressure (sBP) and lengthening of the PR interval, with a NOAEL of 5 mg/kg and a safety margin of ~13x $MRHD_{Cmax}$. In the 27-day pair-fed study #PD001 in spontaneous hypertensive rats (SHR), ertugliflozin-related decreases in blood pressures and heart rate were associated with treatment-related diuresis and activation of the renin-angiotensin-aldosterone-system (RAAS) at 36 mg/kg/day (11x $MRHD_{Cmax}$). Furthermore, based on similar effects observed with a diuretic positive control anti-hypertensive, it's likely that ertugliflozin-related CV effects in the SHR model are, at least in part, secondary to PD-related diuresis.

Respiratory System: In rats, dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed for up to 120 minutes post-dose and correlated with C_{max} at doses of ≥25 mg/kg (~36x $MRHD_{Cmax}$), with a NOAEL of 5 mg/kg (9x $MRHD_{Cmax}$).

Supplemental

Renal/Urinary System: No specific renal safety pharmacology studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes increased urinary glucose excretion and kidney alterations in rats and dogs at clinical exposure levels.

Gastrointestinal System: No GI-specific safety studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs.

Immunotoxicity: There were no indications of immunotoxicity or antigenicity in repeat-dose toxicology studies.

Sitagliptin

Standard CV, neurological and pulmonary safety pharmacology studies for sitagliptin were reviewed under NDA #21995, and are summarized below.

Neurological: No drug-related CNS effects were identified in rat or mouse CNS safety pharmacology studies with functional observational battery (FOB) assessments of CNS activity at doses up to 180 mg/kg in rats and 100 mg/kg in mice. The no observed effect level (NOEL) for neurological effects is >180 mg/kg in rats and >100 mg/kg in mice.

Cardiovascular: Sitagliptin inhibits hERG activity with an IC_{50} of 147 μM, an IC_{20} of ~50 μM, and complete inhibition at 1000 μM, with 80% reversibility. In anesthetized dogs, decreases in blood pressure (↓56 mm Hg) and heart rate (↓40 bpm) were observed with IV infusions of 30 mg/kg and plasma concentrations of 202 μM (253x $MRHD_{Cmax}$), which is associated with a NOAEL of 10 mg/kg (plasma levels ≤59 μM = 74x $MRHD_{Cmax}$). In conscious telemetered dogs, an oral dose of 50 mg/kg (1-hour postdose plasma level of 34 μM = 43x $MRHD_{Cmax}$) was associated with an increase in heart rate (↑30 bpm) and shortening in PR interval in 75% of animals, with a NOAEL of 10 mg/kg (1-hour postdose plasma levels of 7 μM = 9x $MRHD_{Cmax}$). No drug-related changes in QT or other ECG interval were reported. Sitagliptin-related CV risks were evaluated in the CV outcome trial TECOS, wherein the sponsor reported that there was not a drug-related increase in risk of major adverse CV events or the risk of hospitalization for heart failure.

Pulmonary: No meaningful effects on pulmonary parameters were reported in rats at oral doses up to 180 mg/kg. In anesthetized dogs, no changes in respiratory parameters were observed at an IV dose of 10 mg/kg; however, decreases in blood pressure and increases in heart rate of ≤15 minutes were observed.

Renal: No consistent changes in renal function parameters including glomerular filtration rate, effective renal plasma flow, electrolyte excretion, plasma electrolyte concentrations, and filtration fraction were reported in dogs at oral doses up to 10 mg/kg.

Gastrointestinal: No significant effects on GI motility, basal gastric acid secretion, or gastrin-stimulated gastric acid output were reported in dogs at 10 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Ertugliflozin

Ertugliflozin PK parameters were characterized in human, dog, rat, and mouse species. Ertugliflozin protein binding is high in all 4 species, ranging from 92 to 97%. Significant species differences in absorption were associated with oral bioavailability ranging from moderate to high across species and oral absorption ranges of 75-87% in mice, 56-88%

in rats, 94-97% in dogs, and up to 100% in humans. T_{max} is achieved within 30 minutes in mice, 0.7 to 2.3 hours in rats, and 0.8 to 1.5 hours in dogs. In humans, T_{max} is achieved after 1 hour in humans (fasted), but after 2 hours in humans in the fed state, indicating absorption delays in the presence of food. Systemic exposures follow linear pharmacokinetics with a trend for slight increases in female exposures over time at high doses in rodents, indicating a potential gender effect which is likely related to gender differences in metabolism in rodents. Ertugliflozin has a moderate half-life ($t_{1/2}$) of 3 to 4 hours in rodents and 8 hours in dogs, but is 1.5 to 4 times longer in humans ranging from 12 to 18 hours.

Ertugliflozin may be a substrate for the efflux transporter permeability glycoprotein 1/multidrug resistance protein 1 (P-gp/MDR1), but is not affected by P-gp/MDR1 inhibitors; thus, P-gp/MDR1 is unlikely to be a limiting factor in Ertugliflozin absorption. Ertugliflozin has a moderate volume of distribution in rats with preferential distribution into plasma relative to red blood cells. The highest distribution is primarily to organs responsible for drug metabolism and elimination, such as the bladder, liver, and kidney. Ertugliflozin is also highly distributed to rat adrenal gland, Harderian gland, and pancreas. Ertugliflozin crosses the adult blood:brain barrier, but only reaches concentrations 3 to 63-fold lower than that of blood; whereas distribution to the choroid plexus and pituitary gland is 2-fold greater than blood. In fetal rats, ertugliflozin more readily crosses the blood:brain barrier, resulting in significantly more drug exposure to fetal CNS tissues and eyes than in corresponding adult tissues relative to plasma levels. Ertugliflozin is excreted in rat milk at exposures comparable to maternal plasma levels. Ertugliflozin also readily crosses the rat placental barrier, but with fetal exposures remaining lower than maternal plasma levels.

In rats, elimination of radiolabeled drug and metabolites was virtually complete by 168 hours (7 days) postdose. Ertugliflozin is primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans.

The predominant route of elimination of ertugliflozin is via metabolism, wherein glucuronidation is the major metabolic pathway in all species, with minor contributions from oxidative metabolism involving hydroxylation, oxidation, and oxidative desethylation. There are no unique human metabolites; however, the 2-O-β glucuronide M5a (PF-06685948) and the 3-O- β glucuronide M5c (PF-06481944) are disproportional human metabolites, making up 12.2% and 24.1% of total drug in human plasma, respectively.

Sitagliptin

Sitagliptin is absorbed rapidly with an oral bioavailability of 60-90% in rats and dogs, with AUC and C_{max} exposures generally increasing dose-proportionally. Sitagliptin is widely distributed to tissues, with tissue exposures generally higher than plasma, with the exceptions of brain, eyes and bone. Sitagliptin binding to plasma proteins is similar across species, ranging from 32% to 38%. Clearance is moderate in dogs and high in rats, with half-lives of 2 to 5 hours. In Humans, the plasma half-life is 13 hours, which is 3 to 6-fold longer due to slower clearance than both dogs and rats. Sitagliptin is primarily eliminated via urine, possibly by organic anion transporters in the renal tubules and is a substrate for OAT3 transport. Sitagliptin crosses the placenta in pregnant rats and is excreted in the mild of lactating rats. Placental transfer reaches 45% at 2 hours and 80% at 24 hours postdose in rats, and 66% at 2 hours and 30% at 24 hours postdose in rabbits. Sitagliptin is excreted in the milk of lactating rats.

5.2 Toxicokinetics

In rats, PK parameters of ertugliflozin and sitagliptin were not significantly affected by coadministration. Similarly, in the Phase 1 clinical PK study (#P022/1033) in healthy subjects, there were no meaningful differences in ertugliflozin or sitagliptin PK parameters when co-administered together compared to administration of each drug alone. Thus, concomitant administration of ertugliflozin and sitagliptin are not associated with significant changes in drug exposures in humans.

Sponsor's Table 2: Summary of Ertugliflozin PK Parameters with Coadministration in Humans – Study #P022/1033

Parameter (unit)	Parameter Summary Statistics[a] for Ertugliflozin by Treatment	
	Ertugliflozin 15 mg SD	Ertugliflozin 15 mg SD + sitagliptin 100 mg SD
N, n	12, 12	12, 12
AUC_{inf} (ng·hr/mL)	1413 (26)	1445 (25)
AUC_{last} (ng·hr/mL)	1385 (26)	1412 (24)
C_{max} (ng/mL)	262.9 (25)	258.1 (26)
T_{max} (hr)	1.00 (1.00 - 3.00)	1.00 (0.500 - 2.10)
CL/F (mL/min)	177.0 (26)	173.1 (25)
V_z/F (L)	181.4 (41)	203.3 (21)
$t_{½}$ (hr)	12.63 ± 5.15	14.17 ± 4.55

PK parameters are defined in Table S3.

Abbreviations: %CV = percent coefficient of variation; hr = hour(s); N = number of subjects in the treatment group; n = number of subjects contributing to the summary statistics; PK = pharmacokinetic(s); SD = single dose.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean ± standard deviation for $t_{½}$.

Sponsor's Table 3: Summary of Sitagliptin PK Parameters with Coadministration in Humans – Study #P022/1033

Parameter (unit)	Parameter Summary Statistics[a] for Sitagliptin by Treatment	
	Sitagliptin 100 mg SD	Ertugliflozin 15 mg SD +Sitagliptin 100 mg SD
N, n	12, 12	12, 12
AUC_{inf} (uM•hr)	6.882 (21)	6.997 (20)
AUC_{last} (uM•hr)	6.814 (21)	6.912 (21)
C_{max} (nM)	792.0 (24)	805.3 (24)
T_{max} (hr)	2.00 (1.00-4.00)	3.00 (1.00-6.00)
CL/F (mL/min)	594.4 (21)	584.4 (20)
V_z/F (L)	548.2 (28)	579.3 (23)
$t_{½}$ (hr)	11.00 ± 2.89	11.79 ± 2.98

PK parameters are defined in Table S3.
Abbreviations: %CV=percent coefficient of variation; hr = hour(s); N = Number of subjects in the treatment group; n = Number of subjects with reportable $t_{½}$ and AUC_{inf}; SD = single dose
a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean (± standard deviation) for $t_{½}$.

(Tables excerpted from sponsor's package)

6 General Toxicology

6.1 Ertugliflozin

Toxicology studies with administration of ertugliflozin alone were reviewed under NDA #209803 and include pivotal 6-month rat and 9-month dog studies.

Safety margins from the 6 month rat and 9 month dog studies support the proposed 15 mg/day dose of ertugliflozin with safety margins of at least 13x and 46x, respectively, based on AUC exposures at the nonclinical NOAELs. Most findings in the chronic nonclinical toxicology studies can be attributed to drug-related glucosuria and osmotic diuresis. Drug-related gastrointestinal findings in dogs (excessive vomiting, salivation and abnormal feces) and rats (stomach erosion/ulcers, pyloric crypt degeneration and foveolar hyperplasia) are consistent with off-target inhibition of SGLT1. Hyperostosis and changes in calcium regulation have also been observed in rats, and are also likely to be related to SGLT1 inhibition.

Table 2: Ertugliflozin Summary of Pivotal General Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
9-Month + 8-Week Recovery Beagle Dogs **Dose:** 1, 10 & 150 mg/kg ♂ AUC: 6, 63 & 1040 µg·h/mL ♀ AUC: 7, 78 & 767 µg·h/mL	**10 mg/kg** (♂ & ♀)	♂: **46x** ♀: **57x**	**≥1 mg/kg (♂4x/♀6x MRHD):** adrenal gland (↑organ weight & cortex vacuolation), glucosuria **≥10 mg/kg (♂46x/♀57x MRHD):** thyroid mineralization (♀, irreversible) **150 mg/kg (♂754x/♀556x MRHD):** Adverse: GI intolerance (excessive vomiting, diarrhea, salivation), possibly related mortalities, systemic inflammatory response Non-adverse: ↓BW & gain, ↑thymus weight, persistent ↑reticulocytes, ↑urine calcium (partially reversible), irreversible

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
			urine ↑volume
6-Month + 8-Week Recovery Sprague Dawley (SD) Rats **Dose:** 5, 25 & 100 mg/kg ♂ AUC: 18, 128 & 397 μg·h/mL ♀ AUC: 27, 167 & 814 μg·h/mL	**5 mg/kg** (♂ & ♀)	♂: **13x** ♀: **19x**	**≥5 mg/kg (♂13x/♀19x MRHD):** stomach erosion/ulcer, ↓pancreatic zymogen, ↑food consumption, ↓blood glucose, glucosuria, ↓serum electrolytes (minimal), ↑phosphates, possible dehydration, minimal ↑BUN **≥25 mg/kg (♂93x/♀121x MRHD):** Adverse: stomach (pyloric crypt degeneration, discoloration, ↑severity of erosion/ulcer) Non-adverse: minimal-slight kidney findings (pelvic & tubule dilatation, hyperplasia, mineral deposition) **100 mg/kg (♂288x/♀590x MRHD):** bone [severe hyperostosis (♂) & hyperplasia (♀)], digestive tract (stomach hyperplasia, ↑severity of erosions/ulcers & crypt degeneration), ↑severity of kidney findings, adrenal gland (↑organ weight, hypertrophy & cortex vacuolation), ↓BW & gain, ↓RBC parameters, ↓reticulocytes, ↑urine calcium, ↓PTH, significant ↓serum electrolytes (Ca, Na, K, & Cl), mild ↑BUN (1.5-fold)

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL
♂ = males only; ♀ = females only

6.2 Sitagliptin

Sitagliptin target organs of toxicity identified in rats include kidney (necrosis), liver (necrosis), heart (myocardial degeneration) and bone marrow (necrosis); however, these effects were only observed at high doses (~150x MRHD) and were likely due to inhibition of off-target enzymes DPP8/9. Administration of doses up to ~20x MRHD for 6 months in rats was not associated with any significant toxicity findings. In a 12-month dog toxicology study, the NOAEL at 5x MRHD was based on clinical signs of reduced activity, hunched posture, ataxia, tremor, and sporadic emesis at 50 mg/kg (20x MRHD). Respiratory distress was identified in some animals, but no consistent target organs were identified in dogs.

6.3 FDC Ertugliflozin/Sitagliptin

In accordance with ICH and FDA guidances, the sponsor submitted a GLP-compliant 3-month repeat dose toxicity study in rats with coadministration of ertugliflozin and sitagliptin under IND #106447. The sponsor also submitted a preliminary, non-GLP 2-week study under IND #122330.

Study: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats (Study #TT147808 / #8300338 / #14GR162)

Study #	TT147808 / 8300338 / 14GR162
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	7/14/2014
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729: Lot #E010014849, 76.0% purity Sitagliptin: Lot #010X054, 99.6% purity

Key Study Findings

- Ertugliflozin + Sitagliptin Coadministration-related Findings:
  - Potential exacerbation of stomach erosion and discoloration of glandular mucosa in males and incidences of hemorrhage in females
  - Adrenal Gland
    - ↑Adrenal organ weight
    - Exacerbation of zona glomerulosa hypertrophy of the adrenal cortex
  - Prostate mixed cell inflammation (low incidences)
  - Improvement of ertugliflozin-mediated ketonuria
- Ertugliflozin-related Findings:
  - Trends for ↓body weight and ↓weight gain
  - ↑Food consumption
  - Stomach:
    - Discoloration of glandular stomach mucosa
    - Erosion
    - Submucosal inflammation
    - Hemorrhage
  - ↓Pancreatic acinar cell zymogen granules (♂&♀)
  - Clinical chemistry: ↓glucose, ↓Cl, ↓Ca, and ↑BUN
  - Urine: ↑specific gravity, ↑volume, ↑glucose, ↑ketones, and ↓pH
  - Kidney:
    - ↑Kidney organ weight
    - Tubule and pelvic dilatation
  - Adrenal Gland
    - Adrenal cortex hypertrophy
- Sitagliptin-related Findings:
  - No biologically significant findings were attributed primarily to sitagliptin

SD Rat, 13 Weeks	NOAEL (AUC)	Multiple of MRHD*
No significant adverse systemic toxicities	25 mg/kg Ertugliflozin (123 μg ·h/mL) + 60 mg/kg Sitagliptin (24.4 μg ·h/mL)	Ertugliflozin: **89x** Sitagliptin: **9x**

*Based on a maximum once daily dose of 15 mg ertugliflozin / 100 mg sitagliptin with an ertugliflozin exposure of AUC = 1.38 μg·h/mL and sitagliptin exposure of AUC = 2.81 μg·h/mL

METHODS

SD rats were co-administered doses of 0/0, 0/60, 25/0, 5/20, 5/60, 25/20, and 25/60 mg/kg PF-04971729/mg/kg sitagliptin (10/sex/group) via oral gavage daily for 91 days. The vehicle for PF-04971729 was 0.5% MC/10% PEG 400 and the vehicle for sitagliptin was 0.5% MC/5 mM HCl. Animals were evaluated for clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, macroscopic findings, and microscopic findings. Satellite TK groups were included for each dose (4/sex/group).

RESULTS

The NOAEL was set at the high combination dose of 25 mg/kg ertugliflozin and 60 mg/kg sitagliptin due to a lack of significant adverse systemic toxicities. The safety margins for ertugliflozin were 89x $MRHD_{AUC}$ for the proposed clinical high dose of 15 mg/day ertugliflozin and 9x $MRHD_{AUC}$ for a 100 mg/day sitagliptin clinical dose.

Reduced serum glucose, increased urinary volume and glucosuria underlie most of the findings in this study, which include reduced body weight and increased food consumption, as well as histopathology changes in the kidney, adrenal gland, and pancreas. Overall, the key findings associated with PF-04971729 in this study are consistent with administration of PF-04971729 alone for 1, 3, and 6 months in rats. Similarly, findings associated with coadministration of PF-04971729 and sitagliptin, as well as sitagliptin alone, are consistent with findings in the 2-week coadministration study in rats (study #13GR342). Overall, there were no new significant drug-related toxicities and the majority of the findings are considered to be secondary to the PD activity of PF-04971720. Thus, there were no toxicity interactions due to coadministration of PF-04971729 and sitagliptin.

Decreases in body weight gain with reciprocal increases in food consumption were observed in animals receiving ertugliflozin independent of sitagliptin, and are consistent with observations from previous studies with PF-04971729 administration in rats. Thus, these findings are attributable to ertugliflozin. It is noted that treatment-related decreases in body weights and weight gains, as well as increases in food consumption, were not exacerbated with coadministration of sitagliptin coadministration.

PF-04971729-related glucosuria was associated with reciprocal decreases in blood glucose levels, reflecting inhibition of SGLT2 and reduced renal tubular reabsorption of

glucose from the glomerular filtrate. Observed reductions in Ca (↓4-5%) and Cl (↓3-5%) electrolyte concentrations in the blood are consistent with osmotic diuresis. Furthermore, increases in urine volume (↑2 to 3-fold in males, ↑18-37% in females) and urine specific gravity (↑2-4%), as well as decreases in urinary pH (↓3-13%), were also observed with PF-04971729 administration independent of sitagliptin and are also consistent with osmotic diuresis. Since increases in BUN levels correlate with increases in urine volume while creatinine levels were not increased, the observed increases in BUN (↑29-93%) are likely to be secondary to dehydration resulting from glucosuria, rather than kidney toxicity. Ultimately, coadministration of sitagliptin did not exacerbate any of the clinical chemistry findings and there were no clear signs of kidney dysfunction. Overall, the clinical pathology changes observed with coadministration treatment were considered to be non-adverse findings attributable to PD-related effects of PF-04971729.

Drug-related ketonuria was attributed to ertugliflozin administration, but was considered to be non-adverse. The highest incidences and severity of ketonuria were observed at 25 mg/kg ertugliflozin in both males and females, which is consistent with findings in 6-month rat and other 13-week rat toxicology studies with ertugliflozin administration. Importantly, improvement of ketonuria was observed with coadministration of sitagliptin in both males and females. Thus, there was not a negative toxicological interaction regarding ketonuria with co-administration.

Increases in kidney weights were observed in all male (↑14-35%) and female (↑21-35%) PF-04971729 treatment groups, but not in animals treated with sitagliptin alone. Correlating histopathological findings of minimal to marked tubular dilatation in the kidney, consistent with osmotic diuresis, were also observed in animals receiving PF-04971729 but not with sitagliptin alone. Overall, the kidney findings were independent of sitagliptin and attributable to the PD activity of ertugliflozin. Since, there were no indications of kidney dysfunction or toxicity, these findings were considered to be non-adverse. Furthermore, there was no indication of exacerbation of increased kidney weight with coadministration of sitagliptin.

Findings of discolored stomach and/or erosion were observed in animals treated with PF-04971729 in the absence or presence of sitagliptin. Increases in the number of incidences of minimal to mild stomach erosion and discoloration findings were reported in males with sitagliptin coadministration, indicating potential exacerbation. Incidences of stomach hemorrhage were reported in males independent of sitagliptin, but were only observed in females with sitagliptin coadministration. These stomach findings are consistent with previous rat toxicology studies with PF-04971729 administration, and are likely related to off-target inhibition of SGLT1 in this species, which is not likely to occur at clinical exposure levels. It's noted that other members of the DPP4 inhibitor drug class have been associated with stomach findings of necrosis, ulceration and erosion; thus, exacerbation of the stomach findings in coadministration groups may be due to additive toxicities of both PF-04971729 and sitagliptin. Nevertheless, the stomach findings were of low severity and were not dose-limiting; thus, they were not considered to be a significant adverse systemic toxicity in this study.

Pancreatic zymogen depletion was observed in all groups treated with PF-04971729, independent of sitagliptin administration, with increased severity in males. Similar findings were also described in previous toxicology studies with ertugliflozin alone or in combination with sitagliptin, and are considered to be non-adverse findings secondary to PD-related increases in food consumption.

Decreases in blood calcium levels were reported in PF-04971729 treatment groups are attributable to PF-04971729-related glucosuria, and are also consistent with disruption of absorption and calcium homeostasis secondary to off-target inhibition of SGLT1. Although, there were no abnormal bone findings in this study, increased trabecular bone was observed at doses of 25 mg/kg PF-04971729 with longer exposures in males in the 6-month rat toxicology study. Thus, the decreased serum calcium levels observed in this study may be indicative of early drug-related effects on bone in this species. Nevertheless, since PF-04971729-mediated inhibition of SGLT1 is unlikely at clinical exposure levels, this finding is not likely to be clinically relevant.

Adrenal gland hypertrophy was reported in both sexes of PF-04971729 treatment groups and was associated with increased incidence and severity with coadministration of sitagliptin, and also correlated with increased adrenal gland weights. These findings are consistent with previous studies with PF-04971729 alone in rats and dogs, and are likely due to a compensatory response to fluid and electrolyte losses related to the PF-04971729-induced glucose excursion. However, it's also noted that the adrenals are a potential class-related target organ of sitagliptin. The coadministration data indicate that that sitagliptin and ertugliflozin likely work together to increase severity and incidence rates of adrenal gland organ weight increases and hypertrophy. Nevertheless, these effects are not considered to be adverse.

It is noted that there were no abnormal heart organ weight changes or histopathological findings.

Low incidence rates of minimal to moderate prostate mixed cell infiltration were reported in males with coadministration, but not with administration of either drug alone. The DPP4 inhibitor drug class is associated with cellular infiltration of multiple organs. Although this finding was not dose-related and occurred at low incidences of 1 to 2 animals per group, it may be treatment-related with coadministration of PF-04971729 and sitagliptin. Nevertheless, it was also considered to be non-adverse.

Coadministration of PF-04971729 did not affect sitagliptin exposures. PF-04971729 exposures were predominantly unaffected by sitagliptin coadministration. However, it is noted that PF-04971729 exposures were slightly higher on Day 91 by 12-28% with coadministration, indicating a possible trend for accumulation with high doses of both drugs. Nevertheless, given the small amount of increase, it is unclear if this is a significant finding.

Methods

Doses	PF-04971729 (mg/kg) + Sitagliptin (mg/kg): 0+0, 0+60, 25+0, 5+20, 5+60, 25+20, and 25+60
Frequency of dosing	Once daily for 91 days. Animals were dosed 1st with PF-04971729, then dosed with sitagliptin 2nd within 2 minutes.
Route of administration	Oral gavage
Dose volume	5 mL/kg PF-04971729 + 5 mL/kg sitagliptin = 10 mL/kg total
Formulation/Vehicle	Vehicle #1 (PF-04971729): 0.5% (w/v) methylcellulose, 10% (v/v) polyethylene glycol 400 (PEG 400) Vehicle #2 (sitagliptin): 0.5% (w/v) methylcellulose, 5 mM hydrochloric acid
Species/Strain	Crl:CD(SD) rats, (b) (4)
Number/Sex/Group	10/sex/group
Age	6-7 weeks
Weight	♂: 202-282 g ♀: 153-216 g
Satellite groups	TK animals including 4/sex/group
Unique study design	Co-administration of PF-04971729 and sitagliptin
Deviation from study protocol	On some occasions, some animals received only a partial dose; however, the identity of the animals was not reported. Day 17-87: various animals (11 total) across most groups received the sitagliptin dose more than 2 minutes after the PF-04971729 dose.

Study Design

		No. of Animals		PF-04971729		Sitagliptin	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Dose Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	10	10	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Control/ High)	1 (Toxicity)	10	10	0	0	60	12
	2 (Toxicokinetic)	4	4	0	0	60	12
3 (High/ Control)	1 (Toxicity)	10	10	25	5	0	0
	2 (Toxicokinetic)	4	4	25	5	0	0
4 (Low/ Low)	1 (Toxicity)	10	10	5	1	20	4
	2 (Toxicokinetic)	4	4	5	1	20	4
5 (Low/ High)	1 (Toxicity)	10	10	5	1	60	12
	2 (Toxicokinetic)	4	4	5	1	60	12
6 (High/ Low)	1 (Toxicity)	10	10	25	5	20	4
	2 (Toxicokinetic)	4	4	25	5	20	4
7 (High/ High)	1 (Toxicity)	10	10	25	5	60	12
	2 (Toxicokinetic)	4	4	25	5	60	12

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg sitagliptin (or Vehicle Control Article 2, as applicable).

b PF-04971729 dose concentrations were corrected for lot specific potency of 0.760 (76.0%). A correction factor of 1.316 was used for Lot No. E010014849.

c Sitagliptin dose concentrations were corrected for salt content and lot specific potency of 0.996 (99.6%). A correction factor of 1.285 was used for Lot No. 010X054.

d Group 1 received Vehicle Control Article 1 (0.5% [w/v] methylcellulose [4000 cps] with 10% [v/v] polyethylene glycol 400 prepared in reverse osmosis water) and Vehicle Control Article 2 (0.5% [w/v] methylcellulose [4000 cps] with 5 mM hydrochloric acid prepared in reverse osmosis water) only.

Parameters Measured

Clinical Findings	Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. Detailed observations were conducted on all animals prior to dosing on Day 1, weekly during the dosing phase, and on Day 91. Cageside observations were also conducted at 1 hour postdose.
Body weights	Animals were weighed once during the predose phase, prior to dosing of Day 1, weekly thereafter, and on Day 91.
Food consumption	Food consumption was quantified for each cage weekly, beginning on Day 1, for Weeks 1-13 and Days 85-91.
Ophthalmoscopy	Ophthalmic examinations were conducted by a veterinarian using an indirect ophthalmoscope and a mydriatic agent once during the predose phase and during Week 13 of the dosing phase.
EKG	Not evaluated
Hematology	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.2 Hematology Tests** red blood cell (erythrocyte) count hemoglobin hematocrit mean corpuscular volume mean corpuscular hemoglobin mean corpuscular hemoglobin concentration platelet count white blood cell (leukocyte) count differential blood cell count blood smear reticulocyte count mean platelet volume red blood cell distribution width **3.5.1.3 Coagulation Tests** prothrombin time activated partial thromboplastin time
Clinical chemistry	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.4 Clinical Chemistry Tests** glucose urea nitrogen creatinine total protein albumin globulin albumin:globulin ratio cholesterol total bilirubin alanine aminotransferase alkaline phosphatase gamma glutamyltransferase aspartate aminotransferase calcium inorganic phosphorus sodium potassium chloride
Urinalysis	Urine samples were collected at necropsy on Day 92 **3.5.1.5 Urinalysis Tests** appearance (clarity and color) bilirubin blood glucose ketones microscopic examination of sediment pH protein specific gravity urobilinogen volume
Gross pathology	Animals were fasted overnight and necropsied on Day 92. External features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues were examined.
Organ weights	Organ weights were measured (W) according the table below. Paired organs were weighed together.
Histopathology	Tissues were collected from all animals and prepared (P) by preserving in 10% NBF, embedding in paraffin, sectioning, and staining with H&E. All tissues in Groups 1 (0+0), 2 (0+60), 3 (25+0), and 7 (25+60) were examined microscopically. The kidneys, ureter, duodenum, pancreas, glandular stomach, adrenal cortex, and prostate from Groups 4 (5+20), 5 (5+60), and 6 (25+20) were also examined microscopically.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		P,E
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	right upper incisor tooth with root		P,E
epididymis (2)	W	P,E	salivary gland (mandibular [2])		P,E
esophagus		P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
eye (2)[b]		P,E	seminal vesicle		P,E
femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	skin/subcutis {skin and adnexa}		P,E
gross lesions		P,E	spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
gut-associated lymphoid tissue {GALT}		P,E	spleen	W	P,E
Harderian gland[b]		P,E	sternum with bone marrow {sternum}		P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[b]	W	P,E
jejunum		P,E	thymus	W	P,E
kidney (2)	W	P,E	thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
larynx			tongue		P,E
liver	W	P,E	trachea		P,E
lower mandible			ureter		P,E
lungs with large bronchi {lung}		P,E	urinary bladder		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	uterus		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	vagina		P,E
mammary gland (males and females)		P,E			

E = Examined microscopically; P = Processed; W = Weighed.
a Brain was sectioned according to published recommendations (Bolon et al., 2013).
b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Bone marrow smears were prepared from the femur, but were not examined.

Toxicokinetics	Non-fasted blood samples were collected from all groups (2 animals/time point/group) on Days 1 and 91 at 1, 4, 7, and 24 hours postdose.

Observations and Results

Mortality

There were no mortalities.

Clinical Signs

There were no drug-related findings.

Body Weights

Male body weight gains were generally lower (↓6-14%) with administration of ≥5 mg/kg PF-04971729 and were associated with lower final body weights (↓3-8%). However, there was not a clear dose-dependent response. Decreases in body weight gains (↓1-9%) and final body weights (↓1-4%) were less pronounced in females and were also independent of dose. Decreases in body weights and weight gains are consistent with PF-04971729-related findings in rats in multiple other studies and are considered to be drug-related. There were no indications of exacerbation by co-administration with sitagliptin.

Table 3: Body Weights - 13-week Rat Study #14GR162

MALES: Body Weight				
Study Time	Dose (mg/kg+mg/kg)	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	346	-	-
	0+600	347	100.3%	100%
	25+0	325	93.9% (↓6.1%)	96.6% (↓3.4%)
	5+200	302	87.3% (↓12.7%)	92.3% (↓7.7%)
	5+600	307	88.7% (↓11.3%)	93.2% (↓6.8%)
	25+200	298	86.1% (↓13.9%)	92.0% (↓8.0%)
	25+600	318	91.9% (↓8.1%)	95.1% (↓4.9%)
FEMALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	130	-	-
	0+600	133	102.3%	101.0% (↑1.0%)
	25+0	122	93.8% (↓%6.2)	96.8% (↓3.2%)
	5+200	129	99.2% (↓0.8%)	98.7% (↓1.3%)
	5+600	118	90.8% (↓9.2%)	95.9% (↓4.1%)
	25+200	118	90.8% (↓9.2%)	95.9% (↓4.1%)
	25+600	124	95.4% (↓4.6%)	97.8% (↓2.2%)

Sponsor's Figure 1: Body Weights - 13-week Rat Study #14GR162

Figure 7.1: Summary of Body Weight - Males

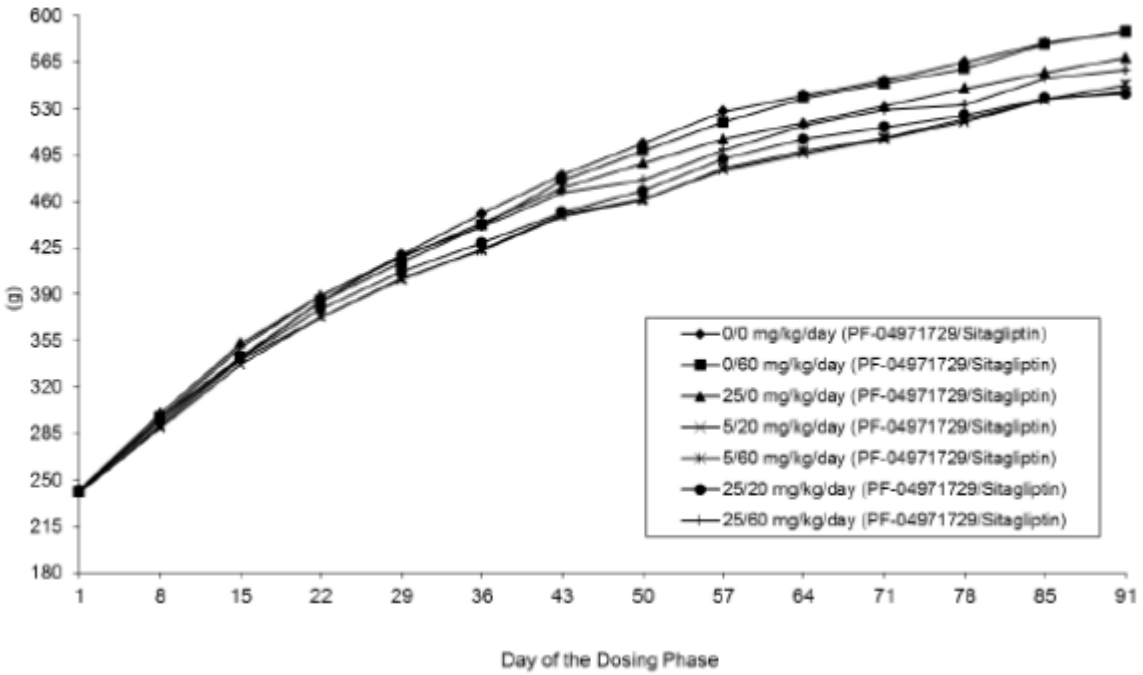
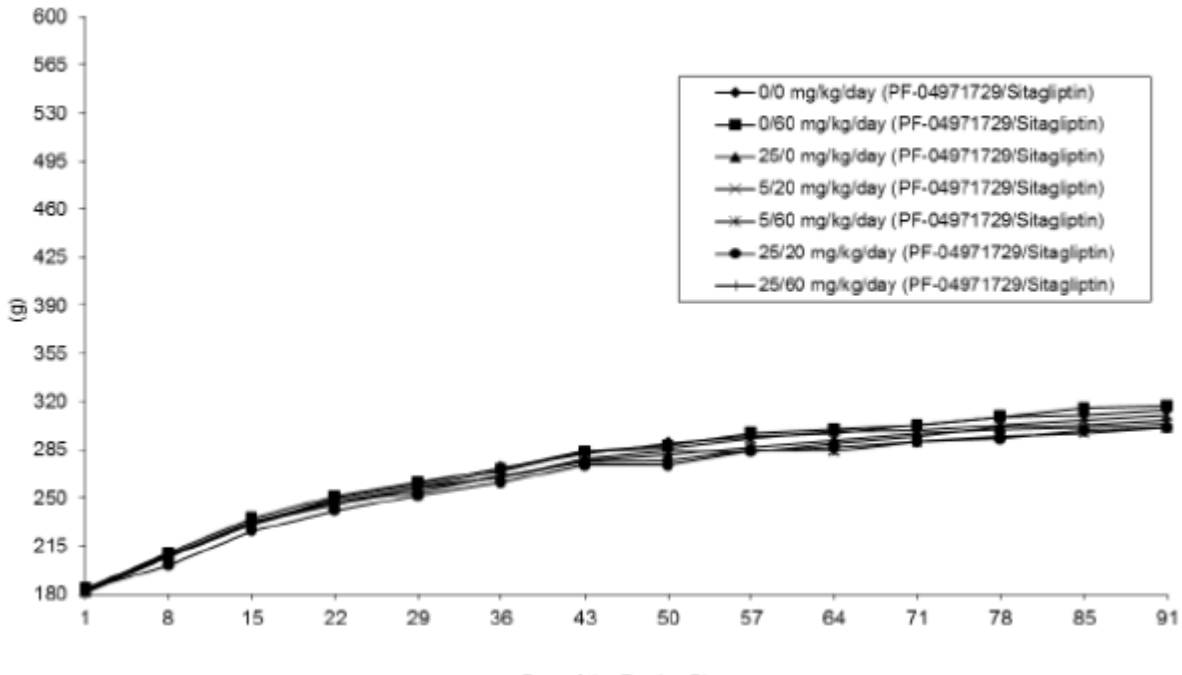


Figure 7.2: Summary of Body Weight - Females



(Figures excerpted from sponsor's package)

Feed Consumption

Food consumption was higher in females (↑11-22%) treated with PF-04971729, but was independent of dose and did not reach statistical significance in all groups due to variability. Similarly, food consumption was also increased in males (↑7-14%) treated with PF-04971729 independent of dose, but did not reach statistical significance in any dose group. Increases in food consumption are consistent with PF-04971729-related findings in rats in multiple other studies and are considered to be drug-related. There were no indications of exacerbation by co-administration with sitagliptin.

Table 4: Food Consumption - 13-week Rat Study #14GR162

Food Consumption				
Dose, mg/kg	Males		Females	
	Consumption (g/animal/day)	% Control	Consumption (g/animal/day)	% Control
0+0	29	-	18	-
0+60	28	96.6%	17	94.4%
25+0	32	110.3%	21*	116.7%
5+20	31	106.9%	20	111.1%
5+60	31	106.9%	21	116.7%
25+20	32	110.3%	22*	122.2%
25+60	33	113.8%	21	116.7%

* p value < 0.05

Ophthalmoscopy

There were no treatment-related findings.

Hematology

There were no biologically significant changes in hematology parameters with either drug treatment or co-administration.

Several statistically significant changes in hematocrit, neutrophil counts and prothrombin time were reported in various treatment groups; however, these findings were considered to be of small magnitude, within the normal biological range, and unlikely to be biologically significant. Small 5% decreases in hematocrit (Hct) were observed in females receiving 25 mg/kg PF-04971729 and sitagliptin, but were not observed in males. Decreases of 25-37% in neutrophils (NEUT) were observed in males treated with PF-04971729, but only reached statistical significance in males receiving 25 mg/kg PF-04971729 co-administered with sitagliptin. An 8% decrease in

pro-thrombin time (PT) was reported for males treated with 25 mg/kg PF-04971729 and 20 mg/kg sitagliptin, but was not dose-dependent nor observed in females.

Table 5: Hematology Parameters - 13-week Rat Study #14GR162

Hematology						
Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	**Hct (%)**		**NEUT (10^3/uL)**		**PT (sex)**	
	♂	♀	♂	♀	♂	♀
0+0	52.5	51.5	2.12	0.82	10.8	9.2
0+60	51.4	51.2	2.39	1.05	10.5	9.3
25+0	51.5	49.9	1.54 (↓27.4%)	0.79	10.4	9.2
5+20	52.2	50.7	2.78	0.74	10.2	9.5
5+60	51.4	49.6	1.60 (↓24.5%)	0.65	10.6	9.0
25+20	51.6	49.1* (↓4.7%)	1.32* (↓37.3%)	1.04	9.9* (↓8.3%)	9.0
25+60	51.9	48.8* (↓5.2%)	1.36* (↓35.8%)	1.02	10.6	9.3

* p value < 0.05

Clinical Chemistry

Drug-related decreases in steady-state fasting serum glucose levels were observed with PF-04971729 treatment in both males (↓17-33%) and females (↓7-28%), but were independent of sitagliptin treatment or co-administration. Thus, maintenance of reduced blood glucose levels was attributed to PF-04971729 administration alone. It is noted that the reduced steady-state blood glucose levels were harvested 24 hours postdose and were at or below the lower limit of normal (LLN), but were not within the hypoglycemic range (<50 mg/dL) for this species.

Drug-related increases in BUN levels were observed with PF-04971729 treatment in males (↑57-93%) and females (↑29-79%), reaching levels above the upper limit of normal (ULN) in males treated with 25 mg/kg PF-04971729. The increases were dose-dependent with regard to PF-04971729, but were independent of sitagliptin treatment or co-administration. Thus, the increases in BUN levels were attributed to Pf-04971729 administration alone.

Statistically significant decreases in total protein levels (↓6-7%) were observed in females treated with 25 mg/kg PF-04971729 alone or in combination with 60 mg/kg sitagliptin. Significant decreases in albumin levels (↓9%) were also observed in females receiving the highest dose combination (25+60). Nevertheless, the decreases in blood protein levels remained within the normal biological range for this species and are not considered to be biologically significant.

Table 6: Clinical Chemistry Parameters - 13-week Rat Study #14GR162

Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	Glucose (mg/dL)		BUN (mg/dL)		TP (g/dL)		ALB (g/dL)	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	102	101	14	14	7.2	8.1	4.4	5.4
0+60	108	111	14	14	7.6	8.1	4.5	5.4
25+0	76* (↓25.5%)	73* (↓27.7%)	27* (↑92.9%)	25* (↑78.6%)	7.1	7.6* (↓6.2%)	4.4	5.0
5+20	85* (↓16.7%)	90 (↓10.9%)	22* (↓57.1%)	20* (↑42.9%)	7.4	7.8 (↓3.7%)	4.4	5.2
5+60	84* (↓17.6%)	94 (↓6.9%)	22* (↑57.1%)	18* (↑28.6%)	7.0	8.1	4.3	5.4
25+20	68* (↓33.3%)	82* (↓18.8%)	27* (↑92.9%)	24* (↑71.4%)	7.1	7.8 (↓3.7%)	4.3	5.2
25+60	70* (↓31.4%)	73* (↓27.7%)	26* (↑85.7%)	24* (↑71.4%)	7.1	7.5* (↓7.4%)	4.3	4.9* (↓9.3%)

* p value < 0.05

Drug-related increases in ALT levels were observed in males (↑24-44%) at ≥5 mg/kg PF-04971729 and in females (↑35-48%) at 25 mg/kg in a dose-dependent manner with regard to PF-04971729, but independent of sitagliptin treatment or co-administration. Thus, the increases in ALT were attributed to PF-04971729 administration; however, the degrees of increase were considered to be minimal and unlikely to be biologically significant.

Statistically significant decreases in cholesterol were observed in females with PF-04971729 administration, but were not consistently dose-dependent and remained within the normal biological range for this species. Although attributed to PF-04971729 treatment, this finding is not considered to be biologically significant.

Drug-related decreases in electrolytes were observed in both sexes. Statistically significant decreases in chloride levels were observed in both males (↓4-5%) and females (↓3%) with PF-04971729 administration in a dose-dependent manner with regard to PF-04971729, but independent of sitagliptin. Statistically significant decreases in calcium levels were observed in both males (↓5%) and females (↓4%) with 25 mg/kg PF-04971729 administration alone or in combination with 20 mg/kg sitagliptin in males (↓4%). The observed decreases in electrolytes are consistent with findings from other studies with PF-04971729 administration in rats and are considered to be drug-related. Nevertheless, it is noted that the mean levels of both calcium and chloride remained within the normal biological range for this species.

Table 7: Clinical Chemistry Parameters Continued - 13-week Rat Study #14GR162

Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	ALT (U/L)		CHOL (mg/dL)		Calcium (mg/dl)		Cl (mmol/L)	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	34	29	79	113	11.4	11.5	103	102
0+60	32	30	84	105	11.4	11.6	102	102
25+0	49* (↑44.1%)	43* (↑48.3%)	76	89* (↓21.2%)	10.8* (↓5.3%)	11.0* (↓4.3%)	98* (↓4.9%)	99* (↓2.9%)
5+20	42* (↑23.5%)	31	72	92 (↓18.6%)	11.2	11.3	99* (↓3.9%)	100 (↓2.0%)
5+60	43* (↑26.5%)	36 (↑24.1%)	66	88* (↓22.1%)	11.0 (↓3.5%)	11.5	99* (↓3.9%)	101 (↓1.0%)
25+20	46* (↑35.3%)	39* (↑34.5%)	80	94 (↓16.8%)	11.0* (↓3.5%)	11.2 (↓2.6%)	98* (↓4.9%)	99* (↓2.9%)
25+60	46* (↑35.3%)	41* (↑41.4%)	70	88* (↓22.1%)	11.1 (↓2.6%)	11.1 (↓3.5%)	98* (↓4.9%)	99* (↓2.9%)

* p value < 0.05

Urinalysis

Moderate to marked glucosuria was reported in both sexes with PF-04971729 administration alone or in combination with sitagliptin.

Statistically significant drug-related increases in specific gravity were reported in males (↑2-3%) and females (↑3-4%) with PF-04971729 administration, but were independent of dose and sitagliptin administration.

Significant drug-related increases in urine volume reaching 2 to 3-fold above concurrent controls were observed in males with PF-04971729 administration and were dose-dependent with regard to PF-04971729, but were independent of sitagliptin administration and dose. A trend for minimal increases (↑18-37%) in urine volume was also observed with 25 mg/kg PF-04971729 administration in females independent of sitagliptin, but did not reach statistical significance.

Trends for decreased urine pH were observed in both males (↓3-8%) and females (↓10-13%), but were less severe with co-administration.

Table 8: Urine Parameters - 13-week Rat Study #14GR162

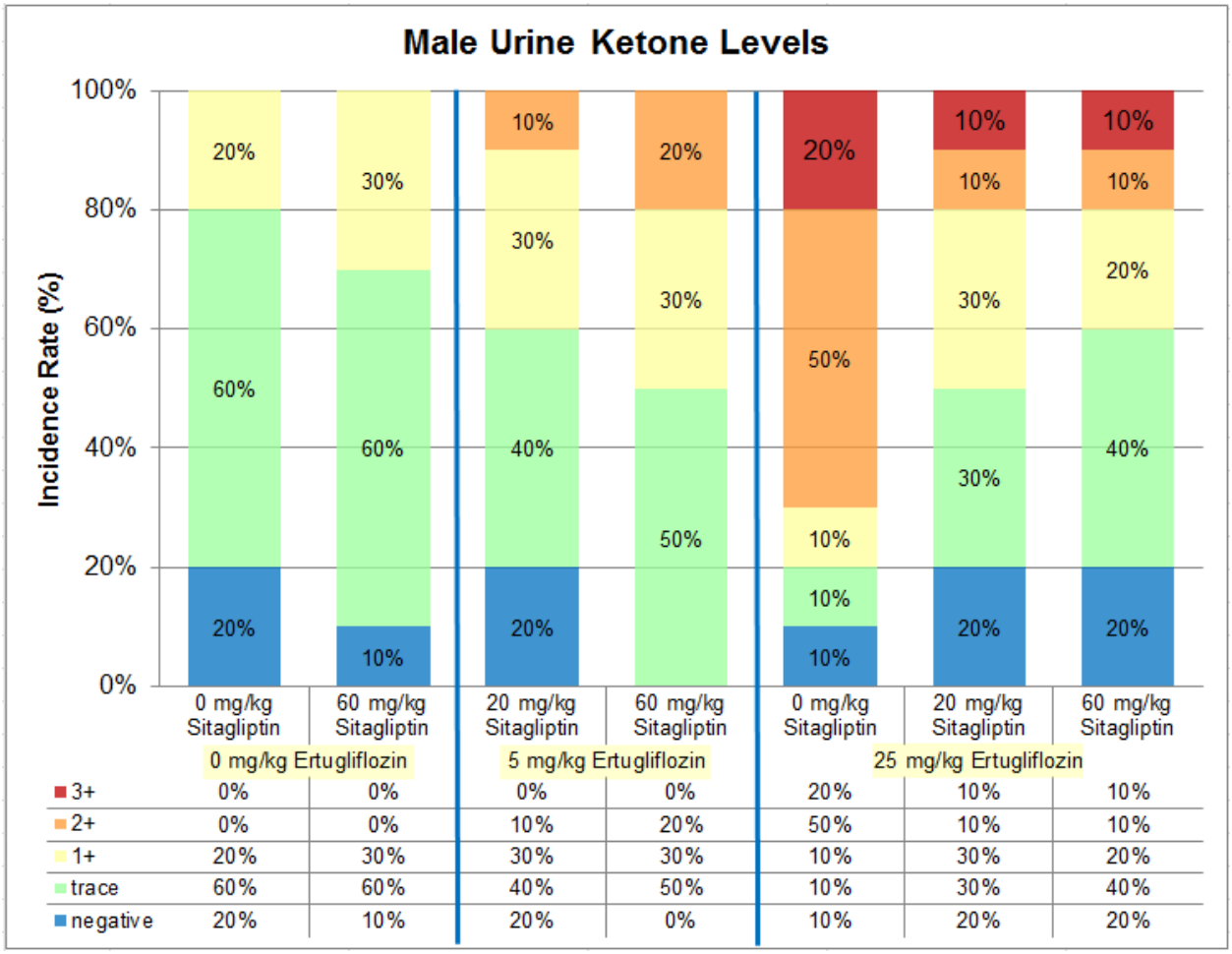
Urine Parameters						
Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	Specific Gravity		Volume (mL)		pH^	
	♂	♀	♂	♀	♂	♀
0+0	1.037	1.015	11.0	15.9	6.7	7.0
0+60	1.040	1.028	8.9	9.0	6.6	6.6
25+0	1.056*	1.053*	32.9*	19.0	6.2	6.1

	(↑1.8%)	(↑3.7%)	(↑3-fold)	(↑19.5%)	(↓7.5%)	(↓12.9%)
5+20	1.063* (↑2.5%)	1.055* (↑3.9%)	21.0* (↑2-fold)	13.9	6.7	6.4
5+60	1.056* (↑1.8%)	1.047* (↑3.2%)	24.1* (↑2-fold)	16.2	6.5	6.4
25+20	1.054* (↑1.6%)	1.056* (↑4.0%)	31.0* (↑3-fold)	18.7 (↑17.6%)	6.4 (↓4.5%)	6.2 (↓11.4%)
25+60	1.051* (↑1.4%)	1.048* (↑3.3%)	34.5* (↑3-fold)	21.8 (↑37.1%)	6.5 (↓3.0%)	6.3 (↓10.0%)

^ Statistical analysis not performed
* p value < 0.05

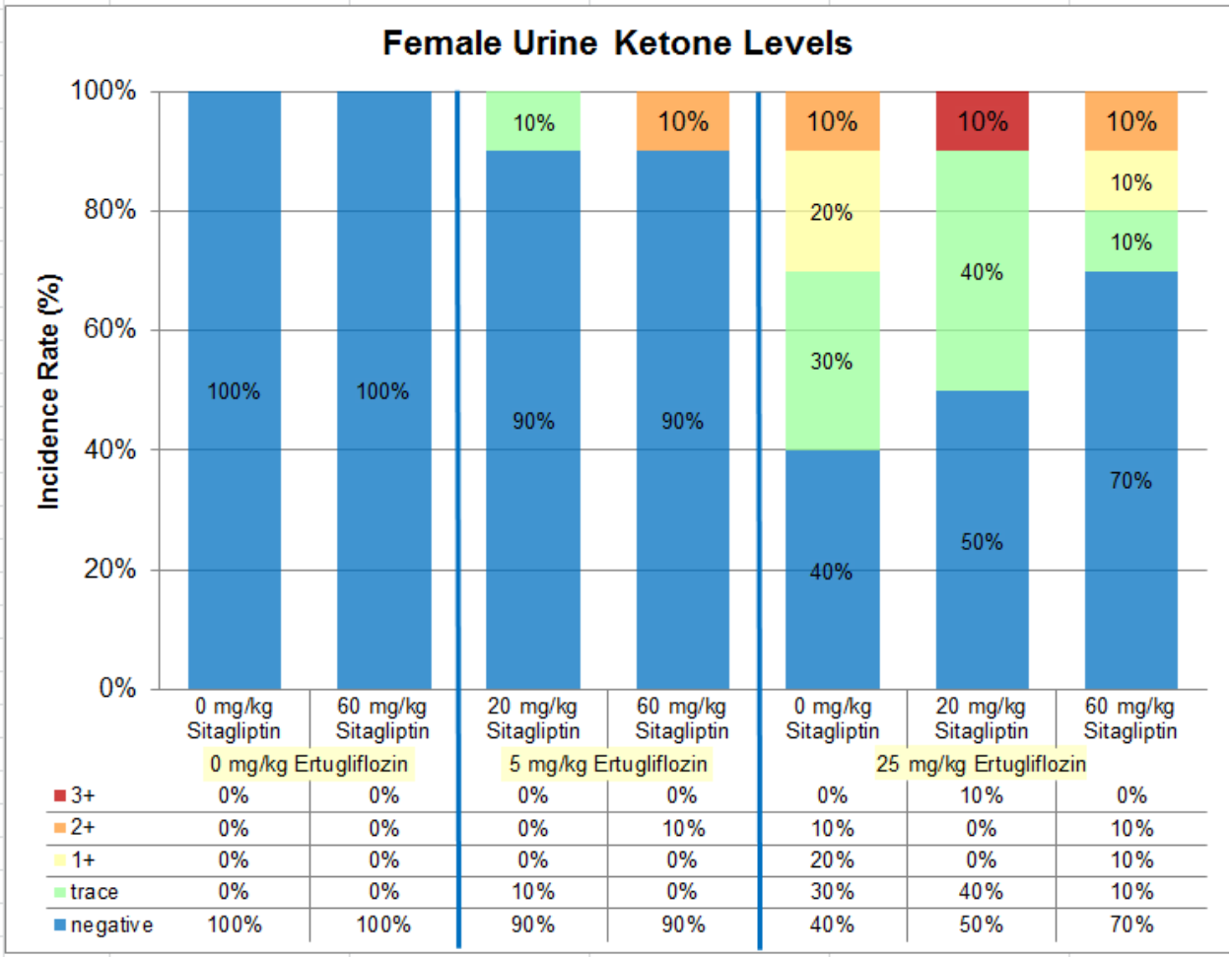
Moderate to marked urine ketone levels of ≥40 mg/dL (2+ and 3+) were observed in 90% of males receiving 25 mg/kg PF-04971729 alone and 20% of males receiving coadministration of 25 mg/kg PF-04971729 and sitagliptin. Decreases in incidence and severity of ketones were observed with co-administration of sitagliptin, which indicates improvement of ketone levels. Importantly, there was not a negative interaction regarding ketonuria with sitagliptin co-administration in male rats.

Figure 1: Male Ketone Urinalysis - 13-week Rat Study #14GR162



In general, urine ketone levels were lower in females, regardless of vehicle or drug administration. Increases in incidence and severity of ketones were observed in females with PF-04971729 administration, and were generally independent of sitagliptin coadministration. The highest incidence rates of females with ketone bodies were observed with 25 mg/kg PF-04971729, with mild (15 mg/dL, 1+) to marked (80 mg/dL, 3+) levels of ketonuria in 30% to 50% of females. It is noted that a decrease in incidence of ketones was observed with co-administration of 60 mg/kg, despite administration of 25 mg/kg PF-04971729, which indicates improvement of ketone levels. Importantly, there was not a negative interaction regarding ketonuria with sitagliptin co-administration in male rats.

Figure 2: Female Ketone Urinalysis - 13-week Rat Study #14GR162



Gross Pathology

Incidences of mucosal discoloration were observed in the glandular stomach were observed in both sexes treated with ≥25 mg/kg PF-04971729. Furthermore, there was an apparent dose-dependent increase in males with co-administration of sitagliptin, indicating exacerbation. In females, the largest incidence of mucosal discoloration was observed in animals treated with 25 mg/kg PF-04971729 alone. Mucosal discoloration

was most often dark red and sometimes black, grey, or white, corresponding with erosion, submucosal inflammation, and/or hemorrhage.

Pelvic enlargement was reported in the kidneys of 2 males treated with 25 mg/kg PF-04971729, which correlated with microscopic findings of renal dilatation.

Sponsor's Table 4: Macroscopic Findings - 13-week Rat Study #14GR162

Table
Incidence of Macroscopic Observations
Terminal Euthanasia (Dosing Phase)

Test Article	(dosage)	1	2	3	4	5	6	7
PF-04971729	mg/kg/day	0	0	25	5	5	25	25
Sitagliptin	mg/kg/day	0	60	0	20	60	20	60

Tissue/ Observation	Group/Subgroup/Sex: Number of Animals:	1/1/M 10	2/1/M 10	3/1/M 10	4/1/M 10	5/1/M 10	6/1/M 10	7/1/M 10
Stomach	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	9	10	9	8	6
Discolored		0	0	1	0	1	2	4
Kidney	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	9	10	10	9	10
Large		0	0	1	0	0	1	0

Table
Incidence of Macroscopic Observations
Terminal Euthanasia (Dosing Phase)

Test Article	(dosage)	1	2	3	4	5	6	7
PF-04971729	mg/kg/day	0	0	25	5	5	25	25
Sitagliptin	mg/kg/day	0	60	0	20	60	20	60

Tissue/ Observation	Group/Subgroup/Sex: Number of Animals:	1/1/F 10	2/1/F 10	3/1/F 10	4/1/F 10	5/1/F 10	6/1/F 10	7/1/F 10
Stomach	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	6	10	10	9	9
Discolored		0	0	4	0	0	1	1

(Tables excerpted from Sponsor's report and highlighted)

Organ Weights

PF-04971729-related increases in kidney weights (absolute, relative body and relative brain weights) were observed in all male (↑14-35%) and female (↑21-35%) PF-04971729 treatment groups, but not in animals treated with sitagliptin alone. There was no indication of exacerbation of increased kidney weight with co-administration of sitagliptin. The increased kidney weights were considered to be due to PF-04971729 treatment, but were independent of sitagliptin administration.

Increases in adrenal weights were observed in both sexes treated with co-administration of 25 mg/kg PF-04971729 and sitagliptin. However, statistical significance was only achieved in weights relative to body weight and only in males (↑32%) at 25+60 and in females (↑20%) at 25+20. Nevertheless, this finding is consistent with other studies involving PF-04971729 administration in rats and is considered likely to be a drug-related finding that is exacerbated by sitagliptin co-administration.

Sponsor's Table 5: Organ Weights - 13-week Rat Study #14GR162

Text Table 4.1: Test Article-Related Changes in Organ Weight Parameters

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/0	0/60	25/0	5/20	5/60	25/20	25/60	0/0	0/60	25/0	5/20	5/60	25/20	25/60
Kidney														
Absolute Weight (g)	3.0101	1.07	1.25*	1.19*	1.14	1.21*	1.23*	1.6836	.99	1.25*	1.21*	1.20*	1.25*	1.22*
Body Weight Ratio (%)	0.5445	1.07	1.33*	1.32*	1.25*	1.35*	1.33*	0.5745	.99	1.33*	1.25*	1.27*	1.35*	1.30*
Brain Weight Ratio (%)	135.2726	1.06	1.27*	1.21*	1.17*	1.22*	1.25*	80.7133	1.03	1.29*	1.25*	1.22*	1.27*	1.27*
Adrenal														
Absolute Weight (g)	0.0612	1.09	1.15	1.01	1.13	1.05	1.23	0.0669	.94	1.07	.94	.95	1.11	1.03
Body Weight Ratio (%)	0.0111	1.08	1.23	1.11	1.24	1.16	1.32*	0.0228	.94	1.14	.98	1.00	1.20*	1.09
Brain Weight Ratio (%)	2.7525	1.07	1.17	1.02	1.16	1.05	1.25	3.2085	.98	1.10	.97	.96	1.12	1.07

* = Statistically significant ($p \leq 0.05$) difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold of control mean value.

(Table excerpted from sponsor's package)

Histopathology

Battery Considered Adequate? Yes
Peer Review Performed? Yes

Increases in incidence and severity of kidney tubule dilatation was observed in males and females with increasing PF-04971729 administration, but were independent of sitagliptin treatment. Furthermore, all animals treated with 25 mg/kg PF-04971729 presented with minimal to mild tubule dilatation. Increased incidence or severity of pelvis dilatation was also observed in males in all PF-04971729 treatment groups except 5+20 and in females at 5+20 and 25+60. Overall, the kidney findings of tubule and pelvic dilatation are considered likely to be drug-related.

Increases of mixed cell inflammation in the prostate were observed in males co-administered both drugs, with an apparent dose-dependence of sitagliptin with 25 mg/kg PF-04971729 co-administration.

Stomach findings of erosion, hemorrhage and inflammation were reported in both sexes. In general, increases in incidence and/or severity of glandular stomach erosion were observed in both sexes. However, there was not a clear dose-dependence in females since this effect was not seen in the highest co-administration group 25+60. Findings of minimal acute submucosal inflammation were also noted in both sexes with PF-04971729 administration alone or in combination with sitagliptin, and with PF-04971729 dose-dependence in males, but not in females. Incidences of minimal hemorrhage were also reported with PF-04971729 administration alone in males and with sitagliptin co-administration with 25 mg/kg PF04971729 in both sexes. Overall, the stomach histopathological findings are consistent with previous findings in rats with PF-04971729 and are considered to be PF-04971729-related, but largely independent of sitagliptin administration.

Hypertrophy in the zona glomerulosa of the adrenal cortex was observed in both sexes with PF-04971729 administration. Furthermore, the increases in incidence and/or severity were increased with sitagliptin co-administration.

Pancreatic zymogen depletion was observed in all groups treated with PF-04971729, independent of sitagliptin administration, with increased severity in males.

Sponsor's Table 6: Histopathology - 13-week Rat Study #14GR162

Text Table 3.3: Incidence and Severity of Test Article-Related Microscopic Findings

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/ 0	0/ 60	25/ 0	5/ 20	5/ 60	25/ 20	25/ 60	0/ 0	0/ 60	25/ 0	5/ 20	5/ 60	25/ 20	25/ 60
Number Examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Kidney														
Dilatation, tubule(s)														
Not Present	6	6	0	0	1	0	0	6	8	0	3	1	0	0
Minimal	4	4	1	9	9	6	5	3	2	5	7	9	4	3
Mild	0	0	9	1	0	4	5	1	0	5	0	0	6	7
Dilatation, pelvis														
Not Present	9	9	7	10	8	4	7	10	10	10	9	10	10	8
Minimal	1	1	3	0	2	6	3	0	0	0	0	0	0	2
Mild	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Prostate														
Inflammation, mixed cell														
Not Present	10	10	10	9	10	9	8	NA	NA	NA	NA	NA	NA	NA
Minimal	0	0	0	0	0	1	2	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA
Stomach, Glandular														
Erosion														
Not Present	9	10	8	10	9	7	6	10	9	9	10	9	8	10
Minimal	1	0	1	0	0	0	4	0	1	0	0	0	2	0
Mild	0	0	1	0	1	3	0	0	0	1	0	1	0	0
Inflammation, acute, submucosa														
Not Present	10	10	8	9	9	6	8	10	10	9	10	9	9	10
Minimal	0	0	2	1	1	4	2	0	0	1	0	1	1	0
Hemorrhage														
Not Present	10	10	8	10	10	7	8	10	10	10	10	10	8	9
Minimal	0	0	2	0	0	3	2	0	0	0	0	0	2	1
Adrenal, Cortex														
Hypertrophy, zona glomerulosa														
Not Present	10	10	1	5	2	1	0	10	9	6	6	4	5	2
Minimal	0	0	8	5	4	6	7	0	1	4	3	3	4	5
Mild	0	0	1	0	4	3	3	0	0	0	1	3	1	3
Pancreas														
Zymogen depletion														
Not Present	10	10	1	0	1	0	0	10	9	2	7	6	2	4
Minimal	0	0	4	8	4	7	5	0	1	8	3	4	8	6
Mild	0	0	5	2	5	3	5	0	0	0	0	0	0	0

NA = Not applicable.

(Table excerpted from sponsor's package and highlighted)

Toxicokinetics

PF-04971729 exposures increased dose-proportionally. Exposures in females tended to be slightly higher, but were not considered to be significant. Exposures were also slightly higher on Day 91 by 12-28% with co-administration, indicating a possible trend for accumulation with high doses of both drugs. T_{max} ranged from 1 to 7 hours postdose, with a trend for delayed T_{max} with high dose co-administration of both drugs (25+60).

Sponsor's Table 7: PF-04971729 Toxicokinetics - 13-week Rat Study #14GR162

6.1. Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
25 / 0	1	Male	7060	4	99100
		Female	10700	4	125000
		Overall	8880	4	112000
	91	Male	8930	4	91200
		Female	9240	4	138000
		Overall	9080	4	114000
5 / 20	1	Male	1920	7	24900
		Female	2350	4	24100
		Overall	1700	4	24000
	91	Male	1400	1	14100
		Female	2970	1	27800
		Overall	2180	1	21000
5 / 60	1	Male	1720	7	23700
		Female	1940	4	25700
		Overall	1800	4	24700
	91	Male	2520	1	21900
		Female	3060	1	27700
		Overall	2790	1	24800
25/20	1	Male	8390	7	120000
		Female	9910	4	120000
		Overall	8930	4	120000
	91	Male	12500	4	157000
		Female	15100	4	150000
		Overall	13800	4	153000
25/60	1	Male	6380	7	89700
		Female	9370	7	131000
		Overall	7870	7	110000
	91	Male	9710	7	122000
		Female	11800	4	124000
		Overall	8240	7	123000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package)

Sitagliptin exposures increased proportionally with dose and were not significantly affected by co-administration of PF-04971729. There were no significant signs of gender effects or accumulation. T_{max} ranged between 1 and 7 hours post-dose.

Sponsor's Table 8: Sitagliptin Toxicokinetics - 13-week Rat Study #14GR162

6.2. Mean Toxicokinetic Parameters for Sitagliptin in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
0 / 60	1	Male	2670	4	27500
		Female	2120	4	19000
		Overall	2400	4	23300
	91	Male	3660	4	37800
		Female	2900	1	22500
		Overall	3140	1	30100
5 / 20	1	Male	778	4	8670
		Female	613	4	5640
		Overall	695	4	7140
	91	Male	853	4	7390
		Female	1000	4	6460
		Overall	929	4	6930
5 / 60	1	Male	3450	7	45300
		Female	2670	4	29300
		Overall	2900	4	37300
	91	Male	4450	1	32700
		Female	3740	1	24500
		Overall	4090	1	28600
25/20	1	Male	382	7	5260
		Female	455	4	5490
		Overall	417	4	5380
	91	Male	1170	4	9050
		Female	807	4	7720
		Overall	989	4	8400
25/60	1	Male	2370	7	28600
		Female	1670	7	22500
		Overall	2020	7	25500
	91	Male	2360	4	26200
		Female	2780	4	22500
		Overall	2570	4	24400

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package)

Dosing Formulation Analysis

PF-04971729 and sitagliptin dose formulations were analyzed using a validated HPLC method. Overall mean concentrations of PF-04971729 and sitagliptin formulations were within ±10% of the target concentrations.

7 Genetic Toxicology

Genetic toxicology studies with the FDC or coadministration of ertugliflozin and sitagliptin have not been conducted, but are not required. Since both ertugliflozin and sitagliptin are not genotoxic; there is not a genotoxic concern with the FDC product.

Ertugliflozin

Based on the weight of evidence, ertugliflozin is not considered to be genotoxic. Ertugliflozin was evaluated for genotoxic potential in a standard battery of valid genotoxicity assays, including in vitro microbial reverse mutation (Ames), in vitro human lymphocyte cytogenetic, and in vivo rat micronucleus assays.

Sitagliptin

There is no evidence of a mutagenic potential for sitagliptin based on in vitro Ames, hepatocyte alkaline elution, and chromosome aberration assays or an in vivo mouse micronucleus induction assay.

8 Carcinogenicity

Combination carcinogenicity studies with the FDC or coadministration of ertugliflozin and sitagliptin have not conducted, but are not required.

Ertugliflozin

Rat and mouse carcinogenicity studies with administration of ertugliflozin alone were reviewed under NDA #209803.

In the 2-year carcinogenicity study conducted in male and female Crl:CD1(ICR) mice, ertugliflozin was administered daily at doses of 5, 15 or 40 mg/kg/day, in accordance with ECAC dosing recommendations. All male groups were terminated during Week 97 and all female groups were terminated during Week 102 due to low survival that was not drug-related. There were no significant drug-related neoplastic findings in male or female mice at any of the doses examined, and the NOAEL for neoplasms was set at the high dose of 40 mg/kg/day (~50x $MRHD_{AUC}$). It is also noted that non-adverse PD-related kidney and bladder findings were considered to be comparable to similar findings observed in shorter toxicology studies.

In the 2-year carcinogenicity study conducted in male and female SD rats, ertugliflozin was administered daily at doses of 1.5, 5, or 15 mg/kg/day ertugliflozin, in accordance with ECAC dosing approval with the exception of exclusion of a saline/water control group. In female rats, there were no statistically significant increases in incidences of benign or malignant neoplasms in any tissues, with a neoplastic NOAEL of 15 mg/kg/day (74x $MRHD_{AUC}$). However, in male rats, drug-related increases in the incidences of adrenal medulla benign pheochromocytoma (PCC) and combined benign + malignant PCC neoplasms were reported at 15 mg/kg/day (66x $MRHD_{AUC}$), resulting in a NOAEL for neoplasms of 5 mg/kg/day (18x $MRHD_{AUC}$). The incidence rates and timing of PCC observations correlated with drug-related increases in adrenal medulla hyperplasia at ≥5 mg/kg/day in a manner that was considered to be consistent with a continuum of tumor development. Thus, the increased incidences of adrenal medulla hyperplasia and PCC observed at ≥5 mg/kg/day were considered possibly drug-related, but were not considered to be unequivocally drug-related.

Sitagliptin

Chronic carcinogenicity studies with sitagliptin were been performed in rats and mice. In rats, increases in the incidence of combined liver adenoma/carcinoma were observed in males and females at 500 mg/kg/day (~62x MRHD), which was considered possibly related to non-genotoxic chronic hepatotoxicity. No drug-related neoplasms were

observed in mice at doses up to 500 mg/kg/day (~72x MRHD). Overall, the carcinogenic risk to humans at clinical doses was considered to be minimal.

9 Reproductive and Developmental Toxicology

Combination reproductive and developmental toxicology studies with the FDC or coadministration of ertugliflozin and sitagliptin have not conducted, but are not required. Based on results from a juvenile toxicology study in rats, ertugliflozin exposure poses a potential risk to human renal development. Thus, the FDC product will also have a potential risk to human renal development and a combination embryonic fetal development (EFD) study is not required, in accordance ICH M3(R2), as a hazard has already been identified for the ertugliflozin component. Therefore labeling for reproductive and developmental hazards of the FDC will be based on each individual drug component. Please see original NDA reviews for ertugliflozin (NDA 209803) and sitagliptin (NDA 21995) for experimental detail.

10 Special Toxicology Studies

No special toxicology studies were conducted the combination.

11 Labeling Review

Only labeling specific to the FDC or the sitagliptin component are captured in this review. Please see the NDA review under #209803 for labeling recommendations regarding the ertugliflozin component.

Section 8 Use in Specific Populations

Section 8.1 Pregnancy

Excerpt 1: Sponsor's Proposed Section 8.1 Text

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to sitagliptin during pregnancy. Health care providers are encouraged to report any prenatal exposure to TRADEMARK by calling the Pregnancy Registry at 1-800-986-8999.

Risk Summary

(b) (4)

(b) (4) TRADEMARK is not recommended during the second and third trimesters of pregnancy.

(b) (4)

In rats and rabbits, sitagliptin doses of 250 and 125 mg/kg, respectively (approximately 30 and 20 times the human exposure at the maximum recommended human dose) did not adversely affect development outcomes of either species.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk

Poorly-controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, spontaneous abortions, preterm delivery, stillbirth, and delivery complications. It can also increase the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Animal Data

Ertugliflozin

In embryo-fetal development studies, ertugliflozin (50, 100 and 250 mg/kg/day) was administered orally to rats on gestation days 6 to 17 and to rabbits on gestation days 7 to 19. Ertugliflozin did not adversely affect developmental outcomes in rats and rabbits at maternal exposures that were (b) (4) (b) (4) the human exposure at the maximum clinical dose of 15 mg/day, based on AUC. At a maternally toxic dose in rats (250 mg/kg/day), lower fetal viability, (b) (4) a higher incidence of a visceral malformation (membranous ventricular septal defect) (b) (4) (b) (4) (b) (4) In the pre- and postnatal development study, decreased postnatal growth (b) (4) (b) (4) were observed in rats administered ertugliflozin gestation day 6 through lactation day 21 at ≥100 mg/kg/day (b) (4) times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC).

When ertugliflozin was orally administered to juvenile rats from PND 21 to PND 90, increased kidney weight, renal tubule and renal pelvis dilatation, and renal mineralization occurred at doses greater than or equal to 5 mg/kg (13-fold human exposures). These effects did not fully reverse within the 1 month recovery period. (b) (4)

Sitagliptin

Sitagliptin administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) did not adversely affect developmental outcomes at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100 mg/day based on AUC comparisons. Higher doses increased the incidence of rib malformations in offspring at 1,000 mg/kg, or approximately 100 times human exposure at the MRHD. (b) (4) (b) (4)

Sitagliptin administered to female rats from gestation day 6 to lactation day 21 decreased body weight in male and female offspring at 1,000 mg/kg. No functional or behavioral toxicity was observed in offspring of rats.

Placental transfer of sitagliptin administered to pregnant rats was approximately 45% at 2 hours and 80% at 24 hours postdose. Placental transfer of sitagliptin administered to pregnant rabbits was approximately 66% at 2 hours and 30% at 24 hours.

(Excerpted from sponsor's package)

Reviewer's Comments

The following statement under the Animal Data section for sitagliptin was removed (b) (4) Although not necessarily inaccurate, this statement is considered to be unnecessary and is not included in the current 01/2017 label for sitagliptin.

Reviewer's Proposed Section 8.1 Text

Sitagliptin

Sitagliptin administered to pregnant female rats and rabbits from gestation day 6 to 20 (organogenesis) did not adversely affect developmental outcomes at oral doses up to 250 mg/kg (rats) and 125 mg/kg (rabbits), or approximately 30- and 20-times human exposure at the maximum recommended human dose (MRHD) of 100 mg/day based on AUC comparisons. Higher doses increased the incidence of rib malformations in offspring at 1,000 mg/kg, or approximately 100 times human exposure at the MRHD. (b) (4)

Sitagliptin administered to female rats from gestation day 6 to lactation day 21 decreased body weight in male and female offspring at 1,000 mg/kg. No functional or behavioral toxicity was observed in offspring of rats.

Placental transfer of sitagliptin administered to pregnant rats was approximately 45% at 2 hours and 80% at 24 hours postdose. Placental transfer of sitagliptin administered to pregnant rabbits was approximately 66% at 2 hours and 30% at 24 hours.

Section 8.2 Lactation

Excerpt 2: Sponsor's Proposed Section 8.2 Text

8.2 Lactation

Risk Summary

There is no information regarding the presence of TRADEMARK, (b) (4) in human milk, the effects on the breastfed infant, or the effects on milk production. Ertugliflozin and sitagliptin are present in the milk of lactating rats *[see Data]*. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney, based on data with ertugliflozin.

(b) (4)

Data

Animal Data

Ertugliflozin

The lacteal excretion of radiolabeled ertugliflozin in lactating rats was evaluated 10 to 12 days after parturition. Ertugliflozin derived radioactivity exposure in milk and plasma were similar, with a milk/plasma ratio of 1.07, based on AUC.

Sitagliptin

Sitagliptin is secreted in the milk of lactating rats at a milk to plasma ratio of 4:1.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's animal data regarding sitagliptin are supported by the available data and are considered to be adequate.

Section 12 Clinical Pharmacology

Section 12.1 Mechanism of Action

Excerpt 3: Sponsor's Proposed Section 12.1 Text

12.1 Mechanism of Action

TRADEMARK

TRADEMARK combines two antihyperglycemic agents with complementary mechanisms of action to improve glycemic control in patients with type 2 diabetes: ertugliflozin, a SGLT2 inhibitor, and sitagliptin, a DPP-4 inhibitor.

Ertugliflozin

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Ertugliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, ertugliflozin reduces renal reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion.

Sitagliptin

Sitagliptin is a DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones. Concentrations of the active intact hormones are increased by sitagliptin, thereby increasing and prolonging the action of these hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal. These hormones are rapidly inactivated by the enzyme, DPP-4. The incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells by intracellular signaling pathways involving cyclic AMP. GLP-1 also lowers glucagon secretion from pancreatic alpha cells, leading to reduced hepatic glucose production. By increasing and prolonging active incretin levels, sitagliptin increases insulin release and decreases glucagon levels in the circulation in a glucose-dependent manner. Sitagliptin demonstrates selectivity for DPP-4 and does not inhibit DPP-8 or DPP-9 activity *in vitro* at concentrations approximating those from therapeutic doses.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's proposed text is supported by the nonclinical data and is considered to be acceptable.

Section 12.3 Pharmacokinetics

Excerpt 4: Sponsor's Proposed Section 12.3 Text

Metabolism

Ertugliflozin

Metabolism is the primary clearance mechanism for ertugliflozin. The major metabolic pathway for ertugliflozin is UGT1A9 and UGT2B7-mediated O-glucuronidation to two glucuronides that are pharmacologically inactive at clinically relevant concentrations. CYP-mediated (oxidative) metabolism of ertugliflozin is minimal (12%).

Sitagliptin

Approximately 79% of sitagliptin is excreted unchanged in the urine with metabolism being a minor pathway of elimination.

Following a [^{14}C]sitagliptin oral dose, approximately 16% of the radioactivity was excreted as metabolites of sitagliptin. Six metabolites were detected at trace levels and are not expected to contribute to the plasma DPP-4 inhibitory activity of sitagliptin. *In vitro* studies indicated that the primary enzyme responsible for the limited metabolism of sitagliptin was CYP3A4, with contribution from CYP2C8.

Elimination

Ertugliflozin

The mean systemic plasma clearance following an intravenous 100 µg dose was 11.2 L/hr. The mean elimination half-life in type 2 diabetic patients with normal renal function was estimated to be 16.6 hours based on the population pharmacokinetic analysis. Following administration of an oral [^{14}C]-ertugliflozin solution to healthy subjects, approximately 40.9% and 50.2% of the drug-related radioactivity was eliminated in feces and urine, respectively. Only 1.5% of the administered dose was excreted as unchanged ertugliflozin in urine and 33.8% as unchanged ertugliflozin in feces, which is likely due to biliary excretion of glucuronide metabolites and subsequent hydrolysis to parent.

Sitagliptin

Following administration of an oral [^{14}C]sitagliptin dose to healthy subjects, approximately 100% of the administered radioactivity was eliminated in feces (13%) or urine (87%) within one week of dosing. The apparent terminal $t_{1/2}$ following a 100-mg oral dose of sitagliptin was approximately 12.4 hours and renal clearance was approximately 350 mL/min.

Elimination of sitagliptin occurs primarily via renal excretion and involves active tubular secretion. Sitagliptin is a substrate for human organic anion transporter-3 (hOAT-3), which may be involved in the renal elimination of sitagliptin. The clinical relevance of hOAT-3 in sitagliptin transport has not been established. Sitagliptin is also a substrate of p-glycoprotein, which may also be involved in mediating the renal elimination of sitagliptin. However, cyclosporine, a p-glycoprotein inhibitor, did not reduce the renal clearance of sitagliptin.

Ertugliflozin

In Vitro Assessment of Drug Interactions

In *in vitro* studies, ertugliflozin and ertugliflozin glucuronides did not inhibit CYP450 isoenzymes (CYPs) 1A2, 2C9, 2C19, 2C8, 2B6, 2D6, or 3A4, and did not induce CYPs 1A2, 2B6, or 3A4. Ertugliflozin was not a time-dependent inhibitor of CYP3A *in vitro*. Ertugliflozin did not inhibit UGT1A6, 1A9, or 2B7 *in vitro* and was a weak inhibitor (IC_{50} >39 µM) of UGT1A1 and 1A4. Ertugliflozin glucuronides did not inhibit UGT1A1, 1A4, 1A6, 1A9, or 2B7 *in vitro*. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of drugs eliminated by these enzymes. Ertugliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and is not a substrate of organic anion transporters (OAT1, OAT3), organic cation transporters (OCT1, OCT2), or organic anion transporting polypeptides (OATP1B1, OATP1B3). Ertugliflozin or ertugliflozin glucuronides do not meaningfully inhibit P-gp, OCT2, OAT1, or OAT3 transporters at clinically relevant concentrations. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of concurrently administered medications that are substrates of these transporters.

Sitagliptin

In Vitro Assessment of Drug Interactions

Sitagliptin is not an inhibitor of CYP isozymes CYP3A4, 2C8, 2C9, 2D6, 1A2, 2C19 or 2B6, and is not an inducer of CYP3A4. Sitagliptin is a p-glycoprotein substrate, but does not inhibit p-glycoprotein mediated transport of digoxin. Based on these results, sitagliptin is considered unlikely to cause interactions with other drugs that utilize these pathways.

Sitagliptin is not extensively bound to plasma proteins. Therefore, the propensity of sitagliptin to be involved in clinically meaningful drug-drug interactions mediated by plasma protein binding displacement is very low.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's nonclinical data regarding sitagliptin are supported by the available data, are consistent with the current sitagliptin label (01/2017), and are considered to be adequate.

Section 13 Nonclinical Toxicology

Section 13.1 Carcinogenicity & Mutagenesis & Impairment of Fertility

Excerpt 5: Sponsor's Proposed Section 13.1 Text

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Ertugliflozin

In the (b) (4) mouse (b) (4) study, ertugliflozin was administered by oral gavage at doses of 5, 15, and 40 mg/kg/day. There were no ertugliflozin-related neoplastic findings at doses up to 40 mg/kg/day (approximately (b) (4) times human exposure at the maximum recommended human dose [MHRD] of 15 mg/day based on AUC). In the (b) (4) rat (b) (4) study, ertugliflozin was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg/day. Ertugliflozin-related neoplastic findings included an increased incidence of (b) (4) adrenal medullary pheochromocytoma in male rats at 15 mg/kg/day. This finding was attributed to carbohydrate malabsorption leading to altered calcium homeostasis (b) (4) to human risk. The no-observed-effect level (NOEL) for neoplasia was 5 mg/kg/day (approximately 16 times human exposure at the MRHD of 15 mg/day).

Sitagliptin

A two year carcinogenicity study was conducted in male and female rats given oral doses of sitagliptin of 50, 150, and 500 mg/kg/day. There was an increased incidence of combined liver adenoma/carcinoma in males and females and of liver carcinoma in females at 500 mg/kg. This dose results in exposures approximately 60 times the human exposure at the maximum recommended daily adult human dose (MRHD) of 100 mg/day based on AUC comparisons. Liver tumors were not observed at 150 mg/kg, approximately 20 times the human exposure at the MRHD.

A two year carcinogenicity study was conducted in male and female mice given oral doses of sitagliptin of 50, 125, 250, and 500 mg/kg/day. There was no increase in the incidence of tumors in any organ up to 500 mg/kg, approximately 70 times human exposure at the MRHD.

Mutagenesis

Ertugliflozin

Ertugliflozin was not mutagenic or clastogenic with or without metabolic activation in the microbial reverse mutation, *in vitro* cytogenetic (human lymphocytes), and *in vivo* rat micronucleus assays.

Sitagliptin

Sitagliptin was not mutagenic or clastogenic with or without metabolic activation in the Ames bacterial mutagenicity assay, a Chinese hamster ovary (CHO) chromosome aberration assay, an *in vitro* cytogenetics assay in CHO, an *in vitro* rat hepatocyte DNA alkaline elution assay, and an *in vivo* micronucleus assay.

Impairment of Fertility

Ertugliflozin

In the rat fertility and embryonic development study, male and female rats were administered ertugliflozin at 5, 25, and 250 mg/kg/day. No effects on fertility were observed at 250 mg/kg/day (approximately (b) (4) times human exposure at the MRHD of 15 mg/day based on AUC comparison).

Sitagliptin

In rat fertility studies with oral gavage doses of 125, 250, and 1000 mg/kg, males were treated for 4 weeks prior to mating, during mating, up to scheduled termination (approximately 8 weeks total) and females were treated 2 weeks prior to mating through gestation day 7. No adverse effect on fertility was observed at 125 mg/kg (approximately 12 times human exposure at the MRHD of 100 mg/day based on AUC comparisons). At higher doses, nondose-related increased resorptions in females were observed (approximately 25 and 100 times human exposure at the MRHD based on AUC comparison).

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's text regarding sitagliptin is reflected in the most recent sitagliptin label (01/2017) and is considered to be appropriate.

Section 13.2 Animal Pharmacology and/or Toxicology

This section was not included in the sponsor's proposed label.

Reviewer's Comments

This section was also not included in the label for ertugliflozin or sitagliptin. Thus, omission of this section is considered to be acceptable.

12 Integrated Summary and Safety Evaluation

This review evaluates the nonclinical safety profile of the NME ertugliflozin FDC with sitagliptin submitted by Merck Sharp and Dohme Corp for the treatment of T2DM. The non-clinical pharmacology, general toxicology, carcinogenicity, reproductive and developmental, and special toxicology studies establishing the safety profile of the NME alone were fully evaluated under NDA #209803, but were also submitted in support of the FDC product under NDA #209805. The approved label for Januvia® (sitagliptin) is also referenced.

Ertugliflozin inhibits SGLT2 resulting in significant glucosuria, which is associated with concomitant decreases in plasma glucose levels despite compensatory increases in food consumption. Sitagliptin suppresses glucagon release and increases insulin secretion; leading to normalization of blood glucose levels. Thus, although both ertugliflozin and sitagliptin reduce blood glucose levels, they act through different mechanisms.

Based on pathways of elimination, absorption and metabolization, significant DFI on PK parameters between ertugliflozin and sitagliptin are not anticipated. Furthermore, concomitant administration of ertugliflozin and sitagliptin were not associated with significant changes in drug exposures in humans.

Based on safety pharmacology studies, both ertugliflozin and sitagliptin may be associated with some concern for CV effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. However, no CV-related toxicities were observed in the combination toxicology studies. Thus, further evaluation of the FDC product in nonclinical safety pharmacology studies is not warranted.

In accordance with ICH guidelines, the sponsor submitted a GLP-compliant 3-month combination toxicology study with coadministration of ertugliflozin and sitagliptin in rats. The majority of the drug-related findings were attributed to PD-related inhibition of SGLT2 mediated by ertugliflozin. Ertugliflozin PD-related kidney tubule dilatations and increased organ weights were not associated with indications of kidney dysfunction or toxicity and were considered likely to be non-adverse. It is noted that similar ertugliflozin-related findings were observed with coadministration of metformin (reference NDA #209806); however, the degree of glucose excursion was lower in animals receiving coadministration of sitagliptin compared to those being co-administered metformin. The DPP4 inhibitor drug class has been associated with decreases in glucose excursion, which may partially counteract the PD activity of ertugliflozin to some extent.

Potential additive effects of ertugliflozin and sitagliptin coadministration were observed in the stomach and adrenal gland. Potential combination-related increases in the incidences of stomach discoloration and minimal to mild erosion/ulcer were observed in male rats. In female rats, potential combination-related increases in incidences of stomach hemorrhage were reported. Since both ertugliflozin and sitagliptin have been associated with stomach findings in previous nonclinical toxicology studies, it's likely that exacerbation of the stomach findings is due to additive toxicities of both PF-04971729 and sitagliptin. However, the stomach findings were of low severity and were not considered to be adverse. Furthermore, since off-target SGLT1 inhibition is not likely in humans, additive ertugliflozin-related effects on the stomach and digestive tract are not likely to be observed clinically. Increases in adrenal gland weights and hypertrophy were considered likely related to additive effects of ertugliflozin and sitagliptin, but were non-adverse. Overall, there were no toxicologically significant adverse synergistic toxicities due to coadministration of ertugliflozin and sitagliptin. Thus, the NOAEL was set at the high combination dose of 25 mg/kg ertugliflozin and 60 mg/kg sitagliptin, with safety margins of 89x $MRHD_{AUC}$ for a high dose of 15 mg/day ertugliflozin 9x $MRHD_{AUC}$ for a high dose of 100 mg/day sitagliptin.

Observations of ertugliflozin-related ketonuria in rats correlate with reductions in body weights and may be secondary to ertugliflozin PD-related inhibition of carbohydrate absorption and decreases in glucose levels, which may be consistent with non-adverse nutritional ketosis. Although ertugliflozin-mediated ketonuria is non-adverse in rats, given that diabetic ketoacidosis (DKA) has been observed clinically in diabetic patients treated with SGLT2 inhibitors, this finding is notable. In the 13-week rat combination toxicology study, improvement of ketonuria was observed with coadministration of sitagliptin in both males and females.

In summary, the 13-week coadministration toxicology study in rats adequately bridges the proposed FDC to the ertugliflozin nonclinical studies and did not identify and new clinically relevant, toxicologically significant toxicities or interactions. Since the safety margins for coadministration of ertugliflozin (111x MRHD) and sitagliptin (9x MRHD) in the 13-week rat study are sufficient, the nonclinical data support clinical dosing of the FDC product at ertugliflozin doses up 15 mg/day ertugliflozin and sitagliptin doses up to 100 mg/day.

FDC Toxicology Summary Table

Table 9: Ertugliflozin + Sitagliptin Coadministration Human Safety Margins

Study	NOAEL (mg/kg)	Human Safety Margin (Based on AUC*)	Findings
2 Week (Non-GLP) **Ertugliflozin/Sitagliptin:** 5/20, 5/60, 25/20, 25/60, 25/0, & 0/60 mg/kg Ertugliflozin AUC: 27.5, 24.2, 133, 132, 163, & - μg·h/mL Sitagliptin AUC: 7.61, 26.7, 7.65, 26.8, -, & 30.6 μg·h/mL	Ertugliflozin / Sitagliptin **25 / 60**	Ertugliflozin: **56x** Sitagliptin: **7x**	*No significant adverse effects.* **5 / 20 mg/kg (20x/3x MRHD):** ↑food consumption, Blood (↓glucose, ↑BUN, ↓Ca), urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight, **5 / 60 mg/kg (18x/10x MRHD):** ↑food consumption, Blood [↓glucose, ↑BUN, ↓protein, <2-fold ↑AST, <2-fold ↑ALT, ↓Ca], urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight, **25 / 20 mg/kg (96x/3x MRHD):** ↑food, Blood (↓glucose, ↑BUN, ↓Cl, ↓Ca), urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight), pancreas (↓zymogen) **25 / 60 mg/kg (96x/10x MRHD):** ↑food, Blood (↓glucose, ↑BUN, ↓Cl, <2-fold ↑AST, <2-fold ↑ALT, ↓Ca), urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight, tubule mineralization in 2 females), pancreas (↓zymogen) **25 / 0 mg/kg (118x/- MRHD):** ↑food consumption, Blood (↓glucose, ↑BUN, ↓protein, ↓Cl, ↓Ca) urine (glucosuria, ↑specific gravity, ↓pH), kidney (↑organ weight), pancreas (↓zymogen) **0 / 60 mg/kg (-/11x MRHD):** Blood (↓protein (♀)

Study	NOAEL (mg/kg)	Human Safety Margin (Based on AUC*)	Findings
13 Week (GLP) **Ertugliflozin/Sitagliptin:** 5/20, 5/60, 25/20, 25/60, 25/0, & 0/60 mg/kg Ertugliflozin AUC: 21, 24.8, 153, 123, 114, & - μg·h/mL Sitagliptin AUC: 6.93, 28.6, 8.4, 24.4, -, & 30.1 μg·h/mL	Ertugliflozin / Sitagliptin **25 / 60**	Ertugliflozin: **66x** Sitagliptin: **5x**	*No significant adverse effects.* **5 / 20 mg/kg (15x/2x MRHD):** ↓Body weight, ↑Food consumption, Blood (↓Cl, ↓glucose, <2-fold ↑BUN, <2-fold ↑ALT), urine [glucosuria, ↑specific gravity (♀), ↑volume (♂)], kidney [↑weight, minimal-mild tubule dilatation, mild pelvis dilatation (1♀)], stomach minimal inflammation (1♂), pancreas (↓zymogen), adrenal hypertrophy **5 / 60 mg/kg (18x/10x MRHD):** ↓Body weight, ↑Food consumption, Blood [↓Cl, ↓glucose, ↓cholesterol (♀),<2-fold ↑BUN, <2-fold ↑ALT], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂)], kidney (↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation (♂)], stomach [mild erosion (1♂), minimal inflammation], pancreas (↓zymogen), adrenal hypertrophy **25 / 20 mg/kg (110x/3x MRHD):** ↓Body weight, ↑Food consumption, Blood (↓glucose, ↓Cl, ↓Ca, <2-fold ↑BUN, <2-fold ↑ALT), urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], stomach (discolored, minimal-mild erosion, minimal inflammation, minimal hemorrhage), kidney [↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation (♂)], pancreas (↓zymogen), adrenal hypertrophy **25 / 60 mg/kg (89x/9x MRHD):** ↓Body weight, ↑Food consumption, Blood [↓glucose, ↓Cl, ↓Ca, ↓protein (♀), ↓cholesterol (♀),<2-fold ↑BUN, <2-fold ↑ALT], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], stomach [discolored, minimal-mild erosion (♂), minimal inflammation, minimal hemorrhage], kidney (↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation), adrenal (↑weight, hypertrophy), pancreas (↓zymogen) **25 / 0 mg/kg (83x/0x MRHD):** ↑↓Body weight, ↑Food consumption, Blood [↓ Cl, ↓Ca (♀), ↓glucose, ↓protein (♀), ↓cholesterol (♀),<2-fold ↑BUN, <2-fold ↑ALT], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], kidney (↑weight, minimal-mild tubule dilatation, minimal pelvis dilatation (♂)], pancreas (↓zymogen), stomach [discolored, minimal-mild erosion, minimal inflammation, minimal hemorrhage (♂)], adrenal hypertrophy **0 / 60 mg/kg (0x/11x MRHD):** Adrenal hypertrophy (1♀), pancreas ↓zymogen (1♀)

* Based on a maximum once daily dose of 15 mg ertugliflozin / 100 mg sitagliptin with a predicted ertugliflozin exposure of $AUC_{0\text{-}24}$ = 1.38 μg·h/mL and sitagliptin exposure of AUC = 2.81 μg·h/mL

♂ = Males only
♀ = Females only

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

/s/

JESSICA HAWES
08/31/2017

RONALD L WANGE
08/31/2017
I concur with Dr. Hawes' recommendation for approval.

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	209806
Supporting document/s:	SDN 1
Applicant's letter date:	12/19/2016
CDER stamp date:	12/19/2016
Product:	Ertugliflozin and Metformin Hydrochloride
Indication:	Type 2 Diabetes Mellitus
Applicant:	Merck Sharpe and Dohme Corp
Review Division:	DMEP
Reviewer:	Jessica J. Hawes, Ph.D.
Supervisor/Team Leader:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Elizabeth Godwin

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209806 are owned by Merck Sharpe and Dohme Corp or are data for which [name of applicant] has obtained a written right of reference. Any information or data necessary for approval of NDA 209806 that Merck Sharpe and Dohme Corp 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 reflected 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 209806.

TABLE OF CONTENTS

Table of Tables

Table of Sponsor's Tables

1 Executive Summary

1.1 Introduction

Merck Sharp and Dohme Corp. has submitted NDA application packages for the new molecular entity (NME) Ertugliflozin (PF-04971729, MK-8835) alone (NDA #209803, IND #106447) and as fixed dose combination (FDC) products with the marketed drugs Metformin (MK-8835B, NDA #209806, IND #122329) and Sitagliptin (MK-8835A, NDA #209805, IND #122330) for the treatment of type 2 diabetes mellitus (T2DM).

The nonclinical profile for the NME, ertugliflozin, was fully evaluated in the Pharm/Tox review under NDA #209803. This review focuses on evaluation of additional information pertinent to the ertugliflozin + metformin hydrochloride FDC product.

1.2 Brief Discussion of Nonclinical Findings

Coadministration of ertugliflozin and metformin in rats for 13-weeks was generally well-tolerated with sufficient margins of safety and was not associated with significant

adverse systemic toxicities. Furthermore, no toxicologically significant new or synergistic toxicities due to coadministration of PF-04971729 and metformin were observed. Thus, the rat combination toxicology study adequately bridges the proposed FDC product to the ertugliflozin nonclinical safety profile under NDA #209803, with sufficient safety margins based on AUC exposures at the maximum recommended high doses ($MRHD_{AUC}$) of 15 mg/day ertugliflozin (66x $MRHD_{AUC}$) and 2000 mg/day metformin (5x $MRHD_{AUC}$). Overall, the nonclinical data were considered to be sufficient and support clinical dosing of the FDC product at ertugliflozin doses up 15 mg/day ertugliflozin and metformin doses up to 2000 mg/day.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of the ertugliflozin/metformin FDC

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Nonclinical labeling recommendations are below. See Section 11 Labeling Review for a full discussion of proposed changes. Only labeling specific to the FDC or the metformin component are captured in this review. Please see the NDA review under #209803 for labeling recommendations regarding the ertugliflozin component.

Section: 8 USE IN SPECIFIC POPULATIONS

Section 8.1 Pregnancy

Metformin hydrochloride

Metformin did not adversely affect development outcomes when administered to rats and rabbits at doses up to 600 mg/kg/day. This represents an exposure of about 2 and 6 times the maximum recommended human daily dose of 2,000 mg based on body surface area comparisons for rats and rabbits, respectively. Determination of fetal concentrations demonstrated a partial placental barrier to metformin.

2 Drug Information

2.1 Drug

CAS Registry Number
Ertugliflozin: 1210344-57-2
Metformin Hydrochloride: 1115-70-4

Generic Name
Ertugliflozin + metformin

Code Name
Ertugliflozin + metformin FDC: MK-8835B
Ertugliflozin: PF-04971729, MK-8835

Ertugliflozin L-pyroglutamic acid (L-PGA) co-crystal form: PF-04971729 (b) (4)

It is noted that the neutral amorphous form was used for most exploratory toxicology studies, whereas the L-PGA co-crystalline form intended for marketing was used in pivotal toxicology and safety pharmacology studies.

Chemical Name

PF-04971729: (1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol

PF-04971729 (b) (4): (1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol L-pyroglutamic acid

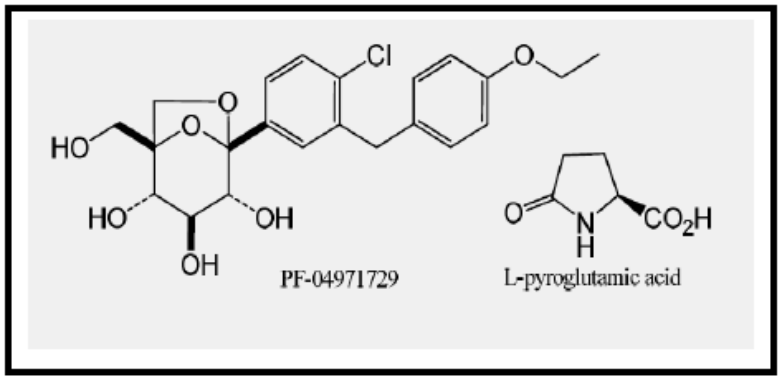
Metformin HCl: 1,1-dimethylbiguanide

Molecular Formula/Molecular Weight

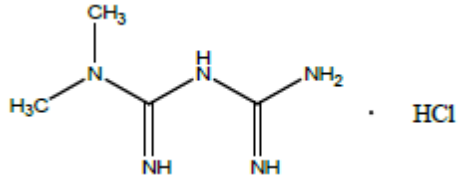
PF-04971729: $C_{22}H_{25}ClO_7$ / 436.88 g/mol
PF-04971729 (b) (4): $C_{27}H_{32}ClNO_{10}$ / 566.00 g/mol
Metformin: $C_4H_{11}N_5$
Metformin Hydrochloride: $C_4H_{12}N_5Cl$

Structure or Biochemical Description

Ertugliflozin L-PGA



Metformin Hydrochloride



Pharmacologic Class

Ertugliflozin: Sodium glucose co-transporter 2 (SGLT2) Inhibitor
Metformin: Biguanide

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA #209803 (IND #106447): MK-8836 Ertugliflozin, Merck Sharp and Dohme Corp

NDA #209805 (IND #122330): MK-8835A (Ertugliflozin + sitagliptin FDC), Merck Sharp and Dohme Corp

NDA #21202 (IND #047342): Metformin HCl (Bristol Myers Squibb)

NDA #63634 (IND #76500): Kombiglyze, combination of Metformin HCl and Saxagliptin (Astrazeneca AB)

2.3 Drug Formulation

The ertugliflozin/metformin FDC will be formulated as film coated tablet in 4 strengths: 2.5 mg ertugliflozin + 500 mg metformin, 2.5 mg ertugliflozin + 1000 mg metformin, 7.5 mg ertugliflozin + 500 mg metformin, and 7.5 mg ertugliflozin + 1000 mg metformin.

Sponsor's Table 1: Ertugliflozin/Metformin FDC Tablet Formulations

Table 1 Ertugliflozin 2.5 mg/Metformin HCl 500 mg Tablet Composition

Component	Quality Reference	Function	Amount per Tablet (mg)
Core Tablet			
Ertugliflozin L-PGA†	In-house	Active	3.238
			(b) (4)
Metformin HCl	In-house	Active	500.0
Povidone	USP-NF	(b) (4)	(b) (4)
Microcrystalline Cellulose	USP-NF		
Crospovidone	USP-NF		
Sodium Lauryl Sulfate	USP-NF		
Magnesium Stearate	USP-NF		
		Core Tablet Weight	
Film Coat			
			(b) (4)
Carnauba Wax	USP-NF		(b) (4)†
			(b) (4)

Table 2 Ertugliflozin 2.5 mg/Metformin HCl 1000 mg Tablet Composition

Component	Quality Reference	Function	Amount per Tablet (mg)
Core Tablet			
Ertugliflozin L-PGA†	In-house	Active	3.238
			(b) (4)
Metformin HCl	In-house	Active	1000.0
Povidone	USP-NF	(b) (4	(b) (4)
Microcrystalline Cellulose	USP-NF		
Crospovidone	USP-NF		
Sodium Lauryl Sulfate	USP-NF		
Magnesium Stearate	USP-NF		
		Core Tablet Weight	
Film Coat			
			(b) (4)
Carnauba Wax	USP-NF		(b) (4)
			(b) (4)

Table 4 Ertugliflozin 7.5 mg/Metformin HCl 500 mg Tablet Composition

Component	Quality Reference	Function	Amount per Tablet (mg)
Core Tablet			
Ertugliflozin L-PGA†	In-house	Active	9.713
			(b) (4)
Metformin HCl	In-house	Active	500.0
Povidone	USP-NF	(b) (4	(b) (4)
Microcrystalline Cellulose	USP-NF		
Crospovidone	USP-NF		
Sodium Lauryl Sulfate	USP-NF		
Magnesium Stearate	USP-NF		
		Core Tablet Weight	
Film Coat			
			(b) (4)
Carnauba Wax	USP-NF		(b) (4)
			(b) (4)

Table 5 Ertugliflozin 7.5 mg/Metformin HCl 1000 mg Tablet Composition

Component	Quality Reference	Function	Amount per Tablet (mg)
Core Tablet			
Ertugliflozin L-PGA†	In-house	Active	9.713
(b) (4)			
Metformin HCl	In-house	Active	1000.0
Povidone	USP-NF	(b) (4)	(b) (4)
Microcrystalline Cellulose	USP-NF		
Crospovidone	USP-NF		
Sodium Lauryl Sulfate	USP-NF		
Magnesium Stearate	USP-NF		
		Core Tablet Weight	
Film Coat			
(b) (4)			
Carnauba Wax	USP-NF		(b) (4)
(b) (4)			

(Tables excerpted from sponsor's package)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

Ertugliflozin-related impurities and degradants were qualified under NDA #209803.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adults with T2DM.

The sponsor recommends individualizing the starting dose based on the patient's current regimen. Administration twice daily with meals is recommended with gradual dose escalation to reduce metformin-related gastrointestinal side effects.

Sponsor's Maximum Recommended Human Dose:

FDC: Twice-daily dose of 7.5 mg ertugliflozin and 1000 mg metformin HCl

- **Total: 15 mg/day ertugliflozin + 2000 mg/day metformin**
  - Ertugliflozin: * **AUC = 1.38 μg·h/mL**, C_{max} = 266 ng/mL ≈ 0.6 μM
    *Based on the clinical population pharmacokinetic (PK) analysis
  - Metformin: ** **AUC_{0-24} = 25 μg·h/mL**, C_{max} = 1.8 μg/mL
    ** Based on study #PB019/1032 (CSR, Table 11) for mean 1000 mg metformin AUC exposures of 12.3 μg·h/mL when co-administered with 15 mg ertugliflozin

Ertugliflozin: The proposed MRHD under NDA #209803 for the NME alone is also 15 mg/day.

Metformin: Approved maximum daily dose of Metformin HCl is 1000 mg twice a day for a total of 2000 mg/day. Reference NDA #020357 and the April 2017 drug label for Glucophage (metformin hydrochloride).

2.7 Regulatory Background

- An IND for Ertugliflozin was originally submitted as PF04971729 in September 2009.
- On 5/9/2014, the sponsor submitted a meeting request and a pre-IND package for the FDC Ertugliflozin + Metformin product. On 5/13/2014, a Pre-IND/Type B meeting was granted with written responses sent to the sponsor on 7/3/2014. Within the pre-IND package, the sponsor submitted 3 clinical questions and one regulatory question, but no non-clinical questions.
- On 8/13/2014, the sponsor submitted and cross-referenced the new IND #122329 for the FDC product MK-8835B containing ertugliflozin and metformin for the treatment of T2DM.

Ertugliflozin

- Ertugliflozin was originally submitted as PF04971729 under IND #106447 in September 2009.
- And NDA package for ertugliflozin as an NME alone (non-FDC) drug formulation was submitted at the same time as the ertugliflozin/metformin FDC NDA on 12/19/2016.

Metformin

Metformin HCl was approved under NDA #020357 as Glucophage (Bristol Myers Squibb) in 1994 with a maximum approved adult dose set at 2000 mg/day. Metformin has been prescribed extensively for long-term treatment of T2DM in patients worldwide for roughly 4 decades. Metformin HCl was later approved as an extended release tablet (Glucophage XR) in 2000. A metformin HCl/saxagliptin combination (Kombiglyze, NDA #200678) was approved in November 2010 and included embryofetal toxicology studies (IND #76500, IND #63634), which were mandated by post marketing requirements under the Onglyza NDA #22350, to specifically address potential treatment-related neural tube defects due to either metformin or the combination of the two drugs. The embryo-fetal studies using metformin HCl were reviewed under the original Metformin IND #47342 in 2011.

3 Studies Submitted

3.1 Studies Reviewed

All nonclinical studies for ertugliflozin were submitted and reviewed under NDA#209803 and IND #106447. All nonclinical coadministration studies have been previously reviewed under IND #106447 and #122329.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Pivotal 2-week and 13-week combination toxicology studies were reviewed in detail in Pharmacology and Toxicology (Pharm/Tox) review #1 under IND#122329 and Pharm/Tox review #7 under IND #106447 by Dr. Jessica Hawes. Summaries of these studies are included in this review.

Table 1: Summaries of Pivotal Previously-Reviewed Nonclinical Studies

Combination Toxicology		
Study #	**Brief Title**	**Primary Review**
TT147809 ((b) (4) #8294466, Pfizer #13GR341)	2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats	IND #122329 Pharm/Tox review #1, Dr. Hawes, 9/5/2014
TT147809 ((b) (4) #8300339, Pfizer #14GR164)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats **(GLP)**	IND #106447 Pharm/Tox review #7, Dr. Hawes, 12/3/2015

4 Pharmacology

4.1 Primary Pharmacology

Ertugliflozin is an inhibitor of SGLT2, thereby blocking the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells and resulting in significant glucosuria. Ertugliflozin is highly selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

Metformin HCl (BMS-207150) is a biguanide class hypoglycemic agent used to treat non-insulin dependent T2DM. Although the molecular mechanisms of metformin are not completely understood, the following mechanisms have been implicated to play a role: inhibition of the mitochondrial respiratory chain (complex I), activation of AMP-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) and consequent activation of protein kinase A (PKA), induced phosphorylation of GLUT4 enhancer factor, and an effect on gut microbiota.

Drug activity related to proposed indication:
Ertugliflozin administration in rats results in concentration-dependent glucosuria, which is directly related to the pharmacodynamic (PD) activity of SGLT2 inhibition. Ertugliflozin administration in rats is associated with compensatory increases in food consumption; however, in food-controlled studies, concomitant decreases in plasma glucose levels are observed. Glucosuria has also been reported in humans.

Metformin suppresses hepatic glucose production, increases insulin sensitivity, enhances peripheral glucose uptake, decreases insulin-induced suppression of fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract.

4.2 Secondary Pharmacology

Ertugliflozin

Nonclinical secondary pharmacology studies for ertugliflozin were fully evaluated in the Pharm/Tox review by Dr. Hawes under NDA #209803.

Briefly, significant drug-drug interactions (DDI) with ertugliflozin administration and drugs metabolized by cytochrome P450 (CYP) enzymes or transported by organic anion transporters (OATs), organic cation transporters (OCTs) or organic anion transporting polypeptides (OATPs) are not likely at clinical exposures. Significant DDI with diphosphate-glucuronosyltransferase (UGT) enzyme inhibition is also unlikely at clinical concentrations.

Metformin

Concomitant use of metformin with drugs that interfere with renal tubular transport systems, such as OCT2, can lead to increases in systemic metformin exposures. Alcohol is known to potentiate the effects of metformin on lactate metabolism. Metformin exposures are increased in the presence of nifedipine due to enhanced absorption.

Ertugliflozin/Metformin FDC

DDI between ertugliflozin and metformin are not anticipated. Ertugliflozin and metformin are eliminated by different mechanisms and are not expected to affect each other's elimination pathways. Ertugliflozin is predominantly eliminated via hepatic metabolism, whereas metformin is not metabolized and is eliminated via filtration at the glomerulus and excreted in the urine unchanged. Ertugliflozin is not anticipated to affect OCT2 activity at clinical exposures; hence ertugliflozin is not anticipated to affect metformin exposures. Metformin does not inhibit or induce metabolizing enzymes involved in ertugliflozin metabolism; thus metformin is not anticipated to affect ertugliflozin exposures. Since metformin is negligibly bound to plasma proteins, it is less likely to interact with highly protein-bound drugs, such as ertugliflozin.

4.3 Safety Pharmacology

Both ertugliflozin and metformin may be associated with some concern for cardiovascular (CV) effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. Given that the margins of safety for CV effects are sufficient for each drug alone and a DDI is not likely, the margins of safety for the FDC product are likely to be sufficient as well. Nevertheless, CV effects with coadministration of ertugliflozin and metformin was investigated in an add-on sub-study of the clinical CV safety study #P004/B1521021.

Ertugliflozin

Standard cardiovascular, neurological and pulmonary safety pharmacology studies were completed for ertugliflozin under IND #106447 and NDA #209803.

Central Nervous System (CNS): At 500 mg/kg ertugliflozin in male rats, drug-related decreases in average body temperature of 0.4°C, and 30-40% decreases in locomotor

activity, were observed at C_{max} exposures approximately 339-fold higher than clinical C_{max} exposure at the maximum recommended high dose ($MRHD_{Cmax}$) of 15 mg/day. The no observed adverse effect level (NOAEL) for CNS effects in rats was set at 25 mg/kg, which is associated with a safety margin of ~36x $MRHD_{Cmax}$.

Cardiovascular System: Ertugliflozin weakly inhibited the human ether-a-go-go-related gene (hERG) potassium channel in vitro with an IC_{50} of 59 μM and an IC_{20} of 8.11 μM in CHO cells, but was a poor inhibitor in human embryonic kidney (HEK293) cells with an IC_{50} value of >300 μM. Ertugliflozin also weakly inhibited Nav1.5 currents with an IC_{50} of 188 μM. Although significant inhibition of hERG and Nav1.5 currents were reported at concentrations ≥30 μM (50x $MRHD_{Cmax}$), significant hERG or Nav1.5 inhibition is not anticipated at biologically relevant exposure levels. In dogs, single doses of 50 mg/kg ertugliflozin (163x $MRHD_{Cmax}$) were associated with moderate decreases in the QTc interval, cardiac contractility, and heart rate corresponding with T_{max}, as well as increases in systolic blood pressure (sBP) and lengthening of the PR interval, with a NOAEL of 5 mg/kg and a safety margin of ~13x $MRHD_{Cmax}$. In the 27-day pair-fed study #PD001 in spontaneous hypertensive rats (SHR), ertugliflozin-related decreases in blood pressures and heart rate were associated with treatment-related diuresis and activation of the renin-angiotensin-aldosterone-system (RAAS) at 36 mg/kg/day (11x $MRHD_{Cmax}$). Furthermore, based on similar effects observed with a diuretic positive control anti-hypertensive, it's likely that ertugliflozin-related CV effects in the SHR model are, at least in part, secondary to PD-related diuresis.

Respiratory System: In rats, dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed for up to 120 minutes post-dose and correlated with C_{max} at doses of ≥25 mg/kg (~36x $MRHD_{Cmax}$), with a NOAEL of 5 mg/kg (9x $MRHD_{Cmax}$).

Supplemental

Renal/Urinary System: No specific renal safety pharmacology studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes increased urinary glucose excretion and kidney alterations in rats and dogs at clinical exposure levels.

Gastrointestinal System: No GI-specific safety studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs.

Immunotoxicity: There were no indications of immunotoxicity or antigenicity in repeat-dose toxicology studies.

Metformin

Neurological
Incidences of headache, confusion, and/or mood swings observed in humans are likely secondary to hypoglycemia.

Cardiovascular
Metformin has been associated with lactic acidosis, which is further associated with CV collapse, acute congestive heart failure, and acute myocardial infarction. Some patients have reported increased heart beat and/or palpitations while on metformin.

Pulmonary
Difficulty breathing is occasionally reported in humans taking metformin, but has not been associated with an adverse clinical outcome.

Renal
Metformin is contraindicated with renal deficiency since it is primarily eliminated via the kidney. Cystic tubular dilation and vacuolization have been observed in mice.

Gastrointestinal
GI upset, diarrhea, cramps, nausea, vomiting and gas.

Other
Metformin-mediated inhibition of hepatic neogenesis leads to decreases in lactate uptake and increases in lactic acid leading to lactic acidosis, which can be fatal. Metformin is also contraindicated in patients with liver dysfunction and acute or chronic metabolic acidosis, including diabetic ketoacidosis. Loss of appetite and a bad taste in the mouth has also been associated with metformin administration in children.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Ertugliflozin

Ertugliflozin PK parameters were characterized in human, dog, rat, and mouse species. Ertugliflozin protein binding is high in all 4 species, ranging from 92 to 97%. Significant species differences in absorption were associated with oral bioavailability ranging from moderate to high across species and oral absorption ranges of 75-87% in mice, 56-88% in rats, 94-97% in dogs, and up to 100% in humans. T_{max} is achieved within 30 minutes in mice, 0.7 to 2.3 hours in rats, and 0.8 to 1.5 hours in dogs. In humans, T_{max} is achieved after 1 hour in humans (fasted), but after 2 hours in humans in the fed state, indicating absorption delays in the presence of food. Systemic exposures follow linear pharmacokinetics with a trend for slight increases in female exposures over time at high doses in rodents, indicating a potential gender effect which is likely related to gender differences in metabolism in rodents. Ertugliflozin has a moderate half-life ($t_{1/2}$) of 3 to 4 hours in rodents and 8 hours in dogs, but is 1.5 to 4 times longer in humans ranging from 12 to 18 hours.

Ertugliflozin may be a substrate for the efflux transporter permeability glycoprotein 1/multidrug resistance protein 1 (P-gp/MDR1), but is not affected by P-gp/MDR1 inhibitors; thus, P-gp/MDR1 is unlikely to be a limiting factor in Ertugliflozin absorption. Ertugliflozin has a moderate volume of distribution in rats with preferential distribution

into plasma relative to red blood cells. The highest distribution is primarily to organs responsible for drug metabolism and elimination, such as the bladder, liver, and kidney. Ertugliflozin is also highly distributed to rat adrenal gland, Harderian gland, and pancreas. Ertugliflozin crosses the adult blood:brain barrier, but only reaches concentrations 3 to 63-fold lower than that of blood; whereas distribution to the choroid plexus and pituitary gland is 2-fold greater than blood. In fetal rats, ertugliflozin more readily crosses the blood:brain barrier, resulting in significantly more drug exposure to fetal CNS tissues and eyes than in corresponding adult tissues relative to plasma levels. Ertugliflozin is excreted in rat milk at exposures comparable to maternal plasma levels. Ertugliflozin also readily crosses the rat placental barrier, but with fetal exposures remaining lower than maternal plasma levels.

In rats, elimination of radiolabeled drug and metabolites was virtually complete by 168 hours (7 days) postdose. Ertugliflozin is primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans.

The predominant route of elimination of ertugliflozin is via metabolism, wherein glucuronidation is the major metabolic pathway in all species, with minor contributions from oxidative metabolism involving hydroxylation, oxidation, and oxidative desethylation. There are no unique human metabolites; however, the 2-O-β glucuronide M5a (PF-06685948) and the 3-O- β glucuronide M5c (PF-06481944) are disproportional human metabolites, making up 12.2% and 24.1% of total drug in human plasma, respectively.

Metformin

Metformin is absorbed slowly with an oral bioavailability of 50-60% under fasting conditions. For the immediate-release formula, C_{max} is reached in 1 to 3 hours; whereas C_{max} is achieved 4 to 8 hours post-dose with the extended-release formula. Steady state is reached in 1 to 2 days. Metformin is not metabolized or subject to biliary excretion, but is a substrate of renal transporter OCT2 and cleared by renal tubular secretion with an elimination half-life of 6.2 hours. However, metformin accumulates in red blood cells where it has an elimination half-life of 17.6 hours. Metformin pharmacokinetics is similar in pediatrics (12 to 16 years) and adults.

5.2 Toxicokinetics

In rats, coadministration of ertugliflozin does not affect metformin exposures; however, increases in metformin dose were associated with dose-dependent decreases in ertugliflozin exposures. Whereas, in the Phase 1 clinical PK study (#P019/1032) in healthy subjects, there were no meaningful differences in ertugliflozin or metformin PK parameters when co-administered together compared to administration of each drug alone. Thus, concomitant administration of ertugliflozin and metformin are not associated with significant changes in drug exposures in humans.

Sponsor's Table 2: Summary of Ertugliflozin PK Parameters with Coadministration in Humans – Study #P019/1032

Parameter (Units)	Parameter Summary Statistics[a] by Treatment	
	Ertugliflozin 15 mg	Ertugliflozin 15 mg + Metformin 1000 mg
N, n	18, 17	18, 17
AUC_{inf} (ng.h/mL)	1363 (24)	1388 (23)
AUC_{last} (ng.h/mL)	1346 (23)	1367 (22)
C_{max} (ng/mL)	272.3 (24)	264.5 (20)
T_{max} (h)	1.02 (1.00, 2.00)	1.29 (1.00, 3.00)
$t_{½}$ (h)	11.79 ± 2.34	13.48 ± 4.65
CL/F (mL/min)	183.8 (24)	180.0 (23)
V_z/F (L)	183.7 (33)	201.7 (31)

AUC_{inf} = area under the plasma concentration-time profile from time 0 extrapolated to infinite time, AUC_{last} = area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last}), C_{max} = maximum observed plasma concentration, CL/F = apparent clearance, N = number of subjects; n = number of subjects for $t_{½}$, AUC_{inf}, CL/F and V_z/F, $t_{½}$ = terminal half-life, T_{max} = time for C_{max}, V_z/F = apparent volume of distribution.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean ±SD for $t_{½}$.

Sponsor's Table 3: Summary of Metformin PK Parameters with Coadministration in Humans – Study #P019/1032

Summary of plasma metformin PK parameters

Parameter (Units)	Parameter Summary Statistics[a] by Treatment	
	Metformin 1000 mg	Ertugliflozin 15 mg + Metformin 1000 mg
N, n	18, 13	18, 13
AUC_{inf} (ng.h/mL)	12770 (27)	12260 (27)
AUC_{last} (ng.h/mL)	12550 (26)	12270 (23)
C_{max} (ng/mL)	1983 (26)	1835 (26)
T_{max} (h)	2.00 (0.50, 4.00)	2.00 (1.00, 3.00)
$t_{½}$ (h)	10.23 ± 2.39	14.47 ± 6.94
CL/F (mL/min)	1305 (27)	1359 (26)
V_z/F (L)	1126 (43)	1577 (51)

AUC_{inf} = area under the plasma concentration-time profile from time 0 extrapolated to infinite time, AUC_{last} = area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last}), C_{max} = maximum observed plasma concentration, CL/F = apparent clearance, N = number of subjects; n = number of subjects for $t_{½}$, AUC_{inf}, CL/F and V_z/F, $t_{½}$ = terminal half-life, T_{max} = time for C_{max}, V_z/F = apparent volume of distribution.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean ±SD for $t_{½}$.

(Excerpted from sponsor's package)

6 General Toxicology

6.1 Ertugliflozin

Toxicology studies with administration of ertugliflozin alone were reviewed under NDA #209803 and include pivotal 6-month rat and 9-month dog studies.

Safety margins from the 6 month rat and 9 month dog studies support the proposed 15 mg/day dose of ertugliflozin with safety margins of at least 13x and 46x, respectively, based on AUC exposures at the nonclinical NOAELs. Most findings in the chronic nonclinical toxicology studies can be attributed to drug-related glucosuria and osmotic diuresis. Drug-related gastrointestinal findings in dogs (excessive vomiting, salivation and abnormal feces) and rats (stomach erosion/ulcers, pyloric crypt degeneration and foveolar hyperplasia) are consistent with off-target inhibition of SGLT1. Hyperostosis

and changes in calcium regulation have also been observed in rats, and are also likely to be related to SGLT1 inhibition.

Table 2: Ertugliflozin Summary of Pivotal General Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
9-Month + 8-Week Recovery Beagle Dogs **Dose:** 1, 10 & 150 mg/kg ♂ AUC: 6, 63 & 1040 μg·h/mL ♀ AUC: 7, 78 & 767 μg·h/mL	**10 mg/kg** (♂ & ♀)	♂: **46x** ♀: **57x**	**≥1 mg/kg (♂4x/♀6x MRHD):** adrenal gland (↑organ weight & cortex vacuolation), glucosuria **≥10 mg/kg (♂46x/♀57x MRHD):** thyroid mineralization (♀, irreversible) **150 mg/kg (♂754x/♀556x MRHD):** Adverse: GI intolerance (excessive vomiting, diarrhea, salivation), possibly related mortalities, systemic inflammatory response Non-adverse: ↓BW & gain, ↑thymus weight, persistent ↑reticulocytes, ↑urine calcium (partially reversible), irreversible urine ↑volume
6-Month + 8-Week Recovery Sprague Dawley (SD) Rats **Dose:** 5, 25 & 100 mg/kg ♂ AUC: 18, 128 & 397 μg·h/mL ♀ AUC: 27, 167 & 814 μg·h/mL	**5 mg/kg** (♂ & ♀)	♂: **13x** ♀: **19x**	**≥5 mg/kg (♂13x/♀19x MRHD):** stomach erosion/ulcer, ↓pancreatic zymogen, ↑food consumption, ↓blood glucose, glucosuria, ↓serum electrolytes (minimal), ↑phosphates, possible dehydration, minimal ↑BUN **≥25 mg/kg (♂93x/♀121x MRHD):** Adverse: stomach (pyloric crypt degeneration, discoloration, ↑severity of erosion/ulcer) Non-adverse: minimal-slight kidney findings (pelvic & tubule dilatation, hyperplasia, mineral deposition) **100 mg/kg (♂288x/♀590x MRHD):** bone [severe hyperostosis (♂) & hyperplasia (♀)], digestive tract (stomach hyperplasia, ↑severity of erosions/ulcers & crypt degeneration), ↑severity of kidney findings, adrenal gland (↑organ weight, hypertrophy & cortex vacuolation), ↓BW & gain, ↓RBC parameters, ↓reticulocytes, ↑urine calcium, ↓PTH, significant ↓serum electrolytes (Ca, Na, K, & Cl), mild ↑BUN (1.5-fold)

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL
♂ = males only; ♀ = females only

6.2 Metformin

Target organs of metformin administration in rats include the heart, liver, lymphoreticular organs, adrenals, salivary gland, and reproductive tissues (Quaile et al., 2010).

6.3 FDC Ertugliflozin/Metformin

In accordance with ICH and FDA guidances, the sponsor submitted a GLP-compliant 3-month repeat dose toxicity study in rats with coadministration of ertugliflozin and metformin under IND #106447. The sponsor also submitted a preliminary, non-GLP 2-week study under IND #122329.

Study: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats (Study #TT147809 / #8300339 / #14GR164)

Study #	TT147809
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	7/14/2014
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729: lot #E010014849, 76.0% potency Metformin: lot #WL00040809, 100% potency

Key Study Findings

- Ertugliflozin + Metformin Coadministration-related Findings:
  - Synergistic exacerbation of ertugliflozin PD-related decreases in body weight and weight gain with coadministration of 600 mg/kg metformin (♂), as well as increased food consumption
  - Discoloration of glandular stomach mucosa
  - Clinical chemistry changes: ↓Na (♀) and ↓Creatinine (♂)
  - ↑Heart organ weight
  - Possible exacerbation of ketonuria in females with coadministration of 600 mg/kg metformin
- Ertugliflozin-related Findings:
  - Minimal erosion/ulcer of glandular stomach
    - Incidence rate possibly exacerbated by metformin
  - ↓Zymogen granules in pancreatic acinar cells (♂)
  - ↑Food consumption and ↓body weights
  - Clinical chemistry: ↓glucose, ↓Cl, ↓Ca (♀), mild ↑BUN
  - Urine: ↑specific gravity, ↑volume, ↑glucose, ↑ketones, and ↓pH
  - Kidney:
    - ↑Kidney organ weight in all ertugliflozin treatment groups
      - possibly exacerbated by 600 mg/kg metformin (♀), but lacking dose-dependence in males
    - Tubule dilatation in all ertugliflozin treatment groups (♀)
  - Adrenal Gland
    - Minimal adrenal cortex hypertrophy with 25 mg/kg ertugliflozin
      - Increased incidence with coadministration of 600 mg/kg metformin (♀)
    - ↑Organ weight in females at the coadministration high dose.
- Metformin-related Findings:
  - Salivary gland hypertrophy and ↓cytoplasmic granules
  - ↑Adrenal gland organ weight at 600 mg/kg
    - exacerbated by 25 mg/kg ertugliflozin (♀)
  - ↑Liver organ weight at 600 mg/kg (♀)
    - exacerbated by 25 mg/kg ertugliflozin

SD Rat, 13 Weeks	NOAEL (AUC)	Multiple of MRHD*
No significant adverse systemic toxicities	25 mg/kg Ertugliflozin (91.2 μg·h/mL) + 600 mg/kg Metformin (135 μg·h/mL)	Ertugliflozin: **66x** Metformin: **5x**

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with an ertugliflozin exposure of AUC = 1.38 μg·h/mL and metformin exposure of AUC = 25 μg·h/mL

METHODS

SD rats were co-administered doses of 0/0 (0.5% MC/10% PEG 400 vehicle), 5/200, 5/600, 25/200, 25/600, 25/0, and 0/600 mg/kg PF-04971729/mg/kg metformin (10/sex/group) via oral gavage daily for 91 days. Animals were evaluated for clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, macroscopic findings, and microscopic findings. Satellite TK groups were included for each dose (4/sex/group).

Reviewer's Comments

The NOAEL was set at the high combination dose of 25 mg/kg PF-04971729 and 600 mg/kg metformin due to a lack of significant adverse systemic toxicities. There were no new or synergistic adverse toxicities due to coadministration of PF-04971729 and metformin. The safety margin for PF-04971729 is 66x $MRHD_{AUC}$ for a high dose of 15 mg/day, and the safety margin for metformin is 5x $MRHD_{AUC}$ based on a high dose of 2000 mg/day.

PD-related findings due to PF-04971729 inhibition of SGLT2 underlie most of the findings in this study. These findings include reduced serum glucose, glucosuria, reduced body weight, increased food consumption, and histopathology changes in the kidney, adrenal gland, and pancreas. These findings are consistent with previous rat toxicology studies in rats with PF-04971729 alone. Overall, there were no new significant drug-related toxicities and the majority of the findings are considered to be secondary to the PD activity of PF-04971720.

Findings of glandular stomach mucosa discoloration, increased heart organ weight, decreases in plasma levels of Na and creatinine were only observed or only achieved statistical significance with coadministration; however, these findings were not associated with correlating observations of dysfunction or toxicity and were considered to be non-adverse.

Decreases in body weight gain with reciprocal increases in food consumption are consistent with the SGLT2 inhibitor drug class and are consistent with a compensatory response to PD-related glucosuria. Increased food consumption has been observed in previous non-clinical studies with PF-04971729 alone and is likely secondary to drug-related decreases in blood glucose levels related to the pharmacodynamic activity of PF-04971729. It's noted that greater decreases in body weights (↓16%) and weight gains (↓27%) in males, as well as greater increases in food consumption (↑23% in

males and ↑39% in females), were reported with coadministration of metformin. Furthermore, the PF-04971729-related effects on reduced body weight, weight gain, and food consumption were exacerbated with coadministration of metformin and were considered likely to be synergistic effects. Nevertheless, these findings are considered to be non-adverse.

Findings of increased kidney weights (↑16-51%) were observed in all PF-04971729 treatment groups and correlated with histopathological findings of minimal to moderate tubular dilatation in the kidney in 70% to 100% of animals in PF-04971729 groups. These findings are consistent with osmotic diuresis and are secondary to the PD activity of PF-04971729. However, it is noted that kidney weights tended to be higher in males (↑14-21% higher) and females (↑9-16% higher) with coadministration of metformin compared to PR-04971729 alone, with metformin dose-dependency further noted in females. Nevertheless, since dilatation of renal tubules reflects a compensatory response to glucosuria and there were no indications of kidney dysfunction, this finding was considered to be non-adverse. It is noted that in longer rat toxicology studies with high doses of PF-04971729, kidney findings included hypertrophy of proximal tubules, tubular mineralization and indications of chronic progressive nephropathy (CPN) at 250 mg/kg.

Increases in glucosuria correlated with reciprocal decreases in blood glucose levels (↓10-38%), consistent with PF-04971729-mediated inhibition of SGLT2 and reduced renal tubular reabsorption of glucose from the glomerular filtrate. Since SGLT2 is a sodium/glucose co-transporter, decreases in plasma Na levels are also consistent with the pharmacodynamic activity of PF-04971729. However, it's noted that decreases in blood Na levels only reached statistical significance (↓1-2%) with coadministration of both drugs, indicating exacerbation of reduced Na levels with metformin coadministration. Statistically significant decreases in plasma chloride levels (↓2-6%) were observed in groups with PF-0497129 administration. During osmotic diuresis, the electrolytes Na, chloride, and potassium are excreted, which is consistent with the observed drug-related reductions in electrolyte concentrations in the blood. Also, the observed increases in urine volume (↑3-fold in males and ↑35-67% in females), urine specific gravity (↑3% in females) and small increases in BUN levels (↑29% to 2-fold in males and ↑25-44% in females), as well as decreases in urinary pH (↓3-9%), are consistent with osmotic diuresis resulting from glucosuria. Decreases in blood creatinine levels were observed with administration of either drug alone in females (↓14%) and were exacerbated in both sexes (17-29%) with coadministration, indicating that at the highest doses, PF-04971729 and metformin may work synergistically or additively together to slightly reduce creatinine levels. Importantly, reduced blood creatinine levels indicate that kidney damage is not present. Since increases in BUN levels correlate with increases in urine volume while creatinine levels were not increased, the observed increases in BUN levels are likely to be secondary to dehydration resulting from glucosuria, rather than related to kidney toxicity. Thus, there were no clear signs of kidney dysfunction. Overall, the metabolic changes observed with PF-04971729 and metformin coadministration were considered to be anticipated PD-related secondary effects and were not considered to be adverse.

Drug-related ketonuria was attributed to ertugliflozin administration and were observed in both males and females, particularly at 25 mg/kg ertugliflozin, which is consistent with findings in 6-month rat and other 13-week rat toxicology studies with ertugliflozin administration. In males, exacerbation of ketonuria was inversely correlated with metformin coadministration; thus, a significant ketonuria interaction between ertugliflozin and metformin was considered to be unlikely. However, in females, incidences and severity of ketonuria were increased in the highest coadministration group, indicative of exacerbation of ertugliflozin-related ketonuria with metformin coadministration. Ketonuria is consistent with ketosis; however, the presence of correlating increases in blood ketone levels and decreases in blood pH are unknown because they were not evaluated. Nevertheless, the incidences of ketonuria were not associated with drug-related increases in adverse clinical signs consistent ketoacidosis; thus, this finding was considered to be non-adverse.

Low incidences of drug-related stomach findings were attributed predominantly to PF-04971729, although gross observations of discolored stomach were only reported in coadministration groups (1 male and 25% of females). In females, 2 incidences of minimal stomach erosion/ulcer were noted with coadministration of 600 mg/kg metformin, but not with PF-04971729 alone. In males, low incidences of minimal stomach erosion/ulcer were noted in 25 mg/kg PF-04971729 groups independent of metformin. Similar stomach findings have been described in previous toxicology studies with PF-04971729, and are likely related to off-target SGLT1 inhibition. Overall, the stomach findings in this study were of low severity, are likely to be reversible, and were not considered to be adverse.

Pancreatic findings of minimal to moderate decreases in acinar cell zymogen granules were reported in males and females treated with PF-04971729 alone or in combination with metformin and are predominantly attributed to PF-04971729. A slight trend for increased severity and incidence was observed in the high dose combination group 25/600, which correlated with a relatively high degree of food consumption. Zymogen granules in the apical region of acinar cells have been shown to reduce in size and/or number after feeding, most likely due to digestive enzyme secretion stimulated by feeding (Ermak & Rothman, *Cell Tissue Res.* 1981; 214: 51-66). Thus, decreases in zymogen granules of pancreatic acinar cells are likely related to PF-04971729-related increases in food consumption and are considered to be non-adverse.

Decreases in blood calcium levels were observed in females (↓4-5%) and are consistent with findings in previous toxicology studies with PF-04971729 alone. Since calcium reabsorption in the proximal tubule follows water reabsorption, glucosuria can be associated with increased calcium excretion. However, changes in calcium absorption and homeostasis are also consistent with off-target SLGT1 inhibition. Although no bone findings were reported in this study, longer exposures to 25 mg/kg PF-0491729 in the 6-month rat toxicology study resulted in increases in trabecular bone and hypertrophy. Thus, the decreased serum calcium levels observed in this study may be indicative of early drug-related bone changes in rats; however, there were no clear indications of a synergistic or additive effect of metformin coadministration.

Incidences of mandibular and sublingual salivary gland duct epithelium hypertrophy and decreases in cytoplasmic granules were dose-dependently observed with metformin administration, independent of PF-04971729 coadministration. Up to 100% of males and females were affected with 600 mg/kg metformin. These findings are known effects of metformin, but are considered to be non-adverse. Furthermore, the salivary gland findings were not exacerbated by PF-04971729 administration.

Adrenal gland hypertrophy was reported in both sexes and was associated with increased incidence and severity at the highest doses of coadministration in females (80%), and also correlated with increased adrenal gland weights (↑33-46%) in high dose females. These findings are consistent with previous studies with PF-04971729 in rats and dogs. The adrenal gland findings are consistent with a compensatory response to fluid and electrolyte losses related to the PF-04971729-induced glucose excursion. However, the adrenals are also known target organs of metformin toxicity, which is consistent with apparent exacerbation of increased incidences and degree of weight increase in adrenal glands with 600 mg/kg metformin coadministration. Overall, it is likely that metformin and PF-04971729 work together to drive adrenal gland hypertrophy and increased organ weight. Nevertheless, there were no indications of dysfunction and these effects were not considered to be adverse.

Increases in liver organ weights were observed with metformin administration, independent of PF-04971729 coadministration; however, the greatest increases were noted in the coadministration high dose group (↑34-47%). Liver disease has been associated with long-term metformin use; however, there were no microscopic findings or clinical chemistry signs of liver damage or dysfunction in this study. Therefore, this finding was considered to be non-adverse.

Increases in heart organ weights (↑17-29%) were observed in females treated with both 25 mg/kg PF-0971729 and 600 mg/kg metformin. Although the heart is a known target organ of metformin administration, significant increases in heart organ weights were not observed in animals treated with metformin alone. Thus, the data indicate that the increase in heart weight was dependent on coadministration of high doses of both drugs and may be an additive effect. However, there were no heart microscopic findings or indications of cardiac hypertrophy, heart damage or dysfunction in this study; thus, the increases in heart weight were considered to be non-adverse. It is noted that heart myonecrosis was observed in both sexes at 250 mg/kg PF-0871729 in the 3-month rat study with a NOAEL of 25 mg/kg, but was not reported in the 6-month rat study, which evaluated doses up to 100 mg/kg. Thus, the NOAEL for PF-0871729-related adverse heart effects is considered to be 100 mg/kg, which is associated with a wide PF-0871729 safety margin of ~300x $MRHD_{AUC}$.

Coadministration of PF-04971729 did not appear to affect metformin exposures. However, PF-04971729 exposures were dose-dependently lowered by up to 60% with increasing metformin dose. Thus, animals in the highest coadministration group

(25+600) had the lowest PF-04971729 exposures of all groups receiving 25 mg/kg PF-04971729. Although the sponsor did not recognize an effect of metformin on PF-04971729 exposures, this observation is consistent with results from the 2-week coadministration study in rats with metformin. The decrease in PF-04971729 exposures with coadministration may explain the decrease in some effects driven by PF-04971729 in groups receiving coadministration.

Methods

Doses	PF-04971729 (mg/kg) + Metformin (mg/kg): 0+0, 0+600, 25+0, 5+200, 5+600, 25+200, and 25+600
Frequency of dosing	Once daily for 91 days. Animals were dosed 1st with PF-04971729, then dosed 2 minutes later with metformin 2nd.
Route of administration	Oral gavage
Dose volume	5 mL/kg PF-04971729 + 5 mL/kg metformin = 10 mL/kg total
Formulation/Vehicle	Vehicle #1 (PF-04971729): 0.5% (w/v) methylcellulose, 10% (v/v) polyethylene glycol 400 (PEG 400) Vehicle #2 (Metformin): 0.5% (w/v) methylcellulose
Species/Strain	Crl:CD(SD) rats, (b) (4)
Number/Sex/Group	10/sex/group
Age	6-7 weeks
Weight	♂: 163-278 g ♀: 155-198 g
Satellite groups	TK animals including 4/sex/group
Unique study design	Co-administration of PF-04971729 and metformin
Deviation from study protocol	Day 14: two 0+600 TK ♂'s and one 25+600 TK ♀ did not receive the dose of metformin. Day 28: one 0+600 TK ♀ and one 25+0 TK ♂ did not receive the dose of PF-04971729 vehicle. Day 41: all 0+0 animals were dosed with vehicle #2 twice. Day 64: one 0+600 TK ♂ did not receive the dose of PF-04971729. Day 6-87: various animals (13 total) across most groups received the metformin dose 9-10 minutes after the PF-04971729 dose instead of after 2 minutes.

Study Design

				PF-04971729		Metformin	
		No. of Animals		Dose		Dose	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	10	10	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Control/	1 (Toxicity)	10	10	0	0	600	120
High)	2 (Toxicokinetic)	4	4	0	0	600	120
3 (High/	1 (Toxicity)	10	10	25	5	0	0
Control)	2 (Toxicokinetic)	4	4	25	5	0	0
4 (Low/	1 (Toxicity)	10	10	5	1	200	40
Low)	2 (Toxicokinetic)	4	4	5	1	200	40
5 (Low/	1 (Toxicity)	10	10	5	1	600	120
High)	2 (Toxicokinetic)	4	4	5	1	600	120
6 (High/	1 (Toxicity)	10	10	25	5	200	40
Low)	2 (Toxicokinetic)	4	4	25	5	200	40
7 (High/	1 (Toxicity)	10	10	25	5	600	120
High)	2 (Toxicokinetic)	4	4	25	5	600	120

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg metformin (or Vehicle Control Article 2, as applicable).
b PF-04971729 dose concentrations were corrected for lot specific potency of 0.760 (76.0%). A correction factor of 1.316 was used for Lot No. E010014849.
c No correction factor was need for metformin dose concentrations. Dose levels and concentrations were expressed as the salt form of Test Article 2.
d Group 1 received Vehicle Control Articles 1 and 2 only.

Parameters Measured

Clinical Findings	Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. Detailed observations were conducted on all animals prior to dosing on Day 1, weekly during the dosing phase, and on Day 91. Cageside observations were also conducted at 1 hour postdose.

Body weights	Animals were weighed once during the predose phase, prior to dosing of Day 1, weekly thereafter, and on Day 91.
Food consumption	Food consumption was quantified for each cage weekly, beginning on Day 1, for Weeks 1-13 and Days 85-91.
Ophthalmoscopy	Ophthalmic examinations were conducted by a veterinarian using an indirect ophthalmoscope and a mydriatic agent once during the predose phase and during Week 13 of the dosing phase.
EKG	Not evaluated
Hematology	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.2 Hematology Tests** red blood cell (erythrocyte) count hemoglobin hematocrit mean corpuscular volume mean corpuscular hemoglobin mean corpuscular hemoglobin concentration platelet count white blood cell (leukocyte) count differential blood cell count blood smear reticulocyte count mean platelet volume red blood cell distribution width **3.5.1.3 Coagulation Tests** prothrombin time activated partial thromboplastin time
Clinical chemistry	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.4 Clinical Chemistry Tests** glucose urea nitrogen creatinine total protein albumin globulin albumin:globulin ratio cholesterol total bilirubin alanine aminotransferase alkaline phosphatase gamma glutamyltransferase aspartate aminotransferase calcium inorganic phosphorus sodium potassium chloride
Urinalysis	Urine samples were collected at necropsy on Day 92 **3.5.1.5 Urinalysis Tests** appearance (clarity and color) bilirubin blood glucose ketones microscopic examination of sediment pH protein specific gravity urobilinogen volume
Gross pathology	Animals were fasted overnight and necropsied on Day 92. External features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues were examined.
Organ weights	Organ weights were measured (W) according the table below. Paired organs were weighed together.
Histopathology	Tissues were collected from all animals and prepared (P) by preserving in 10% neutral-buffered formalin (NBF), embedding in paraffin, sectioning, and staining with hematoxylin and eosin (H&E). All tissues in Groups 1 (0+0), 2 (0+600), 3 (25+0), and 7 (25+600) were examined microscopically. The kidneys, mandibular salivary gland, sublingual salivary gland, pancreas, glandular stomach, and adrenal cortex from Groups 4 (5+200), 5 (5+600), and 6 (25+200) were also examined microscopically.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		P,E
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	salivary gland (mandibular [2])		P,E
epididymis (2)	W	P,E	salivary gland (sublingual [2])		P,E
esophagus		P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
eye (2)[b]		P,E	seminal vesicle		P,E
femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	skin/subcutis {skin and adnexa}		P,E
gross lesions		P,E	spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
gut-associated lymphoid tissue {GALT}		P,E	spleen	W	P,E
Harderian gland[b]		P,E	sternum with bone marrow		P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[b]	W	P,E
jejunum		P,E	thymus	W	P,E
kidney (2)	W	P,E	thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
larynx			tongue		P,E
liver	W	P,E	trachea		P,E
lungs with large bronchi {lung}		P,E	ureter		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	urinary bladder		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	uterus		P,E
mammary gland (males and females)		P,E	vagina		P,E

E = Examined microscopically; P = Processed; W = Weighed.
a Brain was sectioned according to published recommendations (Bolon et al., 2013).
b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Bone marrow smears were prepared from the femur, but were not examined.

Toxicokinetics	Non-fasted blood samples were collected from all groups (2 animals/time point/group) on Days 1 and 91 at 1, 4, 7, and 24 hours postdose.

Observations and Results

Mortality

There were 2 female mortalities, a female in the 25+200 treatment group found dead on Day 74 and a TK female in the 0+600 group found dead on Day 10. There were no clinical signs in either animal before death. Although moderate lung congestion was identified in the 25+200 female, a cause of death was not determined. The cause of death in the TK female could not be determined either. Given the lack of relation to dose in the 2 mortalities and inconsistency with drug-related findings in other animals, it is unlikely that these deaths were drug-related.

Table 3: Mortality - 13-week Rat Study #14GR164

MORTALITY					
Dose Group	**Day**	**ID**	**Cause of Death**	**Clinical signs**	**Pathology**
25+200	74	♀ #B09853	undetermined	Found dead, but no clinical signs	Slight-minimal protein cast and mineralization in kidney, moderate lung congestion, minimal Harderian gland mononuclear cell infiltrate, slight ↓cytoplasmic granules in mandibular salivary gland
0+600	10	TK ♀ #B09806	undetermined	Found dead, but no clinical signs	No macroscopic findings. Histology was not performed

Clinical Signs

No drug-related findings.

Body Weights

In males that received metformin, body weights and body weight gains decreased in a dose-dependent manner as co-administration of PF-04971729 increased, reaching statistical significance in animals co-treated with ≥5 mg/kg PF-04971729 and 600 mg/kg metformin (↓12% and ↓20%). In males that received PF-04971729, body weights and body weight gains decreased in a dose-dependent manner as co-administration of metformin increased, reaching statistical significance in animals co-treated with 600 mg/kg metformin. Furthermore, the decreases in male body weights and body weight gains with co-administration were more than the sum of each drug's effect alone, indicating a synergistic effect.

Female body weights (↓9%) and weight gains (↓19%) were significantly lower in females treated with 25 mg/kg PF-04971729 alone; however, there were no statistically significant decreases in animals receiving both PF-04971729 and metformin. Thus, there was no significant or adverse effect of metformin co-administration on female body weights.

Table 4: Body Weights - 13-week Rat Study #14GR164

MALES: Body Weight				
Study Time	Dose (mg/kg+mg/kg)	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	+330	-	-
	0+600	+301	91.2% (↓8.8%)	94.9% (↓5.1%)
	25+0	+316	95.8% (↓4.2%)	97.8% (↓2.2%)
	5+200	+306	92.7% (↓7.3%)	96.4% (↓3.6%)

	5+600	+263*	79.7% (↓20.3%)	88.0%* (↓12.0%)
	25+200	+296	89.7% (↓10.3%)	95.1% (↓4.90%)
	25+600	+241*	73.0% (↓27.0%)	84.4%* (↓15.6%)
FEMALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	+119	-	-
	0+600	+121	101.7%	100%
	25+0	+96*	80.7% (↓19.3%)	91%* (↓9.0%)
	5+200	+106	89.1% (↓10.9%)	94.3% (↓5.7%)
	5+600	+106	89.1% (↓10.9%)	95.0% (↓5.0%)
	25+200	+119	100%	98.7% (↓1.3%)
	25+600	+108	90.8% (↓9.2%)	96.0% (↓4.0%)

Sponsor's Figure 1: Body Weights - 13-week Rat Study #14GR164

Figure 7.1: Mean Body Weight Data - Males

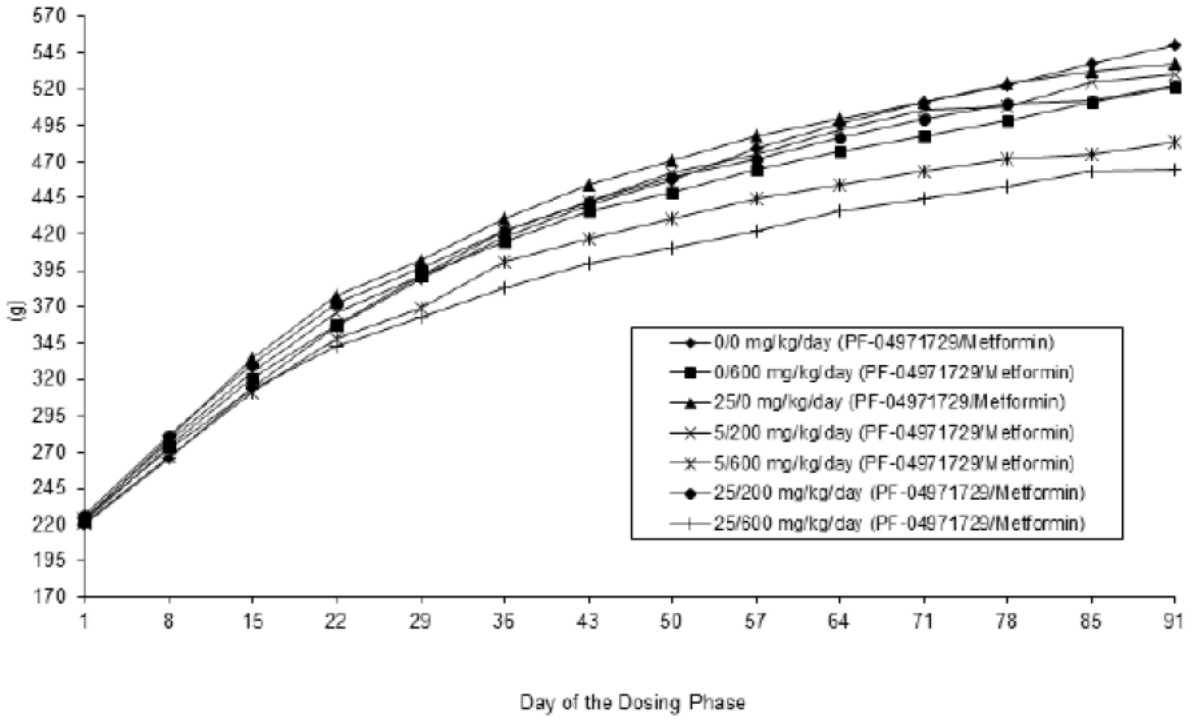
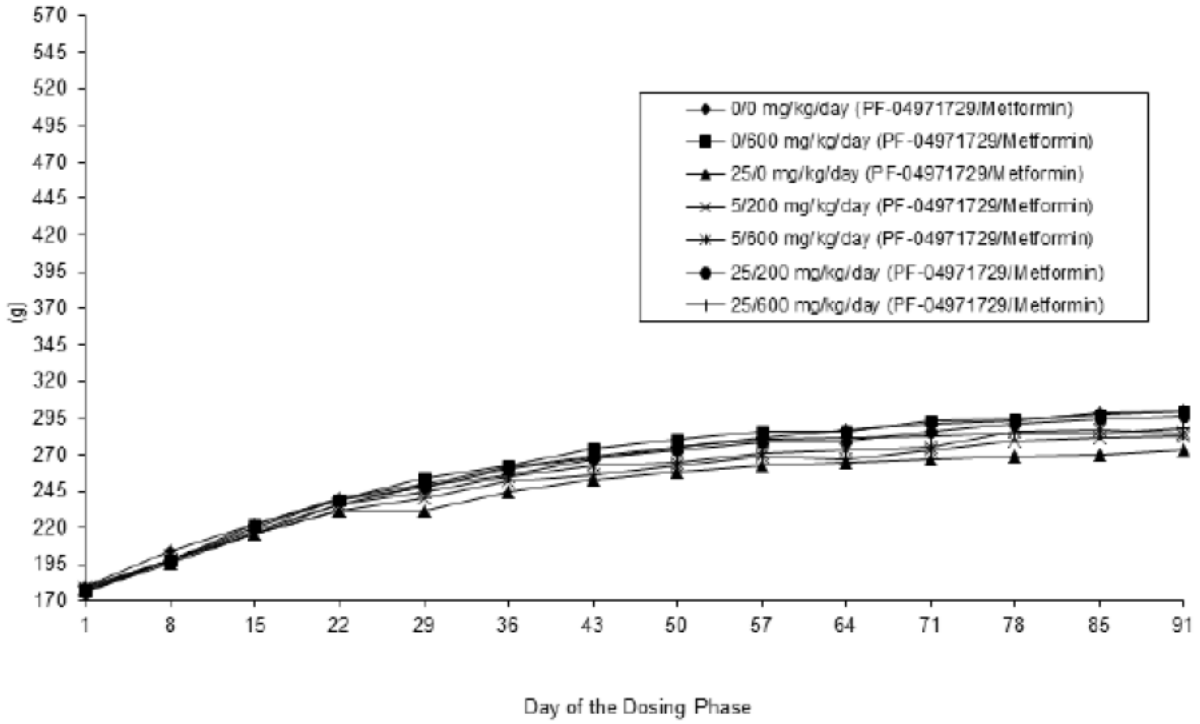


Figure 7.2: Mean Body Weight Data - Females



(Figures excerpted from sponsor's package)

Feed Consumption

After the 1st week of dosing, food consumption was significantly increased in males (↑15-27%) and females (↑11-39%) receiving either PF-04971729 alone or in combination with metformin. In males, increased food consumption was increased dose-dependently with regard to PF-04971729 dose, but was independent of the metformin dose. In females, there was not a direct PF-04971729 dose-dependency; however, there was a dose-dependent increase with regard to the co-administration of both PF-0497179 and metformin.

Table 5: Food Consumption - 13-week Rat Study #14GR164

Food Consumption				
Dose, mg/kg	Males		Females	
	Consumption (g/animal/day)	% Control	Consumption (g/animal/day)	% Control
0+0	26	-	18	-
0+600	27	103.8%	19	105.6%
25+0	33*	126.9%	20*	111.1%

5+200	31*	119.2%	20*	111.1%
5+600	30*	115.4%	21*	116.7%
25+200	33^	126.9%	22*	122.2%
25+600	32*	123.1%	25^	138.9%

* p value <0.05
^ excluded from Day 1 to Day 91 mean statistical analysis, but p value <0.05 at weekly time points

Ophthalmoscopy

No drug-related findings.

Hematology

Statistically significant decreases in red blood cell (RBC) counts (↓5-7%), hematocrit (Hct) percentage (↓4-6%), or hemoglobin (Hg, ↓5%) were noted in females in various groups treated with PF-04971729 alone or in combination with 200 mg/kg; however, there was no clear consistency or dose-dependency in RBC parameter changes, no significant correlating changes in female reticulocyte counts, and no effect in males. On the other hand, a statistically significant decrease in reticulocytes (↓30%) was observed in males in the highest co-treatment group (25+600). Although statistically significant, the observed changes in hematology parameters remained within the normal biological range for this species and are not considered to be biologically significant.

Table 6: Hematology Parameters - 13-week Rat Study #14GR164

RBC Parameters								
Dose (mg/kg PF-04971729 + mg/kg Metformin)	RBC (10^6/uL)		Hct (%)		Hb (g/dL)		Retic. (10^3/µl)	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	9.62	9.21	55.4	54.0	16.6	16.6	198.0	156.7
0+600	9.62	9.27	55.3	54.7	16.5	16.6	194.7	160.9
25+0	9.58	8.89 (↓3.5%)	55.7	50.9* (↓5.7%)	17.1	15.8* (↓4.8%)	172.4	150.3
5+200	9.60	8.74* (↓5.1%)	55.2	51.7* (↓4.3%)	16.7	16.0	178.7	173.7
5+600	9.86	9.26	56.0	54.3	16.9	16.6	175.3 (↓11.5%)	152.0
25+200	9.66	8.60* (↓6.6%)	55.9	51.1* (↓5.4%)	16.8	15.8* (↓4.8%)	189.6	192.2
25+600	9.81	9.05 (↓1.7%)	56.1	54.1	16.9	16.5	139.5* (↓29.5%)	168.1

* p value < 0.05

Clinical Chemistry

Drug-related decreases in blood chloride levels were observed in both sexes, reaching statistically significant changes of ≥2% in PF-04971729 alone (↓2-4%) and combination treatment (↓2-6%) groups. In males, decreases in chloride were primarily dose-related

with regard to PF-04971729, and secondarily dose-related to metformin in the presence of co-treatment with 25 mg/kg PF-04971729. In females, decreases in chloride were dose-dependent with regard to both PF-04971729 and metformin treatment.

Drug-related decreases in blood sodium levels (1.4-2%) were observed in females, reaching statistical significance with co-treatment of both PF-04971729 and metformin. Although decreases in sodium may be anticipated with SGLT2 inhibition by PF-04971729, the data suggest that the decreases in sodium may have been more predominantly driven by metformin than PF-04971729. Furthermore, there were no significant changes in sodium levels in males, indicating a gender effect.

Significant decreases in blood calcium levels were observed in females treated with 25 mg/kg PF-04971729 alone (↓4%) or in combination with metformin (↓4-6%). Furthermore, there was a dose-dependent trend with regard to PF-04971729, as well as exacerbation at the highest doses of both drugs. However, there were no significant changes in calcium levels in males.

Blood levels of inorganic phosphorous (PHOS) were significantly reduced by 15-16% in females co-treated with PF-04971729 and 200 mg/kg metformin. However, PHOS levels were similar in animals treated with PF-04971729 alone, metformin alone, or PF-04971729 in combination with 600 mg/kg metformin. Therefore, although statistically significant, there was not a consistent or dose-dependent decrease in PHOS, nor were there any significant changes in males.

Table 7: Electrolyte Clinical Chemistry - 13-week Rat Study #14GR164

Electrolytes								
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**Chloride (mmol/L)**		**Sodium (mmol/L)**		**Calcium (mg/dl)**		**PHOS (mg/dl)**	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	103	103	147	147	10.6	11.0	7.3	6.7
0+600	102 (↓1.0%)	102 (↓1.0%)	147	145 (↓1.4%)	10.7	11.2	7.6	6.9
25+0	99* (↓3.9%)	101* (↓1.9%)	146	146 (↓0.7%)	10.5	10.6* (↓3.6%)	6.9	6.3 (↓6.0%)
5+200	100* (↓2.9%)	101* (↓1.9%)	147	145 (↓1.4%)	10.5	10.7 (↓2.7%)	7.2	5.6* (↓16.4%)
5+600	100* (↓2.9%)	99* (↓3.9%)	147	144* (↓2.0%)	10.5	10.8 (↓1.8%)	7.5	6.7
25+200	98* (↓4.9%)	99* (↓3.9%)	146	145* (↓1.4%)	10.3	10.6 (↓3.6%)	7.5	5.7* (↓14.9%)
25+600	97* (↓5.8%)	97* (↓5.8%)	146	145* (↓1.4%)	10.5	10.4* (↓5.5%)	7.6	6.7

Decreases in steady-state fasting glucose levels were observed in both sexes with all treatments, reaching statistical significance in animals treated with PF-04971729 alone or in combination with metformin. Decreases in glucose were predominantly driven by

PF-04971729 administration, but were exacerbated by co-administration with metformin in a dose-independent manner.

Significant decreases in the kidney marker creatinine (CREA) were observed in both sexes, reaching a 17% decrease in males and a 29% decrease in females treated with the highest doses of both drugs (25+600). In females, CREA levels were similarly reduced in all drug-treatment groups with the exception of the highest co-administration dose which was exacerbated. In males, CREA levels were only reduced in animals co-treated with PF-04971729 and 600 mg/kg metformin. These data indicate that at the highest doses, PF-04971729 work synergistically or additively together to reduce CREA levels.

Statistically significant increases in BUN levels were observed in both sexes of animals treated with PF-04971729 alone or in combination with metformin. Furthermore, BUN levels were at or above the upper limit of normal (≥20 mg/dL) in groups receiving 25 mg/kg PF-04971729. Although the increases in BUN levels were highest in animals treated with 25 mg/kg PF-04971729 alone (↑2-fold), they were lower with increasing doses of metformin, which also mirrors the decrease in PF-04971729 exposures with co-administration. Thus, these data suggest that increases in BUN levels were primarily driven by PF-04971729 exposure.

Table 8: Glucose & Kidney Clinical Chemistry - 13-week Rat Study #14GR164

Glucose & Kidney Markers						
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**Glucose (mg/dL)**		**CREA (mg/dL)**		**BUN (mg/dL)**	
	♂	♀	♂	♀	♂	♀
0+0	94	95	0.6	0.7	14	16
0+600	88 (↓6.4%)	89 (↓6.3%)	0.6	0.6* (↓14.3%)	13	13
25+0	69* (↓26.6%)	75* (↓21.1%)	0.6	0.6* (↓14.3%)	28* (↑2-fold)	23* (↑43.8%)
5+200	73* (↓22.3%)	86* (↓9.5%)	0.6	0.6* (↓14.3%)	19* (↑35.7%)	16
5+600	70* (↓25.5%)	84* (↓11.6%)	0.5 (↓16.7%)	0.6* (↓14.3%)	18* (↑28.6%)	13
25+200	58* (↓38.3%)	66* (↓30.5%)	0.6	0.6* (↓14.3%)	24* (↑71.4%)	20* (↑25.0%)
25+600	61* (↓35.1%)	68* (↓28.4%)	0.5* (↓16.7%)	0.5* (↓28.6%)	20* (↑42.9%)	17 (↑6.3%)

Minimal, yet statistically significant, increases in ALT levels were reported in males (↑21-50%) and females (↑10-45%) treated with 25 mg/kg PF-04971729 alone or in combination with metformin. However, there was not a clear dose-response. Furthermore, the increases were considered to be minimal and within or near the normal biological range for this species.

A statistically significant decrease in blood cholesterol levels (↓22%) was reported in females at the high doses of both treatments (25+600). Trends for decreases in total protein (↓8%) and albumin (↓10%) were also reported at the highest doses tested (25+600). However, cholesterol and protein levels remained within the normal biological range for this species.

Table 9: Other Clinical Chemistry - 13-week Rat Study #14GR164

ALT, Protein & Cholesterol								
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**ALT (U/L)**		**Total Protein (g/dL)**		**Albumin (g/dL)**		**Cholesterol (10^3/µl)**	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	38	31	7.2	7.9	4.3	5.1	69	97
0+600	32	33	7.2	7.7	4.3	5.0	73	102
25+0	46* (↑21.1%)	34* (↑9.7%)	7.2	7.8	4.4	5.0	71	89
5+200	55 (↑44.7%)	31	7.0	7.8	4.2	5.0	57	85
5+600	48* (↑26.3%)	45* (↑45.2%)	7.0	7.6	4.3	4.9	65	83
25+200	44 (↑15.8%)	36* (↑16.1%)	7.1	7.8	4.3	5.0	53	101
25+600	57* (↑50%)	39* (↑25.8%)	6.8 (↓5.6%)	7.3 (↓7.6%)	4.2	4.6 (↓9.8%)	55 (↓20.3%)	76* (↓21.6%)

Urinalysis

Marked glucose levels were present in nearly all animals treated with ≥5 mg/kg PF-04971729.

Increases in urine specific gravity were reported in all animals treated with ≥5 mg/kg PF-04971729, independent of metformin co-administration, although statistical significance was only achieved in females.

Significant increases in total urine volume were as high as 2.5 to 3.4-fold in males treated with ≥5 mg/kg PF-04971729 independent of metformin co-administration. Trends for smaller increases in urine volume of 35% to 67% were reported in females treated with 25 mg/kg PF-04971729 independent of metformin co-administration, but did not achieve statistical significance.

Decreases in pH were also reported in males (↓3-8%) and females (↓3-9%) treated with PF-0471729 alone or in combination with metformin; however, statistical significance is uncertain since a statistical analysis was not performed.

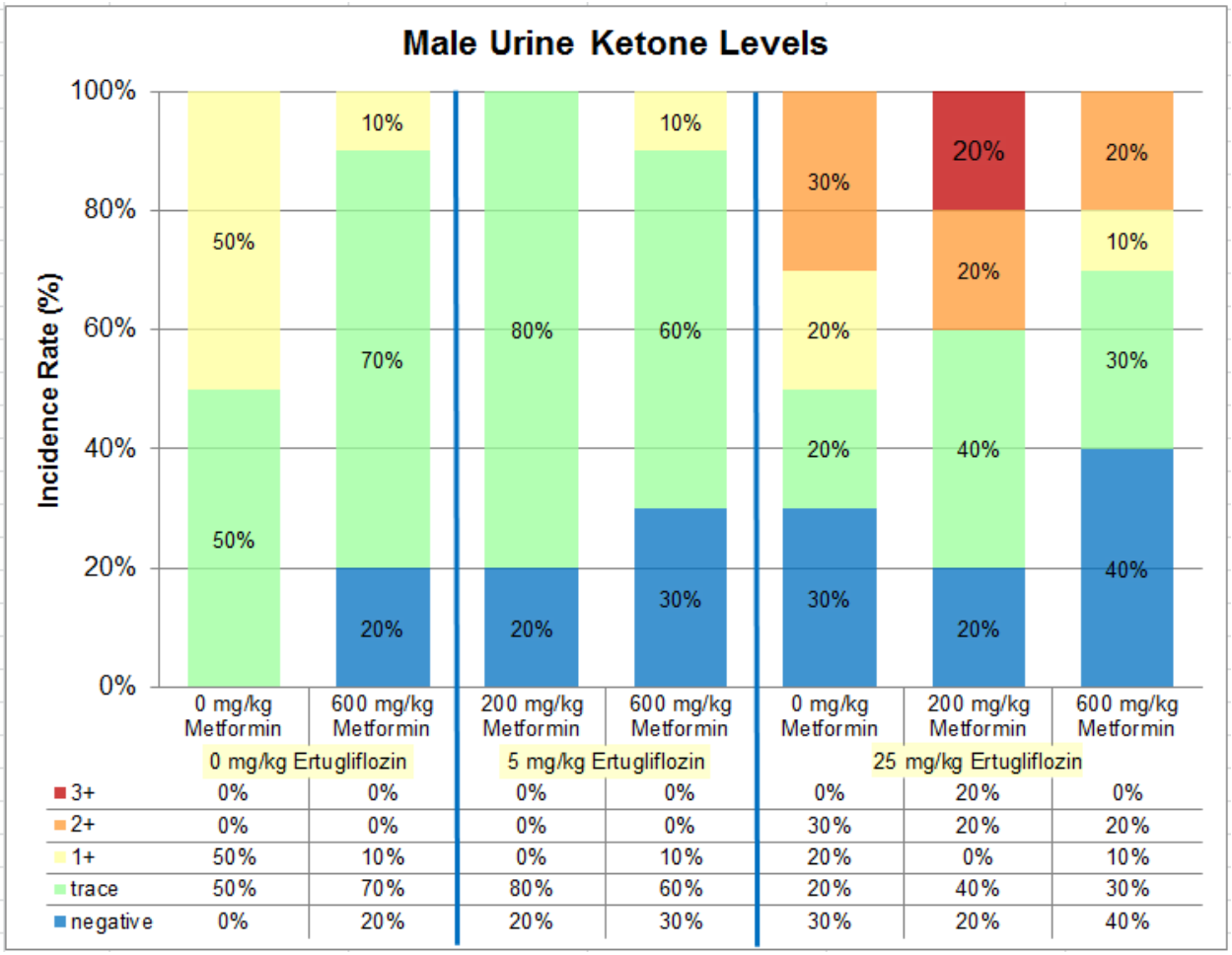
Table 10: Urinalysis - 13-week Rat Study #14GR164

Urine Parameters						
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**Specific Gravity**		**Volume (mL)**		**pH^**	
	♂	♀	♂	♀	♂	♀
0+0	1.037	1.019	9.3	11.6	6.7	6.7
0+600	1.031	1.025	15.1	12.8	6.8	6.4
25+0	1.047 (↑1.0%)	1.048* (↑2.8%)	24.5* (↑2.6-fold)	16.4 (↑41.4%)	6.2 (↓7.5%)	6.3 (↓6.0%)
5+200	1.050 (↑1.3%)	1.051* (↑3.1%)	23.2* (↑2.5-fold)	11.6	6.5 (↓3.0%)	6.4 (↓4.5%)
5+600	1.051 (↑1.4%)	1.044* (↑2.5%)	23.1* (↑2.5-fold)	14.3	6.7	6.5 (↓3.0%)
25+200	1.046 (↑1.0%)	1.048* (↑2.8%)	31.7* (↑3.4-fold)	19.4 (↑67.2%)	6.5 (↓3.0%)	6.2 (↓7.5%)
25+600	1.050 (↑1.3%)	1.051* (↑3.1%)	26.2* (↑2.8-fold)	15.6 (↑34.5%)	6.4 (↓4.5%)	6.1 (↓9.0%)

^ Statistical analysis not performed
* p value < 0.05

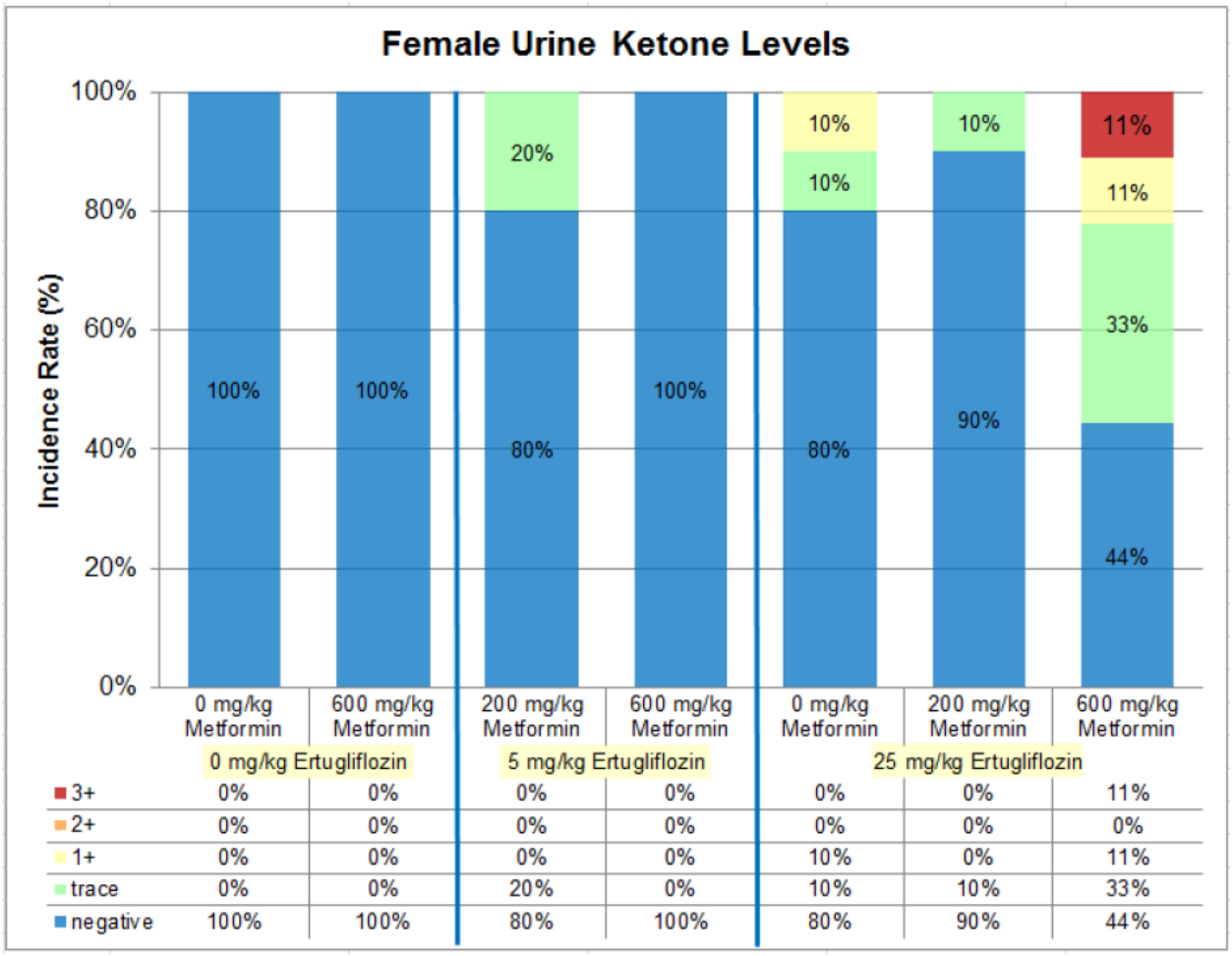
Moderate to marked urine ketone levels of ≥40 mg/dL (2+ and 3+) were observed in 30-40% of males at 25 mg/kg PF-04971729. It is noted that the highest incidence and severity of ketones were observed with co-administration of 200 mg/kg metformin, which may indicate exacerbation; however, incidences and severities were comparable between co-administration of 600 mg/kg metformin and vehicle. Since an increase in metformin coadministration was not associated with exacerbation of ketone incidence rates or severity, there is not a metformin dose-dependent exacerbation of PF-04971729-related increases in urine ketone levels in males. Thus, there is not likely to be a significant interaction regarding kentonuria with metformin co-administration in male rats.

Figure 1: Male Urine Ketone Analysis - 13-week Rat Study #14GR164



Urine ketone levels were lower in females, regardless of vehicle or drug administration. Nevertheless, increases in incidences and severity of urine ketone bodies were apparent with ertugliflozin administration. Slight decreases in incidences and/or severity of ketone bodies were apparent with co-administration of 200 mg/kg metformin. However, the highest incidence rate of females with ketone bodies present (54%) and highest severity (1 female with marked ketones, ≥80 mg/dL, 3+) were observed in the high dose co-administration group with 25 mg/kg PF-04971729 + 600 mg/kg metformin, which may be indicative of exacerbation of ketonuria in female rats.

Figure 2: Female Urine Ketone Analysis - 13-week Rat Study #14GR164



Gross Pathology

Discoloration of the glandular stomach mucosa was observed in 25% of females treated with 25 mg/kg PF-04971729 and ≥200 mg/kg metformin, but not with either drug alone. There was not a drug-related increase in abnormal stomach findings in males compared to concurrent controls.

Macroscopic findings of a large kidney were reported in 3 males and 1 female treated with 25 mg/kg PF-04971729 and 200 mg/kg metformin, but not when PF-04971729 was co-administered with 600 mg/kg metformin. Thus, there is not a clear dose-dependency. Nevertheless, increased kidney size is consistent with drug-related increases in kidney weights and tubule dilatation.

Table 11: Macroscopic Findings - 13-week Rat Study #14GR164

MALES (n=10): Macroscopic Findings		
Tissue	Finding	**Dose Group** (mg/kg PF-04971729 + mg/kg Metformin)

		0+0	0+600	25+0	5+200	5+600	25+200	25+600
Kidney	Large	0	1	1	1	0	3	0
Stomach	Discolored, mucosa, glandular	1	0	0	0	0	0	1

FEMALES (n=10): Macroscopic Findings								
Tissue	Finding	**Dose Group** (mg/kg PF-04971729 + mg/kg Metformin)						
		0+0	0+600	25+0	5+200	5+600	25+200	25+600
Kidney	Large	0	0	0	0	0	1	0
Stomach	Discolored, mucosa, glandular	0	0	0	0	0	2	3

Organ Weights

Drug-related increases in kidney weights were observed in both males (↑17-47%) and females (↑24-51%) treated with ≥5 mg/kg PF-04971729 in the absence or presence of metformin. In females, kidney weights were highest in animals treated with the highest doses of both PF-04971729 and metformin (25+600), reaching up to 38-51% higher than concurrent controls. These data suggest that increases in kidney weights are primarily driven by ≥5 mg/kg PF-04971729, but are exacerbated by 600 mg/kg metformin.

Adrenal gland weights were higher in females (↑19-46%) treated with 600 mg/kg metformin, independent of PF-0971729 co-administration, but only reached statistical significance in all 3 weight parameters (absolute, relative body weight ratio and relative brain weight ratio) at the highest doses of both drug, 25 mg/kg PF-04971729 and 600 mg/kg metformin. Furthermore, the greatest increase was observed at the highest co-administration dose, reaching weights 33-46% higher than concurrent controls. These data suggest that increases in adrenal gland weights are primarily driven by 600 mg/kg metformin, but are exacerbated by 25 mg/kg PF-0971729.

Significant increases in female liver weight parameters were observed in all groups treated with 600 mg/kg metformin in the absence or presence of PF-0971729, as well as animals co-treated with 200 mg/kg metformin and 25 mg/kg PF-0971729. There was not a significant increase in all 3 organ weight parameters in groups treated with PF-0971729 alone or with 5 mg/kg PF-0971729 and 200 mg/kg metformin. Furthermore, the highest weights were observed in animals co-treated with the highest doses of both drugs (25+600), reaching an increase of 34-47% above concurrent controls. Together, these data suggest that increases in liver weights are primarily driven by high doses of metformin, but are exacerbated by 25 mg/kg PF-0971729.

Significant increases in all 3 heart weight parameters were observed in females (↑17-29%) treated with both 25 mg/kg PF-0971729 and 600 mg/kg metformin. Although

statistically significant increases in all 3 organ weight parameters were not observed in any of the other groups, there was a trend for increased heart weight in ¾ of the other groups treated with metformin. Nevertheless, these data suggest that significant increases in heart weights were dependent on co-administration of both drugs at the highest doses in what was likely to be an additive effect.

Sponsor's Table 4: Organ Weights- 13-week Rat Study #14GR164

Text Table 4.1: Test Article-Related Changes in Organ Weight Parameters in Males

Sex		Males					
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Terminal Body Weight (g)	522	0.94x	0.95x	0.94x	0.85x*	0.92x	0.80x*
Kidney							
Absolute Weight (g)	2.9981	1.08x	1.20x*	1.16x	1.17x*	1.28x*	1.19x*
Body Weight Ratio (%)	0.5765	1.15x*	1.26x*	1.22x*	1.38x*	1.40x*	1.47x*
Brain Weight Ratio (%)	138.3293	1.07x	1.18x*	1.12x	1.18x*	1.25x*	1.20x*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for terminal body weight, absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold control mean value.

Text Table 4.2: Test Article-Related Changes in Organ Weight Parameters in Females

Sex		Females					
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Terminal Body Weight (g)	279	0.99x	0.89x*	0.93x	0.93x	0.96x	0.91x*
Kidney							
Absolute Weight (g)	1.5841	1.04x	1.26x*	1.28x*	1.34x*	1.36x*	1.38x*
Body Weight Ratio (%)	0.5707	1.05x	1.41x*	1.37x*	1.44x*	1.41x*	1.51x*
Brain Weight Ratio (%)	79.4099	1.04x	1.24x*	1.24x*	1.34x*	1.35x*	1.40x*
Adrenal							
Absolute Weight (g)	0.0641	1.23x*	1.08x	1.06x	1.19x	1.17x	1.33x*
Body Weight Ratio (%)	0.0231	1.24x	1.21x	1.13x	1.30x*	1.21x	1.46x*
Brain Weight Ratio (%)	3.2064	1.23x*	1.06x	1.02x	1.19x	1.16x	1.35x*
Liver							
Absolute Weight (g)	7.2772	1.16x*	1.04x	1.12x	1.19x*	1.18x*	1.34x*
Body Weight Ratio (%)	2.6176	1.17x*	1.16x*	1.21x*	1.28x*	1.22x*	1.47x*
Brain Weight Ratio (%)	365.1983	1.16x*	1.02x	1.09x	1.18x*	1.17x*	1.36x*
Heart							
Absolute Weight (g)	1.0113	1.12x	0.98x	1.08x	1.10x	1.05x	1.17x*
Body Weight Ratio (%)	0.3640	1.13x*	1.10x	1.15x*	1.18x*	1.09x	1.29x*
Brain Weight Ratio (%)	50.7567	1.12x	0.96x	1.04x	1.09x	1.05x	1.19*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for terminal body weight, absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold control mean value.

(Tables excerpted from sponsor's report and highlighted)

Histopathology

Battery Considered Adequate? Yes
Peer Review Performed? Yes

Drug-related increases in minimal to moderate kidney tubule dilatation characterized by the presence of dilated renal tubules in the outer medulla were observed in 70% to 100% of animals treated with either PF-04971729 alone or in combination with metformin. In both sexes, increases in severity were dose-dependent with regard to PF-04971729, but were largely independent of the metformin dose. These findings indicate that drug-related kidney tubule dilation findings were primarily driven by PF-04971729.

Drug-related increases in incidence and severity of pancreatic zymogen granule decreases, resulting in smaller acinar cells and acini, were observed in both sexes. Furthermore, the increases in both incidence and severity were dose-dependent with regard to PF-04971729, but were largely independent of the metformin dose. These findings suggest that drug-related decreases in pancreatic zymogen granules were primarily driven by PF-04971729.

Hypertrophy of the adrenal cortex associated with increases in cell size and cytoplasmic vacuolation was observed in both sexes. In males, minimal hypertrophy of the zona glomerulosa was observed in up to 40% of animals treated with 25 mg/kg PF-04971729 alone, but lacked a clear dose-dependence or consistency with regard to PF-04971729, metformin, or the combination. In females, hypertrophy of the adrenal cortex was consistently observed in 60-80% of animals in the highest co-administration group (15+600), indicating a potential drug-related finding with regard to co-administration.

In males, minimal erosion and/or ulcer of the glandular stomach was reported in 20% of animals that were administered 25 mg/kg PF-04971729 alone or in combination with 600 mg/kg metformin, but not in combination with 200 mg/kg metformin. In females, minimal erosion and/or ulcer was reported in 20% of animals treated with both 25 mg/kg PF-04971729 and 600 mg/kg metformin. Although, there is not a consistent pattern of dose-dependence with regard to either drug alone or in combination, these findings are consistent with previous PF-04971729 toxicology studies and are likely to be drug-related.

Metformin-related increases in incidence and severity of mandibular and sublingual salivary gland findings were apparent in both sexes. Minimal to marked decreases in cytoplasmic granules in the duct epithelium of the mandibular salivary gland were dose-dependent with regard to metformin in both males and females, but were largely independent of the PF-04971729 dose. Metformin-dependent increased incidences of minimal to moderate sublingual salivary gland hypertrophy of the duct epithelium, characterized by enlarged cuboidal to columnar cells with abundant eosinophilic cytoplasm and nuclei, were also observed in both sexes. However, in males, the severity of the salivary gland hypertrophy was increased at the highest dose of both

metformin and PF-0471729, indicating that co-administration with 25 mg/kg PF-047179 may increase the severity of this predominantly metformin-driven finding.

Sponsor's Table 5: Histopathology- 13-week Rat Study #14GR164

Text Table 4.4: Incidence and Severity of Test Article-Related Microscopic Findings in Males

	PF-04971729/Metformin						
Sex	Males						
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Number Examined	10	10	10	10	10	10	10
Kidney							
Dilatation, tubule(s)							
Total	5	6	10	9	9	8	10
Minimal	5	6	2	3	5	1	0
Mild	0	0	3	5	3	3	4
Moderate	0	0	4	1	0	3	6
Marked	0	0	1	0	1	1	0
Pancreas							
Zymogen granules, decreased							
Total	0	0	10	3	2	9	10
Minimal	0	0	2	3	2	3	2
Mild	0	0	3	0	0	2	5
Moderate	0	0	5	0	0	4	3
Adrenal Cortex							
Hypertrophy, zona glomerulosa							
Total	0	0	4	3	2	2	0
Minimal	0	0	4	3	2	2	0
Stomach, Glandular							
Erosion/ulcer							
Total	0	0	2	0	0	0	2
Minimal	0	0	2	0	0	0	2
Mandibular Salivary Gland							
Decreased cytoplasmic granules, duct epithelium							
Total	0	9	0	1	10	5	10
Minimal	0	3	0	1	0	2	0
Mild	0	4	0	0	2	3	1
Moderate	0	2	0	0	6	0	8
Marked	0	0	0	0	2	0	1
Sublingual Salivary Gland							
Number Examined	10	10	9	10	10	10	10
Hypertrophy, duct epithelium							
Total	0	8	0	0	8	4	9
Minimal	0	5	0	0	8	4	2
Mild	0	3	0	0	0	0	5
Moderate	0	0	0	0	0	0	2

Text Table 4.5: Incidence and Severity of Test Article-Related Microscopic Findings in Females

	PF-04971729/Metformin						
Sex	Females						
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Number Examined[a]	10	10	10	10	10	10	10
Kidney							
Dilatation, tubule(s)							
Total	6	6	10	7	10	7	10
Minimal	6	6	2	6	4	6	3
Mild	0	0	3	1	6	1	4
Moderate	0	0	5	0	0	0	3
Pancreas							
Zymogen granules, decreased							
Total	3	0	10	7	4	8	10
Minimal	2	0	3	3	2	3	0
Mild	1	0	4	4	1	2	6
Moderate	0	0	3	0	1	3	4
Adrenal Cortex							
Hypertrophy, zona glomerulosa							
Total	0	0	4	3	0	2	6
Minimal	0	0	4	3	0	2	6
Hypertrophy							
Total	1	3	0	3	3	1	8
Minimal	1	3	0	3	3	1	8
Stomach, glandular							
Erosion/ulcer							
Total	0	0	0	0	0	0	2
Minimal	0	0	0	0	0	0	2
Mandibular Salivary Gland							
Decreased cytoplasmic granules, duct epithelium							
Total	0	10	0	7	10	9	10
Minimal	0	0	0	2	0	3	0
Mild	0	2	0	5	1	4	1
Moderate	0	5	0	0	3	2	3
Marked	0	3	0	0	6	0	6
Sublingual Salivary Gland							
Number Examined	10	10	10	10	10	9	10
Hypertrophy, duct epithelium							
Total	0	10	0	5	10	4	10
Minimal	0	0	0	5	5	3	1
Mild	0	9	0	0	5	1	9
Moderate	0	1	0	0	0	0	0

a Number examined for all tissues unless noted otherwise.

(Tables excerpted from sponsor's report and highlighted)

Toxicokinetics

In animals treated with 25 mg/kg PF-04971729 alone or in combination with 600 mg/kg, exposures tended to be higher in females. However, given the amount of variability,

there were no clear gender effects. Exposures increased dose-proportionally. However, exposures were dose-dependently lower by up to 60% with increasing metformin dose. Thus, animals in the highest co-administration group (25+600) had the lowest PF-04971729 exposures of all groups receiving 25 mg/kg PF-04971729. T_{max} was generally achieved between 1 and 7 hours postdose, with the exception of 2 animals in the high dose co-administration group (25+600) on Day 1 that achieved T_{max} at 24 hours postdose.

Sponsor's Table 6: PF-04971729 TK - 13-week Rat Study #14GR164

6.1. Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Metformin

Dose PF-04971729 / Metformin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
25 / 0	1	Male	6920	7	99700
		Female	12300	7	172000
		Overall	9610	7	136000
	91	Male	7960	4	104000
		Female	13100	7	186000
		Overall	9740	7	144000
5 / 200	1	Male	1530	4	17300
		Female	1460	4	17100
		Overall	1500	4	17200
	91	Male	2030	1	22200
		Female	2070	1	20200
		Overall	2050	1	21200
5 / 600	1	Male	842	4	9100
		Female	959	4	14100
		Overall	900	4	11600
	91	Male	1600	1	13500
		Female	3770	1	24900
		Overall	2680	1	19200
25 / 200	1	Male	6850	4	83000
		Female	7740	4	87000
		Overall	7290	4	84900
	91	Male	8670	1	110000
		Female	16300	1	130000
		Overall	12500	1	120000
25 / 600	1	Male	4240	24	72600
		Female	4640	4	83200
		Overall	4340	1	77800
	91	Male	10900	1	77900
		Female	10400	1	105000
		Overall	10600	1	91200

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package and highlighted)

Metformin exposures were unaffected by PF-04971729 co-administration. At 600 mg/kg metformin, AUC and C_{max} exposures were 30% and 50-130% higher, respectively, on Day 91 compared to Day 1, indicating potential accumulation at the

high dose. There were no apparent gender effects on metformin exposures. T_{max} was generally achieved between 1 and 4 hours postdose.

Sponsor's Table 7: Metformin TK - 13-week Rat Study #14GR164

6.2. Mean Toxicokinetic Parameters for Metformin in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Metformin

Dose PF-04971729 / Metformin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
0 / 600	1	Male	10700	1	130000
		Female	10900	1	81900
		Overall	10800	1	106000
	91	Male	18300	1.00	157000
		Female	13500	1.00	118000
		Overall	15900	1.00	138000
5 / 200	1	Male	5930	4	45900
		Female	6470	1	51300
		Overall	5640	4	48500
	91	Male	6600	1.00	45700
		Female	8680	1.00	50500
		Overall	7640	1.00	48000
5 / 600	1	Male	9830	4	85000
		Female	9330	4	106000
		Overall	9580	4	95600
	91	Male	14400	1.00	111000
		Female	24100	1.00	134000
		Overall	19200	1.00	122000
25 / 200	1	Male	6290	4	60300
		Female	5210	4	38700
		Overall	5750	4	49500
	91	Male	8840	1.00	70500
		Female	8050	1.00	37100
		Overall	8440	1.00	53800
25 / 600	1	Male	8170	1	104000
		Female	11300	1	103000
		Overall	9710	1	104000
	91	Male	24400	1.00	130000
		Female	20700	1.00	139000
		Overall	22500	1.00	135000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package and highlighted)

Dosing Formulation Analysis

PF-04971729 and metformin dose formulations were analyzed using a validated high-performance liquid chromatography (HPLC) method. Overall mean concentrations of PF-04971729 and metformin formulations were within ±10% of the target concentrations.

7 Genetic Toxicology

Genetic toxicology studies with the FDC or coadministration of ertugliflozin and metformin have not been conducted, but are not required. Since both ertugliflozin and metformin are not genotoxic; there is not a genotoxic concern with the FDC product.

Ertugliflozin

Based on the weight of evidence, ertugliflozin is not considered to be genotoxic. Ertugliflozin was evaluated for genotoxic potential in a standard battery of valid genotoxicity assays, including in vitro microbial reverse mutation (Ames), in vitro human lymphocyte cytogenetic, and in vivo rat micronucleus assays.

Metformin

There is no evidence of a mutagenic potential for metformin in the Ames, mouse lymphoma, in vitro chromosomal aberration, or in vivo mouse micronucleus tests.

8 Carcinogenicity

Combination carcinogenicity studies with the FDC or coadministration of ertugliflozin and metformin have not conducted, but are not required.

Ertugliflozin

Rat and mouse carcinogenicity studies with administration of ertugliflozin alone were reviewed under NDA #209803.

In the 2-year carcinogenicity study conducted in male and female Crl:CD1(ICR) mice, ertugliflozin was administered daily at doses of 5, 15 or 40 mg/kg/day, in accordance with ECAC dosing recommendations. All male groups were terminated during Week 97 and all female groups were terminated during Week 102 due to low survival that was not drug-related. There were no significant drug-related neoplastic findings in male or female mice at any of the doses examined, and the NOAEL for neoplasms was set at the high dose of 40 mg/kg/day (~50x $MRHD_{AUC}$). It is also noted that non-adverse PD-related kidney and bladder findings were considered to be comparable to similar findings observed in shorter toxicology studies.

In the 2-year carcinogenicity study conducted in male and female SD rats, ertugliflozin was administered daily at doses of 1.5, 5, or 15 mg/kg/day ertugliflozin, in accordance with ECAC dosing approval with the exception of exclusion of a saline/water control group. In female rats, there were no statistically significant increases in incidences of benign or malignant neoplasms in any tissues, with a neoplastic NOAEL of 15 mg/kg/day (74x $MRHD_{AUC}$). However, in male rats, drug-related increases in the incidences of adrenal medulla benign pheochromocytoma (PCC) and combined benign + malignant PCC neoplasms were reported at 15 mg/kg/day (66x $MRHD_{AUC}$), resulting in a NOAEL for neoplasms of 5 mg/kg/day (18x $MRHD_{AUC}$). The incidence rates and timing of PCC observations correlated with drug-related increases in adrenal medulla hyperplasia at ≥5 mg/kg/day in a manner that was considered to be consistent with a

continuum of tumor development. Thus, the increased incidences of adrenal medulla hyperplasia and PCC observed at ≥5 mg/kg/day were considered possibly drug-related, but were not considered to be unequivocally drug-related.

Metformin

Long-term carcinogenicity studies have been performed in rats and mice with doses up to 900 mg/kg/day and 1500 mg/kg/day, respectively, with exposure margins approximately 4-fold higher than the maximum recommended human daily dose of 2000 mg based on body surface area (BSA) comparisons (4x $MRHD_{BSA}$). There was no evidence of carcinogenicity in male for female mice. Similarly, no evidence of carcinogenicity was observed in male rats; however, increases in incidences of benign stromal uterine polyps were observed in female rats at the high dose of 900 mg/kg/day (4x $MRHD_{BSA}$).

9 Reproductive and Developmental Toxicology

Combination reproductive and developmental toxicology studies with the FDC or coadministration of ertugliflozin and metformin have not conducted, but are not required. Based on results from a juvenile toxicology study in rats, ertugliflozin exposure poses a potential risk to human renal development. Thus, the FDC product will also have a potential risk to human renal development and a combination embryonic fetal development (EFD) study is not required, in accordance ICH M3(R2), as a hazard has already been identified for the ertugliflozin component. Therefore labeling for reproductive and developmental hazards of the FDC will be based on each individual drug component. Please see original NDA reviews for ertugliflozin (NDA 209803) and metformin (NDA 21202) for experimental detail.

10 Special Toxicology Studies

Ertugliflozin was evaluated for eye and skin irritancy using ex vivo, in vitro and in vivo local tolerance tests. In the bovine corneal opacity and permeability (BCOP) test, the solid form of ertugliflozin was positive for eye irritancy, and ertugliflozin was classified as a category 1 ocular irritant. In human skin 3-dimensional cultures, direct exposure to solid ertugliflozin was corrosive after 1 hour of exposure, but not after acute exposures of ≤3 minutes. However, in the mouse local lymph node assay (LLNA) test, exposure to ertugliflozin solution at a concentration up to ~3-fold higher than the clinical dose was negative for dermal contact hypersensitivity. Thus, ertugliflozin was not classified as a skin sensitizer. Overall, there is not a significant safety concern for skin sensitivity for brief periods of time, such as during oral administration. However, direct eye exposure should be avoided.

Ertugliflozin was not evaluated in phototoxicity assays, but is considered to be negative for potential phototoxicity. Although metformin is associated with photosensitivity, phototoxicity studies are not be required for the combined product FDC.

11 Labeling Review

Only labeling specific to the FDC or the sitagliptin component are captured in this review. Please see the NDA review under #209803 for labeling recommendations regarding the ertugliflozin component.

Section 8 Use in Specific Populations

Section 8.1 Pregnancy

Excerpt 1: Sponsor's Proposed Section 8.1 Text

8.1 Pregnancy

Risk Summary

(b) (4)

(b) (4) TRADEMARK is not recommended during the second and third trimesters of pregnancy.

(b) (4)

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk

Poorly-controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, spontaneous abortions, preterm delivery, stillbirth, and delivery complications. It can also increase the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Human Data

Published data from post-marketing studies have not reported a clear association with metformin and major birth defects, miscarriage, or adverse maternal or fetal outcomes when metformin was used during pregnancy. However, these studies cannot definitely establish the absence of any metformin-associated risk because of methodological limitations, including small sample size and inconsistent comparator groups.

Animal Data

Ertugliflozin

In embryo-fetal development studies, ertugliflozin (50, 100 and 250 mg/kg/day) was administered orally to rats on gestation days 6 to 17 and to rabbits on gestation days 7 to 19. Ertugliflozin did not adversely affect developmental outcomes in rats and rabbits at maternal exposures that were (b) (4) (b) (4) the human exposure at the maximum clinical dose of 15 mg/day, based on AUC. (b) (4) a maternally toxic dose in rats (250 mg/kg/day), (b) (4) fetal viability, (b) (4) a higher incidence of a visceral malformation (membranous ventricular septal defect) (b) (4) (b) (4) (b) (4) In the pre- and postnatal development study, decreased postnatal growth (b) (4) (b) (4) were observed in rats administered ertugliflozin gestation day 6 through lactation day 21 at ≥100 mg/kg/day ((b) (4) times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC).

When ertugliflozin was orally administered to juvenile rats from PND 21 to PND 90, increased kidney weight, renal tubule and renal pelvis dilatation, and renal mineralization occurred at doses greater than or equal to 5 mg/kg (13-fold human exposures). These effects did not fully reverse within the 1 month recovery period. (b) (4)

Metformin hydrochloride

Metformin did not adversely affect development outcomes when administered to rats and rabbits at doses up to 600 mg/kg/day. This represents an exposure of about 2 and 6 times the maximum recommended human dose of 2,000 mg based on body surface area comparisons for rats and rabbits, respectively. Determination of fetal concentrations demonstrated a partial placental barrier to metformin.

(Excerpted from sponsor's package)

Reviewer's Comments

In the Risk Summary section, (b) (4) Similarly, "daily" was added to the Animal Data section for metformin. Clarification of a daily dose of 2000 mg is also included in the metformin label.

Reviewer's Proposed Section 8.1 Text

Metformin hydrochloride

Metformin did not adversely affect development outcomes when administered to rats and rabbits at doses up to 600 mg/kg/day. This represents an exposure of about 2 and 6 times the maximum recommended human daily dose of 2,000 mg based on body surface area comparisons for rats and rabbits, respectively. Determination of fetal concentrations demonstrated a partial placental barrier to metformin.

Section 8.2 Lactation

Excerpt 2: Sponsor's Proposed Section 8.2 Text

8.2 Lactation

Risk Summary

There is no information regarding the presence of TRADEMARK or ertugliflozin in human milk, the effects on the breastfed infant, or the effects on milk production. Limited published studies report that metformin is present in human milk *[see Data]*. However, there is insufficient information on the effects of metformin on the breastfed infant and no available information on the effects of metformin on milk production. Ertugliflozin and metformin are present in the milk of lactating rats *[see Data]*. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney, based on data with ertugliflozin.

(b) (4)

Data

Human Data

Published (b) (4) studies report that metformin is present in human milk which resulted in infant doses approximately 0.11% to 1% of the maternal weight-adjusted dosage and a milk/plasma ratio ranging between 0.13 and 1. (b) (4)

(b) (4)

Animal Data

Ertugliflozin

The lacteal excretion of radiolabeled ertugliflozin in lactating rats was evaluated 10 to 12 days after parturition. Ertugliflozin derived radioactivity exposure in milk and plasma were similar, with a milk/plasma ratio of 1.07, based on AUC.

Metformin hydrochloride

(b) (4)

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's animal data regarding metformin are supported by the available data and are considered to be adequate.

Section 12 Clinical Pharmacology

Section 12.1 Mechanism of Action

Excerpt 3: Sponsor's Proposed Section 12.1 Text

12.1 Mechanism of Action

TRADEMARK

TRADEMARK combines two antihyperglycemic agents with complementary mechanisms of action to improve glycemic control in patients with type 2 diabetes: ertugliflozin, a SGLT2 inhibitor, and metformin hydrochloride, a member of the biguanide class.

Ertugliflozin

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Ertugliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, ertugliflozin reduces renal reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion.

Metformin hydrochloride

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. (b) (4) metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects (except in special circumstances, *[see Warnings and Precautions (5.6)]* and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's proposed text is supported by the nonclinical data and is considered to be acceptable.

Section 12.3 Pharmacokinetics

Excerpt 4: Sponsor's Proposed Section 12.3 Text

Metabolism

Ertugliflozin

Metabolism is the primary clearance mechanism for ertugliflozin. The major metabolic pathway for ertugliflozin is UGT1A9 and UGT2B7-mediated O-glucuronidation to two glucuronides that are pharmacologically inactive at clinically relevant concentrations. CYP-mediated (oxidative) metabolism of ertugliflozin is minimal (12%).

Metformin hydrochloride

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion.

Drug Interaction Studies

TRADEMARK

Pharmacokinetic drug interaction studies with TRADEMARK have not been performed; however, such studies have been conducted with ertugliflozin and metformin, the individual components of TRADEMARK.

Ertugliflozin

In Vitro Assessment of Drug Interactions

In *in vitro* studies, ertugliflozin and ertugliflozin glucuronides did not inhibit CYP450 isoenzymes (CYPs) 1A2, 2C9, 2C19, 2C8, 2B6, 2D6, or 3A4, and did not induce CYPs 1A2, 2B6, or 3A4. Ertugliflozin was not a time-dependent inhibitor of CYP3A *in vitro*. Ertugliflozin did not inhibit UGT1A6, 1A9, or 2B7 *in vitro* and was a weak inhibitor (IC_{50} >39 µM) of UGT1A1 and 1A4. Ertugliflozin glucuronides did not inhibit UGT1A1, 1A4, 1A6, 1A9, or 2B7 *in vitro*. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of drugs eliminated by these enzymes. Ertugliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and is not a substrate of organic anion transporters (OAT1, OAT3), organic cation transporters (OCT1, OCT2), or organic anion transporting polypeptides (OATP1B1, OATP1B3). Ertugliflozin or ertugliflozin glucuronides do not meaningfully inhibit P-gp, OCT2, OAT1, or OAT3 transporters at clinically relevant concentrations. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of concurrently administered medications that are substrates of these transporters.

In Vivo Assessment of Drug Interactions

No dose adjustment of TRADEMARK is recommended when coadministered with commonly prescribed medicinal products. Ertugliflozin pharmacokinetics were similar with and without coadministration of metformin, glimepiride, sitagliptin, and simvastatin in healthy subjects (see Figure 1). Coadministration of ertugliflozin with multiple doses of 600 mg once daily rifampin (an inducer of UGT and CYP enzymes) resulted in approximately 39% and 15% mean reductions in ertugliflozin AUC and C_{max}, respectively, relative to ertugliflozin administered alone. These changes in exposure are not considered clinically relevant. Ertugliflozin had no clinically relevant effect on the pharmacokinetics of metformin, glimepiride, sitagliptin, and simvastatin when coadministered in healthy subjects (see Figure 2). Physiologically-based PK (PBPK) modeling suggests that coadministration of mefenamic acid (UGT inhibitor) may increase the AUC and C_{max} of ertugliflozin by 1.51- and 1.19-fold, respectively. These predicted changes in exposure are not considered clinically relevant.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's data regarding metformin and the PBPK modeling of DDI are supported by the available nonclinical data and are considered to be adequate.

Section 13 Nonclinical Toxicology

Section 13.1 Carcinogenicity & Mutagenesis & Impairment of Fertility

Excerpt 5: Sponsor's Proposed Section 13.1 Text

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Ertugliflozin

In the (b) (4) mouse (b) (4) study, ertugliflozin was administered by oral gavage at doses of 5, 15, and 40 mg/kg/day. There were no ertugliflozin-related neoplastic findings at doses up to 40 mg/kg/day (approximately (b) (4) times human exposure at the maximum recommended human dose [MRHD] of 15 mg/day based on AUC). In the (b) (4) rat (b) (4) study, ertugliflozin was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg/day. Ertugliflozin-related neoplastic findings included an increased incidence of (b) (4) adrenal medullary pheochromocytoma in male rats at 15 mg/kg/day. This finding was attributed to carbohydrate malabsorption leading to altered calcium homeostasis (b) (4) to human risk. The no-observed-effect level (NOEL) for neoplasia was 5 mg/kg/day (approximately 16 times human exposure at the MRHD of 15 mg/day).

Metformin hydrochloride

Long-term carcinogenicity studies have been performed in rats (dosing duration of 104 weeks) and mice (dosing duration of 91 weeks) at doses up to and including 900 mg/kg/day and 1,500 mg/kg/day, respectively. These doses are both approximately four times the maximum recommended human daily dose of 2,000 mg based on body surface area comparisons. No evidence of carcinogenicity with metformin was found in either male or female mice. Similarly, there was no tumorigenic potential observed with metformin in male rats. There was, however, an increased incidence of benign stromal uterine polyps in female rats treated with 900 mg/kg/day.

Mutagenesis

Ertugliflozin

Ertugliflozin was not mutagenic or clastogenic with or without metabolic activation in the microbial reverse mutation, *in vitro* cytogenetic (human lymphocytes), and *in vivo* rat micronucleus assays.

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.

Impairment of Fertility

Ertugliflozin

In the rat fertility and embryonic development study, male and female rats were administered ertugliflozin at 5, 25, and 250 mg/kg/day. No effects on fertility were observed at 250 mg/kg/day (approximately (b) (4) times human exposure at the MRHD of 15 mg/day based on AUC comparison).

Metformin hydrochloride

Fertility of male or female rats was unaffected by metformin when administered at doses as high as 600 mg/kg/day, which is approximately three times the maximum recommended human daily dose based on body surface area comparisons.

(Excerpted from sponsor's package)

Reviewer's Comments

The sponsor's text regarding metformin is reflected in the most recent metformin label (04/2017) and is considered to be appropriate.

Section 13.2 Animal Pharmacology and/or Toxicology

This section was not included in the sponsor's proposed label.

Reviewer's Comments
This section was also not included in the label for ertugliflozin or metformin. Thus, omission of this section is considered to be acceptable.

12 Integrated Summary and Safety Evaluation

This review evaluates the nonclinical safety profile of the NME ertugliflozin FDC with metformin submitted by Merck Sharp and Dohme Corp for the treatment of T2DM. The non-clinical pharmacology, general toxicology, carcinogenicity, reproductive and developmental, and special toxicology studies establishing the safety profile of the NME alone were fully evaluated under NDA #209803, but were also submitted in support of the FDC product under NDA #209806. The sponsor is also referencing the approved label for Glucophage® (metformin).

Ertugliflozin inhibits SGLT2 resulting in significant glucosuria, which is associated with concomitant decreases in plasma glucose levels despite compensatory increases in food consumption. Metformin suppresses hepatic glucose production, increases insulin sensitivity, enhances peripheral glucose uptake, decreases insulin-induced suppression of fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract. Thus, although both ertugliflozin and metformin reduce blood glucose levels, they act through different mechanisms.

Based on pathways of elimination, absorption and metabolization, significant DDI on PK parameters between ertugliflozin and metformin are not anticipated. Although coadministration of metformin was associated in reduced ertugliflozin exposures in rats, there were no meaningful differences in ertugliflozin or metformin PK parameters in clinical studies. Thus, the effect observed in rats is likely to species specific and may correlate with differences in drug metabolism. Regardless, concomitant administration of ertugliflozin and metformin are not associated with significant changes in drug exposures in humans.

Based on safety pharmacology studies, both ertugliflozin and metformin may be associated with some concern for CV effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. Furthermore, CV effects in humans with coadministration of ertugliflozin and metformin was investigated in an add-on sub-study of the clinical CV safety study #P004/B1521021. Thus, further evaluation of the FDC product in nonclinical safety pharmacology studies is not warranted.

In accordance with ICH guidelines, the sponsor submitted a GLP-compliant 3-month combination toxicology study with coadministration of ertugliflozin and metformin in rats. In the 3-month rat study, the majority of the drug-related findings were attributed to PD-related inhibition of SGLT2 mediated by ertugliflozin. Kidney tubule dilatations and increased organ weights were attributed to the PD activity of SGLT2 inhibition mediated by ertugliflozin, but were not associated with indications of kidney dysfunction or toxicity and were considered likely to be non-adverse. Stomach findings were attributed to ertugliflozin and considered likely due to off-target SGLT1 inhibition, but were of minimal severity and were not considered adverse. Non-adverse salivary gland findings were

attributed to known effects of metformin. Potential synergistic effects of the combination were limited to exacerbation of ertugliflozin PD-related effects on body weights and weight gain in males, despite increased food consumption; however, this effect was considered to be non-adverse. Overall, there were no toxicologically significant new or adverse synergistic toxicities due to coadministration of ertugliflozin and metformin. Thus, the NOAEL was set at the high combination dose of 25 mg/kg ertugliflozin and 600 mg/kg metformin. The safety margin for ertugliflozin is 66x MRHD based on AUC ($MRHD_{AUC}$) for a high dose of 15 mg/day, and the safety margin for metformin is 5x $MRHD_{AUC}$ based on a high dose of 2000 mg/day.

It's noted that ertugliflozin-related ketonuria was potentially exacerbated with metformin coadministration in females. Although this finding was considered to be non-adverse in this study, it is notable given that diabetic ketoacidosis (DKA) has been observed clinically in diabetic patients treated with SGLT2 inhibitors. It is also noted that the observations of ketonuria correlate with ertugliflozin-related reductions in body weights and may be secondary to ertugliflozin PD-related inhibition of carbohydrate absorption and decreases in glucose levels, which may then be consistent with non-adverse nutritional ketosis.

In summary, the 13-week coadministration toxicology study in rats adequately bridges the proposed FDC to the ertugliflozin nonclinical studies and did not identify and new toxicologically significant toxicities. Since the safety margins for coadministration of ertugliflozin (66x MRHD) and metformin (5x MRHD) in the 13-week rat study are sufficient, the nonclinical data support clinical dosing of the FDC product at ertugliflozin doses up 15 mg/day ertugliflozin and metformin doses up to 2000 mg/day.

FDC Toxicology Summary Table

Table 12: Ertugliflozin + Metformin Coadministration Human Safety Margins

Study	NOAEL (mg/kg)	Human Safety Margin (Based on AUC*)	Findings
2 Week (Non-GLP) **Ertugliflozin/Metformin:** 5/200, 5/600, 25/200, 25/600, 25/0, & 0/600 mg/kg Ertugliflozin AUC: 26, 15, 109, 77, 124, & - μg·h/mL Metformin AUC: 59, 138, 76, 183, -, & 140 μg·h/mL	Ertugliflozin / Metformin **25 / 600**	Ertugliflozin: **56x** Metformin: **7x**	*No significant systemic adverse effects.* **5 / 200 mg/kg (19x/2x MRHD):** Blood (↓glucose, 2-fold ↑BUN), urine [glucosuria, ↑specific gravity, ↑ketones (♂)], ↑food consumption **5 / 600 mg/kg (11x/6x MRHD):** Blood [↓glucose, 2-fold ↑BUN, <2-fold ↑AST (♂), <2-fold ↑ALT (♂)], (glucosuria, ↑specific gravity, ↑ketones), salivary gland (↓cytoplasmic granules, hypertrophy), ↑food consumption **25 / 200 mg/kg (79x/3x MRHD):** Blood (↓glucose, 2-fold ↑BUN, ↓Cl), urine (glucosuria, ↑specific gravity, ↑ketones), discolored stomach (♂), kidney (tubule hypertrophy), pancreas (↓zymogen), ↓cytoplasmic granules in salivary glands (♂), ↑food consumption **25 / 600 mg/kg (56x/7x MRHD):** Blood (↓glucose, 2-fold ↑BUN, ↓Cl, <2-fold ↑AST, <2-fold ↑ALT), (glucosuria, ↑specific gravity, ↑ketones), stomach (discolored, erosion), kidney (tubule hypertrophy), pancreas (↓zymogen), salivary gland (↓cytoplasmic granules, hypertrophy), ↑food consumption **25 / 0 mg/kg (90x/0x MRHD):** Blood (↓glucose, 2-fold ↑BUN), urine (glucosuria, ↑specific gravity, ↑ketones), stomach (1 discolored, erosion), pancreas (↓zymogen), ↑food consumption, **0 / 600 mg/kg (0x/9x MRHD):** urine (↑ketones), salivary gland (↓cytoplasmic granules, hypertrophy)

Study	NOAEL (mg/kg)	Human Safety Margin (Based on AUC*)	Findings
13 Week (GLP) **Ertugliflozin/Metformin:** 5/200, 5/600, 25/200, 25/600, 25/0, & 0/600 mg/kg Ertugliflozin AUC: 21.2, 19.2, 120, 91.2, 144, & - μg·h/mL Metformin AUC: 48, 122, 53.8, 135, -, & 138 μg·h/mL	Ertugliflozin / Metformin **25 / 600**	Ertugliflozin: **66x** Metformin: **5x**	*No significant systemic adverse effects.* **5 / 200 mg/kg (15x/2x MRHD):** ↑Food consumption, Blood [↓Cl , ↓glucose, ↓Creatinine (♀), ↑36% BUN (♀)], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH], kidney [↑weight (♀), mild-marked tubule dilatation], pancreas (↓zymogen), adrenal hypertrophy, salivary gland [↓cytoplasmic granules, hypertrophy (♀)] **5 / 600 mg/kg (14x/5x MRHD):** ↑Food consumption, Blood [↓Cl, ↓Na (♀), ↓glucose, ↓Creatinine, ↑29% BUN (♀)], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂),↓pH (♂)], ↓BW (♂) & ↓BW gain (♂), kidney (↑weight, mild-marked tubule dilatation), liver (↑weight), pancreas (↓zymogen), adrenal hypertrophy, salivary gland (↓cytoplasmic granules, hypertrophy) **25 / 200 mg/kg (87x/2x MRHD):** ↑Food consumption, Blood [↓glucose, ↓Cl, ↓Na (♀), ↓Ca (♀), ↓Creatinine (♀), ↑BUN (♂ ↑71%, ♀↑ 25%)], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂),↓pH, ↑ketones (♂)] , discolored stomach (♀), kidney (enlarged, ↑weight, mild-marked tubule dilatation), pancreas (↓zymogen), adrenal hypertrophy, salivary gland (↓cytoplasmic granules, hypertrophy), liver ↑weight (♀), **25 / 600 mg/kg (66x/5x MRHD):** ↑Food consumption, Blood [↓glucose, ↓Cl, ↓Na (♀), ↓Ca (♀),↓Creatinine, ↓protein, ↓cholesterol, ↑43% BUN (♀)],urine [glucosuria, ↑specific gravity (♀), ↑volume (♂),↓pH, ↑ketones], ↓BW (♂) & ↓BW gain (♂), stomach (discolored, erosion/ulcer), kidney (↑weight, mild-marked tubule dilatation), adrenal [↑weight (♀), hypertrophy (♀)], liver ↑weight (♀), heart ↑weight (♀),pancreas (↓zymogen) , salivary gland (↓cytoplasmic granules, hypertrophy) **25 / 0 mg/kg (104x/0x MRHD):** ↑Food consumption, Blood [↓ Cl, ↓Ca (♀ , ↓glucose, ↓Creatinine (♀), ↑BUN (♂ ↑2-fold, ♀ ↑44%)], urine [glucosuria, ↑specific gravity (♀), ↑volume (♂), ↓pH, ↑ketones (♂)], kidney (↑weight, mild-marked tubule dilatation), pancreas (↓zymogen), stomach erosion/ulcer (♂), adrenal hypertrophy, ↓BW (♀) **0 / 600 mg/kg (0x/6x MRHD):** Blood [↓Creatinine (♀)], adrenal [↑weight (♀), hypertrophy (♀)], liver ↑weight (♀), salivary gland (↓cytoplasmic granules, hypertrophy)

* Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with ertugliflozin exposure of AUC = 1.38 μg·h/mL and metformin exposure of AUC = 25 μg·h/mL.
♂ = Males only
♀ = Females only

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

/s/

JESSICA HAWES
08/31/2017

RONALD L WANGE
08/31/2017
I concur with Dr. Hawes' recommendation for approval.

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	209803
Supporting document/s:	SDN 3
Applicant's letter date:	12/19/2016
CDER stamp date:	12/19/2016
Product:	Ertugliflozin
Indication:	As an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus
Applicant:	Merck Sharpe and Dohme Corp
Review Division:	DMEP
Reviewer:	Jessica J. Hawes, Ph.D.
Supervisor/Team Leader:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Elizabeth Godwin

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209803 are owned by Merck Sharpe and Dohme Corp or are data for which Merck Sharpe and Dohme Corp has obtained a written right of reference. Any information or data necessary for approval of NDA 209803 that Merck Sharpe and Dohme Corp 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 reflected 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 209803.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Merck Sharp and Dohme Corp. has submitted NDA application packages for the new molecular entity (NME) Ertugliflozin (PF-04971729, MK-8835) alone (NDA #209803, IND #106447) and as fixed dose combination (FDC) products with the marketed drugs Metformin (MK-8835B, NDA #209806, IND #122329) and Sitagliptin (MK-8835A, NDA #209805, IND #122330) for the treatment of type 2 diabetes mellitus (T2DM).

1.2 Brief Discussion of Nonclinical Findings

Ertugliflozin is a potent and selective inhibitor of the sodium-glucose co-transporter 2 (SGLT2), with a lower probability of off-target SGLT1 inhibition in humans than in the nonclinical species used for safety pharmacology and toxicology studies (based on species differences in relative binding affinities). Thus, drug-related effects observed in rats and dogs as a result of off-target inhibition of SGLT1 are less likely to occur in humans.

In humans, ertugliflozin has two disproportional O-glucuronide metabolites, M5a and M5c; however, significant SGLT2 or SGLT1 inhibition by either metabolite is unlikely at clinical exposures. M5a and M5c were sufficiently qualified in pivotal nonclinical toxicology and carcinogenicity studies, and are unlikely to be of significant toxicological concern at clinical exposure levels of a 15 mg/day dose.

Based on nonclinical studies, significant drug-drug interactions (DDI) with ertugliflozin administration and drugs metabolized by cytochrome P450 (CYP) enzymes or transported by organic anion transporters (OATs), organic cation transporters (OCTs) or organic anion transporting polypeptides (OATPs) are not likely at clinical exposures. Significant DDI with diphosphate-glucuronsyltransferase (UGT) enzyme inhibition is also unlikely at clinical concentrations.

In safety pharmacology studies, safety exposure margins for potential ertugliflozin-mediated changes in central nervous system (CNS), cardiovascular (CV) and respiratory parameters were sufficient and there were no significant safety concerns at clinical exposure levels.

Drug-related effects in nonclinical toxicology studies were predominantly observed in the renal system, most likely due to pharmacodynamic (PD)-related glucosuria and osmotic diuresis secondary to SGLT2 inhibition. Drug class-related renal system effects are generally monitorable and treatable with sufficient safety margins for reversibility in adults. However, in juvenile rats exposed to ertugliflozin during renal development corresponding to human late 2nd and 3rd trimesters of pregnancy, irreversibility of renal findings could not be ruled out, similarly to other approved SGLT2 inhibitors, at exposures 17 times higher than clinical exposure levels.

Other drug-related effects predominantly observed in nonclinical studies include gastrointestinal (GI) and bone findings, but are likely due to off-target inhibition of SGLT1 and were associated with sufficient margins of safety. Thus, there is not significant concern for drug-related GI or bone effects at clinical exposures of 15 mg/day.

Ertugliflozin is not genotoxic and was not associated with neoplastic findings in male or female mice or female rats in 2-year carcinogenicity studies. Drug-related increases in adrenal medulla pheochromocytoma (PCC) were observed in male rats at 15 mg/kg/day, with a safety margin of 18x $MRHD_{AUC}$ at the no observed adverse effect level (NOAEL) for neoplasms of 5 mg/kg/day. The molecular mechanisms driving adrenal medulla proliferation and PCC development in rats remains unclear and may be related to species sensitivity to changes in calcium absorption and homeostasis due to off-target SGLT1 inhibition with unclear relevancy to human risk. Furthermore, the neoplastic safety margin is considered to be sufficient and there is not significant concern for carcinogenicity at clinical exposures of 15 mg/day.

In pregnant and lactating rats, fetal and nursing neonatal total drug exposure levels were lower than maternal plasma total drug exposures. In embryonic fetal development (EFD) and pre- and postnatal development (PPND) studies with drug exposures throughout organogenesis, drug-related fetal effects were restricted to doses associated with maternal toxicity and were associated with sufficient margins of safety for teratogenicity. Ertugliflozin did not affect rat fertility parameters in males or females. Thus, aside from PD-related effects on renal development in juvenile rats, as described above, there are no additional concerns for drug-related fetal developmental effects or reproductive fertility at clinical exposure levels.

The commercial formulation of ertugliflozin and associated impurities and degradants were qualified in nonclinical toxicology studies with sufficient margins of safety and were not associated with any new or significant safety concerns. The overall weight of evidence indicates that the potential impurities, degradation products and metabolites in ertugliflozin are unlikely to present a potential risk for genotoxicity, mutagenicity, carcinogenicity, or organ toxicity concerns.

In summary, hazard assessment and characterization of the nonclinical toxicology profile of ertugliflozin is considered to be complete. In general, potential drug-related effects were consistent with the SGLT2 inhibitor drug class and were considered to be monitorable, treatable, reversible, and/or associated with a sufficient margin of safety at the proposed clinical dose of 15 mg/day; with the only exception being that of potential class-related renal developmental effects in juveniles, which will be described in the label. Overall, the nonclinical data support market approval of ertugliflozin.

Recommended changes to the sponsor's proposed labeling primarily add clarification to the safety margins and add consistency with other members of the drug class.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of ertugliflozin

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Nonclinical labeling recommendations are below. See Section 11 Labeling Review for a full discussion of proposed changes.

Section: 8 USE IN SPECIFIC POPULATIONS

Section 8.1 Pregnancy

Risk Summary

Based on animal data showing adverse renal effects, TRADEMARK is not recommended during the second and third trimesters of pregnancy.

The limited available data with TRADEMARK in pregnant women are not sufficient to determine a drug-associated risk for major birth defects or miscarriage. There are risks to the mother and fetus associated with poorly controlled diabetes in pregnancy [see Clinical Considerations].

In animal studies, adverse renal changes were observed in rats when ertugliflozin was administered during a period of renal development corresponding to the late second and third trimesters of human pregnancy. Doses approximately (b) (4) times the maximum clinical dose caused renal pelvic and tubule dilatations and renal mineralization that were not fully reversible. There was no evidence of fetal harm in rats or rabbits at exposures of ertugliflozin approximately 300 times higher than the maximal clinical dose of 15 mg/day when administered during organogenesis [see Data].

The estimated background risk of major birth defects is 6-10% in women with pre-gestational diabetes with a HbA1c >7 and has been reported to be as high as 20-25% in women with HbA1c >10. The estimated background risk of miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk

Poorly-controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, spontaneous abortions, preterm delivery, stillbirth, and delivery complications. (b) (4) increase the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Animal Data

When ertugliflozin was orally administered to juvenile rats from PND 21 to PND 90, increased kidney weight, renal tubule and renal pelvis dilatation, and renal mineralization occurred at doses greater than or equal to 5 mg/kg (17-fold human exposures, based on AUC). These effects occurred with drug exposure during periods of renal development in rats that correspond to the late second and third trimester of human renal development, and did not fully reverse within a 1 month recovery period.

(b) (4)

In embryo-fetal development studies, ertugliflozin (50, 100 and 250 mg/kg/day) was administered orally to rats on gestation days 6 to 17 and to rabbits on gestation days 7 to 19. Ertugliflozin did not adversely affect developmental outcomes in rats and rabbits at maternal exposures that were approximately 300-times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC. A maternally toxic dose (250 mg/kg/day) in rats (707-times clinical dose) (b) (4) was associated with reduced fetal viability (b) (4) and a higher incidence of a visceral malformation (membranous ventricular septal defect) in rats.

In a pre- and postnatal development study in pregnant rats, ertugliflozin was administered from gestation day 6 through lactation day 21 (weaning). Decreased postnatal growth (weight gain) was observed at maternal doses of ≥100 mg/kg/day (greater than or equal to 331 times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC).

Section 8.2 Lactation

Risk Summary

There is no information regarding the presence of TRADEMARK in human milk, the effects on the breastfed infant, or the effects on milk production. Ertugliflozin is present in the milk of lactating rats *[see Data]*. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney.

(b) (4)

Data

Animal Data

The lacteal excretion of radiolabeled ertugliflozin in lactating rats was evaluated 10 to 12 days after parturition. Ertugliflozin derived radioactivity exposure in milk and plasma were similar, with a milk/plasma ratio of 1.07, based on AUC. Juvenile rats directly exposed to TRADEMARK during a developmental period corresponding to human kidney maturation were associated with a risk to the developing kidney (persistent increased organ weight, renal mineralization, and renal pelvic and tubular dilatations).

Section: 12 CLINICAL PHARMACOLOGY

Section 12.3 Pharmacokinetics

Metabolism

Metabolism is the primary clearance mechanism for ertugliflozin. The major metabolic pathway for ertugliflozin is UGT1A9 and UGT2B7-mediated O-glucuronidation to two glucuronides that are (b) (4) pharmacologically ~~in~~active at clinically relevant concentrations. CYP-mediated (oxidative) metabolism of ertugliflozin is minimal (12%).

Drug Interaction Studies

In Vitro Assessment of Drug Interactions

In *in vitro* studies, ertugliflozin and ertugliflozin glucuronides did not inhibit CYP450 isoenzymes (CYPs) 1A2, 2C9, 2C19, 2C8, 2B6, 2D6, or 3A4, and did not induce CYPs 1A2, 2B6, or 3A4. Ertugliflozin was not a time-dependent inhibitor of CYP3A *in vitro*. Ertugliflozin did not inhibit UGT1A6, 1A9, or 2B7 *in vitro* and was a weak inhibitor (IC_{50} >39 µM) of UGT1A1 and 1A4. Ertugliflozin glucuronides did not inhibit UGT1A1, 1A4, 1A6, 1A9, or 2B7 *in vitro*. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of drugs eliminated by these enzymes. Ertugliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and is not a substrate of organic anion transporters (OAT1,

OAT3), organic cation transporters (OCT1, OCT2), or organic anion transporting polypeptides (OATP1B1, OATP1B3). Ertugliflozin or ertugliflozin glucuronides do not meaningfully inhibit P-gp, OCT2, OAT1, or OAT3 transporters, or transporting polypeptides OATP1B1 and OATP1B3, at clinically relevant concentrations. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of concurrently administered medications that are substrates of these transporters.

Section: 13 NONCLINICAL TOXICOLOGY

Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity was evaluated in CD-1 mice and Sprague-Dawley rats. In the mouse study, ertugliflozin was administered by oral gavage at doses of 5, 15, and 40 mg/kg/day for up to 97 weeks in males and 102 weeks in females. There were no ertugliflozin-related neoplastic findings at doses up to 40 mg/kg/day (approximately 50 times human exposure at the maximum recommended human dose [MRHD] of 15 mg/day, based on AUC). In the rat study, ertugliflozin was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg/day for up to 92 weeks in females and 104 weeks in males. Ertugliflozin-related neoplastic findings included an increased incidence of (b) (4) adrenal medullary pheochromocytoma (PCC) in male rats at 15 mg/kg/day. Although the molecular mechanism remains unknown, this finding may be related to carbohydrate malabsorption leading to altered calcium homeostasis, which has been associated with PCC development in rats and has unclear relevancy to human risk. The no-observed-effect level (NOEL) for neoplasia was 5 mg/kg/day (approximately (b) (4) times human exposure at the MRHD of 15 mg/day, based on AUC).

Mutagenesis

Ertugliflozin was not mutagenic or clastogenic with or without metabolic activation in the microbial reverse mutation, *in vitro* cytogenetic (human lymphocytes), and *in vivo* rat micronucleus assays.

Impairment of Fertility

In the rat fertility and embryonic development study, male and female rats were administered ertugliflozin at 5, 25, and 250 mg/kg/day. No effects on fertility were observed at 250 mg/kg/day (approximately 480 and 570 times male and female human exposures, respectively, at the MRHD of 15 mg/day based on AUC comparison).

2 Drug Information

2.1 Drug

CAS Registry Number

1210344-57-2

Generic Name

Ertugliflozin

Code Name

MK-8836
PF-04971729
PF-04971729 (b) (4) = L-pyroglutamic acid (L-PGA) co-crystal form

It is noted that the neutral amorphous form was used for most exploratory toxicology studies, whereas the L-PGA co-crystalline form intended for marketing was used in pivotal toxicology and safety pharmacology studies.

Chemical Name

PF-04971729: (1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol

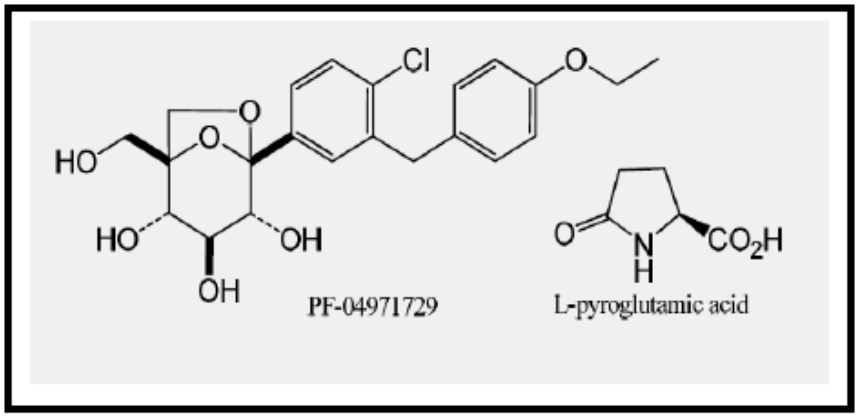
PF-04971729 (b) (4) (1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol L-pyroglutamic acid

Molecular Formula/Molecular Weight

PF-04971729: C22H25ClO7 / 436.88 g/mol

PF-04971729 (b) (4): C27H32ClNO10 / 566.00 g/mol

Structure or Biochemical Description



Pharmacologic Class

Sodium glucose co-transporter 2 (SGLT2) Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND #106447: MK-8836 Ertugliflozin, Merck Sharp and Dohme Corp

IND #122329: MK-8835B (Ertugliflozin + metformin FDC), Merck Sharp and Dohme Corp

IND #122330: MK-8835A (Ertugliflozin + sitagliptin FDC), Merck Sharp and Dohme Corp

2.3 Drug Formulation

Ertugliflozin will be formulated in 5 mg and 15 mg immediate release oral (b) (4) tablets made with the co-crystal form of the active compound ertugliflozin with L-PGA.

Table 1: Ertugliflozin Tablet Compositions

Table 3.2.P.1-1. Composition of Ertugliflozin Immediate Release Film-Coated 5 mg Tablets

Name of Ingredients	Function	Reference to Standard	Unit Formula mg/tablet
Ertugliflozin L-PGA	Active	In-house	6.477*
Microcrystalline Cellulose	(b) (4)	USP/NF	(b) (4)
Lactose Monohydrate		USP/NF	
Sodium Starch Glycolate		USP/NF	
Magnesium Stearate		USP/NF	
Total Weight			
(b) (4)		In-house	
		USP/NF	
Total Finished Tablet			

Note: NF = National Formulary; USP = United States Pharmacopeia

(b) (4)

Table 3.2.P.1-2. Composition of Ertugliflozin Immediate Release Film-Coated 15 mg Tablets

Name of Ingredients	Function	Reference to Standard	Unit Formula mg/tablet
Ertugliflozin L-PGA	Active	In-house	19.431*
Microcrystalline Cellulose	(b) (4)	USP/NF	(b) (4)
Lactose Monohydrate		USP/NF	
Sodium Starch Glycolate		USP/NF	
Magnesium Stearate		USP/NF	
Total Weight			
(b) (4)		In-house	
		USP/NF	
Total Finished Tablet			

Note: NF = National Formulary; USP = United States Pharmacopeia

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients. Excipient levels are below the maximum current Inactive Ingredient list (IIG) levels in previously approved products.

2.5 Comments on Impurities/Degradants of Concern

All actual and potential impurities and degradation products above the qualification threshold were sufficiently evaluated in nonclinical studies and were not associated with any safety concerns.

Previous rat toxicology studies were conducted with test article synthesized via a research process method; however, the synthesis method for the commercial product is associated with 3 novel impurities (b) (4). Thus a 13-week rat bridging toxicology study (#13GR318) was conducted using the commercial formulation. No significant new or impurity-related toxicities were identified and the impurities were considered to be qualified under study #13GR318 and controlled at acceptable levels.

Under extreme storage conditions, there are 3 major degradants, (b) (4) which were qualified in the 13-week rat bridging toxicology study #15GR254 and are controlled at acceptable levels.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adults with T2DM. The recommended starting dose is 5 mg once daily, with or without food, which may be increased to 15 mg once daily.

Maximum recommended high dose (MRHD): **15 mg once daily**

- 15 mg/day Exposures*: AUC = 1.38 μg·h/mL, C_{max} = 266 ng/mL ≈ 0.6 μM
  *Based on the clinical population pharmacokinetic (PK) analysis

- Maximum Unbound** Drug Exposure: AUC ≈ 76.4 ng·h/mL, C_{max} ≈ 17.2 ng/ml ≈ 39 nM
  **based on a 6.4% unbound fraction in humans

  - The sponsor feels that the pharmacological effect of ertugliflozin and its toxicological effects are more closely related to the unbound fraction in plasma rather than the total plasma concentration. Thus, the sponsor calculated safety margins using mean steady state (ss) unbound drug exposures at the 15 mg therapeutic dose with a $C_{max,ss}$ of 0.0172 μg/mL and an $AUC_{24,ss}$ of 0.0764 μg·h/mL. However, percent protein binding is similarly high across species; therefore, there is not considered to be a meaningful difference in protein binding. Thus, ertugliflozin exposure margins in this review were calculated based on total, not free, drug exposure levels.

2.7 Regulatory Background

- An IND for Ertugliflozin was originally submitted as PF04971729 in September 2009.
- An end of phase 2 (EOP2) meeting was held on December 17th 2013.
- The Pediatric Review Committee (PeRC) discussed the sponsor's proposed Pediatric Study Plan (PSP) on April 10th 2013 and a revised PSP was approved after resubmission on June 12th 2013. It was concluded that a juvenile toxicology study in Sprague Dawley (SD) rats would be required prior to initiation of clinical pediatric studies. Ertugliflozin was then discussed at a second PeRC meeting on August 21st 2013 and PeRC concurred with the proposed PSP and approved the sponsor's partial waiver and deferral.
- On December 19, 2013, the Executive CAC (ECAC) recommended that a water or saline control group be added in addition to the vehicle [0.5% MC/10% polyethylene glycol (PEG) 400] control group, rather than inclusion of a second vehicle control group that was proposed by the sponsor.
- On 7/30/2014, the sponsor submitted and cross-referenced the new IND #122330 for the FDC product MK-8835A containing ertugliflozin and sitagliptin for the treatment of T2DM.
- On 8/13/2014, the sponsor submitted and cross-referenced the new IND #122329 for the FDC product MK-8835B containing ertugliflozin and metformin for the treatment of T2DM.
- On January 10, 2014, the sponsor submitted a response to FDA comments regarding the ECAC's recommendations, and included modifications to the proposed control groups which were originally evaluated by ECAC. ECAC communicated with the sponsor that the inclusion of a 0.5% MC only control instead of water or saline is acceptable and that, although the use of two instead of one vehicle control group (0.5% MC/10% PEG400) is not considered necessary, the decision on the number of vehicle controls is at the sponsor's discretion.

- On 6/1/2015, the sponsor submitted a request for ECAC concurrence on a mouse carcinogenicity study amendment regarding [redacted]. ECAC did not agree with the sponsor's plan [redacted]. Since excess mortality had not been observed in the high dose males, it did not appear that ertugliflozin was contributing to the excess deaths in the mid-dose males. However, ECAC conveyed to the sponsor that the male mid-dose group should be terminated when surviving animals fall to 15. However, should the mid-dose males (or any other dose group) drop to 15 surviving animals in Week 100 or later, then all dose groups of that sex (including controls) should be terminated.
- On 6/15/2015, the sponsor submitted a request for ECAC concurrence on plans for early termination of the rat carcinogenicity study. The sponsor proposed to terminate all female groups if the control females drop to ≤20 animals and to terminate all groups of an affected sex if the number of any treatment group drops to ≤15 at Week 100 or later. If the number in a treatment group drops to ≤15 prior to Week 100, the sponsor proposed to terminate only the affected dose group. It was determined that the sponsor's proposal is consistent with current recommendations for early termination of control groups and treatment groups, both prior to and after Week 100. Thus, the sponsor's proposal was acceptable.
- On 11/12/2015, the sponsor submitted a request for ECAC concurrence for early termination of all male mice groups when the first of any of the control group 1 (0.5% MC in water), vehicle control group 2 (0.5% MC/10% PEG 400), low dose group, or mid-dose dose group reaches ≤15 surviving males. ECAC concurred with the sponsor.
- A Type B pre-NDA meeting was approved on 7/27/2016 to discuss the upcoming NDA submission for ertugliflozin and FDCs with metformin and sitagliptin.
- During review of the NDA package, it was noted that the impurity [redacted] was not included in the certificate of analysis for study report #13GR318. In order to verify that the impurity was qualified in the nonclinical study, the sponsor was asked to provide data demonstrating the level of the impurity in the batch used in the study. On 5/10/2017, the sponsor submitted a revised certificate of analysis for Lot #705847-91-10 used in study #13GR318, demonstrating that the impurity [redacted] was present at [redacted]%.

3 Studies Submitted

3.1 Studies Reviewed

Table 2: Nonclinical Studies Reviewed in This Review

Study #	Brief Title
PHARMACOLOGY	
PD002	In Vitro Potency of PF-06481944, a Metabolite of PF-04971729, for Glucose Transport in CHO Cells Expressing Either Human SGLT1 or SGLT2

PD003	In Vitro Potency of PF-06685948, a Metabolite of PF-04971729, for Glucose Transport in CHO Cells Expressing Either Human SGLT1 or SGLT2
PD005	In Vitro Potency of PF-04971729 for Glucose Transport in CHO Cells Transfected with Either Dog SGLT1 or SGLT2
PD007	Effects of PF-04971729 on Glucose, Fluid and Electrolyte Balance in Sprague-Dawley Rats
Safety Pharmacology	
PD008	Effects of PF-04971729 and Furosemide on Blood Pressure in Spontaneously Hypertensive Rats
PD009	Effects of PF-04971729 on Blood Pressure in Spontaneously Hypertensive Rats
TT #16-4705	Electrophysiological Evaluation of hERG Channel Current in CHO Cells
Secondary Pharmacology	
Ertugliflozin (PF-04971729)	
PK050 / PF-04971729/14JUN09/125135	Effect of PF-04971729 on Human Drug Metabolizing Enzymes in Vitro
PK051 / PF-04971729_11Mar15_122850	In Vitro Evaluation of PF-04971729 as a Time-Dependent Inhibitor (TDI) of Cytochrome P450 3A Enzyme Activity in Human Liver Microsomes
PK054 / PF-04971729/19Nov08/125201	An Investigation of the Potential for PF-04971729 to Induce CYP3A4 and CYP1A2 in Human Hepatocytes
PK055 / SNS 14_15214 / 120553	In Vitro Investigation of the Potential for PF-04971729 to Induce Cytochrome P450 (CYP3A4, CYP2B6, and CYP1A2) in Cultured Human Hepatocytes
PK058 / PF-04971729_21May15_113856	In Vitro Evaluation of PF-04971729 as an Inhibitor of UDP-Glucuronosyltransferase (UGT) Enzyme Activities in Human Lier Microsomes
PK059 / PF-04971729_02Mar16_084622	SIMCYP® Prediction of Interaction Between Ertugliflozin (PF-04971729) and UGT Inhibitor Mefenamic Acid
PK071 / PF-04971729_09Mar11_081536	The In Vitro Study of P-Glycoprotein Inhibition by PF-04971729 in CACO-2 Cells
PK063 / PR-04971729_30Mar11_162655	PF-04971729 BCRP Substrate and Inhibition Evaluation
PK072 / PF-04971729_20Dec10_144638	In Vitro Inhibition Assessment of PF-04971729 on Hepatic Transporters, hOATP1B1 and hOATP1B3
PK073 / PF-04971729_10Jun11_140913	PF-04971729: In Vitro Inhibition of Human OCT1
PK074 / #XT108033 / PF-04971729_06Aug10_111034	In Vitro Interaction Studies of PF-04971729 with Human OAT1 and OAT3 Uptake Transporters
M5c (PF-06481944)	
PK052 / XT155074 / #15_18155	In Vitro Evaluation of PF-06481944 as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes
PK056 / SNS 15_18157 / 120049	In Vitro Investigation of the Potential for PF-06481944 to Induce Cytochrome P450 (CYP3A4, CYP2B6, and CYP1A2) in Cultured Human Hepatocytes
PK060 / PF-06481944_29Jun15_151558	In Vitro Evaluation of PF-06481944 as an Inhibitor of UDP-Glucuronosyltransferase (UGT) Enzyme Activities in Human Liver Microsomes
PK075 / PF-06481944_04Nov15_013302	In Vitro Transporter Inhibition of PF-06481944

M5a (PF-06685948)	
PK053 / XT155075 / #15_18161	In Vitro Evaluation of PF-06685948 as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes
PK057 / SNS 15_18163 / 120321	In Vitro Investigation of the Potential for PF-06685949 to Induce Cytochrome P450 (CYP3A4, CYP2B6, and CYP1A2) in Cultured Human Hepatocytes
PK061 / PF-06685948_29Jun15_155303	In Vitro Evaluation of PF-06685948 as an Inhibitor of UDP-Glucuronosyltransferase (UGT) Enzyme Activities in Human Liver Microsomes
PK076 / PF-06685948_04Nov15_013350	In Vitro Transporter Inhibition of PF-06685948
PHARMACOKINETICS / ADME	
Distribution	
PK034 / #8311399	Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Female Sprague Dawley Rats after Oral Administration of [^{14}C]PF-04971729
PK037	Protein Binding of PF-04971729 in Rabbit Plasma
PK062 / PF-04971729_09Mar11_081536	In Vitro Interaction Studies of PF-04971729 with Human MDR1 (ABCB1/P-GP) Transporter on MDCKII-MDRI Monolayers
PK064 / PF-04971729_13Jul11_131709	PF-049717929: In Vitro Assessment of Hepatic Uptake in Human Hepatocyte Suspensions
PK065 / PF-04971729_25Mar14_114705	PF-04971729 – In Vitro Assessment of Uptake and Biliary Excretion using Sandwich Culture Human Hepatocytes (SCHH)
PK066 / PF-04971729_30Sep15_140916	PF-04971729 and Empagliflozin: In Vitro Assessment of Hepatic Uptake in Human Hepatocyte Suspension and Sandwich Culture Human Hepatocytes (SCHH)
PK067 / PF-04971729_10Oct12_115403	In Vitro Assessment of PF-04971729 as Substrate for Human OATP1B1, OATP1B3, and OATP2B1
PK068 / PF-04971729_05Nov15_032208	In Vitro OATP-Mediated Uptake of PF-04971729
PK069 / PF-04971729_06Sep11_153120	PF-04971729:In Vitro Substrate Assay for Human OCT1
PK070 / PF-04971729/18Aug09/143816	In Vitro Renal Transport of PF-04971729
Metabolism	
PK042 / #PF-04971729_04Oct10_145239	Mass Balance, Excretion and Metabolism of PF04971729 in Healthy Male Human Administration Volunteers Following a Single Oral dose of [^{14}C]-PF-04971729
PK041 / #PF-04971729/14Apr10/130940	Mass Balance, Routes of Excretion and Metabolism of PF04971729 Following Oral Administration of [^{14}C]-PF-04971729 to Beagle Dogs
PK043 / #PF-04971729_23Mar16_040523	Structural Assignment of Circulating Glucuronides of Ertugliflozin in human Plasma from Study B1521051
PK044 / #PF-04971729_28Jan16_091450	Assessment of in Vivo Chiral Inversion of PF-04971729 as Observed in Human Plasma from Study B1521051
PK046 / #PF-04971729_06Oct15_130356	Enzyme Kinetics and Reaction Phenotyping of Cytochrome P450 Isoforms Involved in the in Vitro Metabolism of PF-04971729
PK047 / #PF-04971729_01Dec15_044314	Enzyme Kinetics and Identification of UDP-Glucuronosyltransferase (UGT) Isoforms Involved in the In Vitro Metabolism of PF-04971729

Excretion	
PK049 / #8226846	Determination of Mass Balance and Metabolic Profiles of [14C]PF-04971729 in Beagle Dogs Following a Single Oral Dose Administration
TOXICOLOGY	
Repeat-Dose Studies	
TT #13-7809 / 13GR318	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Rats
TT #15-7804 / #8325322 / 15GR254	13-Week Oral Gavage Toxicity and Toxicokinetics Study with PF-04971729 with Degradants in Rats
Carcinogenicity	
TT #14-1003	Two-Year Oral Carcinogenicity Study in Mice
TT #13-7800	104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with PF-04971729 in Rats
Reproductive and Developmental Toxicology	
TT #15-7810 / #20070334 / 14GR472	A Dose Range-Finding Juvenile Toxicity Study of PF-04971729 by Oral (Gavage) in Rats
TT #15-7803 / #20075270 / 15GR084	A Juvenile Toxicity Study of PF-04971729 by Oral (Gavage) in Rats
Special Toxicology	
TT #14-7836 / #506864	In Vitro Skin Corrosion Test withPF-04970729 (b) (4) Using a Human Skin Model
TT #14-7837 / #506868	Assessment of Contact Hypersensitivity to PF-04971729 (b) (4) in the Mouse (Local Lymph Node Assay)
TT #14-7838 / #506865	Screening for the Eye Irritancy Potential of PF-04971729 (b) (4) Using the Bovine Corneal Opacity and Permeability Test (BCOP Test)

3.2 Studies Not Reviewed

Twenty-eight analytical and validation reports were also submitted with the NDA package and summaries were examined, as described in PK section 5.1; however, these reports were not reviewed in detail.

3.3 Previous Reviews Referenced

All other nonclinical studies were reviewed in pharmacology and toxicology (Pharm/Tox) reviews #1, #2, #3, #4, and #5 by Dr. Jeffrey Quinn and Pharm/Tox reviews #6, #7 and #8 by Dr. Jessica Hawes under IND #106447. Summaries of key studies are included in this review.

Table 3: Summaries of Pivotal Previously-Reviewed Nonclinical Studies

Study #	Brief Title	Primary Review
Safety Pharmacology		
TT #09-7883 /	Effect of PF-04971729 on hERG Potassium Current	IND #106447 Pharm/Tox

PF04971729HERG	Stably Expressed in HEK293 Cells	review #1, Dr. Quinn, 10/22/2009
TT #09-7885 / PF04971729NA15	Effect of PF-04971729 on Nav1.5 Sodium Current Stably Expressed in HEK293 Cells	IND #106447 Pharm/Tox review #8, Dr. Hawes, 08/05/2016
TT #08-7887 / 09GR145	Safety Pharmacology – Cardiovascular Assessment of Orally Administered PF-0497172[9] (b) (4) in Male Telemetry-Instrumented Beagle Dogs	IND #106447 Pharm/Tox review #1, Dr. Quinn, 10/22/2009
PD001 / 070904	Effects of PF-04971729 on Blood Pressure in Spontaneously Hypertensive Rats, 19-Nov-2010	IND #106447 Pharm/Tox review #8, Dr. Hawes, 08/05/2016
TT #09-7886 / 09GR146	Safety Pharmacology – Neurofunctional and Pulmonary Assessment of PF0497172[9] (b) (4) in Male Rats	IND #106447 Pharm/Tox review #1, Dr. Quinn, 10/22/2009
Toxicology		
TT #09-7895 / #8222521 / 09GR476	9-Month Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Dogs with an 8-Week Recovery Phase	IND #106447 Pharm/Tox review #5, Dr. Quinn, 10/07/2013
TT #09-7894 / #8215018/ 09GR275	6-Month Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Rats with an 8-Week Recovery Phase	IND #106447 Pharm/Tox review #4, Dr. Quinn, 08/29/2011
Genetic Toxicology		
TT #09-7896 / #8202969 / 09GR181	Bacterial Reverse Mutation Assay with a Confirmatory Assay	IND #106447 Pharm/Tox review #1, Dr. Quinn, 10/22/2009
TT #09-7897 / 09GR182	Genetic Toxicology Human Lymphocyte Assay of PF-04971729	IND #106447 Pharm/Tox review #1, Dr. Quinn, 10/22/2009
TT #09-7890 / 09GR185	1-Month Oral Toxicity Study and Micronucleus Assessment of PF0497172[9] (b) (4) in Rats	IND #106447 Pharm/Tox review #1, Dr. Quinn, 10/22/2009
Reproductive and Developmental Toxicology		
TT #10-7835 / 10GR227	Oral Fertility and Embryonic Development Study of PF-04971729 in Male and Female Rats	IND #106447 Pharm/Tox review #8, Dr. Hawes, 08/05/2016
TT #10-7833 / 10GR058	Oral Embryo-fetal Development Study of PF-04971729 in Rats	IND #106447 Pharm/Tox review #3, Dr. Quinn, 05/23/2011
TT #10-7834 / 10GR059	Oral Embryo-fetal Development Study of PF-04971729 in Rabbits	IND #106447 Pharm/Tox review #3, Dr. Quinn, 05/23/2011
TT #13-7827FIN / 13GR257	A Pre- and Postnatal Developmental Toxicity Study of PF-04971729 by Oral (Gavage) in Rats	IND #106447 Pharm/Tox review #8, Dr. Hawes, 08/05/2016

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action:

Ertugliflozin is an inhibitor of SGLT2, thereby blocking the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells and resulting in significant glucosuria. Ertugliflozin is highly selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

In vitro uptake assays with Chinese Hamster Ovary (CHO) cells stably expressing human SGLT1 or SGLT2 indicate that ertugliflozin selectively inhibits SGLT2-mediated transport of the radiolabeled glucose derivative methyl-α-D glucopyranoside (^{14}C-AMG) with a potency >2200-fold higher than human SGLT1. Ertugliflozin has a similarly high selectivity for dog SGLT2 over SGLT1 of ~2700-fold; however, the potency toward dog SGLT2 is also reduced 7-fold. In rats, ertugliflozin selectivity is roughly 10-fold lower than that of humans, with a rat SGLT2 selectivity of 188-fold over SGLT1. Since ertugliflozin potency at the SGLT1 receptor is 6-fold higher for rat (IC_{50} = 0.352 μM) and dog (IC_{50} = 0.318 μM) than human (IC_{50} = 1.96 μM), potential off-target inhibition of SGLT1 is greater in rats and dogs. Furthermore, C_{max} (0.6 μM) at the therapeutic dose of 15 mg/day, is 3-fold lower than the IC_{50} value for human SGLT1 inhibition in vitro, suggesting that significant SGLT1 inhibition is unlikely at clinical exposures. Overall, ertugliflozin is a highly potent and selective inhibitor of SGLT2, with a lower probability of off-target SGLT1 inhibition in humans than in the nonclinical species used for safety pharmacology and toxicology studies.

Table 4: In Vitro Ertugliflozin IC_{50} Values for SGLT1 and SGLT2

AMG Transport Assay	IC_{50} Geometric Mean	95% Confidence Interval	IC_{50} Arithmetic Mean	Std. Dev	Assay Replicates
Human SGLT2	0.877 nM	0.704 - 1.09 nM	0.927 nM	0.369 nM	10
Human SGLT1	1960 nM	1460 - 2620 nM	2050 nM	642 nM	8
Dog SGLT2	0.118 nM	0.108-0.128 nM	ND	ND	3
Dog SGLT1	318 nM	215-470 nM	ND	ND	3
Rat SGLT2	1.15 nM	0.757 - 1.74 nM	1.18 nM	0.289 nM	4
Rat SGLT1	352 nM	274 - 453	356 nM	54.2 nM	4

AMG = methyl-α-D-glucopyranoside, a non-metabolizable form of glucose; SGLT(1, 2) = Sodium-glucose co-transporter type (1, 2); nM = nanomolar; IC_{50} = 50% inhibitory concentration, ND = Not determined.

(Table excerpted from sponsor's package)

Ertugliflozin is metabolized into 2 major O-glucuronide metabolites, M5a (PF-06685948) and M5c (PF-06481944), which are disproportional in humans, each making up more than 10% of total drug (parent compound + metabolites). In CHO cells overexpressing SGLT2 or SGLT1, SGLT2 inhibitions of 70% and 33% were observed at 1 μM M5a and M5c, respectively, resulting in SGLT2 IC_{50} values of 0.476 μM for M5a and >1 μM for M5c. Inhibition of SGLT1 was not observed with either metabolite at concentrations up to 1 μM. The C_{max} for M5a is 0.102 μM at the therapeutic dose of 15 mg/day, which is 5-fold lower than the IC_{50} value for human SGLT2 inhibition in vitro. The C_{max} for M5c at the therapeutic dose is 0.431 μM, which is 2.3-fold lower than the concentration

associated with 33% SGLT2 inhibition in vitro. Taken together, the reported data suggest that significant SGLT2 or SGLT1 inhibition is unlikely at clinical exposures of metabolites M5a and M5c.

Table 5: Metabolites M5a and M5c IC_{50} Values for Human SGLT1 and SGLT2

Metabolites	SGLT1 IC_{50} (nM)	SGLT2 IC_{50} (nM)
PF-06481944	>1000	>1000
PF-06685948	>1000	476

(Table excerpted from sponsor's package)

Drug activity related to proposed indication:
Ertugliflozin administration in rats results in concentration-dependent glucosuria, which is directly related to the PD activity of SGLT2 inhibition. Ertugliflozin administration in rats is associated with compensatory increases in food consumption; however, in food-controlled studies, concomitant decreases in plasma glucose levels are observed. Glucosuria has also been reported in humans.

4.2 Safety Pharmacology

Brief Safety Pharmacology Summary

Core battery

Central Nervous System: At 500 mg/kg ertugliflozin in male rats, drug-related decreases in average body temperature of 0.4°C, and 30-40% decreases in locomotor activity, were observed at C_{max} exposures approximately 339-fold higher than clinical C_{max} exposure at the therapeutic dose of 15 mg/day (~339x $MRHD_{Cmax}$). The NOAEL for CNS effects in rats was set at 25 mg/kg, which is associated with a safety margin of ~36x $MRHD_{Cmax}$.

Cardiovascular System: Ertugliflozin weakly inhibited the human ether-a-go-go-related gene (hERG) potassium channel in vitro with an IC_{50} of 59 μM and an IC_{20} of 8.11 μM in CHO cells, but was a poor inhibitor in human embryonic kidney (HEK293) cells with an IC_{50} value of >300 μM. Ertugliflozin also weakly inhibited Nav1.5 currents with an IC_{50} of 188 μM. Although significant inhibition of hERG and Nav1.5 currents were reported at concentrations ≥30 μM (50x $MRHD_{Cmax}$), significant hERG or Nav1.5 inhibition is not anticipated at biologically relevant exposure levels. In dogs, single doses of 50 mg/kg ertugliflozin (163x $MRHD_{Cmax}$) were associated with moderate decreases in the QTc interval, cardiac contractility, and heart rate corresponding with T_{max}, as well as increases in systolic blood pressure (sBP) and lengthening of the PR interval, with a NOAEL of 5 mg/kg and a safety margin of ~13x $MRHD_{Cmax}$. In the 27-day pair-fed study #PD001 in spontaneous hypertensive rats (SHR), ertugliflozin-related decreases in blood pressures and heart rate were associated with treatment-related diuresis and activation of the RAAS at 36 mg/kg/day (11x $MRHD_{Cmax}$). Furthermore, based on similar effects observed with a diuretic positive control anti-hypertensive, it's

likely that ertugliflozin-related cardiovascular effects in the SHR model are, at least in part, secondary to PD-related diuresis.

Respiratory System: In rats, dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed for up to 120 minutes post-dose and correlated with C_{max} at doses of ≥25 mg/kg (~36x $MRHD_{Cmax}$), with a NOAEL of 5 mg/kg (9x $MRHD_{Cmax}$).

Supplemental

Renal/Urinary System: No specific renal safety pharmacology studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes increased urinary glucose excretion and kidney alterations in rats and dogs at clinical exposure levels.

Gastrointestinal System: No GI-specific safety studies were performed. However, repeat-dose toxicology studies indicate that ertugliflozin causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs.

Immunotoxicity: There were no indications of immunotoxicity or antigenicity in repeat-dose toxicology studies.

Study: Safety Pharmacology – Cardiovascular Assessment of Orally Administered PF-04971729 (b) (4) in Male Telemetry-Instrumented Beagle Dogs (Study TT #08-7887 / 09GR145)

Key Study Findings

- Ertugliflozin CV NOAEL = 5 mg/kg (~13x $MRHD_{Cmax}$)
  - Decreased corrected QT (QTc) interval, heart rate, and cardiac contractility at 50 mg/kg (163x $MRHD_{Cmax}$)
  - Increased PR interval and sBP at 50 mg/kg

METHODS

Male beagle dogs instrumented with radio-telemetry transmitters were administered 0 (0.5% MC/10% PEG400 vehicle), 1, 5, and 50 mg/kg PF-04971729 via oral gavage, with a 1 week washout period in between doses. Electrocardiographic, hemodynamic, and activity data were recorded continuously in conscious animals between 1 and 24 hours postdose, broken up into 4 monitoring periods. Plasma drug concentrations were determined at 7 hours postdose. At the end of the study, a single oral dose of 50 mg/kg was administered for toxicokinetic profiling at 0.5, 1, 2, 4, 7, and 24 hours postdose.

RESULTS

The study was GLP compliant and considered to be valid.

The NOAEL for CV effects was set at 5 mg/kg (C_{max} ≈ 3.5 μg/mL, based on exposures in study TT #08-7906) due to drug-related effects on heart rate, blood pressure, QT

interval, and cardiac contractility at 50 mg/kg (~C_{max} = 43.4 μg/mL). Moderate decreases in the QTc interval (↓6 msec) and left ventricular +dP/dT, as well as an increase in the PR interval (↑4 msec), were observed during the 3-8 hours postdose monitoring period, which corresponded with the time of C_{max}. Reduced heart rate (↓6 bpm) and associated decreases in RR interval (↑78 msec) and increased sBP pressure (↑6 mmHg) were observed during the 8-16 hours postdose monitoring period. Statistically significant changes in CV parameters were also noted at 1 and 5 mg/kg, but were transient and considered unlikely to be biologically relevant. T_{max} at the 50 mg/kg dose was observed at 4 hours postdose.

Study: Effects of PF-04971729 on Blood Pressure in Spontaneously Hypertensive Rats, 19-Nov-2010 (Study #PD001 / 070904)

Key Study Findings

- ↓Blood pressures (↓mBP and ↓sBP) and ↓heart rate at 36 mg/kg/day (11x $MRHD_{Cmax}$)
  - ~100x $MRHD_{AUC}$, calculated from mean rat exposures (Table 4)
  - 2x $MRHD_{Cmax}$, based on a single supratherapeutic 100 mg/day dose (clinical study #P010/B1521025)
- Treatment-related activation of RAAS (11x $MRHD_{Cmax}$)
  - ↑plasma renin activity, ↑serum aldosterone, ↑plasma angiotensinogen, and ↑urinary angiotensinogen
- Glucose excursion
- Diuresis
  - ↑water intake, ↑urine volume, ↑urine volume/water intake ratio, and ↑hematocrit
- Ertugliflozin-related CV effects may be secondary to PD-related diuresis

METHODS

Male SHR rats instrumented with blood pressure transmitters were administered 36 mg/kg PF-04971729 for 27 days, which was formulated as an admixture in standard rat chow (0.5 mg PF-04971729/g chow), and pair-fed to match food consumption of vehicle controls. Blood samples were collected on Day 23 and 24-hour urine samples were collected and water intake was determined on Day 26. Prior to dosing and on Day 27, dBP and sBP were measured over a 24-hour period and mBP and heart rate were calculated from the arterial pressure and cardiac cycle, respectively.

After a 30-day wash-out period, animals were reallocated based on body weight and sBP, and administered vehicle control (0.5% MC/ 10% PEG400) or the positive control 40 mg/kg hydrochlorothiazide via oral gavage for 24 days. Animals were pair-fed. Blood pressures were determined over a 24-hour period on Day 19, and 24-hour urine samples were collected and water intake was determined on Day 22. Blood samples were collected on Day 24.

Plasma and urine samples were analyzed for angiotensinogen, aldosterone, glucose, and electrolytes. Renin activity was also determined in plasma samples.

RESULTS
PF-04971729 administered in the chow at 36 mg/kg/day reached plasma exposure levels of 2.8 μg/mL (11x $MRHD_{Cmax}$) at 2 hours after feeding. It's noted that mean body weight for PF-04971729-treated animals were 22% lower than pair-fed vehicle controls and may complicate translating the findings from the SHR rat model to humans.

Significant decreases in sBP (↓11%) and mBP (↓12%), as well as heart rate (↓15%), were observed in SHR rats treated with PF-04971729, which returned to levels comparable to controls after the wash-out period. After treatment with the diuretic and anti-hypertensive agent hydrochlorothiazide, a statistically significant decrease in sBP (↓5%) was also reported; however, there were no statistically significant differences from controls in dBP, mBP or mean heart rate. Thus, in SHR rats, greater effects on blood pressure lowering were observed with 36 mg/kg/day PF-04971729 administration via rat chow than with 40 mg/kg hydrochlorothiazide via oral gavage.

Significant, drug-related increases in urinary glucose excretion were over 500-fold higher than pair-fed controls; however, there were no significant differences in plasma glucose levels. Significant increases in water intake (↑67%), urine volume (↑3-fold), urine volume/water intake ratio (↑79%), hematocrit (8%), plasma renin activity (↑3-fold), serum aldosterone (↑2.5-fold), plasma angiotensinogen (↑22%), and urinary angiotensinogen (↑3.3-fold) were also observed in SHR rats treated with PF-04971729. These changes are consistent with a significant diuretic effect. Smaller, yet similar, changes were observed with treatment with 40 mg/kg hydrochlorothiazide, which resulted in increased urine volume (↑33%), urine volume/water intake ratio (↑32%), hematocrit (↑3%), plasma renin activity (↑50%), plasma aldosterone (↑88%), plasma angiotensinogen (↑24%), and urinary angiotensinogen (↑96%), but did not result in increased water intake or decreased body weight. These data indicate diuresis and activation of the RAAS with SGLT2 inhibition by PF-04971729 and, although to a lesser degree, the anti-hypertensive diuretic positive control. Thus, these data further indicate that the observed ertugliflozin-related cardiovascular effects may be at least in part secondary to PD-related diuresis.

Study: Effects of PF-04971729 and Furosemide on Blood Pressure in Spontaneously Hypertensive Rats (Study #PD008)

Key Study Findings
- Similar decreases in blood pressures correlated with similar levels of diuresis in the SHR model
    - Ertugliflozin-related decreases in blood pressure may be secondary to PD-related diuresis more so than RAAS activation

METHODS
Blood pressure transducers were surgically implanted in the abdominal aorta of euglycemic SHR rats. After 2 weeks of recovery, animals were separated into 3 groups, which were balanced in terms of body weight and sBP parameters. Animals were

administered PF-04971729 for 20 days as an admixture in the food at a concentration of 0.0715mg/g chow to deliver a target sub-maximal dose of 6 mg/kg/day PF-04971729. Positive control group animals were administered the loop diuretic furosemide for 20 days in the drinking water at a concentration of 0.4 mg/mL to deliver a target dose of ~54 mg/kg/day. Animals were pair-fed and water intake was measured. Beginning on Day 20, 24-hour urine samples were collected and blood was collected at euthanization on Day 21. Plasma and urine samples were analyzed for angiotensinogen, aldosterone, glucose, and electrolytes, as well as renin activity in plasma. On Day 20, blood pressure recordings were recorded over a 24 hour period.

RESULTS

Based on food consumption and body weights, the actual dose of PF-04971729 was determined to be 6.3 mg/kg/day. Body weights of animals treated with PF-04971729 were decreased by 5% compared to concurrent controls; whereas furosemide did not affect body weights. Urine glucose levels were increased 600-fold in animals treated with PF-04971729, but only increased by less than 3-fold in animals treated with furosemide.

Water intake, urine volume, and the urine volume/water intake ratio were all increased similarly in both PF-04971729 and furosemide treatment groups, indicating similar levels of diuresis. Plasma renin activity and plasma and urinary angiotensinogen levels were significantly increased with furosemide treatment, indicating activation of the RAAS. PF-04971729 treatment was not associated with RAAS activation, despite resulting in a similar level of diuresis.

There were no significant changes in mean heart rate with either treatment. Similar decreases in mean systolic blood pressure (↓9-10%), mean diastolic blood pressure (↓8-9%), and mean blood pressure (↓8-9%) were observed in both PF-04971729 and furosemide treatment groups. These data suggest that similar levels of diuresis are associated with similar decreases in blood pressures. This further suggests that drug-related decreases in blood pressure with PF-04971729 may be secondary to PD-related diuresis more so than RAAS activation.

Table 6: Results Summary - SHR Rat Study #PD008

		Control	6 mg/kg/day PF-04971729	54 mg/kg/day Furosemide
Body Weight	(g)	335 ± 6.4	317 ± 4.9*	324 ± 4.3
Urinary Glucose Excretion	(mg/24 h)	4 ± 0.5	2394 ± 377.4*	11 ± 5.9*
Plasma Glucose	(mg/dL)	174 ± 4.9	163 ± 7.6	174 ± 8.2
Water Intake	(mL/24 h)	29 ± 1.2	59 ± 1.2*	44 ± 2.5*
Urine Volume	(mL/24 h)	13 ± 1.2	37 ± 1.4*	30 ± 2.4*
Urine Volume/Water Intake	(UV/H2O x 100)	43 ± 3.4	63 ± 1.4*	68 ± 5.3*
Hematocrit	(%)	48 ± 0.5	47 ± 0.4	47 ± 0.6
Plasma Renin Activity	(ng A1/mL/h)	5 ± 0.5	6 ± 0.4	9 ± 0.6*
Plasma Angiotensinogen	(pmol/mL)	56 ± 1.3	59 ± 1.2	76 ± 2.6*
Urinary Angiotensinogen	(pmol/24 h)	19 ± 0.8	20 ± 0.7	30 ± 1.3*
Mean Systolic Blood Pressure	(mmHg)	175 ± 2.1	159 ± 2.4*	158 ± 2.3*
Mean Diastolic Blood Pressure	(mmHg)	117 ± 1.8	105 ± 1.3*	108 ± 1.4*
Mean Blood Pressure	(mmHg)	144 ± 1.9	131 ± 1.8*	132 ± 1.7*
Mean Heart Rate	(beats/minute)	304 ± 4.3	295 ± 4.1	307 ± 6.6

A1 = Angiotensin 1; dL = deciliter; g = grams; h = hours; kg = kilogram; mg = milligram; mmHg = millimeter(s) of mercury; ng = nanogram; pmol = picomole; UV = urinary volume; vs = versus; % = percent.
Significance levels are denoted by * $p<0.05$, $p<0.01$, $p<0.001$.

(Table excerpted from sponsor's package)

4.2 Secondary Pharmacology

Brief Secondary Pharmacology Summary

Ertugliflozin has been evaluated for cross-reactivity against a broad panel of receptors, transporters, ion channels, and enzymes using a CEREP Wide Ligand Profile screen (study #7570115) at a concentration of 10 μM (4.3 μg/mL). There were no indications of significant (>50%) inhibition of binding or enzyme activity in any of the targets examined. Overall, no significant off-target binding proteins were identified.

Ertugliflozin does not exhibit reversible or time-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A activities in human liver microsomes (HLMs) in vitro and IC_{50} values for CYP inhibition are restricted to >30 μM, which is at least 50-fold higher than clinical ertugliflozin C_{max} concentrations (0.6 μM). The major metabolites of ertugliflozin, M5a and M5c, also did not significantly inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A enzyme activities in HLMs at concentrations of ≤50 μM M5a and ≤100 μM M5c, which are at least 230 to 490-fold higher than clinical C_{max} concentrations of 0.102 μM M5a and 0.431 μM M5c, respectively. Overall, significant CYP inhibitions by ertugliflozin, M5a or M5c are not likely at clinical exposure levels.

Ertugliflozin-mediated induction of CYP3A4 and/or CYP1A2 may be possible at high concentrations. Ertugliflozin was associated with a mild induction (↑2 to 5-fold) of CYP3A4 mRNA expression ≥10 μM and enzyme activity at ≥50 μM in human hepatocytes (study #PK054). Assuming that human liver concentrations are 8-fold higher than plasma, similarly to rat distributions (study PK #033), these data indicate

that potential ertugliflozin induction of CYP3A4 in hepatocytes may occur at concentrations 2 to 10-fold higher than C_{max} exposures at the 15 mg/day dose. However, ertugliflozin-mediated induction of CYP3A4 was not reproduced in a follow-up study (#PK055) with additional primary hepatocyte cell lines using different methods of determination of enzyme substrate and mRNA expression levels; thus, the evidence indicating drug-related induction of CYP3A4 is considered to be equivocal. Ertugliflozin-mediated inductions of CYP1A2 enzyme activity and mRNA levels were observed in 2 of 6 primary human hepatocyte cell lines at ≥3 μM (study #PK054 and #PK055) or ≥50 μM (study #PK054), indicating that potential ertugliflozin induction of CYP1A2 enzyme activity may occur in hepatocytes at clinically relevant concentrations; however, it is difficult to make direct comparisons between in vitro concentrations and in vivo exposures and the data were not consistent across hepatocyte donors. Thus, the in vitro weight of evidence for ertugliflozin-mediated CYP3A4 and CYP1A2 induction remains equivocal and the biological significance at clinical exposures is considered to be unlikely. M5a and M5c were not associated with induction of CYP3A4, CYP2B6, or CYP1A2 enzymes in human hepatocytes. Overall, significant drug-drug interactions with ertugliflozin administration and drugs metabolized by CYP enzymes are not likely at clinical exposures.

Ertugliflozin is a weak inhibitor of several OAT, OCT, and OATP proteins. Ertugliflozin weakly inhibits human OCT1 (hOCT1) with an IC_{50} value of 53 μM, but only very weakly inhibits human OCT2 (hOCT2) with an IC_{50} value of 917 μM. Ertugliflozin does not inhibit human OAT1 (hOAT1), but weakly inhibits human OAT3 (hOAT3) transport activity with an IC_{50} value of 70 μM. Ertugliflozin also weakly inhibits human OATP1B1 (IC_{50} = 35.4 μM) and OATP1B3 (IC_{50} = 140.7 μM). M5c similarly weakly inhibits human OATP1B1 (IC_{50} = 59.3 μM), but M5a does not. M5a and M5c do not inhibit human OCT2, OAT1, OAT3, or OATP1B3. It is noted that the lowest IC_{50} value associated with ertugliflozin-mediated inhibition of human OATs, OCTs and transporting polypeptides was 35.4 μM, which is approximately 60-fold higher than clinical concentrations. Overall, drug-drug interactions with ertugliflozin administration and drugs transported by OATs, OCTs and transporting polypeptides are not likely at clinical exposures.

Ertugliflozin is a very weak inhibitor of the human efflux transporters breast cancer resistance protein (BCRP) and permeability glycoprotein 1 (P-gp) / multidrug resistance protein 1 (MDR1), with IC_{50} values of ~100 μM and 176 μM, respectively. M5a and M5c did not inhibit either BCRP or P-gp/MDR1 transport. Thus, significant inhibition of BCRP and P-gp/MDR1 efflux transporters is unlikely at clinical exposure levels.

Ertugliflozin is a weak inhibitor of UGT enzymes UGT1A1 and UGT1A4 in HLMs with IC_{50} values of 39 μM and 45 μM, respectively. However, ertugliflozin does not inhibit UGT1A6, UGT1A9 OR UGT2B7 enzymes. Thus, a significant drug-drug interaction with UGT inhibition is unlikely at clinical concentrations. M5a and M5c were both negative for inhibition of UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 enzymes. It is noted that an interaction between ertugliflozin and UGT2B15 was not investigated. Nevertheless, it is noted that the sponsor's physiologically-based pharmacokinetic (PBPK) modeling program predicts that ertugliflozin exposure may

lead to slightly (<2-fold) higher AUC and C_{max} exposures of drugs metabolized by UGT enzymes.

Table 7: Summary of Off-Target Drug-Drug Interaction (DDI) Potential

Table 2.6.4: 11 DDI Potential of Ertugliflozin

Enzyme or Transporter	Substrate (Yes/No)	Inhibition[a]/Induction		DDI Potential
		Ertugliflozin	M5c/M5a	
UGT1A1	No	K_i = 19.5 µM	M5c K_i >50 µM M5a K_i >50 µM	Victim or Perpetrator DDI not expected
UGT1A4	No	K_i = 22.5 µM		
UGT1A6	No	K_i >50 µM		
UGT1A9	Yes f_m UGT1A9 = 0.70[b]	K_i >50 µM		Victim DDI: No clinically meaningful DDI expected (AUC_R = 1.51 and C_{maxR} = 1.19)[d] Perpetrator DDI: not expected
UGT2B7	Yes f_m UGT2B7 = 0.16[c]	K_i >50 µM		
CYP1A2	No	K_i >15 µM, No MDI, Induction: 2 to 3-fold ↑ mRNA at ≥100 µM, No change in activity	M5c K_i >50 µM, No MDI M5a K_i >25 µM, No MDI No Induction	Victim or Perpetrator DDI not expected
CYP2B6	No	K_i >15 µM, No MDI, No Induction		
CYP2C8	Yes f_m CYP2C8 = 0.005[e]	K_i >15 µM, No MDI	M5c K_i >50 µM, No MDI M5a K_i >25 µM, No MDI	
CYP2C9	No	K_i >15 µM, No MDI		
CYP2C19	No	K_i >15 µM, No MDI		
CYP2D6	No	K_i >15 µM, No MDI		
CYP3A4	Yes f_m CYP3A4 = 0.10[f]	K_i >15 µM, No MDI, Induction: 3 to 5-fold ↑ mRNA at ≥50 µM (≤8% of rifampin), No change in activity	M5c K_i >50 µM, No MDI M5a K_i >25 µM, No MDI No Induction	
CYP3A5	Yes f_m CYP3A5 = 0.012[g]	K_i >15 µM, No MDI	M5c K_i >50 µM, No MDI M5a K_i >25 µM, No MDI	
P-gp	Yes	K_i = 176 µM	M5c K_i >100 µM M5a K_i >100 µM	Victim DDI: Not limiting oral absorption in clinical studies[i] Perpetrator DDI: Not expected
BCRP	Yes	K_i ~100 µM[h]		
OATP1B1	No	K_i = 17.7 µM	M5c K_i = 29.7 µM M5a K_i >50 µM	No clinically meaningful DDI anticipated
OATP1B3	No	K_i = 141 µM	M5c K_i >100 µM M5a K_i >100 µM	
OCT1	No	K_i = 53 µM		
OAT1	No	K_i >250 µM		
OAT3	No	K_i = 70 µM		
OCT2	No	K_i = 917 µM		

AUC_R = Area under the concentration-time curve ratio; BCRP = Breast cancer resistance protein; C_{maxR} = Maximum concentration ratio; CYP = Cytochrome P450; DDI = Drug-drug interaction; f_m = Fraction metabolized; IC_{50} = 50% inhibitory concentration; K_i = Inhibition constant; MDI = Metabolism dependent inhibition; mRNA = Messenger ribonucleic acid; OAT = Organic anion transporter; OATP = Organic anion transporting polypeptide; OCT = Organic cation transporter; PBPK = Physiologically based pharmacokinetic; P-gp = P-glycoprotein; UGT = Uridine diphosphate-glucuronosyltransferase.

a. When IC_{50} could not be estimated, K_i was reported as > highest concentration tested.
b. f_m UGT1A9 = 0.86 x 0.81, f_m UGT = 0.86 (81% UGT1A9).
c. f_m UGT2B7 = 0.86 x 0.19, f_m UGT = 0.86 (19% UGT1A9).
d. Victim DDI estimated with Simcyp® PBPK modeling.
e. f_m CYP2C8 = 0.12 x 0.04, f_m CYP = 0.12 (4% CYP2C8).
f. f_m CYP3A4 = 0.12 x 0.85, f_m CYP = 0.12 (85% CYP3A4).
g. f_m CYP3A5 = 0.12 x 0.10, f_m CYP = 0.12 (10% CYP3A5).
h. K_i estimated since 58% inhibition observed 100 µM (highest concentration tested).
i. In humans, the measured bioavailability was approximately 100% and dose proportional increases in exposure over the dose range of 0.5-300 mg were observed (CSR B1521043 and B1521001).

(Table excerpted from sponsor's package)

5 Pharmacokinetics/ADME/Toxicokinetics

Ertugliflozin PK parameters were characterized in human, dog, rat, and mouse species. Ertugliflozin protein binding is high in all species examined (human, dog, rat, and mouse) ranging from 92 to 97%, with no apparent concentration dependence across 1 to 10 μg/mL (2-23 μM). Mean fractions in humans were 6.4% unbound and 93.6% bound, and generally were higher than mouse, rat, and dog, but lower than in rabbit. The sponsor proposes that the toxicological effects are more closely related to the unbound fraction in plasma rather than the total plasma concentration. Thus, the species differences in the unbound plasma fraction of ertugliflozin were incorporated into the sponsor's safety margin calculations. However, since the percent plasma protein binding is similarly high across species, there is not considered to be a meaningful difference in protein binding. Thus, safety margins for Pharm/Tox reviews have been based on total, not free, drug levels.

Significant species differences in absorption were associated with oral bioavailability ranging from moderate to high across species and oral absorption ranges of 75-87% in mice, 56-88% in rats, 94-97% in dogs, and up to 100% in humans. T_{max} is achieved within 30 minutes in mice, 0.7 to 2.3 hours in rats, and 0.8 to 1.5 hours in dogs. In humans, T_{max} is achieved after 1 hour in humans (fasted), but after 2 hours in humans in the fed state, indicating absorption delays in the presence of food. Systemic exposures follow linear pharmacokinetics with a trend for slight increases in female exposures over time at high doses in rodents, indicating a potential gender effect which is likely related to metabolism. Ertugliflozin has a moderate half-life ($t_{1/2}$) of 3 to 4 hours in rodents and 8 hours in dogs, but is 1.5 to 4 times longer in humans ranging from 12 to 18 hours.

Table 8: Ertugliflozin PK parameters Across Nonclinical Species

Species	Dose (mg/kg)	Route	Sex/ n	CL (mL/min/kg)	V_{ss} (L/kg)	$t_{½}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{last} (µg•h/mL)	F (%)[a]
					Ertugliflozin					
Mouse	1.3	IV[b]	M/3	14.3	1.58	2.71	-	-	1.43	-
	6.5	Oral[b]	M/3	-	-	-	0.50	1.47	5.80	75.4
	19.4	Oral[b]	M/3	-	-	-	0.50	3.90	19.5	86.7
Rat	2	IV[c]	M/2	4.04	1.13	4.08	-	-	8.20	-
	2	Oral[c]	M/3	-	-	3.65	1.3	0.772	5.49	66.6
	5	Oral[b]	M/3	-	-	-	1.0	1.94	14.7	71.7[d]
	15	Oral[b]	M/3	-	-	-	1.0	4.88	45.3	73.7[d]
	50	Oral[b]	M/3	-	-	-	0.67	40.5	180	87.8[d]
	500	Oral[b]	M/3	-	-	-	2.3	98.9	1150	56.1[d]
Dog	2	IV[c]	M/2	1.64	0.828	7.63	-	-	18.5	-
	2	Oral[c]	M/2	-	-	8.16	1.5	2.18	17.7	96.6
	2	Oral[b]	M/3	-	-	7.48	0.83	2.50	17.2	93.6

AUC_{last} = Area under the concentration-time curve from time 0 to last measurable concentration; AUC_{inf} = Area under the concentration-time curve from time 0 to infinity; CL = Systemic plasma clearance; C_{max} = Maximum observed concentration; F (%) = Bioavailability; IV = Intravenous; M = Male; n = Number of animals; PK = Pharmacokinetic; $t_{½}$ = Apparent terminal half-life; T_{max} = Time of first occurrence of C_{max}; V_{ss} = Apparent volume of distribution at steady state; - = Data not available.

a. F (%) = ([AUC_{inf} (Oral) × Dose (IV)] / [AUC_{inf} (IV) × Dose (Oral)]) × 100.

b. Ertugliflozin was a co-crystal form.

c. Ertugliflozin was amorphous.

d. F (%) was calculated using AUC_{last} since AUC_{inf} was not reported.

(Table excerpted from sponsor's package)

Table 9: Ertugliflozin PK parameters in Humans (Clinical Study #P009/1023)

Parameters (Units)	Parameter Summary Statistics[a] by Renal Function Group				
	T2DM Normal Renal Function	Mild Renal Function	Moderate Renal Function	Severe Renal Function	Healthy Normal Renal Function
N	6	8	8	6	8
AUC_{inf} (ng•hr/mL)	1199 (42)	1908 (28)	2075 (19)	1895 (23)	1236 (27)
CL/F (mL/min)	208.8 (42)	130.9 (28)	120.4 (19)	132.0 (23)	202.1 (27)
C_{max} (ng/mL)	215.9 (35)	313.1 (30)	305.7 (23)	196.4 (28)	219.3 (26)
T_{max} (hr)	1.00 (1.00-1.50)	1.50 (1.00-2.00)	1.50 (0.500-2.00)	1.51 (0.500-3.02)	1.00 (1.00-2.00)
$t_{½}$ (hr)	14.62 ± 6.37	25.94 ± 13.98	22.89 ± 7.35	24.17 ± 5.98	17.71± 3.53
CL_r (mL/min)	2.092 (28)	0.9872 (45)	0.8024 (34)	0.5360 (23)	1.682 (33)

Source: [Ref. 5.3.3.3: P009].

Renal function groups were based on BSA-unnormalized eGFR.

Abbreviations: %CV=percent coefficient of variation; AUC_{inf} =area under the concentration –time curve from time zero to infinity; CL/F=apparent clearance; CL_r=renal clearance; C_{max}= maximum observed concentration; hr=hour(s); N=number of subjects in the renal function group; SD=standard deviation; $t_{½}$= terminal phase half-life; T2DM=type 2 diabetes mellitus; T_{max}= time to first occurrence of C_{max}.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean (±SD) for $t_{½}$; and arithmetic mean (%CV) for Fu.

(Table excerpted from sponsor's package and highlighted)

Ertugliflozin may be a substrate for P-gp/MDR1-mediated efflux, but is not affected by P-gp inhibitors; thus, P-gp is unlikely to be a limiting factor in Ertugliflozin absorption. Ertugliflozin has a moderate volume of distribution in rats with preferential distribution into plasma relative to red blood cells. The highest distribution is primarily to organs responsible for drug metabolism and elimination, such as the bladder, liver, and kidney. Ertugliflozin is also highly distributed to rat adrenal gland, Harderian gland, and pancreas. Ertugliflozin crosses the adult blood:brain barrier, but only reaches concentrations 3 to 63-fold lower than that of blood; whereas distribution to the choroid plexus and pituitary gland is 2-fold greater than blood. In fetal rats, ertugliflozin more readily crosses the blood:brain barrier, resulting in significantly more drug exposure to fetal CNS tissues and eyes than in corresponding adult tissues relative to plasma levels. Ertugliflozin is excreted in rat milk at exposures comparable to maternal plasma levels. Ertugliflozin also readily crosses the rat placental barrier, but with fetal exposures remaining lower than maternal plasma levels.

In rats, elimination of radiolabeled drug and metabolites was virtually complete by 168 hours (7 days) postdose. Ertugliflozin is primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans.

The predominant route of elimination of ertugliflozin is via metabolism, wherein glucuronidation is the major metabolic pathway in all species, with minor contributions from oxidative metabolism involving hydroxylation, oxidation, and oxidative desethylation. There are no unique human metabolites; however, the 2-O-β glucuronide M5a (PF-06685948) and the 3-O- β glucuronide M5c (PF-06481944) are disproportional human metabolites, making up 12.2% and 24.1% of total drug in human plasma, respectively.

5.1 PK

Quantitation of ertugliflozin PK and toxicokinetic (TK) parameters in plasma was determined using LC-MS/MS methods, which were validated prior to single-dose and repeat-dose toxicology studies with Good Laboratory Practices (GLP) compliance. Internal standards were employed in all GLP-compliant assays and the LC-MS/MS method was validated over an ertugliflozin concentration range of 0.005 to 50.0 μg/mL in dog plasma and 0.005 to 5.0 μg/mL in mouse, rat, and rabbit plasma. Long-term storage stability of ertugliflozin in plasma was verified for up to 285 days in nonclinical species.

5.2.2 Distribution

Study: Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Female Sprague Dawley Rats after Oral Administration of [^{14}C]PF-04971729 (Study #PK034 / #8311399)

Key Study Findings

- Ertugliflozin is present in excreted milk at 24-hour AUC exposure levels similar to maternal plasma

- Ertugliflozin readily crosses the placenta
    - Widespread fetal exposure for at least 24 hours
        - C_{max} = 4 hours for most tissues
        - C_{max} = 24 hours for brain tissues
        - Adrenal gland exposures ~4-fold higher than fetal blood
    - More readily crosses the fetal blood:brain barrier than in adults
        - 4 to 6-fold higher AUC exposures in CNS tissues and eyes
- Radioactivity was eliminated from most maternal and fetal tissues at 24 hours postdose, with the exception of 7 maternal and 5 fetal tissues with radioactivity still present at 48 hours postdose
- In general, [^{14}C]PF-04971729 partitioning to maternal plasma was greater than fetal tissue
    - However, after crossing the placenta, [^{14}C]PF-04971729 partitioning to fetal tissue was greater than fetal blood

METHODS

Lactating and timed-pregnant SD rats were administered an oral dose of 102 mg/kg [^{14}C]PF-04971729-L-pyroglutamic acid 2016 μCi/kg) in a suspension of 0.5% MC/10% PEG 400. Lactating rats were stimulated with a subcutaneous injection of oxytocin, then milk, blood and plasma were collected at 1, 4, 8, and 24 hours postdose [^{14}C]PF-04971729 (3/time point), and total radioactivity was determined using LSC. At 1, 4, 8, 24, and 48 hours postdose [^{14}C]PF-04971729 (1/time point) on gestation day 18 (GD 18), blood was collected from pregnant rats and the carcass was evaluated by quantitative whole-body autoradiography (QWBA). PF-04971729 concentrations were determined in plasma and milk using HPLC. Radioactivity concentrations were determined in fetal and maternal tissues.

RESULTS

C_{max} was achieved at 4 hours postdose in blood, plasma and milk. Elimination half-life ($t_{1/2}$) and $AUC_{0-\infty}$ values were not reported for milk, plasma or blood because study drug was detected at 24 hours post-dose. At C_{max}, milk drug concentrations were lower than that of plasma, but were slightly higher than plasma drug concentrations at ≥8 hours postdose. The milk to plasma ratio for mean 24-hour AUC exposures (AUC_{0-t}) was 1.07, indicating similar total exposure over the 24-hour period.

Table 10: PK Parameters of Ertugliflozin in Rat Milk

Pharmacokinetic parameters for radioactivity in blood, plasma, and milk collected from lactating female Sprague Dawley rats after a single oral administration of [^{14}C]PF-04971729 at 10 to 12 days after parturition (Group 1, 100 mg/kg)

Matrix	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	AUC_{0-t} (ng eq·hours/g)	$AUC_{0-\infty}$ (ng eq·hours/g)	C_{max}blood: C_{max}plasma	C_{max}milk: C_{max}plasma	AUC_{0-t}blood: AUC_{0-t}plasma[a]	AUC_{0-t}milk: AUC_{0-t}plasma[a]
Blood	4	40100	NC	347382	NC	0.633	NA	0.635	NC
Plasma	4	63300	NC	547430	NC	NA	NA	NA	NA
Milk	4	46000	NC	588040	NC	NA	0.727	NA	1.07

eq Equivalents [^{14}C]PF-04971729.
NA Not applicable.
NC Not calculated.
a AUC ratios were calculated using AUC_{0-t} values instead of $AUC_{0-\infty}$ values resulting in a Protocol Deviation. $t_{1/2}$ and $AUC_{0-\infty}$ were not determined because a definitive elimination phase was not apparent

Milk:plasma concentration ratios at specified times after a single oral administration of [^{14}C]PF-04971729 to lactating female Sprague Dawley rats at 10 to 12 days after parturition (Group 1, 100 mg/kg)

	Milk:Plasma Concentration Ratio				
	Animal Number				
Time Point	1	2	3	Mean	SD
1 hour[a]	0.387	0.458	0.433	0.426	0.0362
4 hours[b]	0.729	0.854	0.590	0.724	0.132
8 hours[c]	1.86	1.65	1.91	1.81	0.140
24 hours[d]	1.00	1.54	1.50	1.35	0.297

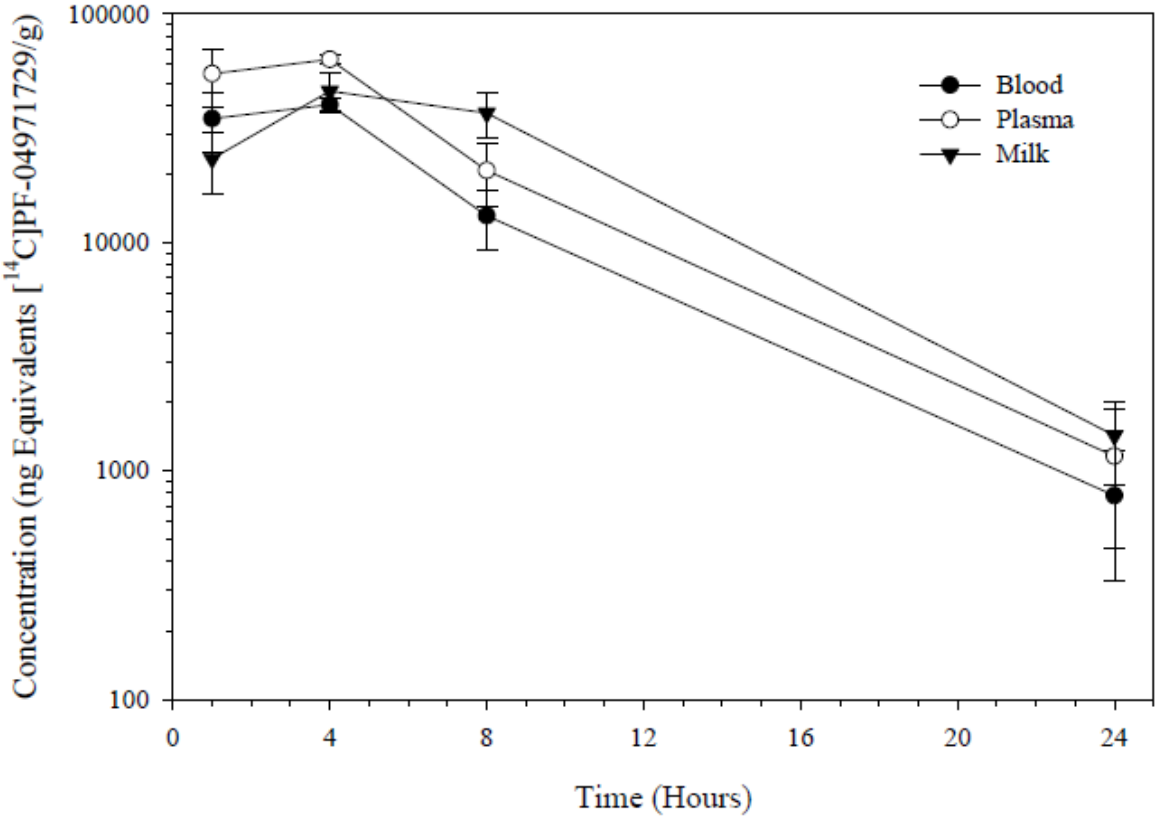
SD Standard deviation.
a Samples collected from Animal Nos. B45415, B45416, and B45417.
b Samples collected from Animal Nos. B45418, B45419, and B45420.
c Samples collected from Animal Nos. B45421, B45422, and B45423.
d Samples collected from Animal Nos. B45424, B45425, and B45426.

(Tables excerpted from sponsor's package)

Figure 1: Radio-labeled Ertugliflozin Concentrations in Milk of Lactating Rats

Figure 1

Mean concentrations of radioactivity in blood, plasma, and milk at specified times after a single oral administration of [^{14}C]PF-04971729 to lactating female Sprague Dawley rats at 10 to 12 days after parturition (Group 1, 100 mg/kg)



(Figure excerpted from sponsor's package)

In fetuses of pregnant rats exposed to [^{14}C]PF-04971729, radioactivity crossed the placenta and was widespread in all fetal tissues examined at the 1, 4, 8, and 24 hours postdose timepoints. By 48 hours postdose, radioactivity was only detectable in fetal adrenal gland, brown fat, gastrointestinal tract (contents and walls), and liver tissues. C_{max} was achieved in most fetal tissues at 4 hours postdose, with the exception of brain cerebellum and olfactory lobe which achieved C_{max} at 24 hours postdose. Ertugliflozin predominantly localized to the fetal adrenal gland, which had the highest concentration at all time points and was ~4-fold higher than fetal blood concentrations. The highest fetal C_{max} levels were observed in the adrenal glands, liver, myocardium, kidneys, and brown fat. The lowest fetal C_{max} levels were observed in the brain, blood, and eye.

Table 11: PK Parameters of Radio-labeled Ertugliflozin in Fetal Rat Tissues

Pharmacokinetic parameters for radioactivity in fetal tissues collected from timed-pregnant female Sprague Dawley rats after a single oral administration of [^{14}C]PF-04971729 on Gestation Day 18 (Group 2, 100 mg/kg)

Tissue	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	AUC_{0-t} (ng eq·hours/g)	$AUC_{0-\infty}$ (ng eq·hours/g)
Fetal adrenal gland(s)	4	53500	7.50	830170	838938
Fetal blood	4	12200	NC	170280	NC
Fetal brain	4	11900	NC	201290	NC
Fetal brain cerebellum	24	7210	NC	NC	NC
Fetal brain cerebrum	4	11000	NC	196274	NC
Fetal brain medulla	4	12900	NC	218830	NC
Fetal brain olfactory lobe	24	9190	NC	NC	NC
Fetal brown fat	4	22200	8.39	450308	457986
Fetal eye(s)	4	15100	NC	200130	NC
Fetal gastrointestinal tract	4	22100	16.2	503850	564669
Fetal kidney(s)	4	23800	NC	280440	NC
Fetal liver	4	26300	8.13	429874	436463
Fetal lung(s)	4	19600	NC	253040	NC
Fetal muscle	4	20500	NC	272630	NC
Fetal myocardium	4	25400	NC	321780	NC
Fetal spinal cord	4	13600	NC	214960	NC
Fetus	4	19800	8.44	345768	352028

Note: Pharmacokinetic parameters were calculated using data from one maternal rat each at 1, 4, 8, 24, and 48 hours postdose.
eq Equivalents [^{14}C]PF-04971729.
NC Not calculated.

(Table excerpted from sponsor's package)

Compared to corresponding maternal tissues, fetal tissues and blood were generally lower, with the exception of CNS tissues, eyes and brown fat. Fetal and maternal brown fat exposures were nearly comparable at 24 hours postdose. Fetal eye and CNS tissues, including cerebrum, medulla, and spinal cord, were 4 to 6-fold higher than corresponding maternal tissues. These data indicate greater partitioning to fetal brain than maternal brain, likely due to incomplete development of the blood:brain barrier in the fetus. However, fetal tissue and blood concentrations generally remained lower than maternal plasma levels, indicating greater [^{14}C]PF-04971729 partitioning to maternal plasma than fetal tissue. After crossing the placenta, greater [^{14}C]PF-04971729 partitioning to fetal tissue than fetal blood was observed.

Radioactivity was present in the amniotic fluid and sac for up to 24 and 48 hours, respectively. Radioactivity was also present in the myometrium and placenta for up to 48 hours.

The elimination $t_{1/2}$ ranged from 7.50 to 8.44 hours postdose for adrenal gland, brown fat, liver and entire fetus. The longest elimination $t_{1/2}$ of 16.2 hours postdose was observed in the liver. Elimination $t_{1/2}$ and $AUC_{0-\infty}$ values were not discernable for all

other fetal tissues. Elimination $t_{1/2}$ values in maternal tissues ranged from 5 hours in the liver to 14.9 hours in the preputial gland. Radioactivity was present in 7 maternal tissues at 48 hours postdose.

Table 12: PK Parameters of Radio-labeled Ertugliflozin in Maternal Rat Tissues

Pharmacokinetic parameters for radioactivity in maternal tissues collected from timed-pregnant female Sprague Dawley rats after a single oral administration of [^{14}C]PF-04971729 on Gestation Day 18 (Group 2, 100 mg/kg)

Tissue	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	AUC_{0-t} (ng eq·hours/g)	$AUC_{0-\infty}$ (ng eq·hours/g)
Adrenal gland(s)	4	142000	NC	1637200	NC
Amniotic fluid	8	989	NC	20357	NC
Amniotic sac	4	55400	9.08	926700	948322
Arterial wall	4	44200	NC	543420	NC
Bile	NC	NC	NC	NC	NC
Blood - LSC	4	41300	5.10	643406	644422
Blood - QWBA	4	43500	NC	540610	NC
Bone	4	1610	NC	10295	NC
Bone marrow	4	36200	NC	558500	NC
Brain cerebellum	4	3220	NC	47950	NC
Brain cerebrum	4	2180	NC	33106	NC
Brain choroid plexus	1	22800	10.7	357640	454180
Brain medulla	4	2820	NC	46418	NC
Brain olfactory lobe	4	2180	NC	33572	NC
Cecum	4	52500	NC	891350	NC
Diaphragm	4	56700	NC	741650	NC
Esophagus	4	40400	NC	588000	NC
Exorbital lacrimal gland	4	88100	NC	1159950	NC
Eye lens	4	741	NC	12358	NC
Eye uveal tract	4	23100	NC	273990	NC
Eye(s)	4	2910	NC	40701	NC
Fat (abdominal)	4	5570	NC	75635	NC
Fat (brown)	1	41800	10.6	587300	746945
Harderian gland	4	124000	7.60	2027040	2050848
Intra-orbital lacrimal gland	4	81900	NC	1020400	NC
Kidney cortex	4	237000	6.46	3892980	3914329
Kidney medulla	4	209000	5.29	3615748	3622270
Kidney(s)	4	221000	5.82	3713940	3725439
Large intestine	4	40100	8.37	947470	960873
Liver	1	238000	5.00	3934632	3940121
Lung(s)	4	50400	NC	745200	NC
Lymph node(s)	8	76200	NC	1046800	NC
Mammary gland	4	33400	NC	564900	NC
Muscle	4	39400	NC	500700	NC

Tissue	T_{max} (hours)	C_{max} (ng eq/g)	$t_{½}$ (hours)	AUC_{0-t} (ng eq·hours/g)	$AUC_{0-\infty}$ (ng eq·hours/g)
Myocardium	4	85500	NC	1176650	NC
Myometrium	4	82500	8.41	1841630	1873052
Nasal turbinates	4	15200	NC	203280	NC
Optic nerve	4	20100	NC	547200	NC
Ovary(ies)	4	53600	NC	604520	NC
Pancreas	4	111000	5.36	1994012	1998080
Peripheral nerve	48	3560	NC	NC	NC
Pituitary gland	4	71100	NC	973050	NC
Placenta	4	47900	6.92	829670	836006
Plasma – LSC	4	62600	4.96	952036	953309
Preputial gland	4	71900	14.9	1629050	1795148
Salivary gland(s)	4	83600	NC	1202800	NC
Skin (nonpigmented)	4	13300	NC	178530	NC
Small intestine	4	49100	NC	704850	NC
Spinal cord	4	3110	NC	51765	NC
Spinal cord grey matter	4	3450	NC	56355	NC
Spinal cord white matter	24	2110	NC	70086	NC
Spleen	4	48400	NC	699000	NC
Stomach	4	56600	NC	905700	NC
Thymus	4	45900	NC	579650	NC
Thyroid	4	58300	NC	786650	NC
Urinary bladder	8	31900	NC	531300	NC
Uterus	4	44900	NC	610390	NC

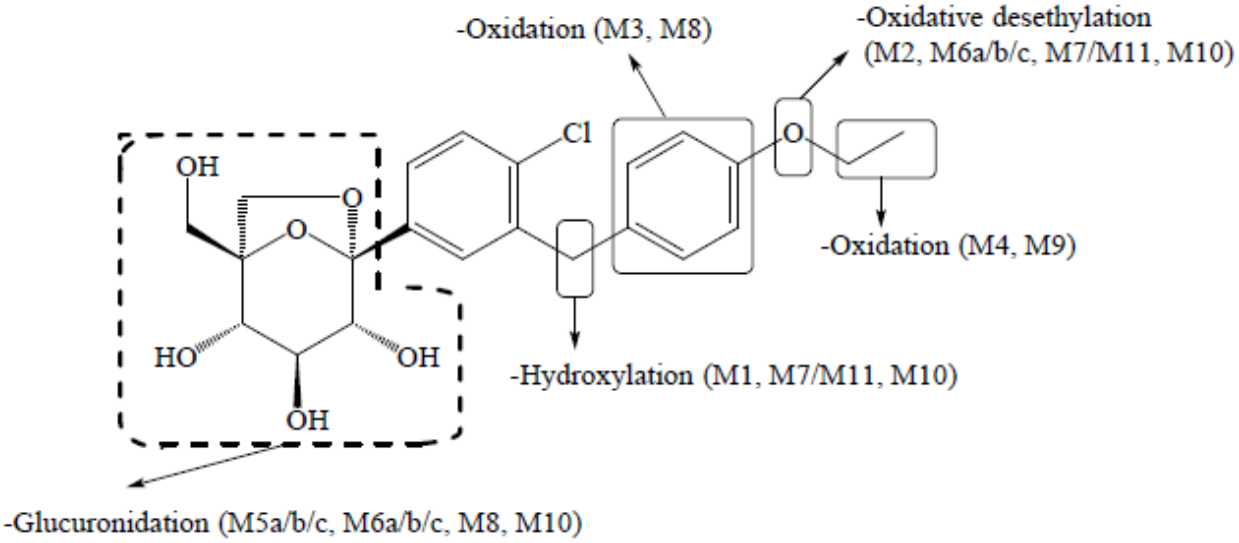
Note: Pharmacokinetic parameters were calculated using data from one maternal rat each at 1, 4, 8, 24, and 48 hours postdose.
eq Equivalents [^{14}C]PF-04971729.
LSC Liquid scintillation counting.
NC Not calculated.

(Tables excerpted from sponsor's package)

5.2.3 Metabolism

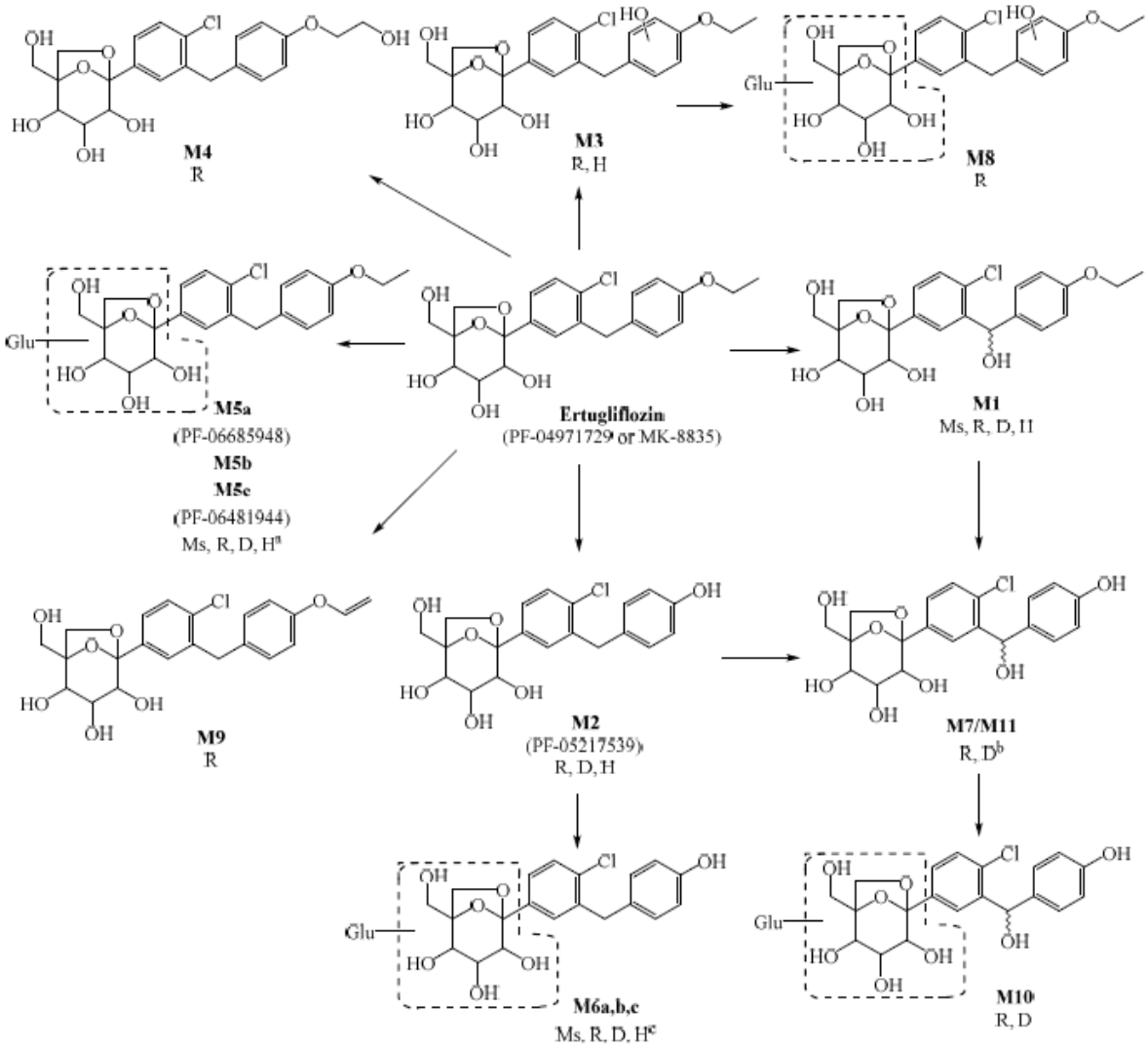
The major metabolic pathway for ertugliflozin is glucuronidation, which is predominately mediated by UGT1A9 and UGT2B7, resulting in glucuronide metabolites M5a/b/c, M6a/b/c, M8, and M10. The minor metabolic pathway is via oxidation, which is predominantly mediated by CYP3A4 with minor contributions from CYP3A5 and CYP2C8. Human metabolites include M2, M3, M5, and M6. There are no unique human metabolites; however, additional oxidative metabolites including M4, M8, and M9 were identified in rats, as well as M1, M7/M11, and M10 that were identified in both rodents and dogs.

Figure 2: Major Ertugliflozin Metabolic Pathways



(Figure excerpted from sponsor's package)

Figure 3: Proposed Ertugliflozin Metabolic Profile across Species



Note: Mice dosed with unlabeled ertugliflozin. Rats, dogs, and humans were dosed with [^{14}C]ertugliflozin. Metabolites detected in plasma from mouse, plasma, urine, feces, and bile from rat and dog, or in plasma, urine, and feces from human. PF-06481944 (M5c) is the 3-O-β glucuronide of ertugliflozin and PF-06685948 (M5a) is the 2-O-β glucuronide of ertugliflozin. Metabolite to metabolite pathways were not confirmed experimentally.

Abbreviations: D = Dog; H = Human; M = Metabolite; Ms = Mouse; R = Rat.

a. M5a = Rat plasma and bile and human plasma and urine; M5b = Rat bile, dog bile, and human plasma and urine; and M5c = Mouse plasma, rat plasma, feces, and bile, dog plasma, urine, feces, and bile, and human plasma and urine.

b. M7 = Rat plasma, urine, and feces and dog urine; M11 = Rat urine and feces.

c. M6a/b = Mouse plasma; M6a = Rat plasma[d], urine, and bile, dog plasma, urine, feces, and bile, and human plasma and urine; M6b = Rat bile and human urine; and M6c = Rat bile[d].

d. Observed by mass spectrometry.

(Figure excerpted from sponsor's package)

In humans, the glucuronide metabolite M5c was the major metabolite, comprising 24.1% of total drug radioactivity, and the glucuronide metabolite M5a comprised 12.2%

of total radioactivity. Thus, both M5a and M5c are disproportional metabolites in humans. In rats, M5a and M5c each comprised only 0.3% and 0.7% of total radioactivity in females and males, respectively. In dogs, M5a was not identified; however, M5c comprised 2.8% and 3.3% of total radioactivity in females and males, respectively.

Table 13: Ertugliflozin, M5a & M5c Percent of Total Drug in Plasma across Species

Species	% Total* Radioactivity			
	Parent (Ertugliflozin)	**Total Drug**	**M5a** (PF-06685948)	**M5c** (PF-06481944)
Human	49.9%	100%	12.2%	24.1%
Rat	♂+♀: 90.3% ♂: 86.5% ♀: 94.0%	100%	♂: 0.7% ♀: 0.3%	♂: 0.7% ♀: 0.3%
Dog	♂: 93.5% ♀: 94.9%	100%	0	♂: 3.3% ♀: 2.8%

* % Total radioactivity in plasma from PK studies in human (study #PK042), rat (study #PK040) and dog (study #PK041)

5.2 Toxicokinetics

In general, systemic exposures increase in a dose-dependent manner in mouse, rat and dog. AUC and C_{max} exposures increased with linear pharmacokinetics in rats at doses ≤100 mg/kg and in dogs at ≤50 mg/kg. However, at higher concentrations, exposure kinetics were non-linear, which may be secondary to decreased absorption efficiency or due to concentration levels beyond the limits of accuracy for the validated analytical LC-MS/MS method (≥50 μg/mL) leading to increased experimental error at higher concentrations. Although trends for slight increases in exposure over time were present at high doses, there were no consistent indications of accumulation over time in combined male and female exposures of rats and dogs. In mice, slight accumulation was observed in females, but not in males, and may indicate a slight gender effect specific to mice. The sponsor did not recognize any significant gender effects on systemic exposure, dose-exposure relationships, or time-dependent changes in rat or dog systemic exposures. However, trends for slightly increased AUC exposures were repeatedly observed in female rats and mice, which may be attributable to rodent-specific gender differences in metabolism. T_{max} was consistently observed at 0.5 hours in mice, and was generally between 1-4 hours in rats and 1 hour in dogs. However, at high doses, T_{max} was often delayed in rats at ≥25 mg/kg, reaching up to 6 hours postdose at 100 mg/kg. Similarly, at high doses in dogs, T_{max} was often delayed to 2-3 hours postdose at 150 mg/kg. The observed delays in T_{max} at high doses correlate with loss of linear pharmacokinetics in exposures and likely indicate that exposures are limited by the rate of absorption at high doses in these species.

Table 14: Ertugliflozin TK Summary in Nonclinical Studies

C_{max}* (µg/mL)														
Species	Sex	Dose (mg/kg)												
		1	1.5	3	5	10	15	25	40	50	100	150	250	500
Mouse	♂	-	-	0.95	1.30	-	4.78	6.60	11.40	-	27.65	-	45.80	-
	♀	-	-	1.34	1.60	-	8.85	8.75	9.99	-	33.55	-	66.00	-
Rat	♂	0.45	0.72	-	2.39	-	7.34	9.46	-	-	34.65	-	53.17	90.20
	♀	0.98	1.08	-	3.33	-	12.70	12.90	-	-	45.00	-	57.70	66.50
Dog	♂	1.00	-	-	6.32	9.60	-	-	-	46.93	-	89.70	-	12.20
	♀	1.08	-	-	6.95	11.41	-	-	-	65.23	-	80.58	-	38.60

* TK data was compiled from 2 mouse, 13 rat, and 6 dog studies. Mean values from exposures at the same doses were averaged.

AUC* (µg·h/mL)														
Species	Sex	Dose (mg/kg)												
		1	1.5	3	5	10	15	25	40	50	100	150	250	500
Mouse	♂	-	-	3.77	4.47	-	19.60	20.10	53.00	-	165.00	-	557.00	-
	♀	-	-	6.67	9.31	-	40.60	45.60	87.20	-	283.50	-	480.00	-
Rat	♂	3.86	6.69	-	20.74	-	91.00	96.40	-	98.80	391.00	-	663.00	1500
	♀	6.38	9.27	-	27.93	-	102.00	143.22	-	-	587.00	-	785.33	-
Dog	♂	7.05	-	-	52.77	71.70	-	-	-	473.00	-	987.80	-	138
	♀	8.57	-	-	57.57	86.20	-	-	-	645.00	-	883.40	-	465

- TK data was compiled from 2 mouse, 12 rat, and 6 dog studies. Mean values from exposures at the same doses were averaged.

AUC exposures for total drug and the disproportional metabolites M5a and M5c were calculated for the pivotal 6-month rat (study #09GR275) and 9-month dog (study #09GR476) toxicology studies based on percent ratios of each component in plasma that was determined in PK studies with administration of radiolabeled ertugliflozin (Table 5). In the pivotal rat toxicology study, M5a exposures approximately 3-fold higher than clinical exposures. M5c exposures approximately 3-fold and at least 23-fold higher than clinical exposures were achieved in the pivotal rat and dog toxicology studies, respectively.

Table 15: Calculated M5a & M5c AUC Exposures in Pivotal Nonclinical Toxicology Studies

Species	Dose (mg/kg/day)	AUC (μg·h//mL)			
		Parent (Ertugliflozin)	Total Drug*	M5a (PF-06685948)	M5c (PF-06481944)
Human	15 (mg/day)	1.38	2.766	0.337	0.667
Rat (6-month)	5	♂: 17.6 ♀: 26.9	♂: 19.5 ♀: 29.8	♂: 0.137 ♀: 0.209	♂: 0.137 ♀: 0.209
	25	♂: 128 ♀: 167	♂: 142 ♀: 185	♂: 0.994 ♀: 0.555	♂: 0.994 ♀: 0.555
	100	♂: 397 ♀: 814	♂: 440 ♀: 901	♂: 3.08 ♀: 2.70	♂: 3.08 ♀: 2.70
Dog (9-month)	1	♂: 6 ♀: 7	♂: 6.4 ♀: 7.4	-	♂: 0.21 ♀: 0.21
	10	♂: 63 ♀: 78	♂: 66.9 ♀: 82.8	-	♂: 2.21 ♀: 2.31
	150	♂: 1040 ♀: 767	♂: 1104 ♀: 814	-	♂: 36.4 ♀: 22.8

* Calculated Total Drug = Parent + all metabolites; based on percent ratios of total radioactivity in plasma from PK studies in human (study #PK042), rat (study #PK040) and dog (study #PK041)
NOAEL = highlighted in yellow

6 General Toxicology

Pivotal GLP-compliant 6-month rat (study #09GR275) and 9-month dog (study #09GR476) toxicology studies were previously reviewed by Dr. Quinn, and have been summarized below. The sponsor also conducted preliminary 3-month and 1-month and/or 2-week toxicology studies in rats, dogs and mice, which will not be discussed in detail in this review.

Two additional repeat-dose toxicology studies were submitted with the NDA package and are reviewed in detail in this review. The sponsor conducted a 13-week bridging toxicology study in rats evaluating ertugliflozin synthesized by the process method used in the commercial formulation to be marketed. The sponsor also conducted a 13-week rat toxicology study evaluating 4 potential ertugliflozin degradation products.

6.2 Repeat-Dose Toxicity

Adverse drug-related effects are predominantly observed in the renal system of all species and are likely to be secondary to the PD activity of SGLT2 inhibition. Drug-related kidney findings include pelvic and tubule dilatation, inflammation, mineral deposits, hypertrophy, chronic progressive nephropathy, and increased organ weight. Other renal system findings such as infection, inflammation, hypertrophy and

hyperplasia of organs of the urinary tract, including the prostate in males, are likely secondary to PD-related glucosuria.

Adverse drug-related effects of the digestive tract have also been observed in both rats and dogs. Digestive tract effects in rats include stomach degeneration/hyperplasia, stomach erosion, ulcers, increased intestinal villi height and GI tract dilatations. In dogs, dose-limiting effects are consistent with GI distress, including clinical signs of salivation, vomiting and diarrhea. Many of the digestive tract findings are consistent with potential off-target inhibition of SGLT1, which can lead to fermentation of unabsorbed glucose in the small and large intestine and subsequent trophic dilatation and villous changes.

Non-adverse drug-related increases in adrenal weights have been reported in both rats and dogs. Correlating increases in adrenal gland hypertrophy and vacuolation were also reported, but were considered likely to be non-adverse and secondary to PD-related diuresis.

Drug-related effects on calcium homeostasis have been reported in rat and dog toxicology studies. Hypercalciuria was observed in rats and dogs, consistent with other members of the SGLT2 drug class and with PD-related osmotic diuresis. Hypercalciuria is also consistent with changes in bone metabolism and parathyroid and thyroid gland dysfunction (Tuchendler 2014). Decreases in PTH correlated with decreases in mean serum calcium levels and increases in trabecular bone and hyperostosis in male rats at 100 mg/kg ertugliflozin (≥288x $MRHD_{AUC}$). Similar bone findings have been reported in rats with other members of the SGLT2 inhibitor drug class, and may be secondary to drug-related effects on calcium absorption/excretion due to off-target SGLT1 inhibition in the gut. Findings in rats indicate that calcium homeostasis and bone health can be disrupted at high doses (≥288x $MRHD_{AUC}$).

It is noted that drug-related liver findings of glycogen depletion, necrosis and fibrosis were reported in the 3-month dog study, but were not present in the 9-month dog study. Absence of these liver findings after 9 months of dosing may be related to hepatic adaptation and regeneration.

Study: 9-Month Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Dogs with an 8-Week Recovery Phase (Study TT #09-7895 / #8222521 / 09GR476)

Study #	#8222521 / 09GR476
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	2/18/2010
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729 (b) (4), Lot #GR02847, 99.9% purity

Key Study Findings

- NOAEL = 10 mg/kg (♂ = 46x $MRHD_{AUC}$, ♀ = 57x $MRHD_{AUC}$)
  - GI intolerance at 150 mg/kg (♂ = 754x $MRHD_{AUC}$, ♀ = 556x $MRHD_{AUC}$)
    - Possibly related to 2 mortalities
    - Systemic inflammatory response
    - ↓body weights and ↓weight gains
    - Persistent ↑reticulocytes
    - Likely secondary to off-target SGLT1 inhibition
- ↑Adrenal gland weights at 150 mg/kg (♂&♀) and adrenal cortex vacuolation at all doses (♂)
- Persistent thyroid mineralization at ≥10 mg/kg (♀)
- ↑Urine calcium at 150 mg/kg, partially reversible after recovery

Reviewer's Comments

Two mortalities at 150 mg/kg were reported as gavage-related; however, they were also associated with severe GI intolerance and it was considered plausible that local tissues may have been damaged and/or weakened by reflux, leading to increased susceptibility to gavage-related injury. Thus, the NOAEL was set at 10 mg/kg based on potentially drug-related mortalities and observations of GI intolerance such as excessive vomiting, salivation and abnormal feces at 150 mg/kg. Drug-related GI intolerance was considered likely due to off-target SGLT1 inhibition, based on consistency with anticipated PD-related effects of SGLT1 inhibition and C_{max} exposure of ≈200 μM, which is >600-fold higher than the IC_{50} value for ertugliflozin-mediated inhibition of dog SGLT1 (Sponsor's Table 2). Correlating lower body weights and weight gains were also observed at 150 mg/kg, but resolved during the recovery period with increased food consumption and weight gain. Microscopic findings indicative of inflammation, increased immune cell numbers, increases in fibrinogen, and elevated thymus weights were considered to be consistent with a systemic inflammatory response at 150 mg/kg. Persistent elevations in reticulocyte counts were observed in both genders, which may be indicative of a regenerative response secondary to digestive tract erosion.

PD-related glucosuria and increased urine volume were observed at all doses. Increases in urinary excretion of calcium, sodium and chloride electrolytes were reported at 150 mg/kg, but were not associated with significant changes in electrolyte

serum concentrations. Increased urine volume persisted throughout the recovery period, indicating persistent changes in kidney function.

Increases in adrenal gland weights at 150 mg/kg in both genders correlated with incidences of adrenal cortex vacuolation in males at all doses, which were not present after recovery.

Persistent thyroid mineralization was reported in females at ≥10 mg/kg, and partially reversible increases in urine calcium were observed at 150 mg/kg. Since hypercalciuria is consistent with changes in bone metabolism and thyroid gland dysfunction (Tuchendler 2014), the thyroid findings may correlate with the observed changes in urinary calcium levels and indicate potential disruption of calcium homeostasis in renal tubules where calcium reabsorption and excretion is regulated. However, increases in calcium excretion are also consistent with osmotic diuresis secondary to the PD activity of ertugliflozin and inhibition of renal tubule glucose reabsorption. .

Male dogs had AUC exposures of 6, 63 and 1040 μg·h/mL with exposure margins of 4x, 46x and 754x $MRHD_{AUC}$. Female AUC exposures were 7, 78 and 767 μg·h/mL with margins of 5x, 57x and 556x $MRHD_{AUC}$. Combined male and female C_{max} exposures were 0.8, 7.72, and 86.4 μg/mL.

Methods

Doses:	0, 1, 10, and 150 mg/kg
Frequency of dosing:	Daily for 9 months
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC/10% PEG 400
Species/Strain:	Beagle Dogs / (b) (4)
Number/Sex/Group:	4/sex/group
Satellite groups:	Recovery groups 2/sex/group for control and 150 mg/k PF-04971729 only

Parameters Measured

Observations and Times	
Mortality Checks Observations	Performed twice daily
Cageside Exams	Performed once daily (1 Hrs Post Dose)
Detailed Exams	3x - Pretreatment, Prior to Day 1 Dose, Weekly thereafter
Body weights	3x - Pretreatment, Prior to Day 1 Dose, Weekly thereafter
Food consumption	Weekly
Ophthalmology	Pre, D139, D272 of Dosing Phase - Ophthalmoscope (indirect)
ECG	2x - Pre, D134, D267 of Dosing Phase – 1-2 Hours PD (Leads I, II, aVF, CV5RL, and CV6LL)
Hematology	Fasted – 2x Pre, Weeks 13, EOD and EOR
Coagulation	
Clinical chemistry	
Urinalysis	Fasted – 1x Pre, Weeks 13, EOD and EOR (Overnight)
Gross pathology	EOD (Day 274), EOR (Day 334)
Organ weights	All Study Animals: Standard Battery
Bone Marrow Slides	Prepared but not evaluated
Histopathology	All tissues (Larynx was not examined)
	Adequate Battery: yes (X), no ()
	Peer review: yes (X), no ()

Observations and Results

Mortality

There were no drug-related mortalities.

Clinical Signs

Vomiting (10 subclasses), abnormal feces (10 subclasses) and excessive salivation increased with dose and were typically more severe at 150 mg/kg/day. These findings tended to resolve during recovery and are consistent with the GI intolerance noted in previous dog studies at the 150 mg/kg/day dose. The relative GI intolerance at the 150 mg/kg/day dose may reflect off-target inhibition of SGLT1, which is important in duodenal absorption of glucose.

Body Weights

High dose dogs (150 mg/kg/day) tended to weigh less than controls despite increased food consumption. These results are consistent with the GI intolerance and increased caloric demand and tended to resolve in HD recovery dogs. The body weight data is consistent with the results from earlier dog studies.

It's noted that body weight loss in low dose females exceeded those observed at the 150 mg/kg/day dose.

Table 16: Body Weights - 9-month Dog Study #09GR476

Body Weight				
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Males	0	700	0%	100%
	1	700	0%	120%
	10	600	-14%	110%
	150	100	- 86%	90%

Body Weight				
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Females	0	1000	0%	100%
	1	300	- 70%	94%
	10	900	- 10%	101%
	150	500	- 50%	95%

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Food Consumption

Food consumption in males dosed at ≥ 1 mg/kg/day was statistical increased from Week 2 forward until the end of the administration period. Food consumption was similarly increased in females although the degrees of change were less often found significant. Increased food consumption persisted through recovery and correlated with increased body weight gain in high dose dogs. Changes in food consumption were less consistent in earlier dog studies and likely reflect the persistent GI intolerance associated with this compound.

Ophthalmologic Examinations

Indirect ophthalmological examinations revealed no visible lesions of the eye.

Electrocardiographic Examinations

Electrocardiographic examinations (Leads I, II, aVF, CV5RL, and CV6LL) did not reveal any abnormal findings at 1-2 hours post dose in dogs administered PF04971729. The selected examination time was consistent with the T_{max} for PF04971729 (1-2 hours). However, ECG data evaluated as part of PF-04971729 toxicology studies have been inconsistent.

Hematology

Reticulocyte counts tended to be elevated in dogs administered PF04971729 although these changes were dose-dependent in females only (Week 39) and were not found to be statistically significant. Increased reticulocyte counts persisted through recovery and may reflect a regenerative response incited by digestive tract erosion, although other RBC parameters were not significantly affected by dosing. Notable changes in reticulocyte counts were not observed in prior dog studies.

WBC counts were minimally increased in dogs administered PF04971729 and these changes were found to be dose-dependent only in males (Week 39). Increased WBC counts trended towards recovery and were not noted in prior dog studies.

Monocyte counts tended to increase dose-dependently in males only (Week 39) with significant changes occurring sporadically in both genders. Monocyte counts normalized following recovery and significant changes were not noted in prior dog studies.

Table 17: Hematology Parameters - 9-month Dog Study #09GR476

Notable Effects of PF04971729 on Reticulocytes

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
Reticulocytes ($10^3/\mu L$)		↑49	↑41	↑48	↑54	↑48	↑24
Week 39							
Reticulocytes ($10^3/\mu L$)		↑6	↑4	↑43	↑19	↑7	↑41
Week 47							
Reticulocytes ($10^3/\mu L$)		-	-	-	-	↑59	↑49
(*p<0.05) (**p<0.01)							

Notable Effects of PF04971729 on White Blood Cells

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
WBC ($10^3/\mu L$)		↑27	↑9	↑11	↑43	↑6	↑17
Week 39							
WBC ($10^3/\mu L$)		↑16	0	↑21	↑12	↑22	↑11
Week 47							
WBC ($10^3/\mu L$)		-	-	-	-	↑11	↑4
(*p<0.05) (**p<0.01)							

Notable Effects of PF04971729 on Monocytes

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
Monocytes ($10^3/\mu L$)		↑44*	0	0	↑61	↑28	↑15
Week 39							
Monocytes ($10^3/\mu L$)		↑33	0	↑53	↑55*	↑60	↑7
Week 47							
Monocytes ($10^3/\mu L$)		-	-	-	-	0	0
(*p<0.05) (**p<0.01)							

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Coagulation

Fibrinogen concentrations tended to increase dose-dependently in males only (Week 39) with significant changes occurring at the high dose during Week 13 (↑54%) and Week 39 (↑47%). Increased fibrinogen concentrations persisted in recovery males (↑42%) and suggest an enduring inflammatory response. Microscopic indications of a systemic inflammatory response were present but limited at the high dose and tended to be minimal or slight in severity.

Significant changes in coagulation parameters were not noted in females during this study and have not been observed in previous dog studies.

Clinical Chemistry

Serum glucose levels tended to decline inversely to dose in males (Week 13) and females (Week 13 & 39) with significant changes occurring sporadically in both genders. Decreased serum glucose levels normalized following recovery and these results are consistent with the pharmacological effect of PF04971729. The unusual reduction in serum glucose in a dose inverted manner replicates the results from the previous 3 month study and indicates a loss of efficacy with prolonged use at higher doses in dogs. Glucose assessment was performed in fasted dogs and the effect of dosing on glucose in non-fasted animals was not ascertained.

Table 18: Clinical Chemistry - 9-month Dog Study #09GR476

Notable Effects of PF04971729 on Serum Glucose Levels

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
Glucose (mg/dL)		↓18	↓32*	↓16	↓20*	↓15	↓11
Week 39							
Glucose (mg/dL)		↓13	↓29*	↓1	↓17*	↓17*	↓13*
Week 47							
Glucose (mg/dL)		-	-	-	-	↑2	↓3
(*p<0.05) (**p<0.01)							

(Table excerpted from Dr. Quinn's Pharm/Tox review)

Serum concentrations of calcium, sodium and chloride were not significantly altered at the high dose despite a significant increase in the urinary excretion of these electrolytes. Homeostatic biomarkers (PTH, Vitamin D, ACTH and aldosterone) were not measured during this study.

Urinalysis

Significant glucosuria was observed at all doses and tended to normalize following recovery. The maximal PD effect was achieved at the LD (1 mg/kg) in females and glucosuria tended to be dose-dependent throughout the administration period in males. An unexplained elevation in urine glucose was observed in control females during the dosing period. Glucosuria is an expected pharmacological effect of SGLT2 inhibition. Urine creatinine levels declined significantly during Week 13 of dosing and remained lower through the end of recovery. Decreased urine creatinine is feasibly related to the corresponding increase in urine volume. Urine glucose to creatinine ratios were significantly elevated (up to 575-fold vs control) through the end of dosing and tended to normalize following recovery.

Table 19: Urine Glucose Levels - 9-month Dog Study #09GR476

Urine Glucose									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
Glucose (mg/dL)	Week 13	ND	1367	2754	7291*	3061	4277*	4354	895
	Week 39	15	502	2699*	3834*	3273*	4162*	3799*	3695*
	Week 47	5	6	ND	ND	ND	ND	ND	56
(*p<0.05) (**p<0.01) ND = No Data									

Creatinine									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
UCRE (mg/dL)	Week 13	95	126	25*	71*	30*	36*	43*	8*
	Week 39	76	65	24	37	28	37	35	31*
	Week 47	96	94	ND	ND	ND	ND	18	22
(*p<0.05) (**p<0.01) ND = No Data									

Glucose:Creatinine Ratios									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
GLCR (Ratio)	Week 13	ND	11	108	104*	109	119*	102	97*
	Week 39	0.2	8	114*	103*	115*	107*	111*	115*
	Week 47	0.1	0.1	ND	ND	ND	ND	ND	2.2
(*p<0.05) (**p<0.01) ND = No Data									

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Increases in urinary excretion of electrolytes were increased at 150 mg/kg. Urine calcium excretion increased (up to 10-fold vs control) at the 150 mg/kg dose during Week 39 and remained slightly elevated (up to 4-fold vs control) through the end of recovery. Urine sodium excretion increased (up to 3-fold vs control) at the 150 mg/kg dose during Week 39 and tended to normalize by the end of recovery. Urine chloride excretion increased (up to 3-fold vs control) at the 150 mg/kg dose during Week 39 and tended to normalize by the end of recovery.

Table 20: Urine Electrolytes - 9-month Dog Study #09GR476

Calcium Excretion									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
CaX (mg)	Week 13	2	5	9	4	11	15	7	12
	Week 39	2	8	12	6	8	13*	20*	32*
	Week 47	4	8	ND	ND	ND	ND	16	19
(*p<0.05) (**p<0.01) ND = No Data									

Sodium Excretion									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
NaX (mg)	Week 13	8	8	10	8	9	11	6	2
	Week 39	5	5	7	3	7	6	16*	11*
	Week 47	5	10	ND	ND	ND	ND	6	15
(*p<0.05) (**p<0.01) ND = No Data									

Chloride Excretion									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
ClX (mg)	Week 13	8	9	9	9	7	12	7	ND
	Week 39	5	7	8	4	9	8	15	13*
	Week 47	7	14	ND	ND	ND	ND	7	13
(*p<0.05) (**p<0.01) ND = No Data									

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

PD-related increases in urine volume (up to 5-fold vs control) were noted at doses ≥1 mg/kg and tended to be dose-dependent in females. However, increases in urine output persisted after recovery, indicative of a persistent change in kidney function.

Table 21: Urine Volume - 9-month Dog Study #09GR476

Urine Volume									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
UVol (mL)	Week 13	72	76	229	121*	298	279*	224	336*
	Week 39	60	130	304*	181	205	269*	282*	425*
	Week 47	69	128	ND	ND	ND	ND	321	431
(*p<0.05) (**p<0.01) ND = No Data									

(Table excerpted from Dr. Quinn's Pharm/Tox review)

There were no drug-related increases in urine ketone levels or specific gravity. There were no consistent or dose-dependent decreases in urine pH.

Gross Pathology

Discoloration of the GI tract was observed in 1 male from the 10 and 150 mg/kg groups and was noted in the single high dose male that died on study. Discoloration of the GI tract occurred independent of dose in females. Lung discoloration was observed in a single high dose recovery male and both high dose dogs whose deaths were attributed to gavage error. The presence of skin abrasions increased in females dosed at ≥10 mg/kg/day.

Table 22: Macroscopic Findings - 9-month Dog Study #09GR476

Gross pathology – Males – End of Dosing						
Tissue	Finding	Main Study				
		dose	0	1	10	150
		n	4	4	4	3
Duodenum	Discolored		0	0	0	1
Colon	Discolored		0	0	1	1
Jejunum	Discolored		0	0	0	1

(Table excerpted from Dr. Quinn's Pharm/Tox review)

Organ Weights

Absolute and relative adrenal weight increased dose-dependently in females at the end of dosing and trended towards recovery following dosing cessation (↑8%). Increased adrenal weights in males were comparable to females at the 150 mg/kg dose. Vacuolation of the adrenal cortex was observed microscopically in male dogs (≥1 mg/kg).

Absolute and relative thymus weight increased dose-dependently in females at the end of dosing and continued to escalate through the recovery period (↑83%). Increased thymus weights in males were comparable to females at the 150 mg/kg dose. PF04971729 dosing-related microscopic changes were not observed in the thymus.

Table 23: Organ Weights - 9-month Dog Study #09GR476

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Organ							
Adrenal (Absolute)		↑10%	↑7%	0	↑16%	↑23%	↑30%
Adrenal (Rel: $\bar{X}$ BrW)		↑2%	↑6%	0	↑22%	↑31%	↑37%
(* = P ≤ 0.05) (** = P ≤ 0.01)							

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Organ							
Thymus (Absolute)		↑47%	↑13%	0	↑14%	↑32%	↑39%
Thymus (Rel: $\bar{X}$ BrW)		↑36%	↑12%	0	↑17%	↑38%	↑47%
(* = P ≤ 0.05) (** = P ≤ 0.01)							

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Heart weight (relative to body weight) decreased dose-dependently in females only. These changes were minimal in nature (↓<10%), not reflected in absolute values and tended to resolve following the recovery period.

Histopathology

Vacuolation of the adrenal cortex was observed at the end of the administration period (males only) and was present in control animals (both genders) following recovery. Hypertrophy of the salivary gland at the high dose is likely related to the excessive salivation that occurred in dogs exposed to PF04971729 and was not present following recovery. Several other tissue types displayed microscopic findings at the high dose in males although the incidence often did not exceed one and the severity was usually minimal to slight in nature.

Thyroid mineralization was observed in females (≥10 mg/kg/day) and persisted in 50% (minimal to slight) of the HD recovery animals. Mineralization may be related to PF04971729-mediated disruption of calcium homeostasis in the renal tubules where calcitonin normally acts to regulate calcium reabsorption and excretion. Calcium excretion remained elevated in HD recovery dogs.

Table 24: Microscopic Findings - 9-month Dog Study #09GR476

Histopathology – Males – End of Dosing						
Tissue	Finding		Main Study			
		dose	0	1	10	150
		n	4	4	4	3
Adrenal	Vacuolation - Cortex		0	2 (1*/1**)	1*	2*
G.A. Lymph	Mineralization		0	0	0	1*
Salivary Gland	Hypertrophy		0	0	0	1**
Epididymis	Chronic Inflammation		0	0	0	1**
Minimal (*) Slight (**) Moderate (***) Marked (****)						

Histopathology – Females – End of Dosing						
Tissue	Finding	Main Study				
		dose	0	1	10	150
		n	4	4	4	3
Thyroid	Mineralization		0	0	1*	2*
Gallbladder	Lymphoid - Follicles		0	0	0	2**
Kidney	Lymp/Macro - Infiltrate		0	0	1*	1*
Urinary Bladder	Chronic Inflammation		0	0	0	1*
Mesen. LN	Sinus Erythrocytes		0	0	1*	1**
Ureter	Chronic Inflammation		0	0	0	1**
Poplit. LN	Sinus Erythrocytes		0	1**	1*	1*
Minimal (*) Slight (**) Moderate (***) Marked (****)						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

It is noted that decreases in glycogen content and liver cell necrosis were reported at 150 mg/kg in the 3-month dog study. Lack of these findings at the end of 9 months of dosing may be related to hepatic adaptation and regeneration.

Toxicokinetics

Mean C_{max} exposures tended to increase in a less than dose proportional manner, while mean AUC_{0-24} exposures increased in proportion to dose between 1 and 150 mg/kg. Systemic exposures tended to be higher in females at ≤10 mg/kg and in males at 150 mg/kg. Mean T_{max} was between 1 and 2 hours post dose. Systemic exposure (C_{max} and AUC_{0-24}) tended to increase at the 150 mg/kg/day dose between Day 1 and Week 39. Systemic exposures were notably lower than those obtained at these doses during the 3-month dog study, especially at ≤10 mg/kg/day.

Table 25: Toxicokinetics - 9-month Dog Study #09GR476

Toxicokinetic Parameters for PF04971729 in Dogs on Day 1 and During Week 39

Dose (mg/kg/ day)	Study Week	Gender	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
1	1	Male	0.751	0.159	4	0.750	0.289	4	6.12	1.55	4
		Female	0.832	0.129	4	1.13	0.629	4	7.41	1.85	4
		Overall	0.791	0.141	8	0.938	0.496	8	6.77	1.72	8
	39	Male	0.743	0.273	4	1.50	1.68	4	5.61	0.364	4
		Female	0.849	0.398	4	1.13	0.629	4	6.99	1.51	4
		Overall	0.796	0.321	8	1.31	1.19	8	6.30	1.26	8
10	1	Male	10.3	1.41	4	0.750	0.289	4	73.9	2.04	4
		Female	8.66	0.742	4	1.00	0.707	4	80.3	4.97	4
		Overall	9.46	1.35	8	0.875	0.518	8	77.1	4.88	8
	39	Male	6.50	2.26	4	1.13	0.629	4	62.9	4.43	4
		Female	8.93	2.24	4	0.875	0.250	4	78.2	21.1	4
		Overall	7.72	2.45	8	1.00	0.463	8	70.5	16.3	8
150	1	Male	51.8	16.5	6	2.17	0.983	6	659	209	6
		Female	57.5	4.34	6	2.33	0.816	6	728	115	6
		Overall	54.6	11.9	12	2.25	0.866	12	693	165	12
	39	Male	98.8	39.5	5	1.80	0.447	5	1040	449	5
		Female	74.0	12.9	5	2.10	1.24	5	767	166	5
		Overall	86.4	30.7	10	1.95	0.896	10	906	351	10

Overall = Male plus female combined

(Table excerpted from Dr. Quinn's Pharm/Tox review)

Study: 6-Month Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Rats with an 8-Week Recovery Phase (Study TT #09-7894 / #8215018/ 09GR275)

Study no.:	09GR275
Study report location:	(SD36 - eCTD 4.2.3.2.1) (DARRTS SDN37)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	15 September 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF04971729-G3, GR02877, 98.4% Active Moiety: 74.4%, GR02546, 99.5%, Active Moiety: 76.0%

Key Study Findings

- NOAEL = 5 mg/kg (♂ = 13x $MRHD_{AUC}$, ♀ = 20x $MRHD_{AUC}$)
  - Digestive tract findings at ≥25 mg/kg (♂ = 93x $MRHD_{AUC}$, ♀ = 121x $MRHD_{AUC}$)
    - Stomach hyperplasia
    - ↑Severity of stomach erosion/ulcer
    - Pyloric crypt degeneration

        - Stomach discoloration
    - Bone findings at 100 mg/kg (♂ = 288x $MRHD_{AUC}$, ♀ = 590x $MRHD_{AUC}$)
        - Severe hyperostosis (♂)
        - Bone hyperplasia (♀)
    - Digestive tract & bone effects are likely secondary to off-target SGLT1 inhibition
- Kidney findings at ≥25 mg/kg
    - Pelvic & tubule dilatation, hyperplasia, mineral deposition
    - Associated with 1.5-fold increase in blood urea nitrogen (BUN) at 100 mg/kg
- ↑Adrenal gland weights, hypertrophy, and vacuolation at 100 mg/kg
- Changes in clinical pathology
    - ↓Parathyroid hormone (PTH) at 100 mg/kg
    - ↑Urine calcium at 100 mg/kg
    - ↑Ketonuria at all doses in males and ≥25 mg/kg in females, with correlating ↓pH
    - ↓Serum electrolyte levels at 100 mg/kg (Ca, Na, K, and Cl)

Reviewer's Comments

The NOAEL was set at 5 mg/kg based on adverse digestive tract findings at ≥25 mg/kg and bone findings at 100 mg/kg.

Drug-related digestive tract findings were predominantly observed in the stomach. Findings of erosions/ulcers were reported at all doses, which increased with dose in incidence and severity. Stomach findings at ≥25 mg/kg were considered to be adverse, which included pyloric crypt degeneration, macroscopic observations of discoloration, and increased severity of erosions/ulcers. At 100 mg/kg, additional stomach findings included hyperplasia. Although macroscopic findings of stomach discoloration persisted in recovery animals at ≥25 mg/kg, all microscopic stomach findings resolved after recovery. The stomach findings were considered to be consistent with findings associated with SGLT1 inhibition in this species. Furthermore, C_{max} levels in plasma were 6 μM at 5 mg/kg, 23 μM at 25 mg/kg and 69 μM at 100 mg/kg, exceeding the IC_{50} value for rat SGLT1 (Sponsor's Table 2) by 17-fold, 65-fold and 200-fold, respectively. Thus, ertugliflozin concentrations were likely to be high enough to achieve off-target inhibition of SGLT1, and the digestive tract findings were considered likely to be secondary to off-target SGLT1 inhibition. Drug-related decreases in pancreatic zymogen granules at all doses were also considered possibly related SGLT1 inhibition or secondary to food consumption, but were considered to be non-adverse.

PD-related kidney findings including dilatation, hyperplasia and mineralization were apparent at ≥25 mg/kg, but were considered unlikely to be adverse and are likely secondary to PD-related osmotic diuresis. Although increases in BUN levels were associated with a 1.5-fold increase at 100 mg/kg, the increase was considered mild and non-adverse in that it was less than 2-fold. Furthermore, concomitant increases in BUN and inorganic phosphorus are consistent with relative dehydration and correlate with decreases in serum electrolytes (calcium, sodium, potassium, and chloride), all of which

may be secondary to PD-related increases in urinary excretion and osmotic diuresis. Thus, there were no clear indications of kidney dysfunction.

Drug-related bone findings at 100 mg/kg included severe bone hyperostosis in males, with minimal to slight increases in trabecular bone, and hyperplasia of the physis in 1 female. After the recovery period, bone changes in the femur were fully resolved; however, changes in the sternum were only partially resolved. It is noted that bone hyperostosis has been hypothesized to be related to inhibition of SGLT1 in the gut and increased calcium absorption. Overall, the bone findings are considered likely due to off-target inhibition of SGLT1.

Decreases in serum calcium correlated with increases in urine calcium levels, which is consistent other members of the SGLT2 drug class and PD-related osmotic diuresis. It is noted that hypercalciuria correlates with decreases in serum PTH levels, which were reported in 31% to 40% of animals at 100 mg/kg (PTH was not assessed at ≤25 mg/kg). Low levels of PTH have been associated with activation of osteoblast activity and inhibition of osteoclast activity, which are consistent with increases in mean serum phosphorous concentrations and increases in bone at 100 mg/kg. Together, these findings are consistent with an adaptive response to increased Ca^{++} absorption secondary to decreases in gut pH due to off-target SGLT1 inhibition and sugar fermentation.

PD-related increases in glucosuria correlated with decreases in blood glucose levels at all doses. Lower body weights and weight gains were observed in 100 mg/kg males, despite compensatory increases in food consumption at all doses in both sexes. The changes in body weights and food consumption are consistent with the SGLT2 inhibitor drug class and are considered to be secondary to the PD activity of ertugliflozin.

Drug-related changes in urine were reported at all doses, including PD-related marked glucosuria and increases in urine volume. Increases in specific gravity were also observed at all doses in both males and females. Increases in severity and incidence of ketones correlated with decreases in pH in males at all doses and in females at 25 mg/kg.

Significant reductions in RBC counts in both sexes corresponded with decreases in reticulocyte counts and red cell distribution widths in males at 100 mg/kg, which did not fully resolve by the end of recovery.

Male rats had AUC exposures of 17.6, 128 and 397 μg·h/mL with exposure margins of 13x, 93x and 288x $MRHD_{AUC}$. Female AUC exposures were 26.9, 167 and 814 μg·h/mL with exposure margins of 19x, 121x and 590x $MRHD_{AUC}$. Male C_{max} exposures during study week 26 were 2.49, 12.9, and 38.9 μg/mL. Female C_{max} exposures during study week 26 were 3.62, 17.6, and 63.7 μg/mL.

Methods

Methods:	
Doses:	0 (vehicle), 5, 25, and 100 mg/kg
Frequency of dosing:	Once Daily for 6 Months + 8 Week Recovery
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	A solution of 0.5% (w/v) methylcellulose with 10 % (v/v) polyethylene glycol 400 (PEG 400)
Species/Strain:	Sprague Dawley (Crl:CD[SD]) (b) (4)
Number/Sex/Group:	20/sex/group
Age:	7 Weeks
Weight:	172-246 g (males) and 135-185 g (females)
TK Satellite groups:	4/sex/group
Unique study design:	None
Deviations from study protocol:	Formulation, Concentration verification, Dose administration, Husbandry, Clinical signs, bioanalytical analysis, Clin Path, organ weights and histology

Parameters Measured

Observational endpoints/timing	
Clinical Findings	2x daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Cage side observations (1x daily) 1 hour postdose during the dosing phase and on toxicity animals (1x daily) during the recovery phase, except on Day 15 of the recovery phase and on days when detailed observations were conducted. Detailed observations 1x predose phase, prior to dosing on Day 1 of the dosing phase, weekly (based on Day 1 of the dosing phase) throughout the dosing and recovery phases, and on the day of scheduled sacrifice (for those animals necropsied).
Body weights	1x Predose, 1x Prior to Dosing D1 and Weekly Thereafter
Food consumption	Dosing and Recovery - Weekly
Ophthalmoscopy	1x during the Predose phase and on toxicity animals once during Week 13 and once during the last 7 days of the dosing phase using an indirect ophthalmoscope.
Toxicokinetics	Weeks 1, 13 and 26 – 1,4 ,7 and 24 Hours Post Dose (Not Fasted)
Hematology	Fasted – Week 13 and at scheduled sacrifices
Clinical chemistry	Fasted – Week 13 and at scheduled sacrifices
Urinalysis	Fasted – Week 13 and at scheduled sacrifices
Parathyroid Hormone	Control and High Dose - Fasted – End of Dosing
Gross pathology	Fasted - End of Dosing and Recovery
Organ weights	End of Dosing and Recovery
Histopathology	Standard Battery
	Adequate Battery: yes (X), no ( )
	Peer review: yes (X), no ( )
Other	Bone Marrow Smears (not evaluated)

Observations and Results

Mortality

Two incidental mortalities were reported, 1 female at 25 mg/kg and 1 male at 100 mg/kg; however, there were no drug-related mortalities.

Clinical Signs

An increase in the incidence of alopecia on the front paws of females dosed at 100 mg/kg/day was observed. The increased clinical incidence of alopecia noted on the front paws of females dosed at 100 mg/kg/day was confirmed at necropsy in a small number of animals. No dose-limiting clinical signs were observed at any dose.

Body Weight

Final BW was 12% lower in 100mg/kg males which reached statistical significance. Final BW in all other groups, male and female, tended to be lower but still within 10% of the control groups.

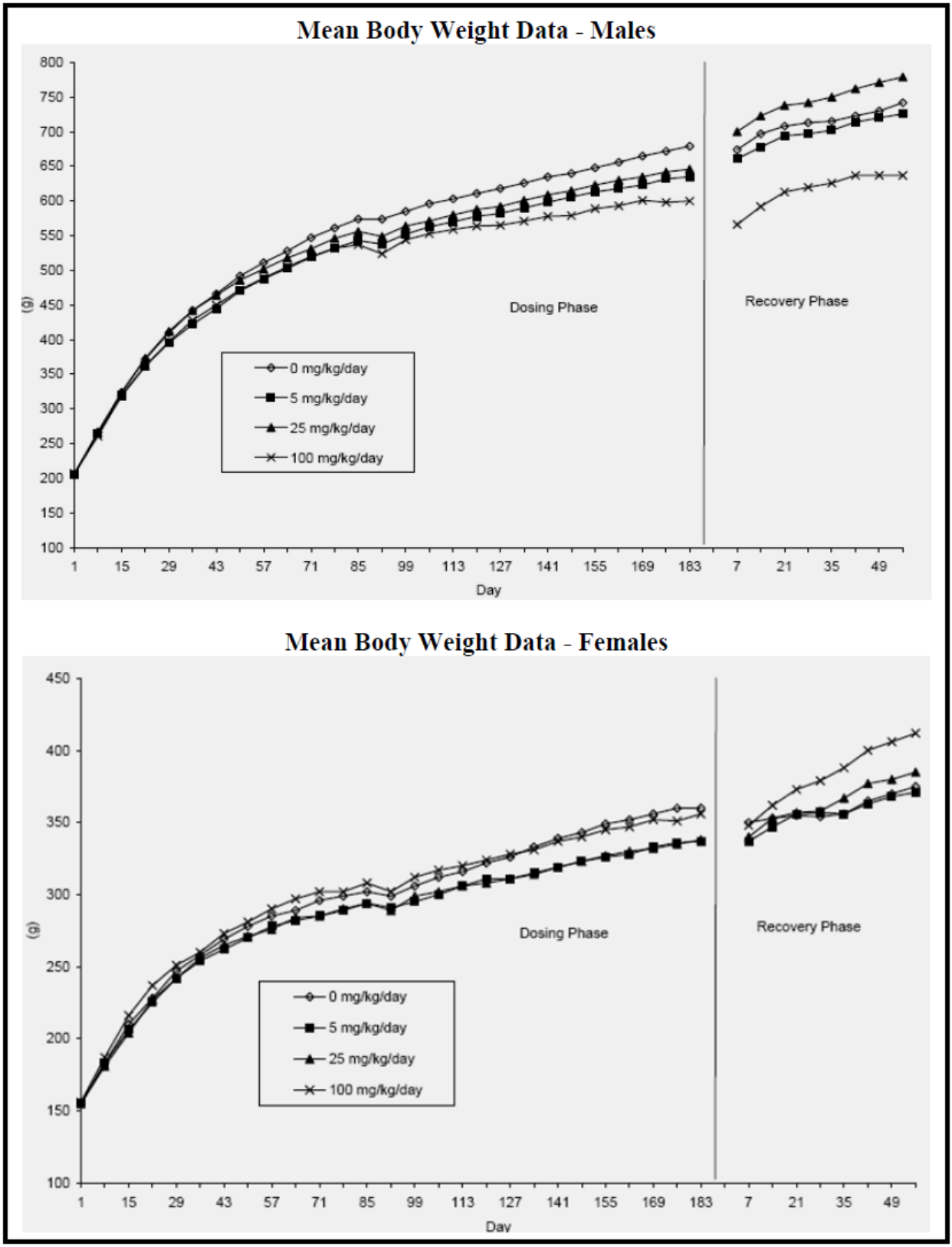
Table 26: Body Weights - 6-month Rat Study #09GR275

Males Body Weight (Dosing)				
Sex	Dose, mg/kg	Starting BW, g	Final BW, g	BW % control
Males	0	205	679	100 %
	5	206	635	93%
	25	206	646	95 %
	100	207	600	88 %*
	(*) $p \leq 0.05$			

Females Body Weight (Dosing)				
Sex	Dose, mg/kg	Starting BW, g	Final BW, g	BW % control
Females	0	155	360	100 %
	5	155	337	94%
	25	155	338	94 %
	100	156	356	99 %
	(*) $p \leq 0.05$			

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Figure 4: Body Weights - 6-month Rat Study #09GR275

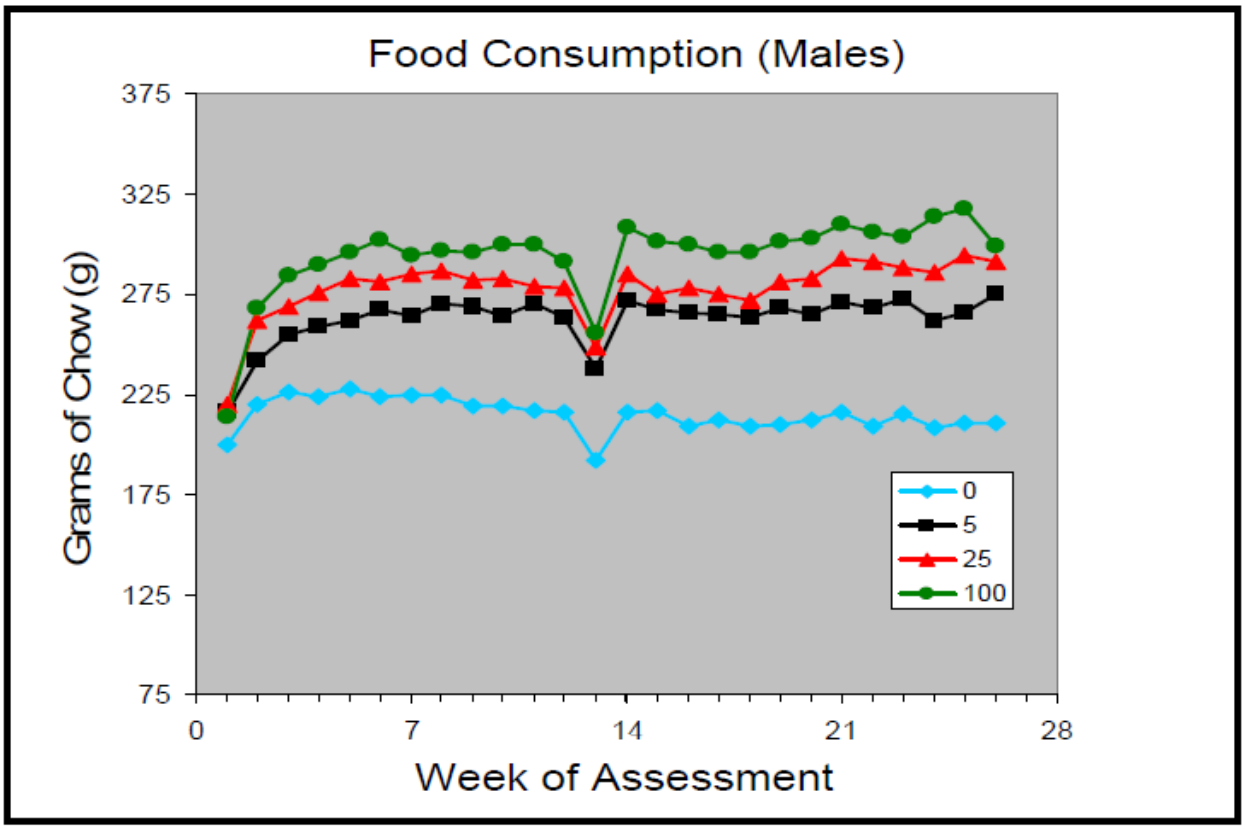


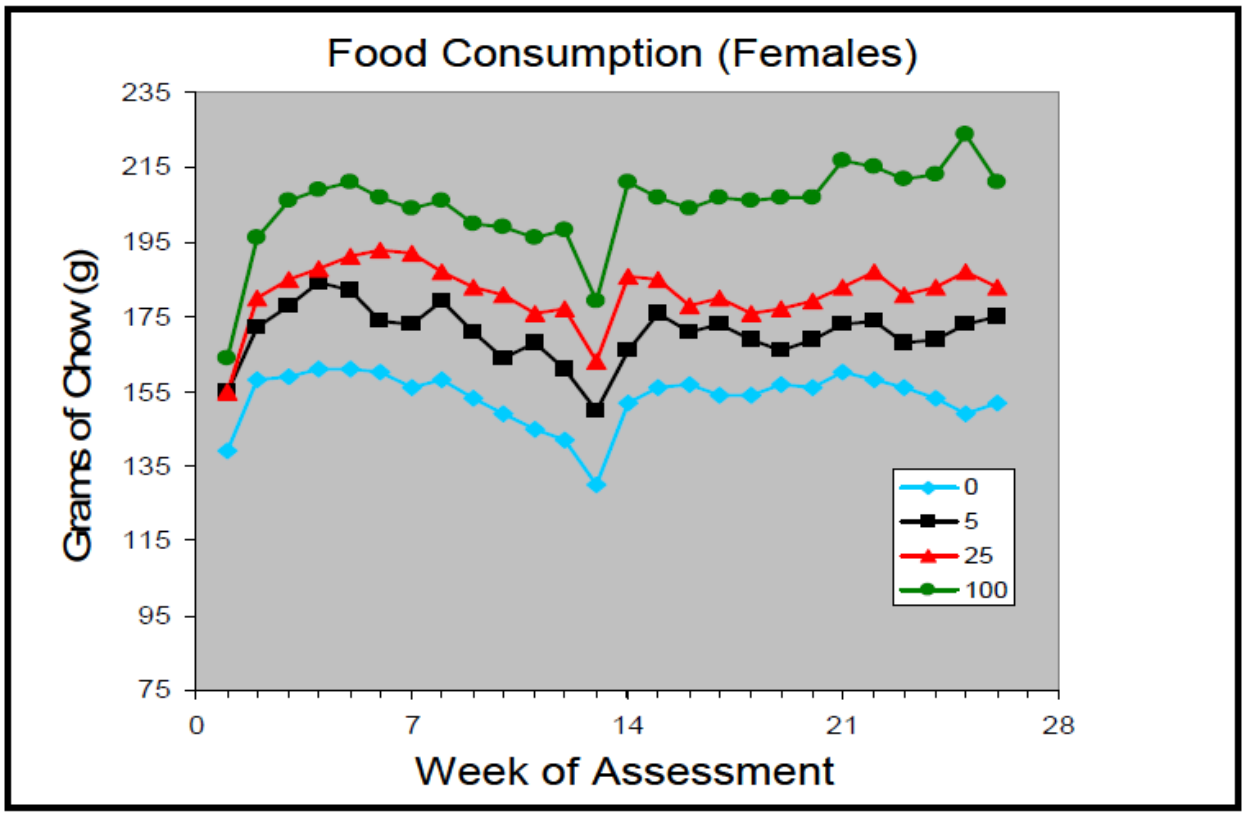
(Figures excerpted from sponsor's package and annotated, reference Dr. Quinn's Pharm/Tox review)

Food Consumption

Significant dose-related increases in food consumption occurred in all male and female dosing groups beginning at Week 1 of the administration phase. The difference was statistically significant during all weeks in females administered ≥ 25 mg/kg and during most weeks for female dosed at 5 mg/kg. At the end of the recovery phase, food consumption in males was not notably different from controls, but remained slightly higher in at ≥25 mg/kg, albeit not significantly different from controls.

Figure 5: Food Consumption - 6-month Rat Study #09GR275





(Figures excerpted from Dr. Quinn's Pharm/Tox review)

Ophthalmoscopy

No visible lesions were noted at the examinations during Weeks 13 and 26 of the dosing phase.

Hematology

Small magnitudes of change occurred in erythrocytic parameters; however, due to the limited number of recovery animals (five/sex/group), reversibility of these toxicities was difficult to assess.

Dose-dependent decreases in RBC counts were observed in males (↓1-4% during weeks 13 and 26) and in females (↓3-4% during week 13) at ≥25 mg/kg. Decreases in reticulocyte counts were observed in males at 100 mg/kg (↓16-19%) during weeks 13 and 26, which tended to remain lower after recovery. Significant changes in reticulocyte counts were not observed in females at any time during this study. Changes in RBC counts were also accompanied by decreases in hemoglobin (↓2-4%) and hematocrit (↓2-5%) in females at ≥25 mg/kg during week 13 and week 26, which tended to remain lower after recovery. Mean corpuscular volumes were elevated in males during week 13 (↑1-2%) and in females during week 26 (↑1%). Mean corpuscular hemoglobin (↑1-5%) and mean corpuscular hemoglobin concentration (↑2-4%) increased significantly during weeks 13 and/or week 26 in males at ≥25 mg/kg, but not in females, and were reversible after recovery. Red cell distribution width declined significantly in males (↓5-9%) at ≥25 mg/kg during weeks 13 and 26, which tended to remain lower after recovery.

A similar trend in red cell distribution width was apparent in females at the end of dosing (↓1-3%).

Platelet counts tended to decline with dose in males at the end of dosing (↓1-7%), which persisted after recovery (↓14 %); whereas, no consistent trend was observed in female platelet counts.

WBC counts tended to decline in males (↓7-29%*) and females (↓1-26%*) at 13 weeks and 26 week, which was reversible after recovery. Lymphocyte counts declined with dose in both males (↓12-37%) and females (↓2-34%) during weeks 13 and 26, which tended to remain lower after recovery. Eosinophil counts tended to decline with dose in both males (↓8-31%) and females (↓33 %) at the end of the dosing period. Basophil counts were lower in males (↓20-60%) and females (↓33-67%) during weeks 13 and 26. A significant decline in leukocyte counts was observed in males (↓33 %*) at 100mg/kg during week 13, but not in females.

Coagulation

Prothrombin times tended to decrease (↓0.3-1.1 seconds) in recovery females previously exposed to PF04971729, but was not observed in females at the end of dosing or in males at any time during this study.

Clinical Chemistry

The most prominent drug-related effect on serum clinical chemistry was lower serum glucose levels, consistent with the pharmacologic activity of PF04971729. Significant decreases in serum glucose levels were observed in both males (↓31-49%) and females (↓9-42%) at all doses during weeks 13 and 26, which remained lower in 100 mg/kg recovery males (↓18%) and females (↓16%).

BUN levels increased significantly with dose in both males (↑69-154%) and females (↑33-140%) at all doses during weeks 13 and 26.

A significant decline in serum creatinine was observed in both males (↓14%) and females (↓25%) at 100 mg/kg during week 26.

Serum cholesterol levels were significantly decreased in males at 100 mg/kg for 13 weeks (↓22%) and 26 weeks (↓26%), remained lower in100 mg/kg recovery males (↓29%). Effects on serum cholesterol and total protein are consistent with secondary changes in lipid and protein metabolism.

Dose-dependent decreases in serum total protein were observed in both males (↓15%) and females (↓1-7%) during weeks 13 and 26. Serum globulin levels decreased with dose in males (↓6-11%) and at 100 mg/kg in females (↓7-8%) during weeks 13 and 26 weeks. The albumin-globulin ratio was increased significantly in males at 100 mg/kg during week 13 only (↑7%).

Serum total bilirubin declined significantly at ≥25 mg/kg in males during week 13 and in females during week 26, which tended to remain lower in recovery females.

A significant increase in AST was observed in males during week 13 (↑24%) at the 100 mg/kg dose and at all doses during week 26 (↑24-39%); whereas significant increases were only observed in females during week 13 (↑26%) at 100 mg/kg. Serum ALT was increased dose-dependently in males during weeks 13 (↑24-32%) and 26 (↑5-21%), but not in females. Dose-dependent increases in serum ALP were observed in males during week 13 (↑5-16%), and in females during weeks 13 (↑2-14) and 26 (↑8-20%).

Significant decreases in serum calcium were observed in males (↓4-6%) and females (↓2-5%) during weeks 13 and 26, with dose-dependency in males.

Table 27: Clinical Chemistry - 6-month Rat Study #09GR275

Males			Females		
	Ca mg/dL			Ca mg/dL	
DSNG 86	DSNG 184	RECO 57	DSNG 86	DSNG 184	RECO 57
11.4	11.2	10.9	11.8	11.4	11.1
0.50	0.36	0.37	0.47	0.44	0.43
20	20	5	20	20	5
10.9*	10.7*	10.9	11.4	11.2*	11.2
0.29	0.30	0.58	0.54	0.40	0.15
20	20	5	20	20	5
10.9*	10.5*	11.0	11.4*	11.1*	11.3
0.36	0.30	0.40	0.52	0.26	0.39
20	20	5	19	19	5
10.8*	10.5*	10.8	11.3*	10.8*	11.2
0.30	0.31	0.35	0.41	0.40	0.54
20	19	5	20	20	5
P	P	P	P	P	P

(Table excerpted from sponsor's package and annotated, reference Dr. Quinn's Pharm/Tox review)

Serum phosphorous levels were significantly elevated in females at 100 mg/kg during week 13 (↑12%) and in both males (↑13%) and females (↑17%) during week 26.

Intermittent decreases in serum sodium levels were observed in both males and females, but never exceeded decreases on more than 2%.

Dose-dependent decreases in serum potassium levels were observed in males (↓2-13%) and females (↓2-15%) during week 13, with significant hypokalemia present in males (↓7-13%) during week 26, which was fully reversible after recovery.

Serum chloride levels tended to decrease in males during weeks 13 (↓2-6%) and 26 (↓3-5%). Significant decreases in serum chloride levels were observed in females at all doses during week 13 (↓2-5%) and at the 100 mg/kg dose during week 26 (↓1%).

Parathyroid Hormone Analysis

Mean serum parathyroid hormone levels were reduced in both sexes at 100 mg/kg PF04971729, although the significance of these changes is difficult to assess because of the variable range of measurements taken within each group. Parathyroid hormone levels decreased in males (↓31%) and females (↓42%) at the end of the administration period. Low levels of PTH are known to lower serum calcium (activation of osteoblast and inhibition of osteoclast activity) and increase serum phosphorus concentrations. The decline PTH levels provides a mechanism of action for the increased trabecular bone (males) and bone hyperplasia (females) observed in rats dosed at 100 mg/kg.

Urinalysis

Significant increases in urine glucose concentrations were observed at all doses in males (↑343-fold to ↑451-fold) and females (↑388-fold to ↑426-fold) at the end of the administration period.

Urine creatinine levels decreased with dose in males (↓54-78%) and females (↓45-74%), which correlated in increases in urine glucose: urine creatine in males (↑1257-fold to ↑1951-fold) and females (↑572-fold to ↑1101-fold).

Total urine protein concentrations decreased with dose in males (↓41-65%), but not in females. However, urine protein: creatine ratios increased with dose in both males (↑33-55%) and females (↑40-247%), which remained notably elevated in males (↑293%) and females (↑964%) in recovery animals at 100 mg/kg.

Urine inorganic phosphorous concentrations decreased with dose in males (↓3-33%) at the end of dosing, but increased with dose in females (↑32-75%) after the recovery period. However, total phosphorous excretion increased with dose at the end of the administration period in both males (↑154-270%) and females (↑81-786%).

Urine sodium concentrations decreased with dose in males (↓11-30%) at the end of the administration period; whereas total sodium excretion increased with dose in males (↑124-288%) and females (↑53-253%).

Urine potassium concentrations declined with dose in males (↓32-56%) and females (↓33-44%) at the end of the administration period; whereas total potassium excretion increased with dose in males (↑65-127%) and females (↑57-248%) at the end dosing, which remained higher in recovery females (↑18-67%).

Urine chloride concentrations declined with dose in males (↓35-54%) and females (↓22-42%) at the end of the administration period; whereas total chloride excretion increased with dose in males (↑95-195%) and females (↑57-179%) at the end of the administration period.

No clear drug-related changes in urine calcium levels were noted, although total calcium excretion increased with dose in both males (↑152-674%) and females (↑104-322%) at the end of dosing.

Table 28: Urine Calcium Excretion - 6-month Rat Study #09GR275

Males

Group/ Sex		CaX mg DGNG 184	CaX mg RECO 57
1M	Mean	0.27	0.30
	SD	0.117	0.085
	N	20	5
2M	Mean	0.69*	0.54
	SD	0.407	0.265
	N	20	5
3M	Mean	0.79*	0.47
	SD	0.628	0.254
	N	19	5
4M	Mean	2.09*	0.33
	SD	1.526	0.187
	N	19	5

Females

Group/ Sex		CaX mg DGNG 184	CaX mg RECO 57
1F	Mean	0.73	0.81
	SD	0.701	0.843
	N	20	5
2F	Mean	1.49*	0.96
	SD	1.002	0.831
	N	20	5
3F	Mean	1.82*	0.97
	SD	1.271	0.349
	N	19	5
4F	Mean	3.09*	0.76
	SD	1.264	0.407
	N	20	5

(Table excerpted from sponsor's package and annotated, reference Dr. Quinn's Pharm/Tox review)

Urine volume was significantly increased with dose during week 13 (Day 86) in males (↑25-123%) and females (↑24-149%) and at the end of the dosing period (Day 184) in males (↑72-233%) and females (↑83-374%).

Statistically significant increases in urine specific gravity were observed at all doses in males and females during the dosing period, but were independent of dose. After the recovery period, specific gravity parameters in all dose groups were comparable to controls.

Statistically significant decreases in urine pH were observed in both males (↓6-9%) and females (↓5-8%) at ≥25 mg/kg, but were comparable to controls after recovery.

Table 29: Urine Parameters - 6-month Rat Study #09GR275

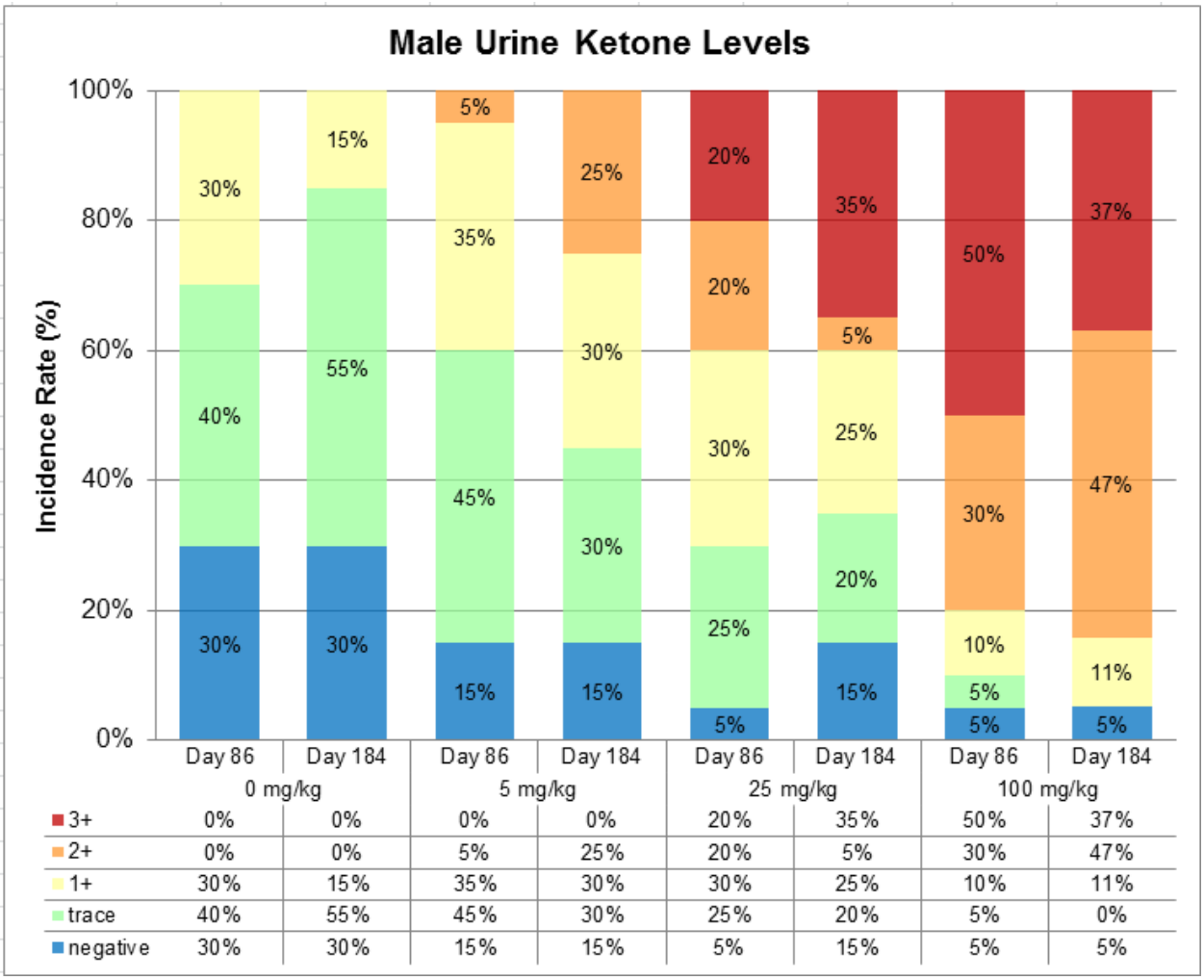
Males Urine Parameters (n=20)						
Dose (mg/kg)	**Specific Gravity**			**pH**		
	Day 86	Day 184	RDay 57	Day 86	Day 184	RDay 57
0	1.033	1.036	1.042	6.7	6.7	6.3

5	1.053* (↑1.9%)	1.056* (↑1.9%)	1.022	6.4 (↓4.5%)	6.4 (↓4.5%)	6.8
25	1.047 (↑1.4%)	1.047* (↑1.1%)	1.039	6.3* (↓6.0%)	6.2* (↓7.5%)	6.5
100	1.048* (↑1.5%)	1.043 (↑0.7%)	1.028	6.1* (↓9.0%)	6.1* (↓9.0%)	6.7
Females Urine Parameters (n=20)						
Dose (mg/kg)	**Specific Gravity**			**pH**		
	Day 86	Day 184	RDay 57	Day 86	Day 184	RDay 57
0	1.030	1.033	1.031	6.5	6.4	6.2
5	1.048* (↑1.7%)	1.056* (↑2.2%)	1.028	6.3 (↓3.1%)	6.4	6.5
25	1.056* (↑2.5%)	1.047* (↑1.4%)	1.025	6.0* (↓7.7%)	6.1* (↓4.7%)	6.4
100	1.048* (↑1.7%)	1.047* (↑1.4%)	1.036	6.0* (↓7.7%)	5.9* (↓7.8%)	6.4

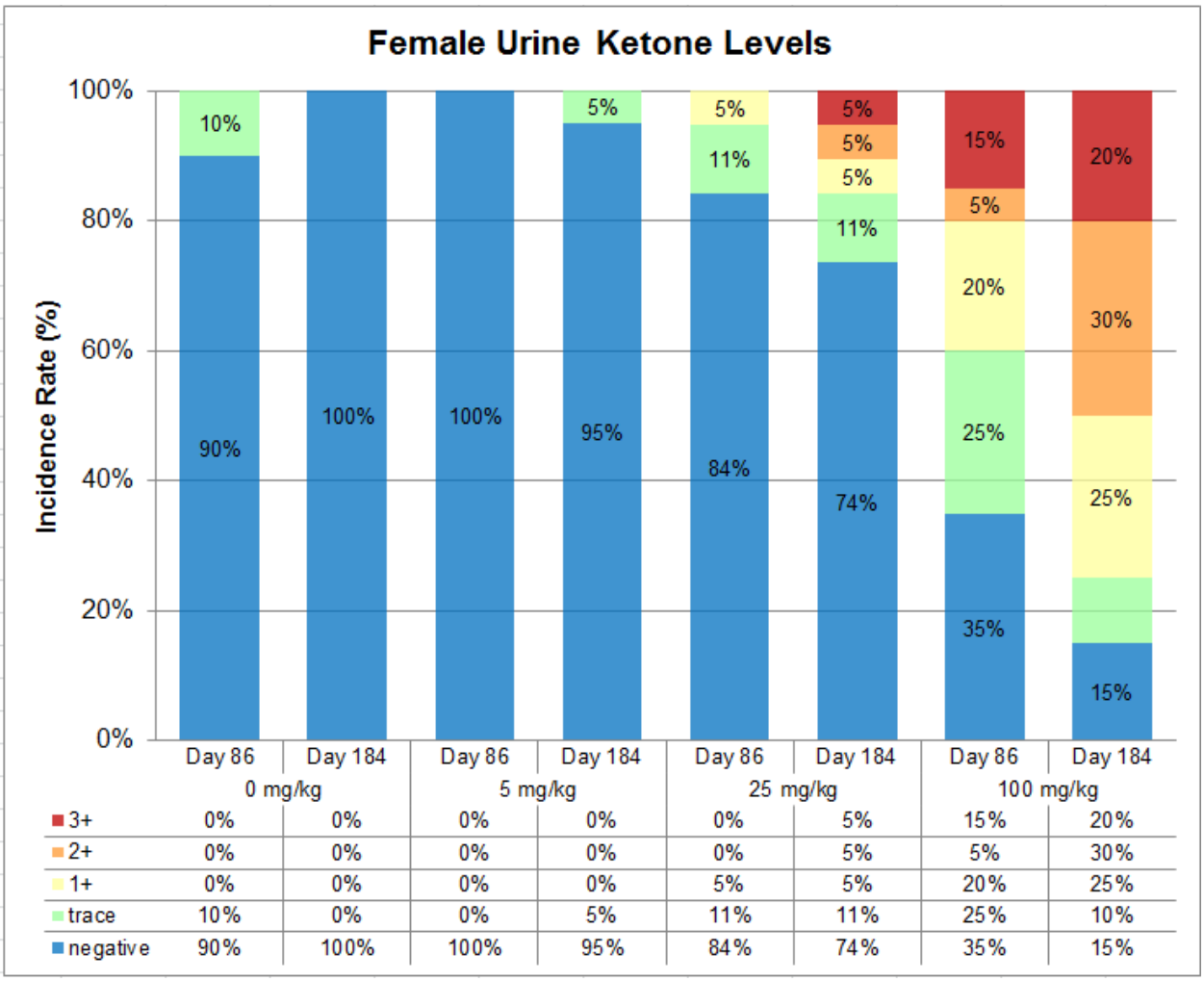
* p value < 0.05

The presence of urine ketone bodies increased with dose and progressed with continued dosing in males at all doses and in females at ≥25 mg/kg. Increased incidences of animals with moderate to marked urine ketone levels of ≥40 mg/dL (2+ and 3+) were observed in males at all doses and in females at ≥25 mg/kg, reaching up to 80% of males and 50% of females at 100 mg/kg by the end of the dosing period. Incidences of marked urine ketone levels of ≥80 mg/dL (3+) were reported at ≥25 mg/kg in 20-50% of males and 5-20% of females. After the recovery period, urine ketone levels were comparable to controls in both males and females, indicating full reversibility.

Figure 6: Urine Ketone Levels - 6-month Rat Study #09GR275



	0 mg/kg Day 86	0 mg/kg Day 184	5 mg/kg Day 86	5 mg/kg Day 184	25 mg/kg Day 86	25 mg/kg Day 184	100 mg/kg Day 86	100 mg/kg Day 184
3+	0%	0%	0%	0%	20%	35%	50%	37%
2+	0%	0%	5%	25%	20%	5%	30%	47%
1+	30%	15%	35%	30%	30%	25%	10%	11%
trace	40%	55%	45%	30%	25%	20%	5%	0%
negative	30%	30%	15%	15%	5%	15%	5%	5%



Gross Pathology

Macroscopic observations of interest that were uniquely noted in dosed animals at the end of the administration period included: 3 males with large renal pelvises, 4 males and 1 female with distended urinary bladders, 1 male with fluid in the urinary bladder, and discolored stomachs in 7 males and 11 females. Changes in the kidneys and urinary bladder were consistent with the changes noted microscopically and those of higher urine volumes described above. Twenty brown, black, red, and/or white discolored foci were noted in the glandular stomachs at the end of the administration phase in 18 dosed animals. Four brown, two black, and a single red foci corresponded to erosions/ulcers microscopically that were observed with an increased incidence in females at the 100 mg/kg dose. One brown and three red foci corresponded microscopically to congestion. The remaining foci had no corresponding microscopic lesions.

An increased incidence of mammary cysts was observed grossly in recovery females at ≥25 mg/kg, but was not reported in females evaluated at the end of the dosing period. Nevertheless, this observation correlated with an increased incidence of cystic dilatation and hyperplasia/hypertrophy of the breast glandular epithelium at 100 mg/kg.

Table 30: Macroscopic Findings - 6-month Rat Study #09GR275

GROSS OBSERVATIONS – MALES – END OF DOSING						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	15	15	15	15
Kidney	Enlarged		0	0	2	1
Urinary Bladder	Distended		0	1	1	2
	Fluid		0	0	1	0
Stomach	Discolored		0	1	4	2
Jejunum	Discolored		0	0	1	0
Prostate	Raised Area		0	1	0	0
Joint	Enlarged		0	0	0	1

GROSS OBSERVATIONS – FEMALES – END OF DOSING						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	15	15	15	15
Urinary Bladder	Distended		0	1	0	0
Stomach	Discolored		0	0	5	6

GROSS OBSERVATIONS – FEMALES - RECOVERY						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	5	5	5	5
Mammary	Cyst		0	0	1	2

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Organ Weights

Statistically significant and dose-related increases in absolute kidney weights were observed in males at ≥5 mg/kg/day and females at 100 mg/kg/day, which tended to remain higher in females after recovery.

Relative adrenal gland weights of males were significantly increased at 100 mg/kg/day (↑22%).

Table 31: Organ Weights - 6-month Rat Study #09GR275

KIDNEY WEIGHTS – TERMINAL SACRIFICE						
	Male			Female		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑18*	↑30*	↑19*	↑8	↑16*	↑9
25	↑22*	↑35*	↑27*	↑9	↑19*	↑8
100	↑31*	↑50*	↑33*	↑27*	↑34*	↑26*
(*) Significant Change						

FEMALE ORGAN WEIGHTS – RECOVERY SACRIFICE						
	Kidney			Spleen		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑4	↑6	-	↑3	↑6	-
25	↑11	↑9	↑8	↑15	↑13	↑12
100	↑31	↑20	↑28	↑28	↑19	↑25
(*) Significant Change						

ADRENAL WEIGHTS – TERMINAL SACRIFICE			
	Male		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑9	↑19	↑9
25	↑3	↑13	↑7
100	↑22	↑39*	↑24*
(*) Significant Change			

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Histopathology

Microscopic findings were noted in the kidneys, adrenal glands, pancreas, bone, and stomach of dosed animals. PF04971729-related findings with the exception of tubular mineralization in the kidney and increased trabecular bone in the sternum, resolved in recovery animals.

Dose-dependent increases in incidences of kidney findings in males at ≥25 mg/kg included hypertrophy of proximal tubule epithelium characterized by an increase in size and eosinophilia of the epithelium, with smaller nuclei more luminal in location. Drug-related increases in the incidence and severity of tubular mineralization were reported in males at all doses, which persisted in recovery males at 100 mg/kg.

Single incidences of slight to moderate mixed cell inflammation were present in the renal pelvis of females at all doses, with severity increasing with dose. Minimal to slight inflammation in the pelvis were also present in the kidneys of 3/5 recovery females at 100 mg/kg/day and 1 recovery female dosed at 25 mg/kg/day, which correlated with transitional cell hyperplasia in some cases.

Table 32: Kidney Microscopic Findings - 6-month Rat Study #09GR275

RENAL HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Kidney	Hypertrophy, Epithelium, Proximal Tubule		0	0	9*	13 (10*/3@)
	Mineralization, Tubule		4*	5*	8*	13 (11*/2@)
	Hyperplasia, Tubular, Focal		0	0	0	1@
	Dilatation, Pelvis		0	0	2P	1P
(P) Present (*) Minimal (@) Slight ($) Moderate						

RENAL HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Kidney	Inflammation, Pelvis, Mixed Cells		0	1@	1@	1$
	Hyperplasia, Transitional Cells		0	0	1*	1*
	Degeneration/Necrosis, Tubule		0	0	0	1*
	Dilatation, Tubule(s), Random		1*	0	1@	4 (1*/3@)
	Edema		0	0	0	1@
(P) Present (*) Minimal (@) Slight ($) Moderate						

RENAL HISTOPATHOLOGY – MALES – END OF RECOVERY						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	5	5	5	5
Kidney	Mineralization, Tubule		1*	2*	5*	5*
(P) Present (*) Minimal (@) Slight ($) Moderate						

RENAL HISTOPATHOLOGY – FEMALES – END OF RECOVERY						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	5	5	5	5
Kidney	Mineralization, Tubule		1*	0	1*	4*
	Inflammation, Pelvis, Mixed Cells		0	0	1*	3 (1*/2@)
	Hyperplasia, Transitional Cells		0	0	0	2@
(P) Present (*) Minimal (@) Slight ($) Moderate						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Single incidences of urinary bladder inflammation and transitional cell hyperplasia were observed at all doses, either at the end of dosing or after recovery, in females. However, there was not dose-dependency or consistency between end of dosing and recovery findings.

Table 33: Urinary Bladder Microscopic Findings- 6-month Rat Study #09GR275

URINARY BLADDER HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Urinary Bladder	Inflammation		0	1@	1@	0
	Hyperplasia, Transitional Cell		0	1@	1@	0
(P) Present (*) Minimal (@) Slight ($) Moderate						

URINARY BLADDER/ URETER HISTOPATHOLOGY – FEMALES – END OF RECOVERY						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	5	5	5	5
Urinary Bladder	Inflammation		0	0	0	1@
	Hyperplasia, Transitional Cell		0	0	0	1@
Ureter	Inflammation		0	0	0	1*
(P) Present (*) Minimal (@) Slight ($) Moderate						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Dose-dependent increases in incidences of minimal hypertrophy and vacuolation of the adrenal gland zona glomerulosa were present in males and females at all doses.

Table 34: Adrenal Gland Microscopic Findings- 6-month Rat Study #09GR275

ADRENAL GLAND HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Adrenal Cortex	Hypertrophy/Vacuolation, Zona Glomerulosa		0	9*	12*	14*
(P) Present (*) Minimal (@) Slight ($) Moderate						

ADRENAL GLAND HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Adrenal Cortex	Hypertrophy/Vacuolation, Zona Glomerulosa		0	7*	9*	14*
	Vacuolation, Zona Fasciculata		0	0	0	1*
(P) Present (*) Minimal (@) Slight ($) Moderate						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Dose-dependent increases in incidences of decreased in pancreatic zymogen granules were present in both males and females at the end of the dosing period.

Table 35: Pancreas Microscopic Findings- 6-month Rat Study #09GR275

PANCREAS HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Pancreas	Decreased Zymogen Granules		2*	4*	6*	8*
(P) Present (*) Minimal (@) Slight ($) Moderate						

PANCREAS HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Pancreas	Decreased Zymogen Granules		0*	1*	5*	8*
(P) Present (*) Minimal (@) Slight ($) Moderate						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Increases in digestive tract microscopic findings were observed in males at all doses and in females at ≥25 mg/kg. Findings of stomach erosions/ulcers corresponded to macroscopic findings of brown/black discoloration and were present in 1 to 2 males in each dose group, 1 female at 25 mg/kg, and 5 (33%) females at 100 mg/kg. Findings of minimal hyperplasia of the glandular stomach foveolar layer were noted in one male and four females at 100 mg/kg. Minimal to slight degeneration of the crypts in the pylorus of the glandular stomach were noted with an increased incidence and severity at ≥25 mg/kg in both males and females. All stomach findings resolved after recovery.

Table 36: Gastro-intestinal Microscopic Findings- 6-month Rat Study #09GR275

GASTRO-INTESTINAL HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Stomach	Hyperplasia, Foveolar Layer		0	0	0	1*
	Degeneration, Crypts, Pylorus		1*	0	5 (4*/1@)	5*
	Erosion/Ulcer		0	1*	2 (1*/1@)	1*
	Inflammation		0	0	0	2
	Dilatation, Gland		1*	1*	2 (1*/1@)	2*
	Hemorrhage		0	0	0	1@
(P) Present (*) Minimal (@) Slight ($) Moderate						

GASTRO-INTESTINAL HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Stomach	Hyperplasia, Foveolar Layer		0	0	0	4*
	Degeneration, Crypts, Pylorus		2*	2*	3 (2*/1@)	8*
	Erosion/Ulcer		0	0	1@	5 (1*/4@)
	Dilatation, Gland		2*	0	2*	4*
(P) Present (*) Minimal (@) Slight ($) Moderate						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Microscopic bone findings including minimal to slight increases in sternal and femur trabecular bone in 86% and 21% of males and hyperplasia of the physis in a single female were reported at 100 mg/kg. After recovery, femur findings had resolved and sternum partially resolved in males, occurring 2 (40%) recovery males.

Table 37: Bone Microscopic Findings- 6-month Rat Study #09GR275

BONE HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Bone Femur	Trabecular Bone, Increased		0	0	0	3 (2*/1@)
Bone Sternum	Trabecular Bone, Increased		1*	1*	1*	12 (11*/1@)
(P) Present (*) Minimal (@) Slight ($) Moderate						

BONE HISTOPATHOLOGY – MALES – END OF RECOVERY						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	5	5	5	5
Bone Sternum	Trabecular Bone, Increased		0	0	0	2*
(P) Present (*) Minimal (@) Slight ($) Moderate						

BONE HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Bone Femur	Hyperplasia, Physis		0	0	0	1*
(P) Present (*) Minimal (@) Slight ($) Moderate						

(Tables excerpted from Dr. Quinn's Pharm/Tox review)

Low incidences of epididymal microscopic findings were noted at 100 mg/kg, an increased incidence (3/15) of mononuclear infiltrates into the epididymal interstitium at the end of dosing and cellular debris of the epididymal lumen and hypospermia in a single recovery male.

Toxicokinetics

Systemic exposures increased approximately dose-proportionally. C_{max} and AUC_{0-24} exposures in females were consistently higher than males at all doses and on all study days. T_{max} was achieved between 3 and 6 hours postdose during Week 1 and between 1 and 3 hours postdose during Week 26, indicating a shorter latency with increased dosing duration. Accumulation of ~2-fold was evident in females during Week 26 at 100 mg/kg.

Table 38: Toxicokinetics - 6-month Rat Study #09GR275

Table 1. Mean Toxicokinetic Parameters for PF-04971729 in Rats after Oral Administration of PF-04971729 on Study Weeks 1, 13, and 26

Dose (mg/kg/ day)	Study Week	Gender	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
5	1	Male	1.56	0.133	4	2.50	1.73	4	19.7	3.19	4
		Female	2.15	0.378	4	3.25	1.50	4	26.6	5.62	4
		Overall	1.86	0.410	8	2.88	1.55	8	23.2	5.61	8
	13	Male	1.70	0.294	4	2.50	1.73	4	17.4	4.79	4
		Female	3.66	0.791	4	1.00	0.00	4	25.3	6.06	4
		Overall	2.68	1.18	8	1.75	1.39	8	21.3	6.57	8
	26	Male	2.49	0.647	4	1.00	0.00	4	17.6	3.84	4
		Female	3.62	0.360	4	1.75	1.50	4	26.9	5.06	4
		Overall	3.06	0.776	8	1.38	1.06	8	22.3	6.46	8
25	1	Male	8.99	1.69	4	5.50	1.73	4	123	32.5	4
		Female	11.0	0.512	4	5.50	1.73	4	147	12.8	4
		Overall	10.0	1.59	8	5.50	1.60	8	135	26.2	8
	13	Male	8.61	1.32	4	4.75	2.87	4	120	19.4	4
		Female	11.3	2.04	4	4.75	1.50	4	147	19.4	4
		Overall	9.97	2.15	8	4.75	2.12	8	134	23.2	8
	26	Male	12.9	3.36	4	2.50	1.73	4	128	31.9	4
		Female	17.6	7.20	4	2.50	1.73	4	167	38.1	4
		Overall	15.2	5.77	8	2.50	1.60	8	148	38.4	8
100	1	Male	26.4	6.84	4	5.50	1.73	4	359	98.0	4
		Female	35.4	5.31	4	6.25	1.50	4	440	173	4
		Overall	30.9	7.41	8	5.88	1.55	8	400	137	8
	13	Male	29.9	5.83	4	3.25	1.50	4	372	24.2	4
		Female	47.7	7.40	4	3.25	2.87	4	612	16.2	4
		Overall	38.8	11.3	8	3.25	2.12	8	492	129	8
	26	Male	38.9	6.42	4	1.00	0.00	4	397	26.6	4
		Female	63.7	10.9	4	2.50	3.00	4	814	113	4
		Overall	51.3	15.6	8	1.75	2.12	8	605	235	8

Overall = Male plus Female combined

(Table excerpted sponsor's package and annotated, reference Dr. Quinn's review #4)

Dosing Formulation Analysis

Stability and homogeneity of prepared doses are reported to be within acceptable specifications. No quantifiable concentrations of PF04971729 were found in plasma samples collected from control animals on Study weeks 1, 13, and 26 with the exception of 1 sample (animal B69659), but this finding does not impact the interpretation of the toxicokinetic data or study integrity.

Study: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Rats (Study TT#13-7809 / 13GR318)

Study #	#8293562 / 13GR318
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	12/02/2013
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729, Lot #705847-91-10, 73.5% purity

Key Study Findings

- NOAEL = 5 mg/kg (♂ = 18x $MRHD_{AUC}$, ♀ = 24x $MRHD_{AUC}$)
  - Adverse drug-related findings in the digestive tract at 25 mg/kg, as well as increased kidney findings, consistent with findings in the 6-month rat study
- The safety profile of the ertugliflozin formulation synthesized by the proposed commercial method was successfully bridged to the safety profile of the previous ertugliflozin formulation
- Impurities (b) (4) were qualified with acceptable margins of safety for a 15 mg/day clinical dose at specifications of NMT (b) (4) %, respectively.
  - Impurity safety margins at the NOAEL of 5 mg/kg and high dose (HD) of 25 mg/kg:
    - (b) (4)

Ertugliflozin (Commercial Formulation)		
Rat, 13-Weeks	**NOAEL (AUC)**	**Multiple of MRHD**
25 mg/kg: Stomach hemorrhage & erosion/ulcer, intestinal tract hemorrhage, and kidney pelvic/tubule dilatations with ↑BUN (↑2-fold), pancreatic acinar atrophy	**5 mg/kg** (♂&♀ = 28.8 µg·h/mL, ♂ = 25.2 µg·h/mL, ♀ = 32.6 µg·h/mL)	**21x** (♂ = 18x, ♀ = 24x)

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 µg·h/mL and C_{max} = 266 ng/mL

Methods

SD rats were administered 0 (0.5% MS/10% PEG 400 vehicle), 1, 5, or 25 mg/kg PF-04971729 via oral gavage once daily for 13 weeks. Main study animals (10/sex/group) were evaluated for mortality, clinical signs, body weights, food consumption, gross pathology, clinical pathology, and histopathology. Toxicokinetic parameters were evaluated in satellite TK animals (6/sex/group). Concentrations of ertugliflozin and the impurity (b) (4) were verified in all dosing formulations administered on Day 90 using a validated HPLC method. Dosing formulations were also assessed for stability and homogeneity.

Results

The NOAEL was set at 5 mg/kg (18x $MRHD_{AUC}$ in males, 24x $MRHD_{AUC}$ in females) based on adverse drug-related findings in the digestive tract and increased kidney findings at 25 mg/kg (76x $MRHD_{AUC}$ in males, 107x $MRHD_{AUC}$ in females).

Adverse drug-related effects of the digestive tract were observed at 25 mg/kg. Adverse stomach findings included mucosa discolorations, hemorrhage, hyperplasia/hyperkeratosis, erosions, and ulcers. In the intestinal tract, cecum hemorrhage of moderate severity was reported in one 25 mg/kg male. These findings are consistent with potential off-target inhibition of SGLT1, which can lead to fermentation of unabsorbed glucose in the small and large intestine and subsequent trophic dilatation and villous changes. In addition, these intestinal findings were predominantly of minimal severity and were not associated with any adverse effects, such as degeneration or hyperplasia. Thus, the drug-related intestinal findings of dilatation and increased villi/mucosa height are considered to be consistent with findings in the 6-month rat toxicology study and are likely secondary to off-target SGLT1 inhibition in rats.

Kidney size increases, increased organ weights, and microscopic findings of pelvis and tubule dilatations are consistent with PD-related osmotic diuresis. Significant increases in incidence rate and severity of kidney findings were associated with increases in BUN levels above the upper limit of normal (ULN) at 25 mg/kg. Although minor dug-related increases in BUN levels at ≤5 mg/kg may be related to dehydration secondary to PD-related osmotic diuresis; the significant increase in BUN elevation at 25 mg/kg, with means reaching elevations up to 2-fold in males, may be partly attributable to drug-related kidney toxicity. Thus, drug-related kidney toxicity could not be ruled out at 25 mg/kg and the kidney NOAEL was set at 5 mg/kg.

Drug-related changes in urine were reported at all doses, including PD-related marked glucosuria and increases in urine volume. Increases in specific gravity (↑2-4%) were also observed at all doses in both males and females. Increases in severity and incidence of ketones correlated with decreases in pH (↓3-9%) in males at all doses and in females at 25 mg/kg, which are consistent with findings in the 6-month rat study.

Drug-related acinus atrophy at 25 mg/kg was likely secondary to zymogen depletion of the acinar cells pancreas. However, there were no indications of pancreatic tissue damage or dysfunction. Furthermore, decreases in pancreatic zymogen are likely to be

secondary to PD-related increases in food consumption. Overall, the pancreatic findings are considered likely to be non-adverse.

Drug-related reductions in weight gains inversely correlated with increases in food consumption are considered to be due to a class-related compensatory response to PD-related increases in glucosuria.

Other drug-related changes in clinical chemistry parameters, including decreases in blood glucose, cholesterol, and/or electrolyte levels, but were considered likely to be related to the PD activity of ertugliflozin and non-adverse.

Hypertrophy of the adrenal cortex zona glomerulus is consistent with findings in other rat studies and stimulation of the renin angiotensin system, which is likely to be an adaptive response to PD-related glucose diuresis. Thus, this finding is considered to be non-adverse.

Systemic exposures increased approximately dose-proportionally. C_{max} and AUC exposures were generally higher in females compared to males by as much as ~2-fold. T_{max} was achieved at 4 hours post-dose in males on Day 1, but at 1 hour postdose on Day 90. Accumulation of ~2-fold was evident in both males and females on Day 90.

Table 39: Toxicokinetics - 13-Week Rat Study #13GR318

Dose (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
1	1	Male	373	4	3540
		Female	468	4	5830
		Overall	420	4	4680
	90	Male	522	1	3880
		Female	1090	1	7350
		Overall	807	1	5610
5	1	Male	1880	4	24400
		Female	2530	4	26400
		Overall	2210	4	25400
	90	Male	4160	1	25200
		Female	4120	4	32600
		Overall	3930	1	28800
25	1	Male	8100	4	93900
		Female	11300	4	147000
		Overall	9680	4	120000
	90	Male	16000	1	105000
		Female	21300	1	190000
		Overall	18600	1	147000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed.

(Table excerpted from sponsor's package)

Concentrations of PF-04971729 and the process-related impurity [redacted] (b) (4) were validated in dosing formulations for homogeneity and stability. HPLC chromatograms indicate that the percent peak area of mean concentrations of [redacted] (b) (4) in dosing formulations ranged between [redacted] (b) (4) % relative to ertugliflozin, which is similar to the relative percentage of [redacted] (b) (4) % [redacted] (b) (4) reported in the certificate of analysis of the starting material. The impurities [redacted] (b) (4) were not evaluated in dosing formulations; however, the certificate of analysis indicates that they

were present in the ertugliflozin starting material at relative concentrations of [redacted] and [redacted], respectively.

Reviewer's Comments

Overall, the safety margins at the NOAEL for males (18x $MRHD_{AUC}$) and females (24x $MRHD_{AUC}$) are comparable to the NOAEL safety margin of 19x $MRHD_{AUC}$ identified in the pivotal 6-month rat toxicology study, which was also set at 5 mg/kg due to similar digestive tract findings at the same doses. Furthermore, no new significant drug-related toxicities were identified. Overall, the nonclinical safety profile of the ertugliflozin formulation intended for marketing, which was synthesized by the new process method containing the process related impurities [redacted] is considered to be comparable to the safety profile of the ertugliflozin formulation used in previous nonclinical toxicology studies. Thus, this study successfully bridges the commercial formulation to the safety profile of the previous ertugliflozin formulation.

A clinical dose containing the maximum amount of impurity [redacted] with a specification limit of NMT [redacted] ([redacted] mg/kg), would have a [redacted] $MRHD_{BSA}$ safety margin at the study's overall NOAEL of 5 mg/kg and a [redacted] $MRHD_{BSA}$ exposure margin at the LOAEL of 25 mg/kg. However, there were no new or impurity-related toxicities at 25 mg/kg with a [redacted] $MRHD_{BSA}$ margin of safety for [redacted]. Thus, the evaluated concentrations were considered to be sufficient to qualify [redacted] in this study. Clinical doses containing the maximum amount of impurity [redacted], each with specification limits of NMT [redacted] ([redacted] mg/kg), would have safety margins of [redacted], respectively, and exposure margins of [redacted] $MRHD_{BSA}$ and [redacted] $MRHD_{BSA}$ at the LOAEL. Overall, all 3 process related impurities were evaluated at relative amounts above anticipated clinical doses and were not associated were any new toxicities. Thus, the impurities [redacted] are considered to be qualified with acceptable margins of safety for a 15 mg/day clinical dose at specifications of NMT [redacted], respectively.

Study: 13-Week Oral Gavage Toxicity and Toxicokinetics Study with PF-04971729 with Degradants in Rats (Study TT #15-7804 / #8325322 / #15GR254)

Study #	#8325322 / 15GR254
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	8/10/2015
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729 with degradants, Lot #705847-234-01, 74.4% purity

Key Study Findings

- Male NOAEL = 5 mg/kg (26x $MRHD_{AUC}$)
  - Mortality: 2 males at 25 mg/kg due to ascending urinary tract infection
  - Adverse drug-related findings in the digestive tract and urinary tract at 25 mg/kg, including increased kidney findings, consistent with findings in the 6-month rat study
- Female NOAEL = 25 mg/kg (91x $MRHD_{AUC}$)
  - No significant adverse findings
- The toxicity profile of ertugliflozin was unchanged by spiking with degradants (b) (4) (Peak 1 and 2), (b) (4)
- The ertugliflozin degradants were qualified with acceptable margins of safety for a 15 mg/day clinical dose at specifications of NMT (b) (4) % each
  - Degradant safety margins at the NOAELs of 5 mg/kg in males and 25 mg/kg in females:
    - (b) (4)

Ertugliflozin + Degradants			
Sex	**Rat, 13-Weeks**	**NOAEL (AUC)**	**Multiple of MRHD**

♂	**25 mg/kg**: Mortality due to ascending urinary tract infection. **Adverse findings:** kidney (large, discolored, dilatation, and inflammation), urinary tract (inflammation, infiltrate, dilatation, and hyperplasia), ↑BUN, and mild glandular stomach erosion. **Non-adverse findings (≤25 mg/kg):** lower severity of kidney and urinary tract dilatation and inflammation, minimal stomach erosion, upper GI tract dilatation and/or increase in villi/mucosa height, pancreatic zymogen depletion, adrenal cortex hypertrophy, and PD-related blood (↓glucose, ↓cholesterol and/or ↓electrolyte) and urine (↑glucosuria, ↓pH, ↑urine volume, ↑specific gravity, and ↑ketones).	**5 mg/kg** (35.7 μg·h/mL)	**26x**
♀	**≤25 mg/kg**: No significant adverse findings. **Non-adverse findings:** ↓body weight, ↓weight gain, minimal stomach erosion, upper GI tract dilatation and/or villi/mucosa ↑height, pancreatic zymogen depletion, adrenal cortex hypertrophy, and PD-related blood (↓glucose, ↓cholesterol and/or ↓electrolyte) and urine (↑glucosuria).	**25 mg/kg** (126 μg·h/mL)	**91x**

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL

Methods

SD rats were administered 0 (0.5% MS/10% PEG 400 vehicle), 1, 5, or 25 mg/kg PF-04971729 spiked with degradants (b) (4) via oral gavage once daily for 13 weeks. Main study animals (10/sex/group) were evaluated for mortality, clinical signs, body weights, food consumption, gross pathology, clinical pathology, and histopathology. Toxicokinetic parameters were evaluated in satellite TK animals (3/sex/group). Ertugliflozin and degradant concentrations were verified in all dosing formulations administered on Days 1, 43 and 91 using a validated HPLC method.

Results

In females, the NOAEL was set at 25 mg/kg (91x $MRHD_{AUC}$) based on the absence of toxicologically significant adverse findings.

In males, the NOAEL was set at 5 mg/kg (26x $MRHD_{AUC}$) based on mortalities due to ascending urinary tract infection, which were associated with macroscopic and microscopic urinary tract and kidney findings at 25 mg/kg (62x $MRHD_{AUC}$). Macroscopic findings of the urinary tract included discoloration and/or enlargement of the kidney, bladder, ureter, prostate, and seminal vesicle. These findings were observed in 10% to 40% of males at 25 mg/kg and were associated with mortalities. Microscopic urinary tract findings included mild to severe mixed cell inflammation (kidney, ureter, bladder, seminal vesicle, and prostate), mononuclear cell infiltrate (ureter and bladder), dilatation, and/or hyperplasia, and are consistent with ascending urinary tract infection

secondary to PD-related glucosuria. Since the mortalities were due to ascending urinary tract infections, the mortalities in this study are also considered to be a consequence of profound glucosuria related to the PD activity of ertugliflozin and do not represent a new drug-related toxicity.

Kidney size increases, increased organ weights, and microscopic findings of pelvis and tubule dilatations are consistent with PD-related osmotic diuresis. Additional kidney findings included pelvis inflammation in 20% of males at 25 mg/kg and females at ≥5 mg/kg. Minimal to marked mixed cell inflammation was also observed in 30% of 25 mg/kg males, consistent with infection. A single incidence of polycystic kidney disease was also reported in 1 male at 25 mg/kg. Increases in incidence rates and severity of kidney findings at 25 mg/kg were also associated with 2-fold increases in BUN levels in males. Overall, the cumulative kidney findings in males at 25 mg/kg were consistent with findings of ascending urinary tract infections and were considered to be adverse.

In females, urinary tract findings at ≥5 mg/kg included mixed cell inflammation and/or mononuclear infiltrate of the ureter and bladder, as well as bladder transitional cell hyperplasia. However, the urinary tract and kidney findings in females were not associated with mortalities or evidence of organ dysfunction, and are considered likely to be non-adverse and secondary to PD-related glucosuria and diuresis. Although adverse urinary tract findings were associated with mortalities in males at 25 mg/kg, there were no drug-related urinary track findings in males at 5 mg/kg.

Drug-related changes in urine were reported at all doses, including PD-related marked glucosuria and increases in urine volume. Increases in specific gravity and severity and incidence of ketones were also observed at all doses in males. Decreases in pH were observed in males (↓2-9%) and females (↓2-6%) at all doses. All urine changes are consistent with similar drug-related findings in the 6-month rat study.

Incidences of minimal focal erosion of the glandular stomach were reported in both sexes at 25 mg/kg, but were considered likely to be non-adverse; whereas focal erosion of increased severity in one 25 mg/kg male was considered adverse. In the upper GI tract, findings of dilatation and increased villi/mucosa height were observed in both sexes. Overall, these findings are consistent with digestive tract observations in the 6-month rat toxicology study and are likely secondary to off-target SGLT1 inhibition in rats.

Drug-related reductions in body weights and weight gains inversely correlate with trends for increased food consumption, which are anticipated class-related effects due to a compensatory response to PD-related increases in glucosuria. Drug-related pancreatic zymogen depletion at ≥5 mg/kg in both sexes was considered likely to be secondary to PD-related increases in food consumption and non-adverse.

Drug-related changes in clinical chemistry parameters, including decreases in blood glucose, cholesterol, and/or electrolyte levels were considered likely to be related to the PD activity of ertugliflozin and non-adverse.

Hypertrophy of the adrenal cortex zona glomerulus reported in both sexes is consistent with findings in other rat studies and stimulation of RAAS, which is likely to be an adaptive response to PD-related glucose diuresis. Thus, this finding is considered to be non-adverse.

Between 1 and 5 mg/kg, mean exposures increased more than dose-proportionally in males, but increased approximately dose-proportionally in females; whereas, exposures increased less than dose-proportionally in both genders between 5 and 25 mg/kg. C_{max} and AUC exposures were generally higher in females compared to males by as much as 2-fold, except at 5 mg/kg for AUC. T_{max} was achieved at 4 hours post-dose in males at ≤5 mg/kg, but at 1 hour postdose in males at 25 mg/kg and females at all doses.

Table 40: Toxicokinetics - 13-Week Rat Study #15GR254

Dose (mg/kg/day)	Day	Sex	C_{max} (ng/mL)	Tmax (h)	AUC_{24} (ng*h/mL)
1	91	Male	381	4	3830
		Female	878	1	5410
		Overall	620	1	4840
5	91	Male	2930	4	35700
		Female	3410	1	31100
		Overall	3010	1	33400
25	91	Male	7920	1	85000
		Female	13500	1	126000
		Overall	10700	1	105000

Notes: Parameters based on mean concentrations
AUC_{24} = Area under the plasma drug concentration-time curve from time 0 to 24 hours
C_{max} = Highest drug concentration observed in plasma
T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined.

(Table excerpted from sponsor's package)

Concentrations of ertugliflozin and each degradant were validated in dosing formulations for homogeneity and stability. Relative chromatogram percent peak areas range was (b) (4) % for (b) (4) At (b) (4) mg/kg, the percent peak areas for (b) (4) were (b) (4) respectively.

Reviewer's Comments

In the pivotal 6-month rat study, the NOAEL was set at 5 mg/kg for both sexes, which is comparable to the male 5 mg/kg NOAEL in this study; however, higher exposures were achieved in this study resulting in a larger safety margin for males (26x $MRHD_{AUC}$). The NOAEL for females in this study was higher than that of the 6-month study and was associated with a significantly higher safety margin (91x $MRHD_{AUC}$). No new drug-related toxicities were identified in this study, and the toxicology results are considered to be comparable to that of previous nonclinical toxicology studies with ertugliflozin. Thus, the ertugliflozin degradants (b) (4) are considered to be qualified with respective concentrations of (b) (4) % of the 1, 5 and 25 mg/kg doses in rats. A clinical dose

containing the maximum amount of [redacted] degradants, with specifications of NMT [redacted]% ([redacted] mg/kg) each, would have respective safety margins of [redacted] $MRHD_{BSA}$ in males at the male 5 mg/kg NOAEL and [redacted] $MRHD_{BSA}$ in females at the female 25 mg/kg NOAEL.

7 Genetic Toxicology

Based on the weight of evidence, ertugliflozin is not considered to be genotoxic. Ertugliflozin was evaluated for genotoxic potential in a standard battery of valid genotoxicity assays, including in vitro microbial reverse mutation (Ames), in vitro human lymphocyte cytogenetic, and in vivo rat micronucleus assays summarized below.

Study: Bacterial Reverse Mutation Assay with a Confirmatory Assay (Study TT #09-7896 / #8202969 / #09GR181)

Ertugliflozin potential to induce mutations was evaluated in 5 bacterial tester strains in the absence or presence of metabolic activation by S9 microsomal enzymes at concentrations up to 5000 μg/plate. Inhibition of growth was observed at concentrations ≥2500 μg/plate, but was evaluated for mutagenicity at non-toxic concentrations up to 1250 μg/plate. There were no significant concentration-related increases in the mean number of revertants with any of the strains tested in the absence or presence of S9. Overall, the study was valid and ertugliflozin was negative for mutagenicity.

Study: Genetic Toxicology Human Lymphocyte Assay of PF-04971729 (Study TT #09-7897 / #09GR182)

Ertugliflozin genotoxic potential was evaluated in human peripheral lymphocyte cultures exposed to concentrations up to 400 μg/mL for 3 hours in the absence or presence of metabolic activation, or up to 213 μg/mL for 24 hours without S9 metabolic activation. Cytotoxicity was observed at concentrations ≥237 μg/mL. Ertugliflozin exposure did not induce structural aberrations under any of the conditions evaluated. However, concentration-dependent increases in polyploidy were reported at ≥156 μg/mL with metabolic activation.

Study: 1-Month Oral Toxicity Study and Micronucleus Assessment of PF04971729 [redacted] in Rats (Study TT #09-7890 / #09GR185)

An in vivo micronucleus assay was conducted as part of a 1-month toxicology study in rats administered 0, 5, 25, or 250 mg/kg daily by oral gavage, reaching AUC_{0-24} exposures of 541 μg·h/mL (392x $MRHD_{AUC}$) and 718 μg·h/mL (567x $MRHD_{AUC}$) in males and females, respectively. There were no statistically significant reductions in polychromatic erythrocytes (PCE), indicating that there was a low incidence of bone marrow toxicity. There were no statistically significant increases in the number of PCEs with micronuclei. Overall, ertugliflozin was negative for the ability to induce chromosomal damage in vivo.

8 Carcinogenicity

A 2-year mouse carcinogenicity study was conducted at doses of 5, 15 and 40 mg/kg, and a 2-year rat carcinogenicity study was conducted at doses of 1.5, 5, and 15 mg/kg. An original high dose of 10 mg/kg/day was agreed upon by ECAC for the rat study based on AUC ratio, but was increased to 15 mg/kg by the sponsor when clinical doses were increased to 15 mg/day.

Study: Two-Year Oral Carcinogenicity Study in Mice (Study TT #14-1003)

Study no.:	TT #14-1003
Study report location:	eDr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01/11/2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ertugliflozin (L-0049215414, MK-8835); Lot #001H016 (Pfizer Lot #E010012479) administered Study Weeks 1 to 22 had 95.4% purity; Lot #001H006 (Pfizer Lot #E010014849) administered Study Weeks 22 to 102 had 98.4% purity.
CAC concurrence:	ECAC concurred with the sponsor's proposed doses of 5, 15 and 40 mg/kg. ECAC concurred with changes requested by the applicant during the conduct of the study, which included early termination.

Key Study Findings

Neoplastic

- **Neoplastic NOAEL** = 40 mg/kg (♂ & ♀)
  - No statistically significant neoplastic findings

Neoplastic Findings			
Sex	**Mouse, 2-year**	**NOAEL (AUC_{24})**	**Multiple of MRHD***
♂ & ♀	**≤40 mg/kg**: No significant neoplastic findings	**40 mg/kg** (~70 μg·h/mL**)	**~50x**

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL
** Day 28 AUC_{24} exposures determined in mouse study #13GR147, which was judged to be similar based on comparisons of C_{max} and 0.5 hour postdose exposures.

Non-neoplastic

- **Non-neoplastic NOAEL** = 40 mg/kg (♂ & ♀)
  - No significant non-neoplastic adverse findings
- **Non-adverse Drug-related Findings**:
  - Potentially drug-related increases in clinical signs of hunched posture, distended abdomen, urine staining, genital/rectal swelling and/or decreased skin turgor
    - Distended abdomen and urine staining likely secondary to PD-activity of SGLT2 inhibition
  - Drug-related decreases in body weights and weight gain in males at all doses, likely secondary to PD activity of SGLT2 inhibition
  - Kidney findings: secondary to PD-activity of SGLT2 inhibition
    - Increase in renal cysts in males at all doses and possibly in females at ≥15 mg/kg.
    - Kidney tubule and pelvic dilatation at all doses in males
  - Urinary bladder findings: secondary to PD-activity of SGLT2 inhibition
    - Increased size and distention in males at all doses

Non-Neoplastic Findings		
Mouse, 2-year	**NOAEL (AUC_{24})**	**Multiple of MRHD**
≥5 mg/kg (~6x MRHD): clinical signs (distended abdomen, urine staining), ↓body weights (♂), ↓weight gain (♂), Kidney cysts (♂), renal tubule & pelvic dilatation (♂), urinary bladder (↑size and distention; ♂) **≥15 mg/kg** (~20x MRHD): renal cysts (♀) **40 mg/kg** (~58x MRHD): **No Significant adverse non-neoplastic findings.**	**40 mg/kg** (~70 μg·h/mL**)	**~51x**

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL
** Day 28 AUC_{24} exposures determined in mouse study #828628, which was judged to be similar based on comparisons of C_{max} and 0.5 hour post-dose exposures.

Adequacy of Carcinogenicity Study

The final study report of a GLP-compliant two year oral gavage carcinogenicity study in the Crl:CD1(ICR mice was reviewed and the results was discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The division considers the mouse study an adequate assessment of carcinogenic potential because ertugliflozin was assessed at AUC ratios of exposure greater than 25-fold higher than clinical exposures at the therapeutic dose of 15 mg/day. Based on approximate C_{max} exposures determined at 0.5 hours postdose, drug exposure levels were considered to be similar to those achieved in the 3-month mouse toxicology study (#8286028). Extrapolated AUC ertugliflozin exposures were considered to have reached an approximate exposure margin of 58x $MRHD_{AUC}$ at the highest dose of 40 mg/kg. During study Week 96, the sponsor reported a trend for a low number of surviving males in one

of the controls and the 40 mg/kg groups. ECAC concurred with plans for early termination of all groups of one sex if control numbers dropped below 15 animals. Subsequently, all male groups were terminated 7 weeks early during Week 97 and all female groups were terminated 2 weeks early during Week 102. Nevertheless, a sufficient number of male and female animals were exposed to the high dose for a sufficient amount of time for the study to be considered adequate.

Appropriateness of Test Models

The sponsor chose doses of ertugliflozin at 5, 15, and 40 mg/kg in males and females, which were considered to be acceptable by the ECAC. ECAC concurred with early termination of all male groups, which occurred during Week 97, due to a low number of surviving male controls (≤20 animals). Females were terminated during Week 102, which was only 2 weeks early and was considered to be acceptable. Dose-limiting toxicities were not observed in either sex up to the highest dose tested (~58x MRHD). The test species and study design are considered appropriate for determining safety margins for chronic ertugliflozin administration.

Evaluation of Tumor Findings

There were no drug-related tumor findings in male or female mice.

Methods

Doses:	0, 5, 15, and 40 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC/10% PEG 400
Basis of dose selection:	AUC ratio in both sexes and MTD in males
Species/Strain:	Mouse / Crl:CD1(ICR)
Number/Sex/Group:	50/sex/group
Age:	5 weeks
Animal housing:	Animals were group-housed, up to 3 mice/box, for the majority of the study. Beginning in Study Week 73, all males were individually housed.
Paradigm for dietary restriction:	Animals were fed PMI Certified Rodent Diet ad libitum throughout the dosing period and were fasted overnight prior to scheduled necropsy.
Dual control employed:	3 vehicle control groups were employed, 1 with 0.5% MC only and 2 with 0.5% MC/10% PEG 400 vehicle.
Interim sacrifice:	None
Satellite groups:	TK groups were included for blood sample collection in Study Week 5 and Week 27.
Deviation from study protocol:	There were no significant deviations affecting the integrity of the study.

Study Design

Table 41: Study Design - Mouse Study TT #14-1003

Carcinogenicity Arm:	Females	Males
Control 1 (vehicle, 0.5% MC)	50	50
Control 2 (vehicle, 0.5% MC/10% PEG 400)	50	50
Control 3 (vehicle, 0.5% MC/10% PEG 400)	50	50
Ertugliflozin (MK-8835)		
5 mg/kg/day	50	50
15 mg/kg/day	50	50
40 mg/kg/day	50	50
Carcinogenicity Replacement Arm[a]:	Females	Males
Control 1-R (vehicle, 0.5% MC)	3	3
Control 2-R (vehicle, 0.5% MC/10% PEG 400)	6	6
Ertugliflozin (MK-8835)		
5 mg/kg/day-R	3	3
15 mg/kg/day-R	3	3
40 mg/kg/day-R	3	3
Toxicokinetic Arm:	Females	Males
Control 1-TK (vehicle, 0.5% MC)	9	9
Control 2-TK (vehicle, 0.5% MC/10% PEG 400)	9	9
Ertugliflozin (MK-8835)		
5 mg/kg/day-TK	9	9
15 mg/kg/day-TK	9	9
40 mg/kg/day-TK	9	9

0.5% MC = 0.5% (w/v) methylcellulose in deionized water.

0.5% MC/10% PEG 400 = 0.5% (w/v) methylcellulose/10% (v/v) polyethylene glycol 400 in deionized water.

a Carcinogenicity Replacement Arm animals were used as potential replacements (R) for Carcinogenicity Arm animals that died during the first approximate 3 months of study due to dosing accidents and/or other physical trauma. Any replacement animal that was not used for replacement was discarded without examination in Study Week 15.

(Table excerpted from sponsor's package)

Observations and Results

Mortality

Decreased survival of both sexes resulted in early termination, with ECAC concurrence, of all remaining male animals in Study Week 97 and female animals in Study Week 102. However, decreased survival was similarly observed in control and treatment groups, in that there were no dose-dependent or statistically significant increases in deaths of treatment groups compared to the 3 concurrent control groups. Furthermore, there was not a significant difference in survival between the 0.5% MC control group 1 and the two 0.5% MC+PEG400 control groups 2 and 3, indicating that the presence of PEG400 in the vehicle did not have affect survival. Overall, there were no drug-related effects on mortalities.

Table 42: Unscheduled Deaths - Mouse Study TT #14-1003

Total Unscheduled Deaths[a]
(Incidence and Percentage of Animals Found Dead or Unscheduled Sacrificed; n=50/sex/group)

Dose Group	Number Dead (Percent)	
	Females[b]	Males[b]
Control 1[c] (0 mg/kg/day)	23 (46%)	34 (68%)
Control 2[d] (0 mg/kg/day)	31 (62%)	29 (58%)
Control 3[d] (0 mg/kg/day)	24 (48%)	22 (44%)
Combined Controls 2 and 3[d]	55 (55%)	51 (51%)
Ertugliflozin		
5 mg/kg/day	27 (54%)	32 (64%)
15 mg/kg/day	32 (64%)	33 (66%)
40 mg/kg/day	27 (54%)	23 (46%)

a Per protocol, Carcinogenicity Arm animals meeting the protocol-defined criteria for replacement (those animals that die during the first approximate 3 months of study due to a dosing accident and/or other physical trauma) were replaced in Study Week 15 and are not reflected in the table. Fourteen mice, generally distributed across gender and all dose groups, were replaced per these criteria.

b Due to decreased survival observed in control and test article-treated dose groups, scheduled termination of all remaining male and female animals occurred in Study Week 97 and Study Week 102, respectively.

c Control 1 vehicle: 0.5% (w/v) methylcellulose in deionized water.

d Control 2 and 3 vehicle: 0.5% (w/v) methylcellulose/10% (v/v) polyethylene glycol 400 in deionized water.

(Table excerpted from sponsor's package)

Clinical Signs

The sponsor reported drug-related increases in clinical observations in both males and females at all doses, which were considered to be mild, equivocal, and/or non-adverse. Observations of hunched posture were increased with regard to the number of affected animals and/or frequencies of incidences in females at all doses; but, were independent of dose and only observed in males at 5 mg/kg. The sponsor also reported drug-related increases in observations of distended abdomen in males at all doses and in females at 15 mg/kg; however, the frequency and incidence rates were only slightly increased compared to concurrent control groups. Slight increases in genital/rectal swelling, urine staining, and decreased skin turgor were also reported in drug-treated males. The clinical observations of distended abdomen and urine staining are consistent with class-related findings and are likely related the PD activity of ertugliflozin. Decreases in skin turgor are likely related to dehydration secondary to increased diuresis.

The sponsor also reported drug-related observations of pale appearance in females and unkempt appearance in males; however, there was not a clear increase in incidence rates compared to concurrent controls.

Table 43: Clinical Observations - Mouse Study TT #14-1003

Clinical Observations (n=50)												
Finding	Male (mg/kg)						Female (mg/kg)					
	0*	0**	0**	5	15	40	0*	0**	0**	5	15	40

Distended Abdomen	10 (7.1)	4 (5.3)	9 (1.8)	12 (3.2)	11 (5.1)	9 (8.7)	18 (14.9)	15 (2.6)	13 (1.9)	15 (2.9)	23 (3.0)	14 (2.5)
Hunched Posture	11 (7.2)	9 (5.6)	15 (6.1)	19 (5.2)	14 (4.1)	8 (5.3)	11 (3.7)	9 (9.4)	10 (3.1)	18 (5.2)	16 (2.3)	15 (12.3)
Genital and rectal signs, Swollen	1 (1.0)	0	1 (4.0)	3 (5.3)	2 (2.0)	3 (7.0)	1 (1.0)	2 (1.5)	1 (2.0)	1 (1.0)	2 (2.0)	1 (1.0)
Urine Staining	11 (10.4)	8 (10.0)	8 (5.8)	15 (3.2)	15 (13.3)	12 (19.0)	5 (1.6)	5 (1.4)	0	2 (2.0)	2 (2.0)	2 (11.5)
↓Skin Turgor	11 (3.2)	8 (1.5)	7 (6.3)	20 (3.4)	13 (3.2)	11 (4.0)	7 (2.3)	9 (1.7)	7 (2.1)	14 (2.6)	11 (2.0)	9 (2.2)
Unkempt Appearance	29 (19.5)	21 (10.0)	19 (8.4)	25 (5.6)	21 (9.7)	19 (9.3)	7 (1.9)	6 (1.8)	8 (4.3)	9 (3.3)	6 (2.8)	5 (3.2)
Pale Appearance	16 (3.2)	9 (2.1)	14 (3.8)	15 (3.7)	10 (4.4)	9 (3.8)	12 (3.5)	15 (2.6)	13 (1.9)	15 (2.9)	23 (3.0)	14 (2.5)

* = 0.5% MC
** = 0.5% MC/10% PEG 400
() = Mean number of days

Body Weights

Drug-related decreases in body weights and weight gain were observed in males at all doses. During Week 62, when stable adult weights are generally achieved in male mice, dose-dependent decreases in body weights (↓4-10%) were apparent in males at all doses. During Week 94, the mean body weights of remaining males were up to 19% lower than mean vehicle controls at ≥15 mg/kg. Decrements in weight gains of 15% to 23% were observed at ≥15 mg/kg, beginning during Week 30 at 40 mg/kg and Week 46 at 15 mg/kg. Over the course of the study, total weight gain was 6% lower at 5 mg/kg compared to concurrent controls. There were no clear drug-related decreases in female body weights or weight gains over the course of the study. Overall, the observed drug-related decreases in male body weights and weight gains are consistent with non-adverse, class-related effects secondary to the PD-activity of SGLT2 inhibition and consequent glucosuria.

Table 44: Body Weight - Mouse Study TT #14-1003

Body Weight				
Males				
Dose (mg/kg)	Week 62		Week 94	
	BW (g)	BW % Control**	BW (g)	BW % Control**
0*	52.3	103.0%	47.5	97.1-0% (↓3.0%)
0**	50.8	-	48.0	-
5	48.6	95.7% (↓4.3%)	46.1	93.9% (↓6.1%)

15	46.9	92.3% (↓7.7%)	44.3	81.2% (↓18.8%)
40	45.7	90.0% (↓10.0%)	44.4	81.2% (↓18.8%)

* = 0.5% MC
** = 0.5% MC/10% PEG 400 (average of control groups 2 and 3)

Table 45: Body Weight Gain - Mouse Study TT #14-1003

Test Article-Related Change in Male[a] Body Weight Gain
(Absolute [grams] and Percent Difference in Mean Values From Concurrent Vehicle Control Means)

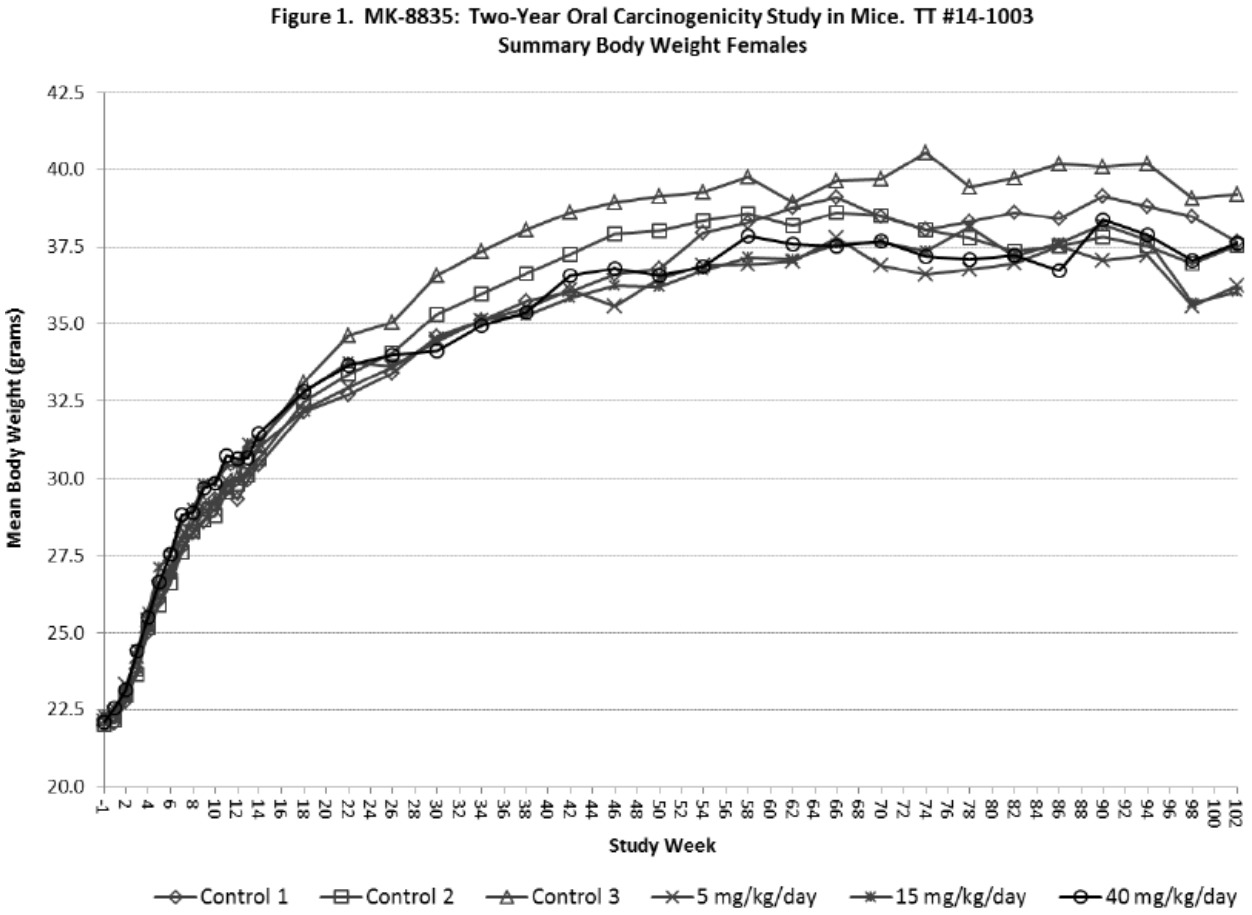
Study Week	Control 2	Control 3	Average of Control 2 and Control 3 Means	Ertugliflozin (mg/kg/day)		
				5	15	40
30	+18.3	+20.5	+19.4	-	-	+15.9 (-18%)
46	+20.8	+23.1	+22.0	-	+18.2 (-17%)	+17.9 (-19%)
62	+21.4	+23.7	+22.6	-	+18.6 (-18%)	+17.3 (-23%)
78	+19.1	+20.8	+20.0	-	+17.1 (-15%)	+16.8 (-16%)
94	+19.3	+20.0	+19.7	-	+16.0 (-19%)	+16.0 (-19%)

- = No test article-related change

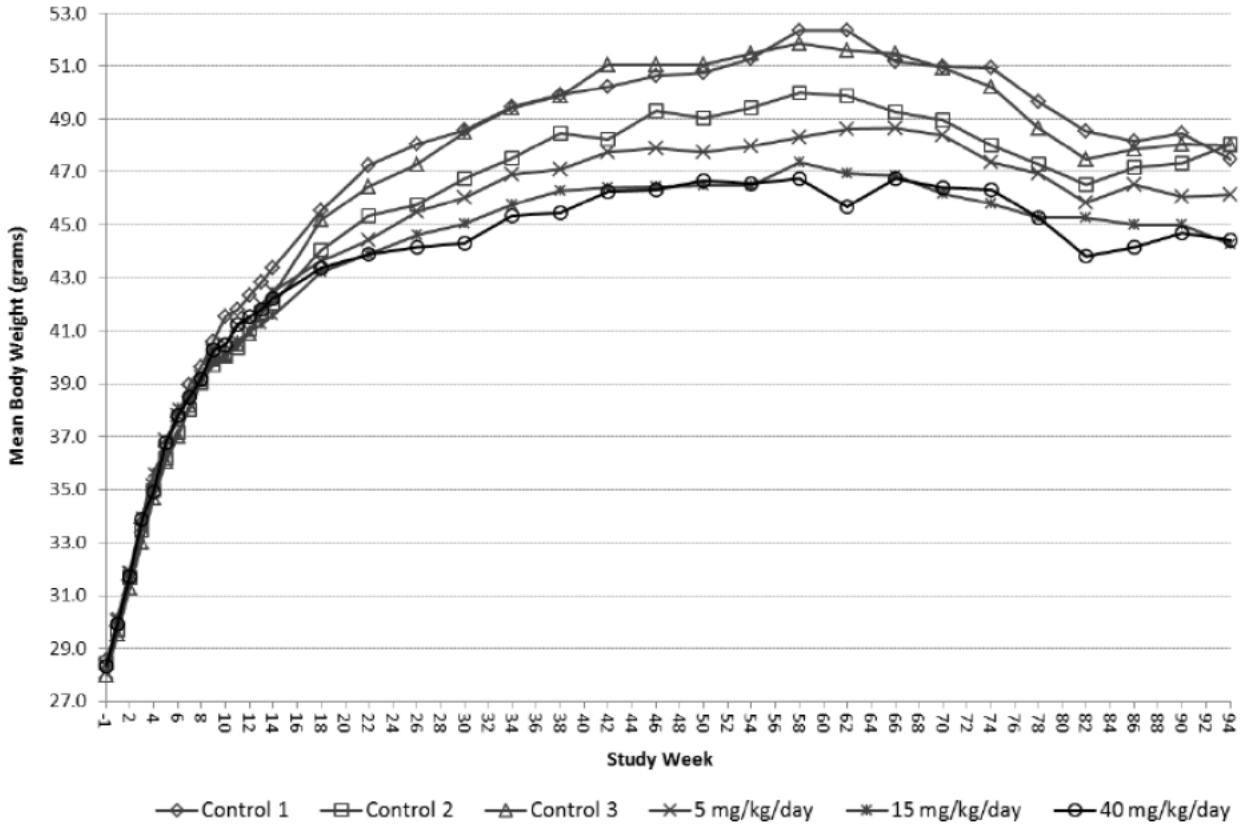
a Due to decreased survival in males from control and test article-treated dose groups, scheduled termination of all remaining male mice from all dose groups occurred in Study Week 97.

(Table excerpted from sponsor's package)

Figure 7: Body Weights – Mouse Study TT #14-1003



Cont. Figure 1. MK-8835: Two-Year Oral Carcinogenicity Study in Mice. TT #14-1003
Summary Body Weight Males



(Figures excerpted from sponsor's package)

Feed Consumption

Not determined.

Gross Pathology

Drug-related renal and urinary bladder macroscopic findings were observed in males. Increased incidences of kidney cysts and dilation were observed in drug-treated males at all doses. Similarly, increased incidences of urinary bladder distention and increased size were also reported in drug-treated males at all doses. Kidney dilation and urinary bladder distention/increased size are consistent with anticipated PD-related effects.

Slight increases in stomach nodules were observed in drug-treated males and 5 mg/kg females. Although the incidence rates were small, they are consistent with stomach findings in previous rodent toxicology studies and may be drug-related.

Table 46: Macroscopic Findings – Mouse Study TT #14-1003

Macroscopic Findings (n=50)			
Tissue		Male (mg/kg)	Female (mg/kg)

		0*	0**	0**	5	15	40	0*	0**	0**	5	15	40
Stomach	Focus	0	0	1	2	1	1	1	1	0	2	0	1
	nodule	1	0	1	3	3	2	1	1	1	4	1	0
Kidney	cyst	6	6	11	23	13	20	3	1	1	2	4	4
	dilation	0	1	0	2	5	5	0	0	0	1	0	0
Urinary Bladder	Increased size	1	2	4	5	6	4	1	1	0	0	3	1
	distention	12	10	8	21	15	13	0	0	1	1	0	0

* = 0.5% MC
** = 0.5% MC/10% PEG 400

There were no drug-related increases in macroscopic bone findings.

Histopathology
Note that an internal statistical analysis of the neoplastic data was performed by Hepei Chen in the Division of Biometrics. Since 3 control groups were included with this study, group 1 with 0.5% MC alone and groups 2 and 3 with 0.5%MC/10% PEG 400, two separate sets of statistical analyses were conducted; one to compare the drug treatment groups (4, 5 and 6) with combined vehicle groups 2+3, and a second to compare the combined vehicle groups 2+3 with the 0.5% MC only control group 1.

Peer Review
All tissues and organs from all animals in all dose groups were peer reviewed.

Neoplastic
Incidences of benign and malignant neoplasms were similar between drug-treated groups and concurrent control groups. In both males and females, there were no dose-dependent increases, statistically significant dose response relationships, or statistically significant pairwise comparisons in incidences of neoplasms in any tissues with drug treatment compared to concurrent control groups 2+3 or control group 1. There were no statistically significant increases in combined neoplasm data at all sites for hemangioma and hemangiosarcoma, mesothelioma, leukemia, or lymphoma. There were also no statistically significant increases in combined neoplasms for any specific tissue. Overall, there were no significant drug-related neoplastic findings in males or females.

Non Neoplastic
Dose-related increases in kidney tubule dilatation were observed at all doses in males. Renal tubular dilatations were characterized by focal to multifocal dilation of tubular lumens, which were lined by cuboidal to slightly flattened tubular epithelial cells and were primarily observed the cortex to outer stripe of the medulla. Renal tubular dilatations frequently corresponded with kidney cysts and sometimes pelvic dilatations of the kidney pelvis and/or urinary bladder, which often correlated with increased kidney size and urinary bladder distention, respectively. There were no dose-related increases in tubule or pelvis dilatations in females.

Table 47: Non-neoplastic Microscopic Findings - Mouse Study TT #14-1003

Test Article-Related Postmortem Findings
Ertugliflozin

	Males				
Dose, mg/kg/day	0[a]	0[b]	5	15	40
Number of Animals	50	50	50	50	50
Histomorphology (Incidence)					
Kidney					
Tubule, dilatation	3	3	7	13	19
Pelvis, dilatation	4	2	9	13	12
Urinary Bladder					
Dilatation	10	8	21	18	15

a 0.5% methylcellulose/10% PEG 400 control (Control 2)
b 0.5% methylcellulose/10% PEG 400 control (Control 3)

(Table excerpted from sponsor's package)

There were no dose-related increases in renal nephropathy in either sex.

There were no drug-related changes in bone microscopic findings in either sex.

There were no dose-related increases in heart microscopic findings in either sex.

Toxicokinetics

Whole blood samples were collected from TK animals at 0.5 and 24 hours postdose on during Study Weeks 6 and 27. Ertugliflozin concentrations were determined in mouse plasma using a validated LC-MS/MS method (SAP.1576).

Mean plasma concentrations were considered to be similar (<2-fold different) in males and females with no consistent gender effect in exposure differences; thus, the TK data for both sexes were combined. Concentrations at 24 hours postdose were less than 1% of T_{max} exposures at 0.5 hours postdose. There were no signs of accumulation at ≤15 mg/kg/day; however, drug concentrations were 1.6 to 2-fold higher at 40 mg/kg on Day 27 compared to Day 6, which may indicate a small amount of accumulation at 40 mg/kg. In general, exposures at 0.5 hours postdose were comparable to C_{max} values previously observed at doses of 5 and 40 mg/kg/day (Study #13GR147), which had Day 28 AUC exposures of 7.24 μg·h/mL and 70.0 μg·h/mL with exposure margins of 5x $MRHD_{AUC}$ and 51x $MRHD_{AUC}$, respectively. At 40 mg/kg, Day 28 AUC exposures were 53.0 μg·h/mL and 87.2 μg·h/mL in males and females, respectively, with gender-specific exposure margins of 38x $MRHD_{AUC}$ and 63x $MRHD_{AUC}$.

Table 48: Toxicokinetics - Mouse Study TT #14-1003

Summary Mean (± SE) Plasma MK-8835[a] Concentrations (ng/ml) in Mice Following Dosing of MK-8835: Study Week 6 and Study Week 27

Dose Group[b]	Dose (mg/kg/day)	Study Week	Time (hr)	
			0.5	24
14	5	6	1760 ± 150	3.23 ± 2.18
		27	1560 ± 281	5.03 ± 3.19
15	15	6	4740 ± 550	17.3 ± 4.15
		27	4540 ± 512	30.2 ± 8.41
16	40	6	8690 ± 405	21.9 ± 3.45
		27	13,500 ± 860	48.7 ± 10.1

a MK-8835 concentrations in plasma from both control treatment group animals (Groups 12 and 13) were below the lower limit of quantitation (LLQ = 5 ng/mL) of the bioanalytical method.

b Toxicokinetic Arm dose groups

(Table excerpted from sponsor's package)

Dosing Solution Analysis

Suspensions were prepared daily on Study Days 1-3 and weekly 7-day preparations were made for Study Days 4-10, 11-17, and 31-37. A 14-day bulk preparation was made for all other weeks in the dosing period. Samples were collected for concentration and/or uniformity (bottom, middle and top portions) analyses on Study Days 1, 4, 5, 11, 18, 36, 37, 51, 64, 78, 148, and 185.

Standard deviations for all uniformity samples were within the target range of ≤10% of target concentration. Most concentration results remained within the range of ±15% target concentration, except for 2 doses on 2 occasions each during the first month. The 15 mg/kg/day formulation was 84-88% of target on Study Day 4 and 81-83% of target on Day 37. The 40 mg/kg/day formulation was 70-87% of target on Study Day 18 and only 61% of target on Day 37. Since formulations out of the acceptable specification range were only observed during the first 5 weeks, there was unlikely to be a significant impact on the integrity of the 2-year study.

Table 49: Dose Formulation Analyses - Mouse Study TT #14-1003

Table 2 Out-of-Specification Dose Formulation Analyses Findings on Study

Assay Results for 40 mg/kg/day (4.0 mg/mL) Suspension – Concentration and Uniformity Analysis		
	Percent of Claim (%) Ertugliflozin	
Study Day of Assays	Original Sample	Duplicate Sample
18	72.3 (Top)	87.0 (Top)
18	70.3 (Middle)	71.7 (Middle)
18	71.9 (Bottom)	71.6 (Bottom)
37	60.8	60.6
Assay Results for 15 mg/kg/day (1.5 mg/mL) Suspension – Concentration Analysis		
	Percent of Claim (%) Ertugliflozin	
Study Day of Assays	Original Sample	Duplicate Sample
4	84.3	88.0
37	80.7	82.9

(Table excerpted from sponsor's package)

Study: 104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with PF-04971729 in Rats (Study TT #13-7800)

Study no.:	TT #13-7800 / 8250936
Study report location:	eDr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09/10/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ertugliflozin (PF-04971729 (b) (4)); Pfizer Lot #E010013903 (ID #000006321) had 75.6% purity; Pfizer Lot #E010015326 (ID #000006321) had 77.1% purity.
CAC concurrence:	ECAC concurred with both the sponsor's proposed original doses of 1, 3 and 10 mg/kg based on AUC and the revised proposed doses of 1.5, 5 and 15 mg/kg, which were increased due to an increase in therapeutic dose. ECAC also recommended addition of a water or saline control group to aid in study interpretation; however, this was not implemented. ECAC concurred with changes requested by the applicant during the conduct of the study, which included early termination.

Key Study Findings

Neoplastic

- **Male Neoplastic NOAEL** = 5 mg/kg (18x $MRHD_{AUC}$)
  - Adrenal medulla neoplasms at 15 mg/kg (66x $MRHD_{AUC}$)
    - Benign pheochromocytoma (PCC)
    - Combined benign + malignant PCC
  - Associated proliferative hyperplasia at ≥5 mg/kg (non-neoplastic)
- **Female Neoplastic NOAEL** = 15 mg/kg (74x $MRHD_{AUC}$)
  - No statistically significant neoplastic findings

Table 50: Neoplastic Summary Table - Rat Study TT#13-7800

Neoplastic Effects			
Sex	**Rat, 2-year**	**NOAEL (AUC_{24})**	**Multiple of MRHD**
♂	**5 mg/kg:** (18x MRHD) Associated adrenal medulla hyperplasia **15 mg/kg**: (66x MRHD) Adrenal medulla neoplasms including benign PCC and combined benign + malignant PCC	**5 mg/kg** (24.4 μg·h/mL)	**18x**
♀	**15 mg/kg**: (74x MRHD) No significant proliferative or neoplastic findings	**15 mg/kg** (102 μg·h/mL)	**74x**

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL

Non-neoplastic

- **Non-neoplastic NOAEL** = not determined in males & 1.5 mg/kg/day in females
  - Due to adverse kidney findings associated with *ascending urinary tract infections* secondary to PD-related glucosuria
    - Adverse in males at all doses and at ≥5 mg/kg in females
    - Kidney:
      - Renal tubule dilatation at ≥5 mg/kg (♂ & ♀)
      - Renal tubule degeneration at 15 mg/kg (♂ & ♀)
      - Pyelonephritis in males (≥1.5 mg/kg) and females (≥5 mg/kg)
        - Correlating thrombus/infarct and death in 1 ♀ at 15 mg/kg
      - Macroscopic findings of large kidney at 15 mg/kg (♂ & ♀)
      - Discolored kidney in males (≥1.5 mg/kg) and females (≥5 mg/kg)
      - Exacerbation of spontaneous nephropathy at ≥5 mg/kg (♂)
    - Urinary Bladder
      - Transitional cell hyperplasia at ≥1.5 mg/kg (♂ & ♀)
      - Inflammation at ≥1.5 mg/kg (♂ & ♀)

        - Presence of yeast and/or hyphae in inflammatory exudate in several animals at ≥5 mg/kg
      - Macroscopic findings of large bladder at all doses (♂) and ≥5 mg/kg (♀)
      - ↑urine volume
    - Ureter
      - Inflammation, dilation and/or transitional cell hyperplasia in males (≥5 mg/kg) and females (≥1.5 mg/kg)
      - Macroscopic findings of large ureter in males (15 mg/kg) and females (≥5 mg/kg)
  - Nonglandular stomach erosion/ulcer and hyperplasia/hyperkeratosis in males at ≥5 mg/kg and females at 15 mg/kg
- **Non-adverse Drug-related Findings**:
  - Tongue
    - Mucosal hyperplasia at all doses (♂ & ♀)
    - Mucosal inflammation at all doses (♂ & ♀)
  - Pancreas
    - Zymogen granule decreases in males at ≥5 mg/kg and all doses in females
    - Slight increase in acinar cell hyperplasia in males at ≥5 mg/kg
    - Inflammation at 15 mg/kg (♂ & ♀)
  - Decreased body weights and weight gains at all doses, with correlating appearance of thinness in 15 mg/kg animals
  - Increased food consumption at all doses

Table 51: Non-neoplastic Summary Table - Rat Study TT#13-7800

Non-Neoplastic Findings			
Sex	**Rat, 2-year**	**NOAEL (AUC_{24})**	**Multiple of MRHD**
♂	**≥1.5 mg/kg**: ***Adverse urinary tract & kidney*** *findings associated with* ***ascending infection***. Kidney (pyelonephritis, discolored), urinary bladder (inflammation, hyperplasia, enlarged, ↑urine volume), tongue (hyperplasia, inflammation), ↓body weight, ↑food consumption **≥5 mg/kg** (18x MRHD): Kidney (tubule & pelvis dilatation, exacerbation of nephropathy), urinary bladder (infection present in inflammatory exudate), ureter (inflammation, dilation, hyperplasia), nonglandular stomach (erosion/ulcer, hyperplasia, hyperkeratosis), pancreas (↓zymogen granules, acinar hyperplasia) **15 mg/kg** (66x MRHD): Kidney (tubule degeneration, enlarged), ureter (enlarged),	**Not Determined** <1.5 mg/kg (<6.69 μg·h/mL)	**<5x**

	pancreas (inflammation), appearance of thinness		
♀	**≥1.5 mg/kg**: Kidney (pelvis dilatation), urinary bladder (inflammation, hyperplasia, ↑urine volume), ureter (inflammation, dilation, hyperplasia), tongue (hyperplasia, inflammation), pancreas (↓zymogen granules), ↓body weight, ↑food consumption **≥5 mg/kg** (28x MRHD): ***Adverse urinary tract & kidney*** *findings associated with* ***ascending infection****.* Kidney (pyelonephritis, tubule dilatation, discolored), urinary bladder (infection present in inflammatory exudate, enlarged), ureter (enlarged) **15 mg/kg** (74x MRHD): Kidney (tubule degeneration, enlarged, thrombus/infarct), nonglandular stomach (erosion/ulcer, hyperplasia, hyperkeratosis), pancreas (inflammation), appearance of thinness	**1.5 mg/kg** (9.27 μg·h/mL)	**7x**

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL

Adequacy of Carcinogenicity Study

The final study report of a GLP-compliant standard two year oral gavage carcinogenicity study in the Sprague Dawley rat was reviewed and the results were discussed at a meeting of the ECAC. The division considers the rat study an adequate assessment of carcinogenic potential because ertugliflozin was assessed at AUC ratios of exposure greater than 25-fold higher than clinical exposures at the therapeutic dose of 15 mg/day. Based on AUC values, ertugliflozin exposures reached a 74x exposure margin in females and a 66x exposure margin in males at the highest doses, which are considered consistent with safety margins predicted from AUC exposures in the 26-week and 13-week toxicology studies. During study Week 88, the sponsor reported a trend for a low number of surviving female control animals and ECAC concurred with plans for early termination of all female groups if control numbers dropped below ≤20 animals. Subsequently, all female groups were terminated 12 weeks early during Week 92. Nevertheless, a sufficient number of male and female animals were exposed to the high dose for a sufficient amount of time for the study to be considered adequate. It is noted that a saline/water control group was recommended by ECAC, but was not included in the study.

Appropriateness of Test Models

The sponsor chose doses of ertugliflozin at 1.5, 5, and 15 mg/kg in males and females, which were considered to be acceptable by the ECAC. All female groups were terminated early during Week 92 due to a low number of surviving female control animals (18 animals); however, 49% of females in the high dose group survived to terminal necropsy during Week 92. There were no dose-limiting toxicities at the highest dose in males (66x MRHD) or females (74x MRHD). Overall, the test species and study design are considered appropriate for determining safety margins for chronic ertugliflozin administration.

Evaluation of Tumor Findings

There were no significant drug-related increases in neoplasms or abnormal proliferation in females. However, significant drug-related neoplasms were observed in the adrenal medulla of male rats.

In males, significant drug-related increases in neoplasms and proliferative hyperplastic effects were restricted to the adrenal medulla. Increases in benign PCC and combined benign+malignant PCC neoplasms at 15 mg/kg are considered to be unequivocally drug-related. Dose-dependent increases in benign PCC in the adrenal medulla were also statistically significant for a dose-response relationship (p value <0.005). At the 5 mg/kg, the sponsor reported that incidences of benign PCC reached statistical significance for a common tumor by pairwise one-sided comparison; however, after poly-k adjustment, the data was slightly outside of statistical significance (p value = 0.0167), see Chen's biostatistics review. The incidence rate at 5 mg/kg was also slightly within the sponsor's historical control dataset for spontaneous benign PCC in this strain of rat, but was greater than historical control incidences after weighted for mortality. Overall, the incidences of PCC at 5 mg/kg were not considered to be statistically significant.

It is noted that the increases in benign PCC at 5 mg/kg correlated with drug-related increases in adrenal medulla hyperplasia, which has been regarded as a PCC precursor lesion by some experts in the literature (Korpershoek et al. 2014). Furthermore, the rat hyperplasia data are consistent with a propensity and/or increased susceptibility toward abnormal proliferation of adrenal medulla chromaffin cells preceding PCC development (see discussion under Adrenal Medulla). Overall, the observed adrenal medulla hyperplasia and PCC are considered to be consistent with a continuum of tumor development. Thus, the increased incidences of adrenal medulla hyperplasia and PCC observed at 5 mg/kg are considered possibly drug-related; however, they are not considered to be unequivocally drug-related.

Methods

Doses:	1.5, 5, and 15 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC / 10% PEG 400
Basis of dose selection:	AUC ratio
Species/Strain:	Rat / Crl:CD(SD), (b) (4)
Number/Sex/Group:	Control & 15 mg/kg groups: 70/sex/group 1.5 & 5 mg/kg groups: 60/sex/group
Age:	6 to 7 weeks
Animal housing:	Group-housed up to 2 animals per cage. Control groups were housed on separate racks.

Paradigm for dietary restriction:	Animals were fed Certified Rodent Diet #2016C ad libitum.
Dual control employed:	None
Interim sacrifice:	None
Satellite groups:	None
Deviation from study protocol:	On Day 511, several males in the 5 mg/kg group were administered the 1.5 mg/kg instead. Tissues for one male (B78008) at 5 mg/kg could not be identified due to mislabeling. Nevertheless, there were no significant deviations affecting the overall integrity of the study.

Study Design

Table 52: Carcinogenicity Study Design - Rat Study TT#13-7800

		No. of Animals		Dose Level	Dose Concentration[b]
Group[a]	Subgroup	Male	Female	(mg/kg/day)	(mg/mL)
1 (Control)	1 (Carcinogenicity)	70	70	0	0
	2 (Toxicokinetic)	6	6	0	0
2 (Low)	1 (Carcinogenicity)	60	60	1.5	0.15
	2 (Toxicokinetic)	6	6	1.5	0.15
3 (Mid)	1 (Carcinogenicity)	60	60	5	0.5
	2 (Toxicokinetic)	6	6	5	0.5
4 (High)	1 (Carcinogenicity)	70	70	15	1.5
	2 (Toxicokinetic)	6	6	15	1.5

a Group 1 received vehicle control article only (0.5% (w/v) methylcellulose (4000 cps) with 10% (v/v) polyethylene glycol 400 prepared in reverse osmosis (RO) water).

b Dose concentrations were corrected for lot specific potency of 0.756 (75.6%) or 0.771 (77.1%), as appropriate. A correction factor of 1.323 was used for lot number E010013903. A correction factor of 1.297 was used for lot number E010015326.

Observations and Results

Mortality

There were no significant differences in survival of males. However, there was a dose-dependent increase in female survival reaching a statistical significance in the number of females surviving to scheduled necropsy at 15 mg/kg (49%) compared to vehicle control (26%), which was verified using the Kaplan-Meier product limit method by Hepei Chen in the Division of Biometrics.. However, the compilation of historical control survival data from 2-year studies by the supplier, (b) (4), indicates that the normal range of survival ranges from ~25% to ~50% in females in this strain of rat (file:///C:/Users/HAWESJ/Documents/My%20Library/animal%20models/Comparison-of-Historical-Control-Data-in-Two-Strai.pdf). Thus, the trend for increased survival in females is likely to be within the normal biological range of percent survival for this strain of rat, and is likely to be an incidental finding. Thus, there a drug-related benefit on female survival is unlikely.

Table 53: Survival at Terminal Necropsy - Rat Study TT#13-7800

	PF-04971729				
Sex	Males				Trend (1,2,3,4)[a]
Dose Level (mg/kg/day)	0	1.5	5	15	NA
Total No. of Animals	70	60	60	70	NA
No. of Surviving Animals (Percentage)	22(31)	15(25)	24(40)	27(39)	NA
Log-Rank P-value	NA	0.6128	0.4285	0.3721	0.2260
Wilcoxon P-value	NA	0.7661	0.5849	0.3746	0.2534

	PF-04971729				
Sex	Females[b]				Trend (1,2,3,4)[a]
Dose Level (mg/kg/day)	0	1.5	5	15	NA
Total No. of Animals	70	60	60	70	NA
No. of Surviving Animals (Percentage)	18(26)	24(40)	26(43)	34(49)*	NA
Log-Rank P-value	NA	0.1776	0.0611	0.0103*†	0.0273*†
Wilcoxon P-value	NA	0.3092	0.0917	0.0194*†	0.0379*†

* = Statistically significant at 5% level ($p \leq 0.05$); † = Significant decrease; NA = Not applicable.

a Trend groups 1,2,3,4 dose response.

b Terminal Euthanasia for females was on Day 645 of the dosing phase.

(Table excerpted from sponsor's package)

Figure 8: Percent Survival at Terminal Necropsy - Rat Study TT#13-7800

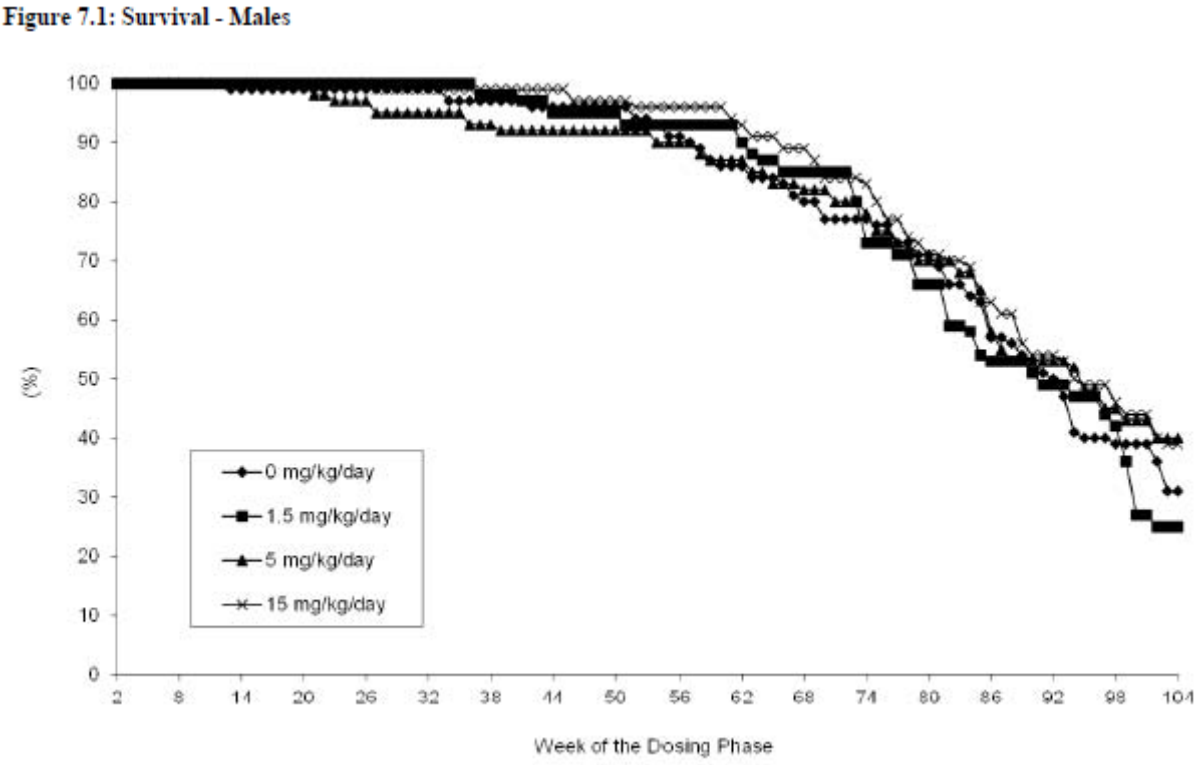
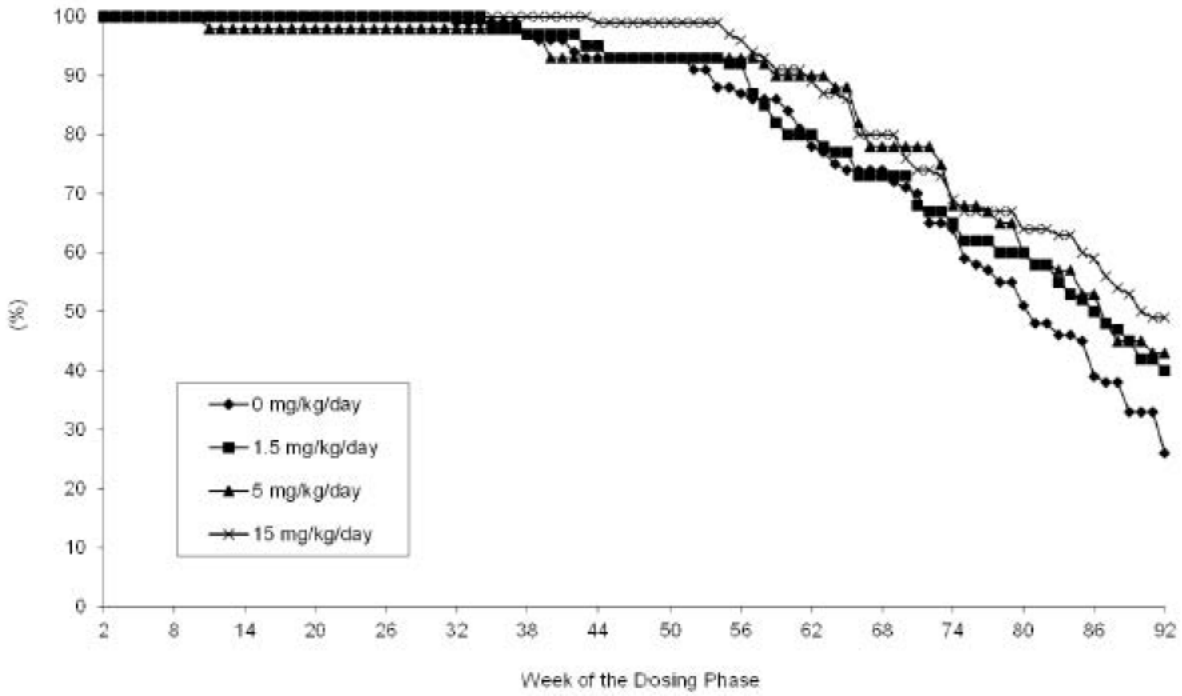


Figure 7.2: Survival - Females



(Figure excerpted from sponsor's package)

Clinical Signs

There were no adverse drug-related clinical observations. Slight trends for increased numbers of animals with the appearance of thinness were apparent at 15 mg/kg in females, and possibly males. Thinness is consistent with drug-related decreases in body weights and weight gain in both sexes; thus, it is considered likely to be treatment-related.

Body Weights

Drug-related decreases in body weights were observed at all doses and in both sexes throughout the majority of the study. Statistically significant decreases in male body weights were observed beginning on Day 57, reaching differences of 4-6% by Week 13 and 11-19% in males surviving to the end of the study (Week 104). Statistically significant decreases in female body weights were observed beginning on Day 29, reaching differences of 2-7% by Week 13 and 8-17% in females surviving to scheduled necropsy (Week 92). Decreases in body weights are consistent with a compensatory response to PD-related increases in glucosuria and are considered to be drug-related, but did not appear to interfere with animal survival.

Table 54: Body Weight - Rat Study TT#13-7800

Body Weight
Males

Dose (mg/kg)	Day 92 (Week 13)		Day 729 (Week 104)	
	BW (g)	BW % Control	BW (g)	BW % Control
0	613	-	940	-
1.5	586*	95.6% (↓4.4%)	838*	89.1% (↓10.9%)
5	585*	95.4% (↓4.6%)	812*	86.4% (↓13.6%)
15	579*	94.5% (↓5.5%)	762*	81.1% (↓18.9%)
Females				
Dose (mg/kg)	Day 92 (Week 13)		Day 645 (Week 92)	
	BW (g)	BW % Control	BW (g)	BW % Control
0	316	-	526	-
1.5	310	98.1% (↓1.9%)	486	92.4% (↓7.6%)
5	301*	95.3% (↓4.7%)	445*	84.6% (↓15.4%)
15	295*	93.4% (↓6.6%)	436*	82.9% (↓17.0%)

* p value < 0.05

Figure 9: Body Weight - Rat Study TT#13-7800

Figure 7.3: Body Weight - Males

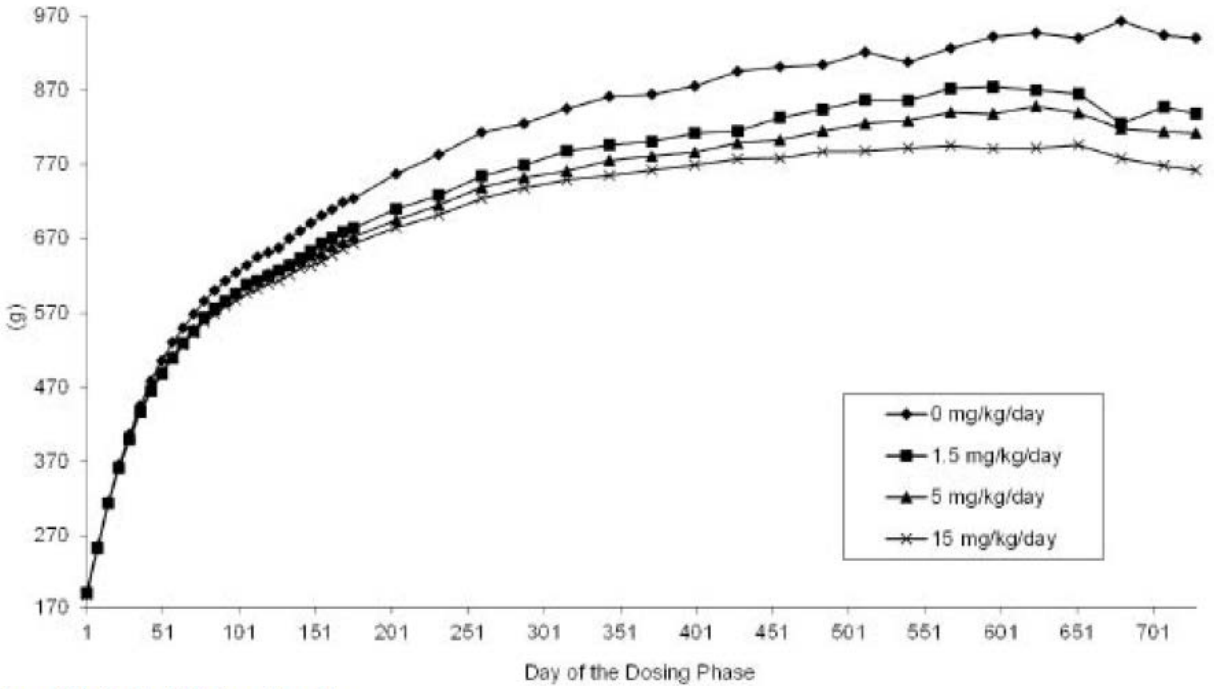
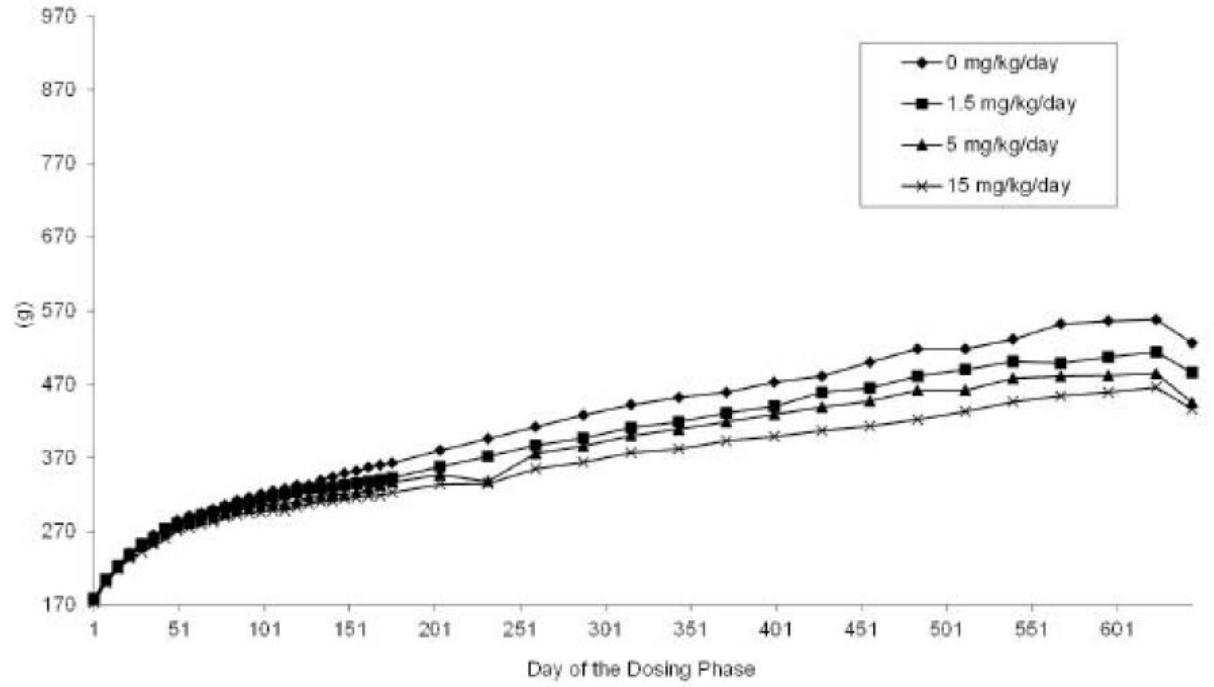


Figure 7.4: Body Weight - Females



(Figures excerpted from sponsor's package)

Feed Consumption

Drug-related increases in food consumption were observed at all doses and in both sexes throughout the entire study. Statistically significant increases in male food consumption intervals were observed beginning with the second week (Day 8-15), reaching differences 18-25% higher than controls by Week 13 and averaging 21-25% higher than controls over the course of the entire study. Statistically significant increases in female food consumption intervals were observed beginning with the second week (Day 8-15), reaching differences 18% higher than controls by Week 13 and averaging 11-17% higher than controls over the course of the entire study. Increases in food consumption are consistent with a compensatory response to PD-related increases in glucosuria and are consistent with the SGLT2 inhibitor drug class.

Table 55: Food Consumption - Rat Study TT#13-7800

Food Consumption				
Males				
Dose (mg/kg)	**Day 85-92 (Week 13)**		**Day 1-729 (entire study duration)**	
	g/animal/day	% Control	g/animal/day	% Control
0	28	-	28	-
1.5	33*	117.9%	34*	121.4%
5	35*	125.0%	34*	121.4%
15	35*	125.0%	35*	125.0%
Females				
Dose (mg/kg)	**Day 92 (Week 13)**		**Day 1-624 (entire study duration)**	
	g/animal/day	% Control	g/animal/day	% Control
0	17	-	18	-
1.5	20*	117.6%	21*	116.7%
5	20*	117.6%	20*	111.1%
15	20*	117.6%	21*	116.7%

* p value < 0.05

Gross Pathology

Drug-related observations of enlarged kidney, urinary bladder and ureter were observed in both males and females. Observations of large bladder in males at all doses and females at ≥5 mg/kg are consistent with PD-related increases in urine volume and also correlated with microscopic findings of inflammation. Observations of large kidney at 15

mg/kg correlated with microscopic findings of pelvic dilatation, nephropathy, and pyelonephritis. Large ureter observations in males at 15 mg/kg and females at ≥5 mg/kg generally correlated with ureter dilatation and/or inflammation. Increased observations of discolored kidney were also reported in 3-7% of males at all doses and in 4-5% of females at ≥5 mg/kg.

Table 56: Macroscopic Findings - Rat Study TT#13-7800

Text Table 4.2: Incidence and Percent Incidence of Test Article-Related Macroscopic Findings - All Animals

	PF-04971729							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	1.5	5	15	0	1.5	5	15
Number Examined	70	60	60	70	70	60	60	70
Kidney								
Large (Percentage)	3(4.3)	3(5.0)	4(6.7)	10(14)	3(4.3)	0(0.0)	3(5.0)	8(11)
Urinary Bladder								
Large (Percentage)	2(2.9)	4(6.7)	4(6.7)	9(13)	1(1.4)	1(1.7)	3(5.0)	2(2.9)
Ureter								
Large (Percentage)	1(1.4)	0 (0.0)	1(1.7)	4(5.7)	0(0.0)	0(0.0)	2(3.3)	3(4.3)

(Table excerpted from sponsor's package)

Histopathology

Note that an internal statistical analysis of the neoplastic data was performed Hepei Chen in the Division of Biometrics using pairwise comparisons of each treatment group against the vehicle control group using the Poly-k method.

Peer Review

Selected tissues from selected animals were peer reviewed by the sponsor.

Neoplastic

Significant drug-related increases in adrenal medulla neoplasms were observed in males, but were not observed in females. Several noteworthy neoplasms were observed in the renal and urinary tract, as well as the liver and brain; however, statistical significance was not achieved and these findings were not considered to be significantly drug-related.

Adrenal Medulla

A statistically significant trend (p value < 0.005) for dose-dependent increases in adrenal medulla benign PCC were observed in males at all doses (10-22%), reaching statistical significance by pairwise one-sided comparison (p value <0.01) at ≥5 mg/kg (15-22%), see Sponsor's Table 60. After adjustment for multiple testing, the increase in benign PCC at 15 mg/kg was statistically significant for a common tumor via analysis for dose response relationship (p value < 0.005) and pairwise comparison (p value <0.01) to vehicle control; however, the increased incidence at 5 mg/kg did not reach statistical significance for a pairwise comparisons test of a common tumor after poly-k adjustment

(see Chen's biostatistics review). Historical control data submitted by the sponsor from 20 carcinogenicity studies indicates that the mean incidence rate for spontaneous benign PCC is 10% in this strain of rat (Sponsor's Table 61), wherein incidence rates as high as 18% were reported, which is slightly above the 15% incidence rate observed at 5 mg/kg. On the other hand, the mortality weighted incidence rate at 5 mg/kg was 21.4% (reference Chen's biostatistics review), which was above that of historical controls. Thus, given the lack of consistent statistical significance for a common tumor (p value < 0.01) and an incidence rate potentially within the range of spontaneous findings in this strain of rat, the increase at 5 mg/kg of benign PCC observations alone may be considered to be equivocally drug-related. However, correlating hyperplastic data (see hyperplasia discussion below) provides supportive evidence that the incidences of PCC at 5 mg/kg may be drug-related. Nevertheless, only the increase in benign PCC at 15 mg/kg is considered to be unequivocally drug-related.

The highest incidence of malignant PCC was observed in 15 mg/kg males (5.8%) and occurred at an incidence rate nearly 2-fold higher than the reported spontaneous incidence range (0-3.08%). However, there was not a statistically significant or dose-dependent increase due to a relatively high incidence rate in concurrent controls (4.2%). Nevertheless, although not significantly drug-related, the incidence of malignant PCC at 15 mg/kg is notable.

A statistically significant dose-response trend (p value < 0.005) for dose-dependent increases in combined benign + malignant PCC were observed in treated males (13-26%), reaching statistical significance by one-sided pairwise comparison at 15 mg/kg (26%). It was noted that one animal had both malignant and benign PCC; therefore, this animal was only counted once in the sponsor's analysis of the combined tumor data. After adjustment for multiple testing, internal statistical analysis also revealed that the increase in combined benign + malignant PCC at 15 mg/kg was statistically significant (p value = 0.0042) via analysis for dose response relationship (p value < 0.005) and pairwise comparison (p value <0.01) to vehicle control, see Chen's biostatistics review. Furthermore, it is noted that the combined PCC incidence rate at 15 mg/kg was considered to be statistically significant, regardless of whether it is considered to be a rare or common tumor type. However, the apparent 2-fold increase in combined benign + malignant PCC at 5 mg/kg compared to concurrent control did not reach statistical significance (p value = 0.0846). Thus, only the combined benign +malignant PCC increase at 15 mg/kg was considered to be significantly drug-related.

Table 57: Adrenal Medulla Hyperplastic and Neoplastic Findings – Rat Study TT#13-7800

Text Table 4.3: Severity, Incidence, and Percent Incidence of Test Article-Related Hyperplastic and Neoplastic Findings - Adrenal Medulla - All Animals

	PF-04971729									
Sex	Males					Females				
Dose Level (mg/kg/day)	0	1.5	5	15	Trend[c] 1.2.3.4	0	1.5	5	15	Trend[c] 1.2.3.4
Number Examined	70	60	60	69		69	59	55	70	
Adrenal Medulla										
Hyperplasia										
Minimal	6	4	6	8	NA	3	0	2	3	NA
Slight	6	5	8	9	NA	3	2	0	2	NA
Moderate	4	5	9	6	NA	1	0	0	1	NA
Marked	0	1	0	3	NA	0	0	0	0	NA
Total (Percentage)	16 (23)	15 (25)	23 (38)	26 (38)	NA	7 (10)	2 (3.4)	2 (3.6)	6 (8.6)	NA
Pheochromocytoma										
Benign (Percentage)	2 (2.9)	6 (10)	9 (15)	15 (22)[a]	NA	2 (2.9)	1 (1.7)	0 (0.0)	3 (4.3)	NA
P value[b]	NA	0.0934	0.0087*§	0.0008*	0.0010†	NA	0.8505	1.0000	0.6072	0.2495
Malignant[¥] (Percentage)	3 (4.2)	2 (3.3)	1 (1.7)	4 (5.8)	NA	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	NA
P value[b]	NA	0.7209	0.9436	0.5711	0.3226	NA	1.0000	1.0000	1.0000	1.0000
Benign + Malignant (Percentage)	5 (7.1)	8 (13)	10 (17)	18[a,d] (26)	NA	3 (4.3)	1 (1.7)	0 (0.0)	3 (4.3)	NA
P value[b]	NA	0.1607	0.0715	0.0022*[d]	0.0025†	NA	0.9365	1.0000	0.7930	0.3989

For common tumors:
* = Pairwise one-sided comparison is statistically significant at the 0.01 level.
† = Trend statistically significant at the 0.005 level.
§ = Considered an aberration and not biologically meaningful.
¥ = No test article-related effect noted.
NA = Not applicable.
a Test article-related.
b Pairwise one-sided comparisons with controls.
c Trend groups 1,2,3,4 dose response.
d Animal B78106 had both benign and malignant pheochromocytomas. There was a total tumor incidence of 19 in a total of 18 animals. The statistical analysis and percentage calculations were based on the animal incidence value of 18.

(Table excerpted from Chen's Biostatistics review)

Table 58: Internal Statistical Analysis of Rat Tumor Data – Rat Study TT#13-7800

Organ name	Tumor name	Ertugliflozin			
		0 mg/kg	1.5 mg/kg	5 mg/kg	15 mg/kg
Adrenal, Medulla	Benign Pheochromocytoma	2/70 (46) p = 0.0018*	6/60 (40) p = 0.0925	9/60 (42) p = 0.0167@	15/69 (51) **p = 0.0010***
	Malignant Pheochromocytoma	3/70 (47) p = 0.2862	2/60 (39) p = 0.4112	1/60 (40) p = 0.6285	4/69 (49) p = 0.5235
	Benign + Malignant Pheochromocytoma	5/70 (47) p = 0.0039*	8/60 (40) p = 0.1791	10/60 (42) p = 0.0846	18/69 (52) **p = 0.0042***

X/YY (ZZ): X = number of tumor bearing animals; YY = unweighted total number of animals observed; ZZ

= mortality weighted total number of animals
* = Statistically significant in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons
@ = Not statistically significant at 0.01 level in common tumor for test of pairwise comparisons

Table 59: Spontaneous Adrenal Medulla PCC in Historical Controls – Rat Study TT#13-7800

Text Table 4.4: Incidence and Range of Benign Pheochromocytoma in Sprague Dawley Rats from 104 Week Studies

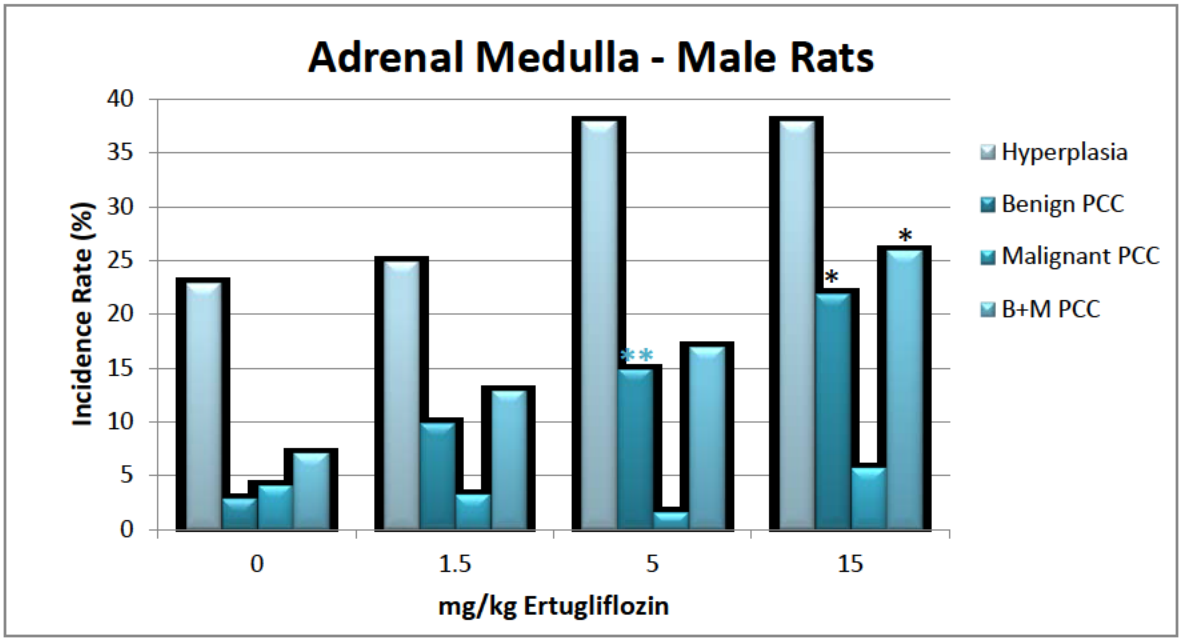
Sex	Males		
	Incidence/Mean (%)	Range (%)	Median (%)
Adrenal Medulla[a]			
Benign pheochromocytoma	144/1404 (10.26)	0-24	9.76
Malignant pheochromocytoma	15/1404 (1.07)	0-3.08	0.38

a Data from 20 studies conducted in (b) (4) from 2005-2015.

(Table excerpted from sponsor's package)

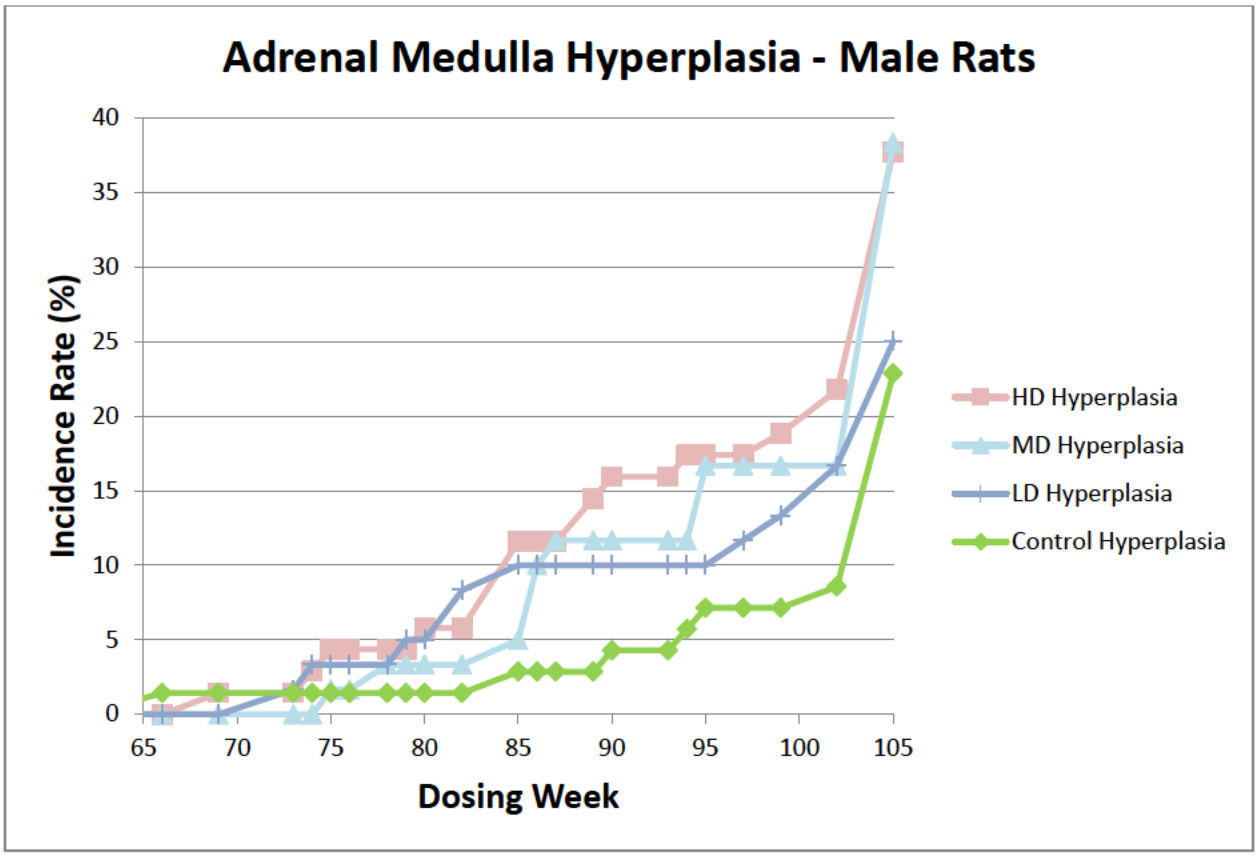
Increases in total incidences of adrenal medulla hyperplasia were observed in males at ≥5 mg/kg, with a trend for increased severity in treatment groups including marked hyperplasia in 1 animal at 1.5 mg/kg and 3 at 15 mg/kg (Sponsor's Table 60). Human data reported in the literature indicate that there is a strong molecular relationship between adrenal medulla hyperplasia and PCC, such that the adrenal medulla hyperplastic lesions "should be regarded as PCC precursor lesions and not as non-neoplastic hyperplasias" (Korpershoek et al. 2014). It is noted that in the rats, the total incidence rate for hyperplasia plateaued at 5 mg/kg, which correlated with an increase in benign and malignant PCC at 15 mg/kg (Figure 6). In addition, incidences of hyperplasia and PCC were observed earlier in treated animals with an apparent dose-dependency, particularly at Weeks ≥86 (Figure 7), with 2- to 2.5-fold increases in incidence rates of hyperplasia in unscheduled deaths (prior to Week 105) compared to concurrent controls. The decreases in latency for hyperplasia observations are consistent with the observed increases in severity of hyperplasia in treated groups. Together, the shortened latency for increases in incidence rates of both PCC and hyperplasia are consistent with a treatment-related propensity and/or increased susceptibility toward abnormal proliferation of adrenal medulla chromaffin cells. Furthermore, the increases in findings of adrenal medulla hyperplasia consistently preceded findings of PCC in all dosing groups and in controls (Figure 8: Adrenal Medulla Hyperplasia vs PCC Time Course), which is consistent with hyperplasia being a precursor lesion to PCC development. Overall, the rat adrenal medulla PCC and hyperplasia data are considered to be consistent with a continuum of tumor development; thus, the increased incidences of adrenal medulla hyperplasia at ≥5 mg/kg may represent PCC precursor lesions. The hyperplastic data provide further support that the increase in PCC incidences at 5 mg/kg is potentially drug-related, despite the equivocal statistical significance in one-sided comparisons with or without poly-k adjustment.

Figure 10: Male Adrenal Medulla Hyperplastic and Neoplastic Findings – Rat Study TT#13-7800



* Unequivocally statistically significant
** Equivocally statistically significant
B+M = benign+malignant

Figure 11: Timing of Adrenal Medulla Hyperplasia and Neoplasia Observations – Rat Study TT#13-7800



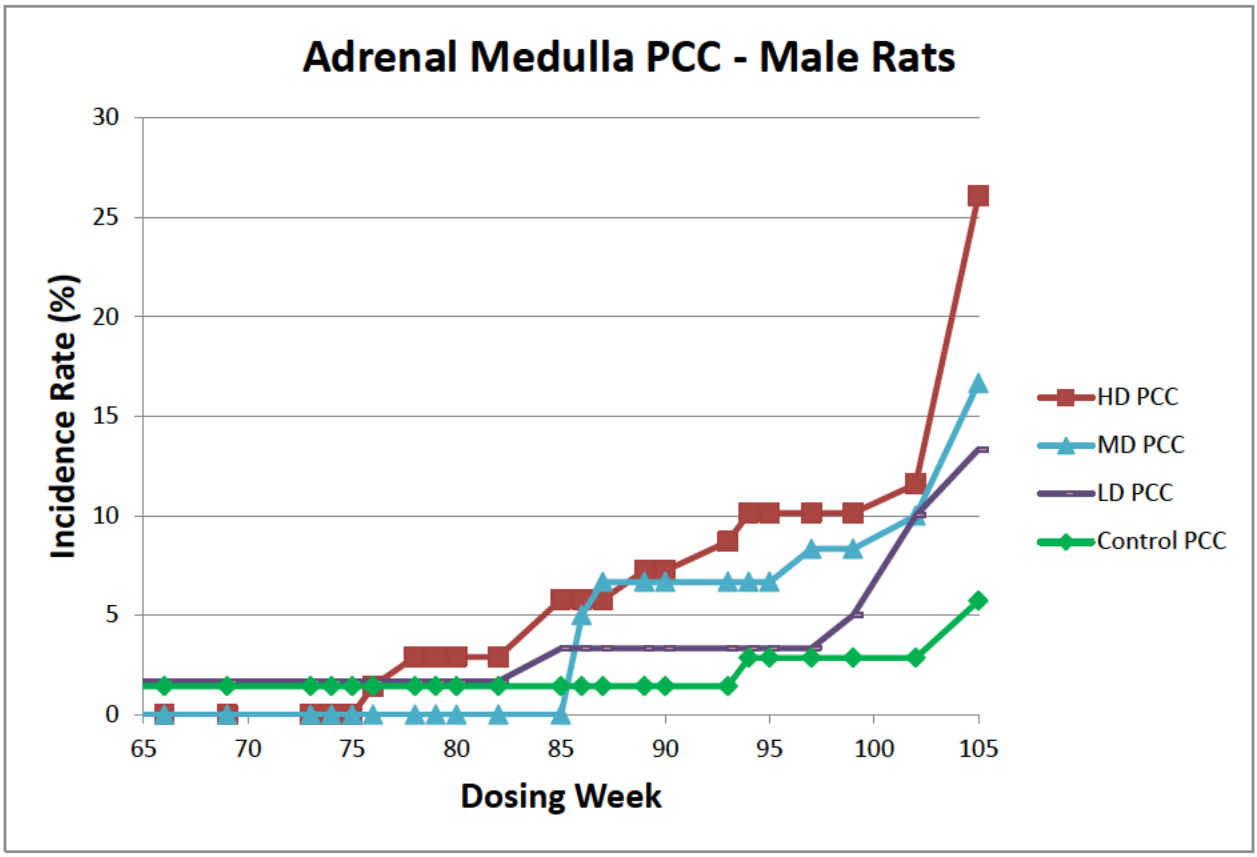
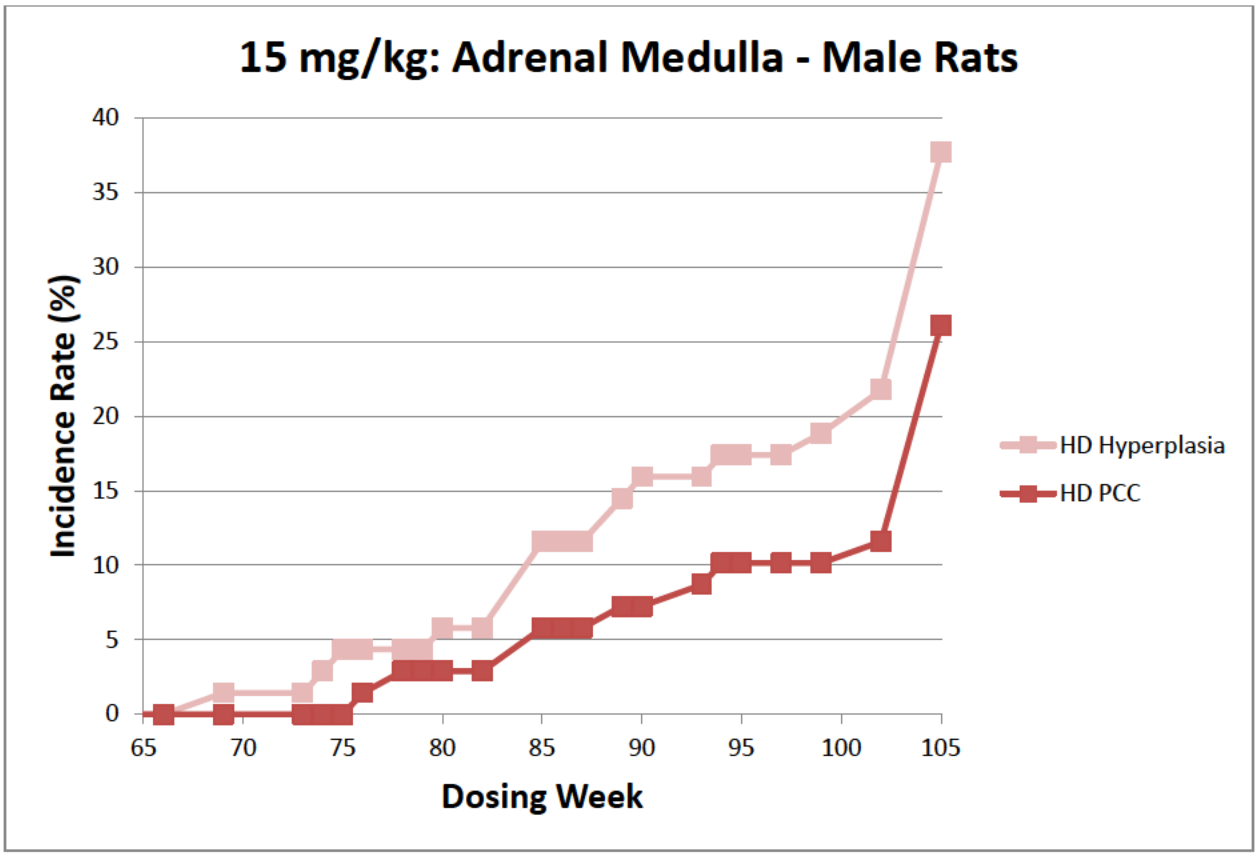
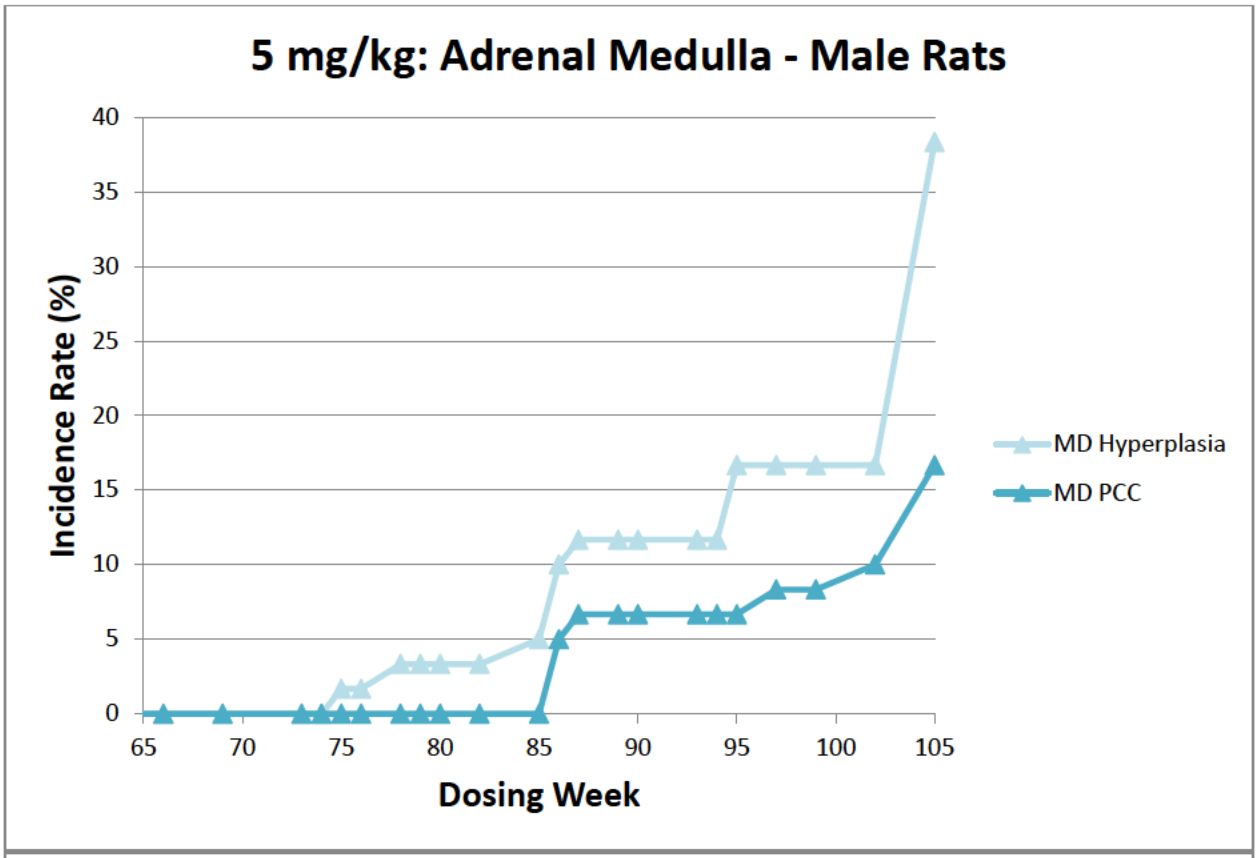
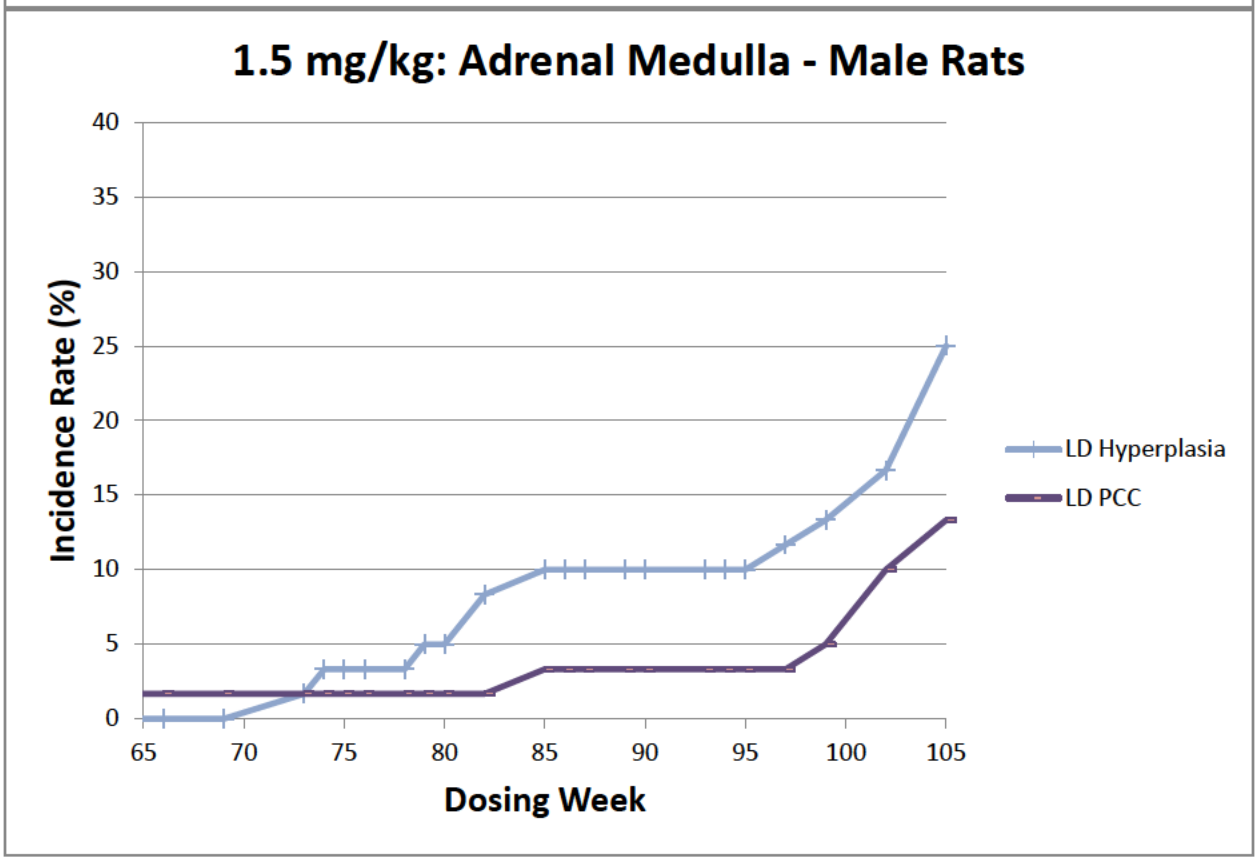
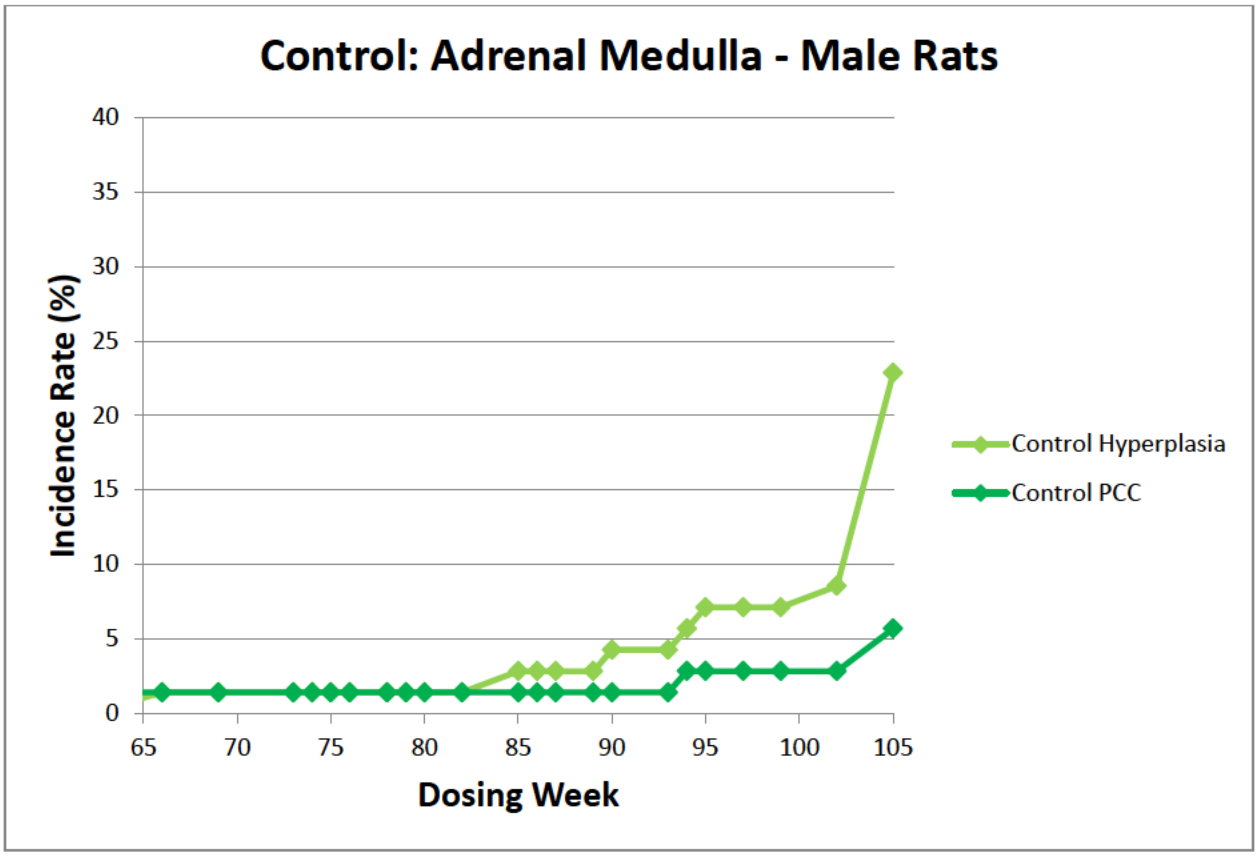

Adrenal Medulla PCC - Male Rats
Incidence Rate (%)
30
25
20
15
10
5
0
65
70
75
80
85
90
95
100
105
Dosing Week
HD PCC
MD PCC
LD PCC
Control PCC

Figure 12: Adrenal Medulla Hyperplasia vs PCC Time Course – Rat Study TT#13-7800




5 mg/kg: Adrenal Medulla - Male Rats
Incidence Rate (%)
0
5
10
15
20
25
30
35
40
65
70
75
80
85
90
95
100
105
Dosing Week
MD Hyperplasia
MD PCC


1.5 mg/kg: Adrenal Medulla - Male Rats
Incidence Rate (%)
0
5
10
15
20
25
30
35
40
65
70
75
80
85
90
95
100
105
Dosing Week
LD Hyperplasia
LD PCC



In females, there were no statistically significant or dose-related increases in incidences or severity of benign PCC or combined benign + malignant PCC. There were also no dose-related increases in adrenal medulla hyperplasia incidence or severity in female rats.

Other Notable Neoplasms

It is noteworthy that incidences of genitourinary neoplasia were associated with 3 unscheduled deaths at 15 mg/kg (2 male and 1 female). Since ertugliflozin treatment has been associated with drug-related effects on the urinary tract, including hyperplasia, the findings of genitourinary neoplasia are considered to be noteworthy. An apparent increase in renal tubule cell carcinoma was also noted in 15 mg/kg females (4.3%) compared to concurrent current controls (1.4%); however, statistical significance was not achieved. Thus, although noteworthy due to the non-neoplastic adverse drug-related effects on the renal system (described below), renal tubule cell carcinoma is not a statistically significant drug-related effect.

An apparent dose-related increase in hepatocellular adenoma and carcinoma was noted in males at ≥5 mg/kg; however, statistical significance was not achieved and this is not considered to be significantly drug-related. Similarly, an apparent increase in brain granular cell tumors, astrocytoma and oligodendrogliomas were noted in treated animals; however, the incidence rates are likely to be within the normal biological range and/or did not reach statistical significance.

Table 60: Notable Non-significant Neoplasm Findings - Rat Study TT#13-7800

Non-significant Neoplasms									
Tissue		Male (mg/kg)				Female (mg/kg)			
		0 (n=70)	1.5 (n=60)	5 (n=60)	15 (n=70)	0 (n=70)	1.5 (n=60)	5 (n=60)	15 (n=70)
Brain	Granular cell tumor	1	0	0	3 (4.3%)	0	0	0	1
	Astrocytoma Benign + Malignant	0	1	1	0	0	0	1	1
	Oligodendroglioma,	0	0	0	1				
	Astrocytoma + oligodendroglioma	0	1 (1.7%)	1 (1.7%)	1 (1.4%)	0	0	1 (1.7%)	1 (1.4%)
Kidney	Tubule cell adenoma	0	2	0	0	1	0	0	0
	Tubule cell carcinoma	1	2	0	0	1	0	0	3
	Adenoma + carcinoma	1 (1.4%)	4 (6.7%)	0	0	1 (1.4%)	0	0	3 (4.3%)
Liver	Hepatocellular adenoma	1	1	1	3	0	1	3	0
	Hepatocellular carcinoma	0	0	1	1	0	0	0	0
	Adenoma + carcinoma	1	1	2 (3.3%)	4 (5.7%)	0	1	3 (5.0%)	0

An apparent dose-related increase in testis B-interstitial cell tumors was noted at ≥5 mg/kg (5-5.7%) compared to concurrent controls (1.4%); however, statistical significance was not achieved and these findings were considered likely to be incidental.

A slight dose-related increase in myeloid hyperplasia in sternum bone marrow was noted in treated females (11.7% to 14.3%) compared to concurrent controls (8.6%); however, statistical significance was not achieved and this finding is not considered to be significantly drug-related.

Non Neoplastic

Drug-related non-neoplastic findings were reported in the renal system (kidney, ureter, and urinary bladder) and alimentary system (tongue, non-glandular stomach, and pancreas). There were no drug-related bone findings, although femur and sternum with bone marrow were examined microscopically for all animals.

Renal System

Evidence of adverse drug-related kidney and ascending urinary tract infection was present in males at all doses and in females at ≥5 mg/kg. Dose-related increases in severity of moderate to marked pyelonephritis were associated with unscheduled deaths in males at all doses and in females at ≥5 mg/kg (Table 23). Chronic-active pyelonephritis was reported at ≥5 mg/kg (Table 22) and was characterized by expansion of the pelvis by inflammatory cells (macrophages, lymphocytes, and neutrophils), cell debris, hyperplastic transitional cells, and fibrosis, as well as evidence of fungal yeast and hyphae infection. Drug-related increases in renal tubule dilatation, characterized by enlarged cortical and medullary tubules due to attenuated epithelial

cells and increased luminal space, were observed in males (17.1-18.3%) and females (21.4-25%) at ≥5 mg/kg, with regard to incidence rates and severities (slight to moderate). Correlating increases in incidence rates and severity of renal tubule degeneration generally accompanied findings of tubular dilation in both males (2.9%) and females (8.6%) at 15 mg/kg, reaching marked severity in one 15 mg/kg male. Furthermore, kidney tubule degeneration was associated with unscheduled deaths in males and females at 15 mg/kg. The findings are consistent with previous toxicology studies with ertugliflozin and other SGLT2 inhibitors, and are considered likely to be secondary to chronic PD-related diuresis. Dose-dependent increases in inflammation and transitional cell hyperplasia were also reported in the urinary bladder of both sexes at all doses, and correlated with incidences of pyelonephritis in animals at ≥5 mg/kg. Urinary bladder inflammation was characterized by luminal, mucosal, submucosal, and transmural accumulation of inflammatory lymphocytes, macrophages and neutrophils. Urinary bladder transitional cell hyperplasia was characterized by thickened mucosa and increased numbers of epithelial cells, often with papillar projections. Ureter microscopic findings of inflammation, dilation, and/or transitional cell hyperplasia were noted in males at ≥5 mg/kg and females at all doses, which correlated with kidney findings of pyelonephritis. It was also noted that yeast and/or hyphae were observed in inflammatory exudate in the bladder and/or kidney of several animals at ≥5 mg/kg. It was also noted that thrombus/infarct was associated with marked pyelonephritis and unscheduled death in one 15 mg/kg female. Overall, inflammation and hyperplasia of the kidney, urinary bladder, and ureters were considered to be due to ascending yeast and/or bacterial infections secondary to PD-related glucosuria.

Table 61: Kidney and Urinary Tract Microscopic Non-neoplastic Findings - All Rats Study TT#13-7800

	PF-04971729							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	1.5	5	15	0	1.5	5	15
Kidney								
Number Examined	70	60	60	70	70	60	60	70
Dilatation, tubule(s)								
Minimal	5	5	8	5	8	4	12	9
Mild	0	0	3	5	0	1	3	2
Moderate	0	0	0	2	1	0	0	4
Total (Percentage)	5(7.1)	5(8.3)	11(18)	12(17)	9(13)	5(8.3)	15(25)	15(21)
Dilatation, pelvis								
Minimal	7	7	6	9	5	5	4	5
Mild	3	1	2	4	0	2	1	4
Moderate	0	0	1	0	0	0	1	2
Total (Percentage)	10(14)	8(13)	9(15)	13(19)	5(7.1)	7(12)	6(10)	11(16)
Degeneration, tubule								
Minimal	0	0	0	0	0	0	0	1
Mild	0	0	0	0	0	0	0	2
Moderate	0	0	0	1	1	0	0	3
Marked	0	0	0	1	0	0	0	0
Total (Percentage)	0(0.0)	0(0.0)	0(0.0)	2(2.9)	1(1.4)	0(0.0)	0(0.0)	6(8.6)
Pyelonephritis, chronic-active								
Mild	0	0	1	0	0	0	0	1
Marked	0	0	0	0	0	0	1	2
Total (Percentage)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	3(4.3)
Urinary Bladder								
Number Examined	70	60	60	70	70	60	59	70
Hyperplasia, transitional call								
Minimal	0	1	1	3	0	1	1	0
Mild	0	5	4	4	0	0	1	3
Moderate	2	1	7	4	0	0	1	2
Marked	1	2	3	6	0	0	0	0
Total (Percentage)	3(4.3)	9(15)	15(25)	17(24)	0(0.0)	1(1.7)	3(5.1)	5(7.1)
Inflammation								
Minimal	0	3	1	2	0	3	1	0
Mild	0	3	6	4	2	1	1	4
Moderate	3	6	6	8	0	0	2	1
Marked	0	0	3	5	0	0	0	0
Total (Percentage)	3(4.3)	12(20)	16(27)	19(27)	2(2.9)	4(6.7)	4(6.8)	5(7.1)

		PF-04971729							
	Sex	Males				Females			
	Dose Level (mg/kg/day)	0	1.5	5	15	0	1.5	5	15
Ureter									
	Number Examined	67	59	60	70	68	58	60	67
Inflammation									
	Minimal	0	0	0	1	0	1	1	3
	Mild	1	2	2	5	1	2	2	2
	Moderate	0	0	0	1	0	0	1	1
	Total (Percentage)	1(1.5)	2(3.3)	2(3.3)	7(10)	1(1.5)	3(5.2)	4(6.7)	6(9.0)
Dilatation									
	Minimal	0	0	0	3	0	1	1	2
	Mild	0	0	0	3	1	1	1	0
	Total (Percentage)	0(0.0)	0(0.0)	0(0.0)	6(8.6)	1(1.5)	2(3.4)	2(3.3)	2(4.5)

Test Article	(dosage)	1	2	3	4
PF-04971729	mg/kg/day	0	1.5	5	15

Tissue/ Observation	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	4/1/M	1/1/F	2/1/F	3/1/F	4/1/F
	Number of Animals:	70	60	60	70	70	60	60	70
Ureter	Number Examined:	67	59	60	70	68	58	60	67
	Unremarkable:	66	57	56	62	66	54	53	61
Hyperplasia, transitional cell									
	finding not present -	67	59	59	69	68	57	57	64
	minimal 1	0	0	0	0	0	1	1	2
	slight 2	0	0	1	1	0	0	2	1
	Total Incidence:	0	0	1	1	0	1	3	3

(Tables excerpted from sponsor's package)

Potentially drug-related increases in severity of nephropathy were observed in drug-treated animals. Increases in moderate severity were reported in 5.7-10% of females and marked severity was reported in 1.4-7.1% of males at all doses, with dose-dependency in males. However, it is noted that the total incidence rates in drug-treated female groups remained within the published spontaneous incidence rate of 61.1% for this species (Brix et al. 2005). The incidence rate in 15 mg/kg males (84.2%) was 23% higher than the spontaneous background rate; however, it remained lower than that of concurrent control males (88.6%), which were considered to be unusually high. Although, there was not an increase in the total number of incidences of nephropathy associated with unscheduled death, there was an increase in severity of nephropathy at death. Given the increases in severity of nephropathy with drug treatment, it is likely that ertugliflozin treatment correlates with exacerbation and/or progression of spontaneous nephropathy in this species.

Other notable kidney findings included renal cysts and the presence of hyaline droplets and/or mineralization. Dose-dependent increases in incidence rates and severity of renal cysts were apparent in females at ≥5 mg/kg, reaching slight to moderate severity in 5.7% of females at 15 mg/kg. Two incidences of marked renal cyst observations also indicated a slight increase in severity in males at ≥5 mg/kg; however, overall incidence rates in males were similar between the control (5.7%) and 15 mg/kg (7.1%) groups. Small increases in incidence rates and/or severity of hyaline droplets or mineralization

were also observed at 15 mg/kg. Although notable, these findings were not considered to be adverse.

Table 62: Kidney Microscopic Non-neoplastic Findings – Unscheduled Deaths Study TT#13-7800

Summary of Severity of Microscopic Observations
Unscheduled Euthanasias and Deaths

Test Article	(dosage)	1	2	3	4
PF-04971729	mg/kg/day	0	1.5	5	15

Tissue/ Observation	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	4/1/M	1/1/F	2/1/F	3/1/F	4/1/F
	Number of Animals:	48	45	36	43	52	36	34	36
Kidney	Number Examined:	48	45	36	43	52	36	34	36
	Unremarkable:	4	4	3	6	15	11	7	8
Degeneration, tubule									
	finding not present -	48	45	36	41	51	36	34	30
	minimal 1	0	0	0	0	0	0	0	1
	slight 2	0	0	0	0	0	0	0	2
	moderate 3	0	0	0	1	1	0	0	3
	marked 4	0	0	0	1	0	0	0	0
	Total Incidence:	0	0	0	2	1	0	0	6
Dilatation, tubule(s)									
	finding not present -	43	41	25	32	45	31	21	22
	minimal 1	5	4	8	5	6	4	10	8
	slight 2	0	0	3	4	0	1	3	2
	moderate 3	0	0	0	2	1	0	0	4
	Total Incidence:	5	4	11	11	7	5	13	14
Pyelonephritis									
	finding not present -	46	42	32	38	42	33	32	32
	minimal 1	0	0	0	1	6	2	1	0
	slight 2	2	1	1	0	2	1	0	1
	moderate 3	0	2	3	2	2	0	1	0
	marked 4	0	0	0	2	0	0	0	3
	Total Incidence:	2	3	4	5	10	3	2	4
Pyelonephritis, chronic-active									
	finding not present -	48	45	35	43	52	36	33	33
	slight 2	0	0	1	0	0	0	0	1
	marked 4	0	0	0	0	0	0	1	2
	Total Incidence:	0	0	1	0	0	0	1	3
Nephropathy, chronic progressive									
	finding not present -	7	7	6	11	22	14	12	22
	minimal 1	20	15	13	8	19	12	11	9
	slight 2	13	17	10	11	10	8	9	3
	moderate 3	8	6	5	11	1	2	2	2
	marked 4	0	0	2	2	0	0	0	0
	Total Incidence:	41	38	30	32	30	22	22	14

(Table excerpted from sponsor's package and cropped and highlighted)

Table 63: Other Kidney Microscopic Non-neoplastic Findings - Rat Study TT#13-7800

Summary of Severity of Microscopic Observations
All Animals

Test Article	(dosage)	1	2	3	4
PF-04971729	mg/kg/day	0	1.5	5	15

Tissue/ Observation	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	4/1/M	1/1/F	2/1/F	3/1/F	4/1/F
	Number of Animals:	70	60	60	70	70	60	60	70
Kidney	Number Examined:	70	60	60	70	70	60	60	70
Cyst									
	finding not present -	66	54	54	65	69	59	58	66
	minimal 1	1	2	0	0	1	1	1	0
	slight 2	1	1	4	4	0	0	1	2
	moderate 3	2	3	1	0	0	0	0	2
	marked 4	0	0	1	1	0	0	0	0
	Total Incidence:	4	6	6	5	1	1	2	4
Hyaline droplet, tubule cell									
	finding not present -	65	60	58	66	70	60	60	69
	slight 2	2	0	0	1	0	0	0	0
	moderate 3	2	0	1	0	0	0	0	1
	marked 4	1	0	1	3	0	0	0	0
	Total Incidence:	5	0	2	4	0	0	0	1
Mineralization									
	finding not present -	69	60	60	67	70	60	60	70
	slight 2	1	0	0	2	0	0	0	0
	moderate 3	0	0	0	1	0	0	0	0
	Total Incidence:	1	0	0	3	0	0	0	0
Nephropathy, chronic progressive									
	finding not present -	8	8	9	11	29	19	20	34
	minimal 1	23	16	16	11	27	25	22	22
	slight 2	19	23	21	20	13	10	14	10
	moderate 3	20	12	12	23	1	6	4	4
	marked 4	0	1	2	5	0	0	0	0
	Total Incidence:	62	52	51	59	41	41	40	36
Pyelonephritis									
	finding not present -	65	55	54	63	57	56	55	64
	minimal 1	2	0	0	1	9	3	2	0
	slight 2	2	2	2	2	2	1	1	1
	moderate 3	1	3	4	2	2	0	2	2
	marked 4	0	0	0	2	0	0	0	3
	Total Incidence:	5	5	6	7	13	4	5	6

(Table excerpted, cropped and high-lighted from sponsor's package)

Alimentary System

Drug-related non-neoplastic findings of the alimentary system were reported in the tongue, nonglandular stomach and pancreas in both males and females. Although some alimentary system findings were evident at all doses, only the increases in incidence and/or severity at doses of ≥5 mg/kg in males and 15 mg/kg in females were considered likely to be toxicologically significant and/or adverse.

Dose-dependent increases in incidence and severity of tongue inflammation (minimal to moderate) and hyperplasia (minimal to marked) were observed at all doses, reaching up

to 17% of males and 7% of females. Tongue mucosal hyperplasia was characterized by basilar papillary extensions into the lamina propria formed by increased numbers of epithelial cells and thickened stratified squamous epithelium. The findings of tongue hyperplasia were accompanied by mucosal inflammation characterized by infiltrating lymphocytes and macrophages at the junction of the mucosa and lamina propria.

Increased incidences of nonglandular stomach erosion/ulcer and hyperplasia/hyperkeratosis were reported in males at ≥5 mg/kg and females at 15 mg/kg, but were independent of dose. Nonglandular stomach erosion was characterized by partial or complete focal loss of the mucosa lining along with neutrophils and cell debris. Incidences of erosion were also often accompanied by findings of hyperplasia characterized increased epithelial thickness with increased cell numbers and multiple layers of surface keratin.

Table 64: Tongue & Stomach Microscopic Non-neoplastic Findings - Rat Study TT#13-7800

		PF-04971729							
	Sex	Males				Females			
	Dose Level (mg/kg/day)	0	1.5	5	15	0	1.5	5	15
Tongue									
	Number Examined	70	59	60	70	70	59	60	70
Hyperplasia, mucosal									
	Minimal	0	1	4	4	0	2	0	2
	Mild	0	0	4	5	0	0	1	1
	Moderate	0	0	2	1	0	0	0	2
	Marked	0	0	0	1	0	0	0	0
	Total (Percentage)	0(0.0)	1(1.7)	10(17)	12(17)	0(0.0)	2(3.4)	1(1.7)	5(7.1)
Inflammation, mucosal									
	Minimal	0	1	5	4	0	3	0	2
	Mild	0	0	3	4	0	0	2	4
	Moderate	0	0	2	2	0	0	1	0
	Total (Percentage)	0(0.0)	1(1.7)	10(17)	10(14)	0(0.0)	3(5.1)	3(5.0)	6(8.6)
Stomach, Nonglandular									
	Number Examined	70	59	60	70	70	60	60	70
Erosion/ulcer									
	Minimal	0	2	8	1	0	0	0	1
	Mild	2	2	6	5	1	1	1	5
	Moderate	1	0	1	1	0	0	0	1
	Total (Percentage)	3(4.3)	4(6.8)	15(25)	7(10)	1(1.4)	1(1.7)	1(1.7)	7(10)
Hyperplasia/hyperkeratosis									
	Minimal	4	6	6	6	1	1	3	5
	Mild	5	4	16	14	2	1	3	5
	Moderate	2	1	0	1	1	0	1	1
	Marked	1	0	0	1	0	0	0	0
	Total (Percentage)	12(17)	11(19)	22(37)	22(31)	4(5.7)	2(3.4)	7(12)	11(16)

(Table excerpted from sponsor's package)

Increased incidences and/or severity of pancreatic zymogen granule decreases, characterized by smaller basophilic acinar cells with fewer eosinophilic granules, were reported in males at ≥5 mg/kg and females at all doses. The sponsor hypothesized that zymogen granule depletion incidences and increased severity in 15 mg/kg females

correlated with increased food consumption and may indicate an adaptive response of increased exocrine pancreatic enzyme turnover. Slight increases in incidence and severity of acinar cell hyperplasia were also apparent in males at ≥5 mg/kg, but not in females. Increased incidences and/severity of pancreas inflammation were also apparent in males and females at 15 mg/kg.

Table 65: Pancreas Microscopic Non-neoplastic Findings - Rat Study TT#13-7800

Summary of Severity of Microscopic Observations
All Animals

Test Article	(dosage)	1	2	3	4
PF-04971729	mg/kg/day	0	1.5	5	15

Tissue/ Observation	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	4/1/M	1/1/F	2/1/F	3/1/F	4/1/F
	Number of Animals:	70	60	60	70	70	60	60	70
Pancreas	Number Examined:	70	59	60	70	70	60	60	70
	Unremarkable:	18	23	23	22	42	40	46	40
Hyperplasia, acinar cell									
	finding not present -	67	55	52	64	66	58	60	68
	minimal 1	1	2	2	0	0	0	0	0
	slight 2	2	2	5	5	1	1	0	1
	moderate 3	0	0	1	1	3	1	0	1
	Total Incidence:	3	4	8	6	4	2	0	2
Inflammation									
	finding not present -	69	58	60	66	69	60	60	68
	minimal 1	0	0	0	3	1	0	0	0
	slight 2	1	1	0	0	0	0	0	1
	moderate 3	0	0	0	1	0	0	0	1
	Total Incidence:	1	1	0	4	1	0	0	2
Zymogen granules, decreased									
	finding not present -	62	54	55	60	67	57	52	53
	minimal 1	5	3	2	3	3	1	4	5
	slight 2	3	2	2	4	0	2	2	11
	moderate 3	0	0	1	3	0	0	2	1
	Total Incidence:	8	5	5	10	3	3	8	17

(Table excerpted from sponsor's package and highlighted)

Other Findings

Small, yet dose-dependent, increases in severity and incidence of heart inflammation were reported in females at ≥5 mg/kg associated with unscheduled deaths, reaching 4.3% of females at 15 mg/kg. Single incidences of endocardium hyperplasia were observed in males at ≥5 mg/kg, which increased in severity with dose. However, given the low incidence rates, these findings may be within the normal biological range for this strain and age of rat.

Table 66: Heart Microscopic Non-neoplastic Findings – Rat Study TT#13-7800

Table
Summary of Severity of Microscopic Observations
All Animals

Test Article	(dosage)	1	2	3	4
PF-04971729	mg/kg/day	0	1.5	5	15

Tissue/ Observation	Group/Subgroup/Sex: Number of Animals:	1/1/M 70	2/1/M 60	3/1/M 60	4/1/M 70	1/1/F 70	2/1/F 60	3/1/F 60	4/1/F 70
Heart	Number Examined:	70	60	60	69	70	60	60	70
	Unremarkable:	7	13	7	14	33	26	25	39
Hyperplasia, endocardium									
	finding not present -	70	60	59	68	70	60	60	70
	slight 2	0	0	1	0	0	0	0	0
	moderate 3	0	0	0	1	0	0	0	0
	Total Incidence:	0	0	1	1	0	0	0	0
Infiltrate, mononuclear cell									
	finding not present -	70	60	60	69	70	59	60	69
	minimal 1	0	0	0	0	0	1	0	0
	slight 2	0	0	0	0	0	0	0	1
	Total Incidence:	0	0	0	0	0	1	0	1
Inflammation									
	finding not present -	69	60	59	69	70	60	59	67
	minimal 1	1	0	0	0	0	0	0	0
	slight 2	0	0	0	0	0	0	1	1
	moderate 3	0	0	1	0	0	0	0	2
	Total Incidence:	1	0	1	0	0	0	1	3

(Table excerpted from sponsor's package and highlighted)

There were no bone microscopic findings.

Given that GI effects have been observed in previous toxicology studies, findings of moderate erosion/ulcer of the cecum reported at 15 mg/kg in 2 males and 1 female with unscheduled deaths were considered to be notable.

Toxicokinetics

Ertugliflozin concentrations were determined in plasma samples using a validated LC-MS/MS method.

AUC and C_{max} exposures increased slightly greater than dose-proportionally. The sponsor did not recognize a gender effect; however, consistent with previous nonclinical studies, female AUC and C_{max} exposures were consistently higher than males by 12 to 60%. Thus, male and female data were not combined for evaluations of safety margins. Exposures generally increased slightly (↑9-27%) during Week 52 compared to Week 26; however, there was not a significant accumulation in exposure over 13-months (52 weeks) of dosing compared to 6 months (Week 26). Furthermore, exposures were considered to be comparable to the previous 13-week and 26-week toxicology studies. T_{max} ranged between 1 and 4 hours postdose, generally increasing with increasing dose. Based on AUC values, at the therapeutic dose of 15 mg/day, ertugliflozin

exposures were associated with AUC ratios of 5x, 18x and 66x MRHD in males and 7x, 28x and 74x MRHD in females.

Table 67: Toxicokinetics - Rat Study #TT#13-7800

7.1. Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Week 26 and 52 after daily Oral Administration of PF-04971729

Dose (mg/kg/day)	Study Week	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
1.5	26	Male	627	1.0	6780
		Female	993	1.0	8010
		Overall	810	1.0	7400
	52	Male	719	1.0	6690
		Female	1080	1.0	9270
		Overall	898	1.0	7530
5	26	Male	2500	4.0	22300
		Female	3630	1.0	30800
		Overall	2900	1.0	26400
	52	Male	2940	1.0	24400
		Female	4310	1.0	39200
		Overall	3490	1.0	31500
15	26	Male	7320	1.0	78000
		Female	12200	4.0	91500
		Overall	9560	4.0	84800
	52	Male	7340	1.0	91000
		Female	12700	4.0	102000
		Overall	9600	4.0	96800

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed.

(Table excerpted from sponsor's package)

Metabolite TK Analysis

Exposures of the disproportional metabolites M5a and M5c were calculated based on previously determined percent ratios of each metabolite in total radioactivity of rat plasma (Table 29).

Table 68: Calculated Metabolite Exposures - Rat Study TT#13-7800

Species	Dose (mg/kg/day)	AUC (μg·h//mL)			
		Parent (Ertugliflozin)	Total Drug*	M5a (PF-06685948)	M5c (PF-06481944)
Human	15 (mg/day)	1.38	2.766	0.337	0.667
Male Rat (2-year)	1.5	6.69	7.41	0.0519	0.0519
	5	24.4	27.0	0.189	0.189
	15	91.0	100.8	0.705	0.705
Female Rat (2-year)	1.5	9.27	10.3	0.0308	0.0308
	5	39.2	43.4	0.130	0.130
	15	102	113.0	0.339	0.339

* Calculated Total Drug = Parent + all metabolites; based on percent ratios of total radioactivity in plasma from PK studies in human (study #PK042), rat (study #PK040) and dog (study #PK041)
Neoplastic NOAEL = highlighted in yellow

Based on the metabolite exposure calculations, safety margins were estimated for anticipated clinical metabolite exposure levels at the therapeutic dose (Table 30). Approximate clinical exposure levels of M5a were achieved at 15 mg/kg in both males (2x $MRHD_{AUC}$) and females (1x $MRHD_{AUC}$). Approximate clinical exposure levels of M5c were also achieved at 15 mg/kg in males (1x $MRHD_{AUC}$), but were not quite achieved in females (0.5x $MRHD_{AUC}$). Nevertheless, the exposure levels of both metabolites at 15 mg/kg were considered to be reasonably adequate.

Table 69: Metabolite Safety Margins - Rat Study TT#13-7800

Species	Dose (mg/kg/day)	Neoplastic $MRHD_{AUC}$		
		Parent (Ertugliflozin)	**M5a** (PF-06685948)	**M5c** (PF-06481944)
Human	15 (mg/day)	1.38	0.337	0.667
Male Rat (2-year)	1.5	5x	0.2x	0.1x
	5	18x	**0.6x**	**0.3**
	15	66x	2x	1x
Female Rat (2-year)	1.5	7x	0.1x	0.1x
	5	121x	0.4x	0.2x
	15	74x	**1x**	**0.5x**

Neoplastic NOAEL = highlighted in yellow

Dosing Solution Analysis

Dosing formulations were prepared at least every 4 weeks. Concentrations of dosing formulations prepared on Day 1, Weeks 26 and 52, Days 476 and 477, and Weeks 70, 78, and 104 were evaluated using a validated LC-MS/MS method. The top, middle and bottom of the dosing formulations prepared on Days 1, 331-336, and Week 53 were analyzed for homogeneity.

Stability was established for storage over a period of 1 month in the refrigerator and for 1 day when at room temperature. All dosing formulations were within ±10% of target concentration and were considered to be within the acceptable limits for target concentration and homogeneity.

8 Reproductive and Developmental Toxicology

Pivotal reproductive and developmental toxicology studies are summarized below and include a fertility and early embryonic development study in rats; two EFD studies in rats and rabbits; and a PPND study in rats. The sponsor also submitted a 10-week juvenile study in Sprague Dawley rats that is reviewed in detail in this review.

9.1 Fertility and Early Embryonic Development

Study: Oral Fertility and Embryonic Development Study of PF-04971729 in Male and Female Rats (Study TT #10-7835 / #10GR227)

Study no.:	TT107835 / 10GR227
Study report location:	eDr
Conducting laboratory and location:	Pfizer Global Research & Development, Groton, CT
Date of study initiation:	7/21/2010
GLP compliance:	Yes (no signature)
QA statement:	Yes
Drug, lot #, and % purity:	PF-04971729 (b) (4), Lot #GR02847, 75.6% purity

Key Study Findings

- Potentially drug-related mortality at 250 mg/kg/day (♂ & ♀)
- Female fertility NOAEL = 250 mg/kg/day
  - No drug-related effects on female mating and fertility parameters
  - Exposure margin = ~570x $MRHD_{AUC}$, based on average exposures in female rats (Table 4)
- Male fertility NOAEL = 250 mg/kg/day
  - No significant difference in male fertility parameters compared to historical controls
  - Exposure margin = ~480x $MRHD_{AUC}$, based on average exposures in male rats (Table 4)

Reviewer's Comments

Two mortalities occurred at 250 mg/kg (1♂ & 1♀), which were associated with clinical signs of hunched posture, decreased activity, cool to the touch, chromorhinorrhea, and/or rough haircoat. Although the causes of death were not determined, potential relationships to drug treatments were not ruled out. Furthermore, the mortalities are consistent with potentially drug-related mortalities observed at the same dose in the juvenile rat study #15GR084.

Observations of transient weight loss in both sexes, decreased body weights in males, and increases in food consumption were consistent with compensatory PD effects of drug-induced glucosuria.

There were no significant differences in fertility parameters of females compared to concurrent controls. Thus, the female fertility NOAEL was set at the high dose of 250 mg/kg.

In males, 10% (2/20) males at 250 mg/kg were reported to have small testes, zero sperm motility, low sperm concentration and failure to produce pregnancies. However, the sponsor submitted historical control data indicating that these findings may be spontaneous events. Thus, a drug-related effect on male fertility at 250 mg/kg was determined to be unlikely and the male fertility NOAEL was set at the high dose of 250 mg/kg.

The initial transient weight loss in both sexes, decreased body weights in males, and increases in food consumption are consistent with compensatory PD effects of drug-induced glucosuria.

Methods

Doses:	0, 5, 25 and 250 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC/10% PEG 400
Species/Strain:	Sprague Dawley rats / Crl:CD®(SD)
Number/Sex/Group:	20/sex/group
Satellite groups:	none
Study design:	• Males were dosed 70 days, beginning 28 days prior to cohabitation and up through the day prior to necropsy. • Females were dosed 14 days prior to mating through Gestation Day (GD) 7.
Deviation from study protocol:	No deviations from the study design were reported

Observations and Results

Mortality

Cageside monitoring for moribundity and mortality was conducted daily for all animals.

A total of 3 mortalities were reported, 2 of which occurred in the HD groups and are likely drug-related. One HD female exhibited clinical signs of hunched posture, decreased activity, cool to the touch, and rough haircoat; however the only macroscopic finding was a dilated cecum. One HD male had clinical signs of chromorhinorrhea and a rough haircoat, and also exhibited a large decrease in body weight prior to being found dead. Although both HD mortalities were considered to be drug-related, a pathological cause of death was not identified.

One LD male was found dead on Day 56 with clinical signs of ↓activity and rough haircoat, as well as enlarged liver, spleen and hepatic lymph nodes; however a cause of death was not determined and the sponsor did not consider it drug-related.

Table 70: Mortality - Rat Study #10GR227

MORTALITY					
Dose Group	**Animal (sex, #)**	**Day**	**Cause of Death**	**Clinical signs**	**Pathology**
LD	♂ #24	56	Unknown	Found dead. Rough haircoat, ↓activity	Enlarged liver, spleen and hepatic

					lymph nodes
HD	♂#78	78	Drug-related	↓Body weight, chromorhinorrhea and rough haircoat	No findings at necropsy
HD	♀ #151	4	Drug-related	Moribund, rough haircoat, hunched posture, ↓activity, & cool to touch	Dilated cecum.

Clinical Signs

Cageside observations were conducted at least once daily for all animals. During the treatment period, animals were observed at least 3 times daily at predose, ~1 hour post-dosing of the cohort, and once at the end of the workday. All gestating females were observed twice daily.

There were no treatment-related findings in surviving animals.

Body Weight

Male body weights were measured twice prior to the dosing period and twice a week throughout the remainder of the study. Female body weights were measured once weekly prior to dosing, twice a week during treatment, and on GD 0 (day of copulation), 3, 7, 10, and 14. Body weights were recorded for all animals on the day of necropsy.

A 3% decrease in body weight was observed in HD males on Day 4 of dosing, which correlated with a transient decrease in food consumption; however, mean body weight gains were comparable to controls by Day 8. Throughout the study, male body weights were lower than concurrent controls across all dose groups and terminal body weights were 8%, 6%, and 9% lower than controls for LD, MD, and HD groups, respectively. Although the decreased body weights were not dose-dependent, they are likely to be drug-related.

A similar, transient decrease in body weight was observed in HD females on Day 4 of dosing; however, mean weight gains rebounded by Day 8 and were within the normal biological range thereafter. There were no significant differences in mean female body weights.

Feed Consumption

Male food consumption was determined twice a week throughout the study other than during the cohabitation period. Female food consumption was measured twice weekly beginning 2 weeks prior to mating, as well as on GD 3, 7, 10 and 14.

After Day 8, dose-related increases in food consumption were observed in both males and females at all doses. During the first measurement period (Day 1-4), a 30% decrease in male food consumption was observed at HD, which correlated with a 3% weight loss; however, food consumption rebounded and was dose-dependently higher than controls by 21-58% throughout the remainder of the study. Food consumption was

consistently higher in males at LD (↑10-25%) and MD (↑14-42%) throughout the entire study. In females, Food consumption was significantly and dose-dependently higher than controls at all doses beginning on Day 8. During gestation, dam food consumption was 13-33% higher at all doses, but only remained significantly higher during GD10-14 at HD (↑29%).

Toxicokinetics

On Day 12 of dosing, blood samples were collected from 4/sex/group at 1 hour post-dose. Plasma C_{max} exposures were determined using HPLC.

Plasma exposures at 1 hour postdose increased approximately dose-proportionally and are consistent with C_{max} exposures from earlier toxicology studies in rats.

Table 71: Toxicokinetics - Rat Study #10GR227

Plasma concentrations at 1 hour post dosing on the 12th day of dose administration						
	Male			Female		
	5 mg/kg	25 mg/kg	250 mg/kg	5 mg/kg	25 mg/kg	250 mg/kg
Mean (µg/mL)	1.66	8.04	36.48	2.75	10.18	50.98
SD	0.87	4.94	8.10	0.53	2.41	18.02
N=4/sex/group; all control samples were below the lower limit of qualification.						

(Table excerpted from sponsor's package)

Dosing Solution Analysis

Suspensions were prepared every 2 weeks using the mortar and pestle method, and stored in a refrigerator, protected from light. Formulations were continuously stirred during dosing. Dose concentrations and homogeneity were assessed using a validated Reversed Phase Liquid Chromatography method with ultraviolet detection.

Formulation concentrations for all preparations administered to animals were within ±10% of target concentration. The homogeneity of top, middle and bottom samples all met acceptance criteria (≤10% RSD).

Necropsy

On GD 14, all surviving dams were euthanized and the abdominal, thoracic and pelvic viscera were grossly examined. External evaluations of the abdominal, thoracic, and pelvic viscera were conducted on all moribund females and pregnant females in which signs of copulation were not observed. The uterus and ovaries were harvested at necropsy and preserved in 10% formalin.

After 70 days of dosing, males were euthanized and the abdominal, thoracic and pelvic viscera were grossly examined. The reproductive organs including the testes, epididymes, seminal vesicles, and prostate were harvested. The left testis and epididymis were fixed in Modified Davidson's solution. The right epididymis and testis were frozen for sperm and spermatid head counts, respectively. The right distal vas deferens was evaluated for sperm motility.

There were no drug-related gross necropsy observations in females. Two (2/20) males at HD were reported to have small testis and epididymis with extremely small testis weights. There were no statistically significant differences in mean testicular organ weights.

Fertility Parameters

Females

To assess the estrous cycle, vaginal smears were collected daily at least 2 weeks prior to dosing until evidence of mating was observed or the end of the cohabitation period. The number of corpora lutea was counted for each ovary. The location and viability of uterine implantation sites were determined. Uteri without implantation sites were treated with 10% ammonium sulfide to determine early embryonic death.

There were no drug-related effects on estrous cyclicity, number of corpora lutea, implantation sites or live fetuses.

Males

Sperm analysis was performed on all males.

Two (2/20) males at HD with small testis had 0 motile sperm, low sperm concentrations, and failed to produce pregnancies. However, all other males at HD had sperm motility and concentrations similar to controls. The 10% incidence rate in the ertugliflozin fertility study is far above the 0.7% and 1.4% incidence rates for the same findings of small testes, 0 sperm motility, and low sperm concentration in historical control and low dose (LD) datasets. It is also 2-fold higher than the estimated incidence rate of 5% for 2 occurrences in 2 different groups of a single study. However, the 10% incidence rate of small testes is within the range of incidence rates of small testis macroscopic findings per study in historical toxicology controls. Thus, it is possible that the 10% incidence rate of small testes that was observed in the ertugliflozin fertility study and associated with infertility is within the range of spontaneous biological variability in this strain. However, it is also noted that the historical data confirm that only low sporadic incidences (<1.5%) of males with small testes also had 0 sperm motility and low sperm concentration in this strain of rat, possibly reaching up to 5% in a single study. Nevertheless, the historical control data indicate that the testes and related fertility findings may not be a potential drug-related effect in the ertugliflozin fertility study #10GR227.

It is also noted that the small testes and sperm effects observed in the fertility study may be consistent with drug-related delayed sexual maturation of F_1 male pups in the ertugliflozin PPND study #13GR257.

9.2 Embryonic Fetal Development

Study: Oral Embryo-fetal Development Study of PF-04971729 in Rats (Study TT #10-7833 / #10GR058)

Study no.:	TT10-7833 / 10GR058
Study report location:	eDr
Conducting laboratory and location:	Pfizer Global Research & Development, Groton, CT
Date of study initiation:	2/21/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04971729 (b) (4), Lot #GR02847, 75.6% purity

Key Study Findings

- Maternal NOAEL = 100 mg/kg (331x $MRHD_{AUC}$)
  - Decreased body weight
  - Increased early resorptions at 250 mg/kg (707x $MRHD_{AUC}$)
- Fetal Developmental NOAEL = 100 mg/kg (maternal 331x $MRHD_{AUC}$)
    - Numerical increase in ***membranous ventricular septum defect*** at 250 mg/kg
  - ***Skeletal variations*** at 250 mg/kg
    - Statistically significant drug-related increases in vertebrae, rib, metatarsal variations and ossification deficits
  - Visceral variation at 250 mg/kg
    - Possibly drug-related increase in **absent innominate artery** above the historical control incidence rate (HCIR)

Reviewer's Comments

The maternal NOAEL was set at 100 mg/kg, based on decreases in maternal body weights and increases in early resorptions at 250 mg/kg. At initial dosing, increased weight loss was associated with decreased food consumption and was considered to be minimal maternal toxicity. Increases in post implantation loss secondary to early resorptions resulted in subsequent decreases in live litter sizes at 250 mg/kg.

The fetal developmental NOAEL was set at 100 mg/kg based on possibly drug-related teratogenic findings including drug-related visceral malformations at 250 mg/kg. It was noted that uterine and fetal weights were not significantly reduced at 250 mg/kg. Total visceral observations, including malformations and variations, were increased 3-fold at 250 mg/kg, affecting 32% of the litters and 10% of the fetuses in each litter. Total skeletal observations, including malformations and variations, were significantly increased 5-fold at 250 mg/kg, affecting 100% of the litters and 92% of the fetuses in each litter.

Visceral malformations of ventricular septum defect were observed in all groups, but were increased 2-fold above concurrent controls and the HCIR at 250 mg/kg. Dose-dependent increases in absent innominate artery above the HCIR at 250 mg/kg were considered potentially drug-related.

Statistically significant increases in multiple skeletal variations were reported at 250 mg/kg, including findings of unossified 7th cervical centrum, incomplete ossification of the thoracic centrum, vertebrae 27th presacral, full supernumerary ribs, short supernumerary ribs, and unossified metatarsal. Although dose-dependency was observed at ≥100 mg/kg in incidence rates of total skeletal observations and variations including the cervical centrum, ribs, and metatarsal, statistical significance was only achieved at 250 mg/kg. Thus, only the skeletal variation findings at 250 mg/kg were considered to be significantly drug-related. There were no drug-related increases in skeletal malformations.

Maternal systemic C_{max} and AUC_{0-24} exposures at 50, 100 and 250 mg/kg on GD17 were 15.4, 31.9 and 66.7 μg/mL and 199, 457 and 975 μg·h/mL, with exposure margins of 144x, 331x and 707x $MRHD_{AUC}$.

Methods

Doses:	0, 5, 100 and 250 mg/kg
Frequency of dosing:	Daily from GD 6 to GD 17
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC/10% PEG 400
Species/Strain:	Sprague Dawley rats / Crl:CD®(SD)
Number/Sex/Group:	20/timed pregnant females/dose group
Satellite groups:	TK groups with 3 pregnant females in the control group and 5/dose PF-04971729

F_0 Generation - Dams

Mortality

Observations of mortality and moribundity were conducted three times daily for all dams.

There were no dosing-related mortalities. A single control group animal was euthanized following an early delivery on GD 21. All other animals survived to the scheduled euthanasia on GD 21.

Clinical Signs

Examinations and/or cageside observations were conducted three times daily for all dams.

There were no dosing-related clinical signs.

Body Weight

Body weights were measured during the pre-dosing period on GD 3 and once daily during the dosing period GD 5 to GD 21.

There were no adverse effects on body weight or body weight gain at 50 mg/kg/day. Decreases in body weights and weight gains were observed intermittently at 100 mg/kg/day. Clear dosing-related decreases in body weight and body weight gain were reported at 250 mg/kg/day, with weight loss during the first 3 days of. Body weight gain was comparable to control from GD 9-15 at all doses, but was slightly reduced from GD 15-18 at 250 mg/kg. Body weight loss and weight gain deficits at 250 mg/kg were reflected in consistent decreases in mean maternal body weights, especially at GD18 with mean body weights 5% lower than controls.

Table 72: Pregnant Dam Body Weights – Rat EFD Study #10GR058

Body Weight – Pregnant Rats				
Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	End BW % control
Females (GD6 – GD9)	0	13.4	0%	100%
	50	15.4	14.9%	101%
	100	9.8	-26.9%	98%
	250	-4.4**	-132.8%	94%**
Females (GD9 – GD12)	0	16.7	0%	100%
	50	23.2	39%	103%
	100	21.1	26.3%	100%
	250	20.8	24.6%	96%
Females (GD12 – GD15)	0	19.1	0%	100%
	50	16.5	-13.6%	102%
	100	16.1	-15.7%	99%
	250	19.7	3.1%	97%
(** $p < 0.01$) (* $p < 0.05$)				

Body Weight – Pregnant Rats				
Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	End BW % control
Females (GD15 – GD18)	0	38	0%	100%
	50	33.3	-12.4%	100%
	100	32.8	-13.7%	98%
	250	29.9*	-21.3%	95%*
Females (GD6 – GD18)	0	87.3	0%	100%
	50	88.5	1.4%	100%
	100	79.8	-8.6%	98%
	250	66.1**	-24.3%	95%*
Females (GD18 – GD21)	0	48.2	0%	100%
	50	46.6	-3.3%	100%
	100	50	3.7%	99%
	250	47.7	-1.0%	96%
Females (GD6 – GD21)	0	133.7	0%	100%
	50	135	1%	100%
	100	129.8	-2.9%	99%
	250	113.8**	-14.9%	96%
(** p < 0.01) (* p < 0.05)				

(Table excerpted from Dr. Quinn's P/T review)

Uterine weights were reduced in the 250 mg/kg group (↓13%) with apparent dose-dependency, although this change was not statistically significant.

Table 73: Uterine Weights – Rat EFD Study #10GR058

Summary of Mean Adjusted Body Weight Change and Uterine Weight (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Uterus weight (g)	Mean	96.53 n	98.40	98.05	84.23
	S.d.	10.08	16.57	19.53	23.89
	N	19	20	20	20
Carcass Weight (g)	Mean	300.84 n	300.61	294.82	295.74
	S.d.	15.97	17.28	15.15	19.58
	N	19	20	20	20
Net weight change (g) From Gestation day 6	Mean	37.21 n	36.62	31.75	29.57
	S.d.	11.70	14.77	11.70	13.85
	N	19	20	20	20

(Table excerpted from Dr. Quinn's P/T review)

Food Consumption

Dose-dependent decreases in mean food consumption were observed at 250 mg/kg from GD 6-9 (↓27%), but rebounded and was similar to or greater than controls thereafter. Similar increases in food consumption were reported at 100 and 50 mg/kg after GD 9.

Table 74: Maternal Food Consumption – Rat EFD Study #10GR058

Summary of Mean Maternal Food Consumption (g/animal/day)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
d 20 -> 21	Mean [g]	25.4 n	28.6	30.4*	32.4**
	S.d.	6.2	5.2	5.5	5.8
	N	20	20	20	20
d 6 -> 9	Mean [g]	22.0 n	23.9	20.0	16.1**
	S.d.	2.5	3.3	2.9	2.8
	N	20	20	19	20
d 9 -> 12	Mean [g]	23.5 n	27.5**	26.2	25.2
	S.d.	2.9	2.6	3.8	4.7
	N	20	20	20	20
d 12 -> 15	Mean [g]	23.7 n	26.0	25.6	27.6**
	S.d.	3.5	4.4	3.6	3.9
	N	20	20	20	20
d 15 -> 18	Mean [g]	25.5 n	27.3	25.9	28.5*
	S.d.	3.0	3.9	4.2	3.2
	N	20	20	20	20
d 6 -> 18	Mean [g]	23.7 n	26.2**	24.3	24.3
	S.d.	2.2	2.5	2.9	2.9
	N	20	20	19	20
d 18 -> 21	Mean [g]	24.1 n	27.4*	30.6**	34.4**
	S.d.	4.2	4.1	3.8	3.8
	N	20	20	20	20
d 6 -> 21	Mean [g]	23.8 n	26.4**	25.6	26.3**
	S.d.	2.3	2.3	2.8	2.7
	N	20	20	19	20

(Table excerpted from Dr. Quinn's P/T review)

Toxicokinetics

T_{max} was variable and occurred from 1 to 7 hours postdose, increasing with dose. Systemic C_{max} and AUC_{0-24} exposures increased approximately dose-proportionally.

Table 75: Toxicokinetics – Rat EFD Study #10GR058

Table 1. Mean Toxicokinetic Parameters for PF-04971729 in Timed-Pregnant Sprague-Dawley Rats after Oral Administration of PF-04971729 on Gestational Day 17

Dose (mg/kg/day)	Gestational Day	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
		Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
50	17	15.4	4.15	5	2.80	1.64	5	199	46.2	5
100		31.9	8.03	5	4.00	2.12	5	457	103	5
250		66.7	11.9	5	5.20	2.68	5	975	173	5

(Table excerpted from sponsor's package)

Reproductive and Postmortem Examinations

Increases in post implantation loss were reported at 250 mg/kg/day (13.1% per litter compared to 4.0% per litter in the control group), which correlated with a 2.7-fold increase in early resorptions and a subsequent reduction in live litter size by 1 pup/litter. There were no dosing-related effects on cesarean section parameters at 100 mg/kg/day and 50 mg/kg/day.

Table 76: Cesarean Section Summary – Rat EFD Study #10GR058

Table 8: Summary of Mean Cesarean Section Values

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	20	20	20
Corpora Lutea	Total	277	282	290	278
No. per animal	Mean	14.6s	14.1	14.5	13.9
	S.d.	2.4	2.2	1.7	2.4
Implantation Sites	Total	253	267	269	264
No. per animal	Mean	13.3s	13.3	13.4	13.2
	S.d.	1.9	1.7	1.9	2.4
Preimplantation Loss	Total	24	15	21	14
No. per animal	Mean	1.3s	0.8	1.1	0.7
	S.d.	1.9	1.2	2.1	0.7
% per animal	Mean	8.0s	4.8	6.5	4.9
	S.d.	9.5	6.7	12.2	5.2
Fetuses	Total	242	251	257	232
No. per animal	Mean	12.7s	12.6	12.8	11.6
	S.d.	1.5	2.5	2.5	3.6
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	Total	242	251	257	232
No. per animal	Mean	12.7s	12.6	12.8	11.6
	S.d.	1.5	2.5	2.5	3.6
Malformed Fetuses (External)	Total	0	0	0	2
No. per animal	Mean	0.0s	0.0	0.0	0.1
	S.d.	0.0	0.0	0.0	0.3
Dead Fetuses	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
% per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Early Resorption	Total	11	16	12	31
No. per animal	Mean	0.6s	0.8	0.6	1.6
	S.d.	0.8	1.5	1.1	2.5
% per animal	Mean	4.0s	6.4	5.3	12.8
	S.d.	5.2	12.4	11.7	22.3
Late Resorption	Total	0	0	0	1
No. per animal	Mean	0.0s	0.0	0.0	0.1
	S.d.	0.0	0.0	0.0	0.2
% per animal	Mean	0.0s	0.0	0.0	0.3
	S.d.	0.0	0.0	0.0	1.5
Not Applicable for Pfizer DART Studies	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Postimplantation Loss	Total	11	16	12	32
No. per animal	Mean	0.6s	0.8	0.6	1.6
	S.d.	0.8	1.5	1.1	2.5
% per animal	Mean	4.0s	6.4	5.3	13.1
	S.d.	5.2	12.4	11.7	22.3

s=DUNN
Preimplantation Loss = Corpora Lutea - Implantation Sites
Postimplantation Loss = Early/Late resorptions + Dead Fetuses

APPEARS THIS WAY ON ORIGINAL

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	20	20	20
Affected Implants	Total	11	16	12	34
No. per animal	Mean	0.6s	0.8	0.6	1.7
	S.d.	0.8	1.5	1.1	2.4
% per animal	Mean	4.0s	6.4	5.3	13.9
	S.d.	5.2	12.4	11.7	22.1

s=DUNN
Affected Implants = Early/Late resorptions + Dead Fetuses + Malformed Fetuses (External)

(Tables excerpted from sponsor's package)

F_1 Generation – Fetal Observations/Measurements

Fetal Body Weights

There were no clear drug effects on fetal weights at necropsy, although the mean weight of fetuses at 250 mg/kg was notably the lowest of all groups.

Table 77: Fetal Body Weights – Rat EFD Study #10GR058

Table 9: Summary of Mean Fetal Body Weights (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Litters, used for calculation	N	19	20	20	19
Fetus Weight	Mean	5.7n	6.0	5.7	5.4
	S.d.	0.4	0.3	0.5	0.4

n=DUNNETT

(Table excerpted from sponsor's package)

External Exams & Gender Ratios

External malformations of omphalocele of the trunk, ectrodactyly of the forepaw, and short tail were noted in two different animals from two different litters at 250 mg/kg. Although these malformations were only observed in the high dose group, they are not of similar origin or structures.

Table 78: Fetal External Evaluations – Rat EFD Study #10GR058

Table 10: Summary of Fetal External Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	242		251	257	232
Litters evaluated		N	19		20	20	19
Total CS External Observation	Litters Affected	N	0	f	0	0	2
		%	0		0	0	10.5
	Fetuses Affected	N	0		0	0	2
	% per Litter	Mean	0.0	s	0.0	0.0	0.9
Trunk *Omphalocele (M)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.4
Forepaw *Ectrodactyly (M)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.5
Tail *Short (M)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.5

f=FISHER-EXACT, s=DUNN, M = Malformation

(Table excerpted from sponsor's package)

Visceral Exams

Dose-dependent increases in incidences of heart membranous ventricular septum defect (mVSD) visceral malformations were observed at ≥100 mg/kg, with an incidence rate of 7%/litter at 250 mg/kg, which is higher than the historical control incidence rate (HICR) of 3.8%/litter. Single incidences of visceral malformations of the right sided aortic arch were also noted at doses ≥100 mg/kg, but occurred at low incidences and were not clearly dose-related; thus, they were not considered likely to be drug-related.

Dose-dependent increases in absent innominate artery were observed at ≥100 mg/kg, reaching an incidence rate of 2.4%/litter at 250 mg/kg, which exceeds the HCIR of 2.1%/litter; thus, this finding was considered potentially drug-related. Single incidences

of several visceral variations were reported at ≥100 mg/kg in the lung and adrenal gland, but were independent of dose and occurred at low frequency; thus, these findings were not considered to be drug-related.

Table 79: Fetal Visceral Findings – Rat EFD Study #10GR058

Table 11: Summary of Fetal Visceral Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	117		121	123	111
Litters evaluated		N	19		20	20	19
Total CS Visceral Observation	Litters Affected	N	2	f	3	3	6
		%	10.5		15.0	15.0	31.6
	Fetuses Affected	N	3		3	3	13
	% per Litter	Mean	3.0	s	2.4	2.3	10.1
Adrenal gland *Hemorrhagic (V)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.8
Aortic arch *Right-sided (M)*	Litters Affected	N	0	f	0	1	1
		%	0		0	5.0	5.3
	Fetuses Affected	N	0		0	1	1
	% per Litter	Mean	0.0	s	0.0	0.8	0.9
Heart *Membranous ventricular septum defect (M)*	Litters Affected	N	2	f	2	1	4
		%	10.5		10.0	5.0	21.1
	Fetuses Affected	N	3		2	1	9
	% per Litter	Mean	3.0	s	1.7	0.7	7.0
Innominate *Absent (V)*	Litters Affected	N	0	f	0	1	3
		%	0		0	5.0	15.8
	Fetuses Affected	N	0		0	1	3
	% per Litter	Mean	0.0	s	0.0	0.8	2.4
Lung	Litters Affected	N	0	f	0	1	0
		%	0		0	5.0	0
	Fetuses Affected	N	0		0	1	0
	% per Litter	Mean	0.0	s	0.0	0.8	0.0
Absent lobe(s) (V)	Litters Affected	N	0	f	0	1	0
		%	0		0	5.0	0
	Fetuses Affected	N	0		0	1	0
	% per Litter	Mean	0.0	s	0.0	0.8	0.0
Absent post caval lobe (V)	Litters Affected	N	0	f	0	1	0
		%	0		0	5.0	0
	Fetuses Affected	N	0		0	1	0
	% per Litter	Mean	0.0	s	0.0	0.8	0.0

f=FISHER-EXACT, s=DUNN, V = Variation, M = Malformation

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	117		121	123	111
Litters evaluated		N	19		20	20	19
Thyroid gland *Hemorrhagic (V)*	Litters Affected	N	0	f	0	1	0
		%	0		0	5.0	0
	Fetuses Affected	N	0		0	1	0
	% per Litter	Mean	0.0	s	0.0	0.7	0.0
Ureter *Dilated (V)*	Litters Affected	N	0	f	1	0	0
		%	0		5.0	0	0
	Fetuses Affected	N	0		1	0	0
	% per Litter	Mean	0.0	s	0.7	0.0	0.0

V = Variation, f=FISHER-EXACT, s=DUNN

(Tables excerpted from sponsor's package and highlighted)

Skeletal Exams

There were no clearly drug-related increases in skeletal malformations. Single incidences of absent metacarpal and fused sternebra, as well as 2 incidences of hemicentric thoracic centrum, were reported at 250 mg/kg, but did not reach statistical significance.

Dose-dependent increases in total skeletal observations were noted at all doses and predominantly driven by variations, but only achieved statistical significance of 250 mg/kg. Skeletal variations with statistically significant increases at 250 mg/kg included full supernumerary rib (8.0%/litter), short supernumerary rib (67.5%/litter), thoracic centrum incomplete ossification (28.9%/litter), 27 presacral vertebra (17.8%/litter), and unossified metatarsal (4.7%/litter). A dose-dependent increase in unossified 7th cervical centrum skeletal variation was also apparent at ≥100 mg/kg (≥2.1 %/llitter), but only reached statistical significance at 250 mg/kg (44.6%/litter). All other skeletal findings were of low incidence and/or unrelated to dose and not considered likely to be drug-related.

Table 80: Fetal Skeletal Findings – Rat EFD Study #10GR058

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F	
Fetuses Examined		N	125		130	134	121	
Litters evaluated		N	19		20	20	19	
Total CS Skeletal Observation	Litters Affected	N	12	f	15	16	19	**
		%	63.2		75.0	80.0	100.0	
	Fetuses Affected	N	22		35	44	112	
	% per Litter	Mean	17.3	s	26.3	30.8	92.2	**
Cervical centrum *7th cervical centrum unossified (V)*	Litters Affected	N	1	f	1	3	16	**
		%	5.3		5.0	15.0	84.2	
	Fetuses Affected	N	1		1	3	58	
	% per Litter	Mean	0.8	s	0.8	2.1	44.6	**
Lumbar centrum *Incomplete ossification (V)*	Litters Affected	N	0	f	0	0	2	
		%	0		0	0	10.5	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.6	
Metacarpal *Absent (M)*	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	1	
	% per Litter	Mean	0.0	s	0.0	0.0	0.8	
Rib	Litters Affected	N	11	f	15	15	19	**
		%	57.9		75.0	75.0	100.0	
	Fetuses Affected	N	18		33	39	90	
	% per Litter	Mean	14.5	s	24.9	27.5	75.5	**
7th Cervical (V)	Litters Affected	N	1	f	2	1	1	
		%	5.3		10.0	5.0	5.3	
	Fetuses Affected	N	1		2	1	2	
	% per Litter	Mean	0.7	s	1.5	0.8	1.5	
Full supernumerary (V)	Litters Affected	N	0	f	0	0	5	*
		%	0		0	0	26.3	
	Fetuses Affected	N	0		0	0	10	
	% per Litter	Mean	0.0	s	0.0	0.0	8.0	**
Short (V)	Litters Affected	N	1	f	0	0	0	
		%	5.3		0	0	0	
	Fetuses Affected	N	1		0	0	0	
	% per Litter	Mean	0.7	s	0.0	0.0	0.0	

f=FISHER-EXACT, ** = $p < 0.01$, s=DUNN, V = Variation, M = Malformation, * = $p < 0.05$

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F	
Fetuses Examined		N	125		130	134	121	
Litters evaluated		N	19		20	20	19	
Short supernumerary (V)	Litters Affected	N	10	f	14	13	19	**
		%	52.6		70.0	65.0	100.0	
	Fetuses Affected	N	16		30	36	80	
	% per Litter	Mean	13.2	s	22.8	25.4	67.5	**
Wavy (V)	Litters Affected	N	0	f	1	2	0	
		%	0		5.0	10.0	0	
	Fetuses Affected	N	0		1	3	0	
	% per Litter	Mean	0.0	s	0.6	1.9	0.0	
Skull *Extra ossification site (V)*	Litters Affected	N	0	f	0	1	2	
		%	0		0	5.0	10.5	
	Fetuses Affected	N	0		0	1	2	
	% per Litter	Mean	0.0	s	0.0	0.6	2.2	
Sternebra	Litters Affected	N	0	f	0	1	2	
		%	0		0	5.0	10.5	
	Fetuses Affected	N	0		0	1	4	
	% per Litter	Mean	0.0	s	0.0	0.8	2.8	
Extra ossification site (V)	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.3	
Fused (M)	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	1	
	% per Litter	Mean	0.0	s	0.0	0.0	0.8	
Unossified #5 and/or #6 (V)	Litters Affected	N	0	f	0	1	1	
		%	0		0	5.0	5.3	
	Fetuses Affected	N	0		0	1	1	
	% per Litter	Mean	0.0	s	0.0	0.8	0.8	
Thoracic centrum	Litters Affected	N	3	f	0	2	14	**
		%	15.8		0	10.0	73.7	
	Fetuses Affected	N	3		0	3	37	
	% per Litter	Mean	2.1	s	0.0	2.0	28.9	**

V = Variation, f=FISHER-EXACT, ** = $p < 0.01$, s=DUNN, M = Malformation

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F	
Fetuses Examined		N	125		130	134	121	
Litters evaluated		N	19		20	20	19	
Hemicentric (M)	Litters Affected	N	0	f	0	0	2	
		%	0		0	0	10.5	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.5	
Incomplete ossification (V)	Litters Affected	N	3	f	0	2	14	**
		%	15.8		0	10.0	73.7	
	Fetuses Affected	N	3		0	3	37	
	% per Litter	Mean	2.1	s	0.0	2.0	28.9	**
Misaligned (V)	Litters Affected	N	0	f	0	0	2	
		%	0		0	0	10.5	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.5	
Unossified (V)	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	1	
	% per Litter	Mean	0.0	s	0.0	0.0	0.8	
Vertebra *27 Presacral (V)*	Litters Affected	N	0	f	0	0	8	**
		%	0		0	0	42.1	
	Fetuses Affected	N	0		0	0	23	
	% per Litter	Mean	0.0	s	0.0	0.0	17.8	**
Metatarsal	Litters Affected	N	1	f	1	1	5	
		%	5.3		5.0	5.0	26.3	
	Fetuses Affected	N	1		1	1	6	
	% per Litter	Mean	0.8	s	0.6	0.8	4.7	
Malpositioned (M)	Litters Affected	N	1	f	0	0	0	
		%	5.3		0	0	0	
	Fetuses Affected	N	1		0	0	0	
	% per Litter	Mean	0.8	s	0.0	0.0	0.0	
Unossified (V)	Litters Affected	N	0	f	1	1	5	*
		%	0		5.0	5.0	26.3	
	Fetuses Affected	N	0		1	1	6	
	% per Litter	Mean	0.0	s	0.6	0.8	4.7	*

M = Malformation, f=FISHER-EXACT, s=DUNN, V = Variation, ** = $p < 0.01$, * = $p < 0.05$

(Tables excerpted from sponsor's package and highlighted)

Study: Oral Embryo-fetal Development Study of PF-04971729 in Rabbits (Study TT #10-7834 / #10GR059)

Study no.:	TT10-7834 / 10GR059
Study report location:	eDr
Conducting laboratory and location:	Pfizer Global Research & Development, Groton, CT
Date of study initiation:	2/28/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04971729 (b) (4), Lot #GR02847, 75.6% purity

Key Study Findings

- Maternal NOAEL was not identified (<50 mg/kg; <150x $MRHD_{AUC}$)
  - Decreased body weight and ↓weight gain at all doses

    - Increased post-implantation loss and decreases in total number of live fetuses at all doses
        - Decreases in embryo-fetal survival were considered to be secondary to maternal toxicity
- Fetal Developmental NOAEL = 100 mg/kg (maternal 307x $MRHD_{AUC}$)
    - Potentially drug-related visceral and skeletal variations at 250 mg/kg

Reviewer's Comments

Drug-related maternal toxicity was observed at all doses. At 250 mg/kg, two main group does and one TK doe aborted between GD19 and 28. Decreases in food consumption and body weight loss were observed prior to abortion in all 3 animals; thus, the abortions were considered to be drug-related and secondary to maternal toxicity. Severe drug-related decreases in maternal body weight and/or weight gain were reported at all doses, with drug-related weight loss during the first week of dosing at ≥100 mg/kg. Statistically significantly lower food consumption over the course of the study was also reported at ≥100 mg/kg, which rebounded after dosing ended. Although within the range of normal historical controls (8.09%), a statistically significant increase in post-implantation loss at 250 mg/kg (5.6%) was associated with a dose-dependent decrease in the total number of live fetuses, reaching a 20% deficit at 250 mg/kg compared to concurrent controls. Given the increase in spontaneous abortions, significant maternal toxicity at 250 mg/kg was considered to adversely affect embryofetal survival. Furthermore, an even greater increase in post-implantation loss occurred at 50 mg/kg (7.5%/animal), indicating potential drug-related embryo lethality at ≥50 mg/kg. Given similar drug-related effects on maternal body weights, it's likely that drug-related maternal toxicity also played a role in increased embryo lethality at 50 mg/kg.

There were no clearly drug-related visceral or skeletal malformations.

Visceral variations of retrocaval ureter and small or absent gallbladder were observed at 250 mg/kg above the maximum incidence rates of historical controls and were considered to be potentially drug-related, even though statistical significance was not achieved. It's further noted that the apparent increases in these findings at 250 mg/kg may be secondary to corresponding maternal toxicity.

A small increase in the incidence rate of the skeletal variation of sternebra with an extra ossification site was observed at 250 mg/kg (2.3% fetuses/litter) and was absent from rabbit historical control data; thus, this finding could not be completely ruled out as being potentially drug- related. It is noted that there was not a statistically significant increase in total skeletal observations with drug treatment; however, the apparent increases at 250 mg/kg may be secondary to corresponding maternal toxicity.

Maternal systemic C_{max} and AUC_{0-24} exposures at 50, 100 and 250 mg/kg on GD19 were 22.5, 46 and 115 μg/mL and 207, 424 and 1150 μg·h/mL, with exposure margins of 150x, 307x and 833x $MRHD_{AUC}$.

Methods

Doses:	0, 5, 100 and 250 mg/kg
Frequency of dosing:	Daily from GD 7 to GD 19
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% MC/10% PEG 400
Species/Strain:	New Zealand White Rabbits / (b) (4)
Number/Sex/Group:	20/timed pregnant females/dose group
Satellite groups:	TK groups with 3 pregnant females in the control group and 5/dose PF-04971729

F_0 Generation - Does

Mortality

Observations of mortality and morbundity were conducted three times daily for all dams.

Two does at 250 mg/kg/day spontaneously aborted (Does #63 and #65) on GD 19 and 21, and were euthanized. In the TK group, a 3rd doe (#95) at 250 mg/kg was also euthanized on GD 28 due to clinical signs suggestive of abortion (blood clots and red fluid in the cage) although no aborted fetuses were noted. All 3 does also had severe decreases in food consumption and body weight losses of 13-22% prior to abortion; thus, the abortions and euthanasia were considered secondary to maternal toxicity.

Clinical Signs

Aside from observations associated with spontaneous abortion of the 3 early euthansias mentioned above, there were no treatment-related clinical signs.

Body Weight

During the 1st 2 weeks of dosing (GD 7-20), doe body weights (↓2-6%) and/or body weight gains (↓48-71%) were significantly lower than controls at all doses. At ≥100 mg/kg, weight losses were observed from GD 7-10 associated with lower overall weight gain throughout the rest of the dosing period, resulting in significantly decreases in mean body weights. A similar pattern was noted at 50 mg/kg, although the body weight decrement for the GD 7-10 interval was less severe.

Table 81: Pregnant Dam Body Weights – Rabbit EFD Study #10GR059

Body Weight – Pregnant Rabbits				
Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	End BW % control
Females (GD7 – GD10)	0	52	0%	100%
	50	1*	-98%	96%
	100	-7**	-113%	97%
	250	-10**	-119%	98%
Females (GD10 – GD13)	0	50	0%	100%
	50	40	-20%	96%
	100	39	-22%	97%
	250	17	-66%	97%
Females (GD13 – GD16)	0	55	0%	100%
	50	48	-12.7%	96%
	100	57	3.6%	97%
	250	16	-71%	95%
Females (GD16 – GD20)	0	79	0%	100%
	50	26**	-67%	95%*
	100	36**	-54.4%	96%
	250	14**	-82.3%	94%*
Females (GD7 – GD20)	0	237	0%	100%
	50	115**	-51.5%	95%*
	100	124**	-47.7%	96%
	250	69**	-70.9%	94%*
Females (GD20 – GD29)	0	190	0%	100%
	50	245	28.9%	96%
	100	229	20.5%	97%
	250	236	24.2%	97%
Females (GD7 – GD29)	0	427	0%	100%
	50	361	-15%	96%
	100	353	-17.3%	97%
	250	334	-21.8%	97%
(** p < 0.01) (* p < 0.05)				

(Table excerpted from Dr. Quinn's P/T review)

Uterine weights tended to be lower (↓8-12%) in drug-treatment groups, but were not dose-related.

Food Consumption

Significant increases in mean food consumption were observed at ≥100 mg/kg/day after cessation of dosing (GD 20-29), resulting in an overall significant increase for the entire measurement period (GD 7-29). These findings are indicative of a rebound in food consumption after dosing ended.

Table 82: Maternal Food Consumption – Rabbit EFD Study #10GR059

Summary of Mean Maternal Food Consumption (g/animal/day)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
d 7 -> 10	Mean [g]	144.7 s	135.0	134.0	137.4
	S.d.	9.2	16.7	23.2	15.8
	N	19	19	20	19
d 10 -> 13	Mean [g]	135.9 s	125.9	128.0	124.5
	S.d.	16.8	23.7	27.2	29.8
	N	19	19	20	19
d 13 -> 16	Mean [g]	123.8 s	134.6	134.2	124.1
	S.d.	35.2	22.6	24.2	45.1
	N	19	19	20	19

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
d 16 -> 20	Mean [g]	141.0 s	143.3	144.2	144.6
	S.d.	14.2	15.3	11.5	9.5
	N	19	19	20	16
d 7 -> 20	Mean [g]	136.7 s	135.3	135.8	139.3
	S.d.	15.6	15.3	19.0	10.6
	N	19	19	20	16
d 20 -> 29	Mean [g]	122.9 s	135.9	142.6**	142.8**
	S.d.	16.8	15.5	10.1	16.6
	N	19	19	20	17
d 7 -> 29	Mean [g]	131.1 s	135.6	138.6*	140.7*
	S.d.	11.1	14.0	13.2	8.9
	N	19	19	20	16

(Tables excerpted from sponsor's package and annotated by Dr. Quinn)

Toxicokinetics

T_{max} ranged from 2 to 4 hours postdose, increasing with dose Systemic C_{max} and AUC_{0-24} exposures increased approximately dose-proportionally. It is noted that pre-dose samples (Time 0) were used in place of 24 hour post-dose time points.

Table 83: Toxicokinetics – Rabbit EFD Study #10GR059

Table 1. Mean Toxicokinetic Parameters for PF-04971729 in Timed-Pregnant New Zealand White Rabbits after Oral Administration of PF-04971729 on Gestational Day 19

Dose (mg/kg/day)	Gestational Day	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
		Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
50	19	22.5	5.91	5	2.40	0.894	5	207	51.6	5
100		46.0	7.67	5	2.40	0.894	5	424	77.6	5
250		115	14.9	4	4.00	0	4	1150	177	4

(Table excerpted from sponsor's package)

Reproductive and Postmortem Examinations

A statistically significant increase in post-implantation loss was noted at 250 mg/kg/day (5.6%/animal), but remained within the HCIR of 8.09%. However, an even greater increase in post-implantation loss occurred at 50 mg/kg (7.5%/animal). The total number of live fetuses also notably decreased with dose, reaching a 20% deficit at 250 mg/kg.

Table 84: Cesarean Section Summary – Rabbit EFD Study #10GR059

Table 8: Summary of Mean Cesarean Section Values

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	19	20	17
Corpora Lutea	Total	188	190	190	164
No. per animal	Mean	9.9s	10.0	9.5	9.6
	S.d.	1.8	1.5	2.0	1.4
Implantation Sites	Total	180	181	164	152
No. per animal	Mean	9.5s	9.5	8.2	8.9
	S.d.	1.7	1.9	1.9	1.8
Preimplantation Loss	Total	8	9	26	12
No. per animal	Mean	0.4s	0.5	1.3	0.7
	S.d.	0.5	1.0	1.5	1.3
% per animal	Mean	4.1s	5.1	13.0	7.3
	S.d.	5.0	11.7	14.0	13.8
Fetuses	Total	179	167	160	143
No. per animal	Mean	9.4s	8.8	8.0	8.4
	S.d.	1.7	2.9	1.8	1.8
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	Total	179	167	160	143
No. per animal	Mean	9.4s	8.8	8.0	8.4
	S.d.	1.7	2.9	1.8	1.8
Malformed Fetuses (External)	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Dead Fetuses	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
% per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Early Resorption	Total	1	12	2	6
No. per animal	Mean	0.1s	0.6	0.1	0.4
	S.d.	0.2	2.3	0.3	0.8
% per animal	Mean	0.5s	6.4	1.2	3.7
	S.d.	2.3	22.9	3.8	8.6
Late Resorption	Total	0	2	2	3
No. per animal	Mean	0.0s	0.1	0.1	0.2
	S.d.	0.0	0.3	0.3	0.4
% per animal	Mean	0.0s	1.1	1.0	1.8
	S.d.	0.0	3.2	2.9	4.1
Not Applicable for Pfizer DART Studies	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Postimplantation Loss	Total	1	14	4	9
No. per animal	Mean	0.1s	0.7	0.2	0.5*
	S.d.	0.2	2.3	0.4	0.8
% per animal	Mean	0.5s	7.5	2.2	5.6*
	S.d.	2.3	22.8	4.5	8.7

s=DUNN; * = $p < 0.05$
Preimplantation Loss = Corpora Lutea - Implantation Sites
Postimplantation Loss = Early/Late resorptions + Dead Fetuses

APPEARS THIS WAY ON ORIGINAL

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	19	20	17
Affected Implants	Total	1	14	4	9
No. per animal	Mean	0.1s	0.7	0.2	0.5*
	S.d.	0.2	2.3	0.4	0.8
% per animal	Mean	0.5s	7.5	2.2	5.6*
	S.d.	2.3	22.8	4.5	8.7

s=DUNN; * = $p < 0.05$
Affected Implants = Early/Late resorptions + Dead Fetuses + Malformed Fetuses (External)

(Table excerpted from sponsor's package and highlighted)

F1 Generation – Fetal Observations/Measurements

Fetal Body Weights

There were no clear drug effects on fetal weights at necropsy, although fetuses obtained from does dosed at 250 mg/kg notably weighed the least of all groups.

Table 85: Fetal Body Weights – Rabbit EFD Study #10GR059

Table 9: Summary of Mean Fetal Body Weights (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Litters, used for calculation	N	19	18	20	17
Fetus Weight	Mean	41.7n	41.4	43.2	41.0
	S.d.	4.3	5.1	4.1	3.7
	Deviation Vs Control		-0.6	3.7	-1.6

n=DUNNETT

(Table excerpted from sponsor's package)

External Exams & Gender Ratios

There were no drug-related external findings.

Visceral Exams

One 250 mg/kg/day fetus (#79-8) presented with 3 related malformations (muscular ventricular septum defect, dilated aortic arch, narrowed pulmonary trunk). These observations are similar to the visceral malformations noted in the rat EFD study at the same dose. However, there were no similar findings in any of the other 142 fetuses at 250 mg/kg; thus, it is likely that this finding was a spontaneous event and was not drug-related.

Increases in visceral variations of absent (3.2%/litter) or small (6.2%) gallbladder observed at 250 mg/kg occurred above the HCIRs of 1.1% and 4.4%, respectively. Although the sponsor feels that these changes are representative of normal biological variation, a relationship to drug has not been ruled out and they are considered potentially drug-related.

An increased incidence of retrocaval ureter variation observed at 250 mg/kg (5.2% fetuses/litter) in 3 litters and 7 fetuses, and exceeded the HCIR of 4.2% fetuses/litter. Although the sponsor feels that this finding is comparable to HCIR, a relationship to drug has not been ruled out and they are considered potentially drug-related

Findings of absent postcaval lung lobe were slightly increased at 250 mg/kg, but were considered to be within the normal biological range for this species and unlikely to be drug-related.

Table 86: Fetal Visceral Findings – Rabbit EFD Study #10GR059

Table 11: Summary of Fetal Visceral Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Total CS Visceral Observation	Litters Affected	N	11	f	8	8	14
		%	57.9		44.4	40.0	82.4
	Fetuses Affected	N	23		18	13	33
	% per Litter	Mean	13.0	s	11.8	7.0	21.8
Gallbladder	Litters Affected	N	6	f	1	1 *	5
		%	31.6		5.6	5.0	29.4
	Fetuses Affected	N	7		2	2	15
	% per Litter	Mean	3.6	s	1.1	1.0	9.3
Absent (V)	Litters Affected	N	1	f	1	0	3
		%	5.3		5.6	0	17.6
	Fetuses Affected	N	1		1	0	5
	% per Litter	Mean	0.6	s	0.6	0.0	3.2
Small (V)	Litters Affected	N	5	f	1	1	5
		%	26.3		5.6	5.0	29.4
	Fetuses Affected	N	6		1	2	10
	% per Litter	Mean	3.0	s	0.6	1.0	6.2
Ureter *Retrocaval (V)*	Litters Affected	N	2	f	3	3	3
		%	10.5		16.7	15.0	17.6
	Fetuses Affected	N	2		3	3	7
	% per Litter	Mean	1.2	s	2.0	1.7	5.2

f=FISHER-EXACT, s=DUNN, M = Malformation, V = Variation, * = $p < 0.05$

(Table excerpted from sponsor's package, cropped and highlighted)

Skeletal Exams

Two skeletal malformations supernumerary cervical centrum and fused rib were observed in the same fetus at 250 mg/kg. A single incidence of misshapen interparietal bone was also noted at 250 mg/kg/day from a separate litter. Since bone effects have been reported with SGLT2 inhibitors, these findings are notable; however, since they were observed in single fetuses, they can be attributed to normal biological variation and are unlikely to be drug-related.

A small increase in the incidence rate of skeletal variations of sternebra with an extra ossification site was apparent at 250 mg/kg (2.3% fetuses/litter), occurring in 2 different litters. Furthermore, this finding was absent from the laboratories' rabbit historical control data. It's noted that the same skeletal variation was observed in 2 fetuses in the rat EFD study at the same dose, but was not statistically significant. Although this finding occurred at a low incidence rate, a relationship to drug cannot be ruled out.

Table 87: Fetal Skeletal Findings – Rabbit EFD Study #10GR059

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Sternebra	Litters Affected	N	13	f	12	15	11
		%	68.4		66.7	75.0	64.7
	Fetuses Affected	N	37		33	31	21
	% per Litter	Mean	20.3	s	18.4	18.5	14.0
Extra ossification site (V)	Litters Affected	N	1	f	0	0	2
		%	5.3		0	0	11.8
	Fetuses Affected	N	1		0	0	3
	% per Litter	Mean	0.7	s	0.0	0.0	2.3

M = Malformation, f=FISHER-EXACT, s=DUNN, V = Variation

(Table excerpted from sponsor's package, cropped and highlighted)

9.3 Prenatal and Postnatal Development

Study: A Pre- and Postnatal Developmental Toxicity Study of PF-04971729 by Oral (Gavage) in Rats (Study TT #13-7827FIN / #13GR257)

Study no.:	TT137827 / 13GR257
Study report location:	eDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/14/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04971729, Lot #E010013903, 98.4%

Key Study Findings

- Maternal NOAEL = 50 mg/kg/day (~144x *$MRHD_{AUC}$)
  - Clinical signs and decreases in body weight and weight gain during gestation at ≥100 mg/kg/day (~331x *$MRHD_{AUC}$)
- Reproductive NOAEL = 250 mg/kg/day (~707x *$MRHD_{AUC}$)
  - No significant effects on maternal reproductive function parameters
- F_1 NOAEL = 50 mg/kg/day (maternal exposure = ~144x *$MRHD_{AUC}$)
  - F_1 pup mortalities on post-natal day (PND) 1 to PND 4 at 250 mg/kg/day (maternal exposure = ~707x *$MRHD_{AUC}$)
    - Associated with lack of nursing
  - Delayed sexual maturation at 250 mg/kg/day
  - Prolonged decreases in F_1 generation body weights at ≥100 mg/kg/day (maternal exposure = ~331x *$MRHD_{AUC}$)
  - Clinical signs of ungroomed coats, bruising, cold to touch, pale and dehydration at ≥100 mg/kg/day.

*Based on GD17 exposures in pregnant rats (EFD study #10GR058)

METHODS

Pregnant SD rats (F_0 generation) were administered 0 (0.5% MC/10% PEG 400), 50, 100, and 250 mg/kg/day ertugliflozin via oral gavage from GD6 through Lactation Day (LD) 20 (~5 weeks total). Pups (F_1 generation) were exposed to the test article via nursing, but were not dosed directly, and were culled on PND 4. After weaning, 1/sex/litter was selected for F_1 neurological (motor activity, Morris water maze, and acoustic startle) and reproduction (mating and fertility) assessments.

RESULTS

The maternal F_0 NOAEL was set at 50 mg/kg/day based on clinical signs of dehydration, decreases in body weights and deficits in weight gain at ≥100 mg/kg/day. Although decreases in body weights and weight gain are common PD-related effects of PF-04971729, they were considered to be adverse in pregnant and lactating dams. Clinical signs of hunched posture, soft/liquid feces and pale ears were also reported at 250 mg/kg.

Although the maternal NOAEL was set at the low dose, there were no drug-related effects on assessments of maternal reproductive function. Therefore, the F_0 Reproductive NOAEL was set at 250 mg/kg/day.

The NOAEL for F_1 generation developmental toxicity was set at 50 mg/kg/day based on mortalities associated with lack of nursing and delayed sexual maturation at 250 mg/kg, as well as prolonged decreases in body weights and clinical signs at ≥100 mg/kg. Adverse F_1 clinical signs included bruising, cold to touch, pale, ungroomed coats, and dehydration at ≥100 mg/kg. Male balano-preputial separation was significantly delayed by 2.5 days and female vaginal patency was significantly delayed by 2.2 days in F_1 pups from dams treated at 250 mg/kg. It was also noted that F_1 pup bodyweight at the time of sexual maturation was significantly reduced by 13% in males and 11% in females.

It is noted that F_1 dehydration, clinical signs, decreased body weights, and mortalities associated with empty stomachs correlated with F_0 maternal observations of not nesting and not nursing. These findings are consistent with deficits in nursing and maternal behavior with drug administration at ≥100 mg/kg/day, which may be related to maternal dehydration secondary to PD-related osmotic diuresis. Thus, the F_1 body weight deficits and clinical signs at 100 mg/kg/day could be secondary to maternal behavior and/or maternal toxicity; however, a direct relationship of drug on F_1 toxicity and development cannot be ruled out in this study.

There were no significant drug-related effects on F_1 neurological assessments or mating and fertility indices.

Methods

Doses:	0, 50, 100 and 250 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage

Formulation/Vehicle:	10% PEG 400 in 0.5% (w/v) methylcellulose
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	22 F_0 females/group, 22 pups/group
Satellite groups:	None
Study design:	Pregnant F0 females were dosed once daily on Gestation Day (GD) 6 through Lactation Day (LD) 20
Deviation from study protocol:	There were no significant deviations that impacted the integrity of the study.

Observations and Results

F_0 Generation - Dams

For females that did not deliver litters, dosing was stopped on GD 24 and necropsy was performed on GD 25. Dosing was continued until LD 20 for all other dams, which were then necropsied on LD 28.

Survival

Animals were observed daily for mortalities.

There were no drug-related mortalities. Two mid-dose (MD) dams were found dead or euthanized early due to gavage accidents.

Clinical Signs

Clinical observations were conducted at 1-2 hours postdose and during parturition. Observations of lactation and maternal behavior were also recorded.

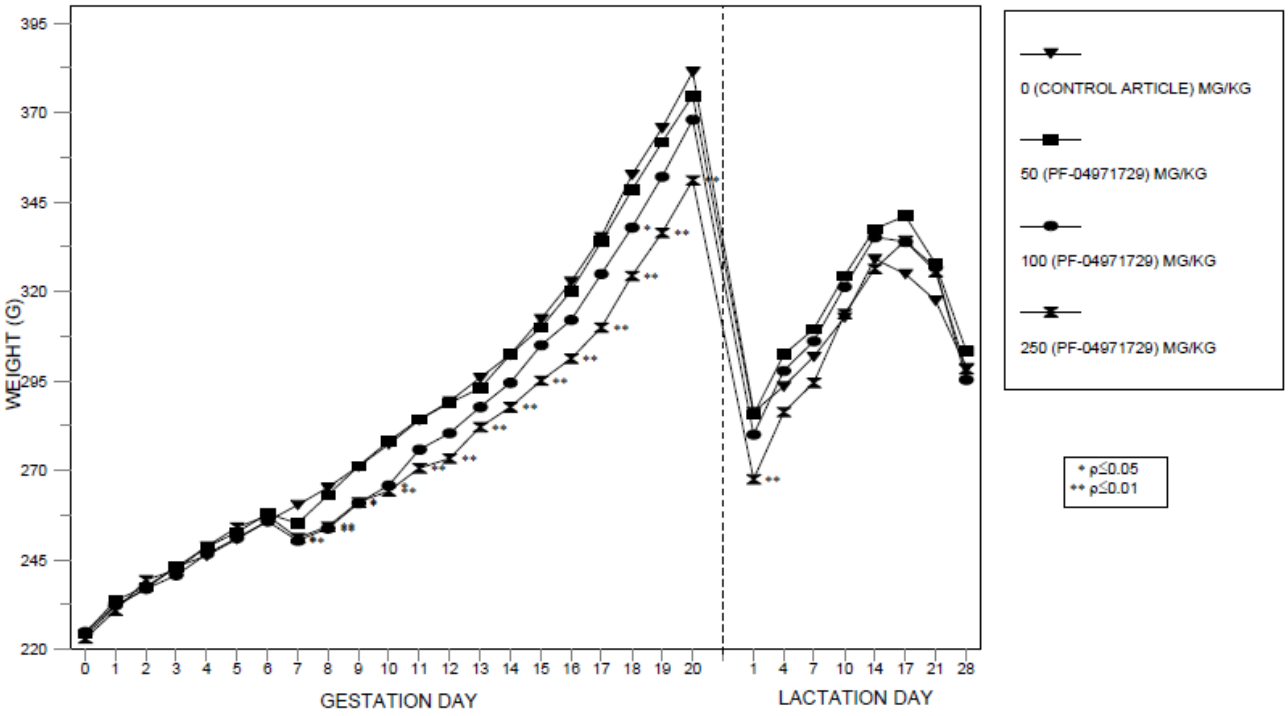
Drug-related clinical signs of mild to moderate dehydration (skin turgor), rales, and urine-stained abdominal fur were observed at ≥MD during gestation, which increased in incidence and severity during the lactation period. Incidences of hunched posture, soft/liquid feces and pale ears were also observed at HD during lactation, and incidences of abdominal distention were reported at ≥MD during lactation.

Body Weight

Dam body weights were measured daily during gestation and on LD 1, 4, 7, 10, 14, 17, 21 and 28.

Transient weight loss was observed at all doses (statistically significant at ≥MD) on GD 7 (Day 2 of dosing) followed by increases in weight gain, although weight gains remained 5-75% lower than controls throughout gestation at ≥MD. Mean body weights remained dose-dependently lower (↓4-8%) at ≥MD throughout gestation and at HD through the first week of lactation. However, during the lactation period, weight gains were dose-dependently, 2-fold higher than controls at ≥MD. By LD 10, body weights in all drug-treated groups were comparable to controls.

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS
Figure 1



Feed Consumption
Dam food consumption was determined on GD 0, 6, 9, 12, 15, 18, 20, and 25, and on LD 1, 4, 7, 10, and 14.

Food consumption was significantly reduced (↓19-32%) at ≥MD during the first interval (GD6-9), but rebounded to 10-40% higher than controls throughout the remainder of gestation and the first 2 weeks of lactation.

Parturition
Dams were allowed to deliver naturally and clinical observations were recorded.

There were no significant drug-related effects on natural delivery parameters, including the number of litter deliveries, duration of gestation, or gestation index. A statistically significant increase in the duration of gestation (↑0.4 days) was reported at HD, but remained within the historical range and was unlikely to be biologically significant.

Uterine Content
Ovarian and uterine examinations were performed for all dams.

There were no drug-related effects on the number of implantation sites per litter.

Necropsy
Macroscopic observations were made at necropsy. Various tissues were collected, but were not examined microscopically.

There were no drug-related macroscopic findings.

Toxicokinetics
Not determined

Dosing Solution Analysis
Test article concentrations were confirmed for all dose groups in the first and last dosing preparations. The first preparation was also assessed for homogeneity.

All mean concentration and homogeneity results were within the acceptance criteria of ±15%

Litter
Litter size and litter viability were determined at delivery.

There were no significant differences in litter sizes, the number of stillborn pups or pup sex ratios. The entire litters of one LD and one HD dams did not survive. However, there was not a consistent dose-dependent effect on the survival of entire litters.

F_1 Generation - Pups
Pups were potentially exposed to the test article via nursing, but were not dosed directly. Pups were culled on Postpartum Day (PD) 4. After weaning, 1/sex/litter was selected for F_1 neurological and reproduction assessments.

Survival
Pup survival was assessed twice daily until weaning.

The number of pups found dead, presumed cannibalized, or moribund was significantly increased at HD during the first 4 days (PD1-4), resulting in an overall 14% decrease in the viability index at HD that is considered to be drug-related. However, after culling on PD 4, there was not a significant drug-related decrease in survival, resulting in comparable lactation indexes (survival postculling PD4-28) between drug-treated and control groups.

Clinical Signs
Clinical signs were assessed once daily.

Drug-related effects were reported at ≥MD. At MD, transient drug-related effects included ungroomed coats in all pups of 2/16 litters on PD17-23 and cold to the touch in ≥1 pups in 3/16 litters on PD 1-3. At HD, increased incidences and frequencies of the number of litters with pups with ungroomed coats (16/21) and cold to touch (5/21) were observed on PD 16-28 and PD 1-7, respectively.

A statistically significant increase in the number of litters with pups that were bruised (purple/black color) and/or pale was observed 29 times in 4 litters at HD. Nine

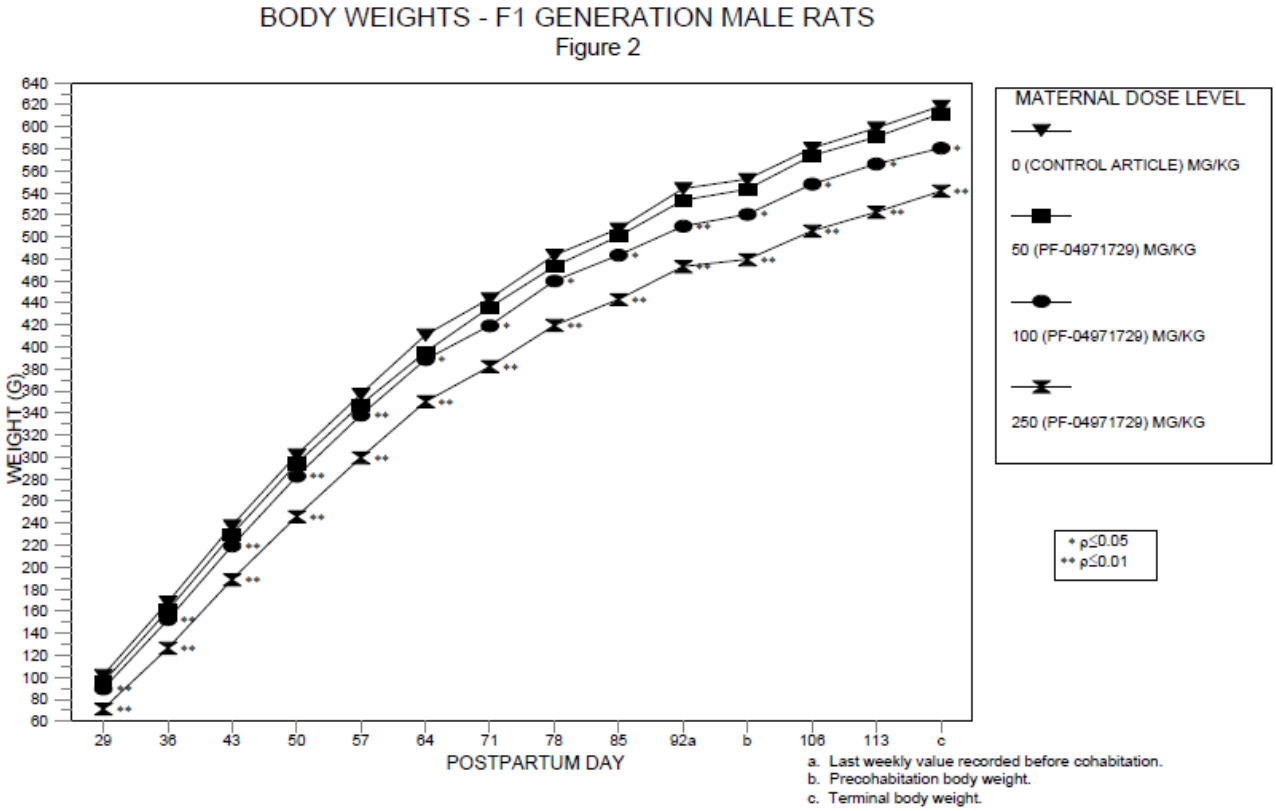
observations of dehydration were also reported in 2 litters at HD. Although, the sponsor considered these findings unrelated to study drug, these findings are considered likely to be drug-related. Occasional observations of not nesting and not nursing were also reported in at least 1 litter and are consistent with pup clinical signs of dehydration.

Body Weight

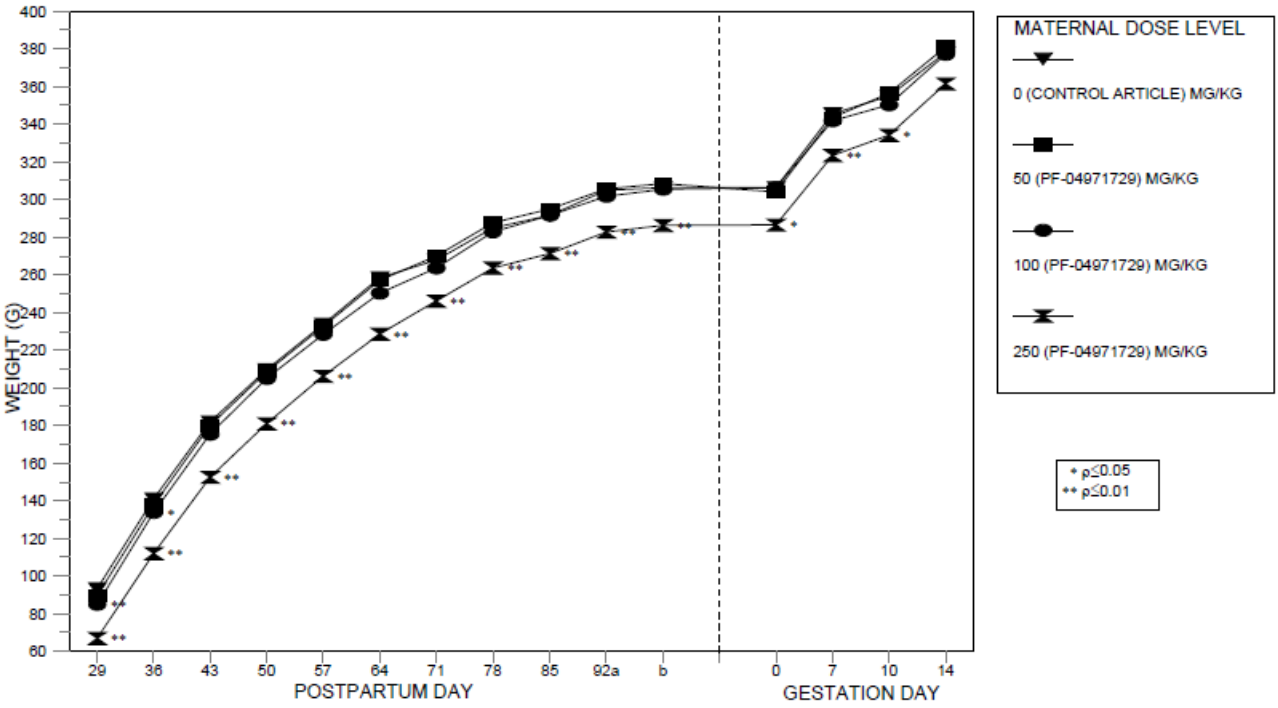
F_1 body weights were determined on PD 1, 4, 7, 10, 14, 17, 21 and 28, followed by once weekly after weaning.

Pup weights were significantly lower at all doses in a dose-dependent manner, reaching 8-17% lower at MD (PD 7-28) and 13-37% lower at HD (PD 1-28). Male F_1 generation body weights remained significantly lower than controls throughout the study (PD 116-120) at ≥MD with periodic weight gain deficits. Female F1 generation body weights remained significantly lower than controls at ≥MD through PD 36 and throughout the remainder of the study (PD 98 + GD 14) at HD. Although the pup weights at LD were significantly 5-9% lower at PD 14-28, they remained within the mean range of control values, in general, were comparable to or greater than controls thereafter, indicating a rebound in growth. It is noted that weight gains were comparable to controls (within 5%) on PD 28 at ≤MD, indicating recovery of weight gain deficits after cessation of drug administration to the dams on LD 20.

Figure 13: F1 Body Weights



BODY WEIGHTS - F1 GENERATION FEMALE RATS
Figure 3



a. Last weekly value recorded before cohabitation.
b. Precohabitation body weight.

(Excerpted from sponsor's package)

Feed Consumption

F_1 feed consumption was determined weekly for all animals. Pregnant F_1 body weights were determined on GD 0, 7, 10 and 14.

Drug-related, dose-dependent decreases in absolute food consumption (↓8-11%) reached statistical significance in males at HD on PD 43 to 57. Although food consumption remained lower throughout PD 92, absolute food consumption was not significantly different from controls after PD 57. On the other hand, food consumption relative to body weight was significantly increased (↑10-40%) at HD throughout PD 29-92.

In females, relative food consumptions were also significantly increased (↑10-20%) at HD between PD 36 to 92, as well as during the F_1 gestation period GD 0 to 14. Although drug-related, the sponsor considered the food consumption changes to be sporadic, minimal and non-adverse in both sexes.

Physical Development

Sexual maturation was assessed in females via vaginal opening beginning on PD 28 and in males via preputial separation beginning on PD 35.

Male balano-preputial separation was significantly delayed by 2.5 days and female vaginal patency was significantly delayed by 2.2 days in F_1 pups from dams treated at

HD. Furthermore, F_1 pup bodyweight at the time of sexual maturation was significantly reduced by 13% in males and 11% in females.

Neurological Assessment
Motor activity (PD 25 and 60), Morris water maze (PD 65 and 80), and acoustic startle testing (PD 80 to 90) were assessed in 1/sex/litter.

There were no significant drug-related findings in motor activity, Morris water maze, or acoustic startle assessments.

Reproduction
On PD 94-98, F_1 male and female pups were cohabitated with other F_1 pups of the opposite sex, but same dose group, for up to 14 days. Estrous cycle was evaluated for 14 consecutive days prior to cohabitation, then until spermatozoa or a copulatory plug was observed.

There were no drug-related findings on mating or fertility indexes.

Gross Pathology
Gross necropsies of the thoracic, abdominal and pelvic viscera were performed for each animal. All lesions were retained, but histopathological evaluations were not conducted. All F_1 pups culled on PD 4 or weaned on PD 28 and not maintained for further tests were necropsied and examined for gross lesions. A single cross-section of the head at the frontal-parietal suture was examined for apparent hydrocephaly. F_1 males used in further studies were necropsied after completion of the 14-day cohabitation period. All F_1 females used in further studies were necropsied on GD 14 and the reproductive tract was dissected for ovarian and uterine examinations.

Necropsies were performed on 27 of the HD pups that were found dead prior to PD28, and 14 were void of milk in the stomach, indicating that they did not nurse. However, 12 were from one litter. There were no other macroscopic findings in any of the other necropsied pups found dead prior to weaning.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	PF-04971729	PF-04971729	PF-04971729
MATERNAL DOSE LEVEL (MG/KG)		0	50	100	250
LITTERS EVALUATED	N	20	21	16	21
TOTAL PUPS STILLBORN OR FOUND DEAD a,b	N	2	4	4	27
STILLBORN	N	1	0	0	2
FOUND DEAD	N	0	4	4	25
UNSCHEDULED EUTHANASIA	N	1	0	0	0
NO MILK IN STOMACH c	N(%)	0(0.0)	1(25.0)	1(25.0)	14(56.0)
APPEARED NORMAL	N(%)	2(100.0)	3(75.0)	3(75.0)	13(48.1)

(Table excerpted from sponsor's package)

In ovarian and uterine examinations of pregnant F_1 females, there were no drug-related differences in litter averages for corpora lutea, implantations or percentage of

preimplantation loss. There were also no drug-related differences in nonviable embryos or percentage of postimplantation loss of F_2 generation embryos.

9.4 Juvenile Toxicology

Study: A Dose Range-Finding Juvenile Toxicity Study of PF-04971729 by Oral (Gavage) in Rats (Study TT #15-7810 / #20070334 / #14GR472)

Key Study Findings

- NOAEL = 250 mg/kg (♂ & ♀)
  - No significant adverse effects
  - 1348x $MRHD_{AUC}$

METHODS

PND 21 juvenile SD rats (5/sex/group) were administered 0.5% MC / 10% PEG 400 vehicle alone or ertugliflozin (lot #2DH0326) doses of 5, 25, 100, or 250 mg/kg (29x, 176x, 217x, and 1348x $MRHD_{AUC}$) once daily by oral gavage (10 mL/kg) for 2 weeks. Main study animals were assessed for viability, clinical observations, body weights, food consumption and macroscopic findings following termination on PND 35. Blood samples were collected from satellite toxicokinetic animals included control (6/sex/group) and drug-treatment groups (12/sex/group) on PND 21/22 at 1, 4, 7, and 24 hours postdose (3/sex/time point).

RESULTS

There no drug-related mortalities. Decreases in skin turgor were reported in females at 250 mg/kg and are likely related to dehydration secondary to increased diuresis. PD-related decreases in body weight gains were observed during both weeks of dosing in males (↓9-18%) and females (↓23-32%) at 250 mg/kg. Overall mean body weight gains over the course of the study were also slightly lower in both males (↓3%) and females (↓8%) at 100 mg/kg. Non-dose-dependent trends for increases in food consumption were apparent in males (↑17-28%) and females (↑10-17%) at doses ≥25 mg/kg, consistent with anticipated PD effects of SGLT2 inhibition. There were no drug-related macroscopic findings. Overall, the NOAEL was set at the high dose based on the absence of any significant adverse effects.

Exposures increased with increasing dose approximately dose-proportionally. There were no consistent indications of gender effects or accumulation. T_{max} ranged between 1 and 7 hours postdose.

Table 88: Juvenile Rat Toxicokinetics - DRF study #14GR472

Table 1. Mean Overall (Male + Female) Toxicokinetic Parameters ± Standard Deviation in Rats

Dose (mg/kg/day)[a]	PND	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•hours/mL)
5	21	3210	4.00	40100
	34	2070 ± 251	4.00 ± 0.00	24500 ± 4740
25	21	16500	7.00	243000
	34	7710 ± 1110	3.50 ± 1.22	79900 ± 12400
100	21	20500	1.00	300000
	34	27900 ± 7080	3.40 ± 1.34	370000 ± 93200
250	21	127000	4.00	1860000
	34	70200 ± 20400	4.60 ± 1.34	816000 ± 117000

Notes: Day 34 are mean of individual parameters. Day 21 are based on mean values since non-serial sampling was used. Group mean TK data is presented in Supportive Table 7.1.
AUC_{24} = Area under the concentration-time curve from time 0 to 24 hours; C_{max} = Maximum observed concentration.
[a]. 12 animals/sex/dose group (non-serial sampling,Day 21) or serial (Day 34) at n = 3/sex/dose group/collection time), except on Day 34 when reduced due to animal death

(Table excerpted from sponsor's package)

Study: A Juvenile Toxicity Study of PF-04971729 by Oral (Gavage) in Rats (Study #15GR084)

Doses: 5, 25, and 250 mg/kg

Study #	TT #15-7803 / #20075270 / #15GR084
Study report location	eDR
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	5/15/2015
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729, lot #E010015326, 77.1% potency

Key Study Findings

- Maximum Tolerated Dose (MTD) = 25 mg/kg/day (81x $MRHD_{AUC}$)
  - Potentially drug-related mortalities at 250 mg/kg (580x $MRHD_{AUC}$)
- **Kidney NOAEL = Not identified (<17x $MRHD_{AUC}$)**
  - Macroscopic findings of renal pelvic dilatation at all doses (♂) and microscopic findings of pelvis dilatation at 250 mg/kg
  - ↑organ weight at all doses
  - Tubular dilatation and mineralization at all doses
  - Cortical fibrosis at ≥25 mg/kg (81x $MRHD_{AUC}$)
  - 2-fold ↑BUN at 250 mg/kg
  - Kidney findings in 100% of animals at 250 mg/kg (580x $MRHD_{AUC}$)

  - Mineralization and tubular/pelvis dilatations
  - ↑organ weight
  - Kidney findings were not fully reversible in 250 mg/kg recovery groups
    - Since, recovery groups at ≤25 mg were not included, irreversibility of kidney findings could not be assessed at ≤25 mg/kg and potential drug-related effects on **renal development/maturation** at all doses (≥17x $MRHD_{AUC}$) cannot be ruled out
- Sexual Maturation NOAEL = 25 mg/kg (81x $MRHD_{AUC}$)
  - Significant delays in sexual maturation at 250 mg/kg by 3 days in males and 5 days in females
- Growth NOAEL = 5 mg/kg (17x $MRHD_{AUC}$)
  - Growth delay indicated by decreased crown rump lengths during the 1st two months of dosing at ≥25 mg/kg, but which resolved by the end of the dosing period
  - Decreased body weights at ≥25 mg/kg
  - Adverse clinical signs consistent with dehydration at ≥25 mg/kg
- Bone NOAEL = 5 mg/kg (17x $MRHD_{AUC}$)
  - Potential delay in bone maturation at ≥25 mg/kg, that was not fully reversible in recovery animals
  - Microscopic findings of minimal to mild increased bone in females at ≥25 mg/kg and in males at 250 mg/kg, with irreversibility in females
  - Changes in bone turnover markers
    - At ≥25 mg/kg, ↓formation and ↓resorption in males, but only ↓resorption in females
  - Femur ↓length in males at ≥25 mg/kg and in females at 250 mg/kg, partial reversibility
  - Femur ↑metaphysis bone mass parameters at ≥250 mg/kg, limited reversibility
  - Femur ↓diaphysis bone mass parameters at 250 mg/kg, limited reversibility
  - Decreases in histomorphometry parameters consistent with decreased bone formation at ≥25 mg/kg, which may be secondary to ↓resorption.
  - Increased urine Ca levels at 250 mg/kg in males and at ≥25 mg/kg in females
  - Bent tails in females at ≥25 mg/kg

Juvenile Rat, 10 Weeks + 4 Week Recovery	NOAEL (AUC)	Multiple of *$MRHD_{AUC}$
≥5 mg/kg: (17x MRHD) No significant adverse findings unrelated to PD activity. PD-related kidney findings (tubular & pelvic dilatation, mineralization, ↑organ weight), and slight ↑BUN (20-30%) ➢ Similar kidney findings at all doses, but only high dose was evaluated after recovery; thus, *irreversible effects on **renal development/maturation** cannot be ruled out*	Kidney **Not Determined** Non-PD-Related **5 mg/kg** (♂: 20.3 µg•h/mL	Non-PD-Related ♂: **15x** ♀: **20x**

≥25 mg/kg: (81x MRHD) Drug-related findings included ↓BW & ↓weight gain, **growth delay**, kidney (cortical fibrosis), **delayed bone maturation** (↑bone, ↓bone formation, ↓bone resorption, ↓length, ↑metaphysis bone mass, ↓diaphysis bone mass, bent tails), urine changes (↑Ca, ↓Na, ↓Cl), and ↓protein blood levels **250 mg/kg**: (580x MRHD) Drug-related effects included **delayed sexual maturation**, possibly drug-related mortalities, non-reversible bone findings (↑bone, ↓length, ↑metaphysis & ↓diaphysis bone mass), **non-reversible kidney** findings (pelvis & tubular dilatation, ↑organ weight, mineralization), clinical pathology changes (2-fold ↑BUN, ↑K)	♀: 28.0 μg•h/mL)	

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 μg·h/mL and C_{max} = 266 ng/mL

Reviewer's Comments

An MTD was set at 25 mg/kg (81x $MRHD_{AUC}$) due to potential drug-related mortalities at 250 mg/kg (580x $MRHD_{AUC}$) in both sexes.

A NOAEL for kidney findings was not identified for both males and females due to kidney findings of renal tubular dilatation, pelvic dilatation, mineralization, and increased organ weight observed at all doses (≥5 mg/kg) that were not fully reversible in high dose animals evaluated after recovery. Although active nephrogenesis is complete in rats by PND 15, kidney maturation is ongoing at the time of dosing (Seely 2017). Thus, irreversibility of the kidney findings is indicative of drug-related effects on kidney development/maturation. However, since recovery animals were only evaluated at the 250 mg/kg dose, but similar renal findings were observed at all doses, similar irreversible drug-related effects on kidney development/maturation cannot be ruled at out doses of 5 and 25 mg/kg. Furthermore, since the juvenile rat dosing period corresponds to human renal development and maturation during the late 2nd and 3rd trimesters of pregnancy, these data further indicate that a risk for drug-related effects on human renal development/maturation cannot be ruled out at exposures similar to the 5 mg/kg dose (17x $MRHD_{AUC}$).

Drug-related kidney findings observed at all doses were considered to be primarily related to PD effects of SGLT2 inhibition. At 250 mg/kg, microscopic kidney findings correlated with significant 2-fold increases in BUN (♂ & ♀), which is consistent with drug-related kidney toxicity; thus, drug-related kidney toxicity could not be ruled out at 250 mg/kg. Other changes in urine and blood parameters could be attributed to dehydration secondary to PD-related osmotic diuresis and there were no findings of kidney damage, nephropathy or degeneration. Drug-related findings of minimal to mild renal tubular dilatation, pelvic dilatation, and mineralization were reported at all doses

and persisted after recovery. In addition, increases in kidney organ weights were not fully reversible after the 4-week recovery period. Although a risk for renal developmental effects were concluded for all doses, it is noted that there were no clear or consistent indications of significant kidney dysfunction at ≤25 mg/kg.

A NOAEL for drug-related effects on growth was set at 5 mg/kg due to growth delays in both males and females at ≥25 mg/kg. Drug-related weight loss during the 1st week of dosing correlated with a transient drug-related decrease in food consumption; however, as food consumption rebounded with continued dosing, body weight gains recovered as well. Thus, although growth delays can be secondary to decrements in food consumption and body weights in pups, it's unclear if the prolonged delay in growth can be fully attributable to transient decreased food consumption during the 1st week of dosing. It's noted that clinical signs consistent with dehydration at 25 mg/kg were also observed during the 1st week of dosing, but were observed throughout the study at 250 mg/kg.

A NOAEL for drug-related effects on sexual maturation was set at 25 mg/kg due to significant delays in sexual maturation in both males and females at 250 mg/kg. It's noted that drug-related deficits in food consumption and weight loss during the 1st week of dosing were reversed approximately 1 week prior to female sexual maturation and 3 weeks prior to male sexual maturation in controls. Thus, there is potential for a drug-related effect on sexual maturation independent of body weight and food consumption deficits.

A NOAEL for drug-related effects on bone was set at 5 mg/kg due to bone findings in both sexes at ≥25 mg/kg that included changes in bone size (decreased femur length), density (most notably trabecular bone density), and bone regulation. Drug-related decreases in biomarkers of both bone formation and resorption were apparent in males; whereas changes in biomarkers in females were more predominantly indicative of decreased resorption, which is consistent with increased incidence rate and severity of microscopic findings of increased bone in females. The drug-related changes in biomarkers are indicative of reduced bone turnover. Increases in mass parameters of the metaphysis, containing the growth plate, corresponded with decreases in diaphysis parameters of the long bone, which may be consistent with delayed or decreased bone maturation. Furthermore, clinical observations of bent tails in rodents at ≥25 mg/kg are consistent with defects in bone regulation (Ouellet and Odent 2013). The changes in bone mass parameters and turnover biomarkers are also consistent with the overall decreases in bone length, as well as bone and whole animal growth and maturation delays. The sponsor did not consider the bone findings to be adverse, claiming that they were of low magnitude and were consistent with increased bone mass, rather than reduction; however, these findings are considered likely to be drug-related effects on bone regulation leading to defects in development, growth and/or maturation in juveniles, which may be irreversible. Thus, these findings were considered to be potentially adverse developmental effects. However, it is noted that bone-related findings may be secondary to off-target SGLT1 inhibition, in which rats are likely to be

more sensitive than humans. Thus, the clinical relevance of these bone findings remains unclear.

Methods

Doses	5, 25, and 250 mg/kg
Frequency of dosing	Once daily for 10 weeks (PND 21 to PND 90)
Route of administration	Oral gavage
Dose volume	10 mL/kg
Formulation/Vehicle	0.5% MC/10% PEG 400
Species/Strain	Rat / Crl:CD(SD)
Number/Sex/Group	10/sex/group
Age	PND 21
Weight	19.2 g to 32.4 g
Satellite groups	Recovery Groups: 5/sex for control and 250 mg/kg Toxicokinetic Groups: 6/sex for control and 15/sex/group for treatment groups.
Unique study design	• Whole litter design was employed, such that 1 litter represented a dose group. All F1 pups were cross-fostered and weaned on PND 21. • All main study groups were administered a slow subcutaneous (SC) injection of bicarbonate buffered calcein green solution on PND 83 and PND 88 • Both recovery groups were administered a slow SC injection of bicarbonate buffered calcein green solution on PND 110 and PND 115
Deviation from study protocol	Crown rump measurements were not determine for 2 control and two 250 mg/kg recovery males, which may have affected the lack of statistical significance in reduced values for treated males after recovery. Nevertheless, the overall impact of the deviation is considered to be minor and the integrity of the study is considered to be acceptable. All other study deviations were considered to be insignificant.

Study Design

Table 89: Study Design – Juvenile Rat Study #15GR084

Group No.	Test Material	PF-04971729 Dose (mg/kg/day)	Dose Volume (mL/kg)	PF-04971729 Dose Concentration (mg/mL)	No. of Animals			
					Main / Recovery Study[a,b]		Toxicokinetic Study[c]	
					Males	Females	Males	Females
1	Vehicle Control Article	0 (Control)	10	0	15	15	6	6
2	PF-04971729	5	10	0.5	10	10	15	15
3	PF-04971729	25	10	2.5	10	10	15	15
4	PF-04971729	250	10	25	15	15	15	15

TK = Toxicokinetic; M = Male; F = Female; - = Not applicable.

[a] Twenty (10) rats/sex/dose group were assigned to Cohort 1 (main study).

[b] Twenty (5) rats/sex were assigned to Cohort 2 (recovery study; Groups 1 and 4 only) and were given an approximately 4 week treatment-free period after the completion of dose administration.

[c] Three (3) rats/sex in Group 1 and 12 rats/sex in Groups 2 through 4 were assigned for blood sample collection following first dose (PND 21/22). Three (3) rats/sex in Group 1 through 4 were assigned for blood sample collection on PND 90/91, when possible.

(Table excerpted from sponsor's package)

Parameters Measured

Clinical Findings	Clinical observations for viability were conducted twice daily and maternal behavior was recorded daily. During the dosing period, evaluations were conducted prior to dosing, at 1-2 hours postdose study drug, at 1-2 hours after calcein green administration, and at the end of the normal work day.
Body weights	Body weights were measured once daily between PND 21 and PND 28, then twice weekly thereafter. Body weights were also recorded on the day of sexual maturation and when crown rump measurements. Terminal weights were determined the day prior to scheduled euthanasia.
Growth & Development	• Crown rump measurements were determined on PND 21, 30, 60, 90, and 117. • Sexual maturation was evaluated daily for signs of vaginal opening beginning on PND 28 or preputial separation beginning on PND 39.
Food consumption	Food consumption was determined once weekly beginning on PND 21.
Ophthalmoscopy	Examinations of the lens and fundus oculi were conducted on all main study and recovery animals during the last week of dosing (PND 84-87) and on recovery animals on PND 118 using an indirect ophthalmoscope with a hand-held lens.
EKG	Not determined
Hematology	Fasted blood samples were collected at scheduled necropsy on PND 91 for main study groups (10/sex/group) or on PND 118 for recovery groups (5/sex/group). The following parameters were examined: Red blood cell count Hemoglobin concentration Hematocrit Mean corpuscular volume Red Blood Cell Distribution Width Mean corpuscular hemoglobin concentration Mean corpuscular hemoglobin Reticulocyte count (absolute) Platelet count White blood cell count Neutrophil count (absolute) Lymphocyte count (absolute) Monocyte count (absolute) Eosinophil count (absolute) Basophil count (absolute) Large unstained cells Other cells (as appropriate)
Clinical chemistry	Fasted blood samples were collected at scheduled necropsy on PND 91 for main study groups (10/sex/group) or on PND 118 for recovery groups (5/sex/group). The following parameters were examined:

	Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Gamma-glutamyltransferase, Creatine kinase, Total bilirubin, Urea nitrogen, Creatinine, Calcium, Phosphorus, Total protein, Albumin, Globulin (calculated), Albumin/globulin ratio, Glucose, Cholesterol, Triglycerides, Sodium, Potassium, Chloride
Urinalysis	Urine samples were collected overnight prior to scheduled euthanasia. The following parameters were examined: Color, Clarity, Specific gravity, Total Volume, pH, Protein, Glucose, Bilirubin, Ketones, Nitrites, Leukocytes, Blood, Urobilinogen Calcium, Phosphorus, Sodium, Potassium, Chloride, Creatinine, Glucose
Hormone & Bone Biomarkers	Remaining fasted blood samples collected on PND 91 from main study animals and PND 118 from recovery animals were evaluated for the bone formation markers N-terminal propeptide of type I procollagen (PINP) and osteocalcin (OC), as well as the bone resorption markers C-terminal telopeptides of type I collagen (CTx I) and the active isoform 5b of the tartrate-resistant acid phosphatase (TRAcP 5b). PTH levels were also determined in remaining fasted blood samples collected from main study animals and recovery animals.
Gross pathology	Complete necropsy evaluations included evaluation of the carcass, musculoskeletal system; all external surfaces and orifices; crania cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities. Necropsy evaluations were performed at scheduled euthanasia of main study groups on PND 90 or recovery groups on PND 118, as well as for any unscheduled deaths. Gross evaluations of unscheduled deaths of TK animals were also performed, but tissues were not retained.
Organ weights	Organ weights were determined at schedule euthanasia of all mains study and recovery animals. Paired organs were weighed together. Weighed organs included the epididymis, prostate gland, seminal vesicle, kidneys, and testis.
Bone	• Femur length was measured for all main study and recovery groups. • Bone densitometry was determined using peripheral quantitative computed tomography (pQCT) on the right femur metaphysis and diaphysis for all main study and recovery groups. • The right proximal tibias of all main study and recovery groups were dehydrated, embedded with methyl-methacrylate (MMA), and evaluated by histomorphometry using calcein green. o Histomorphometric parameters included single label surface, double label surface, mineralizing surface, mineral apposition rate, and BFR surface referent. o Unstained sections were evaluated for cancellous bone. • Bone assessments were not determined for unscheduled deaths
Histopathology	Tissues from all main study and recovery groups were collected and processed for microscopic evaluation or retained for possible future evaluation.

	Bone (left femur, right femur, and right tibia), adrenal gland, prostate gland, seminal vesicle, kidneys, large intestine (cecum, colon, and rectum), small intestine (duodenum, ileum, jejunum), stomach and urinary bladder tissue sections from control and 250 mg/kg main study and recovery groups were processed and stained with H&E for microscopic evaluation. Only bone and kidney from 5 and 25 mg/kg groups were examined microscopically.
	Tissues that were collected, but not processed or examined microscopically included epididymis, esophagus, macroscopic lesions/masses, heart, liver, lung, testis, trachea, and lumbar vertebrae. • Tissues collected from unscheduled necropsies only included esophagus, heart, liver, lung, spleen and trachea. • Testis, lumbar vertebrae, epididymis, and macroscopic lesions/masses were collected from all animals and retained for possible future evaluation.
	Only selected tissues from selected rats were examined by a reviewing pathologist.
Toxicokinetics	Blood samples were collected from TK animals at 1, 4, 7, and 24 hours postdose (3/sex/time point) on PND 21 and PND 90. Plasma PF-04971729 concentrations were determined using a validated LC-MS/MS method.

Observations and Results

Mortality

There were a total of 5 unscheduled deaths, which were not attributed to drug treatment by the sponsor. Deaths of one 5 mg/kg male (#402) and one 250 mg/kg male (#1004) were associated with findings consistent with gavage trauma. Control male #1301 was euthanized based on clinical signs of morbidity, which were considered likely to be related to suspected dehydration, which was associated with bradypnea nad may have been secondary to accidental dosing. Animals #804 and #910 in the 250 mg/kg group exhibited clinical signs indicating failure to thrive after weaning, but were not considered by the sponsor to be drug-related because of "similar" findings for control male #1301. However, there were additional findings observed in the mortalities at 250 mg/kg that were consistent with adverse drug-related clinical signs in other drug-treated animals; thus, a potential contribution from the study drug cannot be ruled out in the mortalities at 250 mg/kg. Furthermore, the mortalities are consistent with potentially drug-related mortalities observed at the same dose in the rat fertility study #10GR227.

Table 90: Mortality – Juvenile Rat Study #15GR084

MORTALITY					
Dose (mg/kg)	**Animal (sex, #)**	**Day**	**Cause of Death**	**Clinical signs**	**Pathology**
0	♂ #1301	PND 22	Undetermined	Suspected dehydration, ↓motor activity, ptosis, coldness to touch, pale extremities, and bradypnea	No macroscopic findings. Histopathology was not evaluated

250	♂ #804	PND 23	Undetermined	Weight loss, suspected dehydration (slight), eyes partly closed	No abnormal findings were reported
250	♀ #910	PND 30	Found dead. Undetermined	Abdominal distention, suspected dehydration, erected fur, and partly closed eyes	Kidney bilateral minimal multifocal tubular basophilia.

Clinical Signs

Adverse drug-related clinical signs were observed in both males and females at ≥25 mg/kg, with presence in all animals at 250 mg/kg. Dose-dependent increases in skin turgor observations consistent with mild to moderate dehydration were reported at ≥25 mg/kg and nearly all (97%) of animals at 250 mg/kg. Dose-dependent increases in abdominal distention and partly closed eyes were also observed in both males and females at ≥25 mg/kg, reaching 97% and 100% penetrance at 250 mg/kg, respectively. Observations of hunched posture and erected fur were also observed at 250 mg/kg in both sexes, but were not present in recovery groups, indicating recovery. Observations of suspected dehydration in males and abdominal distention in both sexes were reduced, indicating a trend towards recovery.

Dose-dependent increases in bent tail observations were reported at ≥25 mg/kg after 7 weeks of dosing. Kinked tails are consistent with defects in bone regulation in rodents (Ouellet and Odent 2013); thus, this findings is considered to be consistent with other drug-related bone effects and is likely to be drug-related.

Table 91: Clinical Signs – Juvenile Rat Study #15GR084

Clinical Observations								
Clinical sign	**Male (mg/kg/day)**				**Female (mg/kg/day)**			
	0 (n=15)	**5** (n=10)	**25** (n=10)	**250** (n=15)	**0** (n=15)	**5** (n=10)	**25** (n=10)	**250** (n=15)
Dosing Phase (PND21-PND91)								
Suspected Dehydration (Skin turgor)	0	2 (1)	4 (16)	15 (92)	3 (3)	1 (3)	5 (21)	14 (81)
Abdominal Distention	0	0	1 (2)	14 (663)	0	0	1 (2)	15 (566)
Hunched Posture	0	0	0	1 (5)	0	0	0	1 (4)
Erected Fur	0	0	0	14 (132)	0	0	0	15 (156)
Partly Closed	0	1	2	15	0	0	2	15

Eyes		(1)	(2)	(37)			(2)	(41)
Bent Tail	0	0	1 (31)	4 (62)	0	0	2 (137)	0
Recovery Phase (n=5) **(PND92-PND119)**								
Suspected Dehydration (Skin turgor)	0	-	-	1 (4)	0	-	-	0
Abdominal Distention	0	-	-	2 (3)	0	-	-	1 (6)
Bent Tail	0	-	-	2 (54)	0	-	-	0

() = total # of observations

Body Weights

In males, drug-related decreases in body weights and weight gains were observed at ≥25 mg/kg, with dose-dependent weight loss noted after the 1st dose in all treatment groups. The decreases in body weights and weight gains are consistent with findings in adults and are attributed to the PD action associated with SGLT2 inhibition. 250 mg/kg male body weights remained significantly lower than concurrent controls after the recovery period; however, weight gains rebounded and were slightly greater than controls after dosing cessation.

In females, dose-dependent weight loss was observed after the 1st dose in all treatment groups with significantly lower weight gains during the 1st week of dosing. Furthermore, 250 mg/kg body weights were significantly lower than controls during the 1st three weeks of dosing. However, overall mean body weights and weight gains at the end of the dosing period were not statistically significant different from controls. Overall, the decreases in body weights and weight gains at 250 mg/kg were considered to be drug-related, but were transient and secondary to the PD activity of ertugliflozin. Although overall weight gains were lower than controls over the course of the recovery period, the difference is likely attributable to variability and there was not a consistent trend for reduced weight gain during recovery.

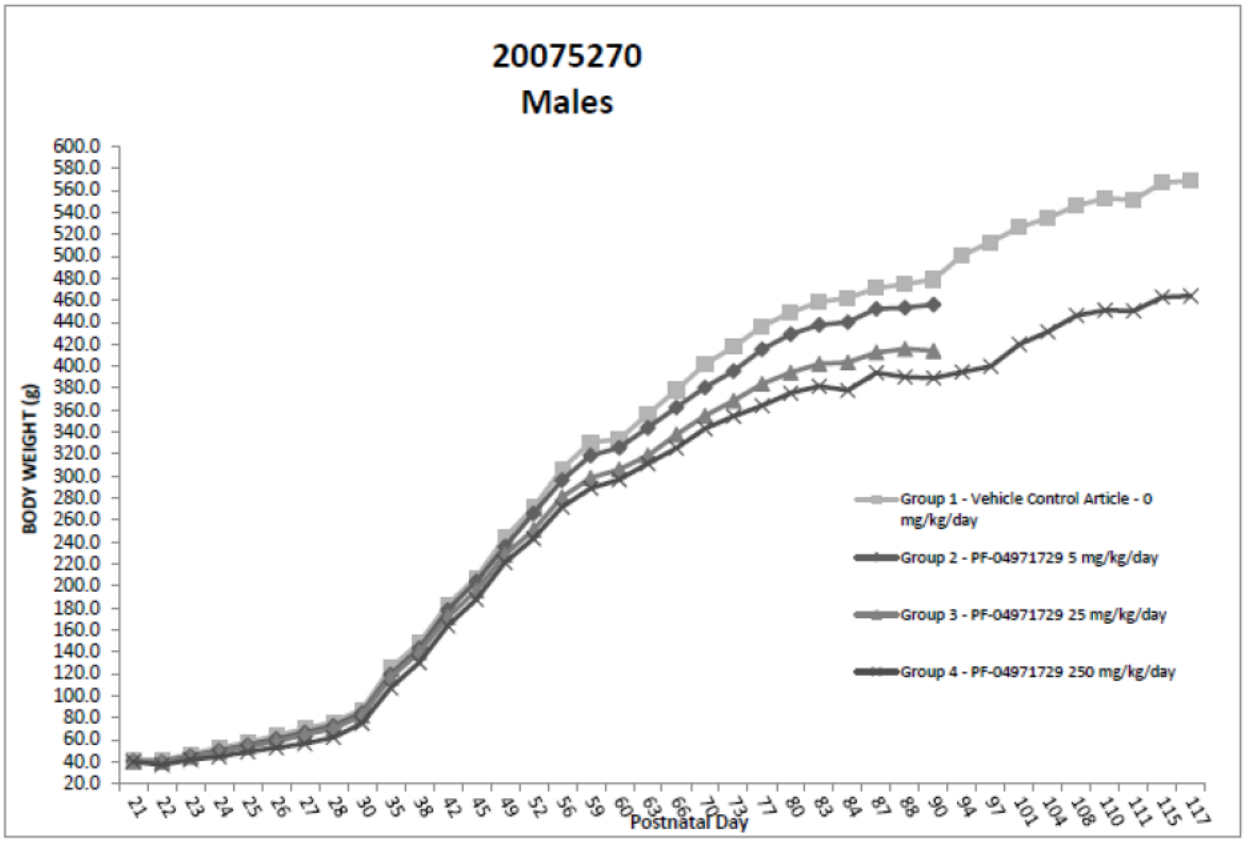
Table 92: Body Weights – Juvenile Rat Study #15GR084

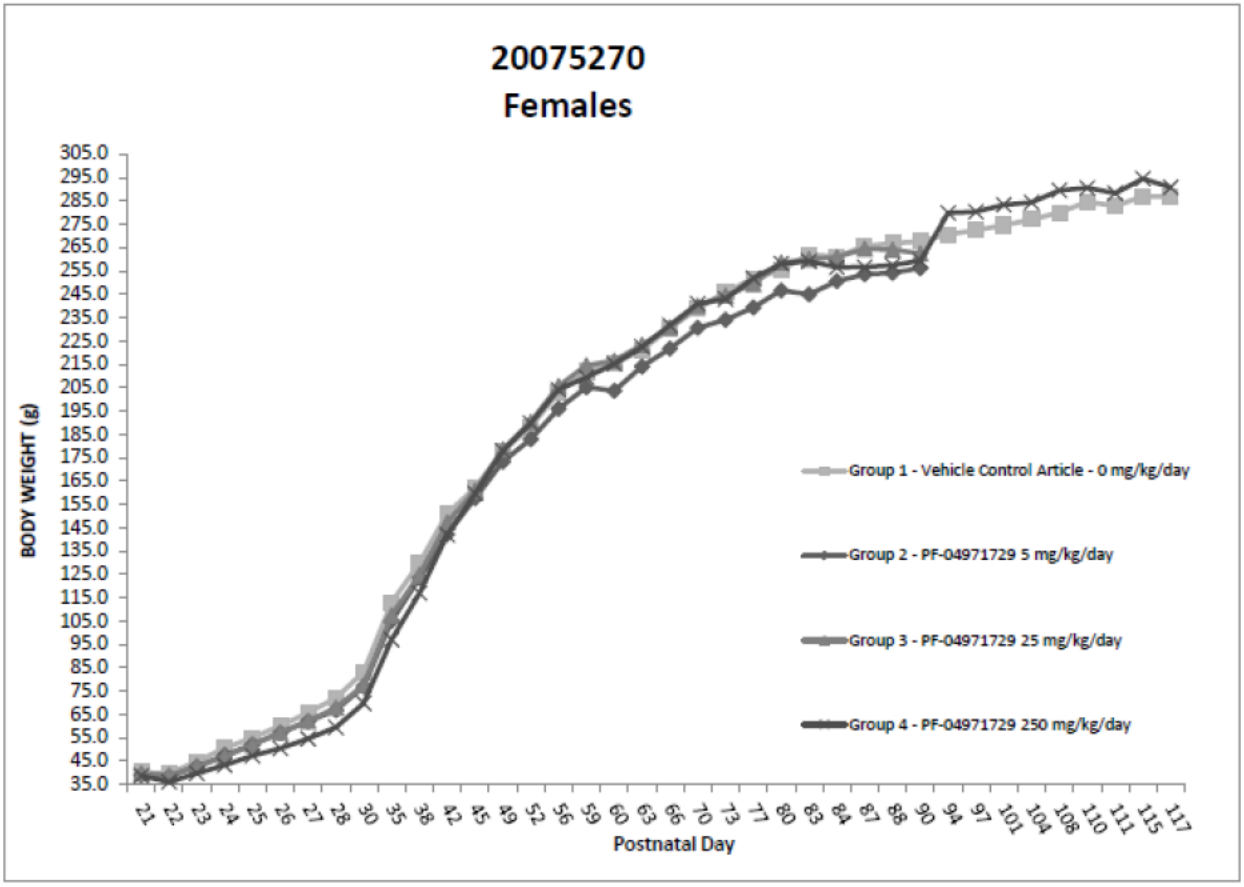
MALES: Body Weight				
Study Time	Dose (mg/kg)	BW gain (g) over study	% Decrement	BW % control
PND 90 (End of Treatment)	0	437.5	-	-
	5	419.9	96.0% (↓4.0%)	95.2% (↓4.8%)
	25	373.6*	85.4% (↓14.6%)	86.4%* (↓13.6%)

	250	348.5*	79.7% (↓20.3%)	81.3%* (↓18.7%)
Week 6 (End of Recovery)	0	67.8	-	-
	250	69.2	102.1%	81.6%* (↓18.4%)
FEMALES: Body Weight				
Study Time	Dose (mg/kg)	BW gain (g) over study	% Decrement	BW % control
PND 90 (End of Treatment)	0	227.4	-	-
	5	217.3	95.6% (↓4.4%)	95.7% (↓4.3%)
	25	222.8	98.0% (↓2.0%)	98.1% (↓1.9%)
	250	220.4	96.9% (↓3.1%)	96.9% (↓3.1%)
Week 6 (End of Recovery)	0	16.4	-	-
	250	11.0	67.1% (↓32.9%)	101.3%

* P value <0.05

Figure 14: Juvenile Rat Body Weights - Study #15GR084





(Figures excerpted from sponsor's package)

Feed Consumption

Drug-related decreases in food consumption were observed in both males (↓21%) and females (↓14%) at 250 mg/kg during the first week of dosing. However, food consumption rebounded and was significantly higher than concurrent controls in males at 250 mg/kg and in females at all doses throughout the last 7 weeks of dosing. Increases in food consumption are consistent with observations in adults and with the SGLT2 inhibitor drug class. During the last week of recovery, food consumption was not significantly different from controls, but tended to remain slightly higher in females.

Table 93: Food Consumption – Juvenile Rat Study #15GR084

Food Consumption						
Dose (mg/kg)	**Dosing Week 1** (PND 21- 28)		**Dosing Weeks 4-10** (PND 42-90)**		**Recovery Week 4** (PND 111-117)**	
	Males	Females	Males	Females	Males	Females
0	9.84	9.04	26.47	17.14	31.50	16.89
5	9.84	9.37	29.42 (↑11.1%)	20.78* (↑21..2%)	-	-
25	9.66	9.34	28.63	21.77*	-	-

			(↑8.2%)	(↑27.0%)		
250	7.75* (↓21.2%)	7.79* (↓13.8%)	31.89* (↑20.5%)	24.90* (↑45.3%)	28.17	20.22 (↑19.7%)

* p value < 0.05
** average of weekly interval mean values

Ophthalmoscopy

There were no treatment-related effects.

Developmental Assessments

Growth – Crown Rump Length

Drug-related decreases in growth were observed in males and females during the first 5 weeks of dosing; however, growth measurements were comparable to controls by the end of the dosing period. During the 2nd week of dosing, crown rump measurements were significantly lower by 7% in both males and females at 250 mg/kg. During dosing Week 5, crown rump lengths were significantly lower at ≥25 mg/kg in both males (↓3-4%) and females (2-4%). However, there were no significant differences in crown rump measurements by the end of the dosing period (PND 90) or the end of the recovery period (PND 117). Overall, the decreases in growth at ≥25 mg/kg were considered to be treatment-related, but resolved with continued dosing.

Table 94: Growth – Juvenile Rat Study #15GR084

GROWTH: Crown Rump Length								
Dose (mg/kg)	**Dosing Week 2** (PND 30)		**Dosing Week 5** (PND 60)		**End of Dosing** (PND 90)		**End of Recovery** (PND 117)	
	Males	Females	Males	Females	Males	Females	Males	Females
0	11.53	11.33	18.97	17.13	20.30	17.87	23.00	18.63
5	11.35	10.95	18.90	16.85	20.35	18.05	-	-
25	11.50	11.05	18.35* (↓3.3%)	16.75 (↓2.2%)	20.00	17.80	-	-
250	10.68* (↓7.4%)	10.53* (↓7.1%)	18.27* (↓3.7%)	16.46* (↓3.9%)	20.12 (↓0.9%)	17.57 (1.7%)	21.50 (↓6.5%)	19.17

* p value < 0.05

Sexual Maturation

Drug-related delays in sexual maturation were observed in both sexes at 250 mg/kg. In 250 mg/kg males, the mean day of balano-preputial separation was delayed by 3 days. In 250 mg/kg females, the mean day of vaginal patency was delayed by 5 days, which also correlated with a significant 15% increase in mean body weight.

Table 95: Sexual Maturation – Juvenile Rat Study #15GR084

Sexual Maturation

Dose (mg/kg)	Males		Females	
	Day	Delay (Days)	Day	Delay (Days)
0	47.8	-	33.9	-
5	47.0	-	34.1	-
25	49.1	1.3	34.5	0.6
250	50.8*	3	39.1*	5.2

* p value < 0.05

Hematology

Non-adverse, treatment-related changes in reticulocyte and platelets were observed at 250 mg/kg in both sexes. Reticulocytes were significantly decreased by 24% in males at 250 mg/kg, but were not observed in females and were not significantly different from controls after recovery. Platelet counts were decreased by 17% in males and females at 250 mg/kg, but only reached statistical significance in females and rebounded after recovery. Although the observed changes in hematology parameters are considered to be small and non-adverse, they are consistent with findings in adult rats and are likely to be drug-related.

It is noted that there were no significant treatment-related changes observed in erythrocyte counts, hemoglobin, hematocrit, or white blood cell counts.

Table 96: Hematology Parameters – Juvenile Rat Study #15GR084

RBC Parameters								
Dose (mg/kg)	Retic. (10^3/µl)				Platelets (10^3/CMM)			
	Males		Females		Males		Females	
	PND 91	PND 118	PND 91	PND 118	PND 91	PND 118	PND 91	PND 118
0	143.8	189.7	129.0	127.7	933	944	899	888
5	146.7	-	153.2	-	914	-	884	-
25	135.2	-	133.1	-	856	-	925	-
250	108.7* (↓24.4%)	175.6	130.6	137.0	774 (↓17.0%)	1007	744* (↓17.2%)	964* (↑8.6%)

* p value < 0.05

Clinical Chemistry

Sporadic, dose-independent and occasionally statistically significant (at the group mean level) increases in GGT were observed. However, all group mean and individual values remained with the normal range for this parameter. In 250 mg/kg males, minimal

increases in ALT (mean ↑43%) and/or ALP were also often observed. Minimal increases in mean ALT levels were also reported in females (↑27%) at 250 mg/kg, but did not reach statistical significance in males and were comparable to controls after recovery. Overall, there was no conclusive evidence of liver toxicity based on increases in liver enzyme levels.

Dose-dependent increases in BUN levels were observed at all doses in both males and females, reaching 2-fold increases at 250mg/kg. However, there were no correlating increases in levels of creatinine, which is a primary marker of kidney dysfunction. The increases in BUN levels may be indicative of dehydration secondary to PD-related increases in urine output and osmotic diuresis; however, it is noted that there were no correlating increases in red blood cell parameters or protein levels. Conversely, slight decreases in albumin, globulin, and/or total protein levels were reported at ≥25 mg/kg in treated males and females, which would argue against dehydration and may be more consistent with kidney or liver dysfunction. However, there were no significant drug-related increases in urinary protein levels.

Dose-dependent increases in potassium levels were observed in 25 mg/kg (↑2%) and 250 mg/kg (↑11%) females, reaching statistical significance at 250 mg/kg. Furthermore, potassium levels remained 14% higher than concurrent controls after recovery, statistical significance was not achieved. Although increases in potassium levels can be indicative of renal dysfunction, they may also be consistent with dehydration secondary to PD-related osmotic diuresis.

Mild dose-dependent decreases in blood glucose levels, ranging between 11% and 29% of concurrent controls, were reported in males at ≥25 mg/kg and in females at all doses. These changes are consistent with the PD activity of SGLT2 inhibition and subsequent glucosuria.

Table 97: Blood Clinical Chemistry - Juvenile Rat Study #15GR084

Text Table 34
Summary of PF-04971729-related Clinical Chemistry Parameter Effects
(Mean Control Values and Ratio Relative to Control) on PND 91

Parameter	Units	Males				Females			
		0	5	25	250	0	5	25	250
ALT	UI/L	35	-	-	**1.43x**	33	-	-	1.27x
ALK PHOS'TASE	UI/L	124	-	-	1.19x	-	-	-	-
GGT, Serum	UI/L	0.01	6x	11x	3x	0.22	**2.23x**	1.50x	**2.77x**
Albumin	g/dL	-	-	-	-	3.31	-	**0.93x**	**0.90x**
Total Protein	g/dL	-	-	-	-	5.52	-	0.95x	0.93x
Globulin	g/dL	2.39	-	-	**0.87x**	-	-	-	-
A/G Ratio	-	1.21	-	-	**1.18x**	-	-	-	-
Glucose	mg/dL	166	-	0.89x	**0.71x**	170	0.86x	**0.75x**	**0.72x**
Urea Nitrogen	mg/dL	15	**1.33x**	**1.67x**	**2.07x**	15	1.20x	**1.60x**	**2.27x**

Doses in mg/kg/day
Control mean values and the ratio of the test article-related findings relative to control means are listed.
Abbreviations: - = Not test article-related; ALK PHOS'TASE = alkaline phosphatase.
Bolded values indicate statistical significance (p≤0.05).

Text Table 35
Summary of PF-04971729-related Clinical Chemistry Parameter Effects
(Mean Control Values and Ratio Relative to Control) on PND 118

Parameter	Units	Males		Females	
		0	250	0	250
Globulin	g/dL	2.37	**0.90x**	-	-
A/G RATIO	-	1.17	**1.12x**	-	-
Urea Nitrogen	mg/dL	21	1.19x	20	1.10x

Doses in mg/kg/day
Control mean values and the ratio of the test article-related findings relative to control means are listed.
Abbreviations: - = Not test article-related; N/A = not applicable as no sample taken for the group.
Bolded values indicate statistical significance ($p \leq 0.05$).

(Tables excerpted from sponsor's package)

Significant increases in triglycerides (↑87%) were observed in females at 250 mg/kg, but were not considered to be adverse or toxicologically significant.

Urinalysis

Significant drug-related glucosuria was observed in all treatment groups, with markedly high glucose concentrations (≥2000 mg/dL) in 96% of males and 80% of females evaluated. Furthermore, urine glucose:creatinine ratios were markedly higher than controls in all treatment groups by 53- to 96-fold in males and 106- to 228-fold in females.

In males, drug-related increases in average urine volume were generally observed at all doses, and were reported in most males at ≥25 mg/kg. In females, drug-related increases in average urine volume were apparent at ≥25 mg/kg. Drug-related increases in urine volume are anticipated PD-related effects secondary to SGLT2 inhibition and glucosuria. After recovery, average urine output was similar to controls in females, but remained higher in males, similarly to irreversible findings in dogs. All other urinalysis parameters were comparable to controls after recovery.

Table 98: Urine Volume - Juvenile Rat Study #15GR084

Urine Volume Average (mL)				
Dose (mg/kg)	**Males**		**Females**	
	PND 91	**PND 118**	**PND 91**	**PND 118**
0	9.8 to* 10.3	10.0 to* 11.0	4.3 to* 6.8	6.0 to* 8.0
5	13.0	-	5.5 to* 6.5	-
25	13.6	-	8.5	-
250	17.2	19.5	9.5 to* 10	5.0 to* 6.5

* Calculated range of group average, based on the presence ≥1 value reported as an inequality

Treatment-related changes in electrolyte levels and electrolyte ratios normalized to creatinine were reported in both males and females at ≥25 mg/kg. Dose-dependent

decreases in urine creatinine concentrations reached statistical significance at 250 mg/kg in both males (↓53%) and females (↓55%), which is indicative of urine dilution and consistent with drug-related increases in urine volume output. Dose-dependent decreases in sodium:creatinine (↓59-73%) and chloride:creatinine (↓45-57%) ratios were reported at ≥25 mg/kg. Dose-dependent decreases in urine potassium levels were also observed, reaching statistical significance at 250 mg/kg in males (↓56%) and a similar non-significant trend in 250 mg/kg females (↓33%). However, statistical significance was lost after normalization to creatinine and the urine potassium:creatinine ratio was only slightly lower in 250 mg/kg males (↓7%). Overall, the observed decreases in sodium, chloride, and, possibly, potassium electrolyte levels are likely to be secondary to PD-related osmotic diuresis and are considered to be non-adverse. After recovery, all urine electrolyte creatinine ratios were comparable to controls.

On the other hand, dose-dependent increases in urine calcium:creatinine ratios were observed in both sexes, reaching statistical significance at 250 mg/kg (↑6-fold) in males and at ≥25 mg/kg (↑3- to 9-fold) in females. Furthermore, increases in urine calcium levels are consistent with ertugliflozin-related findings in adult rats and observations with some other members of the SLGT2 inhibitor drug class. Thus, this finding is considered likely to be drug-related. After recovery, all urine calcium:creatinine ratios were comparable to controls.

Table 99: Urine Clinical Chemistry - Juvenile Rat Study #15GR084

Text Table 36
Summary of PF-04971729-related Urine Chemistry Parameter Effects
(Mean Control Values and Ratio Relative to Control) on PND 91

	Males				Females			
Parameter	0	5	25	250	0	5	25	250
NA/CREAT Ratio	1.37	-	0.44x	0.27x	1.13	-	0.61x	0.37x
CL/CREAT Ratio	1.68	-	0.52x	0.43x	1.34	-	-	0.55x
U GLU/CREAT Ratio	3.62	**53x**	**67x**	**96x**	1.50	**106x**	**168x**	**228x**
U CA/CREAT Ratio	0.08	-	-	**6x**	0.17	2.24x	**2.88x**	**9.41x**

Doses in mg/kg/day.
Control mean values and the ratio of the test article-related findings relative to control means are listed.
Abbreviations: - = Not test article-related; NA/CREAT= urine sodium/creatinine ratio; CL/CREAT = urine chloride/creatinine ratio; U GLU/CREAT = urine glucose/creatinine ratio; U CA/CREAT = urine calcium/creatinine ratio.
Bolded values indicate statistical significance ($p \leq 0.05$).

(Table excerpted from sponsor's package)

Slight, yet dose-dependent increases in urine specific gravity were also reported in all treatment groups, reaching statistical significance at 250 mg/kg in females (↑0.8%) and in males (↑0.4%). Although this change is in the direction consistent with dehydration and drug-related findings in adults, it is considered to be minimal and unlikely to be biologically significant.

Dose-dependent decreases in urine pH were observed in all treatment groups, reaching statistical significance at 250 mg/kg in males (↓11%) and in females (↓16%). Although

drug-related decreases in pH correlated with increases in ketone levels in adults, there were no significant increases in incidences or severity of ketone levels in juveniles.

Table 100: Urine Clinical Chemistry - Juvenile Rat Study #15GR084

Dose (mg/kg)	Specific Gravity				pH			
	Males		Females		Males		Females	
	PND 91	PND 118	PND 91	PND 118	PND 91	PND 118	PND 91	PND 118
0	1.0075	1.0100	1.0065	1.0100	8.00	7.80	7.65	7.50
5	1.0090	-	1.0090	-	7.80	-	7.30 (↓4.6%)	-
25	1.0100	-	1.0100	-	7.78 (↓2.8%)	-	7.40 (↓3.3%)	-
100	1.0113* (↑0.4%)	1.0070	1.0145* (↑0.8%)	1.0113	7.13* (↓10.9%)	8.10	6.45* (↓15.7%)	7.38

* p value < 0.05

There were no drug-related increases in blood, leukocytes, ketones, bilirubin, urobilinogen, nitrite, or protein in the urine. Thus, there were no clear and/or consistent urinary signs of infection or dysfunction.

Gross Pathology

Dose-dependent increases in renal pelvic dilatation were reported in males at all doses, but were not observed in males after the 4-week recovery period. No findings of renal pelvic dilatation were reported in females at the end of the dosing period, but were present in one female after recovery.

Table 101: Macroscopic Findings - Juvenile Rat Study #15GR084

Text Table 27
Summary of Macroscopic Pathology Findings – Main Study (PND 91)

	Males				Females			
	0	5	25	250	0	5	25	250
Kidney (No. Examined)	10	10	10	8	10	10	10	10
Dilatation, Pelvis	0	1	2	3	0	0	0	0

Doses in mg/kg/day.

Text Table 28
Summary of Macroscopic Pathology Findings – Recovery Study (PND 118)

	Male		Females	
	0	250	0	250
Kidney (No. Examined)	5	5	5	4
Dilatation, Pelvis	0	0	0	1

Doses in mg/kg/day.

(Tables excerpted from sponsor's package)

Single incidences of black foci in the stomach mucosa were reported at 250 mg/kg in males and females, and are consistent with stomach findings in adult rats that are likely

related to SGLT1 inhibition. However, there were no abnormal macroscopic stomach findings after recovery, indicating reversibility.

Organ Weights

Treatment-related increases in absolute and relative kidney organ weights were reported in males (↑10-49%) and females (13-31%) at all doses. Increased kidney weights correlate with renal dilatation findings and are attributable to PD-related diuresis. Nevertheless, kidney organ weights remained higher than concurrent controls after the 4-week recovery period.

Treatment-related decreases in absolute (↓20-39%) and relative (↓12-26%) prostate organ weights were also observed in males at all doses.

Table 102: Organ Weights - Juvenile Rat Study #15GR084

Text Table 29
Ratios of PF-04971729-related Mean Absolute and Relative (to Body Weight and Brain Weight) Organ Weights Compared with Mean Controls-Main Study (PND 91)

		Males				Females			
		0	5	25	250	0	5	25	250
Kidney	Absolute (g)	3.301	**1.27x**	1.10x	**1.23x**	1.966	1.13x	**1.19x**	**1.22x**
	OW:BW[a]	0.696	**1.32x**	**1.26x**	**1.49x**	0.730	**1.18x**	**1.22x**	**1.31x**
Prostate	Absolute (g)	1.022	**0.80x**	**0.77x**	**0.61x**	NA	NA	NA	NA
	OW:BW	0.215	**0.84x**	0.88x	**0.74x**	NA	NA	NA	NA

Doses in mg/kg/day.
Abbreviations: - = Not test article-related; BW = Body weight; OW = Organ weight; NA = Not applicable; g = grams.
[a] Organ weight relative to terminal body weight.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – p≤0.05; refer to data tables for actual significance levels and tests used.

Text Table 30
Ratios of PF-04971729-related Mean Absolute and Relative (to Body Weight and Brain Weight) Organ Weights Compared with Mean Controls (PND 118)

		Males		Females	
		0	250	0	250
Kidney	Absolute (g)	3.684	0.98x	1.847	1.21x
	OW:BW[a]	0.651	**1.21x**	0.646	**1.18x**

Doses in mg/kg/day.
Abbreviations: - = Not test article-related; BW = Body weight; OW = Organ weight; g = grams.
[a] Organ weight relative to terminal body weight.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – p≤0.05.

(Tables excerpted from sponsor's package)

Histopathology

Battery Considered Adequate? Yes.

Peer Review Performed? Partially. A full peer review was not conducted since only selected tissues from selected rats were examined by the reviewing pathologist.

Drug-related findings were observed in the kidney and bone of both males and females.

In the kidney, incidences of minimal to mild tubular dilatation were reported in 80% to 100% of surviving males and females at all doses, with dose-dependency in severity apparent in females. After recovery, observations of minimal tubular dilatations remained in 80% of males and 50% of females examined, indicating lack of reversibility after 4 weeks of dosing cessation. Dose-dependent increases in total incidences of minimal to mild pelvis dilatation were reported in both sexes, reaching 75% of surviving males and 40% of females at 250 mg/kg. After recovery, pelvis dilatation was reported in 1 of 4 females. Increases in minimal mineralization were observed at all doses in 30% to 50% of females and 20% to 63% of surviving males, and remained present in 75% to 100% of animals after recovery. Minimal cortical fibrosis was also reported in 2 females at ≥25 mg/kg and one 25 mg/kg male, but was not present in recovery animals. Overall, drug-related kidney findings were observed in both sexes at all doses and were not fully reversible after 4 weeks of recovery. However, there were no drug-related increases in renal cysts, inflammation, or structural damage, such as degeneration, nephropathy or necrosis.

Dose-dependent increases in incidence and severity of minimal to mild increased bone were reported in 60% to 100% of females at ≥25 mg/kg, and remained present in 75% of recovery females. Findings of minimally increased bone were also reported in 33% of 250 mg/kg males, including one animal found dead after a dosing accident, but were not observed in recovery males. These findings are considered to be drug-related and non-reversible in females, and are consistent with changes in bone maturation.

Table 103: Microscopic Findings - Juvenile Rat Study #15GR084

Text Table 31
Summary of PF-04971729-related Microscopic Findings – Main Study (PND 91)

	Males				Females			
	0	5	25	250	0	5	25	250
Kidney (No. Examined)	10	10	10	8	10	10	10	10
Dilatation, tubular	(1)[a]	(8)	(8)	(8)	(5)	(9)	(8)	(9)
Minimal	1	4	6	6	5	9	7	5
Mild	0	4	2	2	0	0	1	4
Dilatation, pelvis	(0)	(1)	(2)	(6)	(0)	(1)	(1)	(4)
Minimal	0	0	1	6	0	1	0	4
Mild	0	1	1	0	0	0	1	0
Mineralization	(0)	(2)	(4)	(5)	(0)	(3)	(3)	(5)
Minimal	0	2	4	5	0	3	3	5
Bone femur (No. Examined)	10	10	10	8	10	10	10	10
Increased bone	(0)	(0)	(0)	(2)	(0)	(0)	(6)	(10)
Minimal	0	0	0	2	0	0	6	9
Mild	0	0	0	0	0	0	0	1

Doses in mg/kg/day.
[a] Numbers in parentheses represent the number of animals with the finding.

Text Table 32
Summary of PF-04971729-related Microscopic Findings – Recovery Study (PND 118)

	Male		Female	
	0	250	0	250
Kidney (No. Examined)	5	5	5	4
Dilatation, tubular	(0)[a]	(4)	(0)	(2)
Minimal	0	4	0	2
Dilatation, pelvis	(0)	(0)	(0)	(1)
Minimal	0	0	0	1
Mineralization	(0)	(5)	(0)	(3)
Minimal	0	5	0	2
Mild	0	0	0	1
Bone femur	5	5	5	4
Increased bone	(0)	(0)	(0)	(3)
Minimal	0	0	0	1
Mild	0	0	0	2

Doses in mg/kg/day.

(Tables excerpted from sponsor's package)

Single incidences of minimal stomach erosion and edema, as well as single cell necrosis of the cecum were reported in females at 250 mg/kg, but were not present in recovery animals. These findings are consistent with those reported in adult rats; thus, a relation to treatment is possible despite the low incidence.

Bone Evaluation

Bone Turnover Biomarkers

Drug-related changes in bone turnover were reported at ≥25 mg/kg. Decreases in bone formation and bone resorption markers were observed in males. However, only statistically significant decreases in the bone resorption maker TRACP-5b were observed in females.

Drug-related decreases in the bone formation markers PINP (↓47%) and osteocalcin (↓20-41%) were observed in males at 250 mg/kg and ≥25 mg/kg, respectively. There were no statistically significant changes in bone formation markers in females. However, similar trends for reduced PINP (↓16%) and osteocalcin (↓24%) were apparent in females at 250 mg/kg. There were no significant differences in PINP or osteocalcin levels after recovery; however, there was a non-significant trend for higher PINP values in treated males (↑37%) and females (↑16%) compared to controls. Similarly, there was a non-significant trend for increased osteocalcin values after recovery (↑9-18%).

Statistically significant, dose-dependent decreases in the bone resorption marker TRACP-5b were reported at ≥25 mg/kg in males (↓16-34%) and females (↓31-52%). There was also an apparent dose-dependent trend for decreases in the bone resorption marker CTx I in males, particularly at 250 mg/kg (↓33%); however, statistical significance was not achieved. Non-significant trends for increased TRACP-5b (↑24%) and CTx I (↑17%) values were apparent in males after recovery.

Table 104: Bone Turnover Markers - Juvenile Rat Study #15GR084

Text Table 26
Summary of PF-04971729-related Bone Formation and Resorption Markers

Parameter	Units	Males				Females			
		0	5	25	250	0	5	25	250
Bone Formation Markers									
PINP	mg/mL	76.550	-	-	**0.53x**	-	-	-	-
OC	ng/mL	639.267	-	**0.80x**	**0.59x**	-	-	-	-
Bone Resorption Markers									
TRACP-5b	U/L	5.696	-	**0.84x**	**0.66x**	6.961	-	**0.69x**	**0.48x**

Doses in mg/kg/day.
Control mean values and the ratio of the test article-related findings relative to control means are listed.
Abbreviations: - = Not test article-related; N-terminal propeptide of type I procollagen (PINP); osteocalcin (OC); osteoclast-derived tartrate-resistant acid phosphatase form 5B (TRACP-5b)
Based upon statistical analysis of group means, values highlighted in **bold** are significantly different from control group – $p \leq 0.05$ or $p \leq 0.01$.

(Table excerpted from sponsor's package)

There were no drug-related changes in PTH hormone levels.

Femur Lengths

Drug-related decreases in ex vivo femur length measurements were observed in males at ≥25 mg/kg (↓3-5%) and in females at 250 mg/kg (↓5%). At the end of the recovery period, there were no statistically significant differences between treated animals and controls; however, it was noted that mean femur lengths remained 4% lower in treated males.

Table 105: Femur Length – Juvenile Rat Study #15GR084

Femur Length (mm)				
Dose (mg/kg)	**Males**		**Females**	
	PND 91	**PND 118**	**PND 91**	**PND 118**
0	38.738	40.522	34.432	35.060
5	38.339	-	33.697	-
25	37.444* (↓3.3%)	-	34.053 (↓1.1%)	-
250	36.915* (↓4.7%)	38.976 (↓3.8%)	32.839* (↓4.6%)	34.990

* p value < 0.05

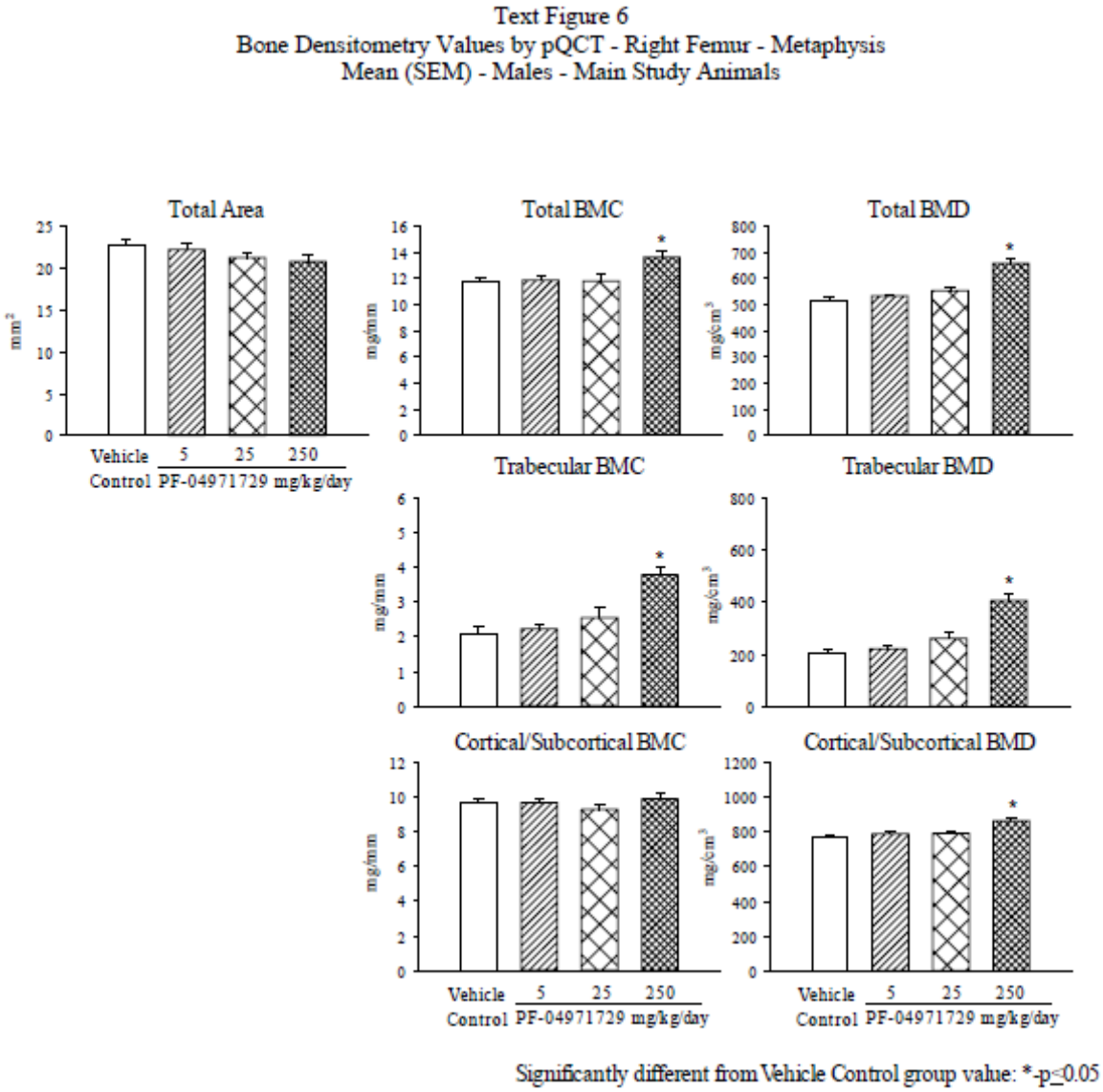
pQCT Analysis

Drug-related changes in bone mass parameters were reported in both males and females at ≥25 mg/kg. Furthermore, full reversibility was not achieved after the 4 week recovery period.

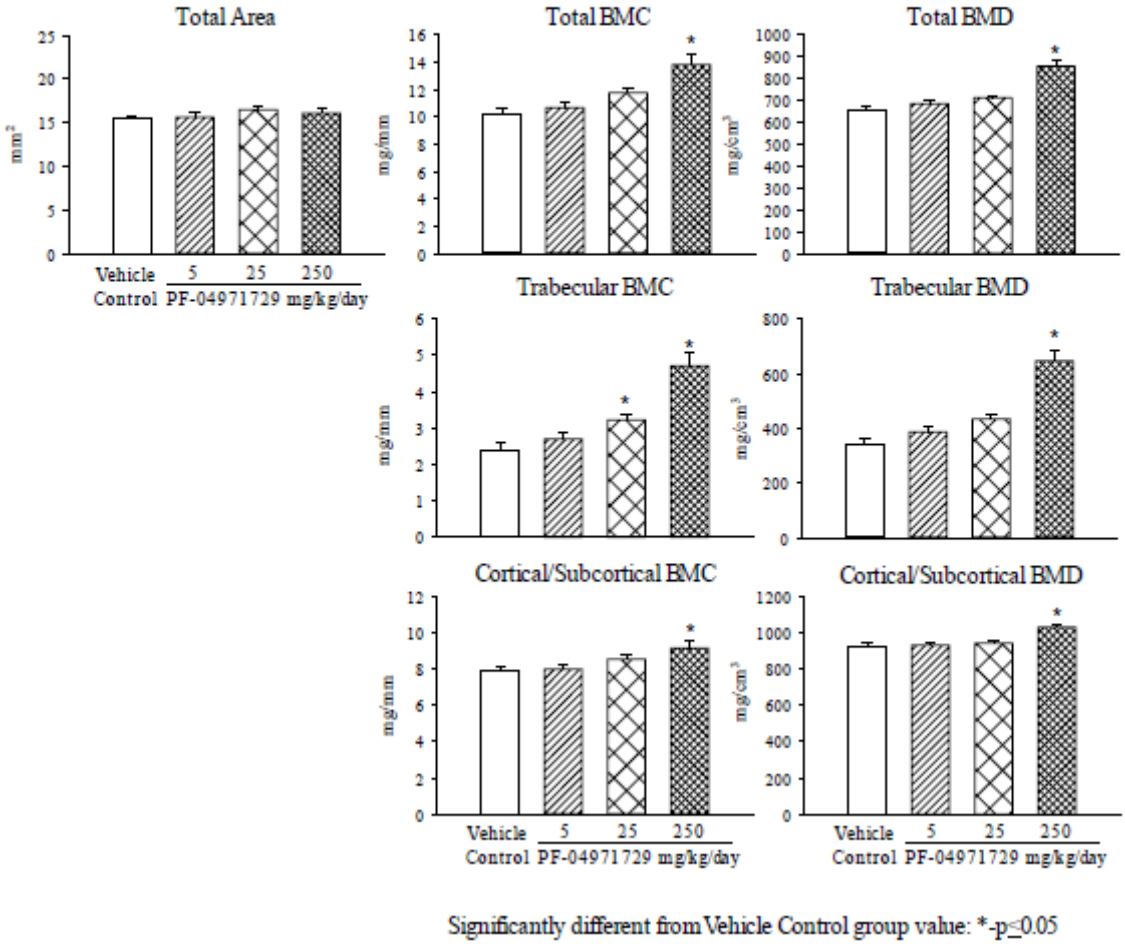
Dose-dependent increases in total, trabecular and cortical/subcortical bone mineral content (BMC) and bone mineral density (BMD) of the femur metaphysis were observed

at ≥25 mg/kg, reaching statistically significance at 250 mg/kg in both sexes. In males at 250 mg/kg, increases in total BMD (↑28%) were attributed primarily to increases in trabecular BMD (↑98%), but also with contributions by increases in cortical/subcortical BMD (↑13%). In females at 250 mg/kg, similar increases in total BMD (↑30%) were also attributed primarily to increases in trabecular BMD (↑89%) with additional contribution by increased cortical/subcortical BMD (↑11%). At 25 mg/kg, trabecular BMC was higher in both males and females, but only achieved statistical significance in females. Increases in trabecular BMD were also observed in both males (↑28%) and females (↑27%) at 25 mg/kg, but did not achieve statistical significance.

Figure 15: Femur Metaphysis Densitometry - Juvenile Rat Study #15GR084



Text Figure 7
Bone Densitometry Values by pQCT - Right Femur - Metaphysis
Mean (SEM) - Females - Main Study Animals

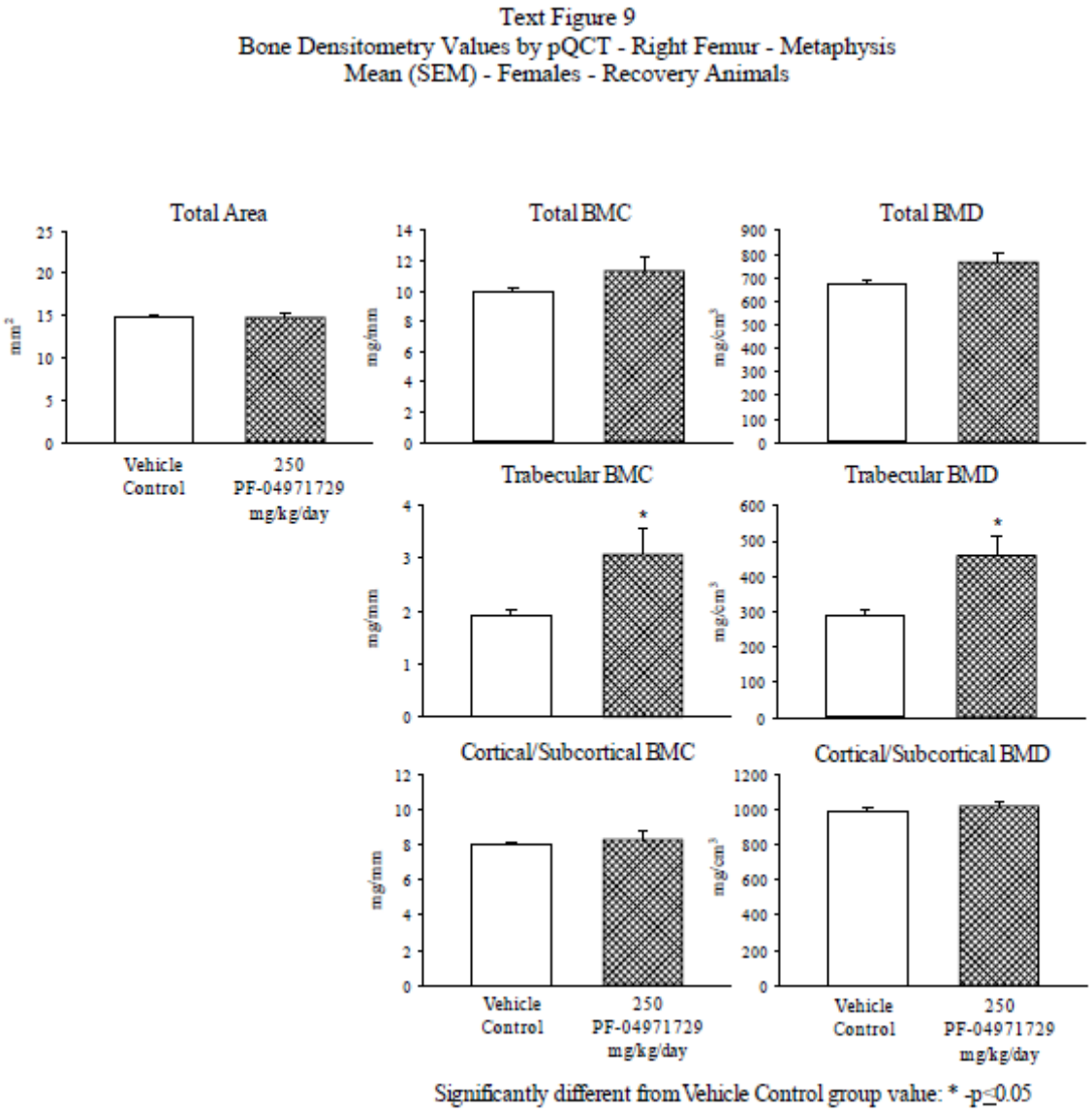


(Figures excerpted from sponsor's package)

In recovery females, statistically significant increases in femur metaphysis trabecular BMC and BMD (↑59%) were reported, indicating a lack of full recovery. It was noted that the magnitude of increases in BMC and BMD parameters were lower than that of females at the end of the dosing period, which may indicate partial recovery.

There were no statistically significant differences in metaphysis bone mass parameters in recovery males, indicating full recovery of drug-related metaphysis findings in males after 4 weeks of recovery.

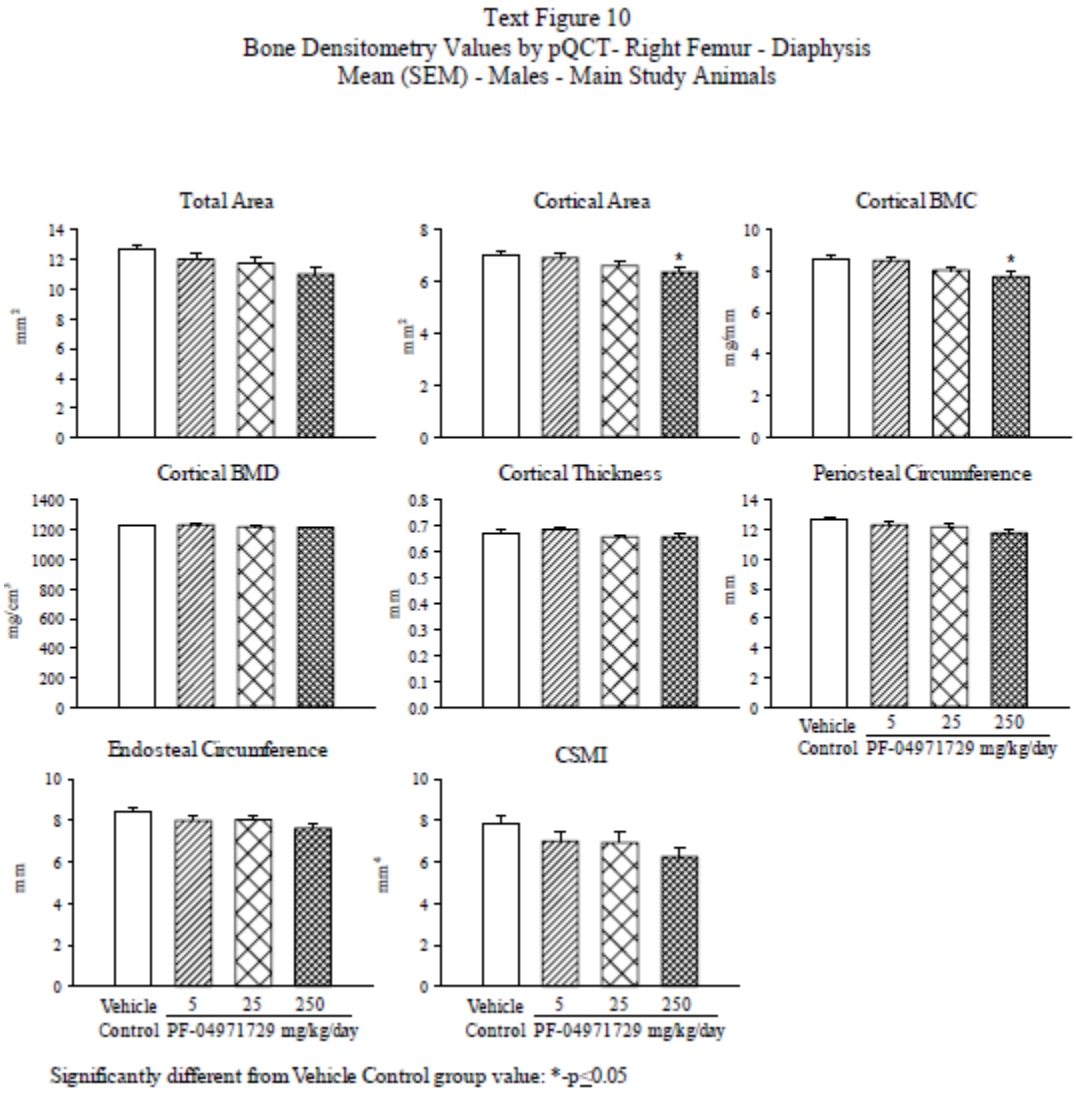
Figure 16: Femur Metaphysis Densitometry in Recovery Females - Juvenile Rat Study #15GR084



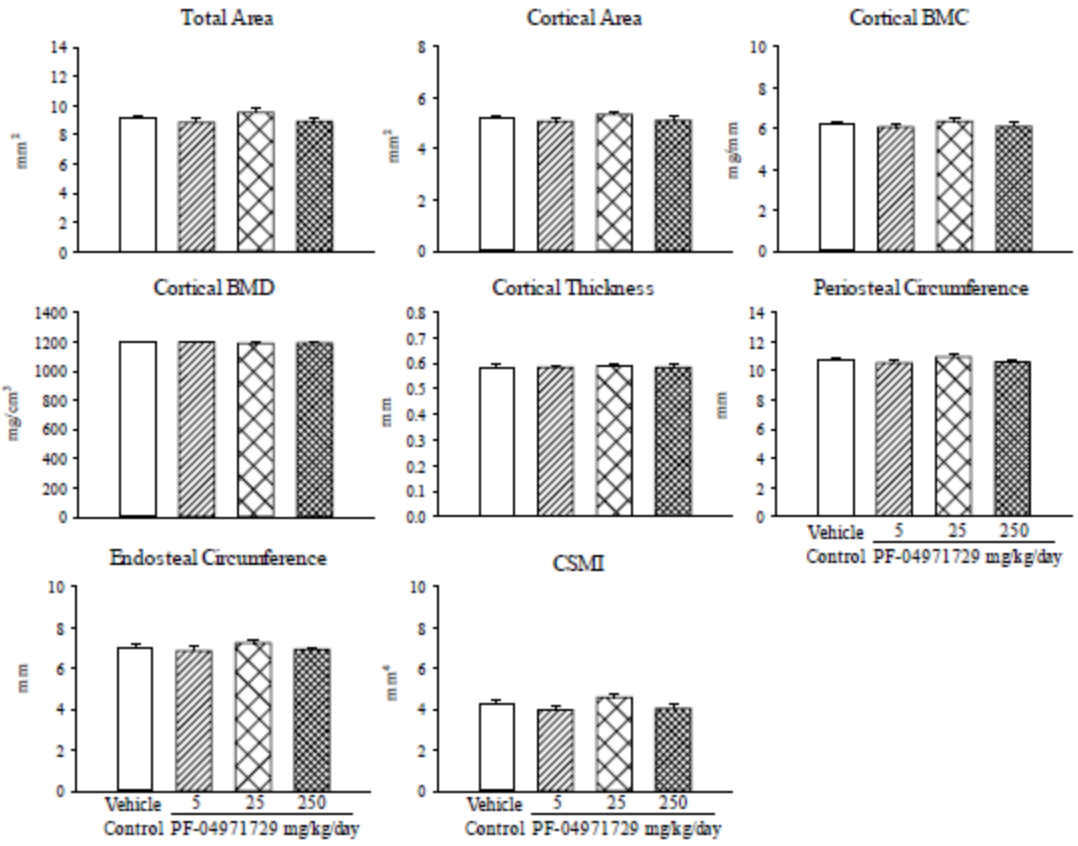
(Figure excerpted from sponsor's package)

In the femur diaphysis, statistically significant decreases in cortical area and BMC (↓10%) were reported in males at 250 mg/kg, along with trends for decreases in total area, periosteal and endosteal circumferences and cross-sectional moment of inertia (CSMI; ↓20%). There were no statistically significant differences compared to concurrent controls in female diaphysis densitometry parameters at the end of the dosing period.

Figure 17: Femur Diaphysis Densitometry - Juvenile Rat Study #15GR084



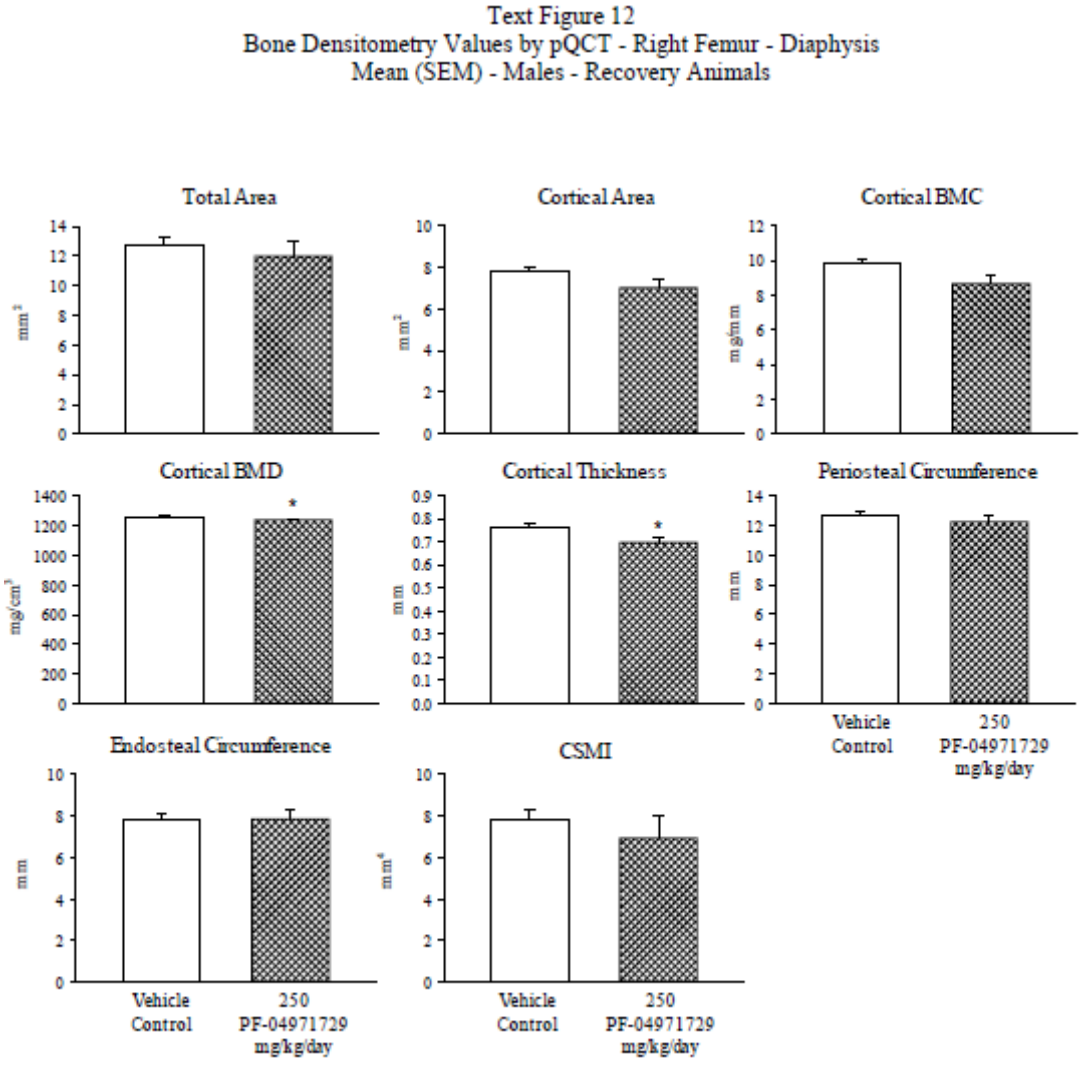
Text Figure 11
Bone Densitometry Values by pQCT - Right Femur - Diaphysis
Mean (SEM) - Females - Main Study Animals



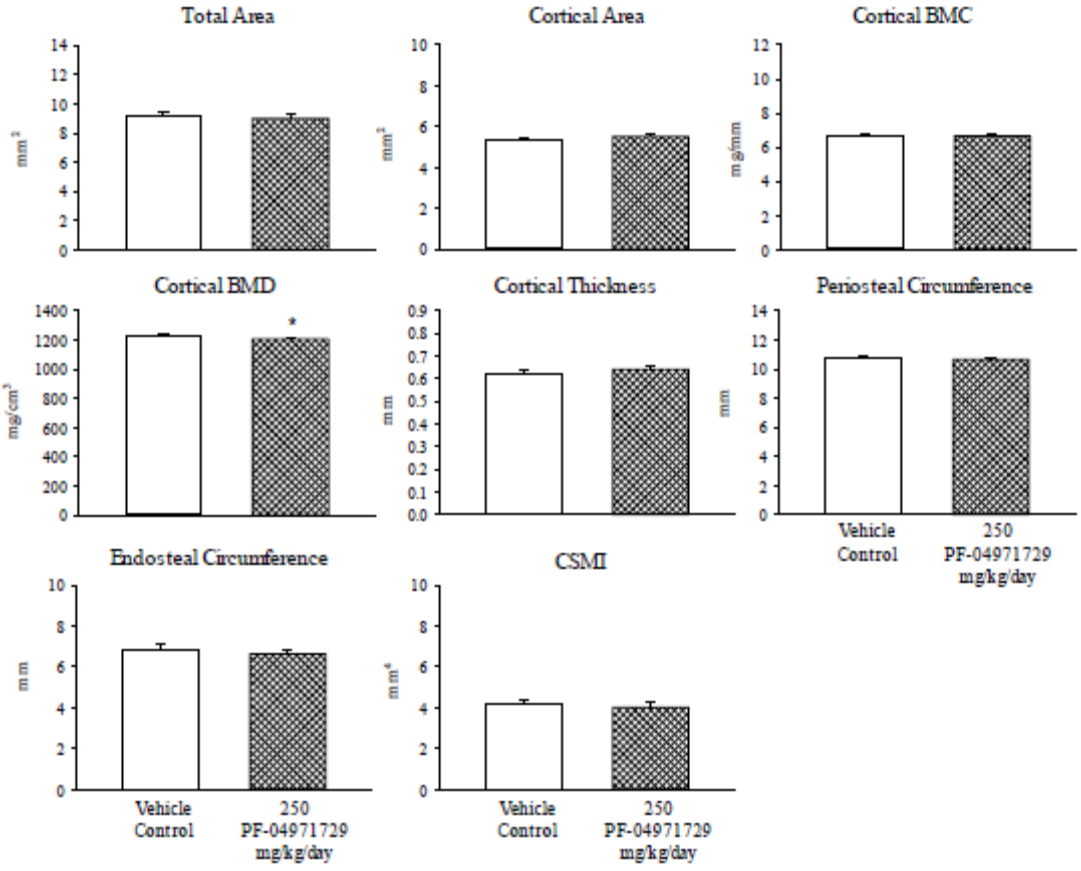
(Figure excerpted from sponsor's package)

In recovery males, slightly lower diaphysis parameters, including cortical BMD (↓2%) and cortical thickness (↓9%) were statistical significant and were associated with trends for lower cortical area (↓10%), BMC (↓12%) and CSMI (↓11%) values. In recovery females, femur diaphysis cortical BMD (↓2%) values were significantly reduced; however, all other diaphysis densitometry parameters were comparable to concurrent controls. Nevertheless, these data indicate lack of full reversibility of drug-related diaphysis findings.

Figure 18: Femur Diaphysis Densitometry in Recovery Groups - Juvenile Rat Study #15GR084



Text Figure 13
Bone Densitometry Values by pQCT - Right Femur - Diaphysis
Mean (SEM) - Females - Recovery Animals



Significantly different from Vehicle Control group value: * -p≤0.05

(Figures excerpted from sponsor's package)

Histomorphometry

Drug-related changes in bone histomorphometry parameters were reported in males and females at ≥25 mg/kg.

At the end of the dosing period, dose-dependent decreases in mineralizing surface (MS/BS), double label surface (dLS/BS), mineral apposition rate (MAR) and bone formation rate, and surface referent (BFR/BS) were reported in males and females at ≥25 mg/kg. These data are consistent with reduced bone formation; however, since resorption precedes formation at the surface of bone trabeculae, these findings may be secondary to reduced resorption. After the recovery period, there were no significant differences between treated animals and controls. Thus, drug-related effects on the evaluated bone remodeling markers were reversible.

Table 106: Histomorphometry Parameters - Juvenile Rat Study #15GR084

Text Table 33
Summary of PF-04971729-related Bone Biomarkers

Parameter	Units	Males				Females			
		0	5	25	250	0	5	25	250
MS/BS	%	39.49	-	0.89x	**0.71x**	33.57	-	**0.69x**	**0.49x**
dLS/BS	%	24.52	-	**0.76x**	**0.38x**	15.10	-	**0.52x**	**0.12x**
MAR	µm/d	2.243	-	0.86x	**0.73x**	1.523	-	**0.85x**	**0.66x**
BFR/BS	$\mu m^3/\mu m^2/yr$	325.49	-	**0.77x**	**0.52x**	185.48	-	**0.62x**	**0.32x**

Doses in mg/kg/day.
Control mean values and the ratio of the test article-related findings relative to control means are listed.
Abbreviations: - = Not test article-related; mineralizing surface (MS/BS); double label surface (dLS/BS); mineral apposition rate (MAR); bone formation rate, surface referent (BFR/BS)
Bolded values indicate statistical significance ($p \leq 0.05$ or $p \leq 0.01$).

(Table excerpted from sponsor's package)

Toxicokinetics

AUC_{24} and C_{max} exposures increased dose-proportionally. There were no apparent gender effects at ≤25 mg/kg and only a small trend for increased exposures in females at 250 mg/kg. Mean T_{max} ranged between 1 and 7 hours postdose, which increased with increasing dose, indicating that the maximum absorption rate was achieved at ≥25 mg/kg.

Table 107: Toxicokinetics - Juvenile Rat Study #15GR084

Text Table 37
Mean Toxicokinetic Parameters for PF-04971729 in Male and Female Sprague-Dawley Rats on PND 21

	Males			Females		
	5	25	250	5	25	250
C_{max} (ng/mL)	3160	14900	117000	3170	14000	147000
T_{max} (h)	1.0	1.0	7.0	1.0	4.0	7.0
AUC_{24} (ng*h/mL)	34900	163000	1710000	34800	205000	1920000

Dose in mg/kg/day.
AUC_{24} = Area under the plasma drug concentration-time curve from time 0 to 24 hours
C_{max} = Highest drug concentration observed in the plasma
T_{max} = Time at which C_{max} was observed
h = hour

Text Table 38
Mean Toxicokinetic Parameters for PF-04971729 in Male and Female Sprague-Dawley Rats on PND 90

	Males			Females		
	5	25	250	5	25	250
C_{max} (ng/mL)	2050	9210	50400	2750	9010	66500
T_{max} (h)	2.0	4.0	4.0	1.0	2.0	5.0
AUC_{24} (ng*h/mL)	20300	116000	696000	28000	97100	939000

Doses in mg/kg/day.
AUC_{24} = Area under the plasma drug concentration-time curve from time 0 to 24 hours
C_{max} = Highest drug concentration observed in the plasma
T_{max} = Time at which C_{max} was observed
h = hour

(Tables excerpted from sponsor's package)

Dosing Formulation Analysis

Concentrations of the first and last dosing formulation preparations from all groups were verified using a validated HPLC method. Homogeneity was confirmed in formulations from the first preparation only.

All mean and individual dosing formulation replicates were within ±15% and ±20%, respectively, of the target concentration. Relative standard deviations of homogeneity samples were ≤5% for each group. Thus, all dosing formulation met protocol specifications.

10 Special Toxicology Studies

The sponsor evaluated potential drug-related skin hypersensitivity using in an vitro human skin model and in vivo using a local lymph node assay in mice. Eye irritancy was also assessed in vitro using the bovine corneal opacity and permeability test.

Ertugliflozin has a molar extinction coefficient less than 1000 L/mol·cm and is considered to be negative for potential phototoxicity.

Study: In Vitro Skin Corrosion Test withPF-04970729 (b) (4) Using a Human Skin Model (Study TT#14-7836 / #506864)

Key Study Findings

- Ertugliflozin was positive for human skin corrosion in vitro after direct exposure of the solid form for 1 hour
  - Non-corrosive after acute exposure for ≤3 minutes

METHODS

A three dimensional epidermal model of human skin culture, EpiDerm (EPI-200), was used to directly expose differentiated human-derived epidermal keratinocyte multi-layer cultures to an amount of solid PF-04971729 (b) (4) (Batch #E010015299) completely covering the skin culture for durations of 3 minutes or 1 hour, each in duplicate. Cytotoxicity was determined as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Relative tissue viability compared to negative control (Milli-Q water) was determined after exposure to drug or 8N KOH positive control. Positive skin corrosion was defined as <15% relative tissue viability compared to negative controls. Inter-tissue variability was determined using 2 tissue sources. The inability of PF-04971729 to directly reduce MTT was also assessed.

RESULTS

PF-04971729 did not directly interact with MTT; thus, the study drug did not interfere with the assay. Values for negative controls were within the range of historical controls. The positive control was positive for cytotoxicity after 3 minute and 1 hour exposures. Overall, the study was considered to be valid.

Acute exposures of the skin cultures to PF-04971729 for 3 minutes were not corrosive. Tissue viability was decreased to only 3% (↓97%) after 1-hour exposure; although, it was noted that the inter-tissue variability in viability between the 2 tissue cultures was ≥50%. Nevertheless, these data indicate that the solid form of PF-04971729 is cytotoxic to a human skin model after direct exposures of at least 1 hour, but not after acute exposures of ≤3 minutes.

Table 108: Mean Tissue Viability - In Vitro Skin Corrosion Test Results

	3-minute application viability (percentage of control)	1-hour application viability (percentage of control)
Negative control	100	100
PF-04971729 (b) (4)	95	3
Positive control	9	7

(Table excerpted from sponsor's package)

Study: Assessment of Contact Hypersensitivity to PF-04971729 (b) (4) in the Mouse (Local Lymph Node Assay) (Study TT#14-7837 / #506868)

Key Study Findings

- Ertugliflozin was negative for skin sensitization in vivo
  - No dermal contact hypersensitivity was observed in mice at concentrations exceeding dermal absorption

METHODS

The ears of female CBA/J mice (5/group) were treated with 0 (N,N-dimethyl formamide vehicle), 10% (18 mM), 25% (44 mM), or 50% (88 mM) w/w PF-04971729 (b) (4) for 3 days (Day 1-3). Animals were observed daily for clinical signs and dermal irritation of the ear was scored daily within 1 hour of dosing. On Day 6, animals were injected with ^{3}H-methyl thymidine and 5 hours later auricular lymph nodes were harvested and pooled for each animal. Incorporation of radioactivity was measured in lymph node DNA using LSC. A concurrent positive control was not included; however, data were compared to reliability tests performed by the study facility (b) (4)) every 6 months using the positive control hexylcinnamaldehyde in acetone/olive oil (4:1 v/v) and the same materials, animal supplier, strain, and procedure.

RESULTS

Comparison to positive control data from regular reliability tests was considered to be acceptable. At the nearest regular reliability check, an EC3 value (effective concentration for stimulation index of 3) of the positive control was calculated to be 17.3% using linear interpolation, which was in the acceptable range for this assay. The maximum concentration of 50% PF-04971729 is approximately 3-fold Log greater than

the clinical dose (0.6 μM) and exceeds maximum dermal absorption. Thus, the high dose of 50% w/w was sufficient and the study was considered to be valid.

There were no mortalities or clinical signs of systemic toxicity at any of the doses. There were no significant changes in body weights or weight gains. On Days 4-6, the ear surfaces at 50% were noted as being stained white, indicating the presence of remnant PF-04971729, which suggests that maximum dermal absorption had been exceeded.

There were no clinical signs of dermal irritation on the ears of any of the treated animals, despite prolonged exposure at 50% due to unabsorbed test article. There were no increases in DNA radioactivity incorporation (DPM) or Stimulation Index (SI) scores compared to concurrent vehicle controls. Since an SI of ≥3 was not achieved for the study drug, PF-04971729 is negative for sensitization.

Table 109: Mouse Local Lymph Node Assay Results

Table 4: Relative size lymph nodes, radioactivity counts (DPM) and Stimulation Index (SI)

group	TS [1] (%)	animal	Size nodes [2] left	Size nodes [2] right	DPM [3] / animal	mean DPM ± SEM [4]	mean SI ± SEM
1	0	1	n	n	639		
		2	n	n	794		
		3	n	n	483	734 ± 106	1.0 ± 0.2
		4	n	n	1112		
		5	n	n	644		
2	10	6	n	n	741		
		7	n	n	1005		
		8	n	n	714	679 ± 102	0.9 ± 0.2
		9	n	n	531		
		10	n	n	403		
3	25	11	n	n	502		
		12	n	n	929		
		13	n	n	594	624 ± 117	0.8 ± 0.2
		14	n	n	822		
		15	n	n	272		
4	50	16	n	n	592		
		17	n	n	820		
		18	n	n	694	657 ± 59	0.9 ± 0.2
		19	n	n	708		
		20	n	n	469		

1. TS = test substance (% w/w).
2. Relative size auricular lymph nodes (-, -- or ---: degree of reduction, +,++ or +++: degree of enlargement, n: considered to be normal).
3. DPM = Disintegrations per minute
4. SEM = Standard Error of the Mean

(Table excerpted from sponsor's package)

Study: Screening for the Eye Irritancy Potential of PF-04971729 (b) (4) Using the Bovine Corneal Opacity and Permeability Test (BCOP Test) (Study TT #14-7838 / #506865)

Key Study Findings

- Ertugliflozin was positive for eye irritancy after direct exposure of the solid form to bovine corneas
  - Category 1 eye irritant, according to the United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS)

METHODS

Corneas were isolated from bovine eyes and cornea with an opacity ≤7 were treated with negative control, 20% Imidazole (positive control), or 313 mg to 330 mg solid PF-04971729 (b) (4) for 240 minutes at 32 °C. Corneas were rinsed with medium containing phenol red in order to assess changes in pH. Cornea opacities were determined using an opacitometer. Na-fluorescein was then used to determine cornea permeability. Mean opacity and permeability values were used to calculate an in vitro irritancy score (IVIS), wherein IVIS scores >55 are category 1 and considered predictive of positive eye irritancy or serious eye damage according to UN GHS.

RESULTS

Negative control values were within the historical control range. Positive control IVIS values were ≥121 and corneas were noted as turbid. Thus, the assay was considered to be valid.

After direct exposure to solid PF-04971729, corneas were turbid and pH changes of the rinsing medium were noted. Study drug IVIS scores were ≥115, similarly to the positive control. Thus, PF-04971729 was positive for ocular irritation and was classified as category 1.

Table 110: BCOP Eye Irritancy Results

Table 1 Summary of opacity, permeability and *in vitro* scores

Treatment	Mean Opacity	Mean Permeability	Mean *in vitro* Irritation Score [1, 2]
Negative control	0	0.000	0.0
Positive control	105	1.501	128
PF-04971729 (b) (4)	38	6.839	140

1 Calculated using the negative control mean opacity and mean permeability values.
2 In vitro irritancy score (IVIS) = mean opacity value + (15 x mean OD_{490} value).

(Table excerpted from sponsor's package)

11 Labeling Review

Section 8 Use in Specific Populations

Section 8.1 Pregnancy

Excerpt 1: Sponsor's Proposed Section 8.1 Text

8.1 Pregnancy

Risk Summary

(b) (4)

(b) (4) TRADEMARK is not recommended during the second and third trimesters of pregnancy.

(b) (4)

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk

Poorly-controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, spontaneous abortions, preterm delivery, stillbirth, and delivery complications. It can also increase the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Animal Data

In embryo-fetal development studies, ertugliflozin (50, 100 and 250 mg/kg/day) was administered orally to rats on gestation days 6 to 17 and to rabbits on gestation days 7 to 19. Ertugliflozin did not adversely affect developmental outcomes in rats and rabbits at maternal exposures that were (b) (4) (b) (4) the human exposure at the maximum clinical dose of 15 mg/day, based on AUC. At a maternally toxic dose in rats (250 mg/kg/day), lower fetal viability, (b) (4) a higher incidence of a visceral malformation (membranous ventricular septal defect) (b) (4) (b) (4) (b) (4) In the pre- and postnatal development study, decreased postnatal growth (b) (4) (b) (4) were observed in rats administered ertugliflozin gestation day 6 through lactation day 21 at ≥100 mg/kg/day ((b) (4) times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC).

When ertugliflozin was orally administered to juvenile rats from PND 21 to PND 90, increased kidney weight, renal tubule and renal pelvis dilatation, and renal mineralization occurred at doses greater than or equal to 5 mg/kg (13-fold human exposures). These effects did not fully reverse within the 1 month recovery period. (b) (4)

(Excerpted from Sponsor's package)

Reviewer's Comments

The sponsor's Risk Summary of nonclinical findings was considered to be repetitive with the Data section. Thus, summary statements regarding overall fetal harm and key juvenile renal findings were recommended for the Risk Summary section.

The sponsor's safety margin calculations were based on the unbound fraction of ertugliflozin, which were corrected for total drug exposure.

A statement was recommended regarding rat fetal exposure and distribution (study #PK034), consistent with other labels of this drug class.

The sponsor's safety margin calculation for absence of developmental outcomes in rabbits was based on the 250 mg/kg dose. However, variations observed at 250 mg/kg were considered to be potentially drug-related and the fetal NOAEL was set at 100 mg/kg. Thus, the corrected rabbit safety margin 307x $MRHD_{AUC}$ was based on the 100 mg/kg dose.

In the rat EFD study, only increases in visceral malformations of membranous ventricular septum defect at 250 mg/kg were statistical significance and unequivocally drug-related. Although only increases in skeletal variations at 250 mg/kg reached statistical significance increases in incidences of absent innominate artery visceral variations at 250 mg/kg were above HCIR, and were considered possibly drug-related. Thus, inclusion of visceral variations at 250 mg/kg is also recommended.

In the rabbit EFD study, drug-related increases in post-implantation loss and reductions in live fetuses at 50mg/kg were attributed to significant maternal toxicity at exposures 150x $MRHD_{AUC}$. Thus, the degree of maternal toxicity was considered to be a confounding factor in fetal survival in rabbits. Furthermore, the addition of this data does not change the overall safety margins or recommendation during pregnancy. Thus, it is acceptable to omit this data from the label.

A statement was added to clarify the correlation between the timing of dosing in the juvenile rat study and corresponding trimesters of human pregnancy encompassing renal development.

The sponsor's PPND study description is acceptable.

Recommendations were made to the juvenile study description to take into account for statistically significant drug-related bone effects and growth delay at ≥25 mg/kg and sexual maturation delays at 250 mg/kg. However, since the growth delays may be related to drug-related effects on bone turnover and/or maturation, which are likely secondary to SGLT1 inhibition in the rat, the human relevancy of these findings remains unclear. Since the addition of this data does not change the overall safety margins or recommendation during pregnancy, it is reasonable to omit this data from the label.

Reviewer's text additions are noted in black and deletions are noted with a strike through the sponsor's blue text.

Reviewer's Proposed Section 8.1 Text

Risk Summary

Based on animal data showing adverse renal effects, TRADEMARK is not recommended during the second and third trimesters of pregnancy.

The limited available data with TRADEMARK in pregnant women are not sufficient to determine a drug-associated risk (b) (4). There are risks to the mother and fetus associated with poorly controlled diabetes in pregnancy [see Clinical Considerations].

In animal studies, adverse renal changes were observed in rats when ertugliflozin was administered during a period of renal development corresponding to the late second and third trimesters of human pregnancy. Doses approximately (b) (4) times the maximum clinical dose caused renal pelvic and tubule dilatations and renal mineralization that were not fully reversible. There was no evidence of fetal harm in rats or rabbits at exposures of ertugliflozin greater than 300 times higher than the maximal clinical dose of 15 mg/day when administered during organogenesis [see Data].

(b) (4)

The estimated background risk of major birth defects is 6-10% in women with pre-gestational diabetes with a HbA1c >7 and has been reported to be as high as 20-25% in women with HbA1c >10. The estimated background risk of miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

(b) (4)

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk

Poorly-controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia, spontaneous abortions, preterm delivery, stillbirth, and delivery complications. It can also increase the fetal risk for major birth defects, stillbirth, and macrosomia related morbidity.

Data

Animal Data

When ertugliflozin was orally administered to juvenile rats from PND 21 to PND 90, increased kidney weight, renal tubule and renal pelvis dilatation, and renal mineralization occurred at doses greater than or equal to 5 mg/kg ((b) (4) fold human exposures, based on AUC). These effects occurred with drug exposure during periods of renal development in rats that correspond to the late second and third trimester of human renal development, and did not fully reverse within a 1 month recovery period.

(b) (4)

In embryo-fetal development studies, ertugliflozin (50, 100 and 250 mg/kg/day) was administered orally to rats on gestation days 6 to 17 and to rabbits on gestation days 7 to 19. Ertugliflozin did not

adversely affect developmental outcomes in rats and rabbits at maternal exposures that were (b) (4) approximately 300-times (b) (4) the human exposure at the maximum clinical dose of 15 mg/day, based on AUC. At a maternally toxic dose (250 mg/kg/day) in rats ~~(250 mg/kg/day),~~ (707-times clinical dose) (b) (4) was associated with reduced fetal viability (b) (4) and (b) (4) a higher incidence of a visceral malformation (membranous ventricular septal defect) (b) (4)

In ~~the~~a pre- and postnatal development study in pregnant rats, ertugliflozin was administered~~,~~ (b) (4) from gestation day 6 through lactation day 21 (weaning). Decreased postnatal growth (weight gain) was observed at maternal doses of ≥100 mg/kg/day (b) (4) greater than or equal to 331 times the human exposure at the maximum clinical dose of 15 mg/day, based on AUC).

(b) (4)

Section 8.2 Lactation

Excerpt 2: Sponsor's Proposed Section 8.2 Text

8.2 Lactation

Risk Summary

There is no information regarding the presence of TRADEMARK in human milk, the effects on the breastfed infant, or the effects on milk production. Ertugliflozin is present in the milk of lactating rats *[see Data]*. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney.

(b) (4)

Data

Animal Data

The lacteal excretion of radiolabeled ertugliflozin in lactating rats was evaluated 10 to 12 days after parturition. Ertugliflozin derived radioactivity exposure in milk and plasma were similar, with a milk/plasma ratio of 1.07, based on AUC.

(Excerpted from Sponsor's package)

Reviewer's Comments

Nonclinical data described in the sponsor's proposed text (Excerpt 2) is supported by nonclinical study #PK034.

A comment was added regarding the potential risk of kidney development in exposure of juveniles during the period of kidney maturation, which is consistent with information included in this section in recently approved drug labels for other SGLT2 class members.

Reviewer's text additions are noted in black and deletions are noted with a strike through the sponsor's blue text.

Reviewer's Proposed Section 8.2 Text

Risk Summary

There is no information regarding the presence of TRADEMARK in human milk, the effects on the breastfed infant, or the effects on milk production. Ertugliflozin is present in the milk of lactating rats *[see*

Data]. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney.

(b) (4)

Data

Animal Data

The lacteal excretion of radiolabeled ertugliflozin in lactating rats was evaluated 10 to 12 days after parturition. Ertugliflozin derived radioactivity exposure in milk and plasma were similar, with a milk/plasma ratio of 1.07, based on AUC. Juvenile rats directly exposed to TRADEMARK during a developmental period corresponding to human kidney maturation were associated with a risk to the developing kidney (persistent increased organ weight, renal mineralization, and renal pelvic and tubular dilatations).

Section 12 Clinical Pharmacology

Section 12.1 Mechanism of Action

Excerpt 3: Sponsor's Proposed Section 12.1 Text

12.1 Mechanism of Action

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Ertugliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, ertugliflozin reduces renal reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion.

(Excerpted from Sponsor's package)

Reviewer's Comments

The sponsor's proposed text (Excerpt 3) is supported by the nonclinical data and is considered to be acceptable.

Section 12.3 Pharmacokinetics

Excerpt 4: Sponsor's Proposed Section 12.3 Text

Metabolism

Metabolism is the primary clearance mechanism for ertugliflozin. The major metabolic pathway for ertugliflozin is UGT1A9 and UGT2B7-mediated O-glucuronidation to two glucuronides that are pharmacologically inactive at clinically relevant concentrations. CYP-mediated (oxidative) metabolism of ertugliflozin is minimal (12%).

Drug Interaction Studies

In Vitro Assessment of Drug Interactions

In *in vitro* studies, ertugliflozin and ertugliflozin glucuronides did not inhibit CYP450 isoenzymes (CYPs) 1A2, 2C9, 2C19, 2C8, 2B6, 2D6, or 3A4, and did not induce CYPs 1A2, 2B6, or 3A4. Ertugliflozin was not a time-dependent inhibitor of CYP3A *in vitro*. Ertugliflozin did not inhibit UGT1A6, 1A9, or 2B7 *in vitro* and was a weak inhibitor (IC_{50} >39 µM) of UGT1A1 and 1A4. Ertugliflozin glucuronides did not inhibit UGT1A1, 1A4, 1A6, 1A9, or 2B7 *in vitro*. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of drugs eliminated by these enzymes. Ertugliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and is not a substrate of organic anion transporters (OAT1, OAT3), organic cation transporters (OCT1, OCT2), or organic anion transporting polypeptides (OATP1B1, OATP1B3). Ertugliflozin or ertugliflozin glucuronides do not meaningfully inhibit P-gp, OCT2, OAT1, or OAT3 transporters at clinically relevant concentrations. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of concurrently administered medications that are substrates of these transporters.

(Excerpted from Sponsor's package)

Reviewer's Comments

The sponsor's discussion regarding metabolism of ertugliflozin are considered to be supported by the data.

Although the O-glucuronide metabolites M5a and M5c have respective 500-fold (study #PD003) and 1000-fold (study #PD002) lower potencies for SGLT2, since the raw inhibition data and IC_{50} curves for M5a study #PD003 and M5c study #PD002 were not submitted for review, it is not possible to completely rule out minimal activity of either metabolite at clinically relevant exposures. Thus, although significant SGLT2 inhibition by M5a and/or M5c is not likely at clinically relevant exposures, the sponsors claim is considered to be an overstatement. Thus, a minor recommendation was added to soften the sponsor's claim.

Ertugliflozin is a weak inhibitor of P-gp/MDR1 (IC_{50} = 176 μM), OCT2 (IC_{50} = 917 μM) and OAT3 (IC_{50} = 70 μM) transporters; however, the sponsor's statement that ertugliflozin and metabolites "do not meaningfully inhibit" these transporters "at clinically relevant concentrations" is considered to be acceptable. It is noted that ertugliflozin is also a weak inhibitor of OATP1B1 (IC_{50} = 35.4 μM) and OATP1B3 (IC_{50} = 140.7 μM), and M5c is a weak inhibitor of OATP1B1 (IC_{50} = 59.3 μM); thus, OATP1B1 and OATP1B3 were added to the description.

Ertugliflozin was demonstrated to be a potential mild inducer of CYP3A4 (study #PK054) and a potential weak inducer of CYP1A2 (study #PK054 and #PK055) in vitro; however, the data were considered to be equivocal with unlikely biological significance at clinical exposure levels. Thus, it is acceptable to leave this information out of the label.

The sponsor's specific descriptions of the other interactions were acceptable.

Reviewer's Proposed Section 12.3 Text

Metabolism

Metabolism is the primary clearance mechanism for ertugliflozin. The major metabolic pathway for ertugliflozin is UGT1A9 and UGT2B7-mediated O-glucuronidation to two glucuronides that are unlikely to be pharmacologically ~~in~~active at clinically relevant concentrations. CYP-mediated (oxidative) metabolism of ertugliflozin is minimal (12%).

Drug Interaction Studies

In Vitro Assessment of Drug Interactions

In *in vitro* studies, ertugliflozin and ertugliflozin glucuronides did not inhibit CYP450 isoenzymes (CYPs) 1A2, 2C9, 2C19, 2C8, 2B6, 2D6, or 3A4, and did not induce CYPs 1A2, 2B6, or 3A4. Ertugliflozin was not a time-dependent inhibitor of CYP3A *in vitro*. Ertugliflozin did not inhibit UGT1A6, 1A9, or 2B7 *in vitro* and was a weak inhibitor (IC_{50} >39 μM) of UGT1A1 and 1A4. Ertugliflozin glucuronides did not inhibit UGT1A1, 1A4, 1A6, 1A9, or 2B7 *in vitro*. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of drugs eliminated by these enzymes. Ertugliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and is not a substrate of organic anion transporters (OAT1, OAT3), organic cation transporters (OCT1, OCT2), or organic anion transporting polypeptides (OATP1B1, OATP1B3). Ertugliflozin or ertugliflozin glucuronides do not meaningfully inhibit P-gp, OCT2, OAT1, or

OAT3 transporters, or transporting polypeptides OATP1B1 and OATP1B3, at clinically relevant concentrations. Overall, ertugliflozin is unlikely to affect the pharmacokinetics of concurrently administered medications that are substrates of these transporters.

Section 13 Nonclinical Toxicology

Section 13.1 Carcinogenicity & Mutagenesis & Impairment of Fertility

Excerpt 5: Sponsor's Proposed Section 13.1 Text

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

In the (b) (4) mouse (b) (4) study, ertugliflozin was administered by oral gavage at doses of 5, 15, and 40 mg/kg/day. There were no ertugliflozin-related neoplastic findings at doses up to 40 mg/kg/day (approximately (b) (4) times human exposure at the maximum recommended human dose [MRHD] of 15 mg/day based on AUC). In the (b) (4) rat (b) (4) study, ertugliflozin was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg/day. Ertugliflozin-related neoplastic findings included an increased incidence of (b) (4) adrenal medullary pheochromocytoma in male rats at 15 mg/kg/day. This finding was attributed to carbohydrate malabsorption leading to altered calcium homeostasis (b) (4) to human risk. The no-observed-effect level (NOEL) for neoplasia was 5 mg/kg/day (approximately 16 times human exposure at the MRHD of 15 mg/day).

Mutagenesis

Ertugliflozin was not mutagenic or clastogenic with or without metabolic activation in the microbial reverse mutation, *in vitro* cytogenetic (human lymphocytes), and *in vivo* rat micronucleus assays.

Impairment of Fertility

In the rat fertility and embryonic development study, male and female rats were administered ertugliflozin at 5, 25, and 250 mg/kg/day. No effects on fertility were observed at 250 mg/kg/day (approximately (b) (4) times human exposure at the MRHD of 15 mg/day based on AUC comparison).

(Excerpted from Sponsor's package)

Reviewer's Comments

Although development of PCC in rats may be related to changes in absorption due to off-target SGLT1 inhibition, the molecular mechanism has not been fully investigated and causality has not been clearly demonstrated. Thus, the sponsor's explanation is considered to be an overstatement. Furthermore, although the human relevancy of rat PCC development as a result of changes in calcium homeostasis is unclear, human risk has not been eliminated. It is noted that ertugliflozin has a higher potency for inhibition of SGTL1 in rats than humans; thus, SGLT1 inhibition is less likely to occur in humans at similar exposures, and is not anticipated at clinical exposures. Overall, PCC development in rats is considered to be possibly related to off-target SGLT1 inhibition and resultant changes in absorption and calcium homeostasis with unclear relevancy to human risk.

The sponsor's safety margin calculations were based on the unbound fraction of ertugliflozin, which were corrected for total drug exposure. Fertility exposure margins were also separated for males and females.

The statements regarding mutagenicity are supported by the nonclinical data and are considered to be acceptable.

Reviewer's text additions are noted in black and deletions are noted with a strike through the sponsor's blue text.

Reviewer's Proposed Section 13.1 Text

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity was evaluated in CD-1 mice and Sprague-Dawley rats. In the (b) (4) mouse (b) (4)-study, ertugliflozin was administered by oral gavage at doses of 5, 15, and 40 mg/kg/day for up to 97 weeks in males and 102 weeks in females. There were no ertugliflozin-related neoplastic findings at doses up to 40 mg/kg/day (approximately (b) (4)50 times human exposure at the maximum recommended human dose [MRHD] of 15 mg/day based on AUC). In the (b) (4) rat (b) (4)-study, ertugliflozin was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg/day for up to 92 weeks in females and 104 weeks in males. Ertugliflozin-related neoplastic findings included an increased incidence of (b) (4) adrenal medullary pheochromocytoma (PCC) in male rats at 15 mg/kg/day. Although the molecular mechanism remains unknown, this finding (b) (4) may be related to carbohydrate malabsorption leading to altered calcium homeostasis, which has been associated with PCC development in rats and has unclear relevancy (b) (4) to human risk. The no-observed-effect level (NOEL) for neoplasia was 5 mg/kg/day (approximately ~~16~~18 times human exposure at the MRHD of 15 mg/day), based on AUC.

Mutagenesis

Ertugliflozin was not mutagenic or clastogenic with or without metabolic activation in the microbial reverse mutation, *in vitro* cytogenetic (human lymphocytes), and *in vivo* rat micronucleus assays.

Impairment of Fertility

In the rat fertility and embryonic development study, male and female rats were administered ertugliflozin at 5, 25, and 250 mg/kg/day. No effects on fertility were observed at 250 mg/kg/day (approximately (b) (4)480 and 570 times male and female human exposures, respectively, at the MRHD of 15 mg/day based on AUC comparison).

Section 13.2 Animal Pharmacology and/or Toxicology

This section was not included in the sponsor's proposed label.

Reviewer's Comments

This section was also not included in recent approved labels for other SGLT2 inhibitor class members, including the 12/2016 label for empagliflozin, the 3/2017 label for dapagliflozin, and the 2/2017 label for canagliflozin. Omission of this section is considered to be acceptable.

12 Integrated Summary and Safety Evaluation

Merck Sharp and Dohme Corp. has submitted NDA application packages for the NME Ertugliflozin alone and as FDC products with the marketed drugs Metformin and Sitagliptin for the treatment of T2DM.

Ertugliflozin is a potent and selective inhibitor of SGLT2, with a lower probability of off-target SGLT1 inhibition in humans than in the nonclinical species used for safety pharmacology and toxicology studies. The PD activity of SGLT2 inhibition blocks glucose transport from the pro-urine across the apical membrane of the proximal epithelial cells, resulting in drug-mediated glucosuria in all species examined. Marked glucosuria is associated with compensatory increases in food consumption; however, concomitant decreases in plasma glucose levels were inconsistently observed across nonclinical studies, but were more clearly evident with controlled food intake. Drug-

related diuresis secondary to PD-related glucosuria has been observed in humans and animal studies. Ertugliflozin administration in rats also leads to activation of RAAS, which is likely secondary to drug-related diuresis.

PK parameters were characterized in humans, rats, dogs, and mice. Species differences were noted in oral PK kinetics, absorption, and metabolism. Systemic exposures generally increase dose-dependently with linear pharmacokinetics, but are likely limited by decreased absorption efficiency at high doses. There were no consistent indications of accumulation over time in rats and dogs, but there may be a gender effect for slight accumulation in female mice. Humans had the highest bioavailability (100%) and a $t_{1/2}$ up to 4 times longer than animal species used in nonclinical studies. Gender effects were observed in rodents, with higher exposures in females that were likely due to greater metabolism in males. Percent plasma protein binding is similarly high across species. Ertugliflozin has a moderate volume of distribution in rats with preferential distribution into plasma relative to red blood cells and is predominantly partitioned to organs responsible for drug metabolism and elimination, such as the bladder, liver, and kidney. The predominant route of elimination is via glucuronidation metabolism mediated by UGT1A9 and UGT2B7. In humans, ertugliflozin is metabolized into 2 major disproportional metabolites, the O-glucuronides M5a and M5c.

Significant CYP inhibition by ertugliflozin, M5a or M5c is not likely at clinical exposure levels. Ertugliflozin-mediated induction of CYP3A4 and/or CYP1A2 was observed in vitro in some hepatocyte donors, but was considered to be equivocal and unlikely to be biologically significant at clinical exposures. M5a and M5c were not associated with induction of CYP3A4, CYP2B6, or CYP1A2 enzymes in vitro. Overall, significant DDI with ertugliflozin administration and drugs metabolized by CYP enzymes are not likely at clinical exposures.

Ertugliflozin was a substrate for P-gp/MDR1 and BCRP transporters in vitro, but is unlikely to be a limiting factor in ertugliflozin absorption. Ertugliflozin is not a substrate for OAT1, OAT3, OCT1, OCT2, OATP1B1 or OATP1B3. Ertugliflozin was a weak to very weak inhibitor of P-gp/MDR1, BCRP, OCT1, OCT2, and OAT3 transporters, as well as OATP1B1 and OATP1B3 polypeptides, in vitro; however, biologically meaningful inhibition is unlikely at clinical exposure levels. M5c similarly weakly inhibited human OATP1B1 at relatively high concentrations. Overall, DDI with ertugliflozin administration and drugs transported by OATs, OCTs and transporting polypeptides are not likely at clinical exposures.

The sponsor's PBPK modeling program predicted that ertugliflozin exposure may lead to slightly (<2-fold) higher AUC and C_{max} exposures of drugs metabolized by UGT enzymes. However, ertugliflozin only demonstrated weak inhibitor activity for UGT1A1 and UGT1A4 enzymes in vitro, and M5a and M5c were negative for inhibition of all UGT enzymes evaluated. Thus, a significant DDI with UGT inhibition is also considered to be unlikely at clinical concentrations.

In nonclinical safety pharmacology studies, sufficient margins of safety were demonstrated for drug-related effects on CNS, CV and respiratory systems. Drug-related decreases in body temperature and locomotor activity were observed in male rats at C_{max} with an exposure margin of ~339x $MRHD_{Cmax}$ and a CNS safety margin of ~36x $MRHD_{Cmax}$ at the NOAEL. In vitro studies indicate that significant hERG or Nav1.5 inhibition is not likely at biologically relevant exposure levels. Drug-related CV effects and related activation of RAAS in rats were observed at C_{max} exposure margins of 11x $MRHD_{Cmax}$, but were likely secondary to PD-related diuresis. In dogs, drug-related CV changes in QTc interval, blood pressure, cardiac contractility, and heart rate corresponded with T_{max} at exposure margins of 163x $MRHD_{Cmax}$, but had a sufficient safety margin of ~13x $MRHD_{Cmax}$ at the NOAEL. Drug-related increases in respiratory rate and volume were observed in rats at C_{max} with an exposure margin of ~36x $MRHD_{Cmax}$ and a respiratory safety margin of ~9x $MRHD_{Cmax}$ at the NOAEL. Overall, based on CNS, CV and respiratory safety pharmacology studies, there were no significant safety concerns at clinical exposure levels.

In nonclinical toxicology studies, drug-related effects were predominantly observed in the renal system of all species, which are considered to be secondary to the PD activity of SGLT2 inhibition and are consistent with other members of this drug class. PD-related renal system findings of pelvic and tubule dilatation, inflammation, mineral deposits, hypertrophy, and increased organ weight were often not associated with a No Observed Effect Level (NOEL), but were not always considered adverse. Increases in the severity of kidney findings with correlating 2-fold increases in BUN, degeneration and/or irreversibility were considered to be adverse. In the 9-month dog study, irreversibility of increased urine volume in recovery animals at 150 mg/kg (≥556x $MRHD_{AUC}$) was considered to be consistent with persistent changes in kidney function, with a NOAEL of 10 mg/kg (47x $MRHD_{AUC}$). Higher exposures were achieved in the 4-week dog study, resulting in adverse findings of renal tubular degeneration at 150 mg/kg/day (≥761x $MRHD_{AUC}$; NOAEL = 51x $MRHD_{AUC}$). In rats, adverse renal system findings also included infiltrate, infection, inflammation, hypertrophy and hyperplasia of organs of the urinary tract, including the prostate in males, which are likely secondary to PD-related glucosuria and untreated UTI. In the pivotal 6-month rat study, irreversibility of kidney findings was observed at ≥25 mg/kg (≥93x $MRHD_{AUC}$), with a NOAEL for reversibility of 5 mg/kg (13x $MRHD_{AUC}$) after 8 weeks of recovery. In the 2-year rat study, similar kidney effects were associated with adverse ascending UTI in males at ≥1.5 mg/kg/day (5x $MRHD_{AUC}$; NOAEL = not determined) and females at ≥5 mg/kg/day (28x $MRHD_{AUC}$; NOAEL = 7x $MRHD_{AUC}$), indicating that adverse kidney effects may be associated with lower exposure margins with chronic dosing. Nevertheless, ertugliflozin-related renal effects are anticipated class-related effects that are monitorable and treatable with sufficient safety margins for reversibility in adults.

Drug-related ketonuria was repeatedly observed in rat toxicology studies, which correlated with decreases in urine pH levels. Ketonuria is consistent with ketosis; however, the presence of correlating increases in blood ketone levels and decreases in blood pH are unknown because they were not evaluated. Nevertheless, there were no clinical signs consistent with an adverse level of ketosis. Furthermore, ketonuria is

consistent with non-adverse nutritional ketosis, which correlates with reductions in body weights, and may be secondary to drug-related inhibition of carbohydrate absorption and decreases in glucose levels. Nevertheless, this finding is notable given that diabetic ketoacidosis (DKA) has been observed clinically in diabetic patients treated with SGLT2 inhibitors.

Adverse drug-related GI effects were observed in pivotal rat and dog toxicology studies. In dogs, adverse findings consistent with GI intolerance included excessive vomiting, salivation, and abnormal feces at 150 mg/kg/day (556x $MRHD_{AUC}$), with a NOAEL of 10 mg/kg/day (46x $MRHD_{AUC}$). In rats, adverse findings of stomach erosion/ulcers, pyloric crypt degeneration and foveolar hyperplasia, were observed at ≥25 mg/kg/day (93x $MRHD_{AUC}$), with a NOAEL of 5 mg/kg/day (13x $MRHD_{AUC}$). In the 2-year rat study, similar adverse GI effects were observed in males at ≥5 mg/kg/day (18x $MRHD_{AUC}$; NOAEL = 5x $MRHD_{AUC}$) and females at 15 mg/kg/day (74x $MRHD_{AUC}$; NOAEL = 28x $MRHD_{AUC}$), indicating that adverse GI effects were observed at similar exposure margins with chronic dosing. Drug-related GI effects were considered likely due to off-target inhibition of SGLT1, which is less likely to occur in humans due to higher selectivity for human SGLT2. It's noted that GI intolerance has been observed in clinical studies with SLGT1/2 mixed inhibitors. Overall, potential drug-related GI effects were associated with sufficient margins of safety and are considered to be monitorable, treatable and reversible.

Drug-related effects on calcium homeostasis were also reported in rat and dog toxicology studies. In pivotal toxicology studies, drug-related hypercalciuria was observed in rats at 100 mg/kg/day (288x $MRHD_{AUC}$; NOAEL = 93x $MRHD_{AUC}$) and dogs at 150 mg/kg/day (556x $MRHD_{AUC}$; NOAEL = 46x $MRHD_{AUC}$), which is consistent with other members of the SGLT2 drug class. Changes in calcium homeostasis are likely partially secondary to PD-related inhibition of renal tubule glucose reabsorption and osmotic diuresis, but plausibly more predominantly secondary to off-target inhibition of SGLT1 in the gut. In male rats at 100 mg/kg ertugliflozin (≥88x $MRHD_{AUC}$; NOAEL = 93x $MRHD_{AUC}$), decreases in PTH correlated with decreases in mean serum calcium levels and increases in trabecular bone and hyperostosis, which is consistent with activation of osteoblast activity and inhibition of osteoclast activity and resultant decreases in serum calcium levels and increased phosphorous levels. Similar bone findings and decreases in PTH and osteocalcin were also reported with at least 2 other members of the SGLT2 drug class. Together, these findings are consistent with an adaptive response to increased Ca absorption secondary to decreases in gut pH due to off-target SGLT1 inhibition and sugar fermentation. It is noted that no drug-related bone findings were observed in rats after 2 years of daily administration of doses up to 15 mg/kg (♂ = 66x $MRHD_{AUC}$, ♀ = 74x $MRHD_{AUC}$). Thus, although the data cumulatively indicate that calcium homeostasis and bone regulation can be disrupted at high doses in rats (≥288x $MRHD_{AUC}$), and possibly in dogs (≥556x $MRHD_{AUC}$), there is a large margin of safety. Furthermore, these effects are likely mediated or exacerbated by off-target SGLT1 inhibition, which is less likely to occur at clinical exposures. Overall, there is not a significant safety concern for ertugliflozin-related effects on calcium homeostasis and/or bone health at clinical exposures of 15 mg/day.

The commercial ertugliflozin formulation synthesized by a new process method, which contained the process related impurities [redacted], was evaluated in a 13-week toxicology bridging study in rats. The NOAEL was set at 5 mg/kg (18x $MRHD_{AUC}$ in males, 24x $MRHD_{AUC}$ in females) based on adverse findings in the digestive tract and increased kidney findings at 25 mg/kg (76x $MRHD_{AUC}$ in males, 107x $MRHD_{AUC}$ in females). Based on exposures, the NOAEL safety margin is considered to be comparable to that of the pivotal 6-month rat toxicology study. Furthermore, no new significant drug-related toxicities were identified. Overall, the nonclinical safety profile of the ertugliflozin formulation intended for marketing is considered to be comparable to the safety profile of the ertugliflozin formulation(s) used in previous nonclinical toxicology studies. Thus, this study successfully bridges the new formulation to the safety profile of the previous ertugliflozin formulation. Furthermore, the 3 process related impurities were evaluated at relative amounts above anticipated clinical doses and were not associated were any new toxicities. Thus, the impurities [redacted] are also considered to be qualified with acceptable margins of safety for a 15 mg/day clinical dose at specifications of NMT [redacted]%, respectively.

The sponsor also conducted a 13-week rat toxicology study evaluating 4 potential ertugliflozin degradation products. In females, the NOAEL was set at 25 mg/kg (91x $MRHD_{AUC}$) based on the absence of toxicologically significant adverse findings. In males, the NOAEL was set at 5 mg/kg (26x $MRHD_{AUC}$) based on mortalities due to ascending urinary tract infection and associated macroscopic and microscopic urinary tract and kidney findings at 25 mg/kg (62x $MRHD_{AUC}$). However, these effects are consistent with previous studies and glucosuria related to the PD activity of ertugliflozin. Based on exposures, the NOAEL safety margins for both males and females were higher than the 6-month rat toxicology study NOAEL safety margin of 19x $MRHD_{AUC}$ and do not represent an increased risk. Overall, the toxicology findings are considered to be comparable to that of previous nonclinical toxicology studies with ertugliflozin and no new significant toxicities were identified. Furthermore, the safety margins for all 4 degradants were considered to be sufficient. Thus, the ertugliflozin degradants [redacted] are considered to be qualified at the specification limits of NMT [redacted]% for a clinical dose of 15 mg/day.

Based on the weight of evidence from a standard battery of valid genotoxicity assays, ertugliflozin is considered to be negative for genotoxic potential.

A 2-year carcinogenicity study was conducted in male and female Crl:CD1(ICR) mice with daily administration of 5, 15 or 40 mg/kg/day ertugliflozin, in accordance with ECAC dosing recommendations. All male groups were terminated during Week 97 and all female groups were terminated during Week 102 due to low survival that was not drug-related. There were no significant drug-related neoplastic findings in male or female mice at any of the doses examined, and the NOAEL for neoplasms was set at the high dose of 40 mg/kg/day (~50x $MRHD_{AUC}$). It is also noted that non-adverse PD-related kidney and bladder findings were considered to be comparable to similar findings

observed in shorter toxicology studies.

A second 2-year carcinogenicity study was conducted in male and female SD rats with daily administration of 1.5, 5, or 15 mg/kg/day ertugliflozin, in accordance with ECAC dosing approval with the exception of exclusion of a saline/water control group. In female rats, there were no statistically significant increases in incidences of benign or malignant neoplasms in any tissues, with a neoplastic NOAEL of 15 mg/kg/day (74x $MRHD_{AUC}$). However, in male rats, drug-related increases in the incidences of adrenal medulla benign PCC and combined benign + malignant PCC neoplasms were reported at 15 mg/kg/day (66x $MRHD_{AUC}$), resulting in a NOAEL for neoplasms of 5 mg/kg/day (18x $MRHD_{AUC}$).

The observation of drug-related neoplasms in rats, but not in mice, is consistent with trends observed with other members of the SGLT2 inhibitor class and further indicates that the rat is the more sensitive species for neoplastic effects of this drug class. It is noted that drug-related changes in calcium homeostasis observed in toxicology studies with ertugliflozin and other SGLT2 inhibitors are consistent with findings with vitamin D analogues, which are also associated with adrenal medulla neoplasms in rats. Thus, it is plausible that the ertugliflozin-related increases in adrenal medulla hyperplastic and neoplastic findings are related to rat sensitivity to changes in calcium homeostasis. It is also noted that the significance of these findings to human susceptibility for adrenal medulla hyperplasia and/or neoplasia is unclear. Given the sufficient margins of safety and lack of clear clinical significance, the adrenal medulla findings do not bar non-clinical support of ertugliflozin approval; however, the increased risk of drug-related proliferative effects at exposures ≥18x $MRHD_{AUC}$ are considered noteworthy in the drug label, particularly for consideration by potentially susceptible populations, such as patients with multiple endocrine neoplasia type 2 (MEN2) syndrome or a history of adrenal medulla hyperplasia or PCC.

Ertugliflozin metabolites were evaluated in accordance with ICH-M3(R2) and the FDA Safety Testing of Drug Metabolites: Guidance for Industry (Nov 2016). Although the O-glucuronide metabolites M5a and M5c are disproportional human metabolites, they are unlikely to be of significant toxicological concern at clinical exposure levels. M5a and M5c are >500-fold to 1000-fold less potent than ertugliflozin at the SGLT2 receptor, with IC_{50} values of 476 nM and >1000 nM, respectively. Given that the reported clinical C_{max} values for M5a and M5c were 102 nM and 431 nM, respectively, after a single 15 mg dose (clinical study #P014/1024), significant inhibition of SGLT2 by either M5a or M5c is not anticipated at clinical exposure levels. Furthermore, both M5a and M5c were qualified in the pivotal 6-month rat and 9-month dog toxicology studies. In the 6-month rat study, exposure margins of 2-3x for M5a and 1-2x for M5c were achieved at 25 mg/kg, which was not associated with any significant toxicities unrelated to PD-related inhibition of SGLT2 or off-target inhibition of SGLT1. Thus, there were no significant metabolite-related toxicities at clinically relevant exposures in rats. M5c was also qualified in the pivotal 9-month dog toxicology study with a safety margin of 3x $MRHD_{AUC}$ in both males and females at the NOAEL of 10 mg/kg. M5a and M5c were also both considered to be qualified in the rat carcinogenicity study, since exposure

levels of both metabolites at 15 mg/kg were considered to be reasonably adequate. Although drug-related neoplasms were observed in the adrenal medulla at doses associated with clinically-relevant metabolite exposures, the neoplasms were considered to be related to the activity of the parent compound, in that adrenal medulla PCC has been observed in rats with other SGLT2 inhibitors and is considered likely to be class-related. Furthermore, since inhibition of SGLT2 or SGLT1 by either metabolite is unlikely at exposure levels achieved in the carcinogenicity study, the metabolites are not likely to be contributing factors to PD-related neoplasms observed in rats. Thus, the metabolites M5a and M5c are considered to be reasonably qualified in the rat carcinogenicity study and are not associated with a significant cause for neoplastic-related concern. Overall, the characterization of ertugliflozin metabolites in toxicology evaluations is considered to be sufficient and the metabolite safety profile is considered to be complete. Thus, no additional studies are required to support the safety of ertugliflozin metabolite exposures at the 15 mg/day therapeutic dose.

In rat studies with radioactively labeled drug, ertugliflozin was present in excreted milk at exposure levels similar to plasma levels in the lactating females. In pregnant rats, radiolabeled drug readily crossed the placenta, but was generally present in fetal blood and tissues at lower levels than maternal plasma levels, indicating greater partitioning to maternal plasma than fetal tissue. However, after crossing the placenta, greater partitioning to fetal tissue than fetal blood was observed. In addition, radiolabeled drug partitioning to fetal brain was higher than maternal brain, indicating that drug more readily crosses the fetal blood:brain barrier, most likely due to incomplete development of the blood:brain barrier in the fetus. Overall, these data indicate that significant total drug exposures can be anticipated in fetuses and nursing neonates.

In the fertility and early embryonic development study in rats, ertugliflozin did not have an effect on male or female fertility at exposure margins up to ~480x and ~570 $MRHD_{AUC}$, respectively. Thus, there are no anticipated adverse effects on fertility at clinical exposure levels.

Fetal development with exposure during the period of organogenesis was evaluated in rats and rabbits. In EFD studies in both species, decreases in embryo-fetal survival correlated with maternal toxicities of decreased body weight and/or decreased weight gain at 250 mg/kg (707x $MRHD_{AUC}$) in rats and 50 mg/kg (150x $MRHD_{AUC}$) in rabbits; although the maternal toxicity was notably minimal in rats. In rat fetuses, drug-related effects on visceral malformations and skeletal variations, as well as possibly visceral variations, were observed at the maternally toxic dose of 250 mg/kg, with a fetal development NOAEL of 100 mg/kg at a maternal exposure margin of 331x $MRHD_{AUC}$. In rabbit fetuses, potentially drug-related effects on skeletal and visceral variations were also observed at 250 mg/kg (833x $MRHD_{AUC}$), with a fetal development NOAEL of 100 mg/kg at a maternal exposure margin of 307x $MRHD_{AUC}$. In a PPND study in rats, drug-related effects on neonatal survival and developmental effects correlated with maternal toxicities of decreased body weight, weight gain and food consumption at maternal doses of ≥100 mg/kg (≥331x $MRHD_{AUC}$), resulting in a pup NOAEL of 50 mg/kg at a maternal exposure margin of ~144x $MRHD_{AUC}$. Decreases in pup size and clinical signs

of decreased maternal care and dehydration were evident at 100 mg/kg, and developmental delays in sexual maturation and increases in pup mortality due to lack of nursing were observed at 250 mg/kg. It's noted that there were no drug-related effects on fetal or neonatal development at doses that were not associated with maternal toxicity in rats; whereas, in rabbits, fetal developmental effects were only observed at doses above the threshold for maternal toxicity. Together, these data indicate that fetal and neonatal developmental safety margins for drug exposure during the period of organogenesis are sufficient and that there is likely to be low fetal risk at drug exposure levels that are not associated with maternal toxicity.

The effect of direct administration of ertugliflozin during renal development/maturation was evaluated in a 10-week juvenile rat study with dosing during the postnatal period corresponding to human renal development during the late 2nd and 3rd trimesters of pregnancy. The NOAEL for PD-related kidney effects was not determined due to similar kidney findings (dilatations, mineralization, and ↑organ weight) at all doses, which were not fully reversible in recovery animals assessed at 250 mg/kg. Thus, the study could not rule out a risk for human renal development at drug exposures margins of 17x $MRHD_{AUC}$ during human pregnancies in the late 2nd and 3rd trimesters. Similar non-reversible kidney effects in juveniles and an associated risk for drug-related effects on human renal development have been described with other SGLT2 inhibitors administered during the same period of renal development, and are considered likely to be class-related.

In the juvenile rat study, a NOAEL for growth delay was set at 5 mg/kg (17x $MRHD_{AUC}$) and a NOAEL for sexual maturation delay was set at 25 mg/kg (81x $MRHD_{AUC}$). . A NOAEL for drug-related effects on bone was set at 5 mg/kg due to bone findings of decreased femur length and changes in density and bone regulation in both sexes at ≥25 mg/kg, which were not fully reversible after recovery. . Drug-related growth delays are likely related to the drug-related effects on bone regulation and maturation, which are consistent with altered bone turnover at ≥25 mg/kg (81x $MRHD_{AUC}$) in both sexes. Overall, the bone findings are consistent with altered bone regulation associated with changes in bone regulation and deficits in bone growth and/or maturation in juveniles. However, since alterations in calcium homeostasis and bone regulation may be secondary to off-target SGLT1 inhibition in rats, which is more probable in rats than in humans, the clinical relevancy of these bone findings, and possibly related growth delays, remain unclear. .

Ertugliflozin was evaluated for eye and skin irritancy using ex vivo, in vitro and in vivo local tolerance tests. In the BCOP test, the solid form of ertugliflozin was positive for eye irritancy, and ertugliflozin was classified as a category 1 ocular irritant. In human skin 3-dimensional cultures, direct exposure to solid ertugliflozin was corrosive after 1 hour of exposure, but not after acute exposures of ≤3 minutes. However, in the mouse LLNA test, exposure to ertugliflozin solution at a concentration up to ~3-fold higher than the clinical dose was negative for dermal contact hypersensitivity. Thus, ertugliflozin was not classified as a skin sensitizer. Ertugliflozin was not evaluated in phototoxicity assays, but is considered to be negative for potential phototoxicity. Overall, there is not

a significant safety concern for skin sensitivity for brief periods of time, such as during oral administration. However, direct eye exposure should be avoided.

All potential and actual impurities above the qualification threshold were evaluated in accordance with ICH M7 Guidance and all unspecified impurities are controlled at specifications consistent with the ICH Q3A qualification threshold for a daily dose of 15 mg. Ertugliflozin-related impurities and degradants were qualified in nonclinical studies and were not associated with any new or significant toxicities. The overall weight of evidence indicates that the potential impurities and degradation products in ertugliflozin are unlikely to present a potential risk for genotoxicity, mutagenicity, carcinogenicity, or organ toxicity concerns.

In summary, characterization of the nonclinical toxicology profile of ertugliflozin is considered to be complete. In general, potential drug-related effects were consistent with the SGLT2 inhibitor drug class and were considered to be monitorable, treatable, reversible, and/or associated with a sufficient margin of safety at the proposed clinical dose of 15 mg/day; the only exception being that of potential class-related renal developmental effects during pregnancy, which will be described in the label. Overall, the nonclinical data support market approval of ertugliflozin.

Toxicology Summary Tables

Table 111: Summary of Pivotal General Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
9-Month + 8-Week Recovery Beagle Dogs **Dose:** 1, 10 & 150 mg/kg ♂ AUC: 6, 63 & 1040 µg·h/mL ♀ AUC: 7, 78 & 767 µg·h/mL	**10 mg/kg** (♂ & ♀)	♂: **46x** ♀: **57x**	**≥1 mg/kg (♂4x/♀6x MRHD):** adrenal gland (↑organ weight & cortex vacuolation), glucosuria **≥10 mg/kg (♂46x/♀57x MRHD):** thyroid mineralization (♀, irreversible) **150 mg/kg (♂754x/♀556x MRHD):** Adverse: GI intolerance (excessive vomiting, diarrhea, salivation), possibly related mortalities, systemic inflammatory response Non-adverse: ↓BW & gain, ↑thymus weight, persistent ↑reticulocytes, ↑urine calcium (partially reversible), irreversible urine ↑volume
6-Month + 8-Week Recovery SD Rats **Dose:** 5, 25 & 100 mg/kg ♂ AUC: 18, 128 & 397 µg·h/mL ♀ AUC: 27, 167 & 814 µg·h/mL	**5 mg/kg** (♂ & ♀)	♂: **13x** ♀: **19x**	**≥5 mg/kg (♂13x/♀19x MRHD):** stomach erosion/ulcer, ↓pancreatic zymogen, ↑food consumption, ↓blood glucose, glucosuria, ↓serum electrolytes (minimal), ↑phosphates, possible dehydration, minimal ↑BUN **≥25 mg/kg (♂93x/♀121x MRHD):** Adverse: stomach (pyloric crypt degeneration, discoloration, ↑severity of erosion/ulcer) Non-adverse: minimal-slight kidney findings (pelvic & tubule dilatation, hyperplasia, mineral deposition) **100 mg/kg (♂288x/♀590x MRHD):** bone [severe hyperostosis (♂) & hyperplasia (♀)], digestive tract (stomach hyperplasia, ↑severity of erosions/ulcers & crypt degeneration), ↑severity of kidney findings, adrenal gland (↑organ weight, hypertrophy & cortex vacuolation), ↓BW & gain, ↓RBC parameters, ↓reticulocytes, ↑urine calcium, ↓PTH, significant ↓serum electrolytes (Ca, Na, K, & Cl), mild ↑BUN (1.5-fold)

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 µg·h/mL and C_{max} = 266 ng/mL
♂ = males only; ♀ = females only

Table 112: Summary of Bridging Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
13-Week SD Rats **Dose:** 1, 5 & 25 mg/kg **Commercial Formulation** (Impurities (b) (4)) ♂ AUC: (b) (4) µg·h/mL ♀ AUC: µg·h/mL	**5 mg/kg** (♂ & ♀)	♂: **18x** ♀: **24x**	**≥1 mg/kg (♂3x/♀5x MRHD):** ↑food consumption, ↑kidney weight, colon dilatation (♂), adrenal gland hypertrophy, pancreatic zymogen depletion, prostate mononuclear cell infiltration, ↓blood glucose, minimal ↓serum Cl, minimal ↑BUN, urine [glucosuria, ↑volume (♂), ↑specific gravity, ↓pH, ↑ketones] **≥5 mg/kg (♂18x/♀24x MRHD):** Stomach [discoloration (♀), erosion/ulcer (♀)], ↓BW gain, GI tract (dilatation, villi/mucosa ↑height) **25 mg/kg (♂76x/♀107x MRHD):** Adverse: Stomach (hemorrhage, erosion/ulcer, discoloration), intestinal tract hemorrhage, ↑severity of kidney findings with ↑BUN (2-fold) Non-adverse: pancreas acinar atrophy (♂), prostate mixed cell inflammation, ↓serum electrolytes (Na, K, Cl)
13-Week SD Rats **Dose:** 1, 5 & 25 mg/kg **+ Degradants** (Degradants (b) (4)) ♂ AUC: (b) (4) µg·h/mL ♀ AUC: µg·h/mL	♂: **5 mg/kg** ♀: **25 mg/kg**	♂: **26x** ♀: **91x**	**≥1 mg/kg (♂3x/♀4x MRHD):** kidney (↑weight, tubule dilatation), adrenal gland hypertrophy, minimal ↑BUN, ↓blood glucose, ↓serum electrolytes (P, Na, K, Cl, & Ca in ♂), ↓cholesterol, urine [glucosuria, ↑volume (♂), ↑specific gravity (♂), ↓pH (♂), ↑ketones (♂)] **≥5 mg/kg (♂26x/♀22x MRHD):** kidney [pelvis inflammation (♀), hyperplasia (♀)], urinary tract [hyperplasia (♀), ureter & bladder mixed cell inflammation (♀)], pancreatic zymogen depletion, **25 mg/kg (♂62x/♀91x MRHD):** Adverse (♂): Mortality secondary to ascending UTI, kidney (large, discolored, dilatation, inflammation), 2-fold ↑BUN, urinary tract (inflammation, infiltrate, dilatation, enlarged, discolored, hyperplasia), ↑blood UN, and mild glandular stomach erosion. Non-adverse: ↓BW & gain (♀), stomach minimal focal erosion, GI tract (dilatation, villi/mucosa ↑height),

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 µg·h/mL and C_{max} = 266 ng/mL
♂ = males only; ♀ = females only

Table 113: Summary of Sub-chronic GLP Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
3-Month Beagle Dogs **Dose:** 1, 10 & 150 mg/kg AUC: 9.8, 92, 1100 µg·h/mL	**10 mg/kg**	**67x**	**≥1 mg/kg (7x MRHD):** liver glycogen depletion **150 mg/kg (797x MRHD):** GI intolerance (excessive vomiting, diarrhea, abnormal feces), ↓BW gain, liver cell necrosis (♀)
4-Week Beagle Dogs **Dose:** 1, 10 & 150 mg/kg ♂ AUC: 7, 77, 1050 µg·h/mL ♀ AUC: 8, 71, 1170 µg·h/mL	**1 mg/kg**	**5x**	**≥1 mg/kg (5x MRHD):** liver glycogen depletion, ↓BW gain **10 mg/kg (51x MRHD):** gallbladder vacuolation **150 mg/kg (761x MRHD):** GI intolerance (vomiting, abnormal feces, salivation), renal tubular degeneration
3-Month SD Rats **Dose:** 5, 25 & 250 mg/kg AUC: 20, 89, 738 µg·h/mL	**<5 mg/kg**	**<14x**	**≥5 mg/kg (14x MRHD):** kidney (pelvic/tubule dilatation, mineral deposition), GI tract dilatation (♂), adrenal gland (↑weight, microscopic findings) **≥25 mg/kg (65x MRHD):** Stomach erosion/ulcer, inflammation ↓prostate weight, hyperostosis (♂), GI tract dilatation (♀) **250 mg/kg (535x MRHD):** pelvic/bladder hyperplasia, ↑severity of nephropathy, heart myonecrosis, hyperostosis (♀)
4-Week SD Rats **Dose:** 5, 25 & 500→250 mg/kg ♂ AUC: 8, 69, 541 µg·h/mL ♀ AUC: 15, 93, 718 µg·h/mL	**25 mg/kg**	♂: **50x** ♂: **67x**	**500→250 mg/kg (♂392x/♀520x MRHD):** mortality, ↑severity of nephropathy, stomach erosion, squamous hyperplasia Note: Doses were reduced to 250 mg/kg on Day 11 due to intolerability at 500 mg/kg

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 µg·h/mL and C_{max} = 266 ng/mL
♂ = males only; ♀ = females only

Table 114: Summary of Reproductive & Developmental Toxicology Studies

Study	NOAEL (mg/kg/day)	Human Safety Margin* ($MRHD_{AUC}$)	Basis
Rat Fertility SD Rats **Dose:** 5, 25 & 250 mg/kg (28 days prior to mating to GD7)	**250 mg/kg**	♂: **~480x** ♀: **~570x**	**250 mg/kg:** No significant drug-related effects of male or female fertility parameters
Rat EFD Pregnant SD Rats **Dose:** 50, 100 & 250 mg/kg (GD6 to GD17) AUC: 15.4, 32, 67 µg·h/mL	Maternal **100 mg/kg** Fetal **100 mg/kg**	Maternal **331x** Fetal **331x**	Maternal **250 mg/kg (707x MRHD):** minimal maternal toxicity (transient weight loss & ↓food consumption), ↑early resorptions, ↑post implantation loss, ↓live litter size Fetal **≥100 mg/kg (331x MRHD):** Dose-dependent (not SS) ↑visceral variations (absent innominate artery, a single right sided aortic arch) and dose-dependent (not SS) ↑skeletal variations **250 mg/kg (707x MRHD):** External, visceral malformations (***ventricular septum defect***, a single right sided *aortic arch*), visceral variation (**absent innominate artery** above HCIR), skeletal malformations (fused sternebra, hemicentric thoracic centrum, absent metacarpal); ***skeletal variations*** (unossified 7th cervical centrum or metatarsal, thoracic centrum incomplete ossification, vertebrae 27th presacral, full or short supernumerary ribs)
Rabbit EFD Pregnant NZ Rabbits **Dose:** 50, 100 & 250 mg/kg (GD7 to GD19) AUC: 207, 424, 1150 µg·h/mL	Maternal **<50 mg/kg** Fetal **100 mg/kg**	Maternal **<150x** Fetal **307x**	Maternal **≥50 mg/kg (150x MRHD):** maternal toxicity (↓BW & ↓BW gain), ↑post implantation loss, ↓live litter size **≥100 mg/kg (307x MRHD):** weight loss, ↓food consumption **250 mg/kg (833x MRHD):** ↑abortions secondary to significant maternal toxicity (↓food consumption & weight loss), ↑post implantation loss, ↓live fetuses Fetal **250 mg/kg (833x MRHD):** visceral malformations (***ventricular septum defect***, dilated *aortic arch*, narrowed *pulmonary trunk*), *visceral variations* above HCIR (retrocaval ureter, absent gallbladder, small gallbladder), skeletal malformations (supernumerary cervical centrum, fused rib, misshapen interparietal bone), skeletal variations (sternebra with extra ossification site)

Rat PPND Pregnant SD Rats **Dose:** 50, 100 & 250 mg/kg (GD6 to LD20)	Maternal **50 mg/kg** Fetal **50 mg/kg**	Maternal **~144x** Fetal **~144x**	Maternal **≥100 mg/kg (~331x MRHD):** ↓BW, ↓BW gain, & ↓food consumption during gestation, dehydration, deficits in nursing and maternal behavior **250 mg/kg (~707x MRHD):** hunched posture, abnormal feces, pale ears Fetal **≥100 mg/kg (~331x MRHD):** prolonged ↓BW, dehydration, bruising, ungroomed coats, cold to touch, pale **250 mg/kg (~707x MRHD):** mortalities (PND 1-4) due to lack of nursing, delayed sexual maturation
10-Week Juvenile Rat PND21 SD Rats **Dose:** 5, 25 & 250 mg/kg (PND21 to PND90) ♂ AUC: 20, 116, 696 µg·h/mL ♀ AUC: 28, 97, 939 µg·h/mL	Kidney **Not Determined** Non-PD-Related **5 mg/kg**	Kidney **<17x** Non-PD-Related **17x**	**≥5 mg/kg (17x MRHD):** No significant adverse findings unrelated to PD activity. PD-related kidney effects (macroscopic dilatation, ↑weight, tubule dilatation, mineralization), associated with slight ↑BUN (20-30%) ➢ Similar kidney findings at all doses, but only high dose was evaluated after recovery; thus, *irreversible effects on **renal development/maturation** cannot be ruled out* **≥25 mg/kg (81x MRHD):** kidney (cortical fibrosis), suspected dehydration, eyes partly closed, abdominal distention, ↓BW & gain (♂), **growth delay**, urine changes (↑Ca, ↓Na, ↓Cl), and ↓protein blood levels, **bone development** (↑bone, ↓bone formation, ↓bone resorption, ↓length, bent tails, ↑metaphysis bone mass & ↓diaphysis bone mass) **250 mg/kg (580x MRHD):** Mortalities, hunched posture, **Irreversible kidney** findings (↑weight tubule/pelvis dilatation, mineralization, ↑organ weight), 2-fold ↑BUN, ↑serum K, **Irreversible bone** findings [↑bone (♀), ↓length (♂), ↑metaphysis (♀) & ↓diaphysis bone mass], ↓BW & gain (♀), ↑food consumption, **sexual maturation developmental delay**.

* Based on a 15 mg/day therapeutic dose with exposures of AUC = 1.38 µg·h/mL and C_{max} = 266 ng/mL
SS = statistically significant; HCIR = historical control incidence rate; GD = gestation day; LD = lactation day; PND = post-natal day; ♂ = males only; ♀ = females only

Metabolite Exposure Margin Summary Tables

Table 115: M5a & M5c Exposure Margins in Pivotal Toxicology Studies

Species	Study		Dose (mg/kg/day)	$MRHD_{AUC}$		
				Parent (Ertugliflozin)	M5a (PF-06685948)	M5c (PF-06481944)
Human			15 (mg/day)	1.38	0.337	0.667
Rat	6-Month	Male	5	13x	0.4x	0.2x
			25	93x	3x	1.5x
			100	288x	9x	5x
		Female	5	19x	0.6x	0.3x
			25	121x	1.6x	0.8x
			100	590x	8x	4x
Dog	9-Month		1	4x	-	0.3x
			10	46x	-	3x
			150	555x	-	34x

NOAEL = highlighted in yellow

Table 116: M5a & M5c Exposure Margins in the Rat Carcinogenicity Study

Species	Dose (mg/kg/day)	Neoplastic $MRHD_{AUC}$		
		Parent (Ertugliflozin)	M5a (PF-06685948)	M5c (PF-06481944)
Human	15 (mg/day)	1.38	0.337	0.667
Male Rat (2-year)	1.5	5x	0.2x	0.1x
	5	18x	**0.6x**	**0.3**
	15	66x	2x	1x
Female Rat (2-year)	1.5	7x	0.1x	0.1x
	5	121x	0.4x	0.2x
	15	74x	**1x**	**0.5x**

Neoplastic NOAEL = highlighted in yellow

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

/s/

JESSICA HAWES
08/17/2017

RONALD L WANGE
08/17/2017
I concur with Dr. Hawes' recommendation for approval.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 209805 **Applicant: Merck Sharpe and Dohme Corp** **Stamp Date: 12/19/2016**

Drug Name: Ertugliflozin and Sitagliptin tablets **NDA/BLA Type: 505(b)(1)**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) 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		Mouse and rat carcinogenicity studies with ertugliflozin alone administration were submitted with the NDA package (reference NDA 209803). The pivotal 3-month combination toxicology study was previously submitted under IND 106447.
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		The sponsor conducted a 13-week toxicology bridging study in rats evaluating ertugliflozin synthesized by the process method used in the commercial formulation to be marketed.
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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The sponsor submitted a 10-week juvenile study in Sprague Dawley rats, which was recommended by the PeRC BPCA

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				subcommittee in 2013.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		The sponsor conducted a 13-week rat toxicology study with ertugliflozin alone to evaluate and qualify 4 potential ertugliflozin degradation products.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			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.

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

/s/

JESSICA HAWES
01/04/2017

RONALD L WANGE
01/05/2017

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 209806 **Applicant: Merck Sharpe and Dohme Corp** **Stamp Date: 12/19/2016**

Drug Name: Ertugliflozin and Metformin tablets **NDA/BLA Type: 505(b)(1)**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) 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		Mouse and rat carcinogenicity studies with ertugliflozin alone administration were submitted with the NDA package (reference NDA 209803). The pivotal 3-month combination toxicology study was previously submitted under IND 106447.
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		The sponsor conducted a 13-week toxicology bridging study in rats evaluating ertugliflozin synthesized by the process method used in the commercial formulation to be marketed.
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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The sponsor submitted a 10-week juvenile study in Sprague Dawley rats, which was recommended by the PeRC BPCA

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				subcommittee in 2013.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		The sponsor conducted a 13-week rat toxicology study with ertugliflozin alone to evaluate and qualify 4 potential ertugliflozin degradation products.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			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.

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

/s/

JESSICA HAWES
01/04/2017

RONALD L WANGE
01/05/2017

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 209803 **Applicant: Merck Sharpe and Dohme Corp** **Stamp Date: 12/19/2016**

Drug Name: Ertugliflozin tablets **NDA/BLA Type: 505(b)(1)**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) 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		Mouse and rat carcinogenicity studies were submitted with the NDA package.
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		The sponsor conducted a 13-week toxicology bridging study in rats evaluating ertugliflozin synthesized by the process method used in the commercial formulation to be marketed.
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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The sponsor submitted a 10-week juvenile study in Sprague Dawley rats, which was recommended by the PeRC BPCA

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				subcommittee in 2013.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		The sponsor conducted a 13-week rat toxicology study evaluating 4 potential degradation products.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			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.

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

/s/

JESSICA HAWES
01/04/2017

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	106447
Review number:	8
Supporting document/s:	87, 98, 115
PDUFA review deadline date:	Click here to enter a date.
CDER stamp date:	1/13/2014, 4/22/2014, 10/6/2014
Product:	Ertugliflozin (MK-8835, PF-04971729)
Indication:	Treatment of Type Two Diabetes Mellitus
Therapeutic area:	Endocrinology, Diabetes, and Metabolism
Sponsor:	Merck Sharp and Dohme Corp
Review Division:	Division of Metabolism and Endocrinology Products (DMEP)
Reviewer:	Jessica Hawes, Ph.D.
Supervisor/Team Leader:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Elizabeth Godwin

Template Version: December 7, 2015

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Ertugliflozin (MK-8835, PK-04971729) is a sodium glucose co-transporter 2 (SGLT2) inhibitor being developed by Merck Sharp and Dohme Corp for the treatment of Type 2 Diabetes Mellitus (T2DM).

The sponsor submitted several nonclinical pharmacology, toxicology and reproductive and developmental toxicology studies that are evaluated in this review.

1.2 Brief Discussion of Nonclinical Findings

No new safety concerns were identified at clinically relevant exposure levels.

Ertugliflozin inhibited Nav1.5 currents and hERG potassium currents; however inhibition of either hERG or Nav1.5 currents are not anticipated at clinically relevant exposure levels. Ertugliflozin activated the renin-angiotensin-aldosterone system (RAAS) in spontaneously hypertensive rats; however, the safety margins for drug-related cardiovascular effects are considered to be sufficient and adverse cardiovascular effects are not anticipated at clinically relevant exposure levels.

Drug-related effects on fertility were observed in male rats, but were associated with sufficient margins of safety (~50x $MRHD_{Cmax@15mg}$, 5x $MRHD_{Cmax@100mg}$) and were not observed in females. Thus, adverse effects on fertility are not anticipated at clinically relevant exposures.

Ertugliflozin was teratogenic and delayed sexual maturation at very high exposures with a NOAEL of 50 mg/kg/day for fetuses and neonates, which is associated with sufficient margins of safety for developmental effects at both the therapeutic 15 mg (~35x $MRHD_{AUC@15mg}$) and the supratherapeutic 100 mg (~16x $MRHD_{AUC@100mg}$) doses of ertugliflozin. Thus, adverse effects on fetal and neonatal development are not anticipated at clinically relevant exposures.

1.3 Internal Comments

Click here to enter text.

1.4 Recommendations

The ongoing clinical studies and the proposed Phase III study # MK-883-004-01/B1521021 are reasonably safe to proceed.

1.4.1 Clinical Study (ies) Safe to Proceed: Yes/No Y

1.4.2 If Not Safe to Proceed

Nonclinical deficiencies

Click here to enter text.

Nonclinical information needed to resolve deficiencies

Click here to enter text.

1.4.3 Additional Recommendation(s) (Non-hold comments/advice to sponsor) *if any*.

- Submit historical background control data in Sprague Dawley rats for incidences of small testis and associated deficits in sperm motility, low sperm count and failure to produce pregnancies.

2 Drug Information

2.1 Drug

CAS Registry Number(s) (Optional): 1210344-57-2

Generic Name: Ertugliflozin

Code Name(s): MK-8835 / PF-04971729

PF-04971729 (b) (4) = L-pyroglutamic acid (L-PGA) co-crystal form

Trade Name: Steglatro

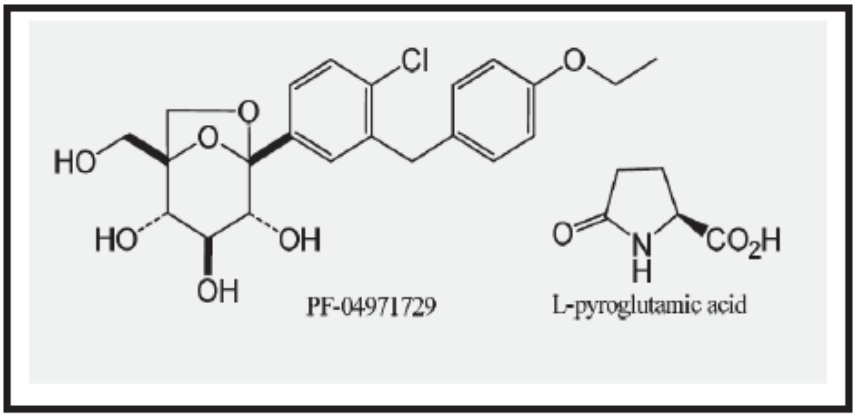
Chemical Name:

PF-04971729:	(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol
PF-04971729 (b) (4)	(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol L-pyroglutamic acid

Molecular Formula/Molecular Weight:

PF-04971729:	C22H25ClO7 / 436.88 g/mol
PF-04971729 (b) (4)	C27H32ClNO10 / 566.00 g/mol

Structure or Biochemical Description:



Pharmacologic Class
Sodium glucose co-transporter 2 (SGLT2) Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND #122329: MK-8835B [Ertugliflozin + metformin fixed dose combination (FDC)], Merck Sharp and Dohme Corp

IND #122330: MK-8835A (Ertugliflozin + sitagliptin FDC), Merck Sharp and Dohme Corp

(b) (4)

2.3 Drug Formulation

Immediate release oral tablets (1 mg, 5 mg, 10 mg, 15 mg, or 25 mg) made with the co-crystal form, Ertugliflozin•L-PGA.

Sponsor's Table 1: Ertugliflozin Tablet Compositions

Table P.1-1. Nominal Unit Composition of 2.5 mg, 5 mg and 10 mg Ertugliflozin•L-PGA Film Coated Tablet

Name of Ingredients	Reference to Standards	Function	Unit Formula (2.5 mg/Tablet)	Unit Formula (5 mg/Tablet)	Unit Formula (10 mg/Tablet)
Core Tablet					
Ertugliflozin•L-PGA [a]	Pfizer	Active Ingredient	(b) (4)	6.48	(b) (4)
Microcrystalline cellulose [b]	NF/Ph.Eur.	(b) (4)		(b) (4)	
Lactose Monohydrate	NF/Ph.Eur.				
Sodium Starch Glycolate	NF/Ph.Eur.				
Magnesium Stearate	NF/Ph.Eur.				
Total Weight					
Film Coated Tablet					
(b) (4)					

Table P.1-1. Nominal Unit Composition of 15 mg Ertugliflozin•L-PGA Film Coated Tablet

Name of Ingredients	Reference to Standards	Function	Unit Formula (mg/Tablet)
Tablet Core			
Ertugliflozin•L-PGA	Pfizer	Active Ingredient	19.431 [a]
Microcrystalline cellulose	NF/Ph.Eur.		(b) (4)
Lactose Monohydrate	NF/Ph.Eur.		
Sodium Starch Glycolate	NF/Ph.Eur.		
Magnesium Stearate	NF/Ph.Eur.		
Tablet Core Weight			
Film Coating			
(b) (4)			
(b) (4)			

2.4 Comments on Novel Excipients

There are no novel excipients. Excipient levels for all 3 formulations are below the maximum current Inactive Ingredient list (IIG) levels in previously approved products.

Table 1: Drug Product Excipient Levels

Excipient	Formulation	Proposed Dose	FDA Maximum Potency
(b) (4)			
Microcrystalline Cellulose	Oral Tablet	(b) (4)	1.3853 g
Lactose Monohydrate	Oral Tablet		760.5 mg
Sodium Starch Glycolate	Oral Tablet		876 mg
Magnesium Stearate	Oral Tablet		256.4 mg

2.5 Comments on Impurities/Degradants of Concern

Impurity (b) (4) was found to be as high as (b) (4) % in the nonclinical Lot #GR02546, but was not detected in clinical Lot GR02694. There are no other identified organic impurities for PF-04971729 (b) (4)

2.6 Proposed Clinical Protocol

First in human: N
Phase: Phase III Trial

Study Title: A Phase 3 randomized, double-blind, placebo-controlled, parallel-group study to assess cardiovascular outcomes following treatment with ertugliflozin in subjects with type 2 diabetes mellitus and established vascular disease, the Vertis CV study

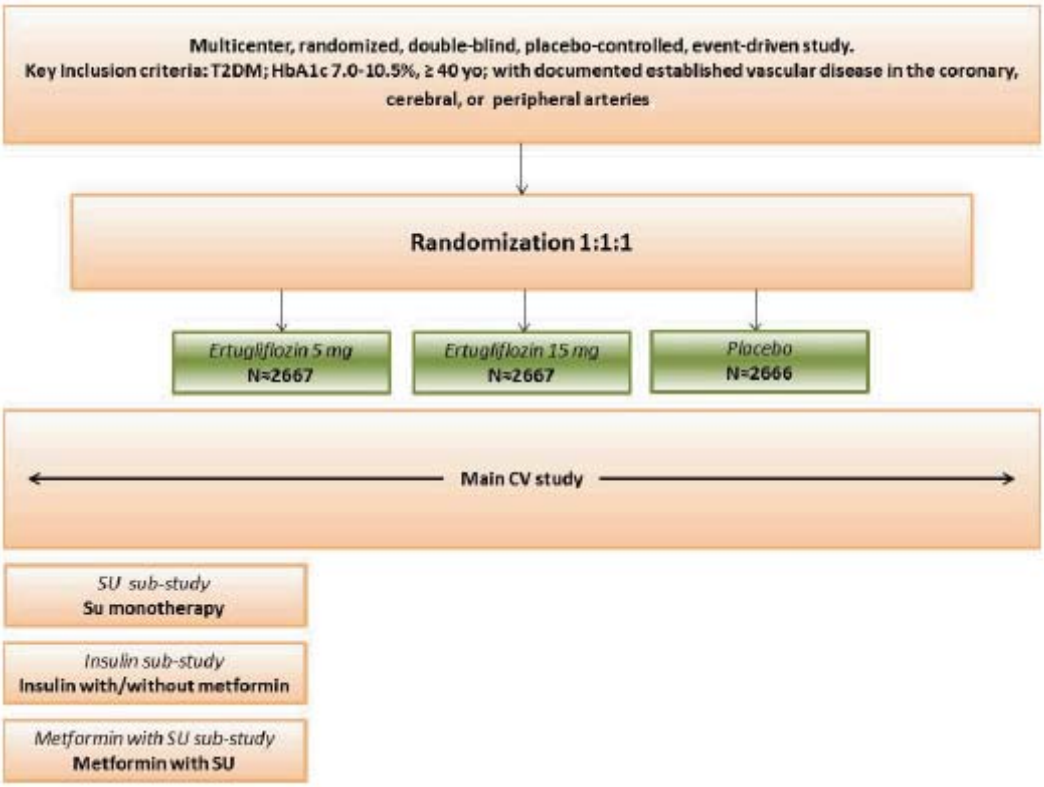
SDN #185, SN #0184

Study ID: MK-883-004-01/B1521021 (P004/1021)

Doses: 5 or 15 mg Ertugliflozin

Study Participants: Approximately 8000 T2DM patients ≥40 years of age with a history of vascular disease involving the coronary, cerebrovascular, or peripheral vascular system. Women of child-bearing potential are included in this study.

Protocol Summary: This study consists of a main 24-month CV study and three 18-week sub-studies, including a sulfonylurea (SU) monotherapy sub-study, an insulin sub-study with and without metformin, and a metformin sub-study in combination with SU.



Primary Endpoints: The primary cardiovascular endpoint for this study will be MACE. Secondary endpoints include cardiovascular death, myocardial infarction, stroke, renal death, hospitalization for heart failure, and all individual components of MACE.

Safety Measures: Subjects will be evaluated every 6-8 weeks for the first 6 months, then every 13 weeks, and finally every 4 months. ECG measurements will be made prior to dosing and on Weeks 18, 52, and Month 24. Vital signs will be assessed at every visit. Clinical labs including clinical chemistry, hematology and urinalysis will be routinely assessed. HbA1c will be assessed at every visit. All subjects will be monitored for adverse events.

Sponsor's Maximum Recommended Human Dose:

Supratherapeutic Dose: *Single-Dose Phase 1 QTc study*

Single oral (tablet) dose of **100 mg/day**: **AUC* = 9.61 μg·h/mL, C_{max}* = 1620 ng/mL = 2.86 μM.**

*AUC and C_{max} exposures were determined in healthy adult subjects following a single oral dose (study #B1521001).

The maximum systemic exposure to unbound** drug: AUC ≈ 615 ng·h/mL, C_{max} ≈ 104 ng/ml ≈ 184 nM

** based on a 6.4% unbound fraction in humans

Therapeutic Dose: *Multiple-Dose Phase 3 Efficacy studies*

Therapeutic oral (tablet) dose of **15 mg/day**: **AUC* = 1.2 μg·h/mL, C_{max}* = 159 ng/mL = 0.3 μM.**

*AUC and C_{max} exposures were extrapolated from 14-day repeat-dose exposure in overweight/obese adult subjects (study #B1521002).

The maximum systemic exposure to unbound** drug: AUC ≈ 79.3 ng·h/mL, C_{max} ≈ 10.2 ng/ml ≈ 18 nM

* * based on a 6.4% unbound fraction in humans

2.7 Previous Clinical Experience Y

If yes, Phase: Phase III Trial

Previous Clinical Experience

Phase 2 clinical studies have been completed. There are 4 additional Phase 1 studies that are ongoing (see Ongoing Phase 1 Clinical Studies), and additional Phase 1 studies that are planned to further assess absolute bioavailability, drug-drug interaction, food effect, and bioequivalence. There are 8 ongoing Phase 3 studies with ertugliflozin (see Ongoing Phase 3 Clinical Studies), but no additional Phase 3 studies are planned.

Ertugliflozin has been administered to humans at doses up to 300 mg in the fasted state and 100 mg in the fed state for up to 14 days. Food decreases the rate of ertugliflozin absorption, but does not affect the overall extent of absorption.

Sponsor's Table 2: Clinical Single-Dose Pharmacokinetic Parameters

Table 6.1-1. Pharmacokinetic Parameters Following Single Oral Doses of PF-04971729

Parameter (Units)	0.5 mg (n=8)	2.5 mg (n=8)	10 mg (n=8)	30 mg (n=8)	100 mg (n=8)	300 mg (n=7)
AUC_{inf}[a] (ng*hr/mL)	45.7 (10)	231 (22)	909 (15)	2810 (18)	9610 (16)	26400 (16)
C_{max}[a] (ng/mL)	7.23 (11)	42.8 (21)	182 (22)	545 (24)	1620 (16)	4330 (20)
T_{max}[a] (hr)	1.0 (0.5-1.5)	1.0 (0.5-1.1)	1.0 (0.5-1.5)	1.0 (0.5-1.5)	1.0 (0.5-1.5)	1.0 (0.5-1.5)
$t_{½}$[a] (hr)	11.4 (19)	13.1 (24)	17.4 (42)	15.2 (33)	16.2 (36)	13.8 (18)

[a] Geometric mean (CV%) for all except: median (range) for T_{max}; arithmetic mean (CV%) for $t_{½}$

Sponsor's Table 3: Clinical Multiple-Dose Pharmacokinetic Parameters

Table 6.1-2. Pharmacokinetic Parameters Following Once-Daily, 14-day Dosing of PF-04971729

Parameter (Units)	1 mg (n=8)	5 mg (n=8)	25 mg (n=8)	100 mg (n=8)
AUC(0-24) [a] (ng*hr/mL)	80.85 (15)	450.5 (35)	2045 (26)	7761 (17)
C_{max} [a] (ng/mL)	10.19 (15)	50.83 (28)	280.8 (28)	1035 (25)
T_{max} [a] (hr)	2.0 (1.0-4.0)	1.50 (1.0-4.03)	2.0 (1.0-2.0)	2.0 (1.0-4.0)
$t_{½}$ [a, b] (hr)	NC	12.28 (24)	14.81 (41)	14.13 (14)
Rac [a]	1.360 (8)	1.247 (7)	1.217 (5)	1.375 (19)

[a] Geometric mean (CV%) for all except: median (range) for T_{max}; arithmetic mean (CV%) for t½
[b] n=0 for 1 mg and n=7 for 25 mg

(Tables excerpted from sponsor's package)

Reoccurring dose-related adverse events (AEs) include headaches, constipation, diarrhea, discolored feces, and folliculitis.

Ongoing Phase 1 Clinical Studies

Study #MK-8835-009/B1521023: An open-label, single-dose study that will evaluate and compare the PK and PD of a single 15 mg dose of ertugliflozin in T2DM subjects with varying degrees of renal impairment to that of subjects with normal renal function.

Study #MK-8835-014/B1521024: An open-label, single-dose study that will evaluate and compare the PK of a single 15 mg dose of ertugliflozin in subjects with hepatic impairment to that of healthy subjects with normal hepatic function.

Study #MK-8835-023/B1521037: A single dose, open-label, randomized, crossover bioequivalence study of the ertugliflozin 15 mg commercial image tablet versus the ertugliflozin Phase 3 tablets in healthy subjects.

Study #MK-8835-035-00/B1521051: A randomized, open label, 2-period, 2-cohort, crossover, steady state evaluation of the PK and PD of once daily and twice daily oral administration of ertugliflozin in healthy subjects.

Ongoing Phase 3 Clinical Studies

Study #P001/B1521016: A randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of the addition of daily treatment with 5 mg and 15 mg ertugliflozin in subjects with T2DM and inadequate glycemic control on standard diabetes therapy(ies) who also have stage 3 chronic kidney disease.

Study #P002/B1521013: A randomized, double-blind, active-controlled study to evaluate the safety and efficacy of the addition of daily treatment with 5 mg and 15 mg ertugliflozin compared with the addition of glimepiride in subjects with T2DM and inadequate glycemic control on a stable dose of metformin (≥1500 mg/day).

Study #P003/B1521022: A randomized, double-blind, placebo- and active-controlled study to evaluate the safety and efficacy of monotherapy treatment with 5 mg and 15 mg ertugliflozin in subjects with T2DM and inadequate glycemic control on diet and exercise alone.

Study #P004/B1521021: A randomized, double-blind, placebo-controlled, event-driven study to evaluate the effect of 5 mg and 15 mg ertugliflozin on cardiovascular outcomes in subjects with T2DM and inadequate glycemic control on standard diabetes therapy(ies) who also have a history of vascular disease.

Study #P005/B1521019: A randomized, double-blind, active-controlled, factorial study to evaluate the safety and efficacy of the addition of combination treatment with 5 mg and 15 mg ertugliflozin and sitagliptin (100 mg QD) compared with the addition of the individual components at corresponding dose strengths in subjects with T2DM and inadequate glycemic control on a stable dose of metformin (≥1500 mg/day).

Study #P006/B1521015: A randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of the addition of treatment with daily 5 mg and 15 mg ertugliflozin in subjects with T2DM and inadequate glycemic control on stable doses of sitagliptin (100 mg QD) and metformin (≥1500 mg/day).

Study #P007/B1521017: A randomized, double-blind, placebo- and active-controlled study to evaluate the safety and efficacy of the addition of treatment with daily 5 mg and 15 mg ertugliflozin in subjects with T2DM and inadequate glycemic control on a stable dose of metformin (≥1500 mg/day).

Study #P017/B1521047: A randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of initial combination treatment with daily 5 mg and 15 mg ertugliflozin and sitagliptin (100 mg QD) in subjects with T2DM and inadequate glycemic control on diet and exercise.

2.8 Regulatory Background

- Ertugliflozin was originally submitted as PF04971729 in September 2009.
- An EOP2 meeting was held on December 17th 2013. The Division encouraged the sponsor to include 2 doses in their phase 3 program, increasing the MRHD to 15 mg/day. The sponsor requested a revision of the proposed rat carcinogenicity study to increase the dose in conjunction with the increased MRHD. Although ECAC did not feel the increase in dose was necessary, it was considered acceptable.
- The PeRC BPCA subcommittee discussed the sponsor's proposed Pediatric Study Plan (PSP) on April 10th 2013 and a revised PSP was approved after resubmission on June 12th 2013. It was concluded that a juvenile toxicology study in Sprague Dawley rats would be required prior to initiation of clinical pediatric studies. Ertugliflozin was then discussed at a second PeRC BPCA

meeting on August 21st 2013 and the PeRC concurred with the proposed PSP and sponsor's plan for a partial waiver and deferral.

- On December 19, 2013, the Executive CAC (ECAC) recommended that a water or saline control group be added in addition to the vehicle [0.5% (w/v) methylcellulose with 20% (v/v) propylene glycol] control group, rather than inclusion of a second vehicle control group that was proposed by the sponsor.
- On 7/30/2014, the sponsor submitted and cross-referenced the new IND #122330 for the FDC product MK-8835A containing ertugliflozin and sitagliptin for the treatment of T2DM.
- On 8/13/2014, the sponsor submitted and cross-referenced the new IND #122329 for the FDC product MK-8835B containing ertugliflozin and metformin for the treatment of T2DM.
- On January 10, 2014, the sponsor submitted a response to FDA comments regarding the committee's recommendations with modifications to the proposed control groups which was evaluated by the ECAC. The ECAC communicated with the sponsor that the inclusion of a 0.5% methylcellulose (MC) control instead of water or saline is acceptable and that, although the use of two instead of one vehicle control group (0.5% MC/10% PEG400) is not considered necessary, the decision on the number of vehicle controls is at the sponsor's discretion.
- On 6/1/2015, the sponsor submitted a request for ECAC (SDN #143, seq. 0142) concurrence for cessation of dosing of the MD males once there are less than 20 surviving male mice, and early termination of the MD male group when there are less than 15 surviving male mice. ECAC did not agree with the sponsor's plan to (b) (4) As excess mortality had not been observed in the high-dose males, it did not appear that ertugliflozin was contributing to the excess deaths in the mid-dose males. ECAC conveyed to the sponsor that the mid-dose male dose group should be terminated when surviving animals fall to 15. However, should the mid-dose males (or any other dose group) drop to 15 surviving animals in Week 100 or later, then all dose groups of that sex (including controls) should be terminated.
- On 6/15/2015, the sponsor submitted a request for ECAC (SDN #145, seq. 0144) concurrence on plans for early termination of the rat carcinogenicity study. The sponsor proposed to terminate all female groups if the control females drop to ≤20 animals and to terminate all groups of an affected sex if the number of any treatment group drops to ≤15 at Week 100 or later. If the number in a treatment group drops to ≤15 prior to Week 100, the sponsor proposed to terminate only the affected dose group. It was determined that the sponsor's proposal is consistent with current recommendations for early termination of control groups and treatment groups, both prior to and after Week 100. Thus, the sponsor's proposal was acceptable.
- A Type C meeting with written responses only was granted on 10/28/2015 regarding statistical and clinical questions, but did not include nonclinical questions.
- On 11/12/2015, the sponsor submitted a request (SDN #170, seq. 0169) for ECAC concurrence for early termination of all male mice groups when the first of any of the control group 1 (0.5% methylcellulose in water), vehicle control group

2 (0.5% methylcellulose/10% polyethylene glycol 400), low dose group, or md dose group reaches ≤15 surviving males (Excerpt 1). ECAC concurred with the sponsor. (Reference Dr. Hawes' nonclinical memo 11/17/2015).

- A Type C meeting with written responses only was granted on 3/7/2016 regarding CMC and pharmaceutical quality questions, but did not include nonclinical questions.
- A Type B pre-NDA meeting was approved on 7/27/2016 to discuss the upcoming NDA submission for ertugliflozin and FDCs with metformin and sitagliptin.

3 Studies Submitted

3.1 Studies Reviewed

Study #	Brief Title
Pharmacology	
PD002 (Pfizer #121450)	Effects of PF-0497129 on Glucose, Fluid and Electrolyte Balance in Sprague-Dawley Rats, 19-Nov-2010
TT097885 (Pfizer #PF04971729NA15)	Effect of PF-04971729 on Nav1.5 Sodium Current Stable Expressed in HEK293 Cells, 27-Sep-2010
PD001 (Pfizer #070904)	Effects of PF-04971729 on Blood Pressure in Spontaneously Hypertensive Rats, 19-Nov-2010
Toxicology	
8291746 (13GR288)	Single-Dose Intravenous Injection Toxicity and Toxicokinetic Study with PF-04971729 in Rats, 13-Mar-2014
TT127803 ((b) (4) #8275450, Pfizer #12GR360)	28-Day Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Wild Type CByB6F1-Tg(HRAS)2Jic Mice, 20-May-2013 **(GLP)**
Reproductive and Developmental Toxicology	
TT107835 (Pfizer #10GR227)	Oral Fertility and Embryonic Development Study of PF-04971729 in Male and Female Rats, 20-May-2011
TT137827FIN (13GR257)	A Pre-and Postnatal Developmental Toxicity Study of PF-04971729 by Oral (Gavage) in Rats, 18-Sep-2014

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Pharmacology and toxicology (Pharm/Tox) reviews #1, #2, #3, #4, and #5 by Dr. Jeffrey Quinn

Pharm/Tox review #6 and #7 by Dr. Jessica Hawes

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action:
Ertugliflozin is an inhibitor of the Sodium Glucose Transporter 2 (SGLT2). Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria. Ertugliflozin is highly selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

Sponsor's Table 4: Ertugliflozin IC_{50} Values

AMG Transport Assay	IC_{50} Geometric Mean	95% Confidence Interval	IC_{50} Arithmetic Mean	Standard Deviation	Assay Replicates
Human SGLT2	0.877 nM	0.704 - 1.09 nM	0.927 nM	0.369 nM	10
Human SGLT1	1960 nM	1460 - 2620 nM	2050 nM	642 nM	8
Rat SGLT2	1.15 nM	0.757 - 1.74 nM	1.18 nM	0.289 nM	4
Rat SGLT1	352 nM	274 - 453 nM	356 nM	54.2 nM	4

AMG = methyl-α-D-glucopyranoside, a non-metabolizable form of glucose; SGLT(1, 2) = sodium-glucose co-transporter type (1, 2); nM = nanomolar; IC_{50} = 50% inhibitory concentration.

(Table excerpted from Investigator's Brochure)

Drug activity related to proposed indication:
Ertugliflozin administration results in concentration-dependent glucosuria in rats. Ertugliflozin acts as a diuretic in rats, increasing urine volume, urinary volume to water intake, and hematocrit levels *in vivo*. Diuretic effects have also been reported in humans.

Table 4-4 Summary of the Effects of Ertugliflozin in Pair-fed Sprague Dawley Rats After 8 Days of Dosing

Measurements	Units	Vehicle	Ertugliflozin
24-h Food	(g)	27 ± 1.0	27
Body Weight	(g)	345 ± 5.8	315 ± 6.1 *
Urinary Glucose Excretion	(mg/24 h)	20 ± 6.2	3612 ± 211.8 *
Plasma Glucose	(mg/dL)	160 ± 5.1	137 ± 8.0 *
Urinary Volume	(mL/24 h)	17 ± 1.6	40 ± 2.7 *
Water Intake	(mL/24 h)	44 ± 3.0	67 ± 3.0 *
Urine Volume/Water Intake	(Uv/H2O x 100)	40 ± 1.3	59 ± 3.5 *
Hematocrit	(%)	45 ± 0.4	48 ± 0.8 *
Urinary Phosphorus	(mg/24h)	11 ± 1.3	19 ± 1.8*
Urinary Potassium	(mmol/24h)	5 ± 0.1	6 ± 0.3*
iPTH	(pg/mL)	456 ± 45.8	415 ± 36.7
Plasma Renin Activity	(ng Ang I/mL/h)	7 ± 2.9	12 ± 2.4*
Serum Aldosterone	(pg/mL)	84 ± 19.0	223 ± 69.1*
Plasma Angiotensinogen	(pmol/mL)	58 ± 2.0	94 ± 3.8*
Urinary Angiotensinogen	(pmol)	5 ± 0.6	10 ± 1.0*

Data are mean ± standard error of the mean, *p ≤0.05 vs Vehicle. Groups were compared using two–sided t-test for independent samples, adjusted for variance as determined by Levene's test.
Ang I = angiotensin I; dL = deciliter(s); g = gram(s); h = hour(s); mg = milligram(s); mL = milliliter(s); mmol = millimole(s); ng = nanogram(s); pg = picogram(s); pmol = picomole(s); Uv = urinary volume; vs = versus; iPTH = intact parathyroid hormone.

Table 4-5 Summary of the Effects of Ertugliflozin in the Spontaneously Hypertensive Rat After 27 Days of Dosing

Measurements	Units	Control	Ertugliflozin
Body Weight	(g)	347 ± 5.7	272 ± 2.8 *
24-h Food	(g)	21 ± 0.4	21
Urinary Glucose Excretion	(mg/24 h)	7 ± 2.9	3636 ± 111.1 *
Plasma Glucose	(mg/dL)	147 ± 21.3	140 ± 11.2
Water Intake	(mL/24 h)	33 ± 1.4	55 ± 1.1 *
Urine Volume	(mL/24 h)	14 ± 1.1	42 ± 1.4 *
Urine Volume/Water Intake	(Uv/H2O x 100)	43 ± 2.3	77 ± 2.8 *
Hematocrit	(%)	49 ± 1	53 ± 1 *
Plasma Renin Activity	(ng Ang I /mL/h)	7 ± 1.1	21 ± 2.9 *
Serum Aldosterone	(pg/mL)	168 ± 64.0	427 ± 87.1 *
Plasma Angiotensinogen	(pmol/mL)	69 ± 3.8	84 ± 5.3 *
Urinary Angiotensinogen	(pmol/24 h)	26 ± 2.1	86 ± 8.8 *
Systolic Blood Pressure	(mmHg)	170 ± 5.0	151 ± 5.0*
Diastolic Blood Pressure	(mmHg)	115 ± 5.0	101 ± 5.0
Mean Blood Pressure	(mmHg)	143 ± 5.0	125 ± 5.0 *
Mean Heart Rate	(beats/minute)	320 ± 6.0	272 ± 5.0 *

Measurements for the treatment period were taken between Day 23 and Day 27; measurements for the washout period were taken between Day 57 and Day 62. Data are mean ± standard error of the mean, *p ≤0.05 vs Control. Groups were compared using two–sided t–test for independent samples, adjusted for variance as determined by Levene's test.
Ang I = angiotensin I; dL = deciliter(s); g = gram(s); h = hour(s); mL = milliliter(s); mg = milligram(s); mmHg = millimeter(s) of mercury; ng = nanogram(s); pg = picogram(s); pmol = picomole(s); Uv = urinary volume; vs = versus.

(Tables excerpted from sponsor's Investigational Brochure)

Effects of PF-0497129 on Glucose, Fluid and Electrolyte Balance in Sprague-Dawley Rats, 19-Nov-2010 (Study #PD002 / Pfizer #121450)

Key Study Findings

- Drug-related increases in glucose excretion
  - ↑urinary glucose, ↓plasma glucose
  - ↑water intake, ↑urine volume, ↑urine volume to water ratio
- ↓Body weight under controlled food intake conditions
- Plasma: ↑plasma renin activity, ↑aldosterone, ↑angiotensinogen
- Urine: ↑phosphorous, ↑potassium

METHODS

Sprague Dawley (SD) rats were evaluated in 2 subsequent study cohorts, one with ad libitum access to food and normal water and the second pair-fed cohort with ad libitum access to 1% deuterated water and with treated animals receiving amounts of food

matching vehicle controls. Animals were administered a 30 mg/kg/day dose of PF-04971729 or vehicle (10% polyethylene glycol 400 in 0.5% methylcellulose) orally for 8 days. On dosing Day 5, fasted blood samples were collected. On Day 7, a urine collection was initiated 2 hours post dose. On Day 8, final urine samples were collected at 2 and 24 hours post-dose. Animals were decapitated on Day 8 and kidneys and blood were collected. Blood and urine samples were analyzed for angiotensinogen, aldosterone, glucose and electrolytes. Estimated glomerular filtration rates (eGFR) were calculated based on urinary creatinine and plasma creatinine ratios. Total RNA was also isolated from kidney tissue and reverse transcribed into cDNA for quantitative PCR of gene expression of SGLT1, SGLT2, SGLT4, SGLT5, SGLT6, PEPCK, beta actin (ACTB), and ribosomal protein L19 (RPL19).

RESULTS

In the first cohort with animals being fed normal water and ad-libitum food, there were significant increases in daily food intake, but no changes in body weight, in animals being treated with study drug. Increases in urinary glucose were also observed in the treated animals, which were likely related to compensatory increases in food intake. However, the increase in food consumption was recognized as a confounding factor to interpret drug-related effects on glucose, electrolyte and fluid balances. Thus, the sponsor initiated the second phase of the study evaluating effects between vehicle control and a pair-fed treatment cohort.

In pair-fed rats treated with PF-04971729, a significant increase in 24-hour urinary glucose excretion (↑180-fold) correlated with a decrease in plasma glucose levels (↓14%) and decreased body weight. Drug-related increases in water intake (↑50%), urine volume (↑2-fold), urine volume to water intake ratio (↑19%), and hematocrit (↑3%) were also observed, consistent with diuresis and volume depletion. There were no drug-related changes on plasma electrolyte (sodium, potassium, magnesium, calcium, and phosphorous) levels, urinary electrolyte (sodium and calcium) levels, or eGFR. Increases in plasma renin activity (↑71%), aldosterone (62%) and angiotensinogen (↑36%) were also observed, but urinary angiotensinogen levels were similar to controls. Drug-related increases in urinary excretion of phosphorous (↑73%) and potassium (↑20%) correlated with drug-related diuresis.

Sponsor's Table 5: Summary of the Effects of PF-04971729 in the Pair-fed Rats

		Vehicle	PF-04971729
24-hr Food	(g)	27 ± 1.0	27
Body Wt.	(g)	345 ± 5.8	315 ± 6.1 *
Urinary Glucose	(mg/24 h)	20 ± 6.2	3612 ± 211.8 *
Plasma Glucose	(mg/dL)	160 ± 5.1	137 ± 8.0 *
Urinary Volume	(mL/24 h)	17 ± 1.6	40 ± 2.7 *
Water Intake	(mL/24 h)	44 ± 3.0	67 ± 3.0 *
UV/H2O	(Uv/H2O x 100)	40 ± 1.3	59 ± 3.5 *
Hematocrit	(%)	45 ± 0.4	48 ± 0.8 *
Urinary Creatinine	(mg/24 h)	13 ± 0.3	13 ± 0.5
Plasma Creatinine	(mg/mL)	0.0023 ± .0002	0.0021 ± 0.0001
eGFR	(mL/min/g Body Weight)	0.0122 ± 0.0009	0.0140 ± 0.0010
Plasma Calcium	(mg/dL)	10 ± 0.1	10 ± 0.1
Plasma Sodium	(mmol/L)	135 ± 0.8	139 ± 0.7
Plasma Magnesium	(mg/dL)	2 ± 0.1	2.2 ± 0.1
Plasma Phosphorus	(mg/dL)	9 ± 0.3	9 ± 0.2
Plasma Potassium	(mmol/L)	7.1 ± 0.3	6.4 ± 0.3
Urinary Calcium	(mg/24h)	2 ± 0.4	3 ± 0.9
Urinary Sodium	(mmol/24h)	2 ± 0.1	3 ± 0.2
Urinary Magnesium	(mg/24h)	4 ± 0.9	6 ± 1.7
Urinary Phosphorus	(mg/24h)	11 ± 1.3	19.0 ± 1.8*
Urinary Potassium	(mmol/24h)	5 ± 0.1	6 ± 0.3*
Plasma Renin Activity	(ng/mL/h)	7.0 ± 2.9	12 ± 2.4*
Serum Aldosterone	(pg/mL)	84 ± 19..0	223 ± 69.1*
Plasma Angiotensinogen	(pmol/mL)	58 ± 2.0	94 ± 3.8*
Urinary Angiotensinogen	(pmol)	5 ± 0.6	10 ± 1*
Fractional Gluconeogenesis	%	49 ± 2	52 ± 2
SGLT1		3108 ± 198.4	3747 ± 290.6
SGLT2		26473 ± 3373.9	22086 ± 4453.8
SGLT4		6155 ± 1058.4	3733 ± 342.9
SGLT5		8073 ± 706.4	10378 ± 924.7
SGLT6		3619 ±192.7	4206 ± 387.2
iPTH	(pg/mL)	456 ± 45.8	415 ± 36.7

dL=deciliter, g=grams, h=hours, mg=milligram,mL=milliliter ng=nanogram, pg=pictogram, pmol=picomole, SGLT(1,2,4,5,6)=sodium-glucose transporter type(1,2,4,5,6), Uv=urinary volume, vs=versus.
(mean ± standard error of the mean, *p ≤ 0.05 vs Vehicle).

(Table excerpted from sponsor's package)

There were no changes in deuterium-labeled glucose, indicating that there were no drug-related effects on hepatic glucose production, gluconeogenesis.

Changes in transcription of examined SGLT isoforms did not reach 50% compared to vehicle controls; thus, there were considered to be no significant transcriptional changes in any of the genes examined in the kidney renal cortex. The sponsor considered the gene expression data to be inconclusive.

4.2 Secondary Pharmacology

Secondary pharmacology of ertugliflozin has been evaluated using *in vitro* binding assays against a broad panel of receptors, transporters, ion channels, and enzyme assays. There were no indications of significant (>50%) inhibition of binding or enzyme activity in any of the targets examined. The ability of ertugliflozin to bind and/or activate estrogen receptors (ERs) has not been investigated.

Ertugliflozin does not exhibit reversible or time-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A activities in human liver microsomes *in vitro* and IC_{50} values for CYP inhibition are restricted to >30 μM. Ertugliflozin was associated with a mild induction (↑2 to 5-fold) of CYP3A4 mRNA expression at high concentrations (>50μM), but did not significantly induce CYP1A2. Overall, drug-drug interactions with drugs metabolized by the CYP enzymes are not anticipated at clinical exposures.

Ertugliflozin is a weak substrate of rat organic anion and organic cation transporters, rOAT3 and rOCT2, but is not a substrate of human organic anion/cation transporters hOAT1, hOAT3 and hOCT2. Ertugliflozin very weakly inhibits hOCT2-mediated uptake of metformin *in vitro* with an IC_{50} value of 917 μM, which is nearly 5000-fold higher than clinical exposure concentrations of free drug. Ertugliflozin also weakly inhibits OAT3 transport activity with an IC_{50} value of 70 μM, which is 24-fold higher than clinical concentrations of total drug and nearly 400-fold higher than free drug concentrations. The IC_{50} values for Ertugliflozin-mediated inhibition of human organic anion transporting polypeptides hOATP1B1 (IC_{50} = 35.4 μM) and hOATP1B3 (IC_{50} = 140.7 μM) are 12 to 48-fold higher than total drug and 200 to 800-fold higher than free drug concentrations at the highest clinical exposures. Overall, drug-drug interactions with drugs transported by OATs, OCTs and transporting polypeptides are not likely at clinical exposures.

4.3 Safety Pharmacology

Brief Safety Pharmacology Summary
The sponsor's safety pharmacology profile is complete and no new safety pharmacology studies are anticipated.

Core battery

Central Nervous System: Male rats dosed with 500 mg/kg of PF-04971729 (b) (4) had a 0.4°C decrease in average body temperature. At 500 mg/kg, PF-04971729 produced decreases in locomotor activity measurements (~30-40%).

Cardiovascular System: PF-04971729 inhibited the hERG channel *in vitro* with an IC_{50} of >300 μM (129 μg/mL) and Nav1.5 currents with an IC_{50} of 188 μM. Although

significant inhibition of hERG and Nav1.5 currents were reported at doses ≥ 30 μM, clinical C_{max} exposures are not expected above 3 μM. Therefore, inhibition is not anticipated at biologically relevant exposure levels. It is noted that 50 mg/kg PF-04971729 (b) (4) in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and heart rate (and associated RR interval shortening), as well as an increase in systolic blood pressure and lengthening of the PR interval, with a NOAEL of 5 mg/kg and a safety margin of 40-fold based on C_{max} of the therapeutic 15 mg/day therapeutic dose. The dog *LOAEL* of 50 mg/kg (AUC_{0-24} = 530 μg·h/mL, C_{max} = 44.7 μg/mL = 80 μM) was observed at exposures 20-fold above the MRHD C_{max} of the clinical QTC study.

Table 2: Cardiovascular Safety Margins

Ertugliflozin	LOAEL	LOAEL MRHD*	NOAEL	NOAEL MRHD*
Total	**50 mg/kg** *C_{max}: 44.7 μg/mL, 80 μM*	28x	**5 mg/kg** *C_{max}: 3.9 μg/mL, 6.8 μM*	**2x**
Unbound	**50 mg/kg** *C_{max}: 1.43 μg/mL, 2.5 μM*	14x	**5 mg/kg** *C_{max}: 0.12 μg/mL, 0.22 μM*	**1x**

* Based on single clinical dose of 100 mg/day: C_{max} = 1620 ng/mL = 2.86 μM

Respiratory System: Dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed in rats at doses ≥25 mg/kg lasting for up to 120 minutes post-dose.

Supplemental

Renal/Urinary System: No renal safety studies were performed although PF-04971729 causes increased urinary glucose excretion and kidney alterations in rats and dogs.

Gastrointestinal System: No GI safety studies were performed although PF-04971729 causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs.

Abuse Liability: Not determined

Effect of PF-04971729 on Nav1.5 Sodium Current Stable Expressed in HEK293 Cells, 27-Sep-2010 (Study #TT097885 / Pfizer #PF04971729NA15)

Key Study Findings

- PF-04971729 inhibits Nav1.5 currents
  - IC_{50} = 188 μM

METHODS

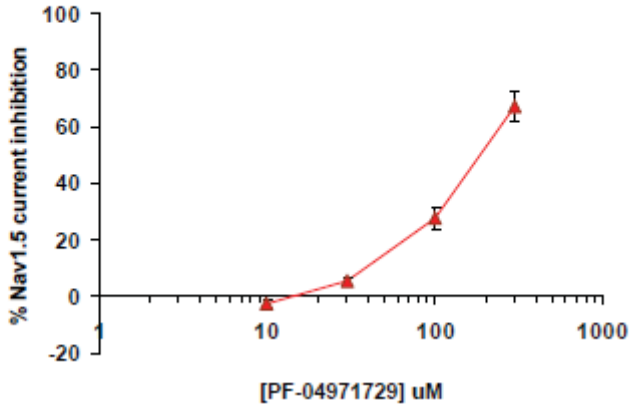
Human embryonic kidney (HEK293) cells stably transfected with the human Nav1.5 gene were suspended in saline and Nav1.5 current were recorded using the whole-cell planar patch clamp technique using a PatchXpress 7000A. The Nav1.5 current was tested in each cell at 1.5 to 3 minute intervals in the presence of 10, 30, 100 and 300 μM PF-04971729 interspersed with exposure to 30 μM amitriptyline to inhibit any remaining current in between PF-04971729 test concentrations. Nav1.5 currents in the presence of the positive control amitriptyline (0.3, 1 and 3 μM) were recorded in a separate experiment.

RESULTS

PF-04971729 inhibited the Nav1.5 current in a concentration-dependent manner with an IC_{50} of 188 μM. Although inhibition reached statistical significance at ≥30 μM, the inhibition at 30 μM was only 20% in magnitude and was considered to be minimal for this assay. Since C_{max} concentrations at the high dose of 100 mg/day in the clinical QTC study are not expected to reach levels higher than 3 μM, PF-04971729-mediated inhibition of Nav1.5 currents are not anticipated at clinical exposures.

Sponsor's Figure 1: PF-04971729 Inhibition of Nav1.5 Current

Figure 1. Concentration-response curve. Data are expressed as the mean ± SEM.



(Figure excerpted from sponsor's package)

Effects of PF-04971729 on Blood Pressure in Spontaneously Hypertensive Rats, 19-Nov-2010 (Study #PD001 / Pfizer #070904)

Key Study Findings

- ↓Blood pressures (mean and systolic) and ↓heart rate at 36 mg/kg/day
  - 100x MRHD, based on clinical dose of 15 mg/day and extrapolated exposures from the 6-month rat toxicology study
  - 14x MRHD, based on clinical QTC study dose of 100 mg/day
- Activation of the renin-angiotensin-aldosterone system (RAAS)

  - ↑plasma renin activity, ↑serum aldosterone, ↑plasma angiotensinogen, and ↑urinary angiotensinogen
- Glucose excursion
  - ↑urinary glucose
- Diuresis
  - ↑water intake, ↑urine volume, ↑urine volume/water intake ratio, and ↑hematocrit

METHODS

Blood pressure parameters were examined in male spontaneous hypertensive rats (SHR) with blood pressure transducers (TA11PA-C40, OpenART telemetry, Data Science International) surgically implanted in the abdominal aorta. Animals were treated for 27 days with 36 mg/kg/day PF-04971729, which was formulated as an admixture in standard rat chow (0.5 mg PF-04971729/g chow), and pair-fed to match food consumption of vehicle controls. On treatment Day 23, blood samples were harvested from the tail vein. On Day 26, 24-hour urine samples were collected and water intake was measured. Prior to dosing and on Day 27, diastolic (dBP) and systolic (sBP) blood pressures were measured over a 24-hour period and mean blood pressure (mBP) and heart rate were calculated from the arterial pressure and cardiac cycle, respectively. Raw data were averaged over 1 minute periods, which were then averaged over 60 minute periods with the exclusion of 1 minute data points where pulse pressure exceeded 100 mmHg, which are assumed to be posturally-induced artifacts associated with eating and grooming. The 60 minute averages were then used to determine 24 hour means.

Animals were then exposed to a 30-day wash-out period with ad libitum access to food and body weights were measured weekly. After the wash-out period, animals were reallocated based on body weight and systolic blood pressure into 2 groups, vehicle control (10% PEG400 and 0.5% methylcellulose) and 40 mg/kg hydrochlorothiazide with approximately equal numbers of animals previously treated with vehicle or PF-04971729. Animals were treated for 24 days via oral gavage and were pair-fed to match food consumption of vehicle controls. Prior to dosing and on Day 19, blood pressures were determined over a 24-hour period, as described above. On Day 22, 24-hour urine samples were collected and water intake was measured. On treatment Day 24, blood samples were harvested from the tail vein.

Plasma and urine samples were analyzed for angiotensinogen, aldosterone, glucose, and electrolytes. Renin activity was also determined in plasma samples.

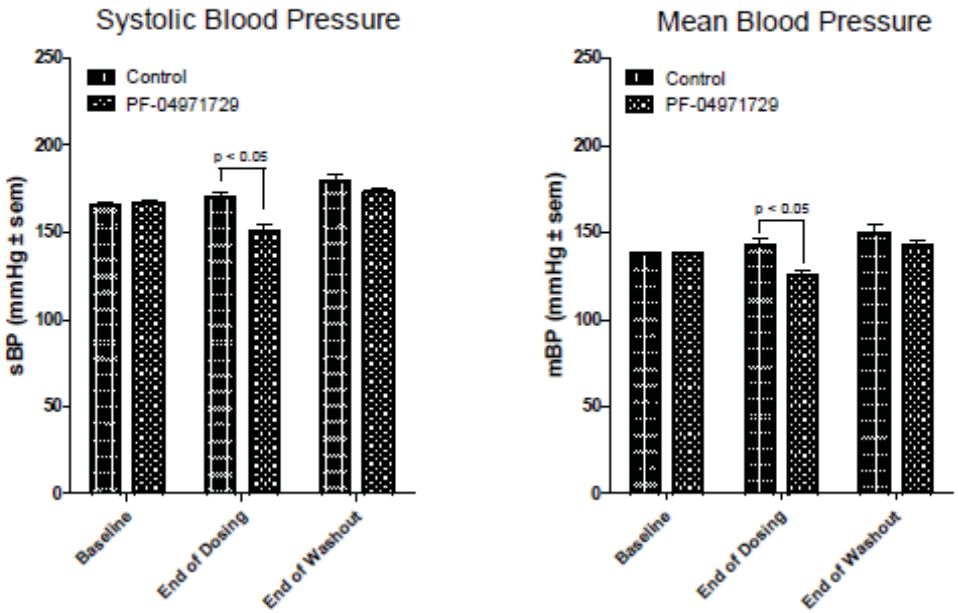
RESULTS

PF-04971729 administered in the chow at 36 mg/kg/day reached plasma exposure levels of 2.8 μg/mL at 2 hours after feeding. It is noted that mean body weight for PF-04971729-treated animals were 22% lower than pair-fed vehicle controls and may complicate translating the findings from the SHR rat model to humans.

Significant decreases in systolic (↓11%) and mean (↓12%) blood pressures, as well as heart rate (↓15%), were observed in SHR rats treated with PF-04971729, which returned to levels comparable to controls after the wash-out period. After treatment with the diuretic and anti-hypertensive agent hydrochlorothiazide, a statistically significant decrease in systolic blood pressure (↓5%) was also reported; however, there were no statistically significant differences from controls in dBP, mBP or mean heart rate. Thus, in SHR rats, greater effects on blood pressure lowering were observed with 36 mg/kg/day PF-04971729 administration than with 40 mg/kg hydrochlorothiazide.

Sponsor's Figure 2: Blood Pressure - PF-04971729 in SHR Rats

Figure 1. PF-04971729 Treatment Caused Decreases in Systolic and Mean Blood Pressure



(Figure excerpted from sponsor's package)

Significant, drug-related increases in urinary glucose excretion were over 500-fold higher than pair-fed controls; however, there were no significant differences in plasma glucose levels. Significant increases in water intake (↑67%), urine volume (↑3-fold), urine volume/water intake ratio (↑79%), hematocrit (8%), plasma renin activity (↑3-fold), serum aldosterone (↑2.5-fold), plasma angiotensinogen (↑22%), and urinary angiotensinogen (↑3.3-fold) were also observed in SHR rats treated with PF-04971729. These changes are consistent with a significant diuretic effect. These data also indicate activation of the renin-angiotensin-aldosterone system with SGLT2 inhibition by PF-04971729. Smaller, yet similar, changes were observed with treatment with 40 mg/kg hydrochlorothiazide, which resulted in increased urine volume (↑33%), urine volume/water intake ration (↑32%), hematocrit (↑3%), plasma renin activity (↑50%), plasma aldosterone (↑88%), plasma angiotensinogen (↑24%), and urinary angiotensinogen (↑96%), but did not result in increased water intake or decreased body weight.

Sponsor's Table 6: PF-04971729 in SHR Rats

Table 1. Summary of the Results from PF-04971729 Study in the Spontaneously Hypertensive Rat

		Treatment		Washout	
		Control	PF-04971729	Control	PF-04971729
Body Weight	(g)	347 ± 5.7	272 ± 2.8 *	378 ± 4.2	370 ± 3.2
24-hr Food	(g)	21 ± 0.4	21	21 ± 0.4	23 ± 0.7
Urinary Glucose	(mg/24 h)	7 ± 2.9	3636 ± 111.1 *	5 ± 3.0	3 ± 0.2
Plasma Glucose	(mg/dL)	147 ± 21.3	140 ± 11.2	151 ± 16.7	145 ± 17.4
Water Intake	(mL/24 h)	33 ± 1.4	55 ± 1.1 *	30 ± 0.4	31 ± 0.4
Urine Volume	(mL/24 h)	14 ± 1.1	42 ± 1.4 *	14 ± 0.4	15 ± 0.4
Urine Volume/Water Intake	(UV/H2O x 100)	43 ± 2.3	77 ± 2.8 *	47 ± 1.1	48 ± 1.1
Hematocrit	(%)	49 ± 1	53 ± 1 *	48 ± 1	48 ± 1
Plasma Renin Activity	(ng/mL/h)	7 ± 1.1	21 ± 2.9 *	7 ± 0.5	7 ± 0.6
Serum Aldosterone	(pg/mL)	168 ± 64.0	427 ± 87.1 *	96 ± 10.7	90 ± 8.1
Plasma Angiotensinogen	(pmol/mL)	69 ± 3.8	84 ± 5.3 *	66 ± 1.6	68 ± 1.4
Urinary Angiotensinogen	(pmol/24 h)	26 ± 2.1	86 ± 8.8 *	26 ± 1.2	27 ± 0.7
Systolic Blood Pressure	(mmHg)	170 ± 5.0	151 ± 5.0*	179 ± 6.4	173 ± 2.8
Diastolic Blood Pressure	(mmHg)	115 ± 5.0	101 ± 5.0	123 ± 6.0	114 ± 2.5
Mean Blood Pressure	(mmHg)	143 ± 5.0	125 ± 5.0 *	150 ± 6.4	143 ± 2.5
Mean Heart Rate	(beats/minute)	320 ± 6.0	272 ± 5.0 *	305 ± 7.4	294 ± 3.2

Measurements for the treatment period were taken between Day 23 and Day 27; measurements for the washout period were taken between Day 57 and Day 62.
dL=deciliter, g=grams, h=hours, mg=milligram, nmHg=millimeter(s) of mercury, ng=nanogram, pg=pictogram, pmol=picomole, Uv=urinaty volume, vs=versus.
(mean ± standard error of the mean, *p≤0.05 vs Control)

Sponsor's Table 7: Hydrochlorothiazide in SHR Rats

Table 2. Summary of the Results from Hydrochlorothiazide Study in the Spontaneously Hypertensive Rat

		Treatment	
		Vehicle	Hydrochlorothiazide
Body Weight	(g)	380 ± 8.9	354 ± 8.0
Food Intake	(g)	22 ± 0.5	22
Urinary Glucose	(mg/24 h)	8 ± 1.0	10 ± 0.9
Plasma Glucose	(mg/dL)	154 ± 6.1	154 ± 4.5
Water Intake	(mL/24 h)	31 ± 1.6	34 ± 2.4
Urine Volume	(mL/24 h)	15 ± 1.0	20 ± 1.0 *
Urine Volume/Water Intake	(Uv/H2O x 100)	47 ± 1.6	62 ± 4.4 *
Hematocrit	(%)	48 ± 0.4	51 ± 0.7 *
Plasma Renin Activity	(ng/mL/h)	8 ± 1.1	12 ± 0.9 *
Plasma Aldosterone	(pg/mL)	126 ± 9.2	237 ± 17.1 *
Plasma Angiotensinogen	(pmol/mL)	67 ± 2.4	83 ± 2.9 *
Urinary Angiotensinogen	(pmol)	28 ± 0.9	55 ± 2.6 *
Systolic Blood Pressure	(mmHg)	182 ± 3.2	173 ± 2.1 *
Diastolic Blood Pressure	(mmHg)	124 ± 2.8	119 ± 3.2
Mean Blood Pressure	(mmHg)	151 ± 3.2	145 ± 2.8
Mean Heart Rate	(beats/minute)	301 ± 9.5	295 ± 7.4

Measurements for the treatment period were taken between Day 19 and Day 24.
dL=deciliter, g=grams, h=hours, mg=milligram, nmHg=millimeter(s) of mercury, ng=nanogram, pg=pictogram, pmol=picomole, Uv=urinaty volume, vs=versus.
(mean ± standard error of the mean, *p≤0.05 vs. Vehicle)

(Tables excerpted from sponsor's package)

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Brief Pharmacokinetics/ADME/Toxicokinetics Summary
The sponsor's nonclinical PK/ADME/TK profile is complete and no further studies are anticipated.

Absorption

- Moderate permeability across Caco-2 cell monolayers
- Ertugliflozin may be a substrate for P-glycoprotein (P-gp)-mediated efflux, but is not affected by P-gp inhibitors. Thus P-gp is unlikely to be a limiting factor in Ertugliflozin absorption.
- Oral absorption of 50% in humans, 78% in rats, and 94% in dogs, indicating significant species differences in absorption
- Oral bioavailability of the co-crystal form is moderate in rats (69%) and mice (75-87%), but relatively high in dogs (94%)
  - Bioavailability of the amorphous form was similar in rats (67%) and dogs (97%), and there are no significant differences in systemic exposure of the 2 forms of ertugliflozin

- Rapid absorption with T_{max} achieved within 30 minutes in mice and 1 hour in humans (fasted)
  - T_{max} is achieved after 2 hours in humans in the fed state, indicating absorption delays in the presence of food.

Distribution

- Moderate volume of distribution in rats
  - C_{max} is achieved in most tissues between 1 and 2 hours post-dose
    - Complete elimination by 168 hours postdose
  - Highest distribution to bladder, liver, kidney, adrenal gland, Harderian gland, and pancreas
  - Ertugliflozin crosses the blood-brain barrier, reaching concentrations 3 to 63-fold lower than that of blood.
    - Eliminated from all brain substructures by 24 hours postdose
  - Distribution to the choroid plexus and pituitary gland is 2-fold greater than blood.

Protein Binding

Ertugliflozin protein binding is high in all species examined (human, dog, rat, and mouse) ranging from 94 to 97%, with no apparent concentration dependence across 1 to 10 μg/mL (2-23 μM). Mean unbound fractions in CD-1 mice (4.5% unbound, 95.5% bound) were slightly lower than that of humans (6.4% unbound, 93.6% bound). Overall, mean unbound fractions were higher in humans than mouse, rat, and dog, but lower than in rabbit. The sponsor proposes that the toxicological effects are more closely related to the unbound fraction in plasma rather than the total plasma concentration. Thus, the species differences in the unbound plasma fraction of ertugliflozin were incorporated into the sponsor's safety margin calculations. However, since the percent (%) plasma protein binding across species ranged from 94% to 98%, which is not considered to be a meaningful difference in protein binding, safety margins for Pharm/Tox reviews have been based on total, not free, drug levels.

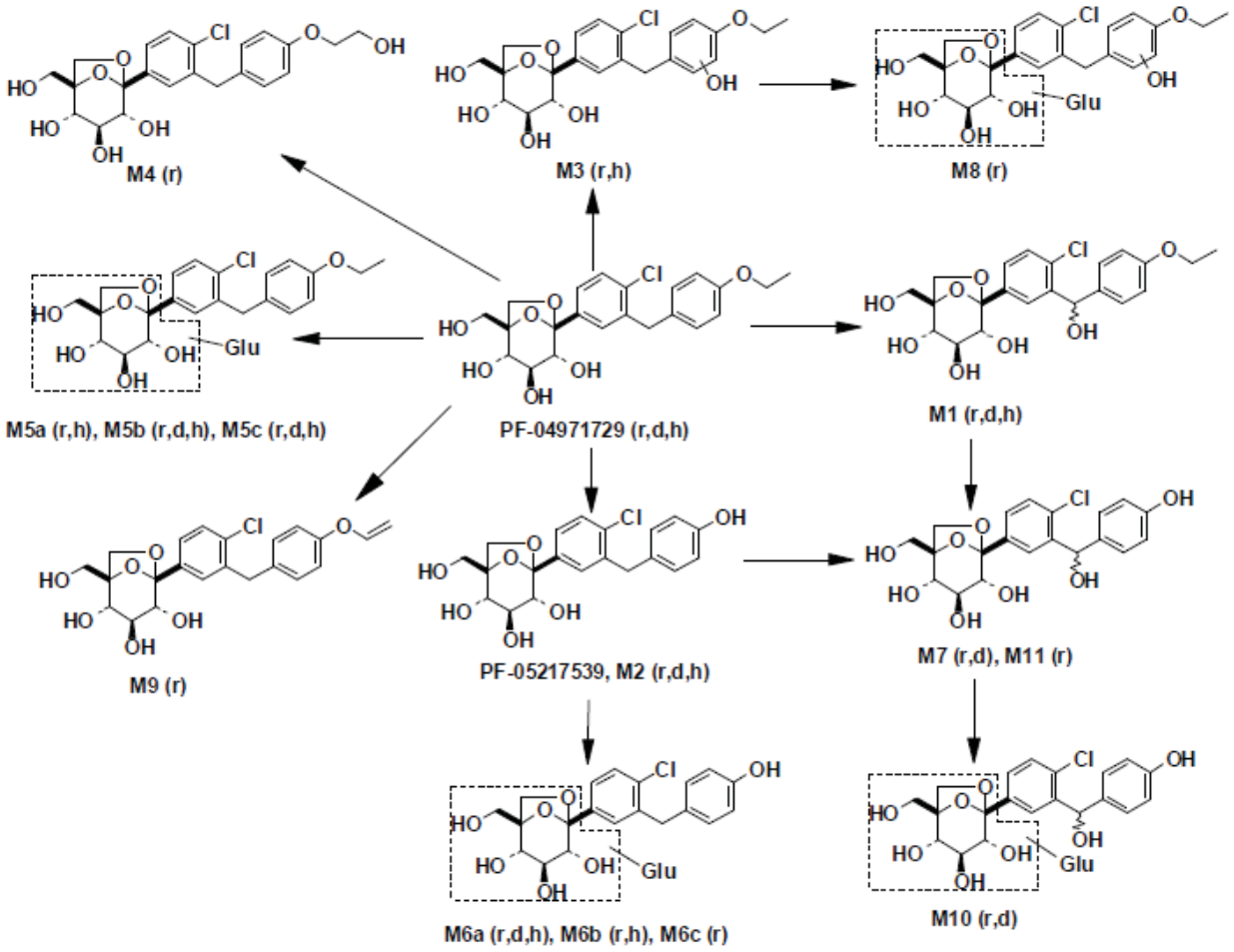
Metabolism

- Ertugliflozin metabolism is catalyzed by CYP3A4, CYP3A5, CYP2D6, UGT1A9, and UGT2B7.
- Similar metabolic profiles in rat, dog and human species
  - No unique human metabolites
- Humans: Glucuronidation is the major metabolic pathway
  - O-glucuronides M5a (12.2%) and M5c (24.1%) are present at >10% of the parent compound in humans.
    - M5a and M5c are O-glucuronides of the parent molecule and glutathione conjugates are normally of low toxicological concern
    - M5c and M5a exposure multiples of 7 to 29-fold in rats dosed at 250 mg/kg
    - M5a and M5c exposures are likely to reach clinical exposure levels in the 2 year rat carcinogenicity study.

        - M5a, M5b (4.1%), M5c and M6a (6%) glucuronides = 46.6% of circulating radioactivity
    - Oxidative metabolites M1 and M2 are limited to 2.5% and 1.3%, respectively.
- CD-1 Mice: Glucuronidation and oxidation are the major metabolic pathways *in vivo* (Study #8286028).
    - After a single dose, the major metabolite in mice is M5c
        - Metabolites M5a and M5c only represent 0.5% of the total drug exposure each in rats.
    - After 3 months of dosing, the major metabolite is M1
    - After both acute and subchronic administration, the major metabolites include M1, M5c, and the O-desethyl glucuronide M6a/b.

Although the O-glucuronide metabolites M5a and M5c are present at levels >10% of the parent compound in humans, they are unlikely to be of significant toxicological concern. Furthermore, they are considered to be qualified in the rat toxicology studies and are anticipated to be qualified in the 2-year rat carcinogenicity study. Thus, the characterization of these metabolites, along with the additional less prominent ertugliflozin metabolites, is considered to be complete and no additional studies are required to support the safety of the metabolites.

Sponsor's Figure 3: Proposed Ertugliflozin Metabolic Pathways



Note: PF-04971729 = ertugliflozin.

(Figure excerpted from sponsor's Investigator's Brochure)

Elimination

- Moderate half-life of 4 to 8 hours in rats and dogs
  - Low systemic clearance in rats
- Long half-life in humans of 12 to 16 hours
- Predominant elimination is via metabolism
- Primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans

5.2 Toxicokinetics

The sponsor did not recognize any gender effects on systemic exposure, dose-exposure relationships, or time-dependent changes in rat or dog systemic exposures. However, trends for slightly increased exposures were repeatedly observed in female rats (see Dr. Quinn's Pharm/Tox reviews) and mice (see Dr. Hawes's Pharm/Tox review

#6). Systemic exposures increased in a dose-dependent manner, indicating linear pharmacokinetics in all 3 species. Although trends for slight increases in exposure over time were present at high doses, there were no consistent indications of accumulation over time in combined male and female exposures of rats and dogs. However, slight accumulation was observed in female mice, but not in males, and may indicate a gender effect. T_{max} was variable and often delayed in both rats (up to 6 hours) and dogs (up to 3 hours) at higher doses, but remained at 0.5 hours in mice at doses up to 100 mg/kg/day. In humans, T_{max} is achieved 1 hour after single dose administration (Sponsor's Table 2) and between 1.5 and 2 hours after multiple doses (Sponsor's Table 3). The half-life is 1.5 to 4 times longer in humans ranging from 12 to 16 hours, as compared to rats and dogs ($T_{1/2}$ = 4 to 8 hours).

Sponsor's Table 8: Rat Toxicokinetic Summary

Species	Dose (mg/kg/day)	Study Day or Week	Cmax (µg/mL)	Tmax (h)	AUC24 (µg·h/mL)
Rats [a,b] (1-month toxicity study)	5	Day 1	1.53	0.50	16.2
		Day 28	1.94	0.50	11.6
	25	Day 1	8.80	4.00	106
		Day 28	10.9	0.50	81.2
	500/250 [c]	Day 1	74.3	4.00	1460
		Day 28	44.4	4.00	611
Rats [b] (3-month toxicity study)	5	Day 1	2.17 ± 0.58	1.00 ± 0.00	16.9 ± 4.76
		Day 91	2.57 ± 0.79	1.00 ± 0.00	19.9 ± 5.28
	25	Day 1	8.25 ± 2.03	2.50 ± 1.60	94.1 ± 19.6
		Day 91	8.11 ± 1.93	2.13 ± 1.55	89.4 ± 17.1
	250	Day 1	74.2 ± 14.9	3.63 ± 2.50	943 ± 182
		Day 91	51.2 ± 9.50	4.38 ± 2.50	738 ± 170
Rats [b] (6-month toxicity study)	5	Week 1	1.86 ± 0.41	2.88 ± 1.55	23.2 ± 5.61
		Week 26	3.06 ± 0.78	1.38 ± 1.06	22.3 ± 6.46
	25	Week 1	10.0 ± 1.59	5.50 ± 1.60	135 ± 26.2
		Week 26	15.2 ± 5.77	2.50 ± 1.60	148 ± 38.4
	100	Week 1	30.9 ± 7.41	5.88 ± 1.55	400 ± 137
		Week 26	51.3 ± 15.6	1.75 ± 2.12	605 ± 235
Pregnant Rats [d] (Embryo-fetal developmental toxicity study)	50	GD 17	15.4 ± 4.15	2.80 ± 1.64	199 ± 46.2
	100	GD 17	31.9 ± 8.03	4.00 ± 2.12	457 ± 103
	250	GD 17	66.7 ± 11.9	5.20 ± 2.68	975 ± 173

Sponsor's Table 9: Dog Toxicokinetic Summary

Species	Dose (mg/kg/day)	Study Day or Week	Cmax (µg/mL)	Tmax (h)	AUC24 (µg·h/mL)
Pregnant Rabbits [d] (Embryo-fetal developmental toxicity study)	50	GD 19	22.5 ± 5.91	2.40 ± 0.894	207 ± 51.6
	100	GD 19	46.0 ± 7.67	2.40 ± 0.894	424 ± 77.6
	500	GD 19	115 ± 14.9	4.00 ± 0.00	1150 ± 177
Dogs [b] (1-month toxicity study)	1	Day 1	1.04 ± 0.12	0.75 ± 0.27	6.34 ± 0.47
		Day 28	1.09 ± 0.13	0.92 ± 0.59	7.35 ± 1.94
	10	Day 1	10.1 ± 1.43	0.83 ± 0.26	62.4 ± 10.9
		Day 28	11.1 ± 1.00	0.92 ± 0.59	74.4 ± 10.6
	150	Day 1	49.0 ± 21.9	2.17 ± 1.57	489 ± 326
		Day 28	99.6 ± 12.1	2.00 ± 0.00	1080 ± 146
Dogs [b] (3-month toxicity study)	1	Day 1	0.95 ± 0.16	0.81 ± 0.26	7.49 ± 1.01
		Day 90	1.23 ± 0.21	0.69 ± 0.26	9.81 ± 1.98
	10	Day 1	10.1 ± 1.26	0.81 ± 0.26	74.3 ± 12.6
		Day 90	12.7 ± 2.74	0.94 ± 0.18	91.9 ± 24.8
	150	Day 1	65.3 ± 22.9	2.75 ± 1.04	910 ± 344
		Day 90	87.0 ± 21.4	2.50 ± 0.93	1100 ± 232
Dogs [b] (9-month toxicity study)	1	Week 1	0.791 ± 0.141	0.938 ± 0.496	6.77 ± 1.72
		Week 39	0.796 ± 0.321	1.31 ± 1.19	6.30 ± 1.26
	10	Week 1	9.46 ± 1.35	0.875 ± 0.518	77.1 ± 4.88
		Week 39	7.72 ± 2.45	1.00 ± 0.463	70.5 ± 16.3
	150	Week 1	54.6 ± 11.9	2.25 ± 0.866	693 ± 165
		Week 39	86.4 ± 30.7	1.95 ± 0.896	906 ± 351

a. Non-serial blood sampling from 3 animals/gender/time point.
b. Data are represented as the combined values for males and females.
c. On Day 11, the high dose was reduced to 250 mg/kg/day.
d. Data from female animals.
AUC_{24} = Area under the concentration-time curve from time zero to 24 hours postdose; C_{max} = Peak concentration; GD = Gestation day; SD = Standard deviation; T_{max} = Time to reach peak concentration.

(Tables excerpted from sponsor's Investigator's Brochure)

Systemic exposures after administration of the co-crystal form, which is used for clinical dosing, were comparable to or slightly higher than systemic exposures after administration of the amorphous form in dogs (Table 3). Overall, systemic exposures of the co-crystal form were generally higher in dogs than in rats at similar doses.

Table 3: Amorphous &. Co-crystal Formulation Toxicokinetics in Rats and Dogs

		Dog (SD) (Amorphous)			Rat (7 Day) (Amorphous)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	5	50	500	5	50	500
	MRHD@	33X	358X	254X	14X	83X	1250X
Parameter	Unit						
C_{MAX}	µg/mL	5	46	25	1	8	90
AUC_{0-24}@	µg.hr/mL	39	430	302	17	99	1500
Shaded = MTD							

		Dog (7 Day) (Amorphous)			Dog (7 Day) (Co-crystal)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	5	50	250/150	5	50	150
	MRHD@	39X	329X	724X	44X	408X	300X
Parameter	Unit						
C_{MAX}	µg/mL	7	47	61	6	42	36
AUC_{0-24}@	µg.hr/mL	47	395	869	53	490	360
Shaded = MTD							

		Dog (1 Month) (Co-crystal)			Rat (1 Month) (Co-crystal)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	1	10	150	5	25	500/250
	MRHD@	5X	52X	408X	13X	88X	1217X
Parameter	Unit						
C_{MAX}	µg/mL	1	10	49	2	9	74
AUC_{0-24}@	µg.hr/mL	6	62	489	16	106	1460
Shaded = NOAEL							

		Dog (3 Month) (Co-crystal)			Rat (3 Month) (Co-crystal)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	1	10	150	5	25	250
	MRHD@	6X	62X	758X	14X	78X	786X
Parameter	Unit						
C_{MAX}	µg/mL	1	10	65	2	8	74
AUC_{0-24}@	µg.hr/mL	7	74	910	17	94	943
Shaded = NOAEL							

(Tables excerpted from Dr. Quinn's review #5)

6 General Toxicology

Overall toxicology summary

The sponsor's nonclinical general toxicology profile is considered to be complete and no further studies are anticipated. The pivotal 6-month rodent and 9-month non-rodent toxicology studies were conducted in rats and dogs, respectively, and were previously reviewed (see Dr. Quinn's Pharm/Tox reviews #4 and #5).

Although the pivotal toxicology studies have already been submitted, the sponsor submitted an additional single-dose intravenous injection study in rats and a 28-day repeat-dose study in wild type HRAS transgenic mice, which are evaluated in this review.

6.1 Single-Dose Toxicity

Single-Dose Intravenous Injection Toxicity and Toxicokinetic Study with PF-04971729 in Rats, 13-Mar-2014 (Study #8291746 / 13GR288) - GLP

Doses: 0.1, 1, 10, & 100 mg/kg

Key Study Findings

- NOAEL = 100 mg/kg
  - 100x $MRHD_{Cmax}$
- No significant drug-related findings at all doses

Single Dose Toxicity in Rats	
Species **Doses and Administration** **# animals** **Follow-up**	**NOAEL : 100 mg/kg ≈ 100x $MRHD_{Cmax}$*** *Based on a single dose of 100 mg/day with C_{max} = 1620 ng/mL
Crl:CD(SD) Rats (♂ & ♀) Doses : 0, 0.1, 1, 10 & 100 mg/kg (MHD & HD groups were dosed 4 & 8 days after controls, respectively) Administration: IV injection Vehicle : 5% PEG 400, 21.85% hydroxypropyl beta cyclodextrin, pH 4-4.5 Main: 10/sex/group TK : 9/sex/group Follow-up: 14 Days observation	**Mortality:** One low dose (LD) toxicokinetic (TK) ♂ was found dead on Day 1 of the dosing phase, but was considered unlikely to be drug-related **Body Weight:** Body weights were dose-dependently higher in ♂'s at MHD (↑7.5%) and HD (↑11.8%), as well as in ♀'s at MHD (↑7.7%) and HD (↑14.9%). However, body weights at MHD and HD may have been confounded by the increased age of the animals at the time of dosing by 4 and 8 days, respectively. Thus, it is not entirely clear if this is a drug-related effect.
Clinical Signs: No drug-related findings	

Organ Weights: Increases at HD were observed in the adrenal glands (↑15-27% ♂'s, ↑9-23% ♀'s), epididymis (↑15-27%) and ovaries (↑12-28%).

Pathology: Microscopic analysis was not conducted. Macroscopic findings of discolored kidney (yellow or tan) were reported in 1/10 ♂/s at ≥MHD. Single incidences of thickened stomach and discolored epididymis (dark red) were observed in 1/10 ♂'s at HD. Given the low incidence rates, these findings are unlikely to be drug-related.

Toxicokinetics: Exposures were consistently slightly higher (↑25-75%) in females compared to males; however, it is unclear if there was a significant gender-related effect due to the degree of individual animal variability. Tmax was observed at 5 minutes post-dose for all groups. Cmax exposures increased slightly greater than dose by 10-30%.

Text Table 4.1: Toxicokinetic Parameters from Mean PF-04971729 Plasma Concentrations in Rats after a Single Intravenous Administration of PF-04971729

Dose (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-24} (ng•h/mL)
0.1	Male	147	0.0833	363
	Female	166	0.0833	514
	Overall	157	0.0833	440
1	Male	1590	0.0833	4360
	Female	1760	0.0833	5480
	Overall	1680	0.0833	4930
10	Male	11800	0.250	41200
	Female	17400	0.0833	51600
	Overall	14500	0.0833	46400
100	Male	167000	0.0833	422000
	Female	187000	0.0833	736000
	Overall	177000	0.0833	579000

AUC_{0-24} = Area under the plasma drug concentration-time curve for 0-24 hours; C_{max} = Highest drug concentration observed in plasma; Overall = Combined Male plus Female; T_{max} = Time at which C_{max} was first observed.

6.2 Repeat-Dose Toxicity

Study title: 28-Day Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Wild Type CByB6F1-Tg(HRAS)2Jic Mice, 20-May-2013 (Study #TT127803 / #8275450 / #12GR360) – GLP

Doses: 0, 3, 15 & 100 mg/kg

Study no.: TT127803 / 8275450 / 12GR360

Study report location: eDr

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/29/2012

Duration: 28

Duration Units: days

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: PF-04971829, GR02694, 73.8%

Target Organs: KIDNEY

Key Study Findings

- NOAEL = 100 mg/kg
- Increased body weight gain in females at HD
- Increased food consumption in both sexes at all doses.
- Pharmacodynamic-related increases in plasma sodium and chloride electrolyte levels in females at ≥MD
- Kidney
    - Increased organ weight in males at all doses
    - Cortical tubule dilatation in males at all doses and in females at ≥MD
    - Basophilic tubules in males at HD and in females at all doses
- Higher drug exposures in females (AUC ↑2-fold)

Drug-related increases in electrolytes are consistent with osmotic diuresis and the pharmacodynamic activity of PF-04971729. Renal findings of basophilic tubules are considered to be consistent with a regenerative response and adaptive changes secondary to glucosuria and osmotic diuresis related to the pharmacodynamic activity of PF-04971729. Therefore, these findings were not considered to be adverse. Therefore, the NOAEL was set at the high dose of 100 mg/kg/day based on the absence of adverse findings.

Methods

Doses:	0, 3, 15, and 100 mg/kg
Frequency of dosing:	Once daily
Route of administration:	ORAL GAVAGE
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 10% polyethylene glycol 400
Species:	MOUSE
Strain:	CB6F1-TgN (RasH2)
Dedicated Juvenile Animal Study:	N
Number/Sex/Group:	10
Age:	8-9 weeks
Weight:	♂: 22.0 to 29.6 g ♀: 17.4 to 22.9g
Satellite groups:	Toxicokinetic group, 9/sex for controls, 12/sex/ group for treated animals
Unique study design:	Limited microscopic evaluation
Deviation from study protocol:	Only minor protocol deviations were reported which had no impact on the integrity of the study.

Observations and Results

Mortality

Animals were checked twice daily for mortality.

There were no drug-related deaths.

There were 2 mortalities at HD (1 ♂ and 1 ♀); however, they were secondary to gavage trauma and were not drug related.

Clinical Signs

Animals were checked twice daily for abnormalities and signs of pain or distress. Cageside observations were performed for main study animals once daily during the dosing phase. Detailed observations were performed for all animals once during the predose phase and prior to dosing on Days 1, 8, 15, 22 and 29.

There were no drug-related findings

Body Weights

Body weights were determined for all animals once during the predose phase and prior to dosing on Days 1, 8, 15, 22 and 29.

Body weight gain was significantly increased in females at HD (↑38.5%), but the final mean final body weight was only 2.2% higher than concurrent controls. There were no statistically significant differences in male body weights or weight gains.

MALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 28 (End of Treatment)	0	2.4	-	-
	3	2.4	0	100.7
	15	2.1	87.5 (↓12.5%)	99.3
	100	2.3	95.8 (↓4.2%)	97.1
FEMALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 28 (End of Treatment)	0	2.6	-	-
	3	2.9	111.5 (↑11.5%)	101.8
	15	2.8	107.7 (↑7.7%)	99.1

	100	3.6*	138.5 (↑38.5%)	102.2 (↑2.2%)

* p value > 0.05

Feed Consumption

Weekly food consumption was quantitated for all main study animals, as well as for toxicokinetic animals for the day intervals of 1-8, 8-15, 15-22, and 22-28.

Food consumption was significantly increased (↑13-19%) in all drug-treated groups beginning on Day 8 in both sexes and continuing throughout the remainder of the study.

Ophthalmoscopy

Not determined

ECG

Not determined

Hematology

Plasma was collected from all surviving, non-fasted animals on Day 29 via cardiac puncture.

Hematology

red blood cell (erythrocyte) count
hemoglobin
hematocrit
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
platelet count
white blood cell (leukocyte) count
blood smear
reticulocyte count
mean platelet volume
red blood cell (erythrocyte) distribution width

Statistically significant increases in MCV (↑1.8%) were reported in males at all doses, along with correlating increases in RDW (↑2.5-3.3%) in males at ≥MD; however, these small changes are likely to be within the normal biological range and were not associated with significant reductions in overall red blood cell counts. Overall, these findings were considered to be minor and not toxicologically significant.

Clinical Chemistry

Plasma was collected from all surviving, non-fasted animals on Day 29 via cardiac puncture.

Clinical Chemistry - Tier 1

glucose
urea nitrogen
creatinine
total bilirubin
alanine aminotransferase
gamma glutamyltransferase
aspartate aminotransferase
total protein
albumin
globulin
albumin-to-globulin ratio

Clinical Chemistry - Tier 2

cholesterol
alkaline phosphatase
calcium
inorganic phosphorus
sodium
potassium
chloride

Increases in plasma sodium (↑1.3-1.9%) and chloride (↑1.7%) levels were reported in females at ≥MD and HD, respectively. The observed increases in electrolytes are consistent with osmotic diuresis, which is related to the pharmacodynamic activity of PF-04971729, and are considered likely to be drug-related.

Urinalysis

Not determined

Gross Pathology

Main study animals were necropsied on Day 29. The external features of the carcass; orifices; abdominal, thoracic, and cranial cavities; organs and tissues were examined for macroscopic abnormalities.

No macroscopic findings were reported.

Organ Weights

Organ weights were determined at necropsied for all main study animals. Paired organs were weighed together and designated with a "(2)".

adrenal (2)
brain
epididymis (2)
heart
kidney (2)
liver with drained gallbladder
ovary (2)
prostate
spleen
testis (2)
thymus

Increases in kidney weights (↑10-12%) were similarly observed in males at all doses, but were independent of dose and did not reach statistical significance in females.

KIDNEY ORGAN WEIGHTS

Dose, mg/kg	♂			♀		
	gram	% BW	% Brain	gram	% BW	% Brain
0	0.4061	1.4461	83.4308	0.2950	1.2801	60.4810
3	0.4431	1.5874* (↑9.8%)	92.9356* (↑11.4%)	0.3126	1.3376	63.303
15	0.4501	1.6187* (↑11.9%)	93.4323* (↑12.0%)	0.3017	1.2729	61.3765
100	0.4351	1.5984* (↑10.5%)	89.7466 (↑7.6%)	0.3173 (↑7.6%)	1.3642 (↑6.6%)	63.9601 (↑5.8%)

Statistically significant increases in mean absolute liver weights (↑7-9%) were observed in females at MD and HD. A statistically significant increase in liver weight relative to body weight was also observed in females at HD. However, there were no statistically significant differences in liver weights relative to brain weight and the increases were

not dose-dependent. Given the lack of dose-dependency and the small amount of increase, these changes are unlikely to be biologically significant.

LIVER ORGAN WEIGHTS						
Dose, mg/kg	♂			♀		
	gram	% BW	% Brain	gram	% BW	% Brain
0	1.4586	5.1993	299.8506	1.1170	4.8470	229.6515
3	1.4975	5.3562	314.4348	1.1945* (↑6.9%)	5.1182 (↑5.6%)	242.3311 (↑5.5%)
15	1.4745	5.3018	306.2757	1.1646 (↑4.3%)	4.9160 (↑1.4%)	237.0155 (↑3.2%)
100	1.4968 (↑2.6%)	5.5002 (↑5.8%)	308.7633 (↑3.0%)	1.2141* (↑8.7%)	5.2246* (↑7.8%)	244.8345 (↑6.6%)

Histopathology

At necropsy, the entire left forelimb, including the scapula, was harvested from each animal, the soft tissue was removed, and the limbs were fixed in 10% neutral-buffered formalin (NBF). Eyes, optic nerves, Harderian glands, and testes were preserved in modified Davidson's fixative and stored in NBF. All other tissues were preserved in 10% NBF.

adrenal (2)	lymph node (inguinal)
aorta	mammary gland (females)
brain	muscle (biceps femoris)
cecum	optic nerve (2)[a,c]
cervix	ovary (2)
colon	oviduct (2)
duodenum	pancreas
epididymis (2)	pituitary gland
esophagus	prostate
eye (2)[a]	salivary gland [mandibular (2)]
femur with bone marrow (articular surface of the distal end to include stifle joint)	sciatic nerve
	seminal vesicle
forelimb (left) including scapula and intact shoulder and elbow joints (scheduled euthanasia only)[b]	skin/subcutis
	spinal cord (cervical, thoracic, and lumbar)
gall bladder (drained)	spleen
gut-associated lymphoid tissue (GALT)	sternum with bone marrow
Harderian gland[a]	stomach
heart	testis (2)[a]
ileum	thymus
jejunum	thyroid (2 lobes) with parathyroid
kidney (2)	tongue
larynx[b]	trachea
lesions	ureter
liver	urinary bladder
lung with large bronchi	uterus
lymph node (mesenteric)	vagina

a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
b Collected but not evaluated. The left forelimb will only be collected at scheduled euthanasia.
c Longitudinal and cross sections will be collected, preserved, and examined.

All tissues, except larynx and the left forelimb, from all animals were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). All tissues from control and HD groups were examined microscopically. Kidneys and macroscopic lesions from LD and MD groups were also examined microscopically.

Bone marrow smears were also prepared from the femur of all main study animals, but were not evaluated.

Adequate Battery
Yes

Peer Review
A formal pathology peer review was conducted. However, only tissues from selected animals were examined microscopically by the reviewing pathologist.

Histological Findings
Drug-related kidney findings were reported in both sexes. Incidences of cortical tubule dilatation characterized by minimal dilatation of cortical tubules were observed in males at all doses and in females at ≥MD. Increased incidences of minimal, multifocal, basophilic cortical tubules were reported in 33% of males at HD and in 100% of females at all doses. Basophilic tubules were primarily bilateral and characterized by proximal

tubules lined by enlarged epithelium with basophilic cytoplasm, enlarged nuclei, rare mitotic figures, and rare luminal debris. These findings are consistent with a regenerative response and adaptive changes secondary to glucosuria and osmotic diuresis related to the pharmacodynamic activity of DS-8500a. Therefore, these findings were not considered to be adverse.

Sponsor's Table 10: Kidney Microscopic Findings

Incidence and Severity of Microscopic Findings - Kidney

	PF-04971729							
Sex	Males				Females			
Dose Level mg/kg/day	0	3	15	100	0	3	15	100
No. Examined	10	10	10	9	10	10	10	9
Kidney								
Cortical tubule dilatation								
Not Present	10	9	4	1	10	10	4	2
Minimal	0	1	6	8	0	0	6	7
Basophilic tubule, multifocal/bilateral								
Not Present	9	9	9	6	10	0	0	0
Minimal	1	1	1	3	0	10	10	9

(Table excerpted from sponsor's package)

Toxicokinetics

Blood samples were collected from nonfasted toxicokinetic animals on Day 28 at 0.5, 4, 7, and 24 hours postdose (3/sex/group/time point).

Exposures increased approximately dose-proportionally. However, female AUC exposures were generally 2-fold higher than that of males. Female C_{max} exposures were 40% to 85% higher than males. T_{max} was consistently achieved at 30 minutes postdose in both sexes and at all doses.

Mean Toxicokinetic Parameters for PF-04971729 in Mice on Day 28 after Daily Oral Administration of PF-04971729

Dose (mg/kg)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(0-24)}$ (ng•h/mL)
3	28	Male	948	0.5	3770
		Female	1340	0.5	6670
		Overall	1140	0.5	5230
15	28	Male	4780	0.5	19600
		Female	8850	0.5	40600
		Overall	6820	0.5	30200
100	28	Male	29600	0.5	178000
		Female	41300	0.5	322000
		Overall	35400	0.5	250000

$AUC_{(0-24)}$ = Area under the plasma drug concentration-time curve for 0-24 hours; C_{max} = Highest drug concentration observed in plasma; Overall = Combined male and female; T_{max} = Time at which C_{max} was first observed.

(Table excerpted from sponsor's package)

Dosing Solution Analysis
Dose formulations were analyzed using a validated high-performance liquid chromatography (HPLC) method. Homogeneity of test article formulations used on Day 1 was verified. Sufficient stability has previously been established for 30 days when stored in a refrigerator and protected from light.

All formulations were within ±10% of target concentrations and were homogeneous.

7 Genetic Toxicology

Ertugliflozin is not considered to be genotoxic. Ertugliflozin was negative for genotoxic potential in a standard battery of valid genotoxicity assays, including *in-vitro* microbial reverse mutation (Ames), *in vitro* human lymphocyte cytogenic, and *in-vivo* rat micronucleus assays.

8 Carcinogenicity

A 2-year rat carcinogenicity study is in progress at doses of 1.5, 5, and 15 mg/kg. An original high dose of 10 mg/kg/day was recommended based on AUC ratio (78x to 119x), but was increased (July 2013) to 15 mg/kg after clinical exposures were increased to 15 mg/day. The AUC ratios at 15 mg/kg are 44x and 67x for males and females, respectively.

9 Reproductive and Developmental Toxicology

Oral fertility and embryonic development, as well as pre- and postnatal development studies were conducted in rats and are evaluated in this review.

The final report for the oral dose-range finding study #TT097899/09GR436 in pregnant rabbits was also submitted. There were no significant differences from the summary described in Dr. Quinn's 2011 review #3.

9.1 Fertility and Early Embryonic Development

Oral Fertility and Embryonic Development Study of PF-04971729 in Male and Female Rats, 20-May-2011 (Study #TT107835 / #10GR227)

Doses: 5, 25 & 250 mg/kg

Study no.:	TT107835 / 10GR227
Study report location:	eDr
Conducting laboratory and location:	Pfizer Global Research & Development, Groton, CT
Date of study initiation:	7/21/2010
GLP compliance:	Yes (no signature)
QA statement:	Yes
Drug, lot #, and % purity:	PF-04971729 (b) (4), Lot #GR02847, 75.6% purity

Key Study Findings

- MTD = 25 mg/kg/day in both sexes
  - Mortality at 250 mg/kg/day (♂ & ♀)
- Female fertility NOAEL = 250 mg/kg/day
  - No drug-related effects on female mating and fertility parameters
- Male fertility NOAEL = 25 mg/kg/day
  - Small testis with zero sperm motility, low sperm count and failure to produce pregnancies were observed in 2/10 (20%) of HD males.
- Transient weight loss at HD (♂ & ♀) on Day 4 correlating with transient decreases in food consumption. Male body weights remained lower than controls at all doses throughout the study.
- By Day 8, drug-related increases in food consumption were observed

The sponsor claims that small testis in 20% of HD males is attributable to a spontaneous preexisting condition because decreased testis is not consistent with expected findings from drug exposure. However, evaluation of historical control data is necessary to support this statement; therefore, a drug-related effect on male fertility has not been ruled out. Nevertheless, since mortalities were observed at HD, the MTD is equivalent to the male fertility NOAEL. Thus, although supporting historical control data could potentially raise the male fertility NOAEL, it would not affect the overall safety NOAEL which is driven by the mortalities at HD.

The initial transient weight loss in both sexes, decreased body weights in males, and increases in food consumption are consistent with compensatory PD effects of drug-induced glucosuria.

Methods

Doses:	0, 5, 25 and 250 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose with 10% (v/v) polyethylene glycol 400
Species/Strain:	Sprague Dawley rats / Crl:CD®(SD)
Number/Sex/Group:	20/sex/group
Satellite groups:	none
Study design:	• Males were dosed 70 days, beginning 28 days prior to cohabitation and up through the day prior to necropsy. • Females were dosed 14 days prior to mating through Gestation Day (GD) 7.
Deviation from study protocol:	No deviations from the study design were reported

Observations and Results

Mortality

Cageside monitoring for moribundity and mortality was conducted daily for all animals.

A total of 3 mortalities were reported, 2 of which occurred in the HD groups and are likely drug-related. One HD female exhibited clinical signs of hunched posture, decreased activity, cool to the touch, and rough haircoat; however the only macroscopic finding was a dilated cecum. One HD male had clinical signs of chromorhinorrhea and a rough haircoat, and also exhibited a large decrease in body weight prior to being found dead. Although both HD mortalities were considered to be drug-related, a pathological cause of death was not identified.

One LD male was found dead on Day 56 with clinical signs of ↓activity and rough haircoat, as well as enlarged liver, spleen and hepatic lymph nodes; however a cause of death was not determined and the sponsor did not consider it drug-related.

MORTALITY

Dose Group	Animal (sex, #)	Day	Cause of Death	Clinical signs	Pathology
LD	♂ #24	56	Unknown	Found dead. Rough haircoat, ↓activity	Enlarged liver, spleen and hepatic lymph nodes
HD	♂#78	78	Drug-related	↓Body weight, chromorhinorrhea and rough haircoat	No findings at necropsy
HD	♀ #151	4	Drug-related	Moribund, rough haircoat, hunched	Dilated cecum.

				posture, ↓activity, & cool to touch	

Clinical Signs

Cageside observations were conducted at least once daily for all animals. During the treatment period, animals were observed at least 3 times daily at predose, ~1 hour post-dosing of the cohort, and once at the end of the workday. All gestating females were observed twice daily.

There were no treatment-related findings in surviving animals.

Body Weight

Male body weights were measured twice prior to the dosing period and twice a week throughout the remainder of the study. Female body weights were measured once weekly prior to dosing, twice a week during treatment, and on GD 0 (day of copulation), 3, 7, 10, and 14. Body weights were recorded for all animals on the day of necropsy.

A 3% decrease in body weight was observed in HD males on Day 4 of dosing, which correlated with a transient decrease in food consumption; however, mean body weight gains were comparable to controls by Day 8. Throughout the study, male body weights were lower than concurrent controls across all dose groups and terminal body weights were 8%, 6%, and 9% lower than controls for LD, MD, and HD groups, respectively. Although the decreased body weights were not dose-dependent, they are likely to be drug-related.

A similar, transient decrease in body weight was observed in HD females on Day 4 of dosing; however, mean weight gains rebounded by Day 8 and were within the normal biological range thereafter. There were no significant differences in mean female body weights.

Feed Consumption

Male food consumption was determined twice a week throughout the study other than during the cohabitation period. Female food consumption was measured twice weekly beginning 2 weeks prior to mating, as well as on GD 3, 7, 10 and 14.

After Day 8, dose-related increases in food consumption were observed in both males and females at all doses. During the first measurement period (Day 1-4), a 30% decrease in male food consumption was observed at HD, which correlated with a 3% weight loss; however, food consumption rebounded and was dose-dependently higher than controls by 21-58% throughout the remainder of the study. Food consumption was consistently higher in males at LD (↑10-25%) and MD (↑14-42%) throughout the entire study. In females, Food consumption was significantly and dose-dependently higher than controls at all doses beginning on Day 8. During gestation, dam food consumption was 13-33% higher at all doses, but only remained significantly higher during GD10-14 at HD (↑29%).

Toxicokinetics

On Day 12 of dosing, blood samples were collected from 4/sex/group at 1 hour post-dose. Plasma C_{max} exposures were determined using HPLC.

Plasma exposures at 1 hour postdose increased approximately dose-proportionally and are consistent with C_{max} exposures from earlier toxicology studies in rats.

Sponsor's Table 11: Toxicokinetics - Rat

Plasma concentrations at 1 hour post dosing on the 12th day of dose administration						
	Male			Female		
	5 mg/kg	25 mg/kg	250 mg/kg	5 mg/kg	25 mg/kg	250 mg/kg
Mean (µg/mL)	1.66	8.04	36.48	2.75	10.18	50.98
SD	0.87	4.94	8.10	0.53	2.41	18.02
N=4/sex/group; all control samples were below the lower limit of qualification.						

(Table excerpted from sponsor's package)

Dosing Solution Analysis

Suspensions were prepared every 2 weeks using the mortar and pestle method, and stored in a refrigerator, protected from light. Formulations were continuously stirred during dosing. Dose concentrations and homogeneity were assessed using a validated Reversed Phase Liquid Chromatography method with ultraviolet detection.

Formulation concentrations for all preparations administered to animals were within ±10% of target concentration. The homogeneity of top, middle and bottom samples all met acceptance criteria (≤10% RSD).

Necropsy

On GD 14, all surviving dams were euthanized and the abdominal, thoracic and pelvic viscera were grossly examined. External evaluations of the abdominal, thoracic, and pelvic viscera were conducted on all moribund females and pregnant females in which signs of copulation were not observed. The uterus and ovaries were harvested at necropsy and preserved in 10% formalin.

After 70 days of dosing, males were euthanized and the abdominal, thoracic and pelvic viscera were grossly examined. The reproductive organs including the testes, epididymes, seminal vesicles, and prostate were harvested. The left testis and epididymis were fixed in Modified Davidson's solution. The right epididymis and testis were frozen for sperm and spermatid head counts, respectively. The right distal vas deferens was evaluated for sperm motility.

There were no drug-related gross necropsy observations in females. Two (2/20) males at HD were reported to have small testis and epididymis with extremely small testis weights. There were no statistically significant differences in mean testicular organ weights.

Fertility Parameters

Females

To assess the estrous cycle, vaginal smears were collected daily at least 2 weeks prior to dosing until evidence of mating was observed or the end of the cohabitation period. The number of corpora lutea was counted for each ovary. The location and viability of uterine implantation sites were determined. Uteri without implantation sites were treated with 10% ammonium sulfide to determine early embryonic death.

There were no drug-related effects on estrous cyclicity, number of corpora lutea, implantation sites or live fetuses.

Males

Sperm analysis was performed on all males.

The two (2/20) males at HD with small testis had 0 motile sperm, low sperm concentrations, and failed to produce pregnancies. However, all other males at HD had sperm motility and concentrations similar to controls. The sponsor argues that the poor reproductive performance of the 2 HD males with small testis is due to a spontaneous preexisting condition. However, there were no coinciding incidences in concurrent controls and historical background data were not submitted to support the claim. Therefore, it is not possible to rule out a drug-related effect at the HD.

9.2 Prenatal and Postnatal Development

A Pre-and Postnatal Developmental Toxicity Study of PF-04971729 by Oral (Gavage) in Rats, 20-May-2011 (Study #TT137827FIN / #13GR257)

Doses: 50, 100 and 250 mg/kg/day

Study no.:	TT137827 / 13GR257
Study report location:	eDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/14/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04971729, Lot #E010013903, 98.4%

Key Study Findings

- Maternal NOAEL = 50 mg/kg/day (~35x *$MRHD_{AUC@15mg}$, ~16x *$MRHD_{AUC@100mg}$)
  - Clinical signs of dehydration, rales, urine-stained fur, and abdominal distention at ≥100 mg/kg/day. Clinical signs of hunched posture, soft/liquid feces and pale ears were also observed at 250 mg/kg/day during lactation.
  - Decreased body weight, body weight gain and food consumption at ≥100 mg/kg/day during gestation.

- Reproductive NOAEL = 250 mg/kg/day (~500x *$MRHD_{AUC@15mg}$, ~60x *$MRHD_{AUC@100mg}$)
  - No significant effects on maternal reproductive function parameters
- F_1 NOAEL = 50 mg/kg/day (~35x *$MRHD_{AUC@15mg}$, ~16x *$MRHD_{AUC@100mg}$)
  - F_1 pup mortality (PD 1-4) at 250 mg/kg/day associated with lack of nursing.
  - Delayed sexual maturation at 250 mg/kg/day
  - Prolonged decreases in F_1 generation body weights at ≥100 mg/kg/day (~225x *$MRHD_{AUC@15mg}$, ~28x *$MRHD_{AUC@100mg}$)
  - Clinical signs of ungroomed coats, bruising, cold to touch, pale and dehydration at ≥100 mg/kg/day.

*Based on extrapolated exposures from 1-month, 3-month and 6-month rat toxicology studies.

Reviewer Comments:
The maternal NOAEL was set at 50 mg/kg/day based on clinical signs of dehydration, decreases in body weights, weight gain deficits and food consumption at ≥100 mg/kg/day. Although decreases in body weights and weight gain are common pharmacodynamic-related effects of PF-04971729, there were considered to be adverse in pregnant and lactating dams.

Although the maternal NOAEL was set at the low dose, there were drug-related effects of assessments of reproductive function. Therefore, the Reproductive NOAEL was set at 250 mg/kg/day.

The NOAEL for F1 generation developmental toxicity was set at 50 mg/kg/day based on mortalities and delayed sexual maturation at 250 mg/kg and decreased body weights and clinical signs at ≥100 mg/kg.

Decreases in F1 generation body weights, pup clinical signs, pup moralities with empty stomachs, observations of not nesting and not nursing, pup dehydration and maternal dehydration are all consistent with deficits in nursing and maternal behavior with drug administration at ≥100 mg/kg/day. Thus, the body weight deficits and clinical signs at 100 mg/kg/day contributing to the F1 NOAEL may be secondary to maternal behavior. Nevertheless, in order to raise the F1 developmental NOAEL to 100 mg/kg/day, a cross-fostering study would be necessary to further assess this possibility and rule out a relationship to a direct developmental effect on the pups.

Methods

Doses:	0, 50, 100 and 250 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	10% PEG 400 in 0.5% (w/v) methylcellulose
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	22 F_0 females/group, 22 pups/group
Satellite groups:	None

Study design:	Pregnant F0 females were dosed once daily on Gestation Day (GD) 6 through Lactation Day (LD) 20
Deviation from study protocol:	There were no significant deviations that impacted the integrity of the study.

Observations and Results

F_0 Dams

For females that did not deliver litters, dosing was stopped on GD 24 and necropsy was performed on GD 25. Dosing was continued until LD 20 for all other dams, which were then necropsied on LD 28.

Survival

Animals were observed daily for mortalities.

There were no drug-related mortalities. Two mid-dose (MD) dams were found dead or euthanized early due to gavage accidents.

Clinical Signs

Clinical observations were conducted at 1-2 hours postdose and during parturition. Observations of lactation and maternal behavior were also recorded.

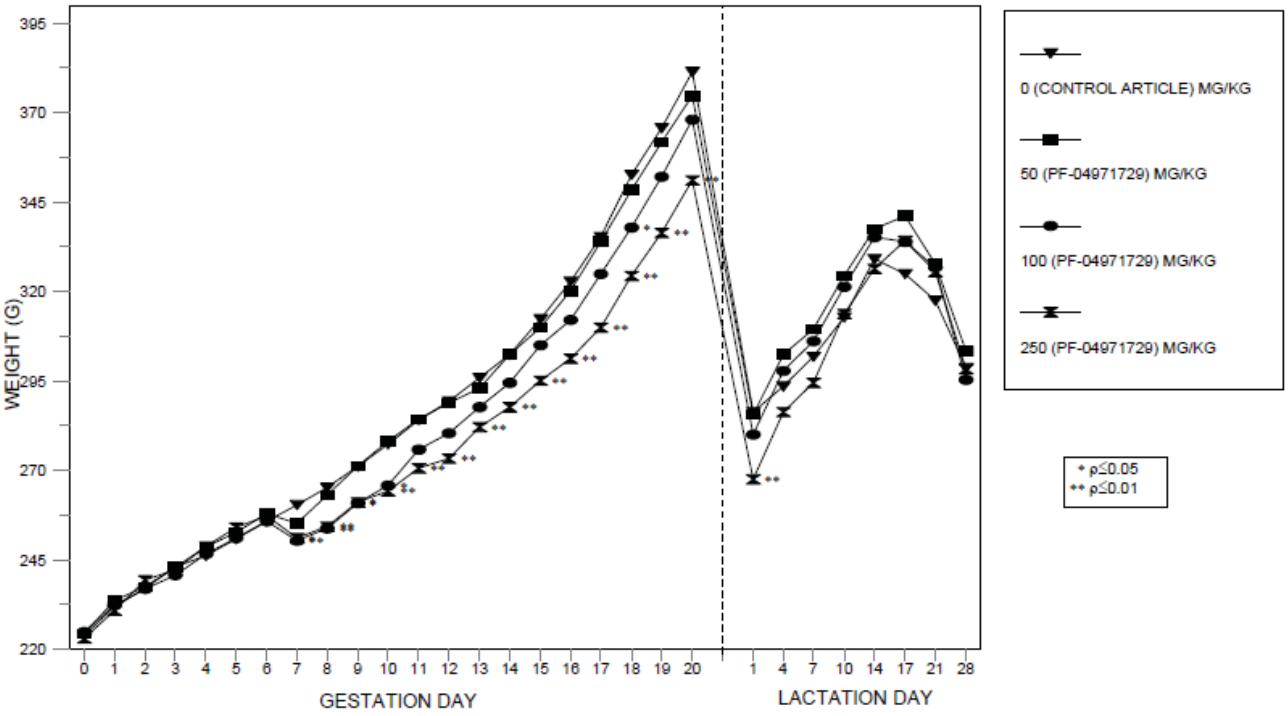
Drug-related clinical signs of mild to moderate dehydration (skin turgor), rales, and urine-stained abdominal fur were observed at ≥MD during gestation, which increased in incidence and severity during the lactation period. Incidences of hunched posture, soft/liquid feces and pale ears were also observed at HD during lactation, and incidences of abdominal distention were reported at ≥MD during lactation.

Body Weight

Dam body weights were measured daily during gestation and on LD 1, 4, 7, 10, 14, 17, 21 and 28.

Transient weight loss was observed at all doses (statistically significant at ≥MD) on GD 7 (Day 2 of dosing) followed by increases in weight gain, although weight gains remained 5-75% lower than controls throughout gestation at ≥MD. Mean body weights remained dose-dependently lower (↓4-8%) at ≥MD throughout gestation and at HD through the first week of lactation. However, during the lactation period, weight gains were dose-dependently, 2-fold higher than controls at ≥MD. By LD 10, body weights in all drug-treated groups were comparable to controls.

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS
Figure 1



Feed Consumption
Dam food consumption was determined on GD 0, 6, 9, 12, 15, 18, 20, and 25, and on LD 1, 4, 7, 10, and 14.

Food consumption was significantly reduced (↓19-32%) at ≥MD during the first interval (GD6-9), but rebounded to 10-40% higher than controls throughout the remainder of gestation and the first 2 weeks of lactation.

Parturition
Dams were allowed to deliver naturally and clinical observations were recorded.

There were no significant drug-related effects on natural delivery parameters, including the number of litter deliveries, duration of gestation, or gestation index. A statistically significant increase in the duration of gestation (↑0.4 days) was reported at HD, but remained within the historical range and was unlikely to be biologically significant.

Uterine Content
Ovarian and uterine examinations were performed for all dams.

There were no drug-related effects on the number of implantation sites per litter.

Necropsy
Macroscopic observations were made at necropsy. Various tissues were collected, but were not examined microscopically.

There were no drug-related macroscopic findings.

Toxicokinetics
Not determined

Dosing Solution Analysis
Test article concentrations were confirmed for all dose groups in the first and last dosing preparations. The first preparation was also assessed for homogeneity.

All mean concentration and homogeneity results were within the acceptance criteria of ±15%

Litter
Litter size and litter viability were determined at delivery.

There were no significant differences in litter sizes, the number of stillborn pups or pup sex ratios. The entire litters of one LD and one HD dams did not survive. However, there was not a consistent dose-dependent effect on the survival of entire litters.

F_1 Generation
Pups were potentially exposed to the test article via nursing, but were not dosed directly. Pups were culled on Postpartum Day (PD) 4. After weaning, 1/sex/litter was selected for F_1 neurological and reproduction assessments.

Survival
Pup survival was assessed twice daily until weaning.

The number of pups found dead, presumed cannibalized, or moribund was significantly increased at HD during the first 4 days (PD1-4), resulting in an overall 14% decrease in the viability index at HD that is considered to be drug-related. However, after culling on PD 4, there was not a significant drug-related decrease in survival, resulting in comparable lactation indexes (survival postculling PD4-28) between drug-treated and control groups.

Clinical Signs
Clinical signs were assessed once daily.

Drug-related effects were reported at ≥MD. At MD, transient drug-related effects included ungroomed coats in all pups of 2/16 litters on PD17-23 and cold to the touch in ≥1 pups in 3/16 litters on PD 1-3. At HD, increased incidences and frequencies of the number of litters with pups with ungroomed coats (16/21) and cold to touch (5/21) were observed on PD 16-28 and PD 1-7, respectively.

A statistically significant increase in the number of litters with pups that were bruised (purple/black color) and/or pale was observed 29 times in 4 litters at HD. Nine

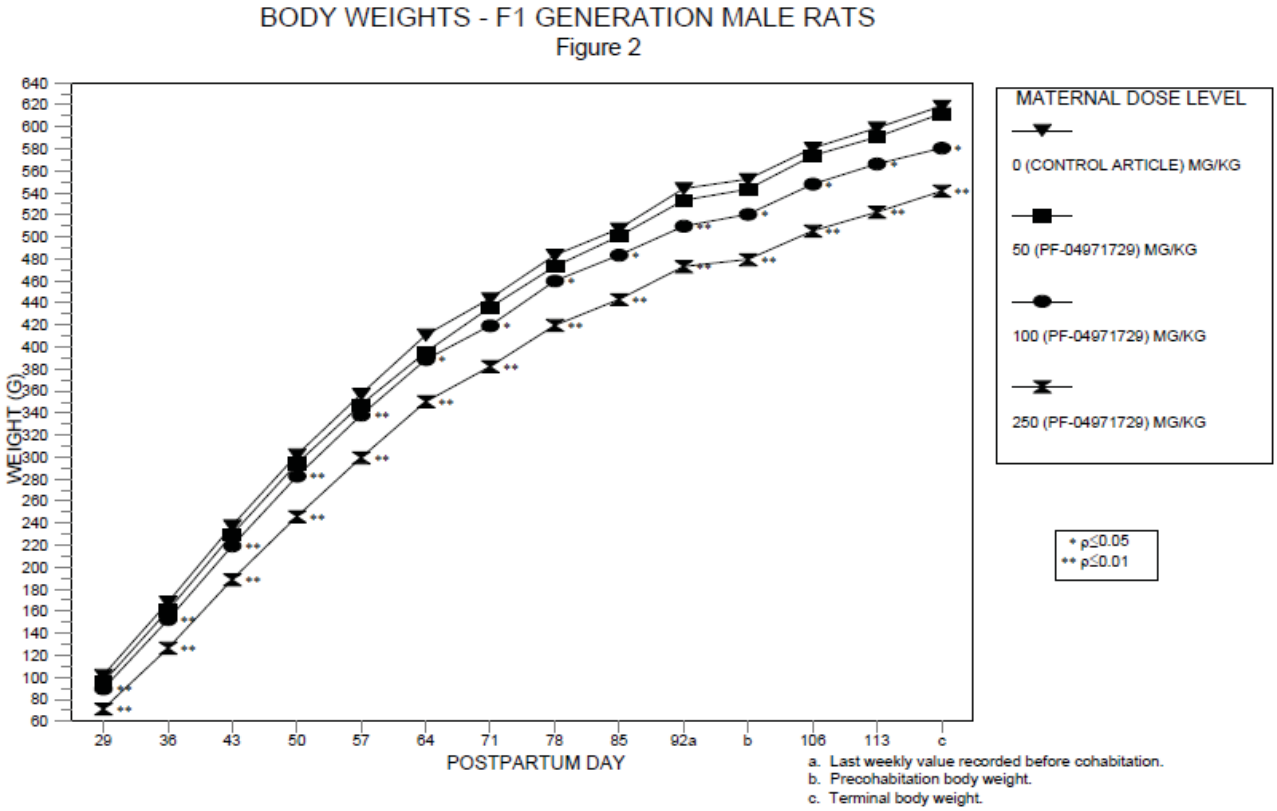
observations of dehydration were also reported in 2 litters at HD. Although, the sponsor considered these findings unrelated to study drug, these findings are considered likely to be drug-related. Occasional observations of not nesting and not nursing were also reported in at least 1 litter and are consistent with pup clinical signs of dehydration.

Body Weight

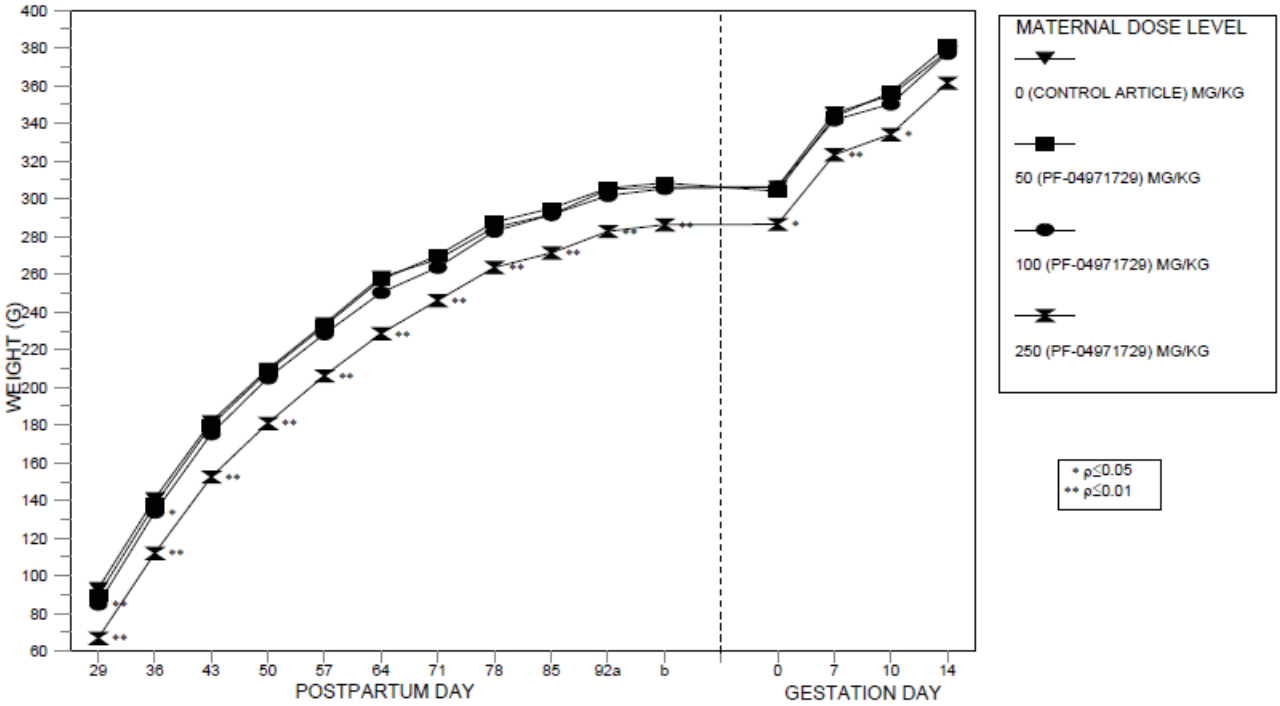
F_1 body weights were determined on PD 1, 4, 7, 10, 14, 17, 21 and 28, followed by once weekly after weaning.

Pup weights were significantly lower at all doses in a dose-dependent manner, reaching 8-17% lower at MD (PD 7-28) and 13-37% lower at HD (PD 1-28). Male F_1 generation body weights remained significantly lower than controls throughout the study (PD 116-120) at ≥MD with periodic weight gain deficits. Female F1 generation body weights remained significantly lower than controls at ≥MD through PD 36 and throughout the remainder of the study (PD 98 + GD 14) at HD. Although the pup weights at LD were significantly 5-9% lower at PD 14-28, they remained within the mean range of control values, in general, were comparable to or greater than controls thereafter, indicating a rebound in growth. It is noted that weight gains were comparable to controls (within 5%) on PD 28 at ≤MD, indicating recovery of weight gain deficits after cessation of drug administration to the dams on LD 20.

Figure 1: F1 Body Weights



BODY WEIGHTS - F1 GENERATION FEMALE RATS
Figure 3



a. Last weekly value recorded before cohabitation.
b. Precohabitation body weight.

(Excerpted from sponsor's package)

Feed Consumption

F_1 feed consumption was determined weekly for all animals. Pregnant F_1 body weights were determined on GD 0, 7, 10 and 14.

Drug-related, dose-dependent decreases in absolute food consumption (↓8-11%) reached statistical significance in males at HD on PD 43 to 57. Although food consumption remained lower throughout PD 92, absolute food consumption was not significantly different from controls after PD 57. On the other hand, food consumption relative to body weight was significantly increased (↑10-40%) at HD throughout PD 29-92.

In females, relative food consumptions were also significantly increased (↑10-20%) at HD between PD 36 to 92, as well as during the F_1 gestation period GD 0 to 14. Although drug-related, the sponsor considered the food consumption changes to be sporadic, minimal and non-adverse in both sexes.

Physical Development

Sexual maturation was assessed in females via vaginal opening beginning on PD 28 and in males via preputial separation beginning on PD 35.

Male balano-preputial separation was significantly delayed by 2.5 days and female vaginal patency was significantly delayed by 2.2 days in F_1 pups from dams treated at

HD. Furthermore, F_1 pup bodyweight at the time of sexual maturation was significantly reduced by 13% in males and 11% in females.

Neurological Assessment
Motor activity (PD 25 and 60), Morris water maze (PD 65 and 80), and acoustic startle testing (PD 80 to 90) were assessed in 1/sex/litter.

There were no significant drug-related findings in motor activity, Morris water maze, or acoustic startle assessments.

Reproduction
On PD 94-98, F_1 male and female pups were cohabitated with other F_1 pups of the opposite sex, but same dose group, for up to 14 days. Estrous cycle was evaluated for 14 consecutive days prior to cohabitation, then until spermatozoa or a copulatory plug was observed.

There were no drug-related findings on mating or fertility indexes.

Gross Pathology
Gross necropsies of the thoracic, abdominal and pelvic viscera were performed for each animal. All lesions were retained, but histopathological evaluations were not conducted. All F_1 pups culled on PD 4 or weaned on PD 28 and not maintained for further tests were necropsied and examined for gross lesions. A single cross-section of the head at the frontal-parietal suture was examined for apparent hydrocephaly. F_1 males used in further studies were necropsied after completion of the 14-day cohabitation period. All F_1 females used in further studies were necropsied on GD 14 and the reproductive tract was dissected for ovarian and uterine examinations.

Necropsies were performed on 27 of the HD pups that were found dead prior to PD28, and 14 were void of milk in the stomach, indicating that they did not nurse. However, 12 were from one litter. There were no other macroscopic findings in any of the other necropsied pups found dead prior to weaning.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	PF-04971729	PF-04971729	PF-04971729
MATERNAL DOSE LEVEL (MG/KG)		0	50	100	250
LITTERS EVALUATED	N	20	21	16	21
TOTAL PUPS STILLBORN OR FOUND DEAD a,b	N	2	4	4	27
STILLBORN	N	1	0	0	2
FOUND DEAD	N	0	4	4	25
UNSCHEDULED EUTHANASIA	N	1	0	0	0
NO MILK IN STOMACH c	N(%)	0(0.0)	1(25.0)	1(25.0)	14(56.0)
APPEARED NORMAL	N(%)	2(100.0)	3(75.0)	3(75.0)	13(48.1)

(Table excerpted from sponsor's package)

In ovarian and uterine examinations of pregnant F_1 females, there were no drug-related differences in litter averages for corpora lutea, implantations or percentage of

preimplantation loss. There were also no drug-related differences in nonviable embryos or percentage of postimplantation loss of F_2 generation embryos.

10 Special Toxicology Studies

No phototoxicity studies have been conducted as UV-visible spectral analysis of PF-04971729 indicated that the compound has a shoulder at 290 nM with a molar extinction coefficient (MEC) of less than 1000 L/mol.cm. Thus, the phototoxicity potential of Ertugliflozin is considered to be low, and phototoxicity studies have not been conducted, nor have they been requested by the division.

11 Integrated Summary and Safety Evaluation

Ertugliflozin (MK-8835, PK-04971729) is a sodium glucose co-transporter 2 (SGLT2) inhibitor being developed by Merck Sharp and Dohme Corp for the treatment of Type 2 Diabetes Mellitus (T2DM).

The sponsor submitted new in vitro and in vivo cardiovascular safety pharmacology studies. In addition to previously identified inhibition of the hERG channel [IC_{50} of >300 μM (129 μg/mL)], ertugliflozin also inhibits Nav1.5 currents with an IC_{50} of 188 μM. Nevertheless, clinical C_{max} exposures are not expected above 3 μM; therefore, inhibition of either hERG or Nav1.5 currents are not anticipated at biologically relevant exposure levels. In spontaneously hypertensive rats, greater effects on blood pressure lowering were observed with 36 mg/kg/day PF-04971729 (100x MRHD) administration than with the anti-hypertensive hydrochlorothiazide. Significant activation of the renin-angiotensin-aldosterone system (RAAS) was also observed. Nevertheless, the safety margins for drug-related cardiovascular effects are considered to be sufficient. Thus, adverse cardiovascular effects are not anticipated at clinically relevant exposure levels.

A 28-day toxicology study in wild-type mice on the HRAS transgenic background did not identify any new significant toxicity findings. Anticipated pharmacodynamic-related increases in food consumption and body weight gain were observed. Drug-related increases in electrolytes were consistent with osmotic diuresis and the pharmacodynamic activity of PF-04971729. Drug-related kidney observations of increased weight, cortical tubule dilatation, and basophilic tubules were considered to be secondary to pharmacodynamic changes in osmotic diuresis and glucosuria. Overall, there were no significant adverse findings unrelated to pharmacodynamic activity and the margin of safety for the 15 mg therapeutic dose of ertugliflozin was considerable (>100x $MRHD_{15mg}$).

In the rat fertility study, drug-related findings were observed at the high dose of 250 mg/kg/day. Small testis with zero sperm motility, low sperm count and failure to produce pregnancies was observed in 20% of males at the high dose resulting in a male fertility NOAEL of 25 mg/kg/day (~50x $MRHD_{Cmax@15mg}$, 5x $MRHD_{Cmax@100mg}$). However, in females, there were no drug-related effects on mating and fertility parameters (female fertility NOAEL = 250 mg/kg/day, ~300x $MRHD_{Cmax@15mg}$, 30x $MRHD_{Cmax@100mg}$). Potentially drug-related mortalities were observed in both sexes at the high dose with a NOAEL of 25 mg/kg/day (~50x $MRHD_{Cmax@15mg}$, 5x $MRHD_{Cmax@100mg}$). Transient weight

losses in both sexes were followed by increases in food consumption, consistent with drug-induced glucosuria and compensatory PD effects. Overall, fertility safety margins are considered to be sufficient at the 15mg therapeutic dose.

In the PPND rat study, adverse drug-related effects were observed in both dams and pups. The maternal NOAEL was set at the low dose of 50 mg/kg/day (~35x $MRHD_{AUC@15mg}$, ~16x $MRHD_{AUC@100mg}$) due to clinical signs of dehydration, decreases in body weights, weight gain deficits and food consumption, which are likely related to the pharmacodynamic activity of the study drug. However, there were no significant effects on maternal reproductive function parameters and the reproductive NOAEL was set at the high dose of 250 mg/kg/day (~500x $MRHD_{AUC@15mg}$, ~60x $MRHD_{AUC@100mg}$). The F1 developmental NOAEL was set at the low dose of 50 mg/kg/day (~35x $MRHD_{AUC@15mg}$, ~16x $MRHD_{AUC@100mg}$) based on mortalities and delayed sexual maturation at 250 mg/kg and prolonged decreases in body weights and clinical signs consistent with dehydration and bruising at ≥100 mg/kg/day (~225x $MRHD_{AUC@15mg}$, ~28x $MRHD_{AUC@100mg}$). It was noted that many of the observed effects in F1 pups are consistent with deficits in nursing and maternal behavior; however, a cross-fostering study would be necessary to distinguish between direct drug-related effects in F1 pups and effects secondary to maternal behavior.

In the previously reviewed embryo-fetal development (EFD) study #10GR058, developmental toxicity safety margins for ertugliflozin were set at 100 mg/kg/day (~380x $MRHD_{AUC@15mg}$, ~200x $MRHD_{Cmax@15mg}$) based on the external, visceral and skeletal malformations observed in rat fetuses at 250 mg/kg/day (reference Dr. Quinn's review #3). In the previously reviewed rabbit EFD study #10GR059, the NOAEL was also set at 100 mg/kg (~290x $MRHD_{AUC@15mg}$, ~350x $MRHD_{Cmax@15mg}$) based on visceral and skeletal malformations and variations at 250 mg/kg.

Based on a compilation of the nonclinical developmental studies, ertugliflozin is teratogenic and delays sexual maturation at very high exposures with the defining NOAEL for fetuses and neonates set at 50 mg/kg/day, which is associated with safety margins of at least at least 35-fold higher than clinical exposures at the therapeutic 15 mg dose (~35x $MRHD_{AUC@15mg}$) and 16-fold higher than the supratherapeutic 100 mg dose (~16x $MRHD_{AUC@100mg}$). Thus, there are sufficient margins of safety for developmental effects at both the therapeutic 15 mg and the supratherapeutic 100 mg doses of ertugliflozin.

In summary, safety margins are considered to be sufficient in all the reviewed nonclinical studies. Thus, the proposed Phase III study is considered reasonably safe to proceed.

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

/s/

JESSICA HAWES
08/05/2016

RONALD L WANGE
08/05/2016

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	IND #106447 (cross-reference IND #122329)
Review number:	7
FDA SDN, Sponsor SN (Vol #), Sponsor letter date, CDER stamp date:	148, 0147, 07/02/2015, 07/02/2015
Product:	MK-8835B (Ertugliflozin / Metformin)
Indication:	Treatment of Type 2 Diabetes Mellitus
Sponsor:	Merck Sharp and Dohme Corp
Review Division:	CDER/DMEP
Reviewer:	Jessica J Hawes, Ph.D.
Supervisor:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Abolade Adeolu
Review completion date:	12/3/2015

DMEP PT Template v.0.04 September 23, 2010

Table of Contents

1 Executive Summary

1.1 Introduction

Merck Sharp and Dohme Corp. is investigating the new molecular entity (NME) MK-8835 (Ertugliflozin, PF-04971729, IND #106447) and the fixed dose combination (FDC) with the marketed drug (MD) Metformin (MK-8835B, IND #122329) for the treatment of type 2 diabetes mellitus.

1.2 Brief Discussion of Nonclinical Findings

Co-administration of ertugliflozin and metformin in rats for 13-weeks was generally well-tolerated with sufficient margins of safety and was not associated with significant adverse systemic toxicities. However, it is noted that the kidney may be a target organ of treatment-related toxicity with longer exposures.

1.3 Recommendations

The proposed clinical ertugliflozin and metformin co-administration studies under both IND #106447 and IND #122329 are safe to proceed. Non-clinical data support administration of the proposed FDC dose of 15 mg/day ertugliflozin + 2000 mg/day metformin (b) (4)

1.3.3 Non-hold Recommendations

None

2 Drug Information

2.1 Drug

CAS Registry Number

None

Product Name

Ertugliflozin
Ertugliflozin/metformin FDC

Code Name

Ertugliflozin + metformin FDC: MK-8835B
Ertugliflozin: PF-04971729, MK-8835
Ertugliflozin L-pyroglutamic acid (L-PGA) co-crystal form: PF-0497172(b) (4)

Chemical Name

(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol compound with (S)-5-oxopyrrolidine-2-carboxylic acid (1:1)

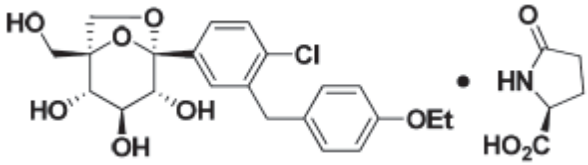
Molecular Formula/Molecular Weight

PF-04971729:	$C_{22}H_{25}ClO_7$	436.88 g/mol
PF-0497172(b) (4):	$C_{27}H_{32}ClNO_{10}$	566.00 g/mol
Metformin:	$C_4H_{11}N_5$	

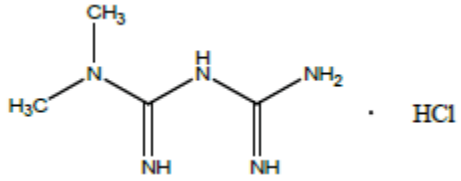
Metformin Hydrochloride: $C_4H_{12}N_5Cl$

Structure or Biochemical Description

Ertugliflozin L-PGA



Metformin Hydrochloride



Pharmacologic Class

Ertugliflozin: Sodium glucose co-transporter 2 (SGLT2) Inhibitor
Metformin: Biguanide

Planned Clinical Route of Administration

Oral tablet administered twice daily with meals. In the past reporting period ending in September of 2015, the sponsor [redacted] (b) (4).

Originally Proposed Tablet Strengths:

1. **0.005 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 2.5 mg / metformin 500 mg FDC tablets

2. **0.0075 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 7.5 mg / metformin 1000 mg FDC tablets

Four New Tablet Strengths:

1. [redacted] (b) (4)

2. **0.0025 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 2.5 mg / metformin 1000 mg FDC tablets

3. **0.015 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 7.5 mg / metformin 500 mg FDC tablets

4. [redacted] (b) (4)

2.2 Relevant INDs, NDAs, and DMFs

IND #122329 – Ertugliflozin + metformin FDC (Merck Sharp & Dohme Corp.)

IND #047342 / NDA #21202 - Metformin HCl (Bristol Myers Squibb)

IND #76500 / NDA #63634 – Kombiglyze, combination of Metformin HCl and Saxagliptin (Astrazeneca AB)

2.3 FDC Drug Formulation

In the past reporting period ending in September of 2015, the sponsor added 4 new strengths of the FDC drug product and replaced the (b) (4).

Sponsor's Table 1: FDC Drug Product Compositions

Table 1 **Drug Product Compositions for MK-8835B FDC Tablets containing 2.5/500 mg, (b) (4) and 2.5/1000 mg of MK-8835 and Metformin Hydrochloride, respectively**

Strength			2.5/500 mg	(b) (4)	2.5/1000 mg
Component	Quality standard	Function	mg/tablet		mg/tablet
MK-8835	In-house	Active	3.24†		(b) (4)
(b) (4) Metformin Hydrochloride (b) (4) Povidone (b) (4)	In-house	Active and binder	(b) (4)		
Microcrystalline cellulose	Compendial*	Diluent			
Crospovidone	Compendial*	Disintegrant			
Sodium lauryl sulfate	Compendial*	Wetting agent			
Magnesium stearate	Compendial*	Lubricant			
Theoretical Core Tablet Weight					(b) (4)

(b) (4)

Table 2 **Drug Product Compositions for MK-8835B FDC Tablets containing 7.5/500 mg, (b) (4) and 7.5/1000 mg of MK-8835 and Metformin Hydrochloride, respectively**

Strength			7.5/500 mg	(b) (4)	7.5/1000 mg
Component	**Quality standard**	**Function**	**mg/tablet**		**mg/tablet**
MK-8835	In-house	Active	9.71†		9.71†
(b) (4) Metformin Hydrochloride (b) (4) Povidone (b) (4)	In-house		(b) (4)		(b) (4)
Microcrystalline cellulose	Compendial*				
Crospovidone	Compendial*				
Sodium lauryl sulfate	Compendial*				
Magnesium stearate	Compendial*				
Theoretical Core Tablet Weight					(b) (4)
					(b) (4)

(Tables excerpted from sponsor's package)

2.4 Comments on Novel Excipients

The only non-compendial excipient is the (b) (4) However, the (b) (4) ingredients meet regulatory and compendial requirements appropriate for their intended use (see Dr. Joseph Leginus's Quality review under IND #122329).

2.5 Comments on Impurities/Degradants of Concern

In the ertugliflozin lot #GR02546 used for previous non-clinical studies, impurity (b) (4) was found to be as high as (b) (4) %. However, this impurity was not detected in clinical Lot #GR02694. There have not been any other reported organic impurities for the ertugliflozin co-crystal.

2.6 IND #106447 Clinical Development Plan

Phase I and II clinical studies under IND (b) (4) have been completed and no further Phase I studies are planned under IND #106447. The 2015 annual report describes eight Phase III studies ongoing in T2DM patients with doses of ertugliflozin up to 15 mg and treatment durations of up to 104 weeks in duration.

Ongoing Clinical Studies under IND #106447

Study #P002/B1521013: A Phase III, multicenter, randomized, double-blind, active-comparator-controlled clinical trial to study the safety and efficacy of the addition of ertugliflozin (MK-8835/PF-04971729) compared with the addition of glimepiride in subjects with type 2 diabetes mellitus who have inadequate glycemic control on metformin. This study is investigating co-administration of once daily ertugliflozin (5 or 15 mg) with metformin (≥1500 mg/day) in TD2M patients for up to 104 weeks.

Study #P002/B1521015: A Phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-Group Clinical Trial to Evaluate the Safety and Efficacy of Ertugliflozin (MK-8835/PF-04971729) in the treatment of subjects with type 2 diabetes mellitus who have inadequate glycemic control on metformin and sitagliptin. This study is investigating co-administration of metformin (≥1500 mg/day) and ertugliflozin (5 or 15 mg once daily) in the absence or presence of daily 100 mg sitagliptin for up to 52 weeks.

Study #P001/B1521016: A phase III, multicenter, randomized, double-blind, placebo-controlled clinical trial to evaluate the efficacy and safety of ertugliflozin (MK-8835/PF-04971729) in subjects with type 2 diabetes mellitus with stage 3 chronic kidney disease who have inadequate glycemic control on background antihyperglycemic therapy. This study involves 2 doses of ertugliflozin administered at doses up to 15 mg/day in patients with T2DM for up to 52 weeks in duration.

Study #P002/B1521017: a randomized, double-blind, placebo- and active- controlled study to evaluate the safety and efficacy of the addition of treatment with ertugliflozin (5 or 15 mg) in subjects with T2DM and inadequate glycemic control on a stable dose of ≥1500 mg/day metformin. This study also examined the effect of ertugliflozin on bone mineral density in post-menopausal women. This study is investigating co-administration of once daily ertugliflozin (5 or 15 mg) with metformin (≥1500 mg/day) in TD2M patients for up to 52 weeks, or up to 104 weeks with extension.

Study #P005/B1521019: a Phase III, randomized, double-blind, multi-center study to evaluate the efficacy and safety of the combination of ertugliflozin (MK-8835/PF-04971729) with sitagliptin compared with ertugliflozin alone and sitagliptin alone, in the treatment of subjects with T2DM with inadequate glycemic control on metformin monotherapy. This study involves co-administration of metformin (≥1500 mg/day) and ertugliflozin (5 or 15 mg once daily) in the absence or presence of daily 100 mg sitagliptin for up to 52 weeks.

Study #P004/B1521021: a randomized, double-blind, placebo-controlled, parallel-group study to assess cardiovascular outcomes following treatment with ertugliflozin (MK-8835/PF-04971729) in subjects with type 2 diabetes mellitus and established vascular disease. This study includes ertugliflozin (5 or 15 mg once daily) co-administration with insulin in the absence or presence of metformin for 18 weeks and includes evaluation of cardiovascular endpoints as well. A sub-study will also evaluate the efficacy and safety of daily ertugliflozin in the absence or presence of sulfonylurea co-administration.

Study #P003/B1521022: a randomized, double-blind, placebo- and active- controlled study to evaluate the safety and efficacy of monotherapy treatment with ertugliflozin 5 mg and 15 mg for up to 52 weeks in subjects with T2DM and inadequate glycemic control on diet and exercise alone.

Study #P002/B1521047: a randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of initial combination treatment with ertugliflozin (5 or 15 mg) and 100 mg sitagliptin for up to 26 weeks in subjects with T2DM and inadequate glycemic control on diet and exercise.

2.7 IND #122329 Clinical Development Plan

Although no clinical reports have been submitted for review under IND #122329, two Phase 1 acute studies have been completed to date, a single-dose Phase 1 PK study protocol in Healthy Subjects (study #MK-8835B-0019-00/B1521032) and a pivotal Phase 1 clinical pharmacology bridging study (#MK-8835-035-00/B1521051) to compare the bioequivalence (BE) of the ertugliflozin/metformin combination FDC tablet to co-administration of ertugliflozin and metformin. There are two Phase 1 studies currently ongoing under IND #122329, including one PK study protocol with ertugliflozin alone (study #MK-8835B-0027-00/B1521051) and another pivotal Phase 1 bioequivalence bridging study with the FDC versus co-administration of ertugliflozin and metformin (#MK-8835-050-00/B152105).

Clinical Plan Outline

1. PK bridging study to evaluate once and twice daily oral administration of ertugliflozin in healthy volunteers (1 study ongoing under IND #122329)
2. Demonstrate bioequivalence (BE) between the FDC product and co-administered ertugliflozin and metformin in the fasted state (2 studies under IND #122329, 1 completed and 1 ongoing)
3. A food interaction study to evaluate the effect of a standard high fat breakfast on the PK of the highest FDC strength (7.5 mg ertugliflozin / metformin 1000 mg) with twice daily dosing
4. Drug interaction studies between metformin and ertugliflozin (5 studies ongoing under IND #106447)
5. The sponsor is not proposing a phase 3 clinical study with the FDC product. The sponsor's development strategy for the twice daily FDC product will be based on bridging studies to once daily ertugliflozin and metformin co-administration.

Completed Clinical Studies under IND #122329

Study #MK-8835B-0019-00/B1521032: A Phase 1, Randomized, Open-Label, 3-Period, 6-Sequence Study to Estimate the Pharmacokinetic Interaction between Ertugliflozin and Metformin in Healthy Subjects. The final study report has not yet been submitted for review; however, a summary of the study results was included in the 2015 annual report.

Sponsor's Table 2: Preliminary Clinical PK Data - Study #MK-8835B-0019-00/B1521032

Table S5. Descriptive Summary of Plasma Ertugliflozin Pharmacokinetic Parameter Values

Parameter (Units)	Parameter Summary Statistics[a] by Treatment	
	Ertugliflozin 15 mg	Ertugliflozin 15 mg + Metformin 1000 mg
N, n	18, 17	18, 17
AUC_{inf} (ng.h/mL)	1363 (24)	1388 (23)
AUC_{last} (ng.h/mL)	1346 (23)	1367 (22)
C_{max} (ng/mL)	272.3 (24)	264.5 (20)
T_{max} (h)	1.02 (1.00, 2.00)	1.29 (1.00, 3.00)
$t_{½}$ (h)	11.79 ± 2.34	13.48 ± 4.65
CL/F (mL/min)	183.8 (24)	180.0 (23)
V_z/F(L)	183.7(33)	201.7(31)

AUC_{inf} = area under the plasma concentration-time profile from time 0 extrapolated to infinite time, AUC_{last} = area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last}), C_{max} = maximum observed plasma concentration, CL/F = apparent clearance, N = number of subjects; n = number of subjects for $t_{½}$, AUC_{inf}, CL/F and V_z/F, $t_{½}$ = terminal half-life, T_{max} = time for C_{max}, V_z/F = apparent volume of distribution.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean ±SD for $t_{½}$.

Table S6. Descriptive Summary of Plasma Metformin Pharmacokinetic Parameter Values

Parameter (Units)	Parameter Summary Statistics[a] by Treatment	
	Metformin 1000 mg	Ertugliflozin 15 mg + Metformin 1000 mg
N, n	18, 13	18, 13
AUC_{inf} (ng.h/mL)	12770 (27)	12260 (27)
AUC_{last} (ng.h/mL)	12550 (26)	12270 (23)
C_{max} (ng/mL)	1983 (26)	1835 (26)
T_{max} (h)	2.00 (0.50, 4.00)	2.00 (1.00, 3.00)
$t_{½}$ (h)	10.23 ± 2.39	14.47 ± 6.94
CL/F (mL/min)	1305 (27)	1359 (26)
V_z/F(L)	1126(43)	1577(51)

AUC_{inf} = area under the plasma concentration-time profile from time 0 extrapolated to infinite time, AUC_{last} = area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration (C_{last}), C_{max} = maximum observed plasma concentration, CL/F = apparent clearance, N = number of subjects; n = number of subjects for $t_{½}$, AUC_{inf}, CL/F and V_z/F, $t_{½}$ = terminal half-life, T_{max} = time for C_{max}, V_z/F = apparent volume of distribution.

a. Geometric mean (geometric %CV) for all except: median (range) for T_{max}; arithmetic mean ±SD for $t_{½}$.

(Tables excerpted from sponsor's package)

The number of total adverse events (AEs) was increased in patients receiving both ertugliflozin and metformin. The AEs were primarily gastrointestinal (abdominal discomfort, distension, pain, diarrhea, nausea) of mild severity.

Sponsor's Table 3: Preliminary Clinical Safety Data - Study #MK-8835B-0019-00/B1521032

Table S7. Summary of Treatment-Emergent Adverse Events, All Causalities (Treatment-Related)

Number of Subjects	Ertugliflozin (15 mg)	Metformin (1000 mg)	Ertugliflozin 15 mg + Metformin 1000 mg
Subjects evaluable for AEs	18	18	18
Number of AEs	1 (1)	4 (3)	10 (10)
Subjects with AEs	1 (1)	4 (3)	7 (7)
Subjects with SAEs	0	0	0
Subjects with severe AEs	0	0	0
Subjects discontinued due to AEs	0	0	0

Included all data collected since the first dose of study drug.
Except for the number of adverse events, subjects were counted only once per treatment in each row.
MedDRA (version 17.1) coding dictionary applied.
AE = Adverse event; MedDRA = Medical Dictionary for Regulatory Activities; SAEs = serious adverse events.

Number of Subjects with AEs by System Organ Class and MedDRA Preferred Term[a]	Ertugliflozin 15 mg (n = 18)	Metformin 1000 mg (n = 18)	Metformin 1000 mg + Ertugliflozin 15 mg (n = 18)
Gastrointestinal disorders	**1 (1)**	**3 (3)**	**6 (6)**
Abdominal discomfort	0	1 (1)	1 (1)
Abdominal distension	0	0	1 (1)
Abdominal pain	0	1 (1)	1 (1)
Constipation	0	0	1 (1)
Diarrhea	1 (1)	0	2 (2)
Gastrointestinal pain	0	0	1 (1)
Nausea	0	0	2 (2)
Vomiting	0	1 (1)	0
Infections and infestations	**0**	**1 (0)**	**0**
Urinary tract infection	0	1 (0)	0
Nervous system disorders	**0**	**0**	**1 (1)**
Headache	0	0	1 (1)
Total preferred term events	1 (1)	4 (3)	10 (10)

Subjects were counted only once per treatment in each row.
Included all data collected since the first dose of study drug.
Abbreviations: AE =adverse event; MedDRA = Medical Dictionary for Regulatory Activities; n = subject number.
a. MedDRA (version 17.1) coding dictionary was used.

(Tables excerpted from sponsor's package)

Study #MK-8835-035-00/B1521041: A pivotal, Phase 1, open-label, randomized, 2-period, 2-sequence single dose crossover study to demonstrate the bioequivalence of ertugliflozin 7.5 mg/metformin 1000 mg FDS tablet to the co-administration of the individual components: ertugliflozin 7.5 mg (administered as one 5 mg tablet + one 2.5 mg tablet) and US-sourced Glucophage® 1000 mg tablet, under fasted conditions, in healthy subjects. The final study report has not yet been submitted for review and a summary of the study results was not available for inclusion in the 2015 annual report.

Sponsor's Table 4: Study #MK-8835-035-00/B1521041 Clinical Design

Sequence	Period 1	Period 2
1 (n = 16)	ERTU+MET-COADM	ERTU/MET-FDC
2 (n = 16)	ERTU/MET-FDC	ERTU+MET-COADM

ERTU+MET-COADM: ertugliflozin (one 5 mg tablet + one 2.5 mg tablet) and US-sourced Glucophage® 1000 mg coadministered under fasted conditions (Reference)
ERTU/MET-FDC: ertugliflozin 7.5 mg /metformin 1000 mg FDC tablet, single dose, under fasted conditions (Test)

(Table excerpted from sponsor's package)

Ongoing Clinical Studies under IND #122329

Study #MK-8835B-0027-00/B1521051: An open-label, randomized, 2-period, 2-cohort, crossover, steady state evaluation of the pharmacokinetics and pharmacodynamics of once daily and twice daily oral administration of ertugliflozin in healthy subjects. This study involves 2 doses of ertugliflozin administered in the absence of metformin at doses up to 15 mg/day.

Sponsor's Table 5: Study #MK-8835B-0027-00/B1521051 Clinical Design

Cohort	Sequence	Period 1 Ertugliflozin	Washout	Period 2 Ertugliflozin
A	1 (n=10)	5 mg QD	≥7 days	2.5 mg BID
	2 (n=10)	2.5 mg BID		5 mg QD
B	1 (n=10)	15 mg QD		7.5 mg BID
	2 (n=10)	7.5 mg BID		15 mg QD

(Table excerpted from sponsor's package)

Study #MK-8835-050-00/B1521058: A pivotal, Phase 1, single dose, open-label, randomized, crossover bioequivalence study of an ertugliflozin 2.5 mg/metformin 500 mg fixed dose combination tablet vs. co-administration of the individual components (ertugliflozin and US-sourced metformin) in healthy subjects.

Sponsor's Table 6: Study #MK-8835-050-00/B1521058 Clinical Design

Sequence	Period 1	Period 2
1 (n = 16)	ERTU+MET-COADM	ERTU/MET-FDC
2 (n = 16)	ERTU/MET-FDC	ERTU+MET-COADM

ERTU+MET-COADM: ertugliflozin 2.5 mg tablet and US-sourced Glucophage® 500 mg co-administered (within 5 minutes of each other with the ertugliflozin administered first) under fasted conditions (Reference)
ERTU/MET-FDC: ertugliflozin 2.5 mg /metformin 500 mg FDC tablet, single dose, under fasted conditions (Test)

(Table excerpted from sponsor's package)

Sponsor's Maximum Recommended Human Dose:

FDC: **Twice-daily dose of 7.5 mg ertugliflozin and 1000 mg metformin HCl**

- **15 mg/day ertugliflozin + 2000 mg/day metformin**
- Ertugliflozin AUC* = 1.37 μg·h/mL
  *Based on preliminary results from study #MK-8835B-0019-00/B1521032
- Predicted Metformin **AUC_{0-24} = 20.5 μg·h/mL

Ertugliflozin: Therapeutic oral dose of 15 mg/day with an exposure of AUC*** = 1.2 μg·h/mL (C_{max}*** = 159 ng/mL) is equivalent to a concentration of 0.3 μM.
*** AUC and C_{max} exposures were extrapolated from 14-day repeat-dose exposure in overweight/obese adult subjects (study #B1521002).

- Maximum systemic exposure to unbound**** drug: AUC ≈ 79.3 ng·h/mL, C_{max} ≈ 10.2 ng/ml ≈ 18 nM
  **** Based on a 6.4% unbound fraction in humans
- Reference IND #106447

Metformin: Approved maximum daily dose of Metformin HCl: 1000 mg twice a day (2000 mg/day)

- Maximum exposure of **AUC 20,544 ng·h/mL
  ** Based on preliminary results from study #MK-8835B-0019-00/B1521032, 1000 mg metformin AUC exposures when co-administered with ertugliflozin were as high as 12.3 μg·h/mL. Thus, 2000 mg/day metformin exposures with the FDC may be expected to reach exposures as high as 25 μg·h/mL.
- C_{max} = 1.8 μg/mL for 2000 mg once-daily dose.
- Reference IND#47342 and the drug label for Glucophage

2.8 Previous Clinical Experience

FDC

The combination FDC product is currently being evaluated in humans in 3 Phase 1 studies under IND #122329, all of which are of <1 month in duration; however, no final study reports have been received yet for review. Phase 3 clinical studies with co-administration of ertugliflozin + Metformin are ongoing under IND #106447.

Ertugliflozin

Phase 1 and 2 clinical studies have been completed for ertugliflozin under IND #106447 and multiple phase 3 studies in T2DM patients are underway (Ertugliflozin + Metformin Co-Administration). However, since data from the phase 3 studies have not yet been submitted for review, ertugliflozin is considered to be an early stage entity, as defined in ICH M3(R2).

Ertugliflozin has been administered to at least 495 humans at doses up to 300 mg in the fasted state, 100 mg in the fed state for up to 14 days, and once-daily doses of 25 mg for up to 12 weeks. Food decreases the rate of ertugliflozin absorption, but does not affect the overall extent of absorption.

Ertugliflozin + Metformin Co-Administration

There are 5 ongoing phase 3 clinical studies incorporating ertugliflozin and metformin co-administration in the trial design under IND #106447 (**Error! Reference source not found.**). Studies #B1521017 and #B1521013 are investigating co-administration of once daily ertugliflozin (5 or 15 mg) with metformin (≥1500 mg/day) in TD2M patients for up to 52 weeks, or up to 104 weeks with extension. Studies #B1521015 and #B1521019 include co-administration of metformin (≥1500 mg/day) and ertugliflozin (5 or 15 mg once daily) in the absence or presence of daily 100 mg sitagliptin for up to 52 weeks. Study #B1521021 includes ertugliflozin (5 or 15 mg once daily) co-administration with insulin in the absence or presence of metformin for 18 weeks and includes evaluation of cardiovascular endpoints as well. The sponsor plans to bridge once daily ertugliflozin co-administration with metformin in the phase 3 studies to the twice daily dosing of the FDC formulation using the PK and BE studies. It is noted that since the phase 3 studies are being conducted globally, the sponsor anticipates that a mixture of metformin sources will be used and that strict bridging to the metformin used in the Phase 1 BE bridging study will not be possible.

Metformin

Metformin has been extensively prescribed for long-term administration in patients worldwide for roughly 4 decades and is thought to be the most widely prescribed antidiabetic drug in the world, with 48 million prescriptions per year in the US alone. The most common adverse reactions to metformin are gastrointestinal, including diarrhea, nausea/vomiting, flatulence, asthenia, indigestion, and abdominal discomfort, as well as headache. Metformin is also associated with risks of hypoglycemia and lactic acidosis when given in excessive doses or patients with contraindications, such as kidney disorders, lung disease and liver disease. Long-term use of metformin has been associated with malabsorption of vitamin B_{12}.

2.9 Regulatory Background

FDC

- On 5/9/2014, the sponsor submitted a meeting request and a pre-IND package for the FDC Ertugliflozin + Metformin product.
- On 5/13/2014, a Pre-IND/Type B meeting was granted with written responses sent to the sponsor on 7/3/2014. Within the pre-IND package, the sponsor submitted 3 clinical questions and one regulatory question, but no non-clinical questions.
- The sponsor submitted the IND package for the FDC product on 8/13/2014 cross-referencing non-clinical pharmacodynamic, pharmacokinetic, toxicology information previously submitted under IND #106447 for ertugliflozin alone.
- An agreement for the sponsor's proposed Pediatric Study Plan (PSP) was reached on 8/20/2015.

Ertugliflozin

Ertugliflozin was originally submitted as PF-04971729 in September 2009.

An EOP2 meeting was held on December 17th 2013. The Division encouraged the sponsor to include 2 doses in their phase 3 program, increasing the MRHD to 15 mg/day. The sponsor requested a revision of the proposed rat carcinogenicity study to increase the dose in conjunction with the increased MRHD. Although ECAC did not feel the increase in dose was necessary, it was considered acceptable.

The PeRC BPCA subcommittee discussed the sponsor's proposed Pediatric Study Plan (PSP) on April 10th 2013 and a revised PSP was approved after resubmission on June 12th 2013. It was concluded that a juvenile toxicology study in Sprague Dawley rats would be required prior to initiation of clinical pediatric studies. Ertugliflozin was then discussed at a second PeRC BPCA meeting on August 21st 2013 and the PeRC concurred with the proposed PSP and sponsor's plan for a partial waiver and deferral.

Metformin

Metformin HCl was approved under NDA #020357 as Glucophage (Bristol Myers Squibb) in 1994 with a maximum approved adult dose set at 2000 mg/day. Metformin has been prescribed extensively for long-term treatment of type II diabetes in patients worldwide for roughly 4 decades. Metformin HCl was later approved as an extended release tablet (Glucophage XR) in 2000. A metformin HCl/saxagliptin combination (Kombiglyze, NDA #200678) was approved in November 2010 and included embryofetal toxicology studies (IND #76500, IND #63634), which were mandated by post marketing requirements under the Onglyza NDA #22350, which specifically addressed potential treatment-related neural tube defects due to either metformin or the combination of the two drugs. Embryo-fetal studies using metformin HCl were cross-referenced to the original Metformin HCl IND #47342 and reviewed by Dr. Jessica Hawes 1/11/2011.

3 Studies Submitted

3.1 Studies Reviewed

No nonclinical studies have been submitted under IND #122329; however, two 13-week co-administration studies in rats were submitted under IND #106447 and are evaluated in this review. The 2015 annual reports for both IND #106447 and IND #122329 are also reviewed in this review.

Study #	Brief Title
Toxicology	
TT147809 ((b) (4) #8300339, Pfizer #14GR164)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats **(GLP)**
TT147808 ((b) (4) #8300338, Pfizer #14GR162)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats **(GLP)**

3.2 Studies Not Reviewed

Reproductive and developmental toxicology studies submitted under IND #106447 will be reviewed in a separate review.

3.3 Previous Reviews Referenced

IND #122329:

- Chemistry review #1 by Dr. Joseph Leginus
- Nonclinical review #1 and #2 by Dr. Jessica Hawes

IND #106447:

- End of phase 2 memo and nonclinical reviews #1, #2, #3, #4, and #5 by Dr. Jeffrey Quinn.
- Nonclinical review #6 by Dr. Jessica Hawes

IND #047342:

- Nonclinical review #1 (2/9/2011) and #2 (9/5/2014) by Dr. Jessica Hawes

4 Pharmacology

4.1 Primary Pharmacology

Ertugliflozin is an inhibitor of SGLT2, which blocks the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in glucosuria. Ertugliflozin is selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

Metformin HCl (BMS-207150) is a biguanide class hypoglycemic agent used to treat non-insulin dependent Type II diabetes. Although the molecular mechanisms of metformin are not completely understood, the following mechanisms have been implicated to play a role: inhibition of the mitochondrial respiratory chain (complex I), activation of AMP-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) and consequent activation of protein kinase A (PKA), induced phosphorylation of GLUT4 enhancer factor, and an effect on gut microbiota.

Drug activity related to proposed indication:
Ertugliflozin administration results in concentration-dependent glucosuria in rats. Ertugliflozin acts as a diuretic in rats, increasing urine volume, urinary volume to water intake, and hematocrit levels *in vivo*. Diuretic effects have also been reported in humans.

Metformin suppresses hepatic glucose production, increases insulin sensitivity, enhances peripheral glucose uptake, decreases insulin-induced suppression of fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract.

4.2 Safety Pharmacology

Ertugliflozin

Standard cardiovascular, neurological and pulmonary safety pharmacology studies have been completed for ertugliflozin under IND #106447.

Neurological: Male rats dosed with 500 mg/kg of PF-04971729(b) (4) had a 0.4°C decrease in average body temperature. At 500 mg/kg, PF-04971729 produced decreases in locomotor activity measurements (~30-40%).

Cardiovascular: PF-04971729 inhibited the hERG channel *in vitro* with an IC_{50} of >300 µM (129 µg/mL). However, significant inhibition of hERG was observed at doses ≥ 30 µM: 2.9% at 30 µM, 8.3% at 100 µM (ranging from 2.9% to 18.1%), and 33.5% at 300 µM. Dosing with PF-04971729(b) (4) at 50 mg/kg in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and heart rate (and associated RR interval shortening), as well as an increase in systolic blood pressure and lengthening of the PR interval, with a NOAEL of 5 mg/kg and an LOAEL of 50 mg/kg ($AUC_{(0-24)}$ = 530 µg·h/mL, C_{max} = 44.7 µg/mL = 80 µM, unbound = 1.43 µg/mL = 2.5 µM, 387x MRHD).

Pulmonary: Dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed in rats at doses ≥25 mg/kg lasting for up to 120 minutes post-dose.

Renal: No renal safety studies were performed although PF-04971729 causes increased urinary glucose excretion and kidney alterations in rats and dogs.

Gastrointestinal: No GI safety studies were performed although PF-04971729 causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs at high exposures.

Metformin

Neurological

Incidences of headache, confusion, and/or mood swings observed in humans are likely secondary to hypoglycemia.

Cardiovascular

Metformin has been associated with lactic acidosis, which is further associated with cardiovascular collapse, acute congestive heart failure, and acute myocardial infarction. Some patients have reported increased heart beat and/or palpitations while on metformin.

Pulmonary

Difficulty breathing is occasionally reported in humans taking metformin, but has not been associated with an adverse clinical outcome.

Renal

Metformin is contraindicated with renal deficiency since it is primarily eliminated via the kidney. Cystic tubular dilation and vacuolization have been observed in mice.

Gastrointestinal

GI upset, diarrhea, cramps, nausea, vomiting and gas.

Other

Metformin-mediated inhibition of hepatic neogenesis leads to decreases in lactate uptake and increases in lactic acid leading to lactic acidosis, which can be fatal. Metformin is also contraindicated in patients with liver dysfunction and acute or chronic metabolic acidosis, including diabetic ketoacidosis. Loss of appetite and a bad taste in the mouth has also been associated with metformin administration in children.

5 Pharmacokinetics/ADME/Toxicokinetics

Drug-drug interactions between ertugliflozin and metformin are not anticipated. Ertugliflozin and metformin are eliminated by different mechanisms and are not expected to affect each other's elimination pathways. Ertugliflozin is predominantly eliminated via hepatic metabolism, whereas metformin is not metabolized and is eliminated via filtration at the glomerulus and excreted in the urine unchanged. Ertugliflozin is not anticipated to affect OCT2 activity at clinical exposures; hence ertugliflozin is not anticipated to affect metformin exposures. Metformin does not inhibit or induce metabolizing enzymes involved in ertugliflozin metabolism; thus metformin is not anticipated to affect ertugliflozin exposures.

Ertugliflozin

Ertugliflozin protein binding is high in all species examined (human, dog, rat, and mouse) ranging from 94 to 97%. Significant species differences are observed with an oral absorption range from 50% in humans to 78% in rats and 94% in dogs, with moderate to high oral bioavailability. T_{max} is achieved within 30 minutes in mice and 1 hour in humans (fasted), but after 2 hours in humans in the fed state, indicating absorption delays in the presence of food. Systemic exposures follow linear pharmacokinetics with a trend for slight increases in female exposures over time at high doses in rodents, indicating a potential gender effect. Ertugliflozin has a moderate half-life of 4 to 8 hours in rats and dogs, but a long half-life in humans of 12 to 16 hours. The predominant route of elimination of ertugliflozin is via metabolism catalyzed by CYP3A4, CYP3A5, CYP2D6, UGT1A9, and UGT2B7 enzymes. Glucuronidation is the major metabolic pathway *in vivo,* with contribution of the O-desethylation pathway in the rat. Ertugliflozin is primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans.

Ertugliflozin is a weak inhibitor of OCT2 with an IC_{50} value of 917 μM, which is 40,000 times higher than the predicted steady state free drug C_{max} of 0.0231 μM at a dose of 15 mg. Ertugliflozin may be a substrate for P-glycoprotein (P-gp)-mediated efflux, but is not affected by P-gp inhibitors. Thus P-gp is unlikely to be a limiting factor in Ertugliflozin absorption. Ertugliflozin has a moderate volume of distribution in rats with the highest distribution to bladder, liver, kidney, adrenal gland, Harderian gland, and pancreas. Ertugliflozin crosses the blood-brain barrier, but only reaches concentrations 3 to 63-fold lower than that of blood; whereas distribution to the choroid plexus and pituitary gland is 2-fold greater than blood.

Metformin

Metformin is absorbed slowly with an oral bioavailability of 50-60% under fasting conditions. For the immediate-release formula, C_{max} is reached in 1 to 3 hours; whereas C_{max} is achieved 4 to 8 hours post-dose with the extended-release formula. Steady state is reached in 1 to 2 days. Metformin is not metabolized or subject to biliary excretion, but is a substrate of renal transporter OCT2 and cleared by renal tubular secretion with an elimination half-life of 6.2 hours. However, metformin accumulates in red blood cells where it has an elimination half-life of 17.6 hours. Metformin pharmacokinetics is similar in pediatrics (12 to 16 years) and adults.

6 General Toxicology

Overall toxicology summary

In accordance with the ICH Guidance for Industry Nonclinical Safety Evaluation of Drug or Biologic Combinations, the sponsor submitted two 3-month repeat dose toxicity study in rats with co-administration of ertugliflozin and metformin or ertugliflozin and sitagliptin under IND #106447.

13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats (Study #TT147809 / #8300339 / #14GR164)

PF-04971729 (mg/kg) + Metformin (mg/kg): 0+0, 0+600, 25+0, 5+200, 5+600, 25+200, & 25+600

Study #	TT147809
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	7/14/2014
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729: lot #E010014849, 76.0% potency Metformin: lot #WL00040809, 100% potency

Key Study Findings

- PF-04971729 + Metformin (specific to the combination):
  - Drug-related ↓body weight and ↓weight gain in males with co-administration of ≥5 mg/kg PF-04971729 and 600 mg/kg metformin in males.
  - Clinical chemistry (♂&♀): ↓Na (♀) and ↓Creatinine (♂)
  - ↑heart organ weight
- PF-04971729:
  - Erosion/ulcer of glandular stomach (♂&♀) and discoloration of glandular stomach mucosa (♀)
  - ↓Pancreatic acinar cell zymogen granules (♂)
  - ↑Food consumption (♂&♀)
  - Clinical chemistry: ↓glucose (♂&♀),↓Cl (♂&♀), ↓Ca (♀),↑BUN (♂&♀)
  - Urine: ↑specific gravity, ↑volume, ↑glucose, and ↓pH
  - Kidney:
    - ↑Kidney organ weight in all PF-04971729 treatment groups (♂ & ♀), exacerbated by HD metformin
    - Tubule dilatation in all PF-04971729 treatment groups (♀)
  - Adrenal Gland
    - Adrenal cortex hypertrophy with 25 mg/kg PF-04971720 (♂ & ♀) and exacerbated by co-administration with 600 mg/kg metformin (♀)
    - ↑Organ weight in females at the co-administration high dose.
- Metformin:
  - Hypertrophy and ↓cytoplasmic granules of salivary gland duct epithelium (♂&♀)
  - ↑Adrenal gland organ weight at HD, exacerbated by HD PF-0971729
  - ↑Liver organ weight at HD (♀), exacerbated by HD PF-0971729

SD Rat, 13 Weeks	NOAEL (AUC)	Multiple of MRHD*
No significant adverse systemic toxicities	25 mg/kg PF-04971729 (91200 ng·h/mL) + 600 mg/kg Metformin (135000 ng·h/mL)	PF-04971729: **67x** Metformin: **5x**

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with an ertugliflozin exposure of AUC = 1.37 μg·h/mL and metformin exposure of AUC = 25 μg·h/mL

Reviewer's Comments

The NOAEL was set at the high combination dose of 25 mg/kg PF-04971729 and 600 mg/kg metformin due to lack of significant adverse systemic toxicities. The safety margins for PF-04971729 are 67x MRHD based on AUC ($MRHD_{AUC}$) for the proposed clinical high dose of 15 mg/day. Although the safety margin for metformin is only 5x $MRHD_{AUC}$, it is consistent with current approved dosage and exposure levels for metformin.

Reduced serum glucose, increased urinary volume and urine glucose excursion (glucosuria) underlie most of the findings in this study, which include reduced body weight and increased food consumption, as well as histopathology changes in the kidney, adrenal gland, and pancreas. Overall, the key findings associated with PF-04971729 in this study are consistent with administration of PF-04971729 alone for 1, 3, and 6 months in rats. Similarly, findings associated with co-administration of PF-04971729 and metformin, as well as metformin alone, are consistent with findings in the 2-week co-administration study in rats (study #8294466/13GR341). Overall, there were no new significant drug-related toxicities and the majority of the findings are considered to be secondary to the pharmacodynamic activity of PF-04971720.

Decreases in body weight gain with reciprocal increases in food consumption are consistent with observations from previous studies with PF-04971729 administration in rats. Thus, these findings are considered to be drug-related. It is noted that decreases in body weights and weight gains were exacerbated with co-administration of metformin. Furthermore, further increases in food consumption were reported with co-administration. Thus, the drug-related effects on reduced body weight and weight gain, as well as increased food consumption, were considered to be exacerbated with co-administration of metformin.

Increases in glucose levels in the urine are indicative of glucosuria and, along with reciprocal decreases in blood glucose levels, reflect PF-04971729-mediated inhibition of SGLT2 and reduced renal tubular reabsorption of glucose from the glomerular filtrate. Since SGLT2 is a sodium (Na)/glucose co-transporter, decreases in plasma Na levels are also consistent with the pharmacodynamic activity of PF-04971729. It is noted that decreases in blood Na levels only reached statistical significance with co-administration of both drugs, indicating exacerbation of reduced Na levels with metformin co-administration. During osmotic diuresis, the electrolytes Na, chloride (Cl), and potassium (K) are excreted, which is consistent with the observed drug-related reductions in electrolyte concentrations in the blood. Also, the observed increases in urine volume, urine specific gravity and blood urea nitrogen (BUN) levels, as well as decreases in urinary pH, are consistent with osmotic diuresis resulting from glucosuria. Decreases in blood creatinine levels were observed with administration of either drug alone in females and were exacerbated in both sexes with co-administration, indicating that at the highest doses, PF-04971729 and metformin work synergistically or additively together to reduce creatinine levels. Importantly, reduction of blood creatinine levels indicates that kidney damage is not present. Since increases in BUN levels correlate with increases in urine volume while creatinine levels were not increased, the observed increases in BUN levels are likely to be secondary to dehydration resulting from glucosuria. Thus, there were no clear signs of kidney dysfunction. Overall, the metabolic changes observed with PF-04971729 treatment and co-administration of metformin were considered to be anticipated pharmacodynamic secondary effects and were not considered to be adverse.

Findings of increased kidney weights and histopathological findings of minimal to marked tubular dilatation in the kidney are also consistent with osmotic diuresis and are considered to be secondary to the pharmacodynamic activity of PF-04971729. Since dilatation of renal tubules reflects a non-toxic compensatory response to glucosuria, this finding is considered to be non-adverse.

Pancreatic and stomach findings were attributed to PF-04971729. Findings of discolored stomach and/or erosion were observed in animals treated with PF-04971729 in the absence or presence of metformin. Similar stomach findings have been described in previous toxicology studies with PF-04971729, but are likely to be reversible and are not considered to be adverse. Decreases in pancreatic zymogen granules were also described in previous toxicology studies with PF-04971729 alone or in combination with metformin, but are considered to be non-adverse. Overall, the stomach and pancreatic findings are likely to be secondary to drug-related increases in food consumption and/or off target inhibition of SGLT1.

Decreased blood calcium levels are consistent with glycosuria and are likely to be secondary to the pharmacodynamic activity of PF-04971729. As calcium reabsorption in the proximal tubule follows water reabsorption, glycosuria is generally associated with increased calcium excretion. Furthermore, there were no abnormal bone findings in this study, unlike many other SGLT2 inhibitors that cause hyperostosis of bone. However, in the 6-month rat toxicology study, increased trabecular bone was observed with

longer exposures to 25 mg/kg PF-0491729 in males. Thus, the decreased serum calcium levels observed in this study may be indicative of early drug-related bone toxicity.

Epithelial hypertrophy and cytoplasmic granule changes in the mandibular and sublingual salivary glands are known non-adverse effects of metformin. Furthermore, the salivary gland findings were not exacerbated by PF-04971729 administration.

Adrenal gland hypertrophy was reported in both sexes and was associated with increased incidence and severity with the highest doses of co-administration in females, which also correlated with increased adrenal gland weights. These findings are consistent with previous studies with PF-04971729 in rats and dogs. The adrenal gland findings may be due to a compensatory response to fluid and electrolyte losses related to the PF-04971729-induced glucose excursion. The adrenals are also known target organs of metformin toxicity, which is consistent with the appearance of the increases in adrenal gland weights being primarily driven by 600 mg/kg metformin. Overall, it is likely that metformin and PF-04971729 work together to increase the adrenal gland organ weights and hypertrophy. Nevertheless, these effects are no considered to be adverse.

Increases in liver organ weights are consistent with previous studies with PF-0971729. Liver disease has been associated with long-term metformin use. However, there were no signs of liver damage or dysfunction in this study. Therefore, this finding is considered to be non-adverse.

Increases in heart organ weights (↑17-29%) were observed in females treated with both 25 mg/kg PF-0971729 and 600 mg/kg metformin. Although the heart is a known target organ of metformin administration, significant increases in heart organ weights were not observed in animals treated with metformin alone. Thus, the data indicate that the increase in heart weight was dependent on co-administration of high doses of both drugs and may be an additive effect. However, there were no signs of cardiac hypertrophy, heart damage or dysfunction in this study and the increase in heart weight was considered to be non-adverse. It is noted that heart myonecrosis was observed in both sexes at 250 mg/kg PF-0871729 in the 3-month rat study with a NOAEL of 25 mg/kg, but was not reported in the 6-month rat study, which evaluated doses up to 100 mg/kg. Thus, the NOAEL for heart myonecrosis is considered to be 100 mg/kg with a wide safety margin of ~300x $MRHD_{AUC}$.

Co-administration of PF-04971729 did not appear to affect metformin exposures. However, PF-04971729 exposures were dose-dependently lowered by up to 60% with increasing metformin dose. Thus, animals in the highest co-administration group (25+600) had the lowest PF-04971729 exposures of all groups receiving 25 mg/kg PF-04971729. Although the sponsor did not recognize an effect of metformin on PF-04971729 exposures, this observation is consistent with results from the 2-week co-administration study in rats with metformin. The decrease in PF-04971729 exposures with co-administration may explain the decrease in some effects driven by PF-04971729 in groups receiving co-administration.

Methods

Doses	PF-04971729 (mg/kg) + Metformin (mg/kg): 0+0, 0+600, 25+0, 5+200, 5+600, 25+200, and 25+600
Frequency of dosing	Once daily for 91 days. Animals were dosed 1st with PF-04971729, then dosed 2 minutes later with metformin 2nd.
Route of administration	Oral gavage
Dose volume	5 mL/kg PF-04971729 + 5 mL/kg metformin = 10 mL/kg total
Formulation/Vehicle	Vehicle #1 (PF-04971729): 0.5% (w/v) methylcellulose, 10% (v/v) polyethylene glycol 400 (PEG 400) Vehicle #2 (Metformin): 0.5% (w/v) methylcellulose
Species/Strain	Crl:CD(SD) rats, (b) (4)
Number/Sex/Group	10/sex/group
Age	6-7 weeks
Weight	♂: 163-278 g ♀: 155-198 g
Satellite groups	TK animals including 4/sex/group
Unique study design	Co-administration of PF-04971729 and metformin
Deviation from study protocol	Day 14: two 0+600 TK ♂'s and one 25+600 TK ♀ did not receive the dose of metformin. Day 28: one 0+600 TK ♀ and one 25+0 TK ♂ did not receive the dose of PF-04971729 vehicle. Day 41: all 0+0 animals were dosed with vehicle #2 twice. Day 64: one 0+600 TK ♂ did not receive the dose of PF-04971729. Day 6-87: various animals (13 total) across most groups received the metformin dose 9-10 minutes after the PF-04971729 dose instead of after 2 minutes.

Study Design

		No. of Animals		PF-04971729 Dose		Metformin Dose	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	10	10	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Control/	1 (Toxicity)	10	10	0	0	600	120
High)	2 (Toxicokinetic)	4	4	0	0	600	120
3 (High/	1 (Toxicity)	10	10	25	5	0	0
Control)	2 (Toxicokinetic)	4	4	25	5	0	0
4 (Low/	1 (Toxicity)	10	10	5	1	200	40
Low)	2 (Toxicokinetic)	4	4	5	1	200	40
5 (Low/	1 (Toxicity)	10	10	5	1	600	120
High)	2 (Toxicokinetic)	4	4	5	1	600	120
6 (High/	1 (Toxicity)	10	10	25	5	200	40
Low)	2 (Toxicokinetic)	4	4	25	5	200	40
7 (High/	1 (Toxicity)	10	10	25	5	600	120
High)	2 (Toxicokinetic)	4	4	25	5	600	120

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg metformin (or Vehicle Control Article 2, as applicable).
b PF-04971729 dose concentrations were corrected for lot specific potency of 0.760 (76.0%). A correction factor of 1.316 was used for Lot No. E010014849.
c No correction factor was need for metformin dose concentrations. Dose levels and concentrations were expressed as the salt form of Test Article 2.
d Group 1 received Vehicle Control Articles 1 and 2 only.

Parameters Measured

Clinical Findings	Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. Detailed observations were conducted on all animals prior to dosing on Day 1, weekly during the dosing phase, and on Day 91. Cageside observations were also conducted at 1 hour postdose.
Body weights	Animals were weighed once during the predose phase, prior to dosing of Day 1, weekly thereafter, and on Day 91.
Food consumption	Food consumption was quantified for each cage weekly, beginning on Day 1, for Weeks 1-13 and Days 85-91.
Ophthalmoscopy	Ophthalmic examinations were conducted by a veterinarian using an indirect ophthalmoscope and a mydriatic agent once during the predose phase and during Week 13 of the dosing phase.
EKG	Not evaluated
Hematology	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.2 Hematology Tests** red blood cell (erythrocyte) count hemoglobin hematocrit mean corpuscular volume mean corpuscular hemoglobin mean corpuscular hemoglobin concentration platelet count white blood cell (leukocyte) count differential blood cell count blood smear reticulocyte count mean platelet volume red blood cell distribution width **3.5.1.3 Coagulation Tests** prothrombin time activated partial thromboplastin time
Clinical chemistry	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.4 Clinical Chemistry Tests** glucose urea nitrogen creatinine total protein albumin globulin albumin:globulin ratio cholesterol total bilirubin alanine aminotransferase alkaline phosphatase gamma glutamyltransferase aspartate aminotransferase calcium inorganic phosphorus sodium potassium chloride
Urinalysis	Urine samples were collected at necropsy on Day 92 **3.5.1.5 Urinalysis Tests** appearance (clarity and color) bilirubin blood glucose ketones microscopic examination of sediment pH protein specific gravity urobilinogen volume
Gross pathology	Animals were fasted overnight and necropsied on Day 92. External features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues were examined.
Organ weights	Organ weights were measured (W) according the table below. Paired organs were weighed together.
Histopathology	Tissues were collected from all animals and prepared (P) by preserving in 10% neutral-buffered formalin (NBF), embedding in paraffin, sectioning, and staining with hematoxylin and eosin (H&E). All tissues in Groups 1 (0+0), 2 (0+600), 3 (25+0), and 7 (25+600) were examined microscopically. The kidneys, mandibular

salivary gland, sublingual salivary gland, pancreas, glandular stomach, and adrenal cortex from Groups 4 (5+200), 5 (5+600), and 6 (25+200) were also examined microscopically.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		P,E
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	salivary gland (mandibular [2])		P,E
epididymis (2)	W	P,E	salivary gland (sublingual [2])		P,E
esophagus		P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
eye (2)[b]		P,E	seminal vesicle		P,E
femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	skin/subcutis {skin and adnexa}		P,E
gross lesions		P,E	spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
gut-associated lymphoid tissue {GALT}		P,E	spleen	W	P,E
Harderian gland[b]		P,E	sternum with bone marrow		P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[b]	W	P,E
jejunum		P,E	thymus	W	P,E
kidney (2)	W	P,E	thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
larynx			tongue		P,E
liver	W	P,E	trachea		P,E
lungs with large bronchi {lung}		P,E	ureter		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	urinary bladder		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	uterus		P,E
mammary gland (males and females)		P,E	vagina		P,E

E = Examined microscopically; P = Processed; W = Weighed.
a Brain was sectioned according to published recommendations (Bolon et al., 2013).
b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Bone marrow smears were prepared from the femur, but were not examined.

Toxicokinetics: Non-fasted blood samples were collected from all groups (2 animals/time point/group) on Days 1 and 91 at 1, 4, 7, and 24 hours postdose.

Observations and Results

Mortality

There were 2 female mortalities, a female in the 25+200 treatment group found dead on Day 74 and a TK female in the 0+600 group found dead on Day 10. There were no clinical signs in either animal before death. Although moderate lung congestion was identified in the 25+200 female, a cause of death was not determined. The cause of death in the TK female could not be determined either. Given the lack of relation to dose in the 2 mortalities and inconsistency with drug-related findings in other animals, it is unlikely that these deaths were drug-related.

MORTALITY					
Dose Group	**Day**	**ID**	**Cause of Death**	**Clinical signs**	**Pathology**
25+200	74	♀ #B09853	undetermined	Found dead, but no clinical signs	Slight-minimal protein cast and mineralization in kidney, moderate lung congestion, minimal Harderian gland mononuclear cell infiltrate, slight ↓cytoplasmic granules in mandibular salivary gland
0+600	10	TK ♀ #B09806	undetermined	Found dead, but no clinical signs	No macroscopic findings. Histology was not performed

Clinical Signs

No drug-related findings.

Body Weights

In males that received metformin, body weights and body weight gains decreased in a dose-dependent manner as co-administration of PF-04971729 increased, reaching statistical significance in animals co-treated with ≥5 mg/kg PF-04971729 and 600 mg/kg metformin (↓12% and ↓20%). In males that received PF-04971729, body weights and body weight gains decreased in a dose-dependent manner as co-administration of metformin increased, reaching statistical significance in animals co-treated with 600 mg/kg metformin. Furthermore, the decreases in male body weights and body weight gains with co-administration were more than the sum of each drug's effect alone, indicating a synergistic effect.

Female body weights (↓9%) and weight gains (↓19%) were significantly lower in females treated with 25 mg/kg PF-04971729 alone; however, there were no statistically significant decreases in animals receiving both PF-04971729 and metformin. Thus, there was no significant or adverse effect of metformin co-administration on female body weights.

MALES: Body Weight				
Study Time	Dose (mg/kg+mg/kg)	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	+330	-	-
	0+600	+301	91.2% (↓8.8%)	94.9% (↓5.1%)
	25+0	+316	95.8% (↓4.2%)	97.8% (↓2.2%)
	5+200	+306	92.7% (↓7.3%)	96.4% (↓3.6%)
	5+600	+263*	79.7% (↓20.3%)	88.0%* (↓12.0%)
	25+200	+296	89.7% (↓10.3%)	95.1% (↓4.90%)
	25+600	+241*	73.0% (↓27.0%)	84.4%* (↓15.6%)
FEMALES: Body Weight				

Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	+119	-	-
	0+600	+121	101.7%	100%
	25+0	+96*	80.7% (↓19.3%)	91%* (↓9.0%)
	5+200	+106	89.1% (↓10.9%)	94.3% (↓5.7%)
	5+600	+106	89.1% (↓10.9%)	95.0% (↓5.0%)
	25+200	+119	100%	98.7% (↓1.3%)
	25+600	+108	90.8% (↓9.2%)	96.0% (↓4.0%)

Sponsor's Figure 1: Body Weights - Co-administration with Metformin

Figure 7.1: Mean Body Weight Data - Males

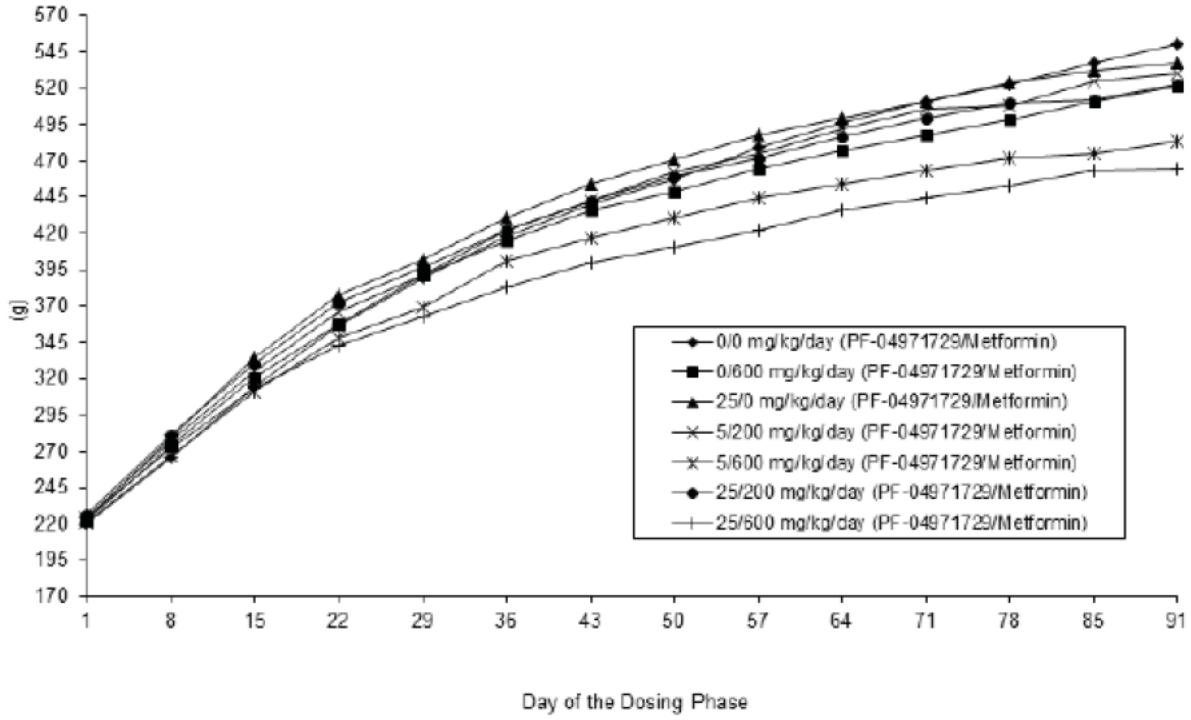
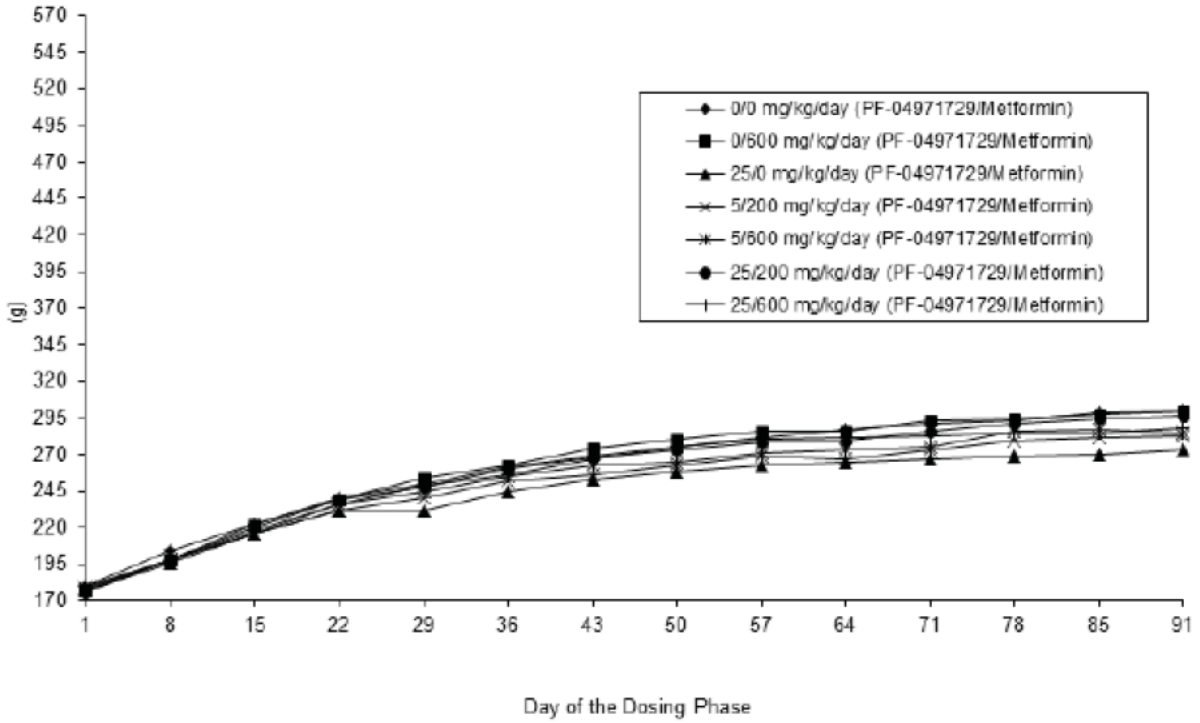


Figure 7.2: Mean Body Weight Data - Females



(Figures excerpted from sponsor's package)

Feed Consumption

After the 1st week of dosing, food consumption was significantly increased in males (↑15-27%) and females (↑11-39%) receiving either PF-04971729 alone or in combination with metformin. In males, increased food consumption was increased dose-dependently with regard to PF-04971729 dose, but was independent of the metformin dose. In females, there was not a direct PF-04971729 dose-dependency; however, there was a dose-dependent increase with regard to the co-administration of both PF-0497179 and metformin.

Food Consumption				
Dose, mg/kg	Males		Females	
	Consumption (g/animal/day)	% Control	Consumption (g/animal/day)	% Control
0+0	26	-	18	-
0+600	27	103.8%	19	105.6%
25+0	33*	126.9%	20*	111.1%
5+200	31*	119.2%	20*	111.1%
5+600	30*	115.4%	21*	116.7%
25+200	33^	126.9%	22*	122.2%

25+600	32*	123.1%	25^	138.9%

* p value <0.05
^ excluded from Day 1 to Day 91 mean statistical analysis, but p value <0.05 at weekly time points

Ophthalmoscopy

No drug-related findings.

Hematology

Statistically significant decreases in red blood cell (RBC) counts (↓5-7%), hematocrit (Hct) percentage (↓4-6%), or hemoglobin (Hg, ↓5%) were noted in females in various groups treated with PF-04971729 alone or in combination with 200 mg/kg; however, there was no clear consistency or dose-dependency in RBC parameter changes, no significant correlating changes in female reticulocyte counts, and no effect in males. On the other hand, a statistically significant decrease in reticulocytes (↓30%) was observed in males in the highest co-treatment group (25+600). Although statistically significant, the observed changes in hematology parameters remained within the normal biological range for this species and are not considered to be biologically significant.

RBC Parameters								
Dose (mg/kg PF-04971729 + mg/kg Metformin)	RBC (10^6/uL)		Hct (%)		Hb (g/dL)		Retic. (10^3/µl)	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	9.62	9.21	55.4	54.0	16.6	16.6	198.0	156.7
0+600	9.62	9.27	55.3	54.7	16.5	16.6	194.7	160.9
25+0	9.58	8.89 (↓3.5%)	55.7	50.9* (↓5.7%)	17.1	15.8* (↓4.8%)	172.4	150.3
5+200	9.60	8.74* (↓5.1%)	55.2	51.7* (↓4.3%)	16.7	16.0	178.7	173.7
5+600	9.86	9.26	56.0	54.3	16.9	16.6	175.3 (↓11.5%)	152.0
25+200	9.66	8.60* (↓6.6%)	55.9	51.1* (↓5.4%)	16.8	15.8* (↓4.8%)	189.6	192.2
25+600	9.81	9.05 (↓1.7%)	56.1	54.1	16.9	16.5	139.5* (↓29.5%)	168.1

* p value < 0.05

Clinical Chemistry

Drug-related decreases in blood chloride levels were observed in both sexes, reaching statistically significant changes of ≥2% in PF-04971729 alone (↓2-4%) and combination treatment (↓2-6%) groups. In males, decreases in chloride were primarily dose-related with regard to PF-04971729, and secondarily dose-related to metformin in the presence of co-treatment with 25 mg/kg PF-04971729. In females, decreases in chloride were dose-dependent with regard to both PF-04971729 and metformin treatment.

Drug-related decreases in blood sodium levels (1.4-2%) were observed in females, reaching statistical significance with co-treatment of both PF-04971729 and metformin. Although decreases in sodium may be anticipated with SGLT2 inhibition by PF-04971729, the data suggest that the decreases in sodium may have been more predominantly driven by metformin than PF-04971729. Furthermore, there were no significant changes in sodium levels in males, indicating a gender effect.

Significant decreases in blood calcium levels were observed in females treated with 25 mg/kg PF-04971729 alone (↓4%) or in combination with metformin (↓4-6%). Furthermore, there was a dose-

dependent trend with regard to PF-04971729, as well as exacerbation at the highest doses of both drugs. However, there were no significant changes in calcium levels in males.

Blood levels of inorganic phosphorous (PHOS) were significantly reduced by 15-16% in females co-treated with PF-04971729 and 200 mg/kg metformin. However, PHOS levels were similar in animals treated with PF-04971729 alone, metformin alone, or PF-04971729 in combination with 600 mg/kg metformin. Therefore, although statistically significant, there was not a consistent or dose-dependent decrease in PHOS, nor were there any significant changes in males.

Electrolytes								
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**Chloride (mmol/L)**		**Sodium (mmol/L)**		**Calcium (mg/dl)**		**PHOS (mg/dl)**	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	103	103	147	147	10.6	11.0	7.3	6.7
0+600	102 (↓1.0%)	102 (↓1.0%)	147	145 (↓1.4%)	10.7	11.2	7.6	6.9
25+0	99* (↓3.9%)	101* (↓1.9%)	146	146 (↓0.7%)	10.5	10.6* (↓3.6%)	6.9	6.3 (↓6.0%)
5+200	100* (↓2.9%)	101* (↓1.9%)	147	145 (↓1.4%)	10.5	10.7 (↓2.7%)	7.2	5.6* (↓16.4%)
5+600	100* (↓2.9%)	99* (↓3.9%)	147	144* (↓2.0%)	10.5	10.8 (↓1.8%)	7.5	6.7
25+200	98* (↓4.9%)	99* (↓3.9%)	146	145* (↓1.4%)	10.3	10.6 (↓3.6%)	7.5	5.7* (↓14.9%)
25+600	97* (↓5.8%)	97* (↓5.8%)	146	145* (↓1.4%)	10.5	10.4* (↓5.5%)	7.6	6.7

Decreases in steady-state fasting glucose levels were observed in both sexes with all treatments, reaching statistical significance in animals treated with PF-04971729 alone or in combination with metformin. Decreases in glucose were predominantly driven by PF-04971729 administration, but were exacerbated by co-administration with metformin in a dose-independent manner.

Significant decreases in the kidney marker creatinine (CREA) were observed in both sexes, reaching a 17% decrease in males and a 29% decrease in females treated with the highest doses of both drugs (25+600). In females, CREA levels were similarly reduced in all drug-treatment groups with the exception of the highest co-administration dose which was exacerbated. In males, CREA levels were only reduced in animals co-treated with PF-04971729 and 600 mg/kg metformin. These data indicate that at the highest doses, PF-04971729 work synergistically or additively together to reduce CREA levels.

Statistically significant increases in BUN levels were observed in both sexes of animals treated with PF-04971729 alone or in combination with metformin. Furthermore, BUN levels were at or above the upper limit of normal (≥20 mg/dL) in groups receiving 25 mg/kg PF-04971729. Although the increases in BUN levels were highest in animals treated with 25 mg/kg PF-04971729 alone (↑2-fold), they were lower with increasing doses of metformin, which also mirrors the decrease in PF-04971729 exposures with co-administration. Thus, these data suggest that increases in BUN levels were primarily driven by PF-04971729 exposure.

Glucose & Kidney Markers						
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**Glucose (mg/dL)**		**CREA (mg/dL)**		**BUN (mg/dL)**	
	♂	♀	♂	♀	♂	♀
0+0	94	95	0.6	0.7	14	16
0+600	88 (↓6.4%)	89 (↓6.3%)	0.6	0.6* (↓14.3%)	13	13
25+0	69* (↓26.6%)	75* (↓21.1%)	0.6	0.6* (↓14.3%)	28* (↑2-fold)	23* (↑43.8%)
5+200	73* (↓22.3%)	86* (↓9.5%)	0.6	0.6* (↓14.3%)	19* (↑35.7%)	16
5+600	70* (↓25.5%)	84* (↓11.6%)	0.5 (↓16.7%)	0.6* (↓14.3%)	18* (↑28.6%)	13
25+200	58* (↓38.3%)	66* (↓30.5%)	0.6	0.6* (↓14.3%)	24* (↑71.4%)	20* (↑25.0%)
25+600	61* (↓35.1%)	68* (↓28.4%)	0.5* (↓16.7%)	0.5* (↓28.6%)	20* (↑42.9%)	17 (↑6.3%)

Minimal, yet statistically significant, increases in ALT levels were reported in males (↑21-50%) and females (↑10-45%) treated with 25 mg/kg PF-04971729 alone or in combination with metformin. However, there was not a clear dose-response. Furthermore, the increases were considered to be minimal and within or near the normal biological range for this species.

A statistically significant decrease in blood cholesterol levels (↓22%) was reported in females at the high doses of both treatments (25+600). Trends for decreases in total protein (↓8%) and albumin (↓10%) were also reported at the highest doses tested (25+600). However, cholesterol and protein levels remained within the normal biological range for this species.

ALT, Protein & Cholesterol								
Dose (mg/kg PF-04971729 + mg/kg Metformin)	**ALT (U/L)**		**Total Protein (g/dL)**		**Albumin (g/dL)**		**Cholesterol (10^3/µl)**	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	38	31	7.2	7.9	4.3	5.1	69	97
0+600	32	33	7.2	7.7	4.3	5.0	73	102
25+0	46* (↑21.1%)	34* (↑9.7%)	7.2	7.8	4.4	5.0	71	89
5+200	55 (↑44.7%)	31	7.0	7.8	4.2	5.0	57	85
5+600	48* (↑26.3%)	45* (↑45.2%)	7.0	7.6	4.3	4.9	65	83
25+200	44 (↑15.8%)	36* (↑16.1%)	7.1	7.8	4.3	5.0	53	101
25+600	57* (↑50%)	39* (↑25.8%)	6.8 (↓5.6%)	7.3 (↓7.6%)	4.2	4.6 (↓9.8%)	55 (↓20.3%)	76* (↓21.6%)

Urinalysis

Marked glucose levels were present in nearly all animals treated with ≥5 mg/kg PF-04971729.

Increases in urine specific gravity were reported in all animals treated with ≥5 mg/kg PF-04971729, independent of metformin co-administration, although statistical significance was only achieved in females.

Significant increases in total urine volume were as high as 2.5 to 3.4-fold in males treated with ≥5 mg/kg PF-04971729 independent of metformin co-administration. Trends for smaller increases in urine volume of 35% to 67% were reported in females treated with 25 mg/kg PF-04971729 independent of metformin co-administration, but did not achieve statistical significance.

Decreases in pH were also reported in males (↓3-8%) and females (↓3-9%) treated with PF-0471729 alone or in combination with metformin; however, statistical significance is uncertain since a statistical analysis was not performed.

Urine Parameters						
Dose (mg/kg PF-04971729 + mg/kg Metformin)	Specific Gravity		Volume (mL)		pH^	
	♂	♀	♂	♀	♂	♀
0+0	1.037	1.019	9.3	11.6	6.7	6.7
0+600	1.031	1.025	15.1	12.8	6.8	6.4
25+0	1.047 (↑1.0%)	1.048* (↑2.8%)	24.5* (↑2.6-fold)	16.4 (↑41.4%)	6.2 (↓7.5%)	6.3 (↓6.0%)
5+200	1.050 (↑1.3%)	1.051* (↑3.1%)	23.2* (↑2.5-fold)	11.6	6.5 (↓3.0%)	6.4 (↓4.5%)
5+600	1.051 (↑1.4%)	1.044* (↑2.5%)	23.1* (↑2.5-fold)	14.3	6.7	6.5 (↓3.0%)
25+200	1.046 (↑1.0%)	1.048* (↑2.8%)	31.7* (↑3.4-fold)	19.4 (↑67.2%)	6.5 (↓3.0%)	6.2 (↓7.5%)
25+600	1.050 (↑1.3%)	1.051* (↑3.1%)	26.2* (↑2.8-fold)	15.6 (↑34.5%)	6.4 (↓4.5%)	6.1 (↓9.0%)

^ Statistical analysis not performed
* p value < 0.05

Gross Pathology

Discoloration of the glandular stomach mucosa was observed in 25% of females treated with 25 mg/kg PF-04971729 and ≥200 mg/kg metformin, but not with either drug alone. There was not a drug-related increase in abnormal stomach findings in males compared to concurrent controls.

Macroscopic findings of a large kidney were reported in 3 males and 1 female treated with 25 mg/kg PF-04971729 and 200 mg/kg metformin, but not when PF-04971729 was co-administered with 600 mg/kg metformin. Thus, there is not a clear dose-dependency. Nevertheless, increased kidney size is consistent with drug-related increases in kidney weights and tubule dilatation.

MALES (n=10): Macroscopic Findings								
Tissue	Finding	Dose Group (mg/kg PF-04971729 + mg/kg Metformin)						
		0+0	0+600	25+0	5+200	5+600	25+200	25+600
Kidney	Large	0	1	1	1	0	3	0
Stomach	Discolored,	1	0	0	0	0	0	1

	mucosa, glandular							

FEMALES (n=10): Macroscopic Findings								
Tissue	Finding	Dose Group (mg/kg PF-04971729 + mg/kg Metformin)						
		0+0	0+600	25+0	5+200	5+600	25+200	25+600
Kidney	Large	0	0	0	0	0	1	0
Stomach	Discolored, mucosa, glandular	0	0	0	0	0	2	3

Organ Weights

Drug-related increases in kidney weights were observed in both males (↑17-47%) and females (↑24-51%) treated with ≥5 mg/kg PF-04971729 in the absence or presence of metformin. In females, kidney weights were highest in animals treated with the highest doses of both PF-04971729 and metformin (25+600), reaching up to 38-51% higher than concurrent controls. These data suggest that increases in kidney weights are primarily driven by ≥5 mg/kg PF-04971729, but are exacerbated by 600 mg/kg metformin.

Adrenal gland weights were higher in females (↑19-46%) treated with 600 mg/kg metformin, independent of PF-0971729 co-administration, but only reached statistical significance in all 3 weight parameters (absolute, relative body weight ratio and relative brain weight ratio) at the highest doses of both drug, 25 mg/kg PF-04971729 and 600 mg/kg metformin. Furthermore, the greatest increase was observed at the highest co-administration dose, reaching weights 33-46% higher than concurrent controls. These data suggest that increases in adrenal gland weights are primarily driven by 600 mg/kg metformin, but are exacerbated by 25 mg/kg PF-0971729.

Significant increases in female liver weight parameters were observed in all groups treated with 600 mg/kg metformin in the absence or presence of PF-0971729, as well as animals co-treated with 200 mg/kg metformin and 25 mg/kg PF-0971729. There was not a significant increase in all 3 organ weight parameters in groups treated with PF-0971729 alone or with 5 mg/kg PF-0971729 and 200 mg/kg metformin. Furthermore, the highest weights were observed in animals co-treated with the highest doses of both drugs (25+600), reaching an increase of 34-47% above concurrent controls. Together, these data suggest that increases in liver weights are primarily driven by high doses of metformin, but are exacerbated by 25 mg/kg PF-0971729.

Significant increases in all 3 heart weight parameters were observed in females (↑17-29%) treated with both 25 mg/kg PF-0971729 and 600 mg/kg metformin. Although statistically significant increases in all 3 organ weight parameters were not observed in any of the other groups, there was a trend for increased heart weight in ¾ of the other groups treated with metformin. Nevertheless, these data suggest that significant increases in heart weights were dependent on co-administration of both drugs at the highest doses in what was likely to be an additive effect.

Sponsor's Table 7: PF-04971729 + Metformin Co-administration - Organ Weights

Text Table 4.1: Test Article-Related Changes in Organ Weight Parameters in Males

Sex		Males						
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Terminal Body Weight (g)	522	0.94x	0.95x	0.94x	0.85x*	0.92x	0.80x*
Kidney							
Absolute Weight (g)	2.9981	1.08x	1.20x*	1.16x	1.17x*	1.28x*	1.19x*
Body Weight Ratio (%)	0.5765	1.15x*	1.26x*	1.22x*	1.38x*	1.40x*	1.47x*
Brain Weight Ratio (%)	138.3293	1.07x	1.18x*	1.12x	1.18x*	1.25x*	1.20x*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for terminal body weight, absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold control mean value.

Text Table 4.2: Test Article-Related Changes in Organ Weight Parameters in Females

Sex		Females						
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Terminal Body Weight (g)	279	0.99x	0.89x*	0.93x	0.93x	0.96x	0.91x*
Kidney							
Absolute Weight (g)	1.5841	1.04x	1.26x*	1.28x*	1.34x*	1.36x*	1.38x*
Body Weight Ratio (%)	0.5707	1.05x	1.41x*	1.37x*	1.44x*	1.41x*	1.51x*
Brain Weight Ratio (%)	79.4099	1.04x	1.24x*	1.24x*	1.34x*	1.35x*	1.40x*
Adrenal							
Absolute Weight (g)	0.0641	1.23x*	1.08x	1.06x	1.19x	1.17x	1.33x*
Body Weight Ratio (%)	0.0231	1.24x	1.21x	1.13x	1.30x*	1.21x	1.46x*
Brain Weight Ratio (%)	3.2064	1.23x*	1.06x	1.02x	1.19x	1.16x	1.35x*
Liver							
Absolute Weight (g)	7.2772	1.16x*	1.04x	1.12x	1.19x*	1.18x*	1.34x*
Body Weight Ratio (%)	2.6176	1.17x*	1.16x*	1.21x*	1.28x*	1.22x*	1.47x*
Brain Weight Ratio (%)	365.1983	1.16x*	1.02x	1.09x	1.18x*	1.17x*	1.36x*
Heart							
Absolute Weight (g)	1.0113	1.12x	0.98x	1.08x	1.10x	1.05x	1.17x*
Body Weight Ratio (%)	0.3640	1.13x*	1.10x	1.15x*	1.18x*	1.09x	1.29x*
Brain Weight Ratio (%)	50.7567	1.12x	0.96x	1.04x	1.09x	1.05x	1.19*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for terminal body weight, absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold control mean value.

(Tables excerpted from sponsor's report and highlighted)

Histopathology

Battery Considered Adequate? Yes
Peer Review Performed? Yes

Drug-related increases in minimal to moderate kidney tubule dilatation characterized by the presence of dilated renal tubules in the outer medulla were observed in 70% to 100% of animals treated with either

PF-04971729 alone or in combination with metformin. In both sexes, increases in severity were dose-dependent with regard to PF-04971729, but were largely independent of the metformin dose. These findings indicate that drug-related kidney tubule dilation findings were primarily driven by PF-04971729.

Drug-related increases in incidence and severity of pancreatic zymogen granule decreases, resulting in smaller acinar cells and acini, were observed in both sexes. Furthermore, the increases in both incidence and severity were dose-dependent with regard to PF-04971729, but were largely independent of the metformin dose. These findings suggest that drug-related decreases in pancreatic zymogen granules were primarily driven by PF-04971729.

Hypertrophy of the adrenal cortex associated with increases in cell size and cytoplasmic vacuolation was observed in both sexes. In males, minimal hypertrophy of the zona glomerulosa was observed in up to 40% of animals treated with 25 mg/kg PF-04971729 alone, but lacked a clear dose-dependence or consistency with regard to PF-04971729, metformin, or the combination. In females, hypertrophy of the adrenal cortex was consistently observed in 60-80% of animals in the highest co-administration group (15+600), indicating a potential drug-related finding with regard to co-administration.

In males, minimal erosion and/or ulcer of the glandular stomach was reported in 20% of animals that were administered 25 mg/kg PF-04971729 alone or in combination with 600 mg/kg metformin, but not in combination with 200 mg/kg metformin. In females, minimal erosion and/or ulcer was reported in 20% of animals treated with both 25 mg/kg PF-04971729 and 600 mg/kg metformin. Although, there is not a consistent pattern of dose-dependence with regard to either drug alone or in combination, these findings are consistent with previous PF-04971729 toxicology studies and are likely to be drug-related.

Metformin-related increases in incidence and severity of mandibular and sublingual salivary gland findings were apparent in both sexes. Minimal to marked decreases in cytoplasmic granules in the duct epithelium of the mandibular salivary gland were dose-dependent with regard to metformin in both males and females, but were largely independent of the PF-04971729 dose. Metformin-dependent increased incidences of minimal to moderate sublingual salivary gland hypertrophy of the duct epithelium, characterized by enlarged cuboidal to columnar cells with abundant eosinophilic cytoplasm and nuclei, were also observed in both sexes. However, in males, the severity of the salivary gland hypertrophy was increased at the highest dose of both metformin and PF-0471729, indicating that co-administration with 25 mg/kg PF-047179 may increase the severity of this predominantly metformin-driven finding.

Sponsor's Table 8: PF-04971729 + Metformin Co-administration - Histopathology

Text Table 4.4: Incidence and Severity of Test Article-Related Microscopic Findings in Males

	PF-04971729/Metformin						
Sex	Males						
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Number Examined	10	10	10	10	10	10	10
Kidney							
Dilatation, tubule(s)							
Total	5	6	10	9	9	8	10
Minimal	5	6	2	3	5	1	0
Mild	0	0	3	5	3	3	4
Moderate	0	0	4	1	0	3	6
Marked	0	0	1	0	1	1	0
Pancreas							
Zymogen granules, decreased							
Total	0	0	10	3	2	9	10
Minimal	0	0	2	3	2	3	2
Mild	0	0	3	0	0	2	5
Moderate	0	0	5	0	0	4	3
Adrenal Cortex							
Hypertrophy, zona glomerulosa							
Total	0	0	4	3	2	2	0
Minimal	0	0	4	3	2	2	0
Stomach, Glandular							
Erosion/ulcer							
Total	0	0	2	0	0	0	2
Minimal	0	0	2	0	0	0	2
Mandibular Salivary Gland							
Decreased cytoplasmic granules, duct epithelium							
Total	0	9	0	1	10	5	10
Minimal	0	3	0	1	0	2	0
Mild	0	4	0	0	2	3	1
Moderate	0	2	0	0	6	0	8
Marked	0	0	0	0	2	0	1
Sublingual Salivary Gland							
Number Examined	10	10	9	10	10	10	10
Hypertrophy, duct epithelium							
Total	0	8	0	0	8	4	9
Minimal	0	5	0	0	8	4	2
Mild	0	3	0	0	0	0	5
Moderate	0	0	0	0	0	0	2

Text Table 4.5: Incidence and Severity of Test Article-Related Microscopic Findings in Females

	PF-04971729/Metformin						
Sex	Females						
Dose Level PF-04971729 (mg/kg/day)	0	0	25	5	5	25	25
Dose Level Metformin (mg/kg/day)	0	600	0	200	600	200	600
Number Examined[a]	10	10	10	10	10	10	10
Kidney							
Dilatation, tubule(s)							
Total	6	6	10	7	10	7	10
Minimal	6	6	2	6	4	6	3
Mild	0	0	3	1	6	1	4
Moderate	0	0	5	0	0	0	3
Pancreas							
Zymogen granules, decreased							
Total	3	0	10	7	4	8	10
Minimal	2	0	3	3	2	3	0
Mild	1	0	4	4	1	2	6
Moderate	0	0	3	0	1	3	4
Adrenal Cortex							
Hypertrophy, zona glomerulosa							
Total	0	0	4	3	0	2	6
Minimal	0	0	4	3	0	2	6
Hypertrophy							
Total	1	3	0	3	3	1	8
Minimal	1	3	0	3	3	1	8
Stomach, glandular							
Erosion/ulcer							
Total	0	0	0	0	0	0	2
Minimal	0	0	0	0	0	0	2
Mandibular Salivary Gland							
Decreased cytoplasmic granules, duct epithelium							
Total	0	10	0	7	10	9	10
Minimal	0	0	0	2	0	3	0
Mild	0	2	0	5	1	4	1
Moderate	0	5	0	0	3	2	3
Marked	0	3	0	0	6	0	6
Sublingual Salivary Gland							
Number Examined	10	10	10	10	10	9	10
Hypertrophy, duct epithelium							
Total	0	10	0	5	10	4	10
Minimal	0	0	0	5	5	3	1
Mild	0	9	0	0	5	1	9
Moderate	0	1	0	0	0	0	0

a Number examined for all tissues unless noted otherwise.

(Tables excerpted from sponsor's report and highlighted)

Toxicokinetics

In animals treated with 25 mg/kg PF-04971729 alone or in combination with 600 mg/kg, exposures tended to be higher in females. However, given the amount of variability, there were no clear gender effects. Exposures increased dose-proportionally. However, exposures were dose-dependently lower by

up to 60% with increasing metformin dose. Thus, animals in the highest co-administration group (25+600) had the lowest PF-04971729 exposures of all groups receiving 25 mg/kg PF-04971729. T_{max} was generally achieved between 1 and 7 hours postdose, with the exception of 2 animals in the high dose co-administration group (25+600) on Day 1 that achieved T_{max} at 24 hours postdose.

Sponsor's Table 9: PF-04971729 TK

6.1. Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Metformin

Dose PF-04971729 / Metformin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
25 / 0	1	Male	6920	7	99700
		Female	12300	7	172000
		Overall	9610	7	136000
	91	Male	7960	4	104000
		Female	13100	7	186000
		Overall	9740	7	144000
5 / 200	1	Male	1530	4	17300
		Female	1460	4	17100
		Overall	1500	4	17200
	91	Male	2030	1	22200
		Female	2070	1	20200
		Overall	2050	1	21200
5 / 600	1	Male	842	4	9100
		Female	959	4	14100
		Overall	900	4	11600
	91	Male	1600	1	13500
		Female	3770	1	24900
		Overall	2680	1	19200
25 / 200	1	Male	6850	4	83000
		Female	7740	4	87000
		Overall	7290	4	84900
	91	Male	8670	1	110000
		Female	16300	1	130000
		Overall	12500	1	120000
25 / 600	1	Male	4240	24	72600
		Female	4640	4	83200
		Overall	4340	1	77800
	91	Male	10900	1	77900
		Female	10400	1	105000
		Overall	10600	1	91200

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package and highlighted)

Metformin exposures were unaffected by PF-04971729 co-administration. At 600 mg/kg metformin, AUC and C_{max} exposures were 30% and 50-130% higher, respectively, on Day 91 compared to Day 1, indicating potential accumulation at the high dose. There were no apparent gender effects on metformin exposures. T_{max} was generally achieved between 1 and 4 hours postdose.

Sponsor's Table 10: Metformin TK

6.2. Mean Toxicokinetic Parameters for Metformin in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Metformin

Dose PF-04971729 / Metformin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
0 / 600	1	Male	10700	1	130000
		Female	10900	1	81900
		Overall	10800	1	106000
	91	Male	18300	1.00	157000
		Female	13500	1.00	118000
		Overall	15900	1.00	138000
5 / 200	1	Male	5930	4	45900
		Female	6470	1	51300
		Overall	5640	4	48500
	91	Male	6600	1.00	45700
		Female	8680	1.00	50500
		Overall	7640	1.00	48000
5 / 600	1	Male	9830	4	85000
		Female	9330	4	106000
		Overall	9580	4	95600
	91	Male	14400	1.00	111000
		Female	24100	1.00	134000
		Overall	19200	1.00	122000
25 / 200	1	Male	6290	4	60300
		Female	5210	4	38700
		Overall	5750	4	49500
	91	Male	8840	1.00	70500
		Female	8050	1.00	37100
		Overall	8440	1.00	53800
25 / 600	1	Male	8170	1	104000
		Female	11300	1	103000
		Overall	9710	1	104000
	91	Male	24400	1.00	130000
		Female	20700	1.00	139000
		Overall	22500	1.00	135000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package and highlighted)

Dosing Formulation Analysis

PF-04971729 and metformin dose formulations were analyzed using a validated high-performance liquid chromatography (HPLC) method. Overall mean concentrations of PF-04971729 and metformin formulations were within ±10% of the target concentrations.

13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats (Study #TT147808 / #8300338 / #14GR162)

PF-04971729 (mg/kg) + Sitagliptin (mg/kg): 0+0, 0+60, 25+0, 5+20, 5+60, 25+20, & 25+60

Study #	TT147808 / 8300338 / 14GR162
Study report location	eDr
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	7/14/2014
GLP compliance statement	Yes
GLP issues identified	None
QA statement	Yes
Drug, lot #, and % purity	PF-04971729: Lot #E010014849, 76.0% purity Sitagliptin: Lot #010X054, 99.6% purity

Key Study Findings

- PF-04971729 + Sitagliptin (specific to the combination):
  - Potential exacerbation of stomach erosion and discoloration of glandular mucosa in males and hemorrhage in females
  - Adrenal Gland (♂&♀)
    - ↑Adrenal organ weight
    - Exacerbation of zona glomerulosa hypertrophy of the adrenal cortex
  - Prostate mixed cell inflammation
- PF-04971729:
  - Trends for ↓body weight and ↓weight gain
  - ↑Food consumption (♂&♀)
  - Stomach (♂&♀):
    - Discoloration of glandular stomach mucosa
    - Erosion
    - Submucosal inflammation
    - Hemorrhage
  - ↓Pancreatic acinar cell zymogen granules (♂&♀)
  - Clinical chemistry (♂&♀): ↓glucose, ↓Cl, ↓Ca, and ↑BUN
  - Urine (♂&♀): ↑specific gravity, ↑volume, ↑glucose, and ↓pH
  - Kidney (♂&♀):
    - ↑Kidney organ weight
    - Tubule dilatation
    - Pelvic dilatation
  - Adrenal Gland
    - Adrenal cortex hypertrophy with 25 mg/kg PF-04971720 (♂ & ♀)
- Sitagliptin:
  - No biologically significant findings were attributed primarily to sitagliptin

SD Rat, 13 Weeks	NOAEL (AUC)	Multiple of MRHD
No significant adverse systemic toxicities	25 mg/kg PF-04971729 (153000 ng·h/mL) + 60 mg/kg Sitagliptin (24400 ng·h/mL)	PF-04971729*: **112x** Sitagliptin**: **36x**

* Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with an ertugliflozin exposure of AUC = 1.37 μg·h/mL and metformin exposure of AUC = 25 μg·h/mL
** Based on a clinical dose of 100 mg/day (1.67 mg/kg)

Reviewer's Comments

The NOAEL was set at the high combination dose of 25 mg/kg PF-04971729 and 60 mg/kg of the dipeptidyl peptidase IV (DPP-4) Inhibitor, sitagliptin, due to lack of significant adverse systemic toxicities.

The safety margins for PF-04971729 are 112x $MRHD_{AUC}$ for the proposed clinical high dose of 15 mg/day. Clinical co-administration of PF-04971729 with sitagliptin is not currently proposed by the sponsor under this IND. However, a sitagliptin clinical dose of 100 mg/day is being evaluated by the sponsor in Phase 3 clinical studies P005/B1521019, P006/B1521015 and P017/B1521047 under IND #106447. Safety margins based on body surface area (BSA) for sitagliptin were 36x $MRHD_{BSA}$ for a 100 mg/day clinical dose.

Reduced serum glucose, increased urinary volume and glucosuria underlie most of the findings in this study, which include reduced body weight and increased food consumption, as well as histopathology changes in the kidney, adrenal gland, and pancreas. Overall, the key findings associated with PF-04971729 in this study are consistent with administration of PF-04971729 alone for 1, 3, and 6 months in rats. Similarly, findings associated with co-administration of PF-04971729 and sitagliptin, as well as sitagliptin alone, are consistent with findings in the 2-week co-administration study in rats (study #8294467/13GR342). Overall, there were no new significant drug-related toxicities and the majority of the findings are considered to be secondary to the pharmacodynamic activity of PF-04971720.

Decreases in body weight gain with reciprocal increases in food consumption are consistent with observations from previous studies with PF-04971729 administration in rats. Thus, these findings are considered to be drug-related. It is noted that treatment-related decreases in body weights and weight gains, as well as increases in food consumption, were not exacerbated with co-administration of sitagliptin.

Increases in glucose levels in the urine are indicative of glucosuria and, along with reciprocal decreases in blood glucose levels, reflect PF-04971729-mediated inhibition of SGLT2 and reduced renal tubular reabsorption of glucose from the glomerular filtrate. Observed reductions in Ca and Cl electrolyte concentrations in the blood are consistent with osmotic diuresis. Furthermore, the observed increases in urine volume, urine specific gravity and BUN levels, as well as decreases in urinary pH, are also consistent with osmotic diuresis resulting from glucosuria. Since increases in BUN levels correlate with increases in urine volume while creatinine levels were not increased, the observed increases in BUN are likely to be secondary to dehydration resulting from glucosuria. Ultimately, co-administration of sitagliptin did not exacerbate any of the clinical chemistry findings and there were no clear signs of kidney dysfunction. Overall, the metabolic changes observed with PF-04971729 treatment were considered to be anticipated pharmacodynamic effects and were not considered to be adverse.

Findings of increased kidney weights and histopathological findings of minimal to marked tubular dilatation in the kidney are also consistent with osmotic diuresis, which is considered to be secondary to the pharmacodynamic activity of PF-04971729. Since pelvic and renal tubule dilatations reflect non-toxic compensatory responses to glucosuria, these findings are considered to be non-adverse.

Findings of discolored stomach and/or erosion were observed in animals treated with PF-04971729 in the absence or presence of sitagliptin, which are consistent with similar stomach findings that were reversible and described in previous toxicology studies with PF-04971729. Increased incidences of stomach erosion and discoloration findings were reported in males with sitagliptin co-administration, indicating potential exacerbation. Similarly, increased incidences of stomach hemorrhage were reported in females with sitagliptin co-administration, also indicating potential exacerbation. Furthermore, the DPP4 inhibitor drug class is associated with stomach findings of necrosis, ulceration and erosion; thus, exacerbation of the stomach findings may be due to additive toxicities of both PF-04971729 and sitagliptin. It is noted that the stomach findings may be related to secondary increases in food consumption and/or off target inhibition of SGLT1 by PF-4071729. Overall, the stomach and findings are likely to be reversible and are not considered to be a significant adverse systemic toxicity.

Decreases in pancreatic zymogen granules were also described in previous toxicology studies with PF-04971729 alone or in combination with sitagliptin, but are considered to be non-adverse. Overall, the stomach and pancreatic findings are likely to be secondary to drug-related increases in food consumption and/or off target inhibition of SGLT1.

Decreased blood calcium levels are consistent with glucosuria and are likely to be secondary to the pharmacodynamic activity of PF04971729. Furthermore, there were no abnormal bone findings in this study, unlike many other SGLT2 inhibitors that cause hyperostosis of bone. However, in the 6-month rat toxicology study, increased trabecular bone was observed with longer exposures to 25 mg/kg PF-0491729 in males. Thus, the decreased serum calcium levels observed in this study may be indicative of early drug-related bone toxicity.

Adrenal gland hypertrophy was reported in both sexes and was associated with increased incidence and severity with co-administration of sitagliptin in both sexes, which also correlates with increased adrenal gland weights. These findings are consistent with previous studies with PF-04971729 in rats and dogs. The adrenal gland findings may be due to a compensatory response to fluid and electrolyte losses related to the PF-04971729-induced glucose excursion. It is noted that the adrenals are not known target organs of sitagliptin or DPP4 inhibitor drug class toxicity. Nevertheless, the data indicate that it is likely that sitagliptin and PF-04971729 work together to increase severity and incidence rates of adrenal gland organ weight increases and hypertrophy. However, these effects are not considered to be adverse.

There were no abnormal heart organ weight changes or histopathological findings.

Small increases in the incidence rates of minimal to moderate prostate mixed cell infiltration were reported in males with co-administration. The DPP4 inhibitor drug class is associated with cellular infiltration of multiple organs. Although this finding was not dose-related, it is considered likely to be treatment-related with co-administration of PF-04971729 and sitagliptin. Nevertheless, it is also considered to be non-adverse.

Co-administration of PF-04971729 did not affect sitagliptin exposures. PF-04971729 exposures were predominantly unaffected by sitagliptin co-administration. However, it is noted that PF-04971729 exposures were slightly higher on Day 91 by 12-28% with co-administration, indicating a possible trend for accumulation with high doses of both drugs. Nevertheless, given the small amount of increase, it is unclear if this is a significant finding.

Overall, there were no significant toxicities observed with administration of sitagliptin alone. Furthermore, sitagliptin co-administration did not exacerbate or induce any adverse systemic toxicities.

Methods

Doses	PF-04971729 (mg/kg) + Sitagliptin (mg/kg): 0+0, 0+60, 25+0, 5+20, 5+60, 25+20, and 25+60
Frequency of dosing	Once daily for 91 days. Animals were dosed 1st with PF-04971729, then dosed with sitagliptin 2nd within 2 minutes.
Route of administration	Oral gavage
Dose volume	5 mL/kg PF-04971729 + 5 mL/kg sitagliptin = 10 mL/kg total
Formulation/Vehicle	Vehicle #1 (PF-04971729): 0.5% (w/v) methylcellulose, 10% (v/v) polyethylene glycol 400 (PEG 400) Vehicle #2 (sitagliptin): 0.5% (w/v) methylcellulose, 5 mM hydrochloric acid
Species/Strain	Crl:CD(SD) rats, (b) (4)
Number/Sex/Group	10/sex/group
Age	6-7 weeks
Weight	♂: 202-282 g ♀: 153-216 g
Satellite groups	TK animals including 4/sex/group
Unique study design	Co-administration of PF-04971729 and sitagliptin
Deviation from study protocol	On some occasions, some animals received only a partial dose; however, the identity of the animals was not reported. Day 17-87: various animals (11 total) across most groups received the sitagliptin dose more than 2 minutes after the PF-04971729 dose.

Study Design

		No. of Animals		PF-04971729		Sitagliptin	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Dose Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	10	10	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Control/ High)	1 (Toxicity)	10	10	0	0	60	12
	2 (Toxicokinetic)	4	4	0	0	60	12
3 (High/ Control)	1 (Toxicity)	10	10	25	5	0	0
	2 (Toxicokinetic)	4	4	25	5	0	0
4 (Low/ Low)	1 (Toxicity)	10	10	5	1	20	4
	2 (Toxicokinetic)	4	4	5	1	20	4
5 (Low/ High)	1 (Toxicity)	10	10	5	1	60	12
	2 (Toxicokinetic)	4	4	5	1	60	12
6 (High/ Low)	1 (Toxicity)	10	10	25	5	20	4
	2 (Toxicokinetic)	4	4	25	5	20	4
7 (High/ High)	1 (Toxicity)	10	10	25	5	60	12
	2 (Toxicokinetic)	4	4	25	5	60	12

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg sitagliptin (or Vehicle Control Article 2, as applicable).

b PF-04971729 dose concentrations were corrected for lot specific potency of 0.760 (76.0%). A correction factor of 1.316 was used for Lot No. E010014849.

c Sitagliptin dose concentrations were corrected for salt content and lot specific potency of 0.996 (99.6%). A correction factor of 1.285 was used for Lot No. 010X054.

d Group 1 received Vehicle Control Article 1 (0.5% [w/v] methylcellulose [4000 cps] with 10% [v/v] polyethylene glycol 400 prepared in reverse osmosis water) and Vehicle Control Article 2 (0.5% [w/v] methylcellulose [4000 cps] with 5 mM hydrochloric acid prepared in reverse osmosis water) only.

Parameters Measured

Clinical Findings	Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. Detailed observations were conducted on all animals prior to dosing on Day 1, weekly during the dosing phase, and on Day 91. Cageside observations were also conducted at 1 hour postdose.
Body weights	Animals were weighed once during the predose phase, prior to dosing of Day 1, weekly thereafter, and on Day 91.
Food consumption	Food consumption was quantified for each cage weekly, beginning on Day 1, for Weeks 1-13 and Days 85-91.
Ophthalmoscopy	Ophthalmic examinations were conducted by a veterinarian using an indirect ophthalmoscope and a mydriatic agent once during the predose phase and during Week 13 of the dosing phase.
EKG	Not evaluated
Hematology	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.2 Hematology Tests** red blood cell (erythrocyte) count hemoglobin hematocrit mean corpuscular volume mean corpuscular hemoglobin mean corpuscular hemoglobin concentration platelet count white blood cell (leukocyte) count differential blood cell count blood smear reticulocyte count mean platelet volume red blood cell distribution width **3.5.1.3 Coagulation Tests** prothrombin time activated partial thromboplastin time
Clinical chemistry	Fasting blood samples were collected from all animals at necropsy on Day 92. **3.5.1.4 Clinical Chemistry Tests** glucose urea nitrogen creatinine total protein albumin globulin albumin:globulin ratio cholesterol total bilirubin alanine aminotransferase alkaline phosphatase gamma glutamyltransferase aspartate aminotransferase calcium inorganic phosphorus sodium potassium chloride
Urinalysis	Urine samples were collected at necropsy on Day 92 **3.5.1.5 Urinalysis Tests** appearance (clarity and color) bilirubin blood glucose ketones microscopic examination of sediment pH protein specific gravity urobilinogen volume
Gross pathology	Animals were fasted overnight and necropsied on Day 92. External features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues were examined.
Organ weights	Organ weights were measured (W) according the table below. Paired organs were weighed together.
Histopathology	Tissues were collected from all animals and prepared (P) by preserving in 10% NBF, embedding in paraffin, sectioning, and staining with H&E. All tissues in Groups 1 (0+0), 2 (0+60), 3 (25+0), and 7 (25+60) were examined microscopically. The kidneys, ureter, duodenum, pancreas, glandular stomach, adrenal cortex, and prostate from Groups 4 (5+20), 5 (5+60), and 6 (25+20) were also examined microscopically.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		P,E
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	right upper incisor tooth with root		P,E
epididymis (2)	W	P,E	salivary gland (mandibular [2])		P,E
esophagus		P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
eye (2)[b]		P,E	seminal vesicle		P,E
femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	skin/subcutis {skin and adnexa}		P,E
gross lesions		P,E	spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
gut-associated lymphoid tissue {GALT}		P,E	spleen	W	P,E
Harderian gland[b]		P,E	sternum with bone marrow {sternum}		P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[b]	W	P,E
jejunum		P,E	thymus	W	P,E
kidney (2)	W	P,E	thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
larynx			tongue		P,E
liver	W	P,E	trachea		P,E
lower mandible			ureter		P,E
lungs with large bronchi {lung}		P,E	urinary bladder		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	uterus		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	vagina		P,E
mammary gland (males and females)		P,E			

E = Examined microscopically; P = Processed; W = Weighed.

a Brain was sectioned according to published recommendations (Bolon et al., 2013).

b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Bone marrow smears were prepared from the femur, but were not examined.

Toxicokinetics	Non-fasted blood samples were collected from all groups (2 animals/time point/group) on Days 1 and 91 at 1, 4, 7, and 24 hours postdose.

Observations and Results

Mortality

There were no mortalities.

Clinical Signs

There were no drug-related findings.

Body Weights

Male body weight gains were generally lower (↓6-14%) with administration of ≥5 mg/kg PF-04971729 and were associated with lower final body weights (↓3-8%). However, there was not a clear dose-dependent response. Decreases in body weight gains (↓1-9%) and final body weights (↓1-4%) were less pronounced in females and were also independent of dose. Decreases in body weights and weight gains are consistent with PF-04971729-related findings in rats in multiple other studies and are considered to be drug-related. There were no indications of exacerbation by co-administration with sitagliptin.

MALES: Body Weight				
Study Time	Dose (mg/kg+mg/kg)	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	346	-	-
	0+600	347	100.3%	100%
	25+0	325	93.9% (↓6.1%)	96.6% (↓3.4%)
	5+200	302	87.3% (↓12.7%)	92.3% (↓7.7%)
	5+600	307	88.7% (↓11.3%)	93.2% (↓6.8%)
	25+200	298	86.1% (↓13.9%)	92.0% (↓8.0%)
	25+600	318	91.9% (↓8.1%)	95.1% (↓4.9%)
FEMALES: Body Weight				
Study Time	Dose, mg/kg	BW gain (g) over study	% Decrement	BW % control
Day 91 (End of Treatment)	0+0	130	-	-
	0+600	133	102.3%	101.0% (↑1.0%)
	25+0	122	93.8% (↓%6.2)	96.8% (↓3.2%)
	5+200	129	99.2% (↓0.8%)	98.7% (↓1.3%)
	5+600	118	90.8% (↓9.2%)	95.9% (↓4.1%)
	25+200	118	90.8% (↓9.2%)	95.9% (↓4.1%)
	25+600	124	95.4% (↓4.6%)	97.8% (↓2.2%)

Sponsor's Figure 2: Body Weights - Co-administration with Sitagliptin

Figure 7.1: Summary of Body Weight - Males

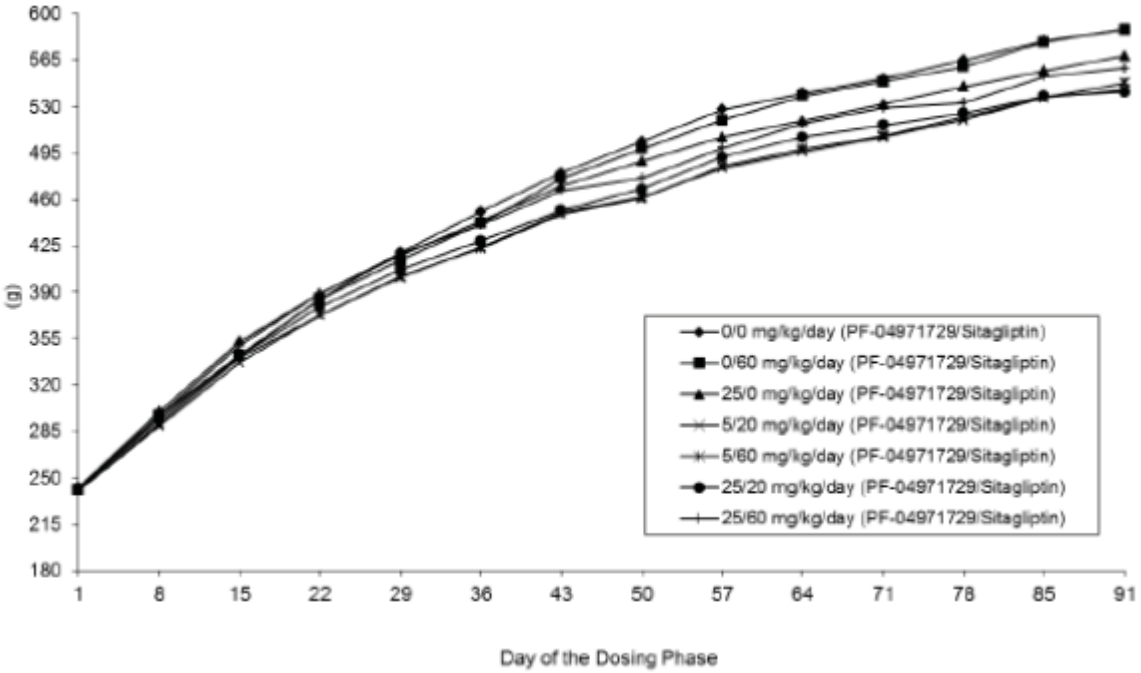
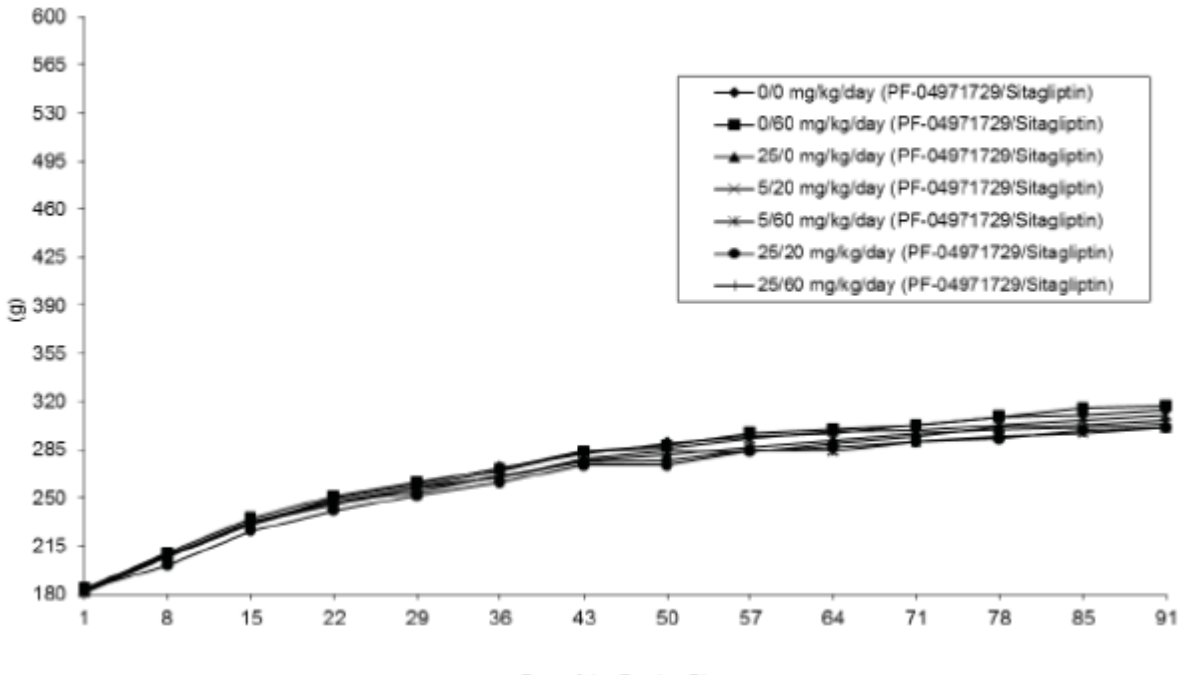


Figure 7.2: Summary of Body Weight - Females



(Figures excerpted from sponsor's package)

Feed Consumption

Food consumption was higher in females (↑11-22%) treated with PF-04971729, but was independent of dose and did not reach statistical significance in all groups due to variability. Similarly, food consumption was also increased in males (↑7-14%) treated with PF-04971729 independent of dose, but did not reach statistical significance in any dose group. Increases in food consumption are consistent with PF-04971729-related findings in rats in multiple other studies and are considered to be drug-related. There were no indications of exacerbation by co-administration with sitagliptin.

Food Consumption

Dose, mg/kg	Males		Females	
	Consumption (g/animal/day)	% Control	Consumption (g/animal/day)	% Control
0+0	29	-	18	-
0+60	28	96.6%	17	94.4%
25+0	32	110.3%	21*	116.7%
5+20	31	106.9%	20	111.1%
5+60	31	106.9%	21	116.7%
25+20	32	110.3%	22*	122.2%
25+60	33	113.8%	21	116.7%

* p value < 0.05

Ophthalmoscopy

There were no treatment-related findings.

Hematology

There were no biologically significant changes in hematology parameters with either drug treatment or co-administration.

Several statistically significant changes in hematocrit, neutrophil counts and prothrombin time were reported in various treatment groups; however, these findings were considered to be of small magnitude, within the normal biological range, and unlikely to be biologically significant. Small 5% decreases in hematocrit (Hct) were observed in females receiving 25 mg/kg PF-04971729 and sitagliptin, but were not observed in males. Decreases of 25-37% in neutrophils (NEUT) were observed in males treated with PF-04971729, but only reached statistical significance in males receiving 25 mg/kg PF-04971729 co-administered with sitagliptin. An 8% decrease in pro-thrombin time (PT) was reported for males treated with 25 mg/kg PF-04971729 and 20 mg/kg sitagliptin, but was not dose-dependent nor observed in females.

Hematology						
Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	**Hct (%)**		**NEUT (10^3/uL)**		**PT (sex)**	
	♂	♀	♂	♀	♂	♀
0+0	52.5	51.5	2.12	0.82	10.8	9.2
0+60	51.4	51.2	2.39	1.05	10.5	9.3
25+0	51.5	49.9	1.54 (↓27.4%)	0.79	10.4	9.2
5+20	52.2	50.7	2.78	0.74	10.2	9.5
5+60	51.4	49.6	1.60 (↓24.5%)	0.65	10.6	9.0
25+20	51.6	49.1* (↓4.7%)	1.32* (↓37.3%)	1.04	9.9* (↓8.3%)	9.0
25+60	51.9	48.8* (↓5.2%)	1.36* (↓35.8%)	1.02	10.6	9.3

* p value < 0.05

Clinical Chemistry

Drug-related decreases in steady-state fasting serum glucose levels were observed with PF-04971729 treatment in both males (↓17-33%) and females (↓7-28%), but were independent of sitagliptin treatment or co-administration. Thus, maintenance of reduced blood glucose levels was attributed to PF-04971729 administration alone. It is noted that the reduced steady-state blood glucose levels were harvested 24 hours postdose and were at or below the lower limit of normal (LLN), but were not within the hypoglycemic range (<50 mg/dL) for this species.

Drug-related increases in BUN levels were observed with PF-04971729 treatment in males (↑57-93%) and females (↑29-79%), reaching levels above the upper limit of normal (ULN) in males treated with 25 mg/kg PF-04971729. The increases were dose-dependent with regard to PF-04971729, but were independent of sitagliptin treatment or co-administration. Thus, the increases in BUN levels were attributed to Pf-04971729 administration alone.

Statistically significant decreases in total protein levels (↓6-7%) were observed in females treated with 25 mg/kg PF-04971729 alone or in combination with 60 mg/kg sitagliptin. Significant decreases in albumin levels (↓9%) were also observed in females receiving the highest dose combination (25+60). Nevertheless, the decreases in blood protein levels remained within the normal biological range for this species and are not considered to be biologically significant.

Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	**Glucose (mg/dL)**		**BUN (mg/dL)**		**TP (g/dL)**		**ALB (g/dL)**	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	102	101	14	14	7.2	8.1	4.4	5.4
0+60	108	111	14	14	7.6	8.1	4.5	5.4
25+0	76* (↓25.5%)	73* (↓27.7%)	27* (↑92.9%)	25* (↑78.6%)	7.1	7.6* (↓6.2%)	4.4	5.0
5+20	85* (↓16.7%)	90 (↓10.9%)	22* (↓57.1%)	20* (↑42.9%)	7.4	7.8 (↓3.7%)	4.4	5.2
5+60	84* (↓17.6%)	94 (↓6.9%)	22* (↑57.1%)	18* (↑28.6%)	7.0	8.1	4.3	5.4

25+20	68* (↓33.3%)	82* (↓18.8%)	27* (↑92.9%)	24* (↑71.4%)	7.1	7.8 (↓3.7%)	4.3	5.2
25+60	70* (↓31.4%)	73* (↓27.7%)	26* (↑85.7%)	24* (↑71.4%)	7.1	7.5* (↓7.4%)	4.3	4.9* (↓9.3%)

* p value < 0.05

Drug-related increases in ALT levels were observed in males (↑24-44%) at ≥5 mg/kg PF-04971729 and in females (↑35-48%) at 25 mg/kg in a dose-dependent manner with regard to PF-04971729, but independent of sitagliptin treatment or co-administration. Thus, the increases in ALT were attributed to PF-04971729 administration; however, the degrees of increase were considered to be minimal and unlikely to be biologically significant.

Statistically significant decreases in cholesterol were observed in females with PF-04971729 administration, but were not consistently dose-dependent and remained within the normal biological range for this species. Although attributed to PF-04971729 treatment, this finding is not considered to be biologically significant.

Drug-related decreases in electrolytes were observed in both sexes. Statistically significant decreases in chloride levels were observed in both males (↓4-5%) and females (↓3%) with PF-04971729 administration in a dose-dependent manner with regard to PF-04971729, but independent of sitagliptin. Statistically significant decreases in calcium levels were observed in both males (↓5%) and females (↓4%) with 25 mg/kg PF-04971729 administration alone or in combination with 20 mg/kg sitagliptin in males (↓4%). The observed decreases in electrolytes are consistent with findings from other studies with PF-04971729 administration in rats and are considered to be drug-related. Nevertheless, it is noted that the mean levels of both calcium and chloride remained within the normal biological range for this species.

Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	**ALT (U/L)**		**CHOL (mg/dL)**		**Calcium (mg/dl)**		**Cl (mmol/L)**	
	♂	♀	♂	♀	♂	♀	♂	♀
0+0	34	29	79	113	11.4	11.5	103	102
0+60	32	30	84	105	11.4	11.6	102	102
25+0	49* (↑44.1%)	43* (↑48.3%)	76	89* (↓21.2%)	10.8* (↓5.3%)	11.0* (↓4.3%)	98* (↓4.9%)	99* (↓2.9%)
5+20	42* (↑23.5%)	31	72	92 (↓18.6%)	11.2	11.3	99* (↓3.9%)	100 (↓2.0%)
5+60	43* (↑26.5%)	36 (↑24.1%)	66	88* (↓22.1%)	11.0 (↓3.5%)	11.5	99* (↓3.9%)	101 (↓1.0%)
25+20	46* (↑35.3%)	39* (↑34.5%)	80	94 (↓16.8%)	11.0* (↓3.5%)	11.2 (↓2.6%)	98* (↓4.9%)	99* (↓2.9%)
25+60	46* (↑35.3%)	41* (↑41.4%)	70	88* (↓22.1%)	11.1 (↓2.6%)	11.1 (↓3.5%)	98* (↓4.9%)	99* (↓2.9%)

* p value < 0.05

Urinalysis

Moderate to marked glucosuria was reported in both sexes with PF-04971729 administration alone or in combination with sitagliptin.

Statistically significant drug-related increases in specific gravity were reported in males (↑2-3%) and females (↑3-4%) with PF-04971729 administration, but were independent of dose and sitagliptin administration.

Significant drug-related increases in urine volume reaching 2 to 3-fold above concurrent controls were observed in males with PF-04971729 administration and were dose-dependent with regard to PF-

04971729, but were independent of sitagliptin administration and dose. A trend for minimal increases (↑18-37%) in urine volume was also observed with 25 mg/kg PF-04971729 administration in females independent of sitagliptin, but did not reach statistical significance.

Trends for decreased urine pH were observed in both males (↓3-8%) and females (↓10-13%), but were less severe with co-administration.

Urine Parameters						
Dose (mg/kg PF-04971729 + mg/kg Sitagliptin)	**Specific Gravity**		**Volume (mL)**		**pH^**	
	♂	♀	♂	♀	♂	♀
0+0	1.037	1.015	11.0	15.9	6.7	7.0
0+60	1.040	1.028	8.9	9.0	6.6	6.6
25+0	1.056* (↑1.8%)	1.053* (↑3.7%)	32.9* (↑3-fold)	19.0 (↑19.5%)	6.2 (↓7.5%)	6.1 (↓12.9%)
5+20	1.063* (↑2.5%)	1.055* (↑3.9%)	21.0* (↑2-fold)	13.9	6.7	6.4
5+60	1.056* (↑1.8%)	1.047* (↑3.2%)	24.1* (↑2-fold)	16.2	6.5	6.4
25+20	1.054* (↑1.6%)	1.056* (↑4.0%)	31.0* (↑3-fold)	18.7 (↑17.6%)	6.4 (↓4.5%)	6.2 (↓11.4%)
25+60	1.051* (↑1.4%)	1.048* (↑3.3%)	34.5* (↑3-fold)	21.8 (↑37.1%)	6.5 (↓3.0%)	6.3 (↓10.0%)

^ Statistical analysis not performed
* p value < 0.05

Gross Pathology

Incidences of mucosal discoloration were observed in the glandular stomach were observed in both sexes treated with ≥25 mg/kg PF-04971729. Furthermore, there was an apparent dose-dependent increase in males with co-administration of sitagliptin, indicating exacerbation. In females, the largest incidence of mucosal discoloration was observed in animals treated with 25 mg/kg PF-04971729 alone. Mucosal discoloration was most often dark red and sometimes black, grey, or white, corresponding with erosion, submucosal inflammation, and/or hemorrhage.

Pelvic enlargement was reported in the kidneys of 2 males treated with 25 mg/kg PF-04971729, which correlated with microscopic findings of renal dilatation.

Sponsor's Table 11: Macroscopic Findings

Table
Incidence of Macroscopic Observations
Terminal Euthanasia (Dosing Phase)

Test Article	(dosage)	1	2	3	4	5	6	7
PF-04971729	mg/kg/day	0	0	25	5	5	25	25
Sitagliptin	mg/kg/day	0	60	0	20	60	20	60
Tissue/ Observation	Group/Subgroup/Sex: Number of Animals:	1/1/M 10	2/1/M 10	3/1/M 10	4/1/M 10	5/1/M 10	6/1/M 10	7/1/M 10
Stomach	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	9	10	9	8	6
Discolored		0	0	1	0	1	2	4
Kidney	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	9	10	10	9	10
Large		0	0	1	0	0	1	0

Table
Incidence of Macroscopic Observations
Terminal Euthanasia (Dosing Phase)

Test Article	(dosage)	1	2	3	4	5	6	7
PF-04971729	mg/kg/day	0	0	25	5	5	25	25
Sitagliptin	mg/kg/day	0	60	0	20	60	20	60

Tissue/ Observation	Group/Subgroup/Sex: Number of Animals:	1/1/F 10	2/1/F 10	3/1/F 10	4/1/F 10	5/1/F 10	6/1/F 10	7/1/F 10
Stomach	Number Examined:	10	10	10	10	10	10	10
	Unremarkable:	10	10	6	10	10	9	9
Discolored		0	0	4	0	0	1	1

(Tables excerpted from Sponsor's report and highlighted)

Organ Weights

PF-04971729-related increases in kidney weights (absolute, relative body and relative brain weights) were observed in all male (↑14-35%) and female (↑21-35%) PF-04971729 treatment groups, but not in animals treated with sitagliptin alone. There was no indication of exacerbation of increased kidney weight with co-administration of sitagliptin. The increased kidney weights were considered to be due to PF-04971729 treatment, but were independent of sitagliptin administration.

Increases in adrenal weights were observed in both sexes treated with co-administration of 25 mg/kg PF-04971729 and sitagliptin. However, statistical significance was only achieved in weights relative to body weight and only in males (↑32%) at 25+60 and in females (↑20%) at 25+20. Nevertheless, this finding is consistent with other studies involving PF-04971729 administration in rats and is considered likely to be a drug-related finding that is exacerbated by sitagliptin co-administration.

Sponsor's Table 12: Ertugliflozin + Sitagliptin Co-administration - Organ Weights

Text Table 4.1: Test Article-Related Changes in Organ Weight Parameters

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/0	0/60	25/0	5/20	5/60	25/20	25/60	0/0	0/60	25/0	5/20	5/60	25/20	25/60
Kidney														
Absolute Weight (g)	3.0101	1.07	1.25*	1.19*	1.14	1.21*	1.23*	1.6836	.99	1.25*	1.21*	1.20*	1.25*	1.22*
Body Weight Ratio (%)	0.5445	1.07	1.33*	1.32*	1.25*	1.35*	1.33*	0.5745	.99	1.33*	1.25*	1.27*	1.35*	1.30*
Brain Weight Ratio (%)	135.2726	1.06	1.27*	1.21*	1.17*	1.22*	1.25*	80.7133	1.03	1.29*	1.25*	1.22*	1.27*	1.27*
Adrenal														
Absolute Weight (g)	0.0612	1.09	1.15	1.01	1.13	1.05	1.23	0.0669	.94	1.07	.94	.95	1.11	1.03
Body Weight Ratio (%)	0.0111	1.08	1.23	1.11	1.24	1.16	1.32*	0.0228	.94	1.14	.98	1.00	1.20*	1.09
Brain Weight Ratio (%)	2.7525	1.07	1.17	1.02	1.16	1.05	1.25	3.2085	.98	1.10	.97	.96	1.12	1.07

* = Statistically significant ($p \leq 0.05$) difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as fold of control mean value.

(Table excerpted from sponsor's package)

Histopathology

Battery Considered Adequate? Yes
Peer Review Performed? Yes

Increases in incidence and severity of kidney tubule dilatation was observed in males and females with increasing PF-04971729 administration, but were independent of sitagliptin treatment. Furthermore, all animals treated with 25 mg/kg PF-04971729 presented with minimal to mild tubule dilatation. Increased incidence or severity of pelvis dilatation was also observed in males in all PF-04971729 treatment groups except 5+20 and in females at 5+20 and 25+60. Overall, the kidney findings of tubule and pelvic dilatation are considered likely to be drug-related.

Increases of mixed cell inflammation in the prostate were observed in males co-administered both drugs, with an apparent dose-dependence of sitagliptin with 25 mg/kg PF-04971729 co-administration.

Stomach findings of erosion, hemorrhage and inflammation were reported in both sexes. In general, increases in incidence and/or severity of glandular stomach erosion were observed in both sexes. However, there was not a clear dose-dependence in females since this effect was not seen in the highest co-administration group 25+60. Findings of minimal acute submucosal inflammation were also noted in both sexes with PF-04971729 administration alone or in combination with sitagliptin, and with PF-04971729 dose-dependence in males, but not in females. Incidences of minimal hemorrhage were also reported with PF-04971729 administration alone in males and with sitagliptin co-administration with 25 mg/kg PF04971729 in both sexes. Overall, the stomach histopathological findings are consistent with previous findings in rats with PF-04971729 and are considered to be PF-04971729-related, but largely independent of sitagliptin administration.

Hypertrophy in the zona glomerulosa of the adrenal cortex was observed in both sexes with PF-04971729 administration. Furthermore, the increases in incidence and/or severity were increased with sitagliptin co-administration.

Pancreatic zymogen depletion was observed in all groups treated with PF-04971729, independent of sitagliptin administration, with increased severity in males.

Sponsor's Table 13: Ertugliflozin + Sitagliptin Co-administration - Histopathology

Text Table 3.3: Incidence and Severity of Test Article-Related Microscopic Findings

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/ 0	0/ 60	25/ 0	5/ 20	5/ 60	25/ 20	25/ 60	0/ 0	0/ 60	25/ 0	5/ 20	5/ 60	25/ 20	25/ 60
Number Examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Kidney														
Dilatation, tubule(s)														
Not Present	6	6	0	0	1	0	0	6	8	0	3	1	0	0
Minimal	4	4	1	9	9	6	5	3	2	5	7	9	4	3
Mild	0	0	9	1	0	4	5	1	0	5	0	0	6	7
Dilatation, pelvis														
Not Present	9	9	7	10	8	4	7	10	10	10	9	10	10	8
Minimal	1	1	3	0	2	6	3	0	0	0	0	0	0	2
Mild	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Prostate														
Inflammation, mixed cell														
Not Present	10	10	10	9	10	9	8	NA	NA	NA	NA	NA	NA	NA
Minimal	0	0	0	0	0	1	2	NA	NA	NA	NA	NA	NA	NA
Moderate	0	0	0	1	0	0	0	NA	NA	NA	NA	NA	NA	NA
Stomach, Glandular														
Erosion														
Not Present	9	10	8	10	9	7	6	10	9	9	10	9	8	10
Minimal	1	0	1	0	0	0	4	0	1	0	0	0	2	0
Mild	0	0	1	0	1	3	0	0	0	1	0	1	0	0
Inflammation, acute, submucosa														
Not Present	10	10	8	9	9	6	8	10	10	9	10	9	9	10
Minimal	0	0	2	1	1	4	2	0	0	1	0	1	1	0
Hemorrhage														
Not Present	10	10	8	10	10	7	8	10	10	10	10	10	8	9
Minimal	0	0	2	0	0	3	2	0	0	0	0	0	2	1
Adrenal, Cortex														
Hypertrophy, zona glomerulosa														
Not Present	10	10	1	5	2	1	0	10	9	6	6	4	5	2
Minimal	0	0	8	5	4	6	7	0	1	4	3	3	4	5
Mild	0	0	1	0	4	3	3	0	0	0	1	3	1	3
Pancreas														
Zymogen depletion														
Not Present	10	10	1	0	1	0	0	10	9	2	7	6	2	4
Minimal	0	0	4	8	4	7	5	0	1	8	3	4	8	6
Mild	0	0	5	2	5	3	5	0	0	0	0	0	0	0

NA = Not applicable.

(Table excerpted from sponsor's package and highlighted)

Toxicokinetics

PF-04971729 exposures increased dose-proportionally. Exposures in females tended to be slightly higher, but were not considered to be significant. Exposures were also slightly higher on Day 91 by 12-28% with co-administration, indicating a possible trend for accumulation with high doses of both drugs. T_{max} ranged from 1 to 7 hours postdose, with a trend for delayed T_{max} with high dose co-administration of both drugs (25+60).

Sponsor's Table 14: Ertugliflozin & Sitagliptin Co-administration - PF-04971729 Toxicokinetics

6.1. Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
25 / 0	1	Male	7060	4	99100
		Female	10700	4	125000
		Overall	8880	4	112000
	91	Male	8930	4	91200
		Female	9240	4	138000
		Overall	9080	4	114000
5 / 20	1	Male	1920	7	24900
		Female	2350	4	24100
		Overall	1700	4	24000
	91	Male	1400	1	14100
		Female	2970	1	27800
		Overall	2180	1	21000
5 / 60	1	Male	1720	7	23700
		Female	1940	4	25700
		Overall	1800	4	24700
	91	Male	2520	1	21900
		Female	3060	1	27700
		Overall	2790	1	24800
25/20	1	Male	8390	7	120000
		Female	9910	4	120000
		Overall	8930	4	120000
	91	Male	12500	4	157000
		Female	15100	4	150000
		Overall	13800	4	153000
25/60	1	Male	6380	7	89700
		Female	9370	7	131000
		Overall	7870	7	110000
	91	Male	9710	7	122000
		Female	11800	4	124000
		Overall	8240	7	123000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package)

Sitagliptin exposures increased proportionally with dose and were not significantly affected by co-administration of PF-04971729. There were no significant signs of gender effects or accumulation. T_{max} ranged between 1 and 7 hours post-dose.

Sponsor's Table 15: Ertugliflozin + Sitagliptin Co-administration - Sitagliptin Toxicokinetics

6.2. Mean Toxicokinetic Parameters for Sitagliptin in Rats on Study Days 1 and 91 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
0 / 60	1	Male	2670	4	27500
		Female	2120	4	19000
		Overall	2400	4	23300
	91	Male	3660	4	37800
		Female	2900	1	22500
		Overall	3140	1	30100
5 / 20	1	Male	778	4	8670
		Female	613	4	5640
		Overall	695	4	7140
	91	Male	853	4	7390
		Female	1000	4	6460
		Overall	929	4	6930
5 / 60	1	Male	3450	7	45300
		Female	2670	4	29300
		Overall	2900	4	37300
	91	Male	4450	1	32700
		Female	3740	1	24500
		Overall	4090	1	28600
25/20	1	Male	382	7	5260
		Female	455	4	5490
		Overall	417	4	5380
	91	Male	1170	4	9050
		Female	807	4	7720
		Overall	989	4	8400
25/60	1	Male	2370	7	28600
		Female	1670	7	22500
		Overall	2020	7	25500
	91	Male	2360	4	26200
		Female	2780	4	22500
		Overall	2570	4	24400

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

(Table excerpted from sponsor's package)

Dosing Formulation Analysis

PF-04971729 and sitagliptin dose formulations were analyzed using a validated HPLC method. Overall mean concentrations of PF-04971729 and sitagliptin formulations were within ±10% of the target concentrations.

11 Integrated Summary and Safety Evaluation

The sponsor's IND #122329 package for the FDC product cross-references non-clinical pharmacodynamic, pharmacokinetic, and toxicology information for the SGLT2 inhibitor ertugliflozin previously submitted under IND #106477. The sponsor is also referencing the approved label for Glucophage® (metformin). In accordance with ICH guidelines, the sponsor submitted a 3 month toxicology bridging study with combination of ertugliflozin and metformin administration in rats to support clinical studies of the FDC for ≥3 months.

Co-administration of PF-04971729 and metformin was associated with minor findings that sometimes reached statistical significance with administration of either drug alone or with co-administration, but all of which were considered to be non-adverse and/or secondary to the pharmacodynamic activity of SGLT2 inhibition and did not contribute to determination of the NOAEL. Findings of minimal to moderate dilated

renal tubules in the outer medulla were not associated with correlative signs of kidney malfunction. Instead, the renal tubule hypertrophy was considered likely to be an adaptive response to the pharmacodynamic activity of PF-04971729 (glucosuria and subsequent osmotic diuresis) in conjunction with workload demands resulting from renal tubular secretion of metformin. It is noted that in longer rat toxicology studies with high doses of PF-04971729, progressive drug-related kidney findings in rats included hypertrophy of proximal tubules, tubular mineralization and indications of chronic progressive nephropathy (CPN) at 250 mg/kg. However, at the doses and duration evaluated in this study, there were no clear or consistent indications of kidney dysfunction. Overall, the kidney findings in the 13-week co-administration bridging study correlates with metabolic changes associated with co-administration of PF-04971279 and metformin, were considered non-adverse, and are associated with sufficient margins of safety (67x MRHD for pF-04971729 and 5x MRHD for metformin). Nevertheless, the risk of more severe kidney toxicities with longer exposures to co-administration of ertugliflozin and metformin has not been ruled out and continued monitoring of kidney function with long-term administration is warranted.

Increases in urine glucose levels (glucosuria) are consistent with the pharmacodynamic activity of SGLT2 inhibitors and previous studies with ertugliflozin alone. Increases in urine specific gravity, urine volume, and BUN levels are consistent with dehydration and osmotic diuresis secondary to glucosuria. Decreases in blood electrolytes levels are also likely to be secondary to PF-04971279-related osmotic diuresis.

Decreases in zymogen granules of pancreatic acinar cells were observed in both 2-week and 13-week studies in animals treated with ertugliflozin in the absence or presence of metformin or sitagliptin. Zymogen granules in the apical region of acinar cells have been shown to reduce in size and/or number after feeding, most likely due to digestive enzyme secretion stimulated by feeding (Ermak & Rothman, *Cell Tissue Res.* 1981; 214: 51-66). Increased food consumption has been observed in previous non-clinical studies with ertugliflozin alone and is likely secondary to drug-related decreases in blood glucose levels related to the pharmacodynamic activity of ertugliflozin. Thus, decreases in zymogen granules of pancreatic acinar cells are likely related to ertugliflozin-related increases in food consumption and are considered to be tertiary to the pharmacodynamic activity of ertugliflozin.

It is noted that both ertugliflozin and metformin may be associated with some concern for cardiovascular effects at high doses; however, both drugs are associated with sufficient margins of safety at the proposed therapeutic doses. Increases in heart organ weights (↑17-29%) were observed in females co-treated with high doses of both drugs, which may reflect an additive effect. However, there were no signs of heart damage or dysfunction in this study and the increase in heart weight was considered to be non-adverse and associated with a sufficient margin of safety. Nevertheless, co-administration of ertugliflozin and metformin will be investigated in the proposed add-on sub-study of the clinical CV safety study #P004/B1521021.

The 13-week co-administration study adequately bridges the proposed FDC to the ertugliflozin nonclinical studies and is in accordance with the ICH Guidance for Industry Nonclinical Safety Evaluation of Drug or Biologic Combinations. Since the safety margins for co-administration of ertugliflozin (67x MRHD) and metformin (5x MRHD) in the 13-week rat study are sufficient, the nonclinical data support clinical exposures of the FDC product (15 mg/day ertugliflozin + 2000 mg/day metformin) for administration (b) (4) in humans.

Similar PF-04971729-related findings were observed with co-administration of sitagliptin. However, the degree of glucose excursion was lower in animals receiving co-administration of sitagliptin compared to those being co-administered metformin. The DPP4 inhibitor drug class has been associated with decreases in glucose excursion, which would be expected to counteract the pharmacodynamic activity of PF-04971729.

Table 1 Human Safety Margins

Study	NOAEL (mg/kg/day)	Human Safety Margin (Based on AUC*)	Basis
2 Week **Ertugliflozin/Metformin:** 5/200, 5/600, 25/200, 25/600, 25/0, & 0/600 mg/kg Ertugliflozin AUC: 26, 15, 109, 77, 124, & - μg·h/mL Metformin AUC: 59, 138, 76, 183, -, & 140 μg·h/mL	Ertugliflozin / Metformin **25 / 600**	Ertugliflozin: **56x** Metformin: **9x**	*No significant systemic adverse effects.* **≥5 / ≥0 mg/kg (10x MRHD):** ↑BUN, ↑food consumption, ↓blood glucose, ↑urine specific gravity, ↑ketones, ↑urine glucose **≥5 / 600 mg/kg (10x/9x MRHD):** ↑ALT, ↑AST **25 / ≥200 mg/kg (56x/7x MRHD):** Minimal renal proximal convoluted tubule hypertrophy, ↓serum electrolytes (Cl) **25 / ≥0 mg/kg (56x MRHD):** stomach erosion, ↓zymogen granules of pancreatic acinar cells **≥0 / 600 mg/kg (9x MRHD):** minimal hypertrophy and ↓cytoplasmic granules of salivary gland duct epithelium
13 Week **Ertugliflozin/Metformin:** 5/200, 5/600, 25/200, 25/600, 25/0, & 0/600 mg/kg Ertugliflozin AUC: 21.2, 19.2, 120, 91.2, 144, & - μg·h/mL Metformin AUC: 48, 122, 53.8, 135, -, & 138 μg·h/mL	Ertugliflozin / Metformin **25 / 600**	Ertugliflozin: **67x** Metformin: **5x**	*No significant systemic adverse effects.* **≥25 / ≥0 mg/kg (76x MRHD):** ↓Body weight (♀), ↑BUN, ↑food consumption, ↓blood glucose, ↑urine specific gravity, ↑urine volume, ↓urine pH, ↑urine glucose, ↓Cl, ↓Ca (♀), small ↑ALT, ↑kidney weight, kidney tubule dilatation, ↓zymogen granules of pancreatic acinar cells, stomach erosion/ulcer (♂), adrenal cortex hypertrophy **25 / ≥200 mg/kg (76x/2x MRHD):** ↓Na (♀), glandular stomach mucosa discoloration, ↑liver weight (♀) **25 / 600 mg/kg (76x/5x MRHD):** stomach erosion/ulcer (♀), ↓protein, ↓cholesterol, ↑adrenal gland weight (♀), ↑heart weight (♀) **≥0 / 600 mg/kg (5x MRHD):** hypertrophy and ↓cytoplasmic granules of salivary gland duct epithelium, ↓creatinine (♂), ↑liver weight (♀)

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with a predicted ertugliflozin exposure of AUC = 1.37 μg·h/mL and metformin exposure of AUC = 25 μg·h/mL

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

/s/

JESSICA J HAWES
12/03/2015

RONALD L WANGE
12/03/2015

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	Pre-IND#122329
Review number:	1
FDA SDN, **Sponsor SN (Vol #),** **Sponsor letter date,** **CDER stamp date:**	2 0000 8/13/2014 8/13/2014
Product:	MK-8835B (Ertugliflozin / Metformin)
Indication:	Treatment of Type 2 Diabetes Mellitus
Sponsor:	Merck Sharp and Dohme Corp
Review Division:	CDER/DMEP
Reviewer:	Jessica J Hawes, Ph.D.
Supervisor:	Ronald Wange, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Abolade Adeolu
Review completion date:	9/5/2014

DMEP PT Template v.0.04 September 23, 2010

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Merck Sharp and Dohme Corp. is investigating the fixed dose combination (FDC, MK-8835B) of their new molecular entity (NME) MK-8835 (Ertugliflozin) with the marketed drug (MD) Metformin for the treatment of type 2 diabetes mellitus.

1.2 Brief Discussion of Nonclinical Findings

Co-administration of ertugliflozin and metformin in rats for 2-weeks was generally well-tolerated with sufficient margins of safety and was not associated with significant adverse systemic toxicities. However, it is noted that the kidney may be a target organ of drug-related toxicity with longer exposures.

1.3 Recommendations

The proposed clinical study is safe to proceed. Non-clinical data support administration of the proposed FDC dose described in the pre-IND package of 15 mg/day ertugliflozin + 2000 mg/day metformin (b) (4)

1.4 Non-hold Recommendations

None

2 Drug Information

2.1 Drug

CAS Registry Number
None

Product Name
Ertugliflozin/metformin FDC

Code Name
Ertugliflozin + metformin FDC: MK-8835B
Ertugliflozin: PF-04971729, MK-8835
Ertugliflozin L-pyroglutamic acid (L-PGA) co-crystal form: PF-04971729(b) (4)

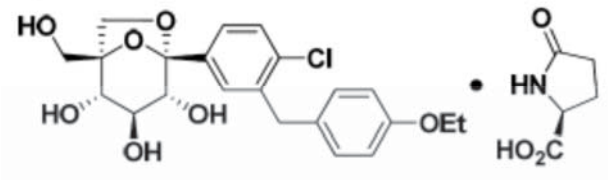
Chemical Name
(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol compound with (S)-5-oxopyrrolidine-2-carboxylic acid (1:1)

Molecular Formula/Molecular Weight

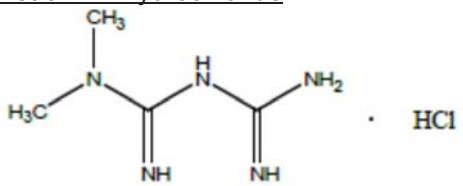
PF-04971729:	$C_{22}H_{25}ClO_7$	436.88 g/mol
PF-04971729 (b) (4)	$C_{27}H_{32}ClNO_{10}$	566.00 g/mol
Metformin:	$C_4H_{11}N_5$	
Metformin Hydrochloride:	$C_4H_{12}N_5Cl$	

Structure or Biochemical Description

Ertugliflozin L-PGA



Metformin Hydrochloride



Pharmacologic Class

Ertugliflozin: Sodium glucose co-transporter 2 (SGLT2) Inhibitor
Metformin: Biguanide

Planned Clinical Route of Administration

Oral tablet administered twice daily with meals

Proposed Tablet Strengths:

1. **0.005 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 2.5 mg / metformin 500 mg FDC tablets

2. **0.0075 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 7.5 mg / metformin 1000 mg FDC tablets

Other potential tablet strengths described in the pre-IND package:

1. **0.0025 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 2.5 mg / metformin 1000 mg FDC tablets

2. **0.015 Ratio Ertugliflozin to metformin:**
   Ertugliflozin 7.5 mg / metformin 500 mg FDC tablets

2.2 Relevant INDs, NDAs, and DMFs

IND #106447 - Ertugliflozin (Merck Sharp & Dohme Corp.)

IND #047342 / NDA #21202 - Metformin HCl (Bristol Myers Squibb)

IND #76500 / NDA #63634 – Kombiglyze, combination of Metformin HCl and Saxagliptin (Astrazeneca AB)

2.3 Drug Formulation

Composition of Ertugliflozin/Metformin HCl Tablets, 2.5 mg/500 mg and 7.5 mg/1000 mg

Component	Weight per Tablet (mg)		Function	Reference to Standard
	2.5/500	7.5/1000		
Core Tablet				
Ertugliflozin (calculated as the L-pyroglutaminc acid cocrystal)	3.2[a]	9.7[b]	Active	Internal
Metformin HCl	(b) (4)	(b) (4)	(b) (4)	Internal
(b) (4)				
Povidone (b) (4)				
Microcrystalline Cellulose				USP
Crospovodone				USP
Sodium Lauryl Sulfate				USP
Magnesium Stearate				USP
Total Core Tablet Weight				
Film Coating				
(b) (4)				

[a] Equivalent to 2.5 mg ertugliflozin
[b] Equivalent to 2.5 mg ertugliflozin
(b) (4)

(Table excerpted from sponsor's IND background package)

2.4 Comments on Novel Excipients

The only non-compendial excipient is the (b) (4). However, the (b) (4) ingredients meet regulatory and compendial requirements appropriate for their intended use (see Dr. Joseph Leginus's Quality review under IND #122329).

2.5 Comments on Impurities/Degradants of Concern

In the ertugliflozin lot #GR02546 used for previous non-clinical studies, impurity (b) (4) was found to be as high as (b) (4)%. However, this impurity was not detected in clinical Lot #GR02694. There have not been any other reported organic impurities for the ertugliflozin co-crystal.

2.6 Proposed Clinical Protocols

The sponsor plans to propose Phase 1 clinical pharmacology studies to bridge the ertugliflozin/metformin combination FDC tablet to co-administration of ertugliflozin and metformin. According to the pre-IND package, the sponsor is planning to propose 6 "stand-alone" studies and addition of 2 sub-studies to the existing CV safety study. Two of the "stand-alone" studies will be add-on to metformin studies (study #P002/B1521013 and #P007/B1521017) and another 2 will include ertugliflozin and metformin in combination with sitagliptin (study #P005/B1521019 and P006/B1521015). The 2 sub-studies will include ertugliflozin with metformin in combination with insulin or sulfonylureas (CV safety study #P004/B1521021), as discussed at the EOP2 meeting for IND #106447 on 12/17/2012.

Only 1 single-dose Phase 1 PK study protocol (described below) was submitted with the IND package on 8/13/2014.

Phase 1 PK Study

Study Title: A Phase 1, Randomized, Open-Label, 3-Period, 6-Sequence Study to Estimate the Pharmacokinetic Interaction between Ertugliflozin and Metformin in Healthy Subjects

Study Objectives:

1. To estimate the effect of metformin on the pharmacokinetics of ertugliflozin following oral administration of a single dose of 15 mg ertugliflozin and 1000 mg metformin in healthy volunteers.
2. To estimate the effect of ertugliflozin on the pharmacokinetics of metformin following oral administration of a single dose of 15 mg ertugliflozin and 1000 mg metformin in healthy volunteers.
3. To evaluate the safety and tolerability of a single dose administration of the combined dose.

Protocol Summary: 18 healthy male and/or female subjects will receive single doses of 15 mg ertugliflozin (Treatment A), 1000 mg metformin (Treatment B) or a single combined dose (Treatment C) of 15 mg ertugliflozin + 1000 mg metformin (single dose of each administered within 5 minutes) with a washout period of at least 5 days between each treatment.

Figure 1. Study Regimen Schematic

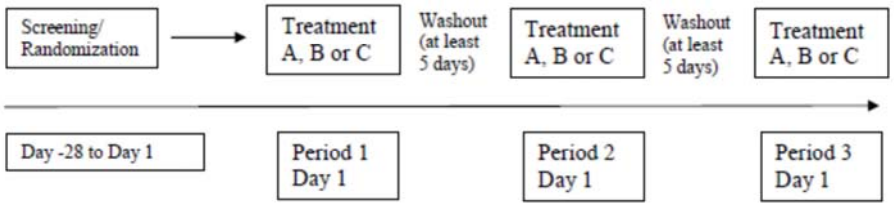


Table 1. Treatment Sequence

Sequence	Period 1	Period 2	Period 3
1	A	B	C
2	A	C	B
3	B	A	C
4	B	C	A
5	C	A	B
6	C	B	A

Treatment A: 15 mg ertugliflozin (single dose).
Treatment B: 1000 mg metformin (single dose).
Treatment C: 15 mg ertugliflozin + 1000 mg metformin (single dose of each administered within 5 minutes of each other).

Primary Endpoints: PK parameters C_{max}, AUC_{inf}, AUC_{last}, T_{max}, $t_{1/2}$, CL/F, and V_z/F.

Sponsor's Table 1: Sponsor's Predicted Exposures - QD vs. BID Ertugliflozin

Table 4: Model predicted mean (SD) steady state exposures for ertugliflozin q.d. and b.i.d. dosing regimens

Total daily dose (mg)	Predicted AUC_{0-24} (ng*hr/mL)	
	b.i.d.	q.d.
5	458 (115)	456 (116)
15	1374 (346)	1366 (347)

(Excerpted from sponsor's pre-IND package)

Other Planned Studies

1. PK bridging study to evaluate once and twice daily oral administration of ertugliflozin in healthy volunteers
2. Demonstrate bioequivalence (BE) between the FDC product and co-administered ertugliflozin and metformin in the fasted state
3. A food interaction study to evaluate the effect of a standard high fat breakfast on the PK of the highest FDC strength (7.5 mg ertugliflozin / metformin 1000 mg) with twice daily dosing
4. A drug interaction study between metformin and ertugliflozin (under IND #106447)

Phase 3 Developmental Plan Overview

In addition to the 5 ongoing ertugliflozin and metformin co-administration studies under IND #106447, the sponsor is planning one add-on sub-study using co-administration of sulfonylurea. The sponsor does not appear to be proposing a phase 3 clinical study with the FDC product (Sponsor's Table 2). The sponsor's development strategy for the twice daily FDC product will be based on bridging studies to once daily ertugliflozin and metformin co-administration.

Sponsor's Table 2: Sponsor's Planned Phase 3 Studies

Table 3: Listing of the Phase 3 Clinical Development Studies

Study # (Merck /Pfizer)	Description	Total N	Background Therapy	Treatment Arms	Duration Phase A/ Total Study[a]	Submission Number[b] and Date
Ertugliflozin in combination with metformin alone						
P007/ B1521017	Placebo-controlled add-on to metformin (includes BMD assessment)	600	Metformin ≥ 1500 mg	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	26 weeks/ 104 weeks	0072 10/9/2013 [2]
P002/ B1521013	Active-controlled (glimepiride) add-on to metformin (non-inferiority)	1230	Metformin ≥ 1500 mg	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Glimepiride	52 weeks/ 104 weeks	0071 10/9/2013 [3]
Ertugliflozin and metformin in combination with other AHAs						
P006/ B1521015	Placebo-controlled add-on to metformin and sitagliptin	405	Metformin ≥ 1500 mg and Sitagliptin 100 mg	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	26 weeks/ 52 weeks	0083 12/18/2013 [4]
P005/ B1521019	Factorial study of ertugliflozin and sitagliptin (add-on to metformin)	1250	Metformin ≥ 1500 mg	• Sitagliptin100 mg • Ertugliflozin 5 mg • Ertugliflozin 15 mg • Sitagliptin 100 mg +Ertugliflozin 5 mg • Sitagliptin 100 mg +Ertugliflozin 15 mg	26 weeks/ 52 weeks	0089 2/3/2014 [5]
P004/ B1521021 sub-study	Add-on to Insulin+/- metformin glycemic efficacy sub-study	At least 450	Insulin +/- Metformin ≥1500 mg	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	18 weeks for A1C efficacy determination	0066 9/6/2013 [6]
P004/ B1521021 sub-study	Add-on to metformin+SU glycemic efficacy sub-study	At least 450	Metformin ≥ 1500 mg +Sulfonylurea	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	18 weeks for A1C efficacy determination	to be discussed under Question 3
Ertugliflozin alone and in combination with other AHAs other than metformin						
P003/ B1521022	Placebo-controlled monotherapy	450	Diet / Exercise	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	26 weeks/ 52 weeks	0064 8/26/2013
P017/ B1521047	Initial combination of sitagliptin and ertugliflozin (on background of diet and exercise)	300	Diet / Exercise	• Sitagliptin 100 mg +Ertugliflozin 5 mg • Sitagliptin 100 mg +Ertugliflozin 15 mg • Placebo	26 weeks/ 26 weeks	To be submitted
P004/ B1521021 sub-study	Add on to SU glycemic efficacy sub-study	120	Sulfonylurea	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	18 weeks for A1C efficacy determination	0066 9/6/2013 [6]

Study # (Merck /Pfizer)	Description	Total N	Background Therapy	Treatment Arms	Duration Phase A/ Total Study[a]	Submission Number[b] and Date
Studies in Special Populations						
P001/ B1521016	Placebo-controlled study in Stage 3 CKD (eGFR 30-<60 mL/min/1.73 m^2) with secondary analyses of Stage 3A(eGFR 45-<60) and 3B CKD (eGFR 30-<45 mL/min/1.73 m^2)	468 (3A CKD: 312; 3B CKD: 156)	Standard of Care	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	26 weeks/ 52 weeks	0068 9/26/2013
P004/ B1521021	Placebo-controlled cardiovascular safety study on a background of standard of care	3900	Standard of Care	• Ertugliflozin 5 mg • Ertugliflozin 15 mg • Placebo	Event-driven	0066 9/6/2013 [6]

a. The interval for collection of the primary and secondary endpoints is denoted as "Phase A." The total duration of some studies duration may be longer due to inclusion of extension periods.
b. Under IND 106,447

(Table excerpted from sponsor's package under IND#106447)

Sponsor's Maximum Recommended Human Dose:

FDC: **Twice-daily dose of 7.5 mg ertugliflozin and 1000 mg metformin HCl**

- **15 mg/day ertugliflozin + 2000 mg/day metformin**
- Sponsor's predicted Ertugliflozin AUC_{0-24} = 1.37 μg·h/mL
- Predicted Metformin AUC_{0-24} = 20.5 μg·h/mL

Ertugliflozin: Therapeutic oral dose of 15 mg/day with an exposure of AUC* = 1.2 μg·h/mL (C_{max}* = 159 ng/mL) is equivalent to a concentration of 0.3 μM.

* AUC and C_{max} exposures were extrapolated from 14-day repeat-dose exposure in overweight/obese adult subjects (study #B1521002).

- Maximum systemic exposure to unbound** drug: AUC ≈ 79.3 ng·h/mL, C_{max} ≈ 10.2 ng/ml ≈ 18 nM

  ** based on a 6.4% unbound fraction in humans
- Reference IND #106447

Metformin: Approved maximum daily dose of Metformin HCl: 1000 mg twice a day (2000 mg/day)

- Maximum exposure of AUC 20,544 ng*h/mL
- C_{max} = 1.8 μg/mL for 2000 mg once-daily dose.
- Reference IND#47342 and the drug label for Glucophage

2.7 Previous Clinical Experience

FDC

The combination FDC product has not been tested in humans. However, clinical studies with co-administration of ertugliflozin + Metformin are ongoing under IND #106447 (see below).

Ertugliflozin

Phase 1 and 2 clinical studies have been completed for ertugliflozin under IND #106447 and multiple phase 3 studies in T2DM patients are underway (Ongoing Clinical Studies: Ertugliflozin + Metformin HCl Co-Administration). However, since data from the phase 3 studies have not yet been submitted for review, ertugliflozin is considered to be an early stage entity, as defined in ICH M3(R2)

Ertugliflozin has been administered to 495 humans at doses up to 300 mg in the fasted state, 100 mg in the fed state for up to 14 days, and once-daily doses of 25 mg for up to 12 weeks. Food decreases the rate of ertugliflozin absorption, but does not affect the overall extent of absorption.

Sponsor's Table 3: Clinical Single-Dose Pharmacokinetic Parameters

Table 6.1-1. Pharmacokinetic Parameters Following Single Oral Doses of PF-04971729

Parameter (Units)	0.5 mg (n=8)	2.5 mg (n=8)	10 mg (n=8)	30 mg (n=8)	100 mg (n=8)	300 mg (n=7)
AUC_{inf} [a] (ng*hr/mL)	45.7 (10)	231 (22)	909 (15)	2810 (18)	9610 (16)	26400 (16)
C_{max} [a] (ng/mL)	7.23 (11)	42.8 (21)	182 (22)	545 (24)	1620 (16)	4330 (20)
T_{max} [a] (hr)	1.0 (0.5-1.5)	1.0 (0.5-1.1)	1.0 (0.5-1.5)	1.0 (0.5-1.5)	1.0 (0.5-1.5)	1.0 (0.5-1.5)
$t_{½}$ [a] (hr)	11.4 (19)	13.1 (24)	17.4 (42)	15.2 (33)	16.2 (36)	13.8 (18)

[a] Geometric mean (CV%) for all except: median (range) for T_{max}; arithmetic mean (CV%) for $t_{½}$

Sponsor's Table 4: Clinical Multiple-Dose Pharmacokinetic Parameters

Table 6.1-2. Pharmacokinetic Parameters Following Once-Daily, 14-day Dosing of PF-04971729

Parameter (Units)	1 mg (n=8)	5 mg (n=8)	25 mg (n=8)	100 mg (n=8)
AUC(0-24) [a] (ng*hr/mL)	80.85 (15)	450.5 (35)	2045 (26)	7761 (17)
C_{max} [a] (ng/mL)	10.19 (15)	50.83 (28)	280.8 (28)	1035 (25)
T_{max} [a] (hr)	2.0 (1.0-4.0)	1.50 (1.0-4.03)	2.0 (1.0-2.0)	2.0 (1.0-4.0)
$t_{½}$ [a, b] (hr)	NC	12.28 (24)	14.81 (41)	14.13 (14)
Rac [a]	1.360 (8)	1.247 (7)	1.217 (5)	1.375 (19)

[a] Geometric mean (CV%) for all except: median (range) for T_{max}; arithmetic mean (CV%) for $t_{½}$
[b] n=0 for 1 mg and n=7 for 25 mg

(Tables excerpted from sponsor's package)

Sponsor's Table 5: Clinical Studies Completed with Ertugliflozin

Study	Design	Ertugliflozin Dose Levels	Number of Subjects Exposed to Ertugliflozin	Duration of Treatment
Phase 1 Studies				
B1521001 (N = 24)	Randomized, double-blind, placebo-controlled, cross-over, single ascending dose study with assessment of food effect, in healthy subjects	0.5, 2.5, 10, 30, 100, 300 mg (food effect dose: 100 mg)	24	Single dose
B1521002 (N = 40)	Randomized, double-blind, placebo-controlled, parallel group, multiple ascending dose study in healthy subjects	1, 5, 25, 100 mg once-daily	32	14 days
B1521003 (N = 6)	Open-label, single period, study to evaluate metabolism and excretion of [^{14}C] PF-04971729 in healthy subjects	25 mg	6	Single dose
B1521005 (N = 12)	Randomized, open-label, 3-period, cross-over study to evaluate relative bioavailability of 3 formulations of PF-04971729 in healthy subjects	10 mg	12	Single dose
B1521007 (N = 52)	Randomized, double-blind, 2-period, cross-over study to evaluate PK-PD effect of QD vs BID dosing in patients with T2DM	1 mg twice-daily 2 mg once-daily 2 mg twice-daily 4 mg once-daily	52	Single day
B1521009 (N = 24)	Randomized, double-blind, cross-over, single and multi-dose study in Japanese healthy subjects plus single-dose in Western healthy subjects	1, 5, 25 mg once-daily	18	Up to 7 days
Phase 2 Studies				
B1521004 (N = 194)	Randomized, double-blind, double-dummy, placebo-controlled, parallel group study in patients with T2DM and inadequate glycemic and blood pressure control (includes hydrochlorothiazide as internal reference standard)	1, 5, 25 mg once-daily	116	28 days
B1521006 (N = 328)	Randomized, double-blind, double-dummy, placebo-controlled, parallel group study in patients with T2DM and inadequate glycemic control (includes sitagliptin as internal reference standard)	1, 5, 10, 25 mg once-daily	219	84 days

N represents number of subjects randomized per study

(Table excerpted from sponsor's package under IND#106447)

Reoccurring dose-related adverse events (AEs) include headaches, constipation, diarrhea, discolored feces, and folliculitis.

Ongoing Clinical Studies: Ertugliflozin + Metformin HCl Co-Administration

There are 5 ongoing phase 3 clinical studies incorporating ertugliflozin and metformin co-administration in the trial design under IND #106447 (Sponsor's Table 2). Studies #B1521017 (Sponsor's Figure 1) and #B1521013 (Sponsor's Figure 2) are investigating co-administration of once daily ertugliflozin (5 or 15 mg) with metformin (≥1500 mg/day) in TD2M patients for up to 52 weeks, or up to 104 weeks with extension. Studies #B1521015 and #B1521019 include co-administration of metformin (≥1500 mg/day) and ertugliflozin (5 or 15 mg once daily) in the absence or presence of daily 100 mg sitagliptin for up to 52 weeks. Study #B1521021 includes ertugliflozin (5 or 15 mg once daily) co-administration with insulin in

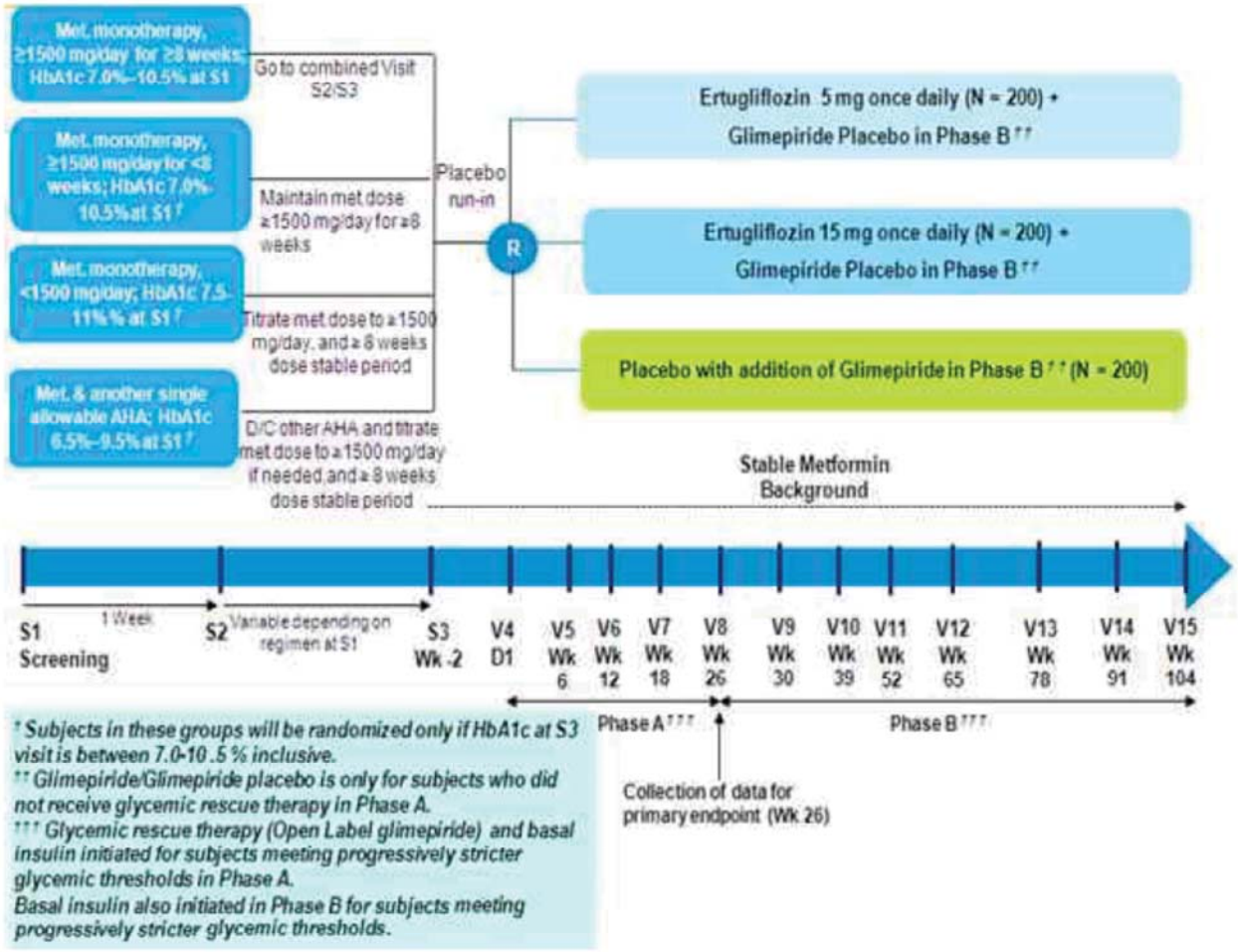
the absence or presence of metformin for 18 weeks and includes evaluation of cardiovascular endpoints as well. The sponsor plans to bridge once daily ertugliflozin co-administration with metformin in the phase 3 studies to the twice daily dosing of the FDC formulation using the PK and BE studies (2.5

Comments on Impurities/Degradants of Concern

In the ertugliflozin lot #GR02546 used for previous non-clinical studies, impurity [redacted] (b) (4) was found to be as high as [redacted] (b) (4) %. However, this impurity was not detected in clinical Lot #GR02694. There have not been any other reported organic impurities for the ertugliflozin co-crystal.

2.6 Proposed Clinical Protocols). It is noted that since the phase 3 studies are being conducted globally, the sponsor anticipates that a mixture of metformin sources will be used and that strict bridging to the metformin used in the Phase 1 BE bridging study will not be possible.

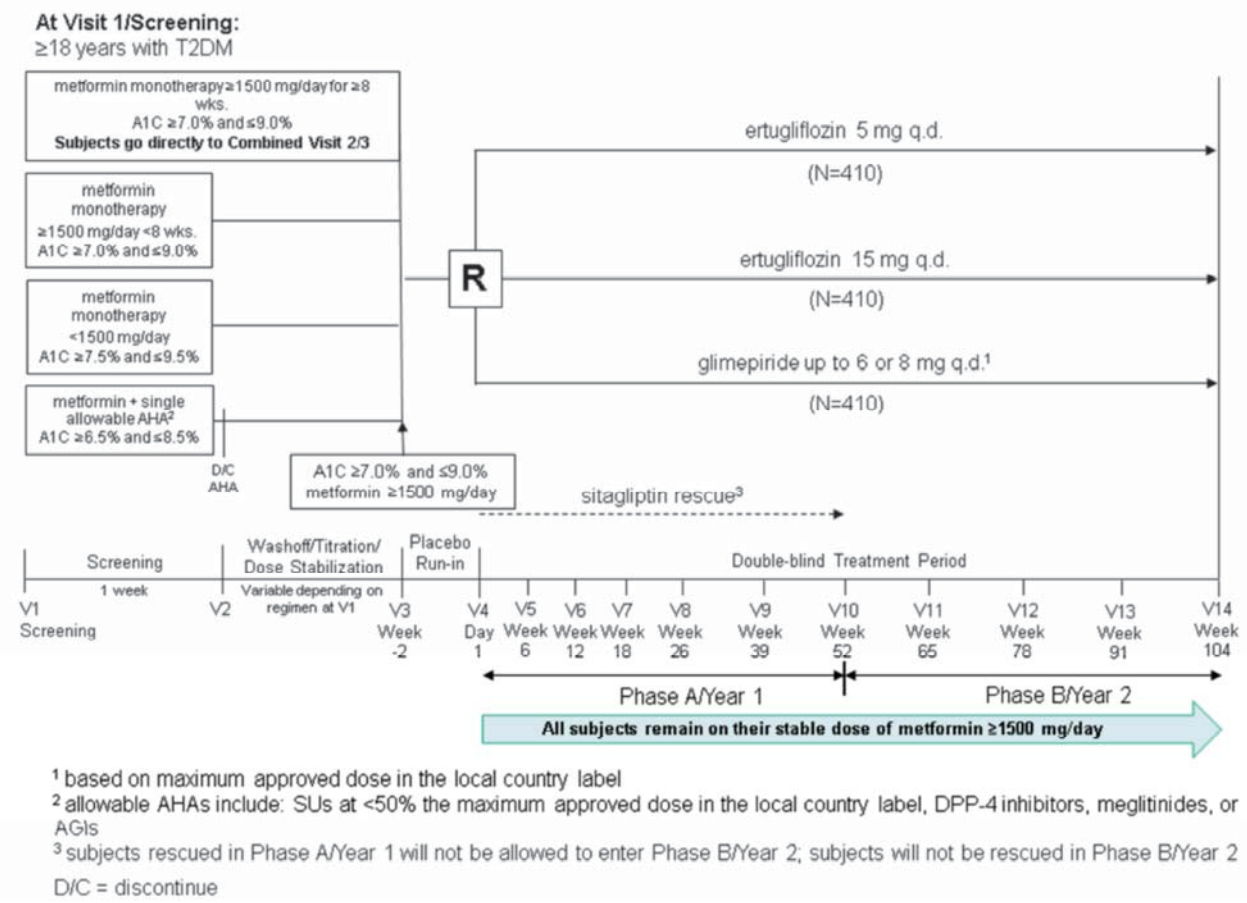
Sponsor's Figure 1: Clinical Study #B1521017 Design



Met=Metformin

(Figure excerpted from sponsor's package under IND#106447)

Sponsor's Figure 2: Clinical Study #B1521013 Design



[1] based on maximum approved dose in the local country label
[2] allowable AHAs include: SUs at <50% the maximum approved dose in the local country label, DPP-4 inhibitors, meglitinides, or AGIs
[3] subjects rescued in Phase A/Year 1 will not be allowed to enter Phase B/Year 2; subjects will not be rescued in Phase B/Year 2
D/C = discontinue

(Figure excerpted from sponsor's package under IND#106447)

Metformin

Metformin has been extensively prescribed for long-term administration in patients worldwide for roughly 4 decades and is thought to be the most widely prescribed antidiabetic drug in the world, with 48 million prescriptions per year in the US alone. The most common adverse reactions to metformin are gastrointestinal, including diarrhea, nausea/vomiting, flatulence, asthenia, indigestion, and abdominal discomfort, as well as headache. Metformin is also associated with risks of hypoglycemia and lactic acidosis when given in excessive doses or patients with contraindications, such as kidney disorders, lung disease and liver disease. Long-term use of metformin has been associated with malabsorption of vitamin B_{12}.

2.8 Regulatory Background

FDC

On 5/9/2014, the sponsor submitted a meeting request and a pre-IND package for the FDC Ertugliflozin + Metformin product. On 5/13/2014, a Pre-IND/Type B meeting was granted with written responses sent to the sponsor on 7/3/2014. Within the pre-IND package, the sponsor submitted 3 clinical questions and one regulatory question, but no non-clinical questions.

The sponsor submitted the IND package for the FDC product on 8/13/2014 cross-referencing non-clinical pharmacodynamic, pharmacokinetic, toxicology information previously submitted under IND #106447 for ertugliflozin alone.

Ertugliflozin

Ertugliflozin was originally submitted as PF04971729 in September 2009.

An EOP2 meeting was held on December 17th 2013. The Division encouraged the sponsor to include 2 doses in their phase 3 program, increasing the MRHD to 15 mg/day. The sponsor requested a revision of the proposed rat carcinogenicity study to increase the dose in conjunction with the increased MRHD. Although ECAC did not feel the increase in dose was necessary, it was considered acceptable.

The PeRC BPCA subcommittee discussed the sponsor's proposed Pediatric Study Plan (PSP) on April 10th 2013 and a revised PSP was approved after resubmission on June 12th 2013. It was concluded that a juvenile toxicology study in Sprague Dawley rats would be required prior to initiation of clinical pediatric studies. Ertugliflozin was then discussed at a second PeRC BPCA meeting on August 21st 2013 and the PeRC concurred with the proposed PSP and sponsor's plan for a partial waiver and deferral.

Metformin

Metformin HCl was approved under NDA #020357 as Glucophage (Bristol Myers Squibb) in 1994 with a maximum approved adult dose set at 2000 mg/day. Metformin has been prescribed extensively for long-term treatment of type II diabetes in patients worldwide for roughly 4 decades. Metformin HCl was later approved as an extended release tablet (Glucophage XR) in 2000. A metformin HCl/saxagliptin combination (Kombiglyze, NDA #200678) was approved in November 2010 and included embryofetal toxicology studies (IND #76500, IND #63634), which were mandated by post marketing requirements under the Onglyza NDA #22350, which specifically addressed potential treatment-related neural tube defects due to either metformin or the combination of the two drugs. Embryo-fetal studies using metformin HCl were cross-referenced to the original Metformin HCl IND #47342 and reviewed by Dr. Jessica Hawes 1/11/2011.

3 Studies Submitted

Exploratory 2 week rat dose range-finding (DRF) studies with ertugliflozin and metformin (study #8294466) or sitagliptin (study #8294467) were submitted under IND #106447 (SDN #105, SN #0104). The sponsor plans to submit a definitive 3-month study with the FDC product under this IND.

8294466; (13GR341)2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats, 09-Jul-2014 (Module 4.2.3.2 - Repeat-Dose Toxicity)

8294467; (13GR342) 2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats, 11-Jul-2014 (Module 4.2.3.2 - Repeat-Dose Toxicity)

3.1 Previous Reviews Referenced

IND #122329:

- Chemistry review #1 by Dr. Joseph Leginus

IND #106447:

- End of phase 2 memo and nonclinical reviews #1, #2, #3, #4, and #5 by Dr. Jeffrey Quinn.
- Nonclinical review #6 by Dr. Jessica Hawes

IND #047342:

- Nonclinical review #1 (2/9/2011) by Dr. Jessica Hawes

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action:

Ertugliflozin is an inhibitor of the Sodium Glucose Transporter 2 (SGLT2). Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in glucosuria. Ertugliflozin is selective for SGLT2 over SGLT1 and other glucose transporters (GLUT1-4).

Metformin HCl (BMS-207150) is a biguanide class hypoglycemic agent used to treat non-insulin dependent Type II diabetes. Although the molecular mechanisms of metformin are not completely understood, the following mechanisms have been implicated to play a role: inhibition of the mitochondrial respiratory chain (complex I), activation of AMP-activated protein kinase (AMPK), inhibition of glucagon-induced elevation of cyclic adenosine monophosphate (cAMP) and consequent activation of protein kinase A (PKA), induced phosphorylation of GLUT4 enhancer factor, and an effect on gut microbiota.

Drug activity related to proposed indication:
Ertugliflozin administration results in concentration-dependent glucosuria in rats. Ertugliflozin acts as a diuretic in rats, increasing urine volume, urinary volume to water intake, and hematocrit levels *in vivo*. Diuretic effects have also been reported in humans.

Metformin suppresses hepatic glucose production, increases insulin sensitivity, enhances peripheral glucose uptake, decreases insulin-induced suppression of fatty acid oxidation, and decreases absorption of glucose from the gastrointestinal tract.

4.2 Safety Pharmacology

Both ertugliflozin and metformin may be associated with some concern for cardiovascular effects at high doses, but are associated with sufficient margins of safety at therapeutic doses. Given that the margins of safety for cardiovascular effects are sufficient for each drug alone, the margins of safety for the FDC product are likely to be sufficient as well. Nevertheless, co-administration of ertugliflozin and metformin will be investigated in the proposed add-on sub-study of the clinical CV safety study #P004/B1521021.

Ertugliflozin

Standard cardiovascular, neurological and pulmonary safety pharmacology studies have been completed for ertugliflozin under IND #106447.

Neurological: Male rats dosed with 500 mg/kg of PF-04971729 (b) (4) had a 0.4°C decrease in average body temperature. At 500 mg/kg, PF-04971729 produced decreases in locomotor activity measurements (~30-40%).

Cardiovascular: PF-04971729 inhibited the hERG channel *in vitro* with an IC_{50} of >300 μM (129 μg/mL). However, significant inhibition of hERG was observed at doses ≥ 30 μM: 2.9% at 30 μM, 8.3% at 100 μM (ranging from 2.9% to 18.1%), and 33.5% at 300 μM. Dosing with PF-04971729 (b) (4) at 50 mg/kg in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and heart rate (and associated RR interval shortening), as well as an increase in systolic blood pressure and lengthening of the PR interval, with a NOAEL of 5 mg/kg and an LOAEL of 50 mg/kg ($AUC_{(0-24)}$ = 530 μg·h/mL, C_{max} = 44.7 μg/mL = 80 μM, unbound = 1.43 μg/mL = 2.5 μM, 387x MRHD).

Pulmonary: Dose-dependent increases in respiratory rate (↑29-40%) and minute volume (↑25-23%) were observed in rats at doses ≥25 mg/kg lasting for up to 120 minutes post-dose.

Renal: No renal safety studies were performed although PF-04971729 causes increased urinary glucose excretion and kidney alterations in rats and dogs.

Gastrointestinal: No GI safety studies were performed although PF-04971729 causes changes in stool quality, vomiting and ulceration of the tongue in rats and dogs at high exposures.

Metformin

Neurological

Incidences of headache, confusion, and/or mood swings observed in humans are likely secondary to hypoglycemia.

Cardiovascular
Metformin has been associated with lactic acidosis, which is further associated with cardiovascular collapse, acute congestive heart failure, and acute myocardial infarction. Some patients have reported increased heart beat and/or palpitations while on metformin.

Pulmonary
Difficulty breathing is occasionally reported in humans taking metformin, but has not been associated with an adverse clinical outcome.

Renal
Metformin is contraindicated with renal deficiency since it is primarily eliminated via the kidney. Cystic tubular dilation and vacuolization have been observed in mice.

Gastrointestinal
GI upset, diarrhea, cramps, nausea, vomiting and gas.

Other
Metformin-mediated inhibition of hepatic neogenesis leads to decreases in lactate uptake and increases in lactic acid leading to lactic acidosis, which can be fatal. Metformin is also contraindicated in patients with liver dysfunction and acute or chronic metabolic acidosis, including diabetic ketoacidosis. Loss of appetite and a bad taste in the mouth has also been associated with metformin administration in children.

5 Pharmacokinetics/ADME/Toxicokinetics

Drug-drug interactions between ertugliflozin and metformin are not anticipated. Ertugliflozin and metformin are eliminated by different mechanisms and are not expected to affect each other's elimination pathways. Ertugliflozin is predominantly eliminated via hepatic metabolism, whereas metformin is not metabolized and is eliminated via filtration at the glomerulus and excreted in the urine unchanged. Ertugliflozin is not anticipated to affect OCT2 activity at clinical exposures; hence ertugliflozin is not anticipated to affect metformin exposures. Metformin does not inhibit or induce metabolizing enzymes involved in ertugliflozin metabolism; thus metformin is not anticipated to affect ertugliflozin exposures.

Ertugliflozin

Ertugliflozin protein binding is high in all species examined (human, dog, rat, and mouse) ranging from 94 to 97%. Significant species differences are observed with an oral absorption range from 50% in humans to 78% in rats and 94% in dogs, with moderate to high oral bioavailability. T_{max} is achieved within 30 minutes in mice and 1 hour in humans (fasted), but after 2 hours in humans in the fed state, indicating absorption delays in the presence of food. Systemic exposures follow linear pharmacokinetics with a trend for slight increases in female exposures over time at high doses in rodents, indicating a potential gender effect. Ertugliflozin has a moderate half-life of 4 to 8 hours in rats and dogs, but a long half-life in humans of 12 to 16 hours. The predominant route of elimination of ertugliflozin is via metabolism catalyzed by CYP3A4, CYP3A5, CYP2D6, UGT1A9, and UGT2B7 enzymes. Glucuronidation is the major metabolic pathway *in vivo,* with contribution of the O-desethylation pathway in the rat. Ertugliflozin is primarily excreted via feces and bile in rats and dogs, but via urine and feces in humans.

Ertugliflozin is a weak inhibitor of OCT2 with an IC_{50} value of 917 μM, which is 40,000 times higher than the predicted steady state free drug C_{max} of 0.0231 μM at a dose of 15 mg. Ertugliflozin may be a substrate for P-glycoprotein (P-gp)-mediated efflux, but is not affected by P-gp inhibitors. Thus P-gp is unlikely to be a limiting factor in Ertugliflozin absorption. Ertugliflozin has a moderate volume of distribution in rats with the highest distribution to bladder, liver, kidney, adrenal gland, Harderian gland, and pancreas. Ertugliflozin crosses the blood-brain barrier, but only reaches concentrations 3 to 63-fold lower than that of blood; whereas distribution to the choroid plexus and pituitary gland is 2-fold greater than blood.

Metformin

Metformin is absorbed slowly with an oral bioavailability of 50-60% under fasting conditions. For the immediate-release formula, C_{max} is reached in 1 to 3 hours; whereas C_{max} is achieved 4 to 8 hours post-dose with the extended-release formula. Steady state is reached in 1 to 2 days. Metformin is not metabolized or subject to biliary excretion, but is a substrate of renal transporter OCT2 and cleared by renal tubular secretion with an elimination half-life of 6.2 hours. However, metformin accumulates in red blood cells where it has an elimination half-life of 17.6 hours. Metformin pharmacokinetics is similar in pediatrics (12 to 16 years) and adults.

6 General Toxicology

In accordance with the ICH Guidance for Industry Nonclinical Safety Evaluation of Drug or Biologic Combinations, in addition to the standard battery of toxicology studies for the NME (ertugliflozin), a 90-day bridging study with the combination product (FDC) in the most appropriate species will be sufficient to support clinical studies of the combination product for durations of ≥3 months. The sponsor plans to conduct a 3-month repeat dose toxicity study in rats with the FDC product.

6.1 Ertugliflozin

Ertugliflozin alone toxicology studies were completed under IND #106447 and include pivotal 6-month rat and 9-month dog studies. No additional general toxicology studies with ertugliflozin alone are anticipated to be required.

Safety margins from the 6 month rat and 9 month dog studies support the proposed 15 mg/day dose of ertugliflozin with 16x and 52x safety margins, respectively, based on AUC exposures at the nonclinical NOAELs. Most findings in the chronic nonclinical toxicology studies can be attributed to drug-related glucosuria, osmotic diuresis and a catabolic state. Drug-related gastrointestinal findings in dogs (excessive vomiting, salivation and abnormal feces) and rats (stomach erosion/ulcers, pyloric crypt degeneration and foveolar hyperplasia) are consistent with off-target inhibition of SGLT1. Hyperostosis was observed in male rats in the 6 month study, which is consistent with the SGLT2 inhibitor drug class, but is associated with a sufficient safety margin (65x MRHD) and can be monitored clinically for changes in bone mass or fractures.

Table 1: Ertugliflozin Human Safety Margins

Species	Study	NOAEL (mg/kg/day)	Human Safety Margin (Based on AUC*)	Basis
Rat	**1 Month** Dose: 5, 25, 500→250 mg/kg AUC: 8, 69, 541 µg·h/mL	25	**50x**	**250 mg/kg:** ↑Severity CPN, stomach erosion, squamous hyperplasia **500 mg/kg:** Mortality
	3 Month Dose: 5, 25, 250 mg/kg AUC: 20, 89, 738 µg·h/mL	ND (≤5)	**<15x**	**≥5 mg/kg:** Kidney pelvic tubule dilatation & mineral deposition, GI tract dilatation, ↑adrenal weight, adrenal histopath. **≥25 mg/kg (65x MRHD):** ↓Prostate weight, inflammation, stomach erosion/ulcer, hyperostosis **100 mg/kg (540x MRHD):** Pelvic bladder hyperplasia, ↑severity CPN, heart myonecrosis
	6 Month Dose: 5, 25, 100 mg/kg AUC: 22, 148, 605 µg·h/mL	5	**16x**	**≥5 mg/kg:** stomach erosion/ulcer, ↑BUN/Phos dehydration, ↓serum electrolytes, ↓pancreatic zymogen, ↑food consumption, ↑urine glucose **≥25 mg/kg (108x MRHD):** Kidney pelvic tubule dilatation/hyperplasia, mineral deposition, & pyloric crypt degeneration. **100 mg/kg (440x MRHD):** ↓BW gain, ↓RBC parameters, ↓PTH, ↑adrenal weight, adrenal cortex hypertrophy/vacuolation, ↑trabecular bone, ↓serum electrolytes, stomach hyperplasia
Dog	**1 Month** Dose: 1, 10, 150 mg/kg AUC: 7, 71, 1050 µg·h/mL	10	**52x**	**≥1 mg/kg:** ↓BW gain, liver glycogen depletion **≥10 mg/kg (52x MRHD):** gallbladder vacuolation **150 mg/kg (770x MRHD):** GI intolerance (vomiting, salivation, abnormal feces), renal tubular degeneration
	3 Month Dose: 1, 10, 150 mg/kg AUC: 9.8, 92, 1100 µg·h/mL	10	**67x**	**≥1 mg/kg (7x MRHD):** Liver glycogen depletion **150 mg/kg (800x MRHD):** GI intolerance (vomiting, diarrhea, mortalities), ↓BW gain, liver cell necrosis (♀)

	9 Month Dose: 1, 10, 150 mg/kg AUC: 6, 71, 606 µg·h/mL	10	**52**	**≥1 mg/kg:** ↓BW gain (♀) **≥10 mg/kg:** thyroid mineralization (♀, persistent) **150 mg/kg (440x MRHD):** GI intolerance (vomiting, diarrhea, salivation), ↓BW gain (♂), ↑reticuloctyes, ↑inflammatory markers, ↑thymus weight, ↑urine volume (persistent), ↑adrenal weight, adrenal cortex vacuolation

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with a predicted ertugliflozin exposure of AUC_{0-24} = 1.37 µg·h/mL

6.2 Metformin

Target organs of metformin administration in rats include the heart, liver, lymphoreticular organs, adrenals, salivary gland, and reproductive tissues (Quaile et al., 2010).

6.3 Ertugliflozin + Metformin

The sponsor submitted one 2-week dose-range finding study with co-administration of ertugliflozin (PF-04971729) and metformin in rats.

2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Metformin in Rats (Study #8294466 / 13GR341)

Doses of PF-04971729/Metformin: 0/0, 5/200, 5/600, 25/200, 25/600, 25/0, and 0/600 mg/kg

Study #	8294466 / 13GR341
Study report location	eDR
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	1/14/2014
GLP compliance statement	No
GLP issues identified	None
QA statement	No
Drug, lot #, and % purity	PF-04971729: Lot #GR02694, 73.9% Metformin: Lot #WL00045745, 100%

Key Study Findings

- PF-04971729 + Metformin:
  - Hypertrophy of renal proximal convoluted tubules (♂&♀)
  - Clinical chemistry (♂&♀): ↑ALT, ↑AST, ↓Cl
- PF-04971729:
  - Stomach erosion (♂&♀)
  - ↓Pancreatic acinar cell zymogen granules (♂)
  - ↑Food consumption (♂&♀)
  - Clinical chemistry (♂&♀): ↓glucose and ↑urea nitrogen
  - Urine (♂&♀): ↑specific gravity, ↑glucose, and ↑ketones
- Metformin:
  - Hypertrophy and ↓cytoplasmic granules of salivary gland duct epithelium (♂&♀)

SD Rat, 2 Weeks	NOAEL (AUC)	Multiple of MRHD*

No significant adverse systemic toxicities	25 mg/kg PF-04971729 (77100 ng·h/mL) + 600 mg/kg Metformin (183000 ng·h/mL)	PF-04971729: **56x** Metformin: **9x**

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with a predicted ertugliflozin exposure of AUC_{0-24} = 1.37 μg·h/mL and metformin exposure of AUC = 20.544 μg·h/mL

Reviewer's Comments

The NOAEL was set at the high combination dose of 25 mg/kg PF-04971729 and 600 mg/kg metformin due to lack of significant adverse systemic toxicities.

Co-administration of PF-04971729 and metformin was associated with minor kidney findings and clinical chemistry changes that were not observed with administration of either drug alone. Findings of minimal renal proximal convoluted tubule hypertrophy characterized by enlargement and increased pallor of epithelial cells extending from the outer cortex to the medulla were observed in both sexes at the PF-04971729 high dose of 25 mg/kg co-administered with both 200 and 600 mg/kg doses of metformin. However, there were no correlative signs of kidney malfunction and the renal tubule hypertrophy was considered likely to be an adaptive response to the pharmacodynamic activity of PF-04971729 (glucosuria and subsequent osmotic diuresis) in conjunction with workload demands resulting from renal tubular secretion of metformin. Mild increases in blood levels of the liver markers ALT and AST were observed with co-administration of 600 mg/kg metformin and ≥5 mg/kg PF-04971729 in males and 25 mg/kg PF-04971729 in females, but there were no other findings consistent with liver toxicity. Decreases in chlorine levels in the blood are likely secondary to osmotic diuresis. Overall, the findings associated with co-administration of PF-04971279 and metformin were considered non-adverse.

Increases in urine glucose levels (glucosuria) are consistent with the pharmacodynamic activity of SGLT2 inhibitors, such as PF-04971729. Increases in urine specific gravity, urine ketone levels, and blood urea nitrogen levels are consistent with dehydration and osmotic diuresis secondary to glucosuria, which are considered to be due to the pharmacodynamic activity of PF-04971729.

Pancreatic and stomach findings were attributed to PF-04971729. Findings of discolored stomach and/or erosion were observed in animals treated with PF-04971729 in the absence or presence of metformin. Similar stomach findings have been described in previous toxicology studies with PF-04971729, but are likely to be reversible and are not considered to be adverse. Decreases in pancreatic acinar cell zymogen granules have not been described in previous toxicology studies with PF-04971729. Nevertheless, since findings were reported in animals treated with PF-04971729 alone, the pancreatic findings are attributed to PF-04971729 and not the co-administration of PF-04971729 and metformin, but are also considered to be non-adverse.

Epithelial hypertrophy and cytoplasmic granule changes in the mandibular and sublingual salivary glands are known non-adverse effects of metformin.

Co-administration of PF-04971729 did not appear to affect metformin exposures. However, PF-04971729 tended to be lower with co-administration of 600 mg/kg metformin, but not 200 mg/kg.

Study Design

				PF-04971729		Metformin	
		No. of Animals			Dose		Dose
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	5	5	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Low/	1 (Toxicity)	5	5	5	1	200	40
Low)	2 (Toxicokinetic)	4	4	5	1	200	40
3 (Low/	1 (Toxicity)	5	5	5	1	600	120
High)	2 (Toxicokinetic)	4	4	5	1	600	120
4 (High/	1 (Toxicity)	5	5	25	5	200	40
Low)	2 (Toxicokinetic)	4	4	25	5	200	40
5 (High/	1 (Toxicity)	5	5	25	5	600	120
High)	2 (Toxicokinetic)	4	4	25	5	600	120
6 (High/	1 (Toxicity)	5	5	25	5	0	0
Control)	2 (Toxicokinetic)	4	4	25	5	0	0
7 (Control/	1 (Toxicity)	5	5	0	0	600	120
High)	2 (Toxicokinetic)	4	4	0	0	600	120

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg metformin (or Vehicle Control Article 2, as applicable).

b PF-04971729 dose concentrations were corrected for lot specific potency of 0.739 (73.9%). A correction factor of 1.353 was used for Lot No. GR02694.

c No correction factor was needed for metformin dose concentrations. Dose levels and concentrations were expressed as the salt form of Test Article 2 (metformin).

d Group 1 received Vehicle Control Article 1 (0.5% [w/v] methylcellulose [4000 cps] with 10% [v/v] polyethylene glycol 400 prepared in reverse osmosis water) and Vehicle Control Article 2 (0.5% [w/v] methylcellulose [4000 cps] prepared in reverse osmosis water) only.

Non-GLP Repeat-Dose Toxicity in Rats	
[Species] [Doses and Administration] [# animals] [Follow-up]	**NOAEL: 25 mg/kg PF-04971729 + 600 mg/kg Metformin**
Crl :CD(SD) Rats **PF-04971729/Metformin**: 0/0, 5/200, 5/600, 25/200, 25/600, 25/0, and 0/600 mg/kg/day Main study : 5/sex/group TK : 4/sex/group	**Toxicokinetics:** **PF-04971729:** **Text Table 4.1: Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 14 after Daily Oral Administration of PF-04971729 and Metformin**

Dose PF-04971729 / Metformin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
5 / 200	1	Male	1560	4	21300
		Female	1990	4	24000
		Overall	1770	4	22600
	14	Male	1620	4	21600
		Female	2340	1	29800
		Overall	1930	4	25600
5 / 600	1	Male	1210	4	13500
		Female	867	4	12800
		Overall	1040	4	13100
	14	Male	1280	4	16600
		Female	1310	1	13500
		Overall	1150	4	15000
25 / 200	1	Male	6470	4	84400
		Female	6970	4	91400
		Overall	6720	4	87800
	14	Male	10600	4	94800
		Female	9120	4	123000
		Overall	9860	4	109000
25 / 600	1	Male	5450	7	82400
		Female	4220	4	56300
		Overall	4510	4	69300
	14	Male	5230	4	72600
		Female	5470	1	81500
		Overall	4720	7	77100
25 / 0	1	Male	6460	4	82200
		Female	12500	7	162000
		Overall	8770	7	122000
	14	Male	8080	4	104000
		Female	10300	7	144000
		Overall	8500	7	124000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed.
Overall = male plus female combined

T_{max} was generally observed at 4 hours post-dose, but ranged between 1 and 7 hours. PF-04971729 exposures increased with dose in the presence of metformin. Exposures were sometimes higher on Day 14, but accumulation was considered to be equivocal due to high inter-animal variability. Overall systemic PF-04971729 exposures were lower when co-administered with metformin, especially in females.

Metformin:

Text Table 4.2: Mean Toxicokinetic Parameters for Metformin in Rats on Study Days 1 and 14 after Daily Oral Administration of PF-04971729 and Metformin

Dose PF-04971729 / Metformin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
5 / 200	1	Male	6560	4	69100
		Female	7660	1	56400
		Overall	6600	1	62700
	14	Male	7380	4	67600
		Female	9050	1	49900
		Overall	7690	1	58700
5 / 600	1	Male	14000	4	131000
		Female	10300	1	149000
		Overall	12000	4	140000
	14	Male	16800	1	154000
		Female	13900	1	122000
		Overall	15300	1	138000
25 / 200	1	Male	7390	4	65100
		Female	8340	4	74200
		Overall	7860	4	69500
	14	Male	10100	4	76000
		Female	6570	1	74000
		Overall	8190	4	75800
25 / 600	1	Male	10600	4	157000
		Female	12100	4	124000
		Overall	11300	4	140000
	14	Male	15900	4	180000
		Female	16300	4	186000
		Overall	16100	4	183000
0 / 600	1	Male	9610	7	145000
		Female	15800	1	130000
		Overall	12300	1	137000
	14	Male	15000	1	132000
		Female	18300	1	148000
		Overall	16600	1	140000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed.
Overall = male plus female combined

T_{max} was generally observed at 1 to 4 hours post-dose. Metformin exposures increased with dose, but with less than dose-proportional increments. Exposures were slightly higher on Day 14, but were not considered to be associated with significant accumulation. Metformin exposures were similar in the absence or presence of PF-04971729 co-administration.

Mortality: Animals were checked twice daily for mortality.
There were no mortalities.

Clinical Signs: Animals were checked twice daily for abnormalities and signs of pain or distress. Cageside observations were conducted once daily at 1 hour postdose. Detailed observations were conducted at predose on Days 1, 8, and 14 for main study animals and before euthanasia on Day 15 of TK animals.
There were no apparent drug-related effects.

Body Weight: Animals were weighed prior to the dosing phase and at predose on Days 1, 8, and 14.
There were no apparent drug-related effects.

Food Consumption: Food consumption over 1 week periods was determined for each cage on Days 1 to 8 and on Days 9 to 14.
Food consumption was generally slightly higher than controls in animals treated with Pf-04971729 in the absence or presence of metformin, but was similar to controls in animals receiving metformin alone.

Hematology: Fasted blood samples were collected at necropsy on Day 15.

red blood cell (erythrocyte) count	white blood cell (leukocyte) count
hemoglobin	differential blood cell count
hematocrit	blood smear
mean corpuscular volume	reticulocyte count
mean corpuscular hemoglobin	mean platelet volume
mean corpuscular hemoglobin concentration	red blood cell distribution width
platelet count	
prothrombin time	activated partial thromboplastin time

There were no apparent drug-related effects on hematology or coagulation parameters.

Clinical Chemistry: Fasted blood samples were collected at necropsy on Day 15.

glucose	alanine aminotransferase
urea nitrogen	alkaline phosphatase
creatinine	gamma glutamyltransferase
total protein	aspartate aminotransferase
albumin	calcium
globulin	inorganic phosphorus
albumin:globulin ratio	sodium
cholesterol	potassium
total bilirubin	chloride

Blood glucose levels were mildly lower with 5 or 25 mg/kg PF-04971729 in the absence or presence of metformin, but were similar to controls with metformin alone. Blood urea nitrogen (BUN) concentration was mildly to moderately higher with PF-04971729 administration (+/- metformin).

Text Table 4.3: Changes in Blood Glucose and Urea Nitrogen Concentration - Day 15

Test Article	PF-04971729/Metformin						
Dose level (mg/kg/day)	0/0	5/200	5/600	25/200	25/600	25/0	0/600
Glucose (mg/dL)							
Males	69	50*[a]	51*[a]	46*[a]	45*[a]	43*[a]	68
Fold Difference from Control	--	0.72x[a]	0.74x[a]	0.67x[a]	0.65x[a]	0.62x[a]	0.99x
Females	76	58*[a]	60*[a]	47*[a]	46*[a]	55*[a]	75
Fold Difference from Control	--	0.76x[a]	0.79x[a]	0.62x[a]	0.61x[a]	0.72x[a]	0.99x
Urea Nitrogen (mg/dL)							
Males	8	16*[a]	14*[a]	19*[a]	18*[a]	21*[a]	8
Fold Difference from Control	--	2.00x[a]	1.75x[a]	2.38x[a]	2.25x	2.63x[a]	0.00x
Females	12	17[a]	16[a]	19*[a]	15[a]	22*[a]	11
Fold Difference from Control	--	1.42x[a]	1.33x[a]	1.58x[a]	1.25x[a]	1.83x[a]	0.92x

-- = Not applicable.
* = Significantly different from control.
a PF-04971729- related effect.

AST and ALT liver markers were increased (<2-fold) in females at 25 mg/kg and males at ≥5 mg/kg PF-04971729 when co-administered with 600 mg/kg metformin, but not with metformin alone. A statistically significant 5% decrease in Cl concentration was observed in females receiving HD PF-04971729 and metformin (both doses), but not with PF-04971729 or metformin alone.

Urinalysis: Overnight urine samples were collected from fasting animals prior to necropsy on Day 15.

appearance (clarity and color)	ketones
volume	bilirubin
specific gravity	urobilinogen
pH	blood
protein	microscopic examination of sediment
glucose	

Minimal increases in urine specific gravity, marked glucose reactivity, and trace to marked ketone reactivity were reported in animals treated with PF-04971729 (+/- metformin), but not with metformin alone. Trace reactivity for ketones was also reported in some animals treated with metformin alone.

Text Table 4.4: Changes in Urinalysis Parameters- Day 15

Test Article	PF-04971729/Metformin						
Dose level (mg/kg/day)	0/0	5/200	5/600	25/200	25/600	25/0	0/600
Urine specific gravity							
Males	1.010	1.045[a]	1.035[a]	1.046[a]	1.033[a]	1.046[a]	1.027
Fold Difference from Control	--	1.03x[a]	1.02x[a]	1.04x[a]	1.02x[a]	1.04x[a]	1.02x
Females	1.024	1.054[a]	1.061*[a]	1.046[a]	1.043[a]	1.062*[a]	1.031
Fold Difference from Control	--	1.03x[a]	1.04x[a]	1.02x[a]	1.02x[a]	1.04x[a]	1.01x
Urine glucose (Negative to 3+)							
Males	Negative	3+[a]	3+[a]	3+[a]	3+[a]	3+[a]	Negative
Females	Negative	3+[a]	3+[a]	3+[a]	3+[a]	3+[a]	Negative
Urine ketones (Negative to 3+)							
Males	Negative to Trace	Negative to 2+	Negative to 2+	2+ to 3+[a]	Negative to 3+[a]	Trace to 3+[a]	Negative to Trace
Females	Negative	Negative	Negative to Trace	Negative to Trace	Negative to 3+[a]	Negative to 2+[a]	Negative to Trace

-- = Not applicable.
* = Significantly different from control.
a PF-04971729-related effect.

Organ Weights: Paired organs were weighed together (see table under Histopathology).
There were no apparent drug-related effects.

Gross Pathology: Examination of external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues was performed at necropsy on Day 15.
Low incidences of discolored stomachs were observed in 1 animal with PF-04971729 alone (25/0), 1 ♂ at 25/200, and 3 animals (♂&♀) at 25/600, but not in animals with metformin alone (0/600).

Histopathology: Tissues from main study animals were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E).
Groups 1, 5, 6, & 7: Tissues (E) in the table below and sublingual salivary glands were examined microscopically.
Groups 3 & 4: macroscopic lesions, kidney, glandular stomach, pancreas (♂ only), mesenteric adipose, mandibular salivary gland, and sublingual salivary gland were examined microscopically.
Group 2: macroscopic lesions, kidney, mandibular salivary gland, and sublingual salivary gland were examined microscopically.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		P,E
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	salivary gland (mandibular [2])		P,E
epididymis (2)	W	P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
esophagus		P,E	seminal vesicle		P,E
eye (2)[b]		P,E	skin/subcutis {skin and adnexa}		P,E
femur with bone marrow (articular surface of the distal end to include the stifle joint)		P,E	spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
gut-associated lymphoid tissue {GALT}		P,E	spleen	W	P,E
Harderian gland[b]		P,E	sternum with bone marrow {sternum}		P,E
heart	W	P,E	stomach		P,E
ileum		P,E	testis (2)[b]	W	P,E
jejunum		P,E	thymus	W	P,E
kidney (2)	W	P,E	thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
larynx			tongue		P,E
lesions		P,E	trachea		P,E
liver	W	P,E	ureter		P,E
lungs with large bronchi {lung}		P,E	urinary bladder		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	uterus		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	vagina		P,E
mammary gland (males and females)		P,E			

E = Examined microscopically; P = Processed; W = Weighed.
a Brain was sectioned according to published recommendations (Bolon et al., 2013).
b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Minimal hypertrophy of renal proximal convoluted tubules (enlargement and increased pallor of epithelial cells extending from the outer cortex to the medulla) was observed in both sexes treated with 25 mg/kg PF-04971729 and metformin (25/200 and 25/600), but not with PF-04971729 alone

or metformin alone.
Minimal to slight stomach erosion was observed in males and females at 25/600 or PF-04971729 alone (25/0). Decreases in pancreatic acinar cell zymogen granules were observed in males treated with 25 mg/kg PF-04971729 (+/- metformin).
Minimal hypertrophy and decreased cytoplasmic granules of the salivary gland duct epithelium were observed in both sexes with 600 mg/kg metformin (+/- PF-04971729).

Text Table 4.5: Incidence and Severity of Test Article-Related Microscopic Findings

	PF-04971729/Metformin													
Sex	Males						Females							
Dose Level (mg/kg/day)	0/ 0	5/ 200	5/ 600	25/ 200	25/ 600	25/ 0	0/ 600	0/ 0	5/ 200	5/ 600	25/ 200	25/ 600	25/ 0	0/ 600
Number Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Kidney														
Number Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Hypertrophy, tubule														
Minimal	0	0	0	4	4	0	0	0	0	0	2	4	0	0
Stomach, Glandular														
Number Examined	5	0	5	5	5	5	5	5	0	5	5	5	5	5
Erosion														
Minimal	0	-	0	0	1	1	0	0	-	0	0	1	0	0
Slight	0	-	0	0	0	0	0	0	-	0	0	1	0	0
Pancreas														
Number Examined	5	0	5	5	5	5	5	5	0	0	0	5	5	5
Zymogen granules, decreased														
Minimal	0	-	0	0	1	2	0	0	-	-	-	0	0	0
Mandibular salivary gland														
Number Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Decreased cytoplasmic granules, duct epithelium														
Minimal	0	0	3	1	4	0	3	0	0	5	0	4	0	4
Sublingual salivary gland														
Number Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Hypertrophy, duct epithelium														
Minimal	0	0	4	0	3	0	4	0	0	3	0	3	0	4

- = Not Examined.

6.4 Ertugliflozin + Sitagliptin

The sponsor submitted one 2-week DRF study with co-administration of ertugliflozin (PF-04971729) and sitagliptin in rats. It is noted that the sponsor does not plan to pursue an FDC product with ertugliflozin and sitagliptin at this time. However, this study provides a comparison for ertugliflozin co-administration with another glucose-lowering agent in rats.

2-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 and Sitagliptin in Rats (Study #8294467 / 13GR342)

Doses of PF-04971729/Sitagliptin: 0/0, 5/20, 5/60, 25/20, 25/60, 25/0, and 0/60 mg/kg

Study #	8294467 / 13GR342
Study report location	eDR
CRO/Laboratory name	(b) (4)
CRO/Laboratory address	
Date of study initiation	1/14/2014
GLP compliance statement	No
GLP issues identified	None
QA statement	No
Drug, lot #, and % purity	PF-04971729: Lot #GR02694, 73.9% Sitagliptin: Lot #010X054, 99.6%

Key Study Findings

- PF-04971729 + Sitagliptin:
  - Increases in PF-04971729 and sitagliptin exposures
  - Minimal increases in AST & ALT
- PF-04971729:
  - ↓Pancreatic acinar cell zymogen granules (♂&♀)
  - ↑Food consumption (♂&♀)
  - Clinical chemistry (♂&♀): ↓glucose, ↑urea nitrogen, ↓electrolytes (Ca, Na, & Cl)
  - Urine (♂&♀): ↑specific gravity, ↑glucose, and ↓pH
  - ↑Kidney weights
- Sitagliptin:
  - No adverse findings

SD Rat, 2 Weeks	NOAEL (AUC)	Multiple of MRHD*
No significant adverse systemic toxicities	25 mg/kg PF-04971729 (132000 ng·h/mL) + 60 mg/kg Sitagliptin (26800 ng·h/mL)	PF-04971729: **96x**

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with a predicted ertugliflozin exposure of AUC_{0-24} = 1.37 µg·h/mL

Reviewer's Comments

The NOAEL was set at the high combination dose of 25 mg/kg PF-04971729 and 60 mg/kg sitagliptin due to lack of significant adverse systemic toxicities.

Increases in urine glucose levels (glucosuria) are consistent with the pharmacodynamic activity of SGLT2 inhibitors, such as PF-04971729. Increases in urine specific gravity, urine ketone levels, and blood urea nitrogen levels are consistent with dehydration and osmotic diuresis secondary to glucosuria. The observed decreases in electrolyte levels (Na, Ca, and Cl) in the blood are also likely to be secondary to PF-04971279-related osmotic diuresis. Thus, these effects are considered to be due to the pharmacodynamic activity of PF-04971729.

Although increases in kidney weights were observed in females and possibly in males, there were no microscopic correlates. Furthermore, the increases in BUN levels are attributable to drug-related diuresis and are not necessarily a sign of kidney dysfunction. Thus, the potential increases in kidney weights are not considered to be significant or adverse drug-related finding in this study. The findings of minimal renal tubule mineralization are likely to be related to ertugliflozin treatment, although increased sensitivity with co-administration cannot be ruled out. Two findings of minimal renal tubule mineralization observed in females at the highest co-administration dose of 25/60 are consistent with similar findings in previous rat toxicology studies with ertugliflozin (reference IND #106447). Nevertheless, since there were no clear

signs of kidney dysfunction, the minimal kidney findings were not considered adverse. It is noted that more significant and/or adverse kidney toxicities may be apparent with longer durations of exposure.

Pancreatic findings were attributed to PF-04971729, but were not considered to be adverse. Decreases in pancreatic acinar cell zymogen granules were reported in animals from most groups, but with increased incidence and/or severity in animals treated with PF-04971729 in combination with sitagliptin or alone. Thus, the pancreatic findings are likely attributable to PF-04971729.

Increases in sitagliptin and PF-04971729 exposures were observed in both sexes with co-administration. Since both PF-04971729 and sitagliptin are predominately eliminated via hepatic metabolism, particularly CYP3A4, it is likely that they compete for metabolization, resulting in increased systemic exposures.

Study Design

		No. of Animals		PF-04971729 Dose		Sitagliptin Dose	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	5	5	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Low/	1 (Toxicity)	5	5	5	1	20	4
Low)	2 (Toxicokinetic)	4	4	5	1	20	4
3 (Low/	1 (Toxicity)	5	5	5	1	60	12
High)	2 (Toxicokinetic)	4	4	5	1	60	12
4 (High/	1 (Toxicity)	5	5	25	5	20	4
Low)	2 (Toxicokinetic)	4	4	25	5	20	4
5 (High/	1 (Toxicity)	5	5	25	5	60	12
High)	2 (Toxicokinetic)	4	4	25	5	60	12
6 (High/	1 (Toxicity)	5	5	25	5	0	0
Control)	2 (Toxicokinetic)	4	4	25	5	0	0
7 (Control /	1 (Toxicity)	5	5	0	0	60	12
High)	2 (Toxicokinetic)	4	4	0	0	60	12

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg sitagliptin (or Vehicle Control Article 2, as applicable).

b PF-04971729 dose concentrations were corrected for lot specific potency of 0.739 (73.9%). A correction factor of 1.353 was used for Lot No. GR02694.

c Sitagliptin dose concentrations were corrected for salt content and lot specific potency of 0.996 (99.6%). A correction factor of 1.285 was used.

d Group 1 received Vehicle Control Article 1 (0.5% [w/v] methylcellulose [4000 cps] with 10% [v/v] polyethylene glycol 400 prepared in reverse osmosis water) and Vehicle Control Article 2 (0.5% [w/v] methylcellulose [4000 cps] with 5 mM hydrochloric acid prepared in reverse osmosis water) only.

Non-GLP Repeat-Dose Toxicity in Rats	
[Species] [Doses and Administration] [# animals] [Follow-up]	**NOAEL: 25 mg/kg PF-04971729 + 60 mg/kg Sitagliptin**
Crl :CD(SD) Rats **PF-04971729/Sitagliptin**: 0/0, 5/20, 5/60, 25/20, 25/60, 25/0, and 0/60 mg/kg/day Main study : 5/sex/group TK : 4/sex/group	**Toxicokinetics:** **PF-04971729:** **Text Table 4.1: Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 14 after Daily Oral Administration of PF-04971729 and Sitagliptin**

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
5 / 20	1	Male	1790	7	23800
		Female	2110	7	30200
		Overall	1950	7	27000
	14	Male	1970	4	22700
		Female	2600	4	32300
		Overall	2280	4	27500
5 / 60	1	Male	1430	4	19300
		Female	2280	7	30900
		Overall	1800	7	25100
	14	Male	1700	4	19000
		Female	2330	4	29500
		Overall	2010	4	24200
25 / 20	1	Male	7720	7	107000
		Female	7650	4	30300
		Overall	7430	4	68900
	14	Male	8420	4	110000
		Female	12800	4	156000
		Overall	10600	4	133000
25 / 60	1	Male	8670	4	99100
		Female	9720	4	86900
		Overall	9190	4	93100
	14	Male	9750	4	110000
		Female	10700	7	155000
		Overall	9760	4	132000
25 / 0	1	Male	8880	7	115000
		Female	13700	7	184000
		Overall	11300	7	150000
	14	Male	8580	4	116000
		Female	14900	7	211000
		Overall	11200	7	163000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

T_{max} was generally observed at 4 to 7 hours post-dose. PF-04971729 exposures increased with dose in the presence of metformin. There were no clear indications of gender-related effects or accumulation. Systemic PF-04971729 exposures increased 3 to 5-fold when co-administered with sitagliptin.

Sitagliptin:

Text Table 4.2: Mean Toxicokinetic Parameters for Sitagliptin in Rats on Study Days 1 and 14 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
5 / 20	1	Male	557	4	7360
		Female	555	4	4820
		Overall	556	4	6090
	14	Male	918	4	9550
		Female	734	4	5880
		Overall	795	4	7610
5 / 60	1	Male	2650	4	33600
		Female	2440	4	23500
		Overall	2540	4	28400
	14	Male	3380	4	31100
		Female	2900	4	22500
		Overall	3140	4	26700
25 / 20	1	Male	449	7	6120
		Female	498	1	5210
		Overall	461	4	5350
	14	Male	625	4	8580
		Female	800	4	6720
		Overall	712	4	7650
25 / 60	1	Male	2490	4	26500
		Female	1710	4	21900
		Overall	2100	4	24100
	14	Male	3080	4	33500
		Female	2260	4	20100
		Overall	2670	4	26800
0 / 60	1	Male	2950	4	36900
		Female	2370	4	19800
		Overall	2660	4	28300
	14	Male	4410	4	39900
		Female	3450	1	21200
		Overall	3660	4	30600

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

T_{max} was generally observed at 4 hours post-dose, but ranged from 1 to 7 hours. Sitagliptin exposures increased with dose, but slightly more than dose-proportional increments. Exposures were slightly higher on Day 14, but were not considered to be associated with significant accumulation. Systemic sitagliptin exposures increased 4 to 5-fold when co-administered with PF-04971729.

Mortality: Animals were checked twice daily for mortality.
There were no mortalities.

Clinical Signs: Animals were checked twice daily for abnormalities and signs of pain or distress. Cageside observations were conducted once daily at 1 hour postdose. Detailed observations were conducted at predose on Days 1, 8, and 14 for main study animals and before euthanasia on Day 15 of TK animals.
There were no apparent drug-related effects.

Body Weight: Animals were weighed at prior to the dosing phase and predose on Days 1, 8, and 14.
There were no significant drug-related effects. Body weights were slightly lower in males during Week 2 in the 25/0 group (PF-04971729 alone), but appeared to be independent of dose.

Food Consumption: Food consumption over 1 week periods was determined for each cage on Days 1 to 8 and on Days 8 to 14.
Food consumption was consistently slightly higher than controls in animals treated with PF-04971729 in the absence or presence of sitagliptin, but was similar to controls in animals receiving sitagliptin alone.

Hematology: Fasted blood samples were collected at necropsy on Day 15.

red blood cell (erythrocyte) count	white blood cell (leukocyte) count
hemoglobin	differential blood cell count
hematocrit	blood smear
mean corpuscular volume	reticulocyte count
mean corpuscular hemoglobin	mean platelet volume
mean corpuscular hemoglobin concentration	red blood cell distribution width
platelet count	
prothrombin time	activated partial thromboplastin time

There were no apparent drug-related effects.

Clinical Chemistry: Fasted blood samples were collected at necropsy on Day 15.

glucose	alanine aminotransferase
urea nitrogen	alkaline phosphatase
creatinine	gamma glutamyltransferase
total protein	aspartate aminotransferase
albumin	calcium
globulin	inorganic phosphorus
albumin:globulin ratio	sodium
cholesterol	potassium
total bilirubin	chloride

Blood glucose levels were significantly lower with PF-04971729 treatment in the absence or presence of sitagliptin, but were similar to controls with sitagliptin alone. Urea nitrogen concentrations were mildly to moderately higher (↑2-fold) with PF-04971729 administration (+/- sitagliptin) in a dose-dependent manner. Total protein (↓10%), albumin (↓9%), and globulin (↓13%) levels were lower in female 5/60, 25/0, and 0/60 groups. AST and ALT liver markers were increased (<2-fold) in females at ≥5 mg/kg and males at 25 mg/kg PF-04971729 when co-administered with sitagliptin, but not with sitagliptin alone. Statistically significant 4 to 6% decreases in Cl concentration were observed in males at combination doses ≥5/60 and PF-04971729 alone and in females with PF-04971729 alone, but not with sitagliptin alone. Significant decreases in calcium levels were observed in males (↓4%) at 25/20 and 25/60 and in females (↓5-9%) in all groups with PF-04971729 administration. Occasionally significant decreases in Na and phosphorus levels were also noted in combination or PF-04971729 groups, but without dose-dependency or a consistent trend.

Urinalysis: Overnight urine samples were collected from fasting animals prior to necropsy on Day 15.

appearance (clarity and color)	pH
bilirubin	protein
blood	specific gravity
glucose	urobilinogen
ketones	volume
microscopic examination of sediment	

Increases in urine specific gravity (↑2-fold), marked glucose reactivity, and mildly lower pH were observed in animals treated with PF-04971729 alone(+/- sitagliptin), but not with sitagliptin alone.

Organ Weights: Paired organs were weighed together (see table under Histopathology).
Mild increases in absolute and relative kidney weights (↑20%) were observed in females with 25 mg/kg PF-04971729 alone, with less severe increases (↑9-16%) when co-administered with sitagliptin. Increases in male kidney:body weight ratios may be equivocal due to slightly lower body weights in males.

Text Table 4.3: Test Article-Related Changes in Kidney Weight Parameters

	PF-04971729/Sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day) PF-04971729/Sitagliptin	0/0	5/20	5/60	25/20	25/60	25/0	0/60	0/0	5/20	5/60	25/20	25/60	25/0	0/60
Kidney														
Absolute Weight (g)	2.3383	110	110	107	115	104	104	1.6327	106	101	104	111	117*	93
Body Weight Ratio (%)	0.7195	120*	116	119*	125*	115	107	0.7909	110*	103	110*	116*	122*	95
Brain Weight Ratio (%)	115.9305	107	109	111	115*	99	105	83.5880	107	102	109	115*	123*	97

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage control mean value.

Gross Pathology: Examination of external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues was performed at necropsy on Day 15.
There were no significant drug-related effects.

Histopathology: Tissues from main study animals were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). A peer review evaluation was performed by the sponsor. Tissues indicated in the table below from animals in Groups 1, 5, 6, & 7 were examined microscopically (E).

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle (biceps femoris) {skeletal muscle}		P,E
animal identification			optic nerve (2)[b,c]		P,E
aorta		P,E	ovary (2)	W	P,E
brain[a]	W	P,E	oviduct (2)		
cecum		P,E	pancreas		P,E
cervix		P,E	pituitary gland		P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	right upper incisor tooth with root		P,E
epididymis (2)	W	P,E	salivary gland (mandibular [2])		P,E
esophagus		P,E	sciatic nerve (2)[c] {peripheral nerve}		P,E
eye (2)[b]		P,E	seminal vesicle		P,E
femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	skin/subcutis {skin and adnexa}		P,E
gut-associated lymphoid tissue {GALT}			spinal cord (cervical, thoracic, and lumbar) {spinal cord}		P,E
Harderian gland[b]		P,E	spleen	W	P,E
heart	W	P,E	sternum with bone marrow {sternum}		P,E
ileum		P,E	stomach		P,E
jejunum		P,E	testis (2)[b]	W	P,E
kidney (2)	W	P,E	thymus	W	P,E
larynx			thyroid (2 lobes) with parathyroid {thyroid, parathyroid}		P,E
lesions		P,E	tongue		P,E
liver	W	P,E	trachea		P,E
lower mandible			ureter		P,E
lungs with large bronchi {lung}		P,E	urinary bladder		P,E
lymph node (mesenteric) {mesenteric lymph node}		P,E	uterus		P,E
lymph node (inguinal) {inguinofemoral lymph node}		P,E	vagina		P,E
mammary gland (males and females)		P,E			

E – Examined microscopically; P – Processed; W – Weighed.
a Brain was sectioned according to published recommendations (Bolon et al., 2013).
b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
c Longitudinal and cross sections were collected, preserved, and examined. For the sciatic nerve, only the left sciatic nerve was examined.

Decreases in pancreatic acinar cell zymogen granules were observed in both sexes treated with 25 mg/kg PF-04971729 (+/- sitagliptin).

Text Table 4.4: Incidence and Severity of Test Article-Related Microscopic Findings

	PF-04971729/sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day)	0/0	5/20	5/60	25/20	25/60	25/0	0/60	0/0	5/20	5/60	25/20	25/60	25/0	0/60
Number Examined	5	0	5	5	5	5	5	5	0	5	5	5	5	5
Pancreas														
Zymogen granules, decreased														
Minimal	1	-	1	3	2	2	1	2	-	1	2	2	3	1
Slight	1	-	2	0	2	0	0	0	-	0	1	0	0	1
Moderate	0	-	0	0	1	1	0	1	-	0	0	2	1	0

-= Not examined.

Minimal renal tubule mineralization was reported in 2/5 females at 25/60, but not in any of the other groups.

7 Genetic Toxicology

Since both ertugliflozin and metformin are not considered to be genotoxins, the FDC is not expected to be genotoxic. Genetic toxicology studies with FDC are not considered necessary.

Ertugliflozin

Ertugliflozin is not considered to be genotoxic. Ertugliflozin was negative for genotoxic potential in a standard battery of valid genotoxicity assays, including *in-vitro* microbial reverse mutation (Ames), *in vitro* human lymphocyte cytogenic, and *in-vivo* rat micronucleus assays.

Metformin

There is no evidence of a mutagenic potential for metformin in the Ames, mouse lymphoma, *in vitro* chromosomal aberration, or *in vivo* mouse micronucleus tests.

8 Carcinogenicity

Carcinogenicity studies with the combined product FDC are not considered to be necessary.

Ertugliflozin

Rat and mouse carcinogenicity studies are in progress for ertugliflozin under IND#106447. In the 2-year rat study, doses of 1.5, 5, and 15 mg/kg are being examined with AUC ratios at the high dose of 44x and 67x in males and females, respectively. Doses of 5, 15, and 40 mg/kg are being examined in mice with exposure multiples of 4x, 15x, and 44x in males and 8x, 25x, and 73x in females, in which the high dose exposure multiples are reduced to 30x in males and 50x in females after correction for the minor difference in protein binding between mice (95.5% bound) and humans (96.3%).

Metformin

In metformin carcinogenicity studies, there was no evidence of carcinogenicity in rats or mice aside from benign stromal uterine polyps in female rats.

9 Reproductive and Developmental Toxicology

Metformin is a Pregnancy category B compound and ertugliflozin is not anticipated to be a category “D” or “X” compound, based on segment II studies. Thus, in accordance with the FDA Guidance for Industry Nonclinical Safety Evaluation of Drug or Biologic Combinations, one embryonic fetal development study in the most appropriate species would be required at the time of submission of the NDA application for the ertugliflozin + metformin combination product FDC. Given that the unbound fraction of ertugliflozin is higher in rats similarly to humans, but lower in rabbits, the PK profile in rats is likely to be more similar to humans and the rat is considered to be the more appropriate species. However, since the ICH M3(R2) guidance states that …”If nonclinical embryo-fetal studies have indicated that neither agent poses a potential human developmental risk, combination studies are not recommended unless concerns exist, based on the properties of individual components, that their combination could give rise to a hazard for humans. In circumstances when the individual agents have been tested in embryo-fetal studies but embryo-fetal studies of the drug combination are warranted, the study(ies) of the combination should be available to support the marketing application.” Although the two guidances contradict each other, the ICH M3(R2) guidance supercedes recommendations of the FDA guidance. Since ertugliflozin and metformin are individually not likely to pose a significant risk for reproductive and developmental toxicities at clinical exposures, as well as a drug-drug interaction between the two is not anticipated, an additional segment 2 study with the FDC product is not warranted at this time, based on the ICH M3(R2) stipulations. However, since segment 3 studies for ertugliflozin alone have not yet been submitted for review, the reproductive and developmental safety profile of ertugliflozin has not been fully established.

Ertugliflozin

A rat fertility and embryonic development study (#10GR227) is described in the Investigator’s Brochure, but has not been reviewed. The sponsor reports a NOAEL of 250 mg/kg/day with no effects on estrous cycling, mating, fertility, male reproductive organ weights, or sperm motility and concentration. Segment II rat and rabbit embryo-fetal development studies have been reviewed. In rats, the maternal and fetal NOAEL was 100 mg/kg (>100x MRHD, total drug) with major findings including maternal toxicity and fetal malformations (external/visceral/skeletal) at 250 mg/kg/day. In rabbits, the fetal NOAEL was 100

mg/kg/day (>100x MRHD, total drug) due to visceral and skeletal malformations at 250 mg/kg/day. The rabbit maternal NOAEL was not determined due to toxicities at the lowest dose of 50 mg/kg/day. Drug transfer to the fetuses was not assessed in rats or rabbits. Although malformations were reported, the margins of safety are sufficient and adverse fetal effects are unlikely at the clinical therapeutic dose of 15 mg/day ertugliflozin.

Metformin

Metformin is not teratogenic in rats or rabbits at doses up to 2 to 6X the maximum adult dose of 2000 mg/day ($MRHD_{2000mg/day}$), and is not expected to reach high enough levels in humans to cause teratogenic effects independent of hypoglycemia and/or folic acid deficiency.

Metformin is a hydrophilic, low molecular mass molecule with low protein binding that freely crosses the placental barrier. While one report states that fetal concentrations only reach 40-50% of that of the mother, another study suggests that fetal exposures can be as high as or higher than maternal exposures. Metformin is a Pregnancy category B compound because it was not considered teratogenic in regulatory embryo fetal development studies and was not anticipated to have adverse effects on human development, but there are no adequate or well-controlled studies in pregnant humans. Published reports of rodent studies indicate that metformin may have weak teratogenic properties despite a partial placental barrier to metformin. Wistar rats treated with metformin during pregnancy have increased rates of resorption and low incidences of severe axial/neural tube malformations, including craniorachischisis and double monster, situs inversus/levocardia, hydronephrosis, absent kidneys, shortened/missing digits, and forelimb flexure abnormalities, along with hematoma and edema. Whole mouse embryo cultures treated with doses similar to human exposure levels were reported to cause 10% incidence rate of delayed neural tube closure. Metformin-related neural tube teratogenic effects observed in vitro are associated in part with the folate pathway and that the teratogenic effects of metformin seen at lower doses (approximately 2x $MRHD_{2000mg/day}$) are partially preventable with co-treatment of folinic acid. Since prolonged treatment with metformin leads to subnormal vitamin B12 levels in 7% of patients, prevention of metformin-induced teratogenic effects, although only partial, is a significant finding. However, at higher doses, metformin-induced exencephaly neural tube defects (estimated 4x $MRHD_{2000mg/day}$) are independent of the folate pathway, but are unlikely to occur at human exposure levels. Conversely, i*n vivo* studies suggest that metformin, alone or in combination with saxagliptin, is not associated with significant development of teratogenic effects above historical controls at doses with up to a 10-fold safety margin. Further implying that exposure levels are unlikely to reach high enough levels *in vivo* to result in metformin-induced teratogenic effects seen *in vitro*. Therefore, metformin HCl is not likely to be teratogenic at exposure levels seen in humans. This conclusion is supported by the lack of teratogenic incidences in humans despite the wide, global use of metformin HCl.

Although adequate clinical studies have not been conducted with metformin in pregnant women, humans have been exposed to metformin for roughly 4 decades and metformin has not been linked with teratogenic effects in humans. Literature reports of multiple studies in humans suggest that metformin is not associated with teratogenic effects in humans, however not all studies were properly balanced or associated with treatment during the first trimester, which is the critical stage for proper neural tube development. Perhaps the most informative study to date, published in December 2010, was a multi-center study of metformin versus placebo during treatment throughout the first trimester in women with polycystic ovary syndrome, which found that preeclampsia was more prevalent in the metformin group (7.4%) compared to placebo (3.7%), but that metformin was not associated with any malformations or severe neonatal hypoglycemia. Although preeclampasia can be a significant health risk to both the fetus and the mother and the only treatment is delivery, it is not a teratogenic effect. A separate study reports that treatment with oral hypoglycemic agents, including metformin, reduces the rate of infants with hypoglycemia compared to those treated with insulin during pregnancy. These studies suggest that there is not risk of teratogenic effects, including neural tube defects, in humans. Furthermore, these studies suggest that there is an added benefit to the fetus with treatment of maternal diabetes with metformin over maternal treatment with insulin.

10 Special Toxicology Studies

The phototoxicity potential of Ertugliflozin is considered to be low; thus, phototoxicity studies have not been conducted, nor have they been requested by the division. Although metformin is associated with photosensitivity, phototoxicity studies will not be required for the combined product FDC.

11 Integrated Summary and Safety Evaluation

The sponsor's IND package for the FDC product cross-references non-clinical pharmacodynamic, pharmacokinetic, toxicology information for the SGLT2 inhibitor ertugliflozin previously submitted under IND #106477. The sponsor is also referencing the approved label for Glucophage® (metformin).

Co-administration of PF-04971729 and metformin was associated with minor findings that were not observed with administration of either drug alone and which were considered to be non-adverse and did not contribute to determination of the NOAEL. Findings of minimal renal proximal convoluted tubule hypertrophy characterized by enlargement and increased pallor of epithelial cells extending from the outer cortex to the medulla were not associated with correlative signs of kidney malfunction. Instead, the renal tubule hypertrophy was considered likely to be an adaptive response to the pharmacodynamic activity of PF-04971729 (glucosuria and subsequent osmotic diuresis) in conjunction with workload demands resulting from renal tubular secretion of metformin. Although the renal tubule hypertrophy findings were not considered adverse in the 2-week study, based on kidney findings of tubule dilatation with hyperplasia and mineral deposition in 3 and 6-month rat studies with ertugliflozin alone, more severe kidney toxicities may become evident with longer exposures to co-administration of ertugliflozin and metformin.

Increases in urine glucose levels (glucosuria) are consistent with the pharmacodynamic activity of SGLT2 inhibitors and previous studies with ertugliflozin alone. Increases in urine specific gravity, urine ketone levels, and blood urea nitrogen levels are consistent with dehydration and osmotic diuresis secondary to glucosuria. Decreases in electrolyte levels (Na, Ca, and Cl) in the blood are also likely to be secondary to PF-04971279-related osmotic diuresis.

Decreases in zymogen granules of pancreatic acinar cells were observed in both 2-week studies in animals treated with ertugliflozin in the absence or presence of metformin or sitagliptin. Zymogen granules in the apical region of acinar cells have been shown to reduce in size and/or number after feeding, most likely due to digestive enzyme secretion stimulated by feeding (Ermak & Rothman, *Cell Tissue Res.* 1981; 214: 51-66). Increased food consumption has been observed in previous non-clinical studies with ertugliflozin alone and is likely secondary to drug-related decreases in blood glucose levels related to the pharmacodynamic activity of ertugliflozin. Thus, decreases in zymogen granules of pancreatic acinar cells are likely related to ertugliflozin-related increases in food consumption and are considered to be tertiary to the pharmacodynamic activity of ertugliflozin.

Safety margins for co-administration of ertugliflozin (56x MRHD) and metformin (9x MRHD) based on the 2-week rat study are sufficient to support clinical exposures of the FDC product (15 mg/day ertugliflozin + 2000 mg/day metformin) (b) (4).

In accordance with ICH guidelines, the sponsor plans to submit a 3 month toxicology bridging study with combination of ertugliflozin and metformin administration in rats to support clinical studies of the FDC for ≥3 months.

Table 2: Ertugliflozin + Metformin Co-Administration Human Safety Margins

Study	NOAEL (mg/kg/day)	Human Safety Margin (Based on AUC*)	Basis
2 Week **Ertugliflozin/Metformin:** 5/200, 5/600, 25/200, 25/600, 25/0, & 0/600 mg/kg Ertugliflozin AUC: 26, 15, 109, 77, 124, & - µg·h/mL Metformin AUC: 59, 138, 76, 183, -, & 140 µg·h/mL	Ertugliflozin / Metformin **25 / 600**	Ertugliflozin: **56x** Metformin: **9x**	*No significant systemic adverse effects.* **≥5 / ≥0 mg/kg (10x MRHD):** ↑BUN, ↑food consumption, ↓blood glucose, ↑urine specific gravity, ↑ketones, ↑urine glucose **≥5 / 600 mg/kg (10x/9x MRHD):** ↑ALT, ↑AST **25 / ≥200 mg/kg (56x/7x MRHD):** Minimal renal proximal convoluted tubule hypertrophy, ↓serum electrolytes (Cl) **25 / ≥0 mg/kg (56x MRHD):** stomach erosion, ↓zymogen granules of pancreatic acinar cells **≥0 / 600 mg/kg (9x MRHD):** minimal hypertrophy and ↓cytoplasmic granules of salivary gland duct epithelium

*Based on a maximum twice daily dose of 7.5 mg ertugliflozin / 1000 mg metformin with a predicted ertugliflozin exposure of AUC_{0-24} = 1.37 µg·h/mL and metformin exposure of AUC = 20.544 µg·h/mL

12 Appendix/Attachments

Table 3: Non-Clinical Study Checklist

Safety Pharmacology (NME only)	PK/ADME (NME only)
✓ **Cardiovascular** ✓ **Respiratory** ✓ **CNS Neurological** ☐ ~~Abuse Liability~~ ✓ Pharmacodynamic	✓ **Protein Binding** ✓ Pharmacokinetics ✓ Absorption ✓ Distribution ✓ **Metabolism** ✓ Elimination

Species	Toxicity
Rat Dog	**NME: Single-dose** (or multiple dose <24 hours) **IV & intended route** (+14 days observation) → *support clinical single-dose Ph I clinical studies.* ✓ 2 species - OR - ✓ DRF/escalation MTD study in 1 species
Mouse, Rat, Dog	**NME:** 2-wk repeat-dose study → *support clinical studies up to 2 weeks*
Rat: 1-mo, 3-mo Mouse: 3-mo, Dog: 1-mo, 3-mo	**NME:** 2-wk to 6-mo repeat-dose study → *support clinical studies of ≤ duration of nonclinical study*
(planned in rats)	**Combination (MD+NME)**: 90-day Bridging study in most appropriate species → *support clinical studies > 1 month*
Rat	**NME: 6**-mo rodent repeat-dose study → *support clinical studies > 6 months**
Dog (+ 8-wk Recovery)	**NME:** 9-mo non-rodent repeat-dose study → *support clinical studies > 6 months**

Genetic Toxcity (NME only)	Carcinogenicity (NME only)
✓ *In Vitro* Mutation Assay (Ames) ✓ *In Vitro* Chromosome Aberration ✓ *In Vivo* Clastogenicity Assay	☐ 2-yr Rat ✓ Protocol submitted ☐ 18-mo Mouse ✓ Protocol submitted

Repro Tox

☐ Segment I: Fertility and Early Embryonic Development (NME only) – Rat Study Needs Review

✓ Segment II: Embryonic Fetal Development
- ✓ Rat
- ✓ Rabbit

☐ Segment III: Prenatal and Postnatal Development (NME only)

* Both rodent and non-rodent chronic studies required for clinical studies > 6 months.

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

/s/

JESSICA J HAWES
09/05/2014

RONALD L WANGE
09/05/2014
I concur

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

Application number:	IND 122,330
Review Number (Completion Date):	#1 (28 August, 2014)
Supporting document/s:	SDN-2(N-000)
Sponsor's letter date:	7/30/14
CDER stamp date:	7/30/14
Product:	Ertugliflozin/sitagliptin FDC
Indication:	Type 2 diabetes mellitus
Sponsor:	Merck (partnership with Pfizer (ertugliflozin))
Review Division:	Metabolism and Endocrinology Products
Reviewer:	David B. Carlson, Ph.D.
Supervisor/Team Leader:	Todd Bourcier, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Bola Adeolu

Review Notes and Abbreviations/Key – Data tables and figures from the Sponsor have been included and cited in this review; original tables and figures by this reviewer are also noted. All drug-related trends are discussed in relation to concurrent vehicle control groups in each study unless otherwise noted.

Key: Fixed-dose combination (FDC), once daily dosing (QD); dosing groups – LD (low dose), MD (mid dose), LMD (low mid dose), HMD (high mid dose), HD (high dose); mg/kg (mg/kg/day); 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);
DPP4 (dipeptidyl peptidase 4); SGLT2 (sodium glucose co-transporter 2)

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Merck is partnering with Pfizer to develop a FDC tablet of ertugliflozin, a SGTL2 inhibitor, and sitagliptin, a DPP4 inhibitor, for treatment of type 2 diabetes mellitus. The complementary mechanisms are expected to provide more robust treatment than the single agents alone. Sitagliptin is currently indicated for T2DM and ertugliflozin is in Phase 3 trials. Merck plans to reference the Januvia® label for evidence of sitagliptin safety and effectiveness and IND 106,447 for all ertugliflozin supporting clinical and nonclinical PD, PK, and toxicology data.

1.2 Brief Discussion of Nonclinical Findings (Internal Comments)

Several DDP4 inhibitors and SGLT2 inhibitors are approved for treatment of type 2 diabetes. Inhibition of DPP4 prevents, or delays, inactivation of postprandial incretins which improve insulin sensitivity and glucose control. SGLT2 inhibition prevents glucose reuptake in kidney proximal tubules, thus facilitating urinary glucose excretion in T2DM. DPP4 inhibitors typically have high margins of safety between therapeutic doses and exposures that cause nonclinical toxicity. SGLT2 inhibitor class-related risks are well known, including potential kidney and bone-related toxicity and gastrointestinal distress. Other DPP4 inhibitor/SGLT2 inhibitor FDC drugs are under investigation and there are no predictable interactions suggesting specific risks with DPP4 inhibitor and SGLT2 inhibitor FDC drug products.

Nonclinical toxicity investigations with sitagliptin and ertugliflozin coadministration are limited. Merck submitted an exploratory, non-GLP, two week rangefinding study in rats which they will use to set doses for a definitive three month rat combination toxicity study. Thus, this Pharmacology/Toxicology Review is limited in scope to addressing any obvious toxicity concerns with combined sitagliptin and ertugliflozin treatment.

There were no apparent toxicokinetic (TK) or toxicity interactions in the two-week combination rat study. Drug exposures were similar between sexes and there were no apparent exposure differences from drugs given alone or in combination. Most toxicity was due to ertugliflozin and consistent with the SGLT2 inhibitor class. Toxicity included increased kidney-related effects (20% ↑ kidney weight, 2X ↑ serum urea nitrogen, 10% ↓ serum proteins), minimal increases in serum transaminases (↑ 30-80%) and minimal to mild changes in serum calcium (↓ 4-9%), sodium (↓ 2%), and chloride (↓ 3-4%).

The drug targets, DPP4 and SGLT2, are both expressed in kidney proximal tubules. Sitagliptin and other DPP4 inhibitors for T2DM are intended to act on soluble DPP4 in plasma, however, drug will theoretically inhibit any available DPP4. Therefore there is a theoretical risk for exacerbated toxicity in kidney proximal tubules due to combined DPP4 and SGLT2 inhibition or unintended toxicity from exaggerated pharmacology.

1.3 Recommendations

The proposed single dose clinical pharmacokinetic study is reasonably safe to proceed.

1.4 External Comments to Sponsor

Non-hold recommendations

- There are inconsistencies in the 2-week combination rat study report toxicokinetic data and study report summary and conclusions ((b) (4) Study No. 8294467, Sponsor Ref. No. 13GR342). Please clarify discrepancies between toxicokinetic data tables (Text Tables 4.1 and 4.2) and conclusions in the original toxicokinetic report ((b) (4) TK Report No. 0455-13326-1) which show no TK interaction between ertugliflozin and sitagliptin and the Sponsor's conclusions that coadministration of ertugliflozin and sitagliptin affects plasma levels of individual drugs (Summary, pp. 8-9; Results, Section 4.3, Toxicokinetics, p. 24).

    - Ensure that any clinical information (protocol, IB, informed consent) are consistent with the current nonclinical data which show no apparent drug interactions on exposures in rat.

- It is expected that results of the 3-month combination toxicology study in rats will be submitted prior to initiation of Phase 3 trials or prior to dosing of patients beyond 3 month's duration.

2 Drug Information

2.1 Drug

2.1.2 Generic Name

Sitagliptin phosphate (sitagliptin)
Ertugliflozin L-pyroglutamic acid (ertugliflozin)

2.1.3 Code Name

MK-0431 (sitagliptin)
PF-04971729 (ertugliflozin); PF-04971729 (b) (4) (ertugliflozin L-pyroglutamic acid co-crystal form)
MK-8835A (ertugliflozin/sitagliptin FDC)

2.1.7 Pharmacologic class

DPP4 inhibitor (sitagliptin) and SGLT2 inhibitor (ertugliflozin)

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

NDA 21995 (sitagliptin; Januvia®)
IND 106,447 (ertugliflozin)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Drugs will initially be coadministered in the opening clinical trial. A fixed dose combination (FDC) is planned for the clinical development program.

Ertugliflozin and sitagliptin FDC in a film-coated (b) (4) tablet with proposed tablet strengths of 100/5 and 100/15 mg sitagliptin (MK-0431) and ertugliflozin (MK-8835). Drug product composition is shown in the Sponsor's summary table, below.

Drug Product Composition

Strength			100/5 mg	100/15 mg
Component	Quality Standard	Function	mg/tablet	mg/tablet
MK-0431†	In-house	Drug substance	128.48	128.48
MK-8835‡	In-house	Drug substance	6.48	19.43
Microcrystalline Cellulose	Compendial§	(b) (4)		
Dibasic calcium phosphate (anhydrous)	Compendial§			
Croscarmellose Sodium	Compendial§			
Sodium Stearyl Fumarate	Compendial§			
Magnesium Stearate	Compendial§			
Theoretical Core Tablet Weight				
(b) (4)				

2.3.2 Comments on Novel Excipients

No novel excipients in current drug product formulation.

2.4 Proposed Clinical Population and Dosing Regimen

Sitagliptin has been approved for treatment of T2DM for several years as monotherapy, FDC, or on top of various background therapies. Ertugliflozin is in Phase 3 development for treatment of T2DM. Importantly, at least three Phase 3 trials have been initiated under the ertugliflozin development program that involve up to 26 weeks of coadministration with sitagliptin (with or without background metformin therapy).

Merck states the overall objective of the sitagliptin/ertugliflozin FDC program "is to establish bioequivalence to co-administration of the tablets for sitagliptin (Januvia®) and ertugliflozin used in Phase 3 studies, thus bridging all relevant safety and efficacy data from clinical studies in T2DM…".

Sponsor's General Investigational Plan
Over the next year it is anticipated that up to three studies will be conducted under this IND:

1) a descriptive, two-way, pharmacokinetic drug interaction study between sitagliptin and ertugliflozin, the IND opening study

2) a food effect study with the higher strength FDC tablet (sitagliptin 100 mg/ertugliflozin 15 mg)

3) a pivotal bioequivalence study (or studies) in which the selected FDC tablets will be tested in the fasted state against the individual components used in the Phase 3 trials.

A phase 1, randomized, open-label, 3-period, 6-sequence study to estimate the pharmacokinetic interaction between ertugliflozin and sitagliptin in healthy subjects (Protocol MK-8835-022-00/B1521033)

Sponsor's summary
The study will be a Phase 1, open-label, randomized, 3-period, 6-sequence single dose crossover drug-drug interaction study to estimate the pharmacokinetic interaction between ertugliflozin and sitagliptin in healthy subjects. Approximately 12 healthy male and/or female subjects will be enrolled in the study. Each subject will receive 3 treatments in a randomized manner according to one of six sequences. These treatments are: **Treatment A:** 15 mg ertugliflozin (single dose), **Treatment B:** 100 mg sitagliptin (single dose), and **Treatment C:** 15 mg ertugliflozin + 100 mg sitagliptin (single dose of each) administered simultaneously. Eligible subjects will be admitted to the Clinical Research Unit (CRU) on Day 0. Subjects will receive the assigned trial treatment in the morning of Day 1 in each period after an overnight fast of at least 8 hours. Serial blood samples for determination of ertugliflozin and/or sitagliptin concentrations will be collected from each subject for 72 hours post-dose. Dosing in each period will be separated by a washout period of at least 5 days. Subjects will be monitored for adverse events and laboratory abnormalities during the conduct and at the conclusion of the trial.

The primary objective(s) of this study is/are.

- To estimate the effect of sitagliptin on the pharmacokinetics of ertugliflozin following oral administration of a single dose of 15 mg ertugliflozin and 100 mg sitagliptin in healthy volunteers.

- To estimate the effect of ertugliflozin on the pharmacokinetics of sitagliptin following oral administration of a single dose of 15 mg ertugliflozin and 100 mg sitagliptin in healthy volunteers.

2.5 Regulatory Background

NDA 21995 – sitagliptin is currently listed by Merck as Januvia® for treatment of T2DM
IND 106,447 – ertugliflozin is under development by Pfizer for treatment of T2DM

2.5.1 Previous Clinical Experience

Ertugliflozin is currently in Phase 3 clinical trials as monotherapy for treatment of T2DM.

2.5.2 History of Regulatory Submission

A pre-IND meeting request was submitted and meeting minutes and written responses from DMEP are archived in CDER's electronic tracking system, DAARTS.

3 Studies Submitted

3.1 Studies Reviewed

2-Week combination rat rangefinding (cross-referenced from IND 106,447)

3.2 Studies Not Reviewed

None.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: 2-Week oral gavage toxicity and toxicokinetic study with PF-04971729 and sitagliptin in rats (Sponsor No. 13GR342, TT #14-7801, (b) (4) Study 8294467)

Study no.:	Sponsor No. 13GR342, TT #14-7801, (b) (4) Study 8294467
Study report location:	N-000 (cross-reference IND 106,447, SDN-104, 7/21/14, eCTD)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/14/14
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PF-04971729, Lot GR02694, 73.9% potency; Sitagliptin, Lot No. 010X054, 99.6% potency

Key Study Findings:

- Study was limited in scope to identify doses for a definitive 3-month combination toxicity study
- There were no apparent drug interactions that affected toxicity from individual drugs
- No apparent TK interactions or sex differences in exposure with PF-04971729 (ertugliflozin) and sitagliptin coadministration
    - Note – the study report erroneously concluded there were TK interactions with coadministration, based on an apparent misinterpretation of dose-related increases in exposure (not drug interactions) in the original TK report
- Ertugliflozin-related toxicity, generally dose-related and consistent with SGLT2 inhibitor class effects, included:
    - Clinical pathology markers
        - Marked glucosuria, moderately lower serum glucose (↓ 20-40%, and urine changes (higher specific gravity, lower pH)
            - Glucose-related changes consistent with expected pharmacology
        - Mildly (~10%) lower serum protein (total, albumin, globulin)
        - Minimal increased AST, ALT (↑ 30-80%)
        - Minimal to mild decreased serum calcium (↓ 4-9%)
        - Minimal decreased serum sodium (female; ↓ 2%) and chloride (↓ 3-4%)
    - Mild kidney-related changes
        - Increased urea nitrogen (2X), decreased serum proteins (female)
        - Increased kidney weights (↑ ~20%, HD females) without histological correlate
    - Pancreas decreased zymogen granules, increased incidence and severity with increased dose
- Sitagliptin-related toxicity was limited to “mildly lower” (9%) total protein and albumin concentrations (HD females)

Methods

Doses:	0/0, 5/20, 5/60, 25/20, 25/60, 25/0, 0/60 mg/kg/d PF-04971729 / sitagliptin
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg PF-04971729 + 5 ml/kg sitagliptin
Formulation/Vehicle:	PF-04971729 – 0.5% w/v methylcellulose (4000 cps)/10% w/v PEG-400; Sitagliptin – 0.5% w/v methylcellulose (4000 cps)/5 mM HCL
Species/Strain:	Sprague Dawley rat / Crl:CD(SD)
Number/Sex/Group:	5
Age:	7 Weeks
Weight:	Male – 209 – 267 g / Female – 152 – 203 g
Satellite groups:	4/sex/group TK
Unique study design:	Histopathology of suspected target organs (macroscopic lesions and pancreas)
Deviation from study protocol:	Several protocol amendments were documented, none that affected results (n.b. non-GLP study, no QA report)

Study Design Summary

		No. of Animals		PF-04971729 Dose		Sitagliptin Dose	
Group[a]	Subgroup	Male	Female	Dose Level (mg/kg/day)	Concentration[b] (mg/mL)	Dose Level (mg/kg/day)	Concentration[c] (mg/mL)
1 (Control)[d]	1 (Toxicity)	5	5	0	0	0	0
	2 (Toxicokinetic)	4	4	0	0	0	0
2 (Low/ Low)	1 (Toxicity)	5	5	5	1	20	4
	2 (Toxicokinetic)	4	4	5	1	20	4
3 (Low/ High)	1 (Toxicity)	5	5	5	1	60	12
	2 (Toxicokinetic)	4	4	5	1	60	12
4 (High/ Low)	1 (Toxicity)	5	5	25	5	20	4
	2 (Toxicokinetic)	4	4	25	5	20	4
5 (High/ High)	1 (Toxicity)	5	5	25	5	60	12
	2 (Toxicokinetic)	4	4	25	5	60	12
6 (High/ Control)	1 (Toxicity)	5	5	25	5	0	0
	2 (Toxicokinetic)	4	4	25	5	0	0
7 (Control / High)	1 (Toxicity)	5	5	0	0	60	12
	2 (Toxicokinetic)	4	4	0	0	60	12

a Animals received two consecutive doses via oral gavage daily: 5 mL/kg PF-04971729 (or Vehicle Control Article 1, as applicable) followed by 5 mL/kg sitagliptin (or Vehicle Control Article 2, as applicable).

b PF-04971729 dose concentrations were corrected for lot specific potency of 0.739 (73.9%). A correction factor of 1.353 was used for Lot No. GR02694.

c Sitagliptin dose concentrations were corrected for salt content and lot specific potency of 0.996 (99.6%). A correction factor of 1.285 was used.

d Group 1 received Vehicle Control Article 1 (0.5% [w/v] methylcellulose [4000 cps] with 10% [v/v] polyethylene glycol 400 prepared in reverse osmosis water) and Vehicle Control Article 2 (0.5% [w/v] methylcellulose [4000 cps] with 5 mM hydrochloric acid prepared in reverse osmosis water) only.

Observations and Results (additional or not described above)

Mortality – None.

Clinical Signs – Unremarkable.

Body Weights – Slight decreased mean body weight (ss) Week 2 in HD ertugliflozin males. Very slight body weight decreases (nss) in ertugliflozin groups ± sitagliptin. Slight body weight differences were considered "not test article related" by the Sponsor but the decreases are consistent with the SGLT2 inhibitor class.

Feed Consumption – Slight trend of increased food consumption in ertugliflozin ± sitagliptin, consistent with the SGTL2 inhibitor class.

Hematology – Unremarkable.

Gross Pathology – Unremarkable.

Histopathology

Incidence and Severity of Test Article-Related Microscopic Findings

	PF-04971729/sitagliptin													
Sex	Males							Females						
Dose Level (mg/kg/day)	0/ 0	5/ 20	5/ 60	25/ 20	25/ 60	25/ 0	0/ 60	0/ 0	5/ 20	5/ 60	25/ 20	25/ 60	25/ 0	0/ 60
Number Examined	5	0	5	5	5	5	5	5	0	5	5	5	5	5
Pancreas														
Zymogen granules, decreased														
Minimal	1	-	1	3	2	2	1	2	-	1	2	2	3	1
Slight	1	-	2	0	2	0	0	0	-	0	1	0	0	1
Moderate	0	-	0	0	1	1	0	1	-	0	0	2	1	0

-= Not examined.

Toxicokinetics

TK analyses were limited to four satellite animals per treatment and only two samples time point. Total ($AUC_{24\ h}$) ertugliflozin seemed to be higher in females than males (+60-80%) while sitagliptin seemed higher in males than females(+88%), however, no trends could be established based on limited sample endpoints. There were no apparent differences in drug exposures after single dosign (day 1) and multiple dosing (day 14) for any individual drug or combination treatment. Data are shown in the Sponsor's summary TK tables, below.

Mean Toxicokinetic Parameters for PF-04971729 in Rats on Study Days 1 and 14 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
5 / 20	1	Male	1790	7	23800
		Female	2110	7	30200
		Overall	1950	7	27000
	14	Male	1970	4	22700
		Female	2600	4	32300
		Overall	2280	4	27500
5 / 60	1	Male	1430	4	19300
		Female	2280	7	30900
		Overall	1800	7	25100
	14	Male	1700	4	19000
		Female	2330	4	29500
		Overall	2010	4	24200
25 / 20	1	Male	7720	7	107000
		Female	7650	4	30300
		Overall	7430	4	68900
	14	Male	8420	4	110000
		Female	12800	4	156000
		Overall	10600	4	133000
25 / 60	1	Male	8670	4	99100
		Female	9720	4	86900
		Overall	9190	4	93100
	14	Male	9750	4	110000
		Female	10700	7	155000
		Overall	9760	4	132000
25 / 0	1	Male	8880	7	115000
		Female	13700	7	184000
		Overall	11300	7	150000
	14	Male	8580	4	116000
		Female	14900	7	211000
		Overall	11200	7	163000

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

Mean Toxicokinetic Parameters for Sitagliptin in Rats on Study Days 1 and 14 after Daily Oral Administration of PF-04971729 and Sitagliptin

Dose PF-04971729 / Sitagliptin (mg/kg/day)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{24} (ng•h/mL)
5 / 20	1	Male	557	4	7360
		Female	555	4	4820
		Overall	556	4	6090
	14	Male	918	4	9550
		Female	734	4	5880
		Overall	795	4	7610
5 / 60	1	Male	2650	4	33600
		Female	2440	4	23500
		Overall	2540	4	28400
	14	Male	3380	4	31100
		Female	2900	4	22500
		Overall	3140	4	26700
25 / 20	1	Male	449	7	6120
		Female	498	1	5210
		Overall	461	4	5350
	14	Male	625	4	8580
		Female	800	4	6720
		Overall	712	4	7650
25 / 60	1	Male	2490	4	26500
		Female	1710	4	21900
		Overall	2100	4	24100
	14	Male	3080	4	33500
		Female	2260	4	20100
		Overall	2670	4	26800
0 / 60	1	Male	2950	4	36900
		Female	2370	4	19800
		Overall	2660	4	28300
	14	Male	4410	4	39900
		Female	3450	1	21200
		Overall	3660	4	30600

AUC_{24} = Area under the plasma drug concentration-time curve for 0-24 h; C_{max} = Highest drug concentration observed in plasma; T_{max} = Time at which C_{max} was first observed. Overall = male plus female combined

---

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
08/28/2014
Reasonably safe to proceed

TODD M BOURCIER
08/28/2014
I concur

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	106447
Review number:	5
FDA SDN/ Sponsor SN/ Sponsor letter date/	SDN 65/SN 64 (8/26/2013) 26 Week T2DM Clin Prot SDN 66/SN 65 (9/5/2013) Chronic Dog Tox SDN 67/SN 66 (9/6/2013) Cardiovas. Outcomes Trial
Drug:	PF04971729 (b) (4) (SGLT2 Inhibitor)
Indication:	T2DM
Sponsor:	Merck Sharp & Dohme Corp (co-sponsor Pfizer)
Review Division:	DMEP
Reviewer:	Jeffrey Quinn
Supervisor:	Todd Bourcier
Division Director:	Jean Marc Guettier
Project Manager(s):	Abolade Adeolu
Review completion date:	October 7th 2013
Comments:	No External Comments To Sponsor

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

PF04971729 (Ertugliflozin/SGLT2i) was submitted by Pfizer in September 2009, for the treatment of type 2 diabetes mellitus (T2DM). The sponsor was officially changed to Merck Sharp & Dohme Corp on June 21st 2013, although several communications since that date indicate that IND 106447 is being co-developed by both Merck and Pfizer.

Merck submitted a new clinical protocol on August 26th 2013 entitled,"A Phase 3, Randomized, Double-Blind, Placebo-Controlled, 26-Week Multicenter Study with a 26-Week Extension to Evaluate the Efficacy and Safety of Ertugliflozin Monotherapy in the Treatment of Subjects with Type 2 Diabetes Mellitus and Inadequate Glycemic Control Despite Diet and Exercise".

The sponsor was notified by the Division on August 30th 2013 that there was insufficient nonclinical information to support this clinical protocol and that submission of the 9-month toxicology study in dogs was required to avoid a partial clinical hold. The 9 month dog study was emailed by the sponsor and officially submitted to the Division on September 6th 2013.

A cardiovascular outcomes trial entitled, "Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Assess Cardiovascular Outcomes Following Treatment with Ertugliflozin in Subjects with Type 2 Diabetes Mellitus and Established Vascular Disease" was submitted the same day (September 6th 2013) as part of the Phase 3 program of IND 106447. The sponsor is seeking the Agency's input regarding their plan to forgo expedited reporting of serious adverse events that meet their pre-specified criteria.

A review of the 9 month dog study, the sponsor's preclinical program and how they pertain to the proposed clinical dose are discussed herein.

1.2 Brief Discussion of Nonclinical Findings (Internal Comments)

- Safety assessment was based on the toxicological data from the 6 month rat and the 9 month dog studies which support the proposed 15 mg/day dose based on the 19X and 59X safety margins (AUC) to the rat and dog NOAELs, respectively and the duration of the completed toxicology studies.

- Most of the findings noted during the chronic toxicity studies can reasonably be attributed to drug induced glucosuria, osmotic diuresis and a catabolic state. It should be noted that NOAELs were not established in the chronic toxicology studies for adrenal cortex hypertrophy (dog and rat), stomach erosions (rat) and kidney mineralization (rat).

- Gastrointestinal findings in dogs (excessive vomiting, salivation and abnormal feces) and rats (stomach erosion/ulcers, pyloric crypt degeneration and foveolar hyperplasia) are arguably related to off-target inhibition of SGLT1. Similar GI intolerance has been observed in clinical studies with SGLT1/2 mixed inhibitors and should be monitorable.

- Hyperostosis was observed in male rats after 6 months of dosing and occurs with a safety margin of 107X relative to the NOAEL for PF04971729. This effect is observed with several other SGLT2 inhibitors and monitoring of bone mass and for fractures has been incorporated into Phase 3 programs when deemed necessary (canagliflozin).

1.3 Recommendations

Pharm/Tox recommends that the proposed 15 mg/day dose is sufficiently supported by preclinical data, based on the 19X and 59X safety margins (AUC) to the rat and dog NOAELs, respectively and the duration of the completed toxicology studies.

2 Drug Information

2.1 Drug

Ertugliflozin

CAS Registry Number

N/A

Code Name

PF04971729 or PF04971729 (b) (4)

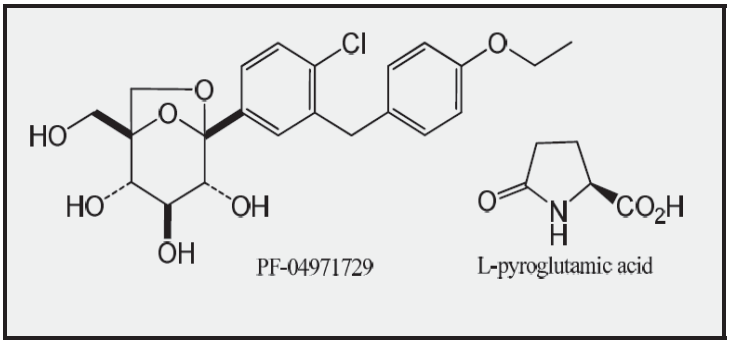
Chemical Name

((1S, 2S, 3S, 4R, 5S)-5-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-1-hydroxymethyl-6,8-dioxabicyclo [3.2.1]octane-2,3,4-triol

Molecular Formula/Molecular Weight

PF04971729 (amorphous form) $C_{22}H_{25}ClO_7$ / 436.88 Daltons
PF04971729 (b) (4) (L-pyroglutamic acid co-crystal form) $C_{27}H_{32}ClNO_{10}$ / 566.00 Daltons

Structure or Biochemical Description



PF04971729 L-pyroglutamic acid co-crystal form

Pharmacologic Class

PF04971729 is a sodium glucose co-transporter2 (SGLT2) inhibitor.

Planned Clinical Route of Administration

Oral

2.2 Relevant INDs, NDAs, and DMFs

(b) (4)

[redacted]

2.3 Drug Formulation

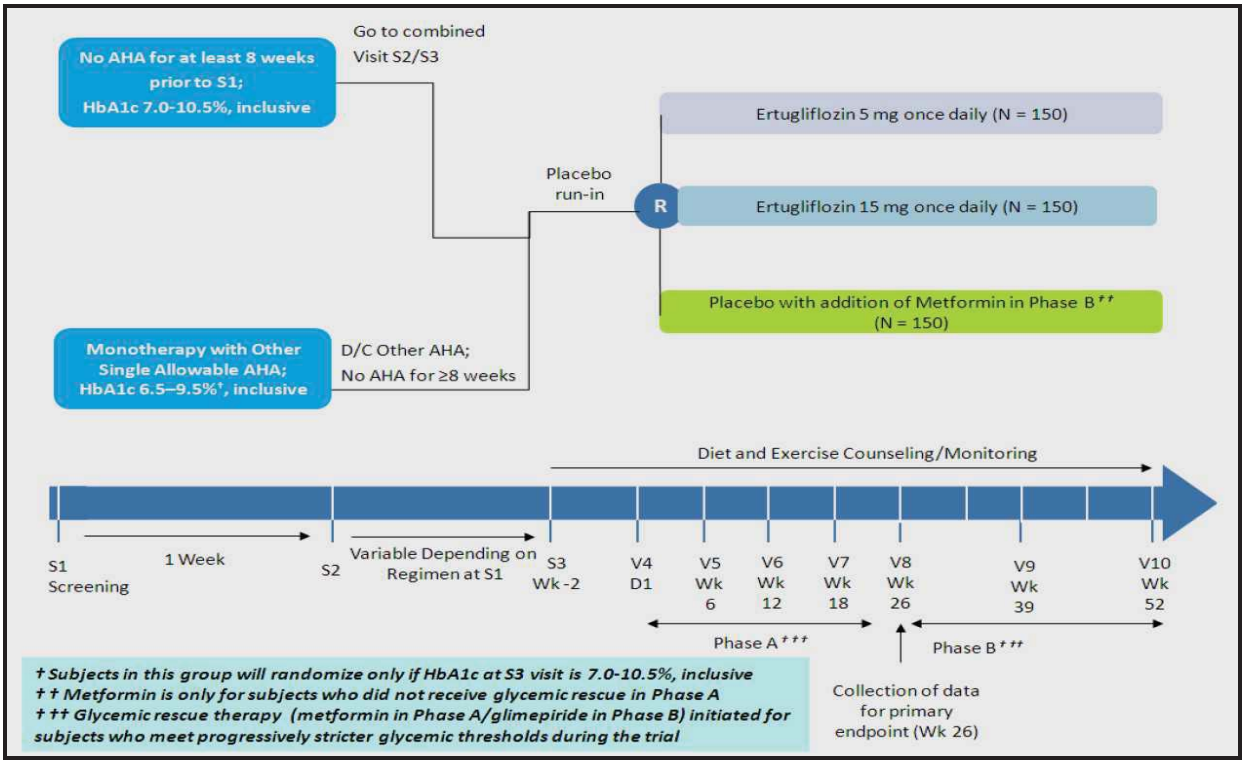
PF04971729 will be supplied as 5 mg and 10 mg tablets.

2.4 Proposed Clinical Protocol(s)

A Phase 3, Randomized, Double-Blind, Placebo-Controlled, 26-Week Multicenter Study with a 26-Week Extension to Evaluate the Efficacy and Safety of Ertugliflozin Monotherapy in the Treatment of Subjects with Type 2 Diabetes Mellitus and Inadequate Glycemic Control despite Diet and Exercise (Protocol MK-8835-003-00/B1521022)

The primary objectives of this study are:

- To assess the effect on HbA1c of 15 mg ertugliflozin as compared with placebo.
- To assess the effect on HbA1c of 5 mg ertugliflozin as compared with placebo.
- To assess the safety and tolerability of ertugliflozin



Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Assess Cardiovascular Outcomes Following Treatment with Ertugliflozin (MK-8835/PF-04971729) in Subjects with Type 2 Diabetes Mellitus and Established Vascular Disease (Protocol MK-8835-004-00/B1521021)

The primary objective of this study is:

- To demonstrate the non-inferiority of ertugliflozin compared with a non-ertugliflozin comparator group on the time to first occurrence of any of the components of the composite endpoint of cardiovascular death, non-fatal myocardial infarction or non-fatal stroke.

As part of the Phase 3 program for ertugliflozin, this protocol will contribute safety data to a program-wide meta-analysis of major adverse cardiovascular events. An Endpoint Adjudication Committee (EAC) will be established to review and adjudicate potential specific cardiovascular events and all deaths in this trial (including events from subjects who continue to be followed after discontinuation of investigational product), and across the Phase 2 and 3 program studies for ertugliflozin.

Note: Merck/Pfizer is proposing that the following cardiovascular SAEs will not be subject to expedited reporting under 21 CFR 312.32 (i.e., if reported as related to study drug by the investigator), unless and until the event is reviewed by the EAC and found not to meet the specified criteria in the EAC charter for that event type:

1. Cardiovascular deaths;
2. Non-fatal myocardial infarction; hospitalization for chest pain or to rule out myocardial infarction, or other hospitalization due to suspected myocardial ischemia where myocardial infarction needs to be ruled out;
3. Non-fatal stroke; TIA, reversible ischemic neurologic deficit (RIND) or other acute ischemic cerebrovascular event where stroke needs to be ruled out;
4. Hospitalization for unstable angina.

This trial will utilize an external Data Monitoring Committee (E-DMC) to periodically review safety data. All SAEs, including confirmed adjudicated cardiovascular events, will be reviewed and monitored by an E-DMC unblinded to treatment as part of the overall assessment of safety for ertugliflozin. Based upon their regular review of unblinded safety results, the E-DMC is empowered by the E-DMC charter to make recommendations with regard to trial conduct to assure the continuing appropriate safety of the subjects participating in the study.

Merck requests agency concurrence with this plan

Sponsor's Maximum Recommended Human Dose (MRHD)

The Maximum Recommended Human Dose (**MRHD**) for PF04971729 is: **15 mg/day**

This dose represents the anticipated maximum clinical phase III dose (Therapeutic Dose).

Human exposure at 15 mg/day was extrapolated from PK data obtained from the 14 day repeat dose MAD study (B1521002) in otherwise healthy overweight and obese adult subjects (C_{max} 159 ng/mL = 0.3 μM) and (AUC 1.2 μg.h/mL).

2.7 Previous Clinical Experience

Dosing with PF04971729 has been completed in five Phase 1 and two Phase 2 clinical studies.

The highest doses administered to humans to date are 300 mg (Fasted) and 100 mg (Fed). The longest duration of PF04971729 dosing in humans is 100 mg (Fed) for 14 days.

Reoccurring dosing related adverse events (AEs) are headaches, constipation, diarrhea, discolored feces, and folliculitis.

2.8 Regulatory Background

On July 8th 2011, Pfizer requested a EOP2 meeting with the FDA to obtain the Division's input on their proposed Phase 3 development program relevant to the planned indications for type 2 diabetes mellitus and to receive input on their submitted briefing document questions. Following the dapagliflozin Advisory Committee Meeting, Pfizer decided to re-evaluate the different potential scenarios regarding the strategy and design of their proposed Phase 3 program and cancelled the meeting request on September 6th 2012.

A meeting request was resubmitted with a set of newly revised briefing document questions on September 13th 2012. The background briefing document was received on November 16 h 2012 with modified briefing document questions and the sponsor's Phase 3 clinical plan.

The sponsor was informed on December 12th 2012 that although the completed toxicology package appeared to be sufficient in scope to support the Phase 3 clinical studies, the chronic toxicity study in dogs had not been submitted to the Division for review.

Following the Division's assessment of the EOP2 meeting document/questions information related to the SGLT2 inhibitor class was provided to the sponsor that pertained directly to the carcinogenicity and reproductive/developmental toxicity assessments of PF04971729.

The Agency informed to sponsor at the EOP2 meeting that the submission of a Pediatric Study Plan (PSP) was required within 60 days and that an extension could not be granted. The PSP was submitted on February 14th 2013 and was discussed during the PeRC BPCA subcommittee meeting on April 10th 2013 where it was determined that the sponsor would need to revise their PSP. A revised PSP was submitted on June 12th 2013 and ultimately approved. The study plan included a requirement for a juvenile toxicology study in Sprague Dawley rats prior to the initiation of clinical pediatric studies.

At the EOP2 meeting the sponsor was encouraged by the Division to include two doses in their Phase 3 program. While the sponsor agreed to the dosing changes, the decision prompted a request for input regarding a revision of dosing to their proposed rat carcinogenicity study which they had determined was necessary with the increased MRHD. ECAC had concurred with the sponsor's original doses (September 2011) and felt it was not necessary but acceptable to increase the doses in the rat carcinogenicity study, as proposed.

Most recently the sponsor submitted a new clinical protocol entitled, "A Phase 1, Non Randomized, Open-Label, Single Dose Study to Evaluate the Effect of Renal Impairment on the Pharmacokinetics, Pharmacodynamics, Safety and Tolerability of Ertugliflozin in Subjects with Type 2 Diabetes Mellitus (MK-8835-009-00/B1521023). The proposed 15 mg/day single dose (MRHD) was supported by the safety margins and duration of the relevant toxicology studies.

3 Studies Submitted

3.1 Studies Reviewed

- **Study 09GR476:** 9-Month Oral Gavage Toxicity and Toxicokinetic Study with PF-04971729 in Dogs with an 8-Week Recovery Phase

3.2 Studies Not Reviewed

All nonclinical data submitted to date has been reviewed by the Division.

3.3 Previous Reviews Referenced

Pharmacology/Toxicology Reviews 1-4

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

PF04971729 is an inhibitor of the Sodium Glucose Transporter 2 (SGLT2). Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

Drug activity related to proposed indication

PF04971729 dosing results in a concentration-dependent glucosuria in rats. *In vitro*, PF04971729 acts as a potent inhibitor of rat and human SGLT2 and possesses a high selectivity against glucose transport via human and rat SGLT1 and several other glucose transporters (GLUT1-4).

5 Pharmacokinetics/ADME/Toxicokinetics

5.2 Toxicokinetics

Single Dose – Cross-Species and Formulation Comparison

		Dog (SD) (Amorphous)			Rat (7 Day) (Amorphous)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	5	50	500	5	50	500
	MRHD@	33X	358X	254X	14X	83X	1250X
Parameter	Unit						
C_{MAX}	µg/mL	5	46	25	1	8	90
AUC_{0-24}@	µg.hr/mL	39	430	302	17	99	1500
Shaded = MTD							

		Dog (7 Day) (Amorphous)			Dog (7 Day) (Co-crystal)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	5	50	250/150	5	50	150
	MRHD@	39X	329X	724X	44X	408X	300X
Parameter	Unit						
C_{MAX}	µg/mL	7	47	61	6	42	36
AUC_{0-24}@	µg.hr/mL	47	395	869	53	490	360
Shaded = MTD							

		Dog (1 Month) (Co-crystal)			Rat (1 Month) (Co-crystal)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	1	10	150	5	25	500/250
	MRHD@	5X	52X	408X	13X	88X	1217X
Parameter	Unit						
C_{MAX}	µg/mL	1	10	49	2	9	74
AUC_{0-24}@	µg.hr/mL	6	62	489	16	106	1460
Shaded = NOAEL							

		Dog (3 Month) (Co-crystal)			Rat (3 Month) (Co-crystal)		
	Dose	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
	mg/kg	1	10	150	5	25	250
	MRHD@	6X	62X	758X	14X	78X	786X
Parameter	Unit						
C_{MAX}	µg/mL	1	10	65	2	8	74
AUC_{0-24}@	µg.hr/mL	7	74	910	17	94	943
Shaded = NOAEL							

6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.2 Repeat-dose Non-Rodent Studies

9 Month Oral Toxicity Study in Dogs with a 2-Month Recovery Period

Study no.:	09GR476 (8222521)
Study report location:	(SDN 66/SN 65) September 5th 2013
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 18th 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF04971729 (b) (4), GR02847, 99.9%

Key Study Findings

- After 39 weeks of dosing, male dogs administered 1, 10, and 150 mg/kg/day had exposures of 6, 63 and 1040 μg.h/mL (5X, 53X and 867X MRHD, μg.h/mL basis). In females exposures were 7, 78 and 767μg.h/mL, respectively (6X, 65X and 639X MRHD).
- Two dogs dosed at 150 mg/kg/day died on study and examinations indicated that death was caused by gavage-related instillation of PF04971729 into the lungs. Excessive vomiting, salivation and abnormal feces increased with dose and were more severe at 150 mg/kg/day. These findings tended to resolve during recovery and are consistent with the GI intolerance observed in previous dog studies. GI intolerance may reflect off-target inhibition of SGLT1.
- HD dogs tended to weigh less than controls despite an increase in food consumption. The results correlate with GI intolerance and are consistent with the results from the previous 3 month dog study. Lower body weight at the HD tended to resolve in the presence of increased food consumption and weight gain observed during recovery.
- Persistent elevations in reticulocyte counts were observed in both genders and plausibly reflect a regenerative response incited by digestive tract erosion. Fibrinogen tended to increase in males and persisted through recovery. This finding is suggestive of a systemic inflammatory response and correlates with microscopic data indicative of inflammation, increased immune cell number and elevated thymus weight.
- Glucosuria and increased urine volume were observed at all doses and are an expected pharmacological effect of SGLT2 inhibition. Serum concentrations of calcium, sodium and chloride were not significantly altered at the HD despite a significant increase in the urinary excretion of these electrolytes. Urine volume did not normalize at the HD following the 2 month recovery period and is indicative of a persistent change in kidney function.
- Adrenal weight increased at 150 mg/kg/day (both genders) and correlated with vacuolation of the adrenal cortex in males (≥ 1 mg/kg/day) at the end of dosing.
- Thyroid mineralization was noted in females (≥ 10 mg/kg/day) and persisted through recovery. Mineralization is plausibly related to disruption of calcium homeostasis in the renal tubules where calcitonin normally acts to regulate calcium reabsorption and excretion. Urine calcium remained slightly elevated at the HD following the 2 month recovery period.

Reviewer Comments: The sponsor concluded a NOAEL of 150 mg/kg/day, the high dose in this study, citing the myriad of clinical findings related to GI intolerance and the associated loss in body weight as being non-adverse. Gavage-related mortalities in all dog studies up to this point have been isolated to the 150 mg/kg/day dose and have consistently been accompanied by severe GI intolerance. This correlation plausibly represents a propensity for gavage-related injuries to occur in animals where local tissues have been damaged and/or weakened by reflux. Based on the excessive vomiting, salivation and abnormal feces at 150 mg/kg/day the reviewer concludes that the 10 mg/kg/day dose better represents the NOAEL in this study.

Dog (9 Month) (1, 10 and 150 mg/kg/day)	NOAEL	Safety Margin 15 mg/day (1.2 μg.h/mL)
Mortality (Gavage Related)	10 mg/kg/day (71 μg.h/mL)	59X
GI Intolerance (Excessive Vomiting and Salivation) (Abnormal Feces and/or Diarrhea)	10 mg/kg/day (71 μg.h/mL)	59X
Body Weight Loss (Presence of Increased Food Consumption)	10 mg/kg/day M: (63 μg.h/mL)	53X
	< 1 mg/kg/day F: (< 7μg.h/mL)	< 6X
Reticulocyte Elevations (Persistent)	10 mg/kg/day (71 μg.h/mL)	59X
Systemic Inflammatory Response (M: Persistent Fibrinogen Elevations) (M: Increased WBCs + Monocytes) (Elevated Thymus Weight) Microscopic Indications of Chronic Inflammation	10 mg/kg/day (71 μg.h/mL)	59X
Glucosuria (Reversible) Increased Urine Volume (Persistent) Decreased Urine Creatinine (Persistent)	< 1 mg/kg/day (< 7 μg.h/mL)	< 6X
Increased Urine Ca (Partially Reversed) Increased Urine Na and Cl (Reversible)	10 mg/kg/day (71 μg.h/mL)	59X
Adrenal Weight Increased	10 mg/kg/day (71 μg.h/mL)	59X
Adrenal Cortex Vacuolation (Males Only)	< 1 mg/kg/day (< 6 μg.h/mL)	< 5X
Thyroid Mineralization (Persistent) (Females Only)	1 mg/kg/day (7 μg.h/mL)	6X

Methods	
Doses	0, 1, 10, 150 mg/kg/day for 9 Months + 2M Recovery
Species/source	Beagle Dogs, (b) (4)
Age / Weight	13-14 Months / M: 7.6 to 10kg F: 6.8 to 8.5kg
n/sex/group (main)	4/sex/group
n/sex/group (recovery)	2/sex - Control and HD Only
Toxicokinetics	Weeks 1+39 - 0.5, 1, 2, 4, 7, and 24 hrs p.d. (Not Fasted)
Route, formulation, volume	Oral, 0.5% (w/v) methylcellulose (4000 cps) with 10% (v/v) PEG 400, 5 mL/kg – Concentrations corrected for Potency

Observations and Times	
Mortality Checks Observations	Performed twice daily
Cageside Exams	Performed once daily (1 Hrs Post Dose)
Detailed Exams	3x - Pretreatment, Prior to Day 1 Dose, Weekly thereafter
Body weights	3x - Pretreatment, Prior to Day 1 Dose, Weekly thereafter
Food consumption	Weekly
Ophthalmology	Pre, D139, D272 of Dosing Phase - Ophthalmoscope (indirect)
ECG	2x - Pre, D134, D267 of Dosing Phase – 1-2 Hours PD (Leads I, II, aVF, CV5RL, and CV6LL)
Hematology	Fasted – 2x Pre, Weeks 13, EOD and EOR
Coagulation	
Clinical chemistry	
Urinalysis	Fasted – 1x Pre, Weeks 13, EOD and EOR (Overnight)
Gross pathology	EOD (Day 274), EOR (Day 334)
Organ weights	All Study Animals: Standard Battery
Bone Marrow Slides	Prepared but not evaluated
Histopathology	All tissues (Larynx was not examined)
	Adequate Battery: yes (X), no ()
	Peer review: yes (X), no ()

Results

Mortality

Animal #	Gender	Dose (mg/kg/day)	Day of Death	Cause of Death
H04830	Male	150	55	Gavage Accident
H04847	Female	150	64	Gavage Accident

Clinical signs prior to sacrifice, findings at necropsy, and findings upon microscopic examination were consistent with gavage-related instillation of PF04971729 into the lungs. Two females in the prior 1 month dog study died at the 150 mg/kg/day dose from gavage-related errors.

Clinical Observations

Vomiting (10 subclasses), abnormal feces (10 subclasses) and excessive salivation increased with dose and were typically more severe at 150 mg/kg/day. These findings tended to resolve during recovery and are consistent with the GI intolerance noted in previous dog studies at the 150 mg/kg/day dose. The relative GI intolerance at the 150 mg/kg/day dose may reflect off-target inhibition of SGLT1, which is associated with duodenal absorption of glucose.

Body Weight

High dose dogs (150 mg/kg/day) tended to weigh less than controls despite increased food consumption. These results are consistent with the GI intolerance and increased caloric demand and tended to resolve in HD recovery dogs. The body weight data is consistent with the results from earlier dog studies.

Body Weight				
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Males	0	700	0%	100%
	1	700	0%	120%
	10	600	-14%	110%
	150	100	- 86%	90%

Body weight loss in low dose females exceeded those observed at the 150 mg/kg/day dose.

Body Weight				
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Females	0	1000	0%	100%
	1	300	- 70%	94%
	10	900	- 10%	101%
	150	500	- 50%	95%

Food Consumption

Food consumption in males dosed at ≥ 1 mg/kg/day was statistical increased from Week 2 forward until the end of the administration period. Food consumption was similarly increased in females although the degrees of change were less often found significant. Increased food consumption persisted through recovery and correlated with increased body weight gain in high dose dogs. Changes in food consumption were less consistent in earlier dog studies and likely reflect the persistent GI intolerance associated with this compound.

Ophthalmologic Examinations

Indirect ophthalmological examinations revealed no visible lesions of the eye.

Electrocardiographic Examinations

Electrocardiographic examinations (Leads I, II, aVF, CV5RL, and CV6LL) did not reveal any abnormal findings at 1-2 hours post dose in dogs administered PF04971729. The selected examination time was consistent with the T_{max} for PF04971729 (1-2 hours). Electrocardiogram results have been inconsistent (1 Month) or negative (3 Month) in previous dog studies.

Hematology

Reticulocyte counts tended to be elevated in dogs administered PF04971729 although these changes were dose-dependent in females only (Week 39) and were not found to be statistically significant. Increased reticulocyte counts persisted through recovery and may reflect a regenerative response incited by digestive tract erosion, although other RBC parameters were not significantly affected by dosing. Notable changes in reticulocyte counts were not observed in prior dog studies.

Notable Effects of PF04971729 on Reticulocytes

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
Reticulocytes ($10^3/\mu L$)		↑49	↑41	↑48	↑54	↑48	↑24
Week 39							
Reticulocytes ($10^3/\mu L$)		↑6	↑**4**	↑43	↑**19**	↑7	↑**41**
Week 47							
Reticulocytes ($10^3/\mu L$)		-	-	-	-	↑59	↑49
(*p<0.05) (**p<0.01)							

WBC counts were minimally increased in dogs administered PF04971729 and these changes were found to be dose-dependent only in males (Week 39). Increased WBC counts trended towards recovery and were not noted in prior dog studies.

Notable Effects of PF04971729 on White Blood Cells

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
WBC ($10^3/\mu L$)		↑27	↑9	↑11	↑43	↑6	↑17
Week 39							
WBC ($10^3/\mu L$)		**↑16**	0	**↑21**	↑12	**↑22**	↑11
Week 47							
WBC ($10^3/\mu L$)		-	-	-	-	↑11	↑4
(*p<0.05) (**p<0.01)							

Monocyte counts tended to increase dose-dependently in males only (Week 39) with significant changes occurring sporadically in both genders. Monocyte counts normalized following recovery and significant changes were not noted in prior dog studies.

Notable Effects of PF04971729 on Monocytes

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
Monocytes ($10^3/\mu L$)		↑44*	0	0	↑61	↑28	↑15
Week 39							
Monocytes ($10^3/\mu L$)		**↑33**	0	**↑53**	↑55*	**↑60**	↑7
Week 47							
Monocytes ($10^3/\mu L$)		-	-	-	-	0	0
(*p<0.05) (**p<0.01)							

Coagulation

Fibrinogen concentrations tended to increase dose-dependently in males only (Week 39) with significant changes occurring at the high dose during Week 13 (↑54%) and Week 39 (↑47%). Increased fibrinogen concentrations persisted in recovery males (↑42%) and suggest an enduring inflammatory response. Microscopic indications of a systemic inflammatory response were present but limited at the high dose and tended to be minimal or slight in severity.

Significant changes in coagulation parameters were not noted in females during this study and have not been observed in previous dog studies.

Clinical Chemistry

Serum glucose levels tended to decline inversely to dose in males (Week 13) and females (Week 13 & 39) with significant changes occurring sporadically in both genders. Decreased serum glucose levels normalized following recovery and these results are consistent with the pharmacological effect of PF04971729. The unusual reduction in serum glucose in a dose inverted manner replicates the results from the previous 3 month study and indicates a loss of efficacy with prolonged use at higher doses in dogs. Glucose assessment was performed in fasted dogs and the effect of dosing on glucose in non-fasted animals was not ascertained.

Notable Effects of PF04971729 on Serum Glucose Levels

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Analyte		(%) Change vs. control mean					
Week 13							
Glucose (mg/dL)		↓18	↓32*	↓16	↓20*	↓15	↓11
Week 39							
Glucose (mg/dL)		↓13	↓29*	↓1	↓17*	↓17*	↓13*
Week 47							
Glucose (mg/dL)		-	-	-	-	↑2	↓3
(*p<0.05) (**p<0.01)							

Serum concentrations of calcium, sodium and chloride were not significantly altered at the high dose despite a significant increase in the urinary excretion of these electrolytes. Homeostatic biomarkers (PTH, Vitamin D, ACTH and aldosterone) were not measured during this study.

Urinalysis

Significant glucosuria was observed at all doses and tended to normalize following recovery. The maximal PD effect was achieved at the LD (1 mg/kg) in females and glucosuria tended to be dose-dependent throughout the administration period in males. An unexplained elevation in urine glucose was observed in control females during the dosing period. Glucosuria is an expected pharmacological effect of SGLT2 inhibition.

Urine Glucose									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
Glucose (mg/dL)	Week 13	ND	1367	2754	7291*	3061	4277*	4354	895
	Week 39	15	502	2699*	3834*	3273*	4162*	3799*	3695*
	Week 47	5	6	ND	ND	ND	ND	ND	56
(*p<0.05) (**p<0.01) ND = No Data									

Urine creatinine levels declined significantly during Week 13 of dosing and remained lower through the end of recovery. Decreased urine creatinine is feasibly related to the corresponding increase in urine volume.

Creatinine									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
UCRE (mg/dL)	Week 13	95	126	25*	71*	30*	36*	43*	8*
	Week 39	76	65	24	37	28	37	35	31*
	Week 47	96	94	ND	ND	ND	ND	18	22
(*p<0.05) (**p<0.01) ND = No Data									

Urine glucose to creatinine ratios were significantly elevated (up to 575-fold vs control) through the end of dosing and tended to normalize following recovery.

Glucose:Creatinine Ratios									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
GLCR (Ratio)	Week 13	ND	11	108	104*	109	119*	102	97*
	Week 39	0.2	8	114*	103*	115*	107*	111*	115*
	Week 47	0.1	0.1	ND	ND	ND	ND	ND	2.2
(*p<0.05) (**p<0.01) ND = No Data									

Urine calcium excretion increased (up to 10-fold vs control) at the 150 mg/kg/day dose during Week 39 and remained slightly elevated (up to 4-fold vs control) through the end of recovery.

Calcium Excretion									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
CaX (mg)	Week 13	2	5	9	4	11	15	7	12
	Week 39	2	8	12	6	8	13*	20*	32*
	Week 47	4	8	ND	ND	ND	ND	16	19
(*p<0.05) (**p<0.01) ND = No Data									

Urine sodium excretion increased (up to 3-fold vs control) at the 150 mg/kg/day dose during Week 39 and tended to normalize by the end of recovery.

Sodium Excretion									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
NaX (mg)	Week 13	8	8	10	8	9	11	6	2
	Week 39	5	5	7	3	7	6	16*	11*
	Week 47	5	10	ND	ND	ND	ND	6	15
(*p<0.05) (**p<0.01) ND = No Data									

Urine chloride excretion increased (up to 3-fold vs control) at the 150 mg/kg/day dose during Week 39 and tended to normalize by the end of recovery.

Chloride Excretion									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
ClX (mg)	Week 13	8	9	9	9	7	12	7	ND
	Week 39	5	7	8	4	9	8	15	13*
	Week 47	7	14	ND	ND	ND	ND	7	13
(*p<0.05) (**p<0.01) ND = No Data									

An increase in urine volume (up to 5-fold vs control) was noted at doses ≥ 1 mg/kg/day and tended to be dose-dependent in females and non-recoverable in both genders. These findings are indicative of a persistent change in kidney function.

Urine Volume									
		0		1		10		150	
Analyte	Duration	M	F	M	F	M	F	M	F
UVol (mL)	Week 13	72	76	229	121*	298	279*	224	336*
	Week 39	60	130	304*	181	205	269*	282*	425*
	Week 47	69	128	ND	ND	ND	ND	321	431
(*p<0.05) (**p<0.01) ND = No Data									

Gross Pathology

Gross pathology – Males – End of Dosing						
Tissue	Finding	Main Study				
		dose	0	1	10	150
		n	4	4	4	3
Duodenum	Discolored		0	0	0	1
Colon	Discolored		0	0	1	1
Jejunum	Discolored		0	0	0	1

Discoloration of the GI tract was observed in 1 male from the 10 and 150 mg/kg/day groups and was noted in the single HD male that died on study. Discoloration of the GI tract occurred independent of dose in females. Lung discoloration was observed in a single HD recovery male and both HD dogs whose deaths were attributed to gavage error. The presence of skin abrasions increased in females dosed at ≥ 10 mg/kg/day.

Organ Weights

Absolute and relative adrenal weight increased dose-dependently in females at the end of dosing and trended towards recovery following dosing cessation (↑8%). Increased adrenal weights in males were comparable to females at the 150 mg/kg/day dose. Vacuolation of the adrenal cortex was observed microscopically in male dogs (≥ 1 mg/kg/day).

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Organ							
Adrenal (Absolute)		↑10%	**↑7%**	0	**↑16%**	↑23%	**↑30%**
Adrenal (Rel: $\bar{X}$ BrW)		↑2%	**↑6%**	0	**↑22%**	↑31%	**↑37%**
(* = P ≤ 0.05) (** = P ≤ 0.01)							

Absolute and relative thymus weight increased dose-dependently in females at the end of dosing and continued to escalate through the recovery period (↑83%). Increased thymus weights in males were comparable to females at the 150 mg/kg/day dose. PF04971729 dosing-related microscopic changes were not observed in the thymus.

	Dose (mg/kg/dose)	1		10		150	
	Gender	M	F	M	F	M	F
Organ							
Thymus (Absolute)		↑47%	**↑13%**	0	**↑14%**	↑32%	**↑39%**
Thymus (Rel: $\bar{X}$ BrW)		↑36%	**↑12%**	0	**↑17%**	↑38%	**↑47%**
(* = P ≤ 0.05) (** = P ≤ 0.01)							

Heart weight (relative to body weight) decreased dose-dependently in females only. These changes were minimal in nature (↓<10%), not reflected in absolute values and tended to resolve following the recovery period.

Histopathology

Vacuolation of the adrenal cortex was observed at the end of the administration period (males only) and was present in control animals (both genders) following recovery. Hypertrophy of the salivary gland at the high dose is likely related to the excessive salivation that occurred in dogs exposed to PF04971729 and was not present following recovery. Several other tissue types displayed microscopic findings at the high dose in males although the incidence often did not exceed one and the severity was usually minimal to slight in nature.

Histopathology – Males – End of Dosing						
Tissue	Finding		Main Study			
		dose	0	1	10	150
		n	4	4	4	3
Adrenal	Vacuolation - Cortex		0	2 (1*/1**)	1*	2*
G.A. Lymph	Mineralization		0	0	0	1*
Salivary Gland	Hypertrophy		0	0	0	1**
Epididymis	Chronic Inflammation		0	0	0	1**
Minimal (*) Slight (**) Moderate (***) Marked (****)						

Thyroid mineralization was observed in females (≥ 10 mg/kg/day) and persisted in 50% (minimal to slight) of the HD recovery animals. Mineralization may be related to PF04971729-mediated disruption of calcium homeostasis in the renal tubules where calcitonin normally acts to regulate calcium reabsorption and excretion. Calcium excretion remained elevated in HD recovery dogs.

Histopathology – Females – End of Dosing						
Tissue	Finding		Main Study			
		dose	0	1	10	150
		n	4	4	4	3
Thyroid	Mineralization		0	0	1*	2*
Gallbladder	Lymphoid - Follicles		0	0	0	2**
Kidney	Lymp/Macro - Infiltrate		0	0	1*	1*
Urinary Bladder	Chronic Inflammation		0	0	0	1*
Mesen. LN	Sinus Erythrocytes		0	0	1*	1**
Ureter	Chronic Inflammation		0	0	0	1**
Poplit. LN	Sinus Erythrocytes		0	1**	1*	1*
Minimal (*) Slight (**) Moderate (***) Marked (****)						

Surprisingly the decreased glycogen content observed in the liver at the end of the preceding dog studies (≤ 3 months) was not observed here and likely represents a metabolic adaptation that circumvents the utilization of hepatic glycogen. Liver cell necrosis was present in 75% of the females previously dosed at 150 mg/kg/day for 3 months. Lack of this finding at the end of 9 months of dosing is presumably related to hepatic adaptation and regeneration.

Toxicokinetics

After 9 months of dosing, mean C_{max} tended to increase in a less than dose proportional manner, while mean AUC_{0-24} increased in proportion to dose between 1 and 150 mg/kg/day,

Systemic exposures tended to be higher in females at doses ≤ 10 mg/kg/day and in males at the 150 mg/kg/day dose.

Mean T_{max} was between 1 and 2 hours post dose.

Systemic exposure (C_{max} and AUC_{0-24}) tended to increase at the 150 mg/kg/day dose between Day 1 and Week 39. Systemic exposures were lower than those obtained at these doses during the previous 3 month study, especially at ≤ 10 mg/kg/day.

Toxicokinetic Parameters for PF04971729 in Dogs on Day 1 and During Week 39

Dose (mg/kg/ day)	Study Week	Gender	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
1	1	Male	0.751	0.159	4	0.750	0.289	4	6.12	1.55	4
		Female	0.832	0.129	4	1.13	0.629	4	7.41	1.85	4
		Overall	0.791	0.141	8	0.938	0.496	8	6.77	1.72	8
	39	Male	0.743	0.273	4	1.50	1.68	4	5.61	0.364	4
		Female	0.849	0.398	4	1.13	0.629	4	6.99	1.51	4
		Overall	0.796	0.321	8	1.31	1.19	8	6.30	1.26	8
10	1	Male	10.3	1.41	4	0.750	0.289	4	73.9	2.04	4
		Female	8.66	0.742	4	1.00	0.707	4	80.3	4.97	4
		Overall	9.46	1.35	8	0.875	0.518	8	77.1	4.88	8
	39	Male	6.50	2.26	4	1.13	0.629	4	62.9	4.43	4
		Female	8.93	2.24	4	0.875	0.250	4	78.2	21.1	4
		Overall	7.72	2.45	8	1.00	0.463	8	70.5	16.3	8
150	1	Male	51.8	16.5	6	2.17	0.983	6	659	209	6
		Female	57.5	4.34	6	2.33	0.816	6	728	115	6
		Overall	54.6	11.9	12	2.25	0.866	12	693	165	12
	39	Male	98.8	39.5	5	1.80	0.447	5	1040	449	5
		Female	74.0	12.9	5	2.10	1.24	5	767	166	5
		Overall	86.4	30.7	10	1.95	0.896	10	906	351	10

Overall = Male plus female combined

11 Integrated Summary and Safety Evaluation

PF04971729 (Ertugliflozin) is being co-developed by Merck and Pfizer for the treatment of type 2 diabetes mellitus (T2DM) and is an inhibitor of the Sodium Glucose Transporter 2 (SGLT2). Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

The proposed Phase 3 clinical trail is designed as a 26 week multiple ascending oral dose study in subjects with T2DM and is intended to evaluate the efficacy and safety of PF04971729 up to 15 mg/day. Safety margins to the 15 mg/day dose were based on an exposure value (1.2μg.hr/mL) extrapolated from the PK data associated with a 14 day repeat dose MAD study of PF04971729 in otherwise healthy overweight and obese adult subjects.

Safety assessment was based on the toxicological data from the 6 month rat and the 9 month dog studies which support the proposed 15 mg/day dose based on the 19X and 59X safety margins (AUC) to the rat and dog NOAELs, respectively and the duration of the completed toxicology studies. It should be noted that NOAELs were not established for adrenal cortex hypertrophy (dog + rat), stomach erosions/ulcers and kidney mineralization in the rat.

The 9 month toxicology study confirms the evidence of GI intolerance (excessive vomiting, salivation and abnormal feces) in dogs (750X MRHD) and likely reflects off-target inhibition of SGLT1. In the rat, GI intolerance manifests as stomach erosion/ulcers (≥ 19X MRHD), crypt degeneration (≥ 123X MRHD) and hyperplasia (500X MRHD). Consistent with previous dog studies GI intolerance was accompanied by weight loss in the presence of increased food consumption and tended to resolve during recovery. Severe GI intolerance at the HD may have precipitated digestive tract injury/bleeding as evidenced by persistent elevations in reticulocyte counts and indications of inflammation including: increased fibrinogen, immune cell counts and elevated thymus weight.

Glucosuria and elevations in urine volume during dosing were consistent with SGLT2 inhibition and the results from prior dog studies. In contrast to previous studies, elevated urine volumes persisted through the recovery period and are arguably related to changes in renal function. Excretion of calcium in the urine was increased at the HD (750X MRHD) and remained slightly elevated through the end of recovery. Serum concentrations of calcium, sodium and chloride were not significantly altered at the HD despite the significant increase in the urinary excretion of these electrolytes. Persistent thyroid mineralization was observed in females (≥ 65X MRHD) and may be related to disruption of calcium homeostasis in the renal tubules where calcitonin normally acts to regulate calcium reabsorption and excretion. While there were no notable bone affects in dogs, increased trabecular bone in rats indicates that calcium homeostasis and bone health can be disrupted at high doses (500X MRHD).

Decreased liver glycogen content noted in prior dog studies (≥ 8X MRHD) was not observed in the 9 month study and likely represents a metabolic adaptation that circumvents the utilization of hepatic glycogen. Liver cell necrosis observed in HD females (980X MRHD) during the 3 month study was notably absent in dogs administered PF04971729 for 9 months. The absence of this finding is consistent with the lower exposures obtained at this dose during the 9 month study (639X MRHD) and is plausibly related to hepatic adaptation and regeneration.

Adrenal weights were minimally increased in dogs (750X MRHD) and correlated with adrenal cortex vacuolation in males (≥ 5X MRHD). These findings were not observed in previous dog studies but are consistent with the adrenal cortex hypertrophy and/or vacuolation observed during the chronic rat toxicity study (≥ 19X MRHD).

Species Toxicology Studies – Chronic Toxicology			
Species/ Study (Exposure)	**NOAEL**	**Multiple MRHD (AUC*)**	**Basis**
Rat Duration: 6 Month (GLP) Dose: 5, 25, 100 mg/kg Co-crystalline Drug Form Average exposure: 22, 148, 605 μg.hr mL	**5 mg/kg** Ave: (22 μg.hr/mL) M: 17.6 μg.hr/mL F: 26.9 μg.hr/mL	19X	**≥ 5 mg/kg:** ↑Food Consumption ↓Serum Glucose ↑Urinary Glucose ↑BUN/Phos Dehydration ↓Serum electrolytes Stomach Erosion/Ulcer ↓Pancreatic zymogen **≥ 25 mg/kg:** Kidney Pelvic Tubule Dilatation/Hyperplasia Mineral Deposition Pyloric Crypt Degeneration **100 mg/kg:** M: ↓BW gain ↓RBC Parameters ↓PTH ↑Adrenal Weight Adrenal Cortex (Hypertrophy/Vacuolation) M: ↑Trabecular Bone ↑BUN/Phos Dehydration ↓Serum electrolytes Stomach Hyperplasia
Dog Duration: 9 Month (GLP) Dose: 1, 10, 150 mg/kg Co-crystalline Drug Form Average exposure: 6, 71, 906 μg.hr mL	**10 mg/kg** Ave: (71 μg.hr/mL) M: 63 μg.hr/mL F: 78 μg.hr/mL	59x	**≥ 1 mg/kg:** F: ↓BW gain **≥ 10 mg/kg:** F: Thyroid Mineralization (Persistent) **150 mg/kg:** GI Intolerance (Vomiting/Diarrhea/Salvation) M: ↓BW gain ↑Reticulocytes M: ↑Inflammatory Markers ↑Thymus Weight ↑Urine Volume (Persistent) ↑Adrenal Weight Adrenal Cortex Vacuolation

*AUC at MRHD (15 mg/day): 1.2μg.hr/mL

Species Toxicology Studies – 3 Month			
Species/ Study (Exposure)	**NOAEL**	**Multiple MRHD (AUC*)**	**Basis**
Rat Duration: 3 Month (GLP) Dose: 5, 25, 250 mg/kg Co-crystalline Drug Form Average exposure: 19.9, 89.4, 738 μg.hr mL	**< 5 mg/kg** Ave: (< 20 μg.hr mL)	< 17x	**≥ 5 mg/kg:** Pelvic Tubule Dilatation Mineral Deposition M: GI Tract Dilatation ↑Adrenal Weight Adrenal Histopath **≥ 25 mg/kg:** ↓Prostate Weight Inflammation Stomach Erosion Ulcer M: Hyperostosis F: GI Tract Dilatation **250 mg/kg:** Pelvic/Bladder Hyperplasia ↑Severity CPN Heart Myonecrosis F: Hyperostosis
Dog Duration: 3 Month (GLP) Dose: 1, 10, 150 mg/kg Co-crystalline Drug Form Average exposure: 9.79, 91.9, 1100 μg.hr mL	**10 mg/kg** Ave: (91.9 μg.hr/mL) M: 74.8 μg.hr/mL F: 109 μg.hr/mL	77x	**≥ 1 mg/kg:** Glycogen Depletion (Liver) **150 mg/kg:** GI Intolerance (exceeding MTD) (Vomiting) (Diarrhea) (Liquid/Mucoid feces) ↓BW gain Liver Cell Necrosis (Females)

*AUC at MRHD (15 mg/day): 1.2μg.hr/mL

(Previous Review) Species Toxicology Studies (1 Week and 4 Week)			
Species/ Study	**NOAEL**	**Multiple MRHD (AUC)***	**Basis**
Rat 1 Week (non-GLP) 5, 50, 500 mg/kg M:17, 99, 1500 μg.hr/mL	50 mg/kg (99 μg.hr/mL)	83X	**500 mg/kg:** Loose Stool ↓BW ↓WBCs Histological changes Pancreas and Liver
4 Week (GLP) 5, 25, 500→(D11) 250 mg/kg M: 8, 69, 541 μg.hr/mL F: 15, 93, 718 μg.hr/mL	25 mg/kg (81μg.hr/mL)	68X	**500 → (D11) 250 mg/kg:** Mortality, ↑ severity CPN, Stomach erosion Squamous hyperplasia.
Dog Single Dose (non-GLP) 5, 50, 500 mg/kg M: 27, 386, 138 μg.hr/mL F: 51, 474, 465 μg.hr/mL	50 mg/kg (430 μg.hr/mL)	358X	**500 mg/kg:** Emesis F: Salivation
1 Week (non-GLP) 5, 50, 250→(D3) 150 mg/kg M: 55, 373, 1150 μg.hr/mL F: 63, 627, 789 μg.hr/mL Amorphous Drug Form	50 mg/kg (500μg.hr/mL)	417X	**250→(D3) 150 mg/kg:** Emesis Abnormal Feces
1 Week (non-GLP) 5, 50, 150 mg/kg M: 77, 660, 679 μg.hr/mL F: 59, 834, 511 μg.hr/mL Co-crystalline Drug Form	50 mg/kg (750μg.hr/mL)	625X	**150 mg/kg:** Emesis
4 Week (GLP) 1, 10, 150 mg/kg M: 7, 77, 1050 μg.hr/mL F: 8, 71, 1170 μg.hr/mL	1 mg/kg (8 μg.hr/mL)	7X	**≥ 1 mg/kg:** ↓BW gain Glycogen Depletion (Liver) **≥ 10 mg/kg:** Gallbladder Vacuolation **150 mg/kg:** Renal tubular Degeneration Emesis, Salivation Abnormal Feces

*AUC at MRHD (15 mg/day): 1.2μg.hr/mL

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

/s/

JEFFREY A QUINN
10/07/2013

TODD M BOURCIER
10/07/2013
I concur

PHARMACOLOGY/TOXICOLOGY REVIEW

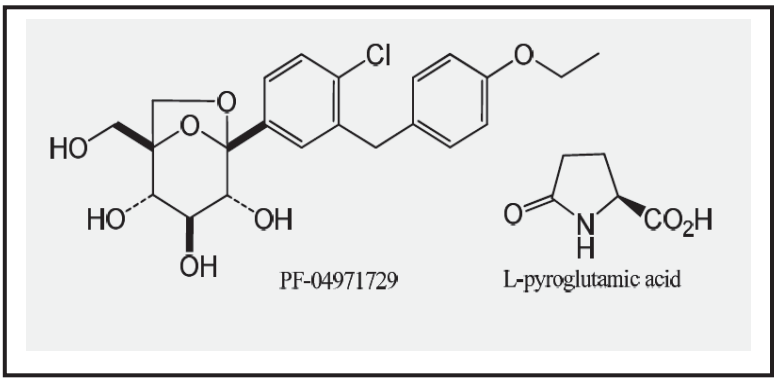
2 INTRODUCTION AND DRUG HISTORY

IND number:	106447
Review number:	4
Sequence number/date/type of submission:	SDN37 / SN36 / 10 June 2011 / SPA1 SDN39 / SN38 / 28 July 2011 / SPA2
Information to sponsor:	Yes ( ) No (X)
Sponsor and/or agent:	Pfizer
Manufacturer for drug substance:	Pfizer
Reviewer name:	Jeffrey Quinn, Ph.D.
Division name:	Metabolism and Endocrinology Products
HFD #:	510
Review completion date:	August 29th 2011

Drug:

Trade name:	None
Generic name:	None
Code name:	PF04971729 or PF04971729 (b) (4)
Chemical name:	((1*S*, 2*S*, 3*S*, 4*R*, 5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-1-hydroxymethyl-6,8-dioxabicyclo [3.2.1]octane-2,3,4-triol
CAS registry number:	None
Molecular formula/M.W.:	PF04971729 (amorphous form) $C_{22}H_{25}ClO_7$ / 436.88 Daltons. PF04971729 (b) (4) (L-pyroglutamic acid co-crystal form) $C_{27}H_{32}ClNO_{10}$ / 566.00 Daltons

Structure: The pharmaceutical preparation of PF04971729 is an (b) (4) of the L-pyroglutamic acid co-crystal form.



Relevant INDs/NDAs/DMFs:

(b) (4)

Drug class: PF04971729 is a sodium glucose co-transporter 2 (SGLT2) inhibitor.

Intended clinical population: Treatment of Type 2 Diabetes Mellitus (T2DM)

Clinical formulation: PF04971729 will be supplied as 1 mg, 5 mg and 25 mg tablets.

Route of administration: Oral

Previous Clinical Experience:

Dosing with PF04971729 has been completed in five Phase 1 clinical studies, and two Phase 2 studies are ongoing now.

The highest doses used in humans to date are (300 mg – Fasted Condition) and (100 mg – Fed Condition).

The longest duration of PF04971729 dosing in humans is (100 mg – Fed) for 14 days.

Reoccurring dosing related adverse events (AEs) are headaches, constipation, diarrhea, discolored feces, and folliculitis.

Adverse events requiring pharmacological management were limited to constipation.

Sponsor's Maximum Recommended Human Dose:

The Maximum Recommended Human Dose (**MRHD**) for PF04971729 is: (b) (4) **mg/day.**

(b) (4)

3 STUDIES SUBMMITED

3.1 Studies Reviewed

Study Number (143527) RBC to Plasma Partitioning of PF04971729

Study Number (102539) Quantitative Whole Body Autoradiography in LE Rats

Study Number (141524) Mass Balance, Excretion and Metabolism in SD Rats

Study Number (B1521003) Mass Balance, Excretion and Metabolism in Male Humans

Study Number (09GR275) 6-Month Oral Toxicity of PF04971729 in SD Rats

3.2 Studies Previously Reviewed (Recapitulated and Updated for Reference)

Study Number (09GR320) 3-Month Oral Toxicity of PF04971729 in SD Rats

Study Number (09GR185) 1-Month Oral Toxicity of PF04971729 in SD Rats

TABLE OF CONTENTS

4 PHARMACOLOGY

Background:

Pfizer submitted the original IND for PF04971729 in September, 2009, for the treatment of type 2 diabetes mellitus. Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

Brief summary

PF04971729 is a selective SGLT2 inhibitor that results acutely in a concentration-dependent glucosuria in rats. *In vitro*, PF04971729 is a highly potent inhibitor of rat and human SGLT2 and possesses a high selectivity against glucose transport via human and rat SGLT1 and several other glucose transporters (GLUT1-4).

Studies on the secondary pharmacology evaluated *in vitro* binding activity of PF04971729 against a broad panel of receptors, transporters, ion channels, and enzyme assays, and the results indicated no significant inhibition (> 50%) of binding or enzyme activity. The ability of PF04971729 to bind and/or activate estrogen receptors (ERs) has not been investigated by the sponsor.

5 PHARMACOKINETICS/ADME/TOXICOKINETICS

5.1 PK/ADME

Distribution

Red Blood Cell to Plasma Partitioning of PF04971729 in Rat, Dog and Human Whole Blood (143527)

Key study findings:

- PF04971729 at a nominal concentration of 1 µg/mL (2.3 µM) distributed preferentially into plasma over red blood cells in whole blood with blood-to-plasma concentration ratios (Cb/Cp) of 0.66 and 0.66 in rat and human, respectively.

Study no.: 143527
Volume # and page #: EDR (4.2.2.3.1)
Conducting laboratory and location: Pfizer Global Research and Development Groton, CT USA.
Date of study initiation: Not Stated
GLP compliance: No
QA report: No
Drug, lot #, and % purity: PF04971729, PF04971729-00-0001

Methods: This report describes the partitioning of PF04971729 at a concentration of 1 µg/mL (equivalent to 2.3 µM) between red blood cells and plasma in rat, dog, and human whole blood.

Results: Individual and mean whole blood and plasma concentrations and blood to plasma concentration ratios of PF04971729 in rat, dog, and human are shown in the table below. At a concentration of 1 µg/mL (2.3 µM) PF04971729, blood to plasma ratios (Cb/Cp) were 0.66 ± 0.01, 0.58 ± 0.02, and 0.66 ± 0.07 for rat, dog, and human, respectively.

Species	PF-04971729 Concentration (µg/mL) / (µM)	Replicate	Whole Blood Concentration (Cb) (ng/mL)	Plasma Concentration (Cp) (ng/mL)	Blood to Plasma Ratio (Cb/Cp)
Rat	1.0 / 2.3	1	914.6	1414	0.65
		2	881.1	1361	0.65
		3	902.7	1344	0.67
		Mean	900	1400	0.66
		SD	17	37	0.01
Dog	1.0 / 2.3	1	859.4	1523	0.56
		2	867.3	1454	0.60
		3	854.1	1484	0.58
		Mean	860	1500	0.58
		SD	6.6	35	0.02
Human	1.0 / 2.3	1	806.5	1317	0.61
		2	835.8	1330	0.63
		3	881.3	1202	0.73
		Mean	840	1300	0.66
		SD	38	70	0.07

SD = Standard Deviation; Molecular weight (MW) of PF-04971729 = 436.888

Quantitative Whole-Body Autoradiography and Eye Excision of Male Long Evans Rats Following Oral Administration of [^{14}C] PF04971729

Key study findings:

- Following a single 25 mg/kg oral dose of [^{14}C] PF04971729 to LE rats the distribution of drug-derived radioactivity was extensive with C_{max} occurring at 1-2 hrs postdose in most tissues. [^{14}C] PF04971729-derived radioactivity was sustainable in most tissues for at least 8 hours postdose with measurable amounts of radioactivity present in only 12 tissues by 24 hours postdose. Measurable amounts of radioactivity was present in only the kidney cortex at the last sampling time, indicating that the elimination of [^{14}C] PF04971729-derived radioactivity was virtually complete by 168 hours postdose.

- Excluding bile and urine, the tissues with the highest C_{max} concentrations of radioactivity were measured in the urinary bladder, liver, kidney medulla, and kidney, with the liver and kidney being the primary eliminating tissues responsible for the concentrations of PF04971729-derived radioactivity within the bile and urine.

- The uveal tract achieved maximum concentrations of [^{14}C] PF04971729-derived radioactivity at 2 hours postdose and was devoid of radioactivity by 48 hours postdose. [^{14}C] PF04971729-derived radioactivity levels in the lens of the eye were BLQ or ND throughout the course of this study. [^{14}C] PF04971729-derived radioactivity was present in eyes (LSC analysis) excised from Group 1 rats from 1 through 96 hours postdose with concentrations declining to BLQ by 168 hours postdose. Eyes (LSC analysis) excised from Group 2 rats were devoid of radioactivity at both sampling times of 336 and 672 hours postdose. Ocular and skin concentration data illustrated that [^{14}C] PF04971729-derived radioactivity did not exhibit an affinity for pigmented tissues containing melanin.

- [^{14}C] PF04971729-derived radioactivity was present at low levels in the non-circumventricular CNS tissues for at least 8 hours postdose. The data illustrated that [^{14}C] PF04971729-derived radioactivity penetrated the blood: brain barrier in the male LE rat.

- The presence of radioactivity in bile and urine illustrated that biliary and renal excretion were routes of elimination for [^{14}C] PF04971729-derived radioactivity. Excluding bile and urine, the urinary bladder, kidney (including the cortex and medulla), liver, adrenal gland, Harderian gland, and pancreas had the highest $AUC_{0-\alpha}$ values. Elimination $t_{1/2}$ values ranged from 1.52 hrs in bone to 158 hrs in kidney. Blood had an elimination $t_{1/2}$ of 2.14 hours. Tissues with the longest $t_{1/2}$ values were in the order of kidney > kidney cortex > urinary bladder > eye (LSC) > testis > brain medulla > uveal tract (158 to 5.01 hrs). Tissues with the shortest $t_{1/2}$ values were in order of bone < thyroid < brown fat < stomach mucosa < bone marrow (1.52 to 2.11 hrs).

Reviewer Comments:

The epididymis, kidney, kidney cortex, testis, and liver were the tissues expected to be exposed to the highest doses of radiation. In humans, these tissues are estimated to be exposed to 11.1, 4.27, 3.96, 3.43, and 3.39 mRad or mrem, respectively, at the expected target dose of 100 µCi. These exposure values are approximately 450 to 1480-fold lower than the allowable exposure limit of 5000 mrem established by the FDA.

Based on data derived from partially pigmented male rats, the whole-body radiation dose (based on the effective dose) in a 70-kg man following administration of a single 100 µCi dose of [^{14}C] PF04971729 was calculated to be 2.38 mrem. This value is approximately 1260-fold lower than the FDA exposure limit of 3000 mRad or mrem after a single dose for human isotope studies. Based on the pharmacokinetic and dosimetry data, administration of a single oral 100 µCi dose of [^{14}C] PF04971729 would not be expected to represent a significant radiation risk in human male subjects.

Study no.: 102539
Volume # and page #: EDR (4.2.2.3.1)
Conducting laboratory and location: (b) (4)
Date of study initiation: January 25th 2010
GLP compliance: No
QA report: Yes
Drug, lot #, and % purity: [^{14}C] PF04971729 (b) (4) (99.4% Chemical Purity) (96.8% Radio Purity)

Methods: The objective of this study was to evaluate the tissue distribution of drug-derived radioactivity in Long Evans (LE) male rats following oral administration of [^{14}C] PF04971729 by using quantitative whole-body autoradiography (QWBA). Male LE rats were orally administered [^{14}C] PF04971729 in 0.5% methylcellulose and 40% polyethylene glycol (PEG) at a target dose level of 25 mg/kg and 200µCi/kg. Group 1 LE rats were sacrificed at 1, 2, 4, 8, 24, 48, 96, and 168 hours postdose; the right eye from each rat was collected, and the carcasses were then prepared for QWBA. Concentrations of radioactivity were determined in tissues, organs, and biological fluids for Group 1 rats by QWBA. For Group 2, both eyes were removed from one rat at 336 and 672 hours postdose. Eyes from Groups 1 and 2 rats were analyzed for content of radioactivity by liquid scintillation counting (LSC). Pharmacokinetic parameters in tissues were determined and radiation dosimetry parameters were calculated.

Group	Number of Male Animals	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1	8	Oral	25	10	Carcass for WBA and Right Eye for Radioanalysis
2	2	Oral	25	10	Eyes for Radioanalysis

WBA Whole-body autoradiography.
Note: The dose was approximately 200 µCi/kg.

Results:

Clinical signs:

All animals appeared healthy and exhibited no overt signs of toxicity throughout the study.

Tissue Distribution of Radioactivity:

The distribution of [^{14}C] PF04971729-derived radioactivity following oral administration of a 25 mg/kg target dose level to Long Evans (LE) male rats was of measurable concentrations in most tissues, bile, and urine at 1 hour postdose. Concentrations of [^{14}C] PF04971729-derived radioactivity in blood were measurable from 1 through 8 hours postdose with a C_{max} value of 8430 ng equivalents [^{14}C] PF04971729 (eq)/g) occurring 1 hour postdose. Blood consistently contained lower concentrations of radioactivity than those observed for most other tissues. By 24 hours and for all subsequent time points, radioactivity concentrations in blood were BLQ or ND.

Concentrations of [^{14}C] PF04971729-derived radioactivity achieved C_{max} levels at 1 or 2 hours postdose in most tissues, blood, bile, and urine. Radioactivity in most tissues thereafter declined over time. Levels of radioactivity were still of measurable concentrations in most tissues at 8 hours postdose. Approximately 77% of the tissues that contained measurable amounts of radioactivity at 8 hours declined to levels that were BLQ or ND by 24 hours. From 48 through 168 hours, only the kidney and kidney cortex contained measurable amounts of radioactivity illustrating that the elimination of radioactivity was virtually complete by 7 days postdose following a single orally administered bolus dose of [^{14}C] PF04971729 to LE male rats. Radioactivity levels in the lens of the eye were BLQ or ND throughout the course of this study.

Radioactivity was observed in gastrointestinal contents and bile, which is consistent with an orally administered dose, transit of [^{14}C] PF04971729 through the digestive tract, and biliary excretion. The presence of radioactivity in bile and urine illustrated that biliary and urinary excretion were routes of elimination for [^{14}C] PF04971729-derived radioactivity. Excluding bile and urine, the tissues with the highest C_{max} concentrations of radioactivity were measured in the urinary bladder, liver, kidney medulla, and kidney, with the liver and kidney being the primary eliminating tissues responsible for the concentrations for [^{14}C] PF04971729-derived radioactivity within the bile and urine. The lowest C_{max} values were observed in non-circumventricular central nervous system (CNS) tissues, bone, eye (WBA analysis), testis, and abdominal fat.

Radioactivity concentrations of [^{14}C] PF04971729-derived radioactivity were present in the non-circumventricular CNS tissues (e.g. cerebrum, cerebellum, medulla, olfactory lobe, and spinal cord) from 1 hour through 8 hours postdose with C_{max} values observed at 2 or 4 hours. Concentrations of radioactivity were also observed in two regions of the brain that are not protected by a blood: brain barrier (i.e. choroid plexus and pituitary gland). Maximum concentrations of [^{14}C] PF04971729-derived radioactivity in the choroid plexus and pituitary gland occurred at 1 hour postdose. Concentrations of radioactivity in the choroid plexus and pituitary gland were similar to or were approximately 2-fold greater than blood concentrations, respectively. Concentrations of [^{14}C] PF04971729-derived radioactivity were BLQ or ND in all brain substructures by 24 hours postdose.

[^{14}C] PF04971729-derived radioactivity was detected in the uveal tract from 1 through 24 hours postdose and in the eye (WBA) from 1 through 8 hours postdose. Concentrations of radioactivity attained C_{max} at 2 hours in both the uveal tract and the eye (WBA analysis). Radioactivity concentrations thereafter declined over time to BLQ in the eye (WBA analysis) by 24 hours postdose and ND in the uveal tract by 48 hours postdose. Pigmented and non-pigmented skin contained similar concentrations of radioactivity with C_{max} levels occurring at 1 hour postdose for non-pigmented skin and at 2 hours postdose for pigmented skin. Concentration of radioactivity in non-pigmented skin and pigmented skin were BLQ by 24 hours postdose. The skin and ocular data illustrated that [^{14}C] PF04971729-derived radioactivity did not exhibit an affinity for pigmented tissues containing melanin.

Pharmacokinetics of Radioactivity and Dosimetry Parameters in Tissues:

The area under the concentration-time curve from 0 to infinity ($AUC_{0-\alpha}$) and elimination half-life ($t_{1/2}$) values were estimated for all tissues, bile, blood, and urine having discernable levels of radioactivity. Excluding bile and urine, tissues with the greatest ($AUC_{0-\alpha}$) were the urinary bladder, kidney (including the cortex and medulla), liver, adrenal gland, Harderian gland, and pancreas. Bone, non-circumventricular CNS tissues, eye (WBA), abdominal fat, and meninges had the lowest ($AUC_{0-\alpha}$) values. Tissues with the longest ($t_{1/2}$) values were kidney > kidney cortex > urinary bladder > eye (LSC) > testis > brain medulla > uveal tract (158 to 5.01 hrs).

[^{14}C] PF04971729-derived radioactivity was eliminated from blood in male LE rats with an estimated elimination ($t_{1/2}$) value of 2.14 hours. Tissues with the shortest ($t_{1/2}$) values were in order of bone < thyroid < brown fat < stomach mucosa < bone marrow (1.52 to 2.11 hours).

Tissues with the greatest projected exposure to [^{14}C] PF04971729 radioactivity in human research subjects were epididymis > kidney > kidney cortex > testis > liver (11.1 to 3.39 mRad or mrem). The estimated absorbed dose of radiation to the epididymis was 11.1 mRad or mrem which represented 0.223% of the allowable 5000 mrem single dose limit. Bone, bone marrow (blood forming organs), and testis (gonads) had estimated absorbed doses of 0.00786, 0.0468 and 3.43 mRad or mrem, which represented 0.000262, 0.00156, and 0.114% of the allowable exposure limits, respectively. Radioactivity levels in the lens of the eye were BLQ or ND.

The estimated absorbed dose of radiation to the uveal tract in the partially pigmented rat was 1.31 mRad or mrem which represented 0.0262% of the allowable 5000 mrem exposure limit. Pigmented skin had an estimated absorbed dose which represented 0.0224% of the allowable 5000 mrem exposure limit. In comparison, non-pigmented skin had an estimated absorbed dose that was 0.0174% of the allowable exposure limit. The exposure data provided evidence that [^{14}C] PF04971729-derived radioactivity did not selectively associate with skin or ocular melanin. The overall whole-body effective dose equivalent exposure was calculated to be 2.38 mRad or mrem, approximately 0.0793% of the allowable 3000 mrem exposure limit established by the FDA. The exposure of healthy human research subjects to an oral dose containing 100 μCi of [^{14}C] PF04971729-derived radioactivity was projected to be within the allowable radiation safety exposure limits for a single dose of carbon-14 radioactivity, and would not be expected to represent a significant radiation safety exposure risk (See autorad images in EDR).

	Radioactivity (ng eq/g)							
	Animal Number (Sacrifice Time)							
Tissue	B19965 (1 Hour)	B19966 (2 Hours)	B19967 (4 Hours)	B19968 (8 Hours)	B19969 (24 Hours)	B19970 (48 Hours)	B19971 (96 Hours)	B19972 (168 Hours)
Adrenal gland	32100	23500	16900	3810	BLQ	BLQ	ND	ND
Bile	91900	93500	52600	13200	ND	ND	ND	ND
Blood	8430	6660	4150	986	BLQ	ND	ND	ND
Bone	915	326	207	BLQ	ND	ND	ND	ND
Bone marrow	9450	7970	4770	1140	BLQ	ND	ND	ND
Brain cerebellum	334	581	433	251	ND	ND	ND	ND
Brain cerebrum	169	393	338	159	ND	ND	ND	ND
Brain choroid plexus	7940	3830	2820	656	ND	ND	ND	ND
Brain medulla	307	617	554	286	ND	ND	ND	ND
Brain olfactory lobe	134	456	461	166	ND	ND	ND	ND
Cecum	10000	10600	5190	2460	BLQ	BLQ	ND	ND
Diaphragm	10700	14400	8210	2050	BLQ	ND	ND	ND
Epididymis	3180	4930	5130	3750	312	ND	ND	ND
Esophagus	10800	9000	6040	1660	BLQ	ND	ND	ND
Exorbital lacrimal gland	17500	18600	13000	3210	181	ND	ND	ND
Eye	739	1170	943	383	BLQ	ND	ND	ND
Eye lens	ND	ND	ND	BLQ	BLQ	ND	ND	ND
Eye uveal tract	4980	6760	5440	1480	266	ND	ND	ND
Fat (abdominal)	1060	1880	1350	585	ND	ND	ND	ND
Fat (brown)	13100	13600	7940	1670	BLQ	ND	ND	ND
Harderian gland	10100	16200	13500	5140	602	ND	ND	ND
Intra-orbital lacrimal gland	16500	18900	11600	NR	151	ND	ND	ND
Kidney	53900	35400	20400	6510	607	503	435	BLQ
Kidney cortex	52900	38300	21300	7280	813	650	547	189
Kidney medulla	54300	27900	20000	4240	BLQ	BLQ	BLQ	BLQ
Large intestine	14000	10500	6150	2100	BLQ	BLQ	ND	ND
Liver	67200	40200	27700	7600	176	BLQ	BLQ	ND
Lung	10800	9760	6000	1490	BLQ	ND	ND	ND
Lymph nodes	8470	9110	5470	1500	BLQ	ND	ND	ND
Meninges	3990	2140	1100	415	ND	ND	ND	ND
Muscle	7350	9010	6190	1710	BLQ	ND	ND	ND
Myocardium	19800	16300	9810	2680	BLQ	ND	ND	ND
Nasal turbinates	2190	3530	1810	681	ND	ND	ND	ND
Pancreas	27900	23900	14200	3460	BLQ	ND	ND	ND
Pituitary gland	17600	13100	8810	2160	BLQ	ND	ND	ND
Preputial gland	4630	5170	6350	1790	BLQ	ND	ND	ND
Prostate gland	6930	8780	7520	1770	150	ND	ND	ND
Salivary gland	22000	16900	12500	2590	BLQ	ND	ND	ND
Seminal vesicle	2190	2230	1750	821	BLQ	BLQ	ND	ND
Skin (nonpigmented)	5320	4900	3620	983	BLQ	BLQ	ND	ND
Skin (pigmented)	5800	6710	4610	1350	BLQ	BLQ	BLQ	BLQ
Small intestine	9750	8670	7330	2860	BLQ	ND	ND	ND
Spinal cord	254	789	654	305	BLQ	ND	ND	ND
Spleen	11900	9160	5950	1450	BLQ	ND	ND	ND
Stomach	5260	13700	5970	1880	BLQ	ND	ND	ND
Stomach mucosa	27400	20000	11400	2730	ND	ND	ND	ND
Stomach wall	6280	7040	5240	1230	ND	ND	ND	ND
Testis	1380	1470	1810	1170	349	BLQ	ND	ND
Thymus	4740	7800	6230	1720	BLQ	ND	ND	ND
Thyroid	17800	13500	7010	1290	ND	ND	ND	ND
Urinary bladder	7680	185000	189000	1630	263	178	ND	ND
Urine	34100	102000	47700	11500	211	BLQ	ND	ND

BLQ Below limit of quantitation (<121 ng equivalents [^{14}C]PF-04971729/g).
ND The sample shape was not discernible from background. Thus, the level of radioactivity was below the limit of quantitation based on visual observation rather than being quantitatively measured using Imaging Research Inc. AIS software.

Excretion

Mass Balance, Routes of Excretion and Metabolism of PF04971729 Following Oral Administration of [^{14}C] PF04971729 to Sprague-Dawley Rats

Key study findings:

- Following oral administration of a 25 mgA/kg dose of [^{14}C] PF04971729 to Sprague-Dawley rats, the radioactive dose was quantitatively recovered over 168 hours postdose with total recoveries of 92.4±1.5% and 95.3±1.8% in male and female rats, respectively. Approximately 85% of the excreted radioactivity recovery occurred in the first 24 hours. The majority of radioactivity was excreted in feces. The radioactivity recovered in feces accounted for 64.8%±0.8% and 58.7±5.1% of the administered radioactive dose in male and female rats, respectively. The radioactivity recovered in urine accounted for 26.6%±1.1% and 35.2±5.7% of the administered radioactive dose in male and female rats, respectively.

- Following oral administration of a 25 mgA/kg dose of [^{14}C] PF04971729 to bile duct-cannulated rats, the radioactive dose was quantitatively recovered over 48 hours postdose with the total recoveries of 93.7% and 92.3% in male and female rats, respectively. The percentage of radioactive dose recovered in feces, urine and bile of male rats was 11.8%, 34.6 % and 46.8%. In female rats, the recoveries were 14.4%, 43.8% and 32.5%, respectively. Estimated by the summation of recovered radioactivity in urine and bile, the absorption of administered dose was no less than 81.4% and 76.3% in male and female rats, respectively.

- Following oral administration to rats, concentrations of radioactivity in plasma reached mean C_{max} at 0.8 hour and 0.7 hour postdose in male and female rats, respectively. Concentrations of radioactivity in plasma declined after reaching C_{max} and remained measurable through 24 hours postdose in males and 48 hours postdose in females. Mean C_{max} and $AUC_{0-\alpha}$ values were 12200±1300 ng eq/g and 59000±3000 ng eq.h/g in males and 16600±800 ng eq/g and 118000±35000 ng eq.h/g in females, respectively. The $t_{1/2}$ of radioactivity in plasma was slightly longer in females (4.4 hrs) when compared to males (3.3 hrs).

- Following oral administration to rats, a total of 15 metabolites and PF04971729 were detected by LC-MS. The metabolic profiles in both plasma and excreta were qualitatively similar between male and female rats. The major metabolic pathways were glucuronidation and O-deethylation. The glucuronidation occurred on the hydroxy groups of the modified glucose moiety of PF04971729 and its O-deethylated product (M2), and the glucuronides formed were mainly excreted in bile. PF04971729 was detected in urine and accounted for 5% and 14.4% of the radioactive dose in male and female rats, respectively. In plasma, PF04971729 was predominant and accounted for 86.5% and 94.0% of circulating radioactivity, in male and female rats, respectively.

Study no.: 141524
Volume # and page #: EDR (4.2.2.5.1)
Conducting laboratory and location: Pfizer - Groton, CT
(b) (4) Mass Balance)
Date of study initiation: Unknown
GLP compliance: No
QA report: No
Drug, lot #, and % purity: [^{14}C] PF04971729, 00701380-070-01, 98.9%

Methods:

The purpose of this study was to determine the radiolabeled mass balance, routes of excretion, and metabolic profiles of PF04971729 in Sprague-Dawley rats following oral administration of a single 25 mg/kg; 200 µCi/kg dose of [^{14}C] PF04971729.

A group of three male and three female Sprague-Dawley rats (175-187g) were housed individually in stainless steel metabolic cages and were administered a single 25 mgA/kg oral dose of [^{14}C] PF04971729 (Group 1). Urine was collected at predose, and 0-8, 8-24, and at 24-hour intervals through 168 hours postdose. Feces were collected at predose, and at 24-hour intervals through 168 hours postdose.

Another group of two male and two female bile duct cannulated rats (279-326g) were orally administered a 25 mgA/kg dose of [^{14}C] PF04971729 (Group 2). Urine and bile were collected for 2 days at 0-8, 8-24 and 24-48 hours postdose. Feces were collected at 0-24 and 24-48 hours postdose.

To identify circulating metabolites of PF04971729, a third group of rats (173-190g) (2/gender/timepoint) were sacrificed by exsanguination (cardiac puncture) under isoflurane anesthesia at 1, 4, 8, 12, and 24 hours postdose (Group 3).

A fourth group of three male and three female jugular-vein cannulated rats (178-195g) were orally dosed at 25 mgA/kg, blood was collected at 0.25, 0.50, 1, 2, 4, 6, 8, 12,24 and 48 hours postdose.

Results:

Mass Balance (b) (4)

The mean percentage of radioactivity recovered in male and female rats (Group 1) and the mean percentage of radioactivity recovered in male and female bile duct cannulated rats (Group 2).

Excretion Route: Males	Urine	Feces	Total (a)
Time	% of Dose	% of Dose	% of Dose
0-24 hours	25.7	59.6	85.3
24-48 hours	0.7	4.7	5.4
48-72 hours	0.1	0.3	0.4
72-168 hours	0.2	0.2	0.4
Total	26.6	64.8	92.4*
Excretion Route: Females	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0-24 hours	33.8	54.1	87.9
24-48 hours	0.9	4.2	5.1
48-72 hours	0.1	0.2	0.3
72-96 hours	0.4	0.2	0.6
Total	35.2	58.7	95.3*
Combined Total (M+F)	30.9	61.8	93.9*

(a) *Includes cage wash

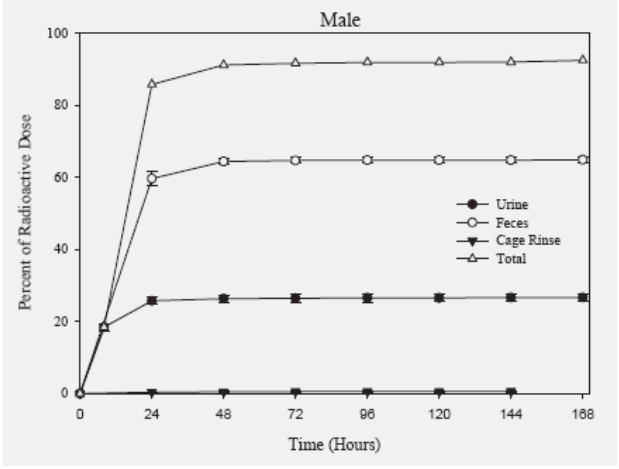
Group 1

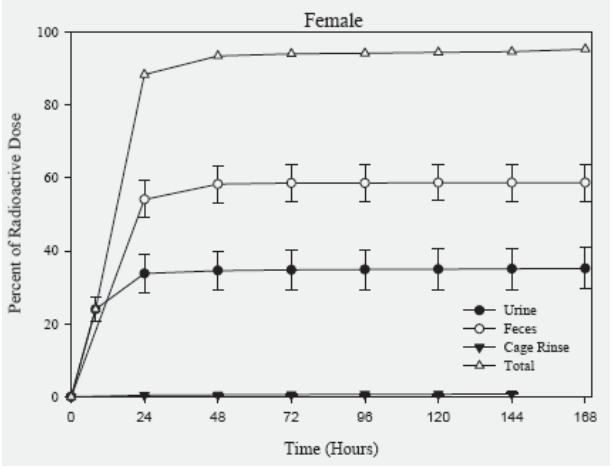
Excretion Route: Males	Urine	Feces	Bile	Total (a)
Time	% of Dose	% of Dose	% of Dose	% of Dose
0-24 hours	33.5	9.7	45.9	89.1
24-48 hours	1.1	2.0	0.9	4.0
Total	34.6	11.7	46.8	93.7
Excretion Route: Females	Urine	Feces	Bile	Total
Time	% of Dose	% of Dose	% of Dose	% of Dose
0-24 hours	42.3	13.0	31.7	87.0
24-48 hours	1.4	1.4	0.8	3.6
Total	43.8	14.4	32.5	92.3
Combined Total (M+F)	39.2	13.1	39.6	95.9

(a) *Includes cage wash, rinse and cage wipe

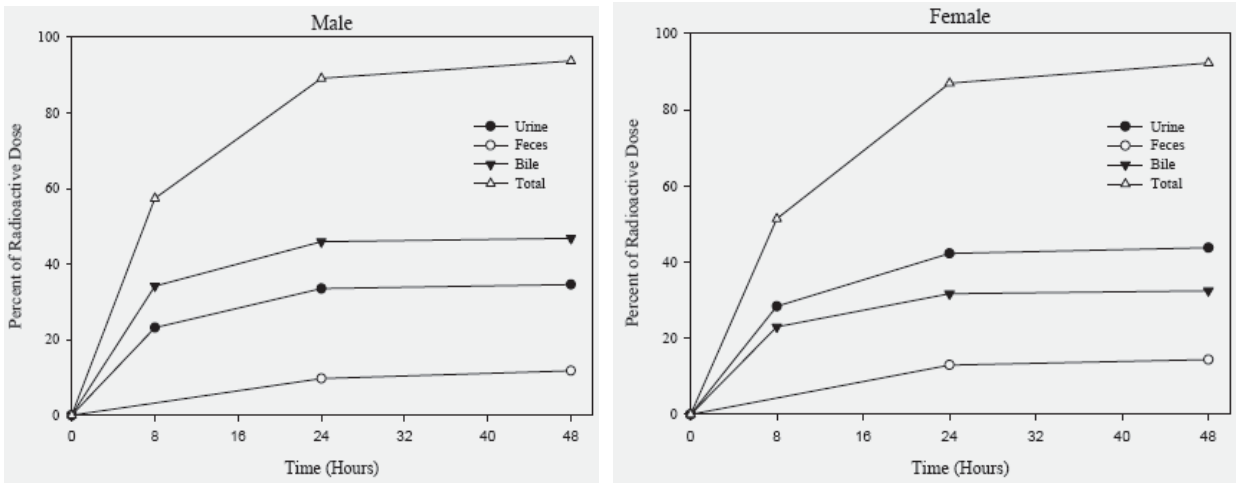
Group 2

The mean cumulative recovery of administered dose in urine and feces (Group 1) and as well as the sum of urine, feces and bile over time (Group 2) are graphically depicted below.





Group 1



Group 2

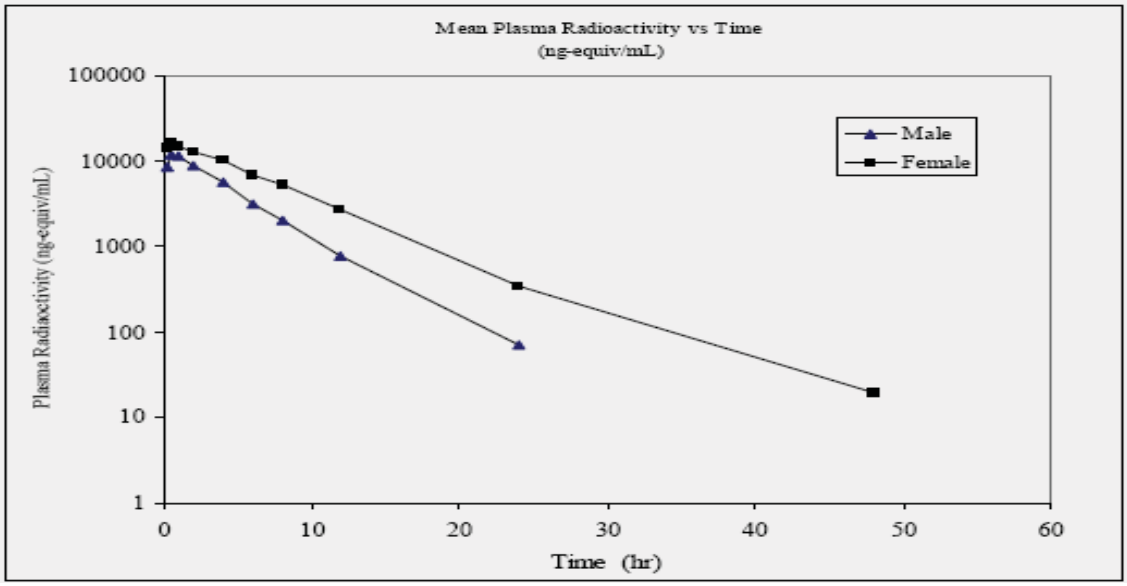
After a single oral dose of [^{14}C] PF04971729 to rats (Group 1), the radioactive dose was quantitatively recovered over 168 hours postdose with the total recoveries of 92.4±1.5 % and 95.3±1.8% in male and female rats, respectively. Approximately 85% of the excreted radioactivity recovery occurred in the first 24 hours. The majority of radioactivity was excreted in feces. The radioactivity recovered in feces accounted for 64.8%±0.8 and 58.7±5.1% of administered radioactive dose in male and female rats, respectively. The radioactivity recovered in urine accounted for 26.6%±1.1% and 35.2±5.7% of administered radioactive dose in male and female rats, respectively.

After a single oral dose of [^{14}C] PF04971729 to bile duct cannulated rats (Group 2), the radioactive dose was quantitatively recovered over 48 hours with the total recoveries of 93.7% and 92.3% in male and female rats, respectively. The recovery of the administered radioactive dose in feces, urine and bile of male rats were 11.8%, 34.6 % and 46.8%, in female rats, the recoveries were 14.4%, 43.8% and 32.5%, respectively. Estimated by the summation of recovered radioactivity in urine and bile, the absorption of administered dose was no less than 81.4% and 76.3% in male and female rats, respectively.

Pharmacokinetics of Radioactivity in plasma

Following oral administration to rats (Group 4), concentrations of radioactivity in plasma reached mean C_{max} at 0.8 hr and 0.7 hr postdose in male and female rats, respectively. Concentrations of radioactivity in plasma declined after reaching C_{max} and remained measurable through 24 hrs postdose in males and 48 hrs postdose in females. Mean C_{max} and $AUC_{0-\alpha}$ values were 12200±1300 ng eq/g and 59000±3000 ng eq.h/g in males and 16600±800 ng eq/g and 118000±35000 ng eq.h/g in females, respectively. The $t_{1/2}$ of radioactivity in plasma was slightly longer in female (4.4 hours) when compared to males (3.3 hours).

Animal Number	C_{max} (ng eq/g)	T_{max} (hours)	$t_{½}$ (hours)	$AUC_{(0-24h)}$ (ng eq•hour/g)	$AUC_{(0-t)}$ (ng eq•hour/g)	$AUC_{(0-\infty)}$ (ng eq•hour/g)
Male						
11	12900	0.5	3.4	57900	59400	59800
12	12900	1	3.2	60300	61200	61500
13	10700	1	3.2	54700	55400	55700
Mean	12200	0.8	3.3	57600	58700	59000
SD	1300	0.3	0.1	2810	3000	3000
Female						
14	15900	1	4.9	114000	119000	119000
15	17400	0.5	2.9	80800	82700	83000
16	16500	0.5	5.2	140000	151000	152000
Mean	16600	0.7	4.4	112000	118000	118000
SD	800	0.3	1.3	29700	34000	35000



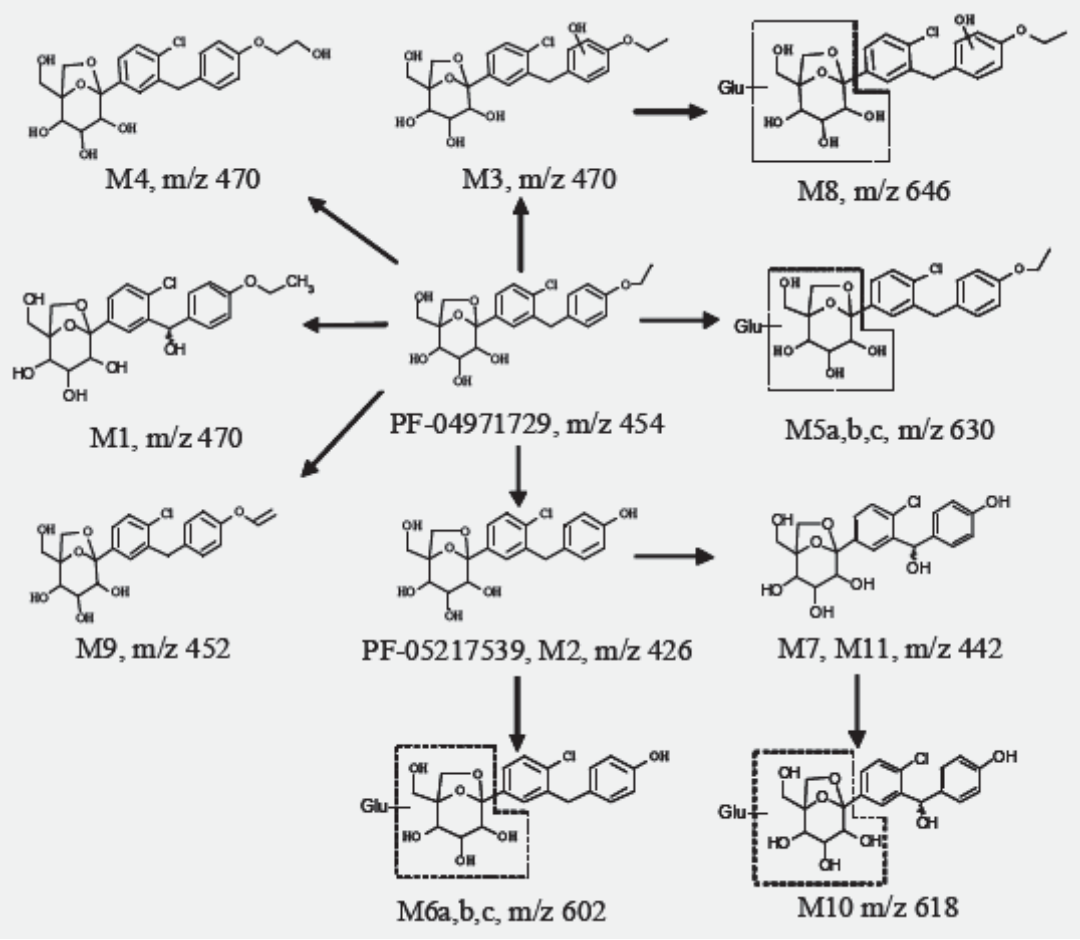
Metabolic Profiles

A total of 15 metabolites and PF04971729 were detected, the summary of mean percentage of metabolites detected in urine, feces and bile (% of dose) and in plasma (% of radioactivity) is shown below.

Sample Analyzed for Metabolites	Urine	Feces	Bile[b]	Plasma
Total Time Period of Collection	0 to 96 h	0 to 96 h	0 to 48 h	0 to 24 h
Sample Analyzed for Metabolites	0 to 24 h	0 to 48 h	0 to 24 h	0 to 12 h
Compound (as % of Dose or % of Sample)	% of Dose	% of Dose	% of Dose	% of Sample
Males:	Mean ± SD	Mean ± SD	Mean	Mean
% of Total Dose Excreted (0-24hr or 0-48h)	25.7±1.0	65.1±1.0	45.9	N/A[a]
PF-04971729	5.0±0.3	22.0±2.8	1.7	86.5
M1	0.9±0.2	5.3±1.1	1.5	2.8
M2	12.5±1.9	27.9±4.6	2.0	4.2
M3		1.2±1.1		
M4		1.2±0.2		
M5a			1.6	0.7
M5b			1.8	
M5c		0.8±0.5	15.8	0.7
M6a	0.6±0.3		16.3	0.0[c]
M6b			2.7	
M6c			0.0[c]	
M7	6.1±1.0	3.0±0.7		2.3
M8			1.1	
M9	0.2±0.0	1.4±0.3		2.8
M10			0.8	
M11	0.2±0.1	3.2±1.0		
Females:	Mean ± SD	Mean ± SD	Mean	Mean
% of Total Dose Excreted (0-24hr or 0-48h)	33.8±5.3	58.8±5.1	31.7	N/A[a]
PF-04971729	14.4±3.8	27.0±6.7	3.1	94.0
M1	0.3±0.0	1.6±0.2	0.4	0.6
M2	13.3±4.2	25.5±2.4	0.1	2.3
M3		0.1±0.1		
M4		0.4±0.2		
M5a			1.2	0.3
M5b			2.4	
M5c			17.3	0.3
M6a	0.3±0.2		6.3	0.0[c]
M7	0.6±0.2	0.3±0.2		0.3
M8			0.3	
M9	0.5±0.0	1.5±0.3		2.3
M11	0.1±0.1	1.8±0.1		

a: N/A; not applicable, b: SD
b:data derived from bile duct cannulated rats (Group 2)
c:detected by mass spectrometry

Proposed Metabolic Pathways of PF04971729 in Rats



Mass Balance, Excretion and Metabolism of PF04971729 in Healthy Male Human Volunteers Following a Single Oral Dose of [^{14}C] PF04971729

Key study findings:

- After a single 25 mgA; 100µCi oral dose of PF04971729 to healthy human subjects, the total recovery of administered radioactivity ranged from 83.7% to 96.6%. The mean total recovery of radioactivity for all subjects was 91.0 ± 4.6%, of which 40.9 ± 7.1% was in urine and 50.2 ± 10.1% was in feces.

- The excretion of radioactivity in urine was rapid, at 24 hours postdose, the mean cumulative recovery was 40.0 ± 7.0%, accounting for approximately 80% of total radioactivity recovered in urine; at 48 hours postdose, the mean cumulative recovery was 46.1% ± 8.7, constituting approximately 92% of total radioactivity recovered in urine.

- The excretion of radioactivity in feces was prolonged due to irregular bowel movement observed in some subjects. At 24, 48, 72 and 96 hours postdose, the mean cumulative recovery was 4.5 ± 9.8%, 11.4 ± 16.7%, 20.9 ±1 7.8% and 28.3 ± 17.5% which accounted for approximately 11%, 28%, 51% and 69% of radioactivity recovered in feces, respectively.

- A total of 8 metabolites were detected by LC/MS/MS with radiochemical detection, all of these metabolites had been identified previously in nonclinical species.

- PF04971729 underwent minimal phase I metabolism; the major metabolic pathway was glucuronidation. The glucuronidation occurred on the hydroxyl groups of the modified glucose moiety of PF04971729 and its des-ethyl metabolite, M2.

- Glucuronides were primarily excreted in urine. Isomeric glucuronides of PF04971729, i.e. M5a, M5b, M5c, and those of M2, i.e. M6a, and M6b were the major radioactivity constituents in urine. Collectively they accounted for 43.9% of the administered dose, and 87.8% of radioactivity excreted in urine. Glucuronides M5a, M5b, M5c, and M6a were also the major circulating metabolites, representing 12.2%, 4.1%, 24.1% and 6.0% of total radioactivity in plasma. Collectively they accounted for 46.4% of circulating radioactivity.

- Unchanged PF04971729 accounted for 49.9% of total radioactivity in plasma and 1.5% of the radioactive dose in urine.

Clinical Study no.:	B1521003
Volume # and page #:	EDR (5.3.3.1.1)
Clinical Research Site and location:	Charles River Clinical Services, Tacoma, WA Tandem Labs (West Trenton, NJ) - PK
Date of study initiation:	Unknown
Drug, lot #, and % purity:	[^{14}C] PF04971729, #121591-111-YSZ001, 99%

Methods:

The purpose of this study was to determine the routes of excretion and metabolic profiles of PF04971729 in healthy male human subjects following oral administration of a single 25 mgA; 100 μCi dose of [^{14}C] PF04971729.

Six healthy male subjects between the ages of 20 and 50 years participated in the study. The subjects entered the clinical research site (Charles River Clinical Services, Tacoma, WA) 12 hours before dosing, and remained there for up to 288 hours after dosing under continuous medical observation. All subjects had fasted at least 8 hours prior to administration of a single 25 mgA; 100 μCi oral dose of [^{14}C] PF04971729.

After dosing, urine samples were collected at 0-12, 12-24 hours and then at 24-hour intervals for up to 216 hours for subject Nos. 10011002, 10011001, 10011008, 10011018 and 10011017, or up to 288 hours for Subject No. 10011011. Fecal samples were collected at 24-hour intervals for up to 216 hours for subject Nos. 10011002, 10011001, 10011008, 10011018 and 10011017, or up to 288 hours for Subject No. 10011011.

A total of three sets of blood samples were collected for the PK analysis of PF04971729 and total radioactivity, and for the profiling and identification of metabolites.

Blood samples for plasma PK analysis of PF04971729 and for the measurement of total plasma radioactivity were collected at predose, 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 36, 48, 72, 96, 120 and 144 hours postdose for all subjects. Plasma AUC exposure data was not included in this study report.

Blood samples for profiling and identification of metabolites in plasma were collected at predose and at 1, 3, 6, 12 and 24 hours following dosing.

Results:

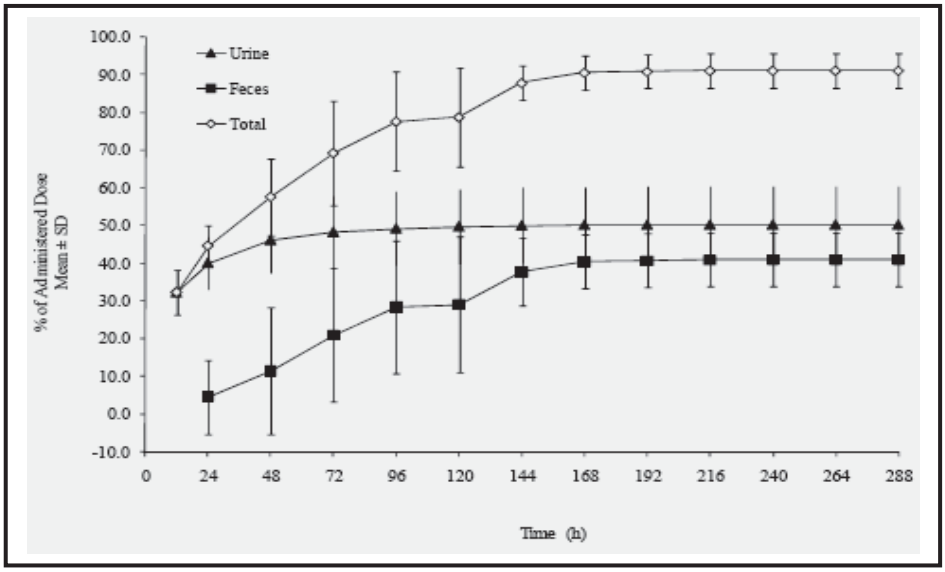
Mass Balance

The percentage of total radioactive dose excreted in urine and feces for each male subject is shown in the table below.

Subject	SSID	Urine	Feces (%Dose)	Total
1001	10011002	56.4	35.7	92.1
1002	10011001	42.2	45.8	88.0
1003	10011008	55.0	41.7	96.6
1004[a]	10011011	35.1	48.6	83.7
1005	10011018	62.7	29.3	92.0
1006	10011017	49.8	44.0	93.8
Mean		50.2	40.9	91.0
±SD		10.1	7.1	4.6

SD: Standard Deviation
a: sample collected up to 288 hours

The mean cumulative recovery of radioactivity in urine, feces and the sum of these two is graphically depicted below.



After a single 25 mgA; 100 µCi oral dose of PF04971729 to human subjects (n=6), the total recovery of administered radioactivity ranged from 83.7% to 96.6%. The mean total recovery of radioactivity for all subjects was 91.0±4.6%. The mean fecal recovery was 40.9±7.1% and the mean urinary recovery was 50.2±10.1%. The excretion of radioactivity in urine was rapid; at 24 hours postdose, the mean cumulative recovery was 40.0±7.0%, accounting for approximately 80% of radioactivity recovered in urine; at 48 hours postdose, the mean cumulative recovery was 46.1%±8.7, constituting approximately 92% of radioactivity recovered in urine. The excretion of radioactivity in feces was prolonged due to irregular bowel movement observed in some subjects. At 24, 48 72 and 96 hours postdose, the mean cumulative recovery was 4.5±9.8%, 11.4±16.7%, 20.9±17.8 and 28.3%±17.5 which accounted for approximately 11%, 28%, 51% and 69% of radioactivity recovered in feces, respectively.

Excreted Metabolites

Urine

The percentage of urinary metabolites, expressed as percent of dose, are presented in the table below.

	SSID		10011002	10011001	10011008	10011011	10011018	10011017		
Metabolite	Rt (min)	m/z	1001	1002	1003	1004	1005	1006	Mean	±SD
						(%Dose)				
M6a	21.4	602	2.2	1.6	0.7	1.0	1.0	0.8	1.2	0.6
M6b	26.7	602	5.1	3.0	3.3	2.2	3.1	3.6	3.4	1.0
M2	27.2	426	0.4	0.3	0.2	0.4	0.2	0.9	0.4	0.3
M1	27.7	470	1.0	0.4	1.2	0.5	0.4	0.6	0.7	0.3
M5a	29.4	630	8.5	5.1	5.9	3.6	6.5	4.7	5.7	1.7
M5b	31.3	630	2.0	1.6	1.8	1.5	2.5	1.9	1.9	0.4
M5c	34.2	630	30.9	27.9	34.6	21.9	43.0	32.1	31.7	7.0
Parent	36.4	454	1.9	1.2	1.8	1.0	1.3	1.5	1.5	0.4

PF04971729 and a total of 7 metabolites were detected in radiochromatograms. Unchanged PF04971729 was a minor urinary radioactivity constituent, accounting for 1.5% of the administered dose. Isomeric glucuronides of PF04971729, i.e. M5a, M5b, M5c, and those of M2, i.e. M6a, and M6b were the major radioactivity constituents in urine. Collectively they accounted for 43.9% of the administered dose, and 87.8% of radioactivity excreted in urine.

Feces

The percentages of fecal metabolites, expressed as percent of dose, are presented below.

	SSID		10011002	10011001	10011008	10011011	10011018	10011017		
Metabolite	Rt (min)	m/z	1001	1002	1003	1004	1005	1006	Mean	±SD
						(%Dose)				
M2	27.4	426	1.3	1.4	0.5	0.5	0.6	1.4	1.0	0.5
M1	27.8	470	1.3	3.3	1.3	1.8	1.2	1.5	1.7	0.8
M3	29.8	470	1.3	1.6	0.9	1.5	1.6	1.4	1.4	0.3
Parent	36.7	454	29.5	36.0	32.8	42.9	23.5	38.1	33.8	6.8

PF04971729 and a total of 3 metabolites were detected in radiochromatograms. Unchanged PF04971729 was the major radioactivity component in feces; accounting for approximately 90% of recovered radioactivity in feces, and for 33.8% of the administered dose.

Circulating Metabolites

The percentages of circulating metabolites are presented below.

	SSID		10011002	10011001	10011008	10011011	10011018	10011017		
Metabolite	Rt (min)	m/z	1001	1002	1003	1004	1005	1006	Mean	±SD
						(%Radioactivity)				
M6a	21.3	602	9.7	6.7	4.9	6.1	3.4	5.0	6.0	2.2
M2	26.6	426	1.3	1.4	1.3	1.0	0.9	1.7	1.3	0.3
M1	27.5	470	2.6	3.2	2.9	1.0	1.7	3.7	2.5	1.0
M5a	29.4	630	11.6	12.8	13.3	13.2	10.5	11.9	12.2	1.1
M5b	31.3	630	4.6	4.0	2.7	4.9	4.3	3.9	4.1	0.8
M5c	34.3	630	14.8	15.6	30.5	23.6	32.3	27.7	24.1	7.5
Parent	36.4	454	55.4	56.3	44.2	50.2	47.0	46.1	49.9	5.0

PF04971729 and a total of 6 metabolites were detected in radiochromatograms. PF04971729 accounted for 49.9% of circulating radioactivity. Glucuronides M5a, M5b, M5c, and M6a were the major circulating metabolites, representing 12.2%, 4.1%, 24.1% and 6.0% of total radioactivity in plasma. Collectively they accounted for 46.4% of circulating radioactivity.

Metabolic Profile of PF04971729

	% Male Metabolite / % Female Metabolite (Rat)						Metabolite Detection			
Metabolite	Urine	Urine BDC	Feces	Feces BDC	Bile BDC	Plasma	Human Males	Human *In Vitro*	Canine *In Vitro*	Rat *In Vitro*
M1	3x	2.1x	3.3x	2.6x	3.8x	1.6x	U,F,P	M,H	M,H	M,H
M2	0.9x	3.1x	1.1x	1.7x	20x	1.3x	U,F,P	M,H	M,H	M,H
M3	-	-	12x	-	-	-	F	M	M	M,H
M4	-	-	1x	-	-	-	-	M	M	M,H
M5a	-	-	-	-	1.3x	1.4x	U,P	H	H	H
M5b	-	-	-	-	0.8x	-	U,P	H	H	H
M5c	-	-	1.3x	-	0.9x	1.4x	U,P	-	-	H
M6a	2x	2.7x	-	-	2.6x	BLQ	U,P	H	H	H
M6b	-	-	-	-	5.4x	-	U	H	H	H
M6c	-	-	-	-	-	-	-	H	H	H
M7	10x	♂	10x	-	-	1.8x	-	-	-	H
M8	-	-	0.8x	-	3.6x	-	-	-	-	H
M9	0.4x	0.8x	0.9x	1x	-	1.1x	-	-	-	-
M10	-	-	-	-	♂	-	-	-	-	-
M11	2x	-	1.7x	-	-	-	-	-	-	-
Parent	0.35x	0.4x	-	0.8x	0.5x	0.96x	U,F,P	M,H	M,H	M,H

BDC = Bile Duct Cannulated

U = Urine, F = Feces, P = Plasma

M = Liver Microsomes, H = Hepatocytes

♂ = Detected in Males Rats Only

BLQ = Below Limit Quantification

6 TOXICOLOGY

6.2 REPEAT-DOSE TOXICITY

1-Month Oral Toxicity Study and Micronucleus Assessment of PF04971729 (b) (4) in Rats

Key study findings:

- After one month of dosing, female rats administered 5, 25, and 500 → (D11)250 mg/kg/day had exposures of 16.2, 93.0 and 718 µg.h/mL (36X, 206X and 1592X MRHD, µg/h/mL basis) compared to males 5, 25, and 500 → (D11)250 mg/kg/day had exposures of 8.4, 69.3 and 541 µg.h/mL (19X, 154X and 1200X MRHD, µg/h/mL basis).

- Significant clinical signs in HD animals included: (Abdomen distended, reduction activity, anogenital staining, fecal discoloration and/or softness and changes in hair quality. Several animals at the original HD of 500 mg/kg/day were euthanized in moribund condition or found dead within the first 3 weeks. There were no significant clinical signs ≤ 25 mg/kg.

- Males administered ≤ 25 mg/kg lost 4% more weight than controls, at ≥ 250 mg/kg males lost 28%. Females lost on the average 16% more weight than controls across all dosing levels. Weight loss occurred despite substantial increases in food consumption.

- Decreases (2.3% to 6.0% vs. control) in group mean red blood cell count (RBC), hematocrit (HCT), and/or hemoglobin (HGB) was observed in all rats at 500/250 mg/kg.

- Increased BUN levels were accompanied by decreased creatinine levels (14%-18% vs. control) in male and (6%-10% vs. control) female rats. There were dose-dependent increases in group mean absolute and relative kidney weights (10% to 40%). In the kidney, dosing related dilatation of renal tubules (mainly distal nephron segments) was noted in the cortex and medulla of males administered ≥ 5 mg/kg and females at ≥ 25 mg/kg.

- AST and ALT increased 2 to 3-fold at 250 mg/kg, with smaller increases occurring at 5 and 25 mg/kg. No change was observed in total bilirubin or in liver histology.

Reviewer Comments: Throughout this study, females had higher exposures relative to males, which may have contributed to the more severe clinical signs and increased mortality. Most of the findings in this study could reasonably be attributed to PF04971729-induced glucosuria, osmotic diuresis and a catabolic state. The relatively modest (≤ 3 fold) increase in ALT and AST were not accompanied by bilirubin elevation or adverse liver histology, but should nevertheless be monitored in clinical studies. Of note, all doses were associated with adrenal hypertrophy (males), pancreatic zymogen depletion, and atrophy of adipose tissue. Dilatation of renal tubules also occurred at all doses in males and at doses ≥ 25mg/kg in females. These findings were not considered as a basis for a NOAEL as they reasonably

reflect a non-toxic compensatory response to glucosuria. Rather, the NOAEL is based on the clearly evident toxic effects of moribundity at 500 mg/kg and increased severity of chronic progressive nephropathy and stomach erosion/squamous hyperplasia in females at 250 mg/kg.

PF04971729 (b) (4) did not alter bone in the stifle joint or the sternum, unlike many other SGLT2 inhibitors that cause hyperostosis of bone. Decreased serum calcium levels may be indicative of early PF04971729 (b) (4) induced bone toxicity.

Rat – (1 month) (5 and 25) (500 → (D11) 250 mkd)	**NOAEL**	**Multiple of Phase 3 Therapeutic Dose (Current MRHD)** (b) (4)
Mortality	250 mg/kg (611 µg.h/mL)	
Increased Chronic Nephropathy	25 mg/kg (81 µg.h/mL)	

3-Month Oral Toxicity Study of PF04971729 (b) (4) in Rats – No Recovery Period

Key Study Findings:

- After 91 days of dosing, male rats administered 5, 25, and 250 mg/kg/day had exposures of 15.2, 81.4 and 694 µg.h/mL (34X, 180X and 1539X MRHD) and female exposures were 24.5, 97.4 and 781 µg.h/mL (54X, 216X and 1732X MRHD), respectively (µg.h/mL basis).

- Oral administration of PF04971729 to rats for 3 months was tolerated at doses up to 250 mg/kg. Significant decreases in body weight and sporadic occurrence of soft feces were noted in males only at 250 mg/kg with increases in food consumption at doses ≥ 5 mg/kg in both sexes. Serum glucose was decreased and urinary glucose increased markedly at all doses, consistent with effects on BW and the PD activity of PF04971729.

- Reduced reticulocytes (males) and WBCs (both genders) were observed at all doses.

- Increased kidney weights (both sexes, all doses) were associated with histopathology that included: pelvic/tubule dilatation and mineral deposition at all doses (M), progressing to include pelvic inflammation at 25 mg/kg, and finally to include pelvic hyperplasia and increased severity of CPN at 250 mg/kg.

- Dilatation, hyperplasia, and inflammation were observed in the bladder at 250 mkd.

- Increased adrenal weight (females, all doses) and associated histopathology (both sexes, all doses) were observed.

- Reduced prostate weight (all doses) and associated inflammation (≥ 25 mg/kg).
- Hyperostosis was observed in the long bones and sternum at ≥ 25 mg/kg in males and at the 250 mg/kg dose in females.
- G.I. tract dilatation and increased villi height (males, all doses and females ≥ 25 mg/kg), and changes in the stomach (erosions/ulcers ≥ 25 mg/kg) were noted.
- Heart myonecrosis was observed at increased incidence in both genders at 250 mg/kg.

Reviewer Comments: NOAELs were identified for the findings of greatest severity, notably the pelvic and urinary bladder hyperplasia (198x), stomach erosion (44x), and heart myonecrosis (198x). Bone hyperostosis occurred with a NOAEL of (34x to 216x), depending on gender.

Reduced serum glucose, increased urinary volume and glucose excursion underlie most of the findings in this study, including reduced BW and increased food consumption, histopathology changes in the kidney, bladder, and adrenal gland, and zymogen depletion in the pancreas.

The gastrointestinal effects are plausibly related to inhibition of SGLT1. PF04971729 concentrations at the lowest dose in this study was 4µM, exceeding the IC_{50} for rat SGLT1 (0.35 µM). This is not expected in human subjects where the IC_{50} for human SGLT1 is (2 µM).

Bone hyperostosis is consistent with stimulation of osteoblast driven calcium deposition and inhibition of osteoclast removal of calcium from the bone, although serum parathyroid hormone levels were not measured during this study. It has been hypothesized that inhibition of SGLT1 in the gut increases calcium absorption. Although SGLT1 was most likely inhibited in this study, serum calcium levels were not changed. Bone biomarkers should be monitored by the sponsor.

Reduced reticulocytes and WBC may be secondary effects of altered glucose metabolism, although the mechanism is unclear. These parameters are monitored during clinical studies, which is adequate. The mechanism behind the heart myonecrosis toxicity is unknown, but because it occurred at 200x the clinical dose, it is not considered a clinically relevant toxicity.

Rat – (3 Month) **(5, 25 and 250 mg/kg/day)**	**NOAEL**	**Multiple of Phase 3 Therapeutic Dose (Current MRHD)** (b) (4)
Pelvic/tubule dilatation	< 5mg/kg	
Pelvic and bladder hyperplasia ↑ Severity of CPN	25 mg/kg (89.4 µg.h/mL)	
Increased Adrenal Weight Associated Histopathology	< 5mg/kg	
Decreased Prostate Weight Associated Inflammation	5 mg/kg (15.2 µg.h/mL)	
Gastro-Intestinal Tract Dilatation	M: < 5mg/kg	

Increased Villi Height	F : 5 mg/kg (24.5 µg.h/mL)	(b) (4)
Heart myonecrosis	25 mg/kg (89.4 µg.h/mL)	
Stomach Erosion/Ulcer	5 mg/kg (19.9 µg.h/mL)	
Hyperostosis (Long Bones and Sternum)	M: 5 mg/kg (15.2 µg.h/mL)	
	F : 25 mg/kg (97.4 µg.h/mL)	

6-Month Oral Toxicity Study with a 8-Week Recovery Phase of PF04971729 (b) (4) in Rats

Study no.:	09GR275
Study report location:	(SD36 - eCTD 4.2.3.2.1) (DARRTS SDN37)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	15 September 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF04971729 (b) (4), GR02877, 98.4% Active Moiety: 74.4%, GR02546, 99.5%, Active Moiety: 76.0%

Key Study Findings:

- After 26 weeks of dosing, male rats administered 5, 25, and 100 mg/kg/day had exposures of 17.6, 128 and 397 µg.h/mL (39X, 284X and 880X MRHD, µg.h/mL basis). In females exposures were 26.9, 167 and 814µg.h/mL, respectively (60X, 370X and 1805X MRHD, µg.h/mL basis).

- Oral administration of PF04971729 to rats for 6 months was tolerated at doses up to 100 mg/kg. Significant decreases in body weight gain and final body weights were noted in males only at 100 mg/kg with significant increases in food consumption at doses ≥ 5 mg/kg in both genders. Serum glucose was decreased and urinary glucose increased at all doses, consistent with the effects on BW and the PD activity of PF04971729.

- A significant reduction in RBC counts (both genders), reticulocyte counts (males) and red cell distribution widths (males) were observed at 100 mg/kg. Male absolute reticulocyte counts and red cell distribution widths did not resolve fully by the end of recovery.

- Decreased serum parathyroid hormone and adrenal hypertrophy/vacuolation associated with increased adrenal weights were observed in both genders dosed at 100 mg/kg. Increased trabecular bone (femur and sternum) was observed in males dosed at 100 mg/kg indicating that decreased PTH levels may drive serum calcium levels down through the stimulation of osteoblast and inhibition of osteoclast activity in the bone. Hypertrophy of adrenal gland zona glomerulosa may have been associated with increased aldosterone production in response to fluid and electrolyte losses.

- Increased blood urea nitrogen and inorganic phosphorus were consistent with relative dehydration and lower serum calcium, sodium, potassium, and chloride correlated with increased urinary excretion. The pharmacologic activity of PF04971729 (reduced renal tubular reabsorption of glucose from the glomerular filtrate) and subsequent osmotic diuresis and systemic metabolic changes were plausibly responsible for the majority of urinalysis and urine chemistry effects. These effects correlated with pathological changes noted in the kidney (hypertrophy of proximal tubules, tubular mineralization, and tubular dilatation) and bone (increased trabecular bone).

- Discoloration of the stomach noted at the end of dosing in both genders corresponded to microscopic findings of erosions/ulcers. An increased incidence of foveolar hyperplasia (100 mg/kg) and crypt degeneration in the pylorus was present in both genders (≥ 25 mg/kg/day). A decrease in pancreatic zymogen granules was present at all dose levels. Changes in the stomach and pancreas resolved in recovery animals and were consistent with increased food consumption and/or off target inhibition of SGLT1.

Reviewer Comments*:* NOAELs were identified for pelvic/tubule dilatation and/or hyperplasia (49x MRHD), and stomach hyperplasia (328x MRHD). Severe bone hyperostosis was noted at the 100 mg/kg dose and was confined to male rats (284x MRHD), while female rats presented with bone hyperplasia of the physis at the 100 mg/kg dose (370x MRHD).

Reduced serum glucose and increased urinary volume and glucose excursion underlie most of the findings in this study, including reduced BW and increased food consumption, histopathology changes in the kidney, bladder, and adrenal gland, and zymogen depletion in the pancreas.

The gastrointestinal effects are plausibly related to inhibition of SGLT1. PF04971729 concentrations at the lowest dose in this study were 5µM, exceeding the IC_{50} for rat SGLT1 (0.35µM). This is not expected in human subjects where the IC_{50} for human SGLT1 is (2 µM).

It has been hypothesized that bone hyperostosis is related to inhibition of SGLT1 in the gut and increased calcium absorption. In addition, low levels of PTH are known to lower serum calcium levels through the activation of osteoblast and inhibition of osteoclast activity. A concurrent increase in serum phosphorus at the 100 mg/kg dose supports the hypothesis that low PTH levels were affecting the concentration of circulating cations.

It is plausible that the observed reduction in reticulocyte and WBC counts may be secondary to the effect of altered glucose metabolism, although this hypothesis remains unproven. These parameters are monitored during clinical studies, which is adequate to assess this toxicity.

An increased incidence of mammary cysts was observed grossly in recovery females previously dosed at ≥ 25 mg/kg (NOAEL, 60x MRHD, AUC basis). This observation correlated with an increased incidence of cystic dilatation and hyperplasia/hypertrophy of the breast glandular epithelium in high dose recovery females (100 mg/kg). It is interesting to note that this class of drug has recently been associated with an increased incidence of breast cancer in humans.

Rat – (6 Month) **(5, 25 and 100 mg/kg/day)**	**NOAEL**	**Multiple of Phase 3 Therapeutic Dose (Current MRHD)** (b) (4)
Decreased RBCs Decreased Reticulocytes (M)	25 mg/kg (148 µg.h/mL)	
Kidney - Pelvic/Tubule (Dilatation and/or Hyperplasia)	5 mg/kg (22.3 µg.h/mL)	
Kidney - Mineralization	< 5 mg/kg (< 22.3 µg.h/mL)	
Stomach – Hyperplasia (Foveolar Layer)	25 mg/kg (148 µg.h/mL)	
Stomach - Erosion/Ulcer	< 5 mg/kg (< 22.3 µg.h/mL)	
Adrenal Cortex (Hypertrophy/Vacuolation)	< 5 mg/kg (< 22.3 µg.h/mL)	
Bone Hyperostosis (Femur and Sternum)	M: 25 mg/kg (128 µg.h/mL)	
Bone Hyperplasia (Physis)	F: 25 mg/kg (167 µg.h/mL)	
Mammary Cysts (Recovery Females)	F: 5 mg/kg (26.9 µg.h/mL)	

Methods:	
Doses:	0 (vehicle), 5, 25, and 100 mg/kg
Frequency of dosing:	Once Daily for 6 Months + 8 Week Recovery
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	A solution of 0.5% (w/v) methylcellulose with 10 % (v/v) polyethylene glycol 400 (PEG 400)
Species/Strain:	Sprague Dawley (Crl:CD[SD]) (b) (4)
Number/Sex/Group:	20/sex/group
Age:	7 Weeks
Weight:	172-246 g (males) and 135-185 g (females)
TK Satellite groups:	4/sex/group
Unique study design:	None
Deviations from study protocol:	Formulation, Concentration verification, Dose administration, Husbandry, Clinical signs, bioanalytical analysis, Clin Path, organ weights and histology

Observational endpoints/timing	
Clinical Findings	2x daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Cage side observations (1x daily) 1 hour postdose during the dosing phase and on toxicity animals (1x daily) during the recovery phase, except on Day 15 of the recovery phase and on days when detailed observations were conducted. Detailed observations 1x predose phase, prior to dosing on Day 1 of the dosing phase, weekly (based on Day 1 of the dosing phase) throughout the dosing and recovery phases, and on the day of scheduled sacrifice (for those animals necropsied).
Body weights	1x Predose, 1x Prior to Dosing D1 and Weekly Thereafter
Food consumption	Dosing and Recovery - Weekly
Ophthalmoscopy	1x during the Predose phase and on toxicity animals once during Week 13 and once during the last 7 days of the dosing phase using an indirect ophthalmoscope.
Toxicokinetics	Weeks 1, 13 and 26 – 1,4 ,7 and 24 Hours Post Dose (Not Fasted)
Hematology	Fasted – Week 13 and at scheduled sacrifices
Clinical chemistry	Fasted – Week 13 and at scheduled sacrifices
Urinalysis	Fasted – Week 13 and at scheduled sacrifices
Parathyroid Hormone	Control and High Dose - Fasted – End of Dosing
Gross pathology	Fasted - End of Dosing and Recovery
Organ weights	End of Dosing and Recovery
Histopathology	Standard Battery
	Adequate Battery: yes (X), no ( )
	Peer review: yes (X), no ( )
Other	Bone Marrow Smears (not evaluated)

Results

Mortality

One female dosed at 25 mg/kg/day was found dead on Day 80 of the dosing phase. No clinical signs preceded death, and no observation was made at necropsy. One male administered 100 mg/kg/day was sacrificed in moribund condition on Day 93 of the dosing phase. This animal had a swollen left hind paw, rough and red hair coat, and clear oral discharge and was sacrificed due to general debilitation and humane care and use concerns. These deaths were likely incidental and not related to dosing.

Clinical Signs

An increase in the incidence of alopecia on the front paws of females dosed at 100 mg/kg/day was observed. The increased clinical incidence of alopecia noted on the front paws of females dosed at 100 mg/kg/day was confirmed at necropsy in a small number of animals. No dose-limiting clinical signs were observed at any dose.

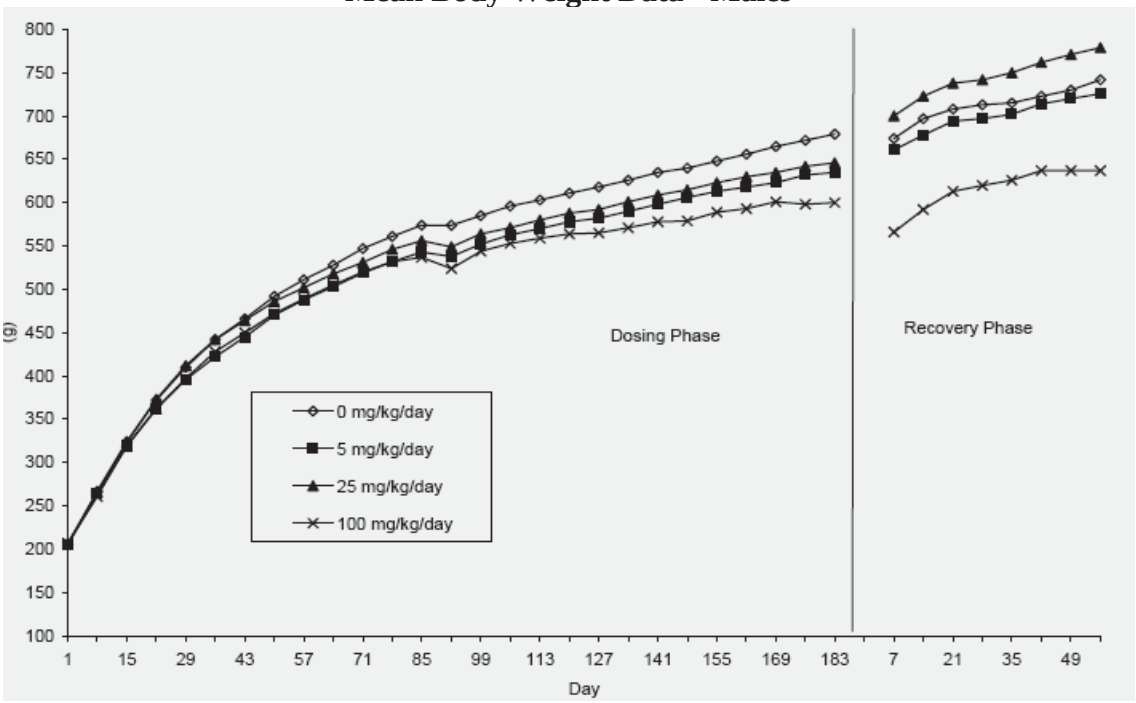
Body Weights

Final BW was 12% lower in 100mg/kg males which reached statistical significance. Final BW in all other groups, male and female, tended to be lower but still within 10% of the control groups.

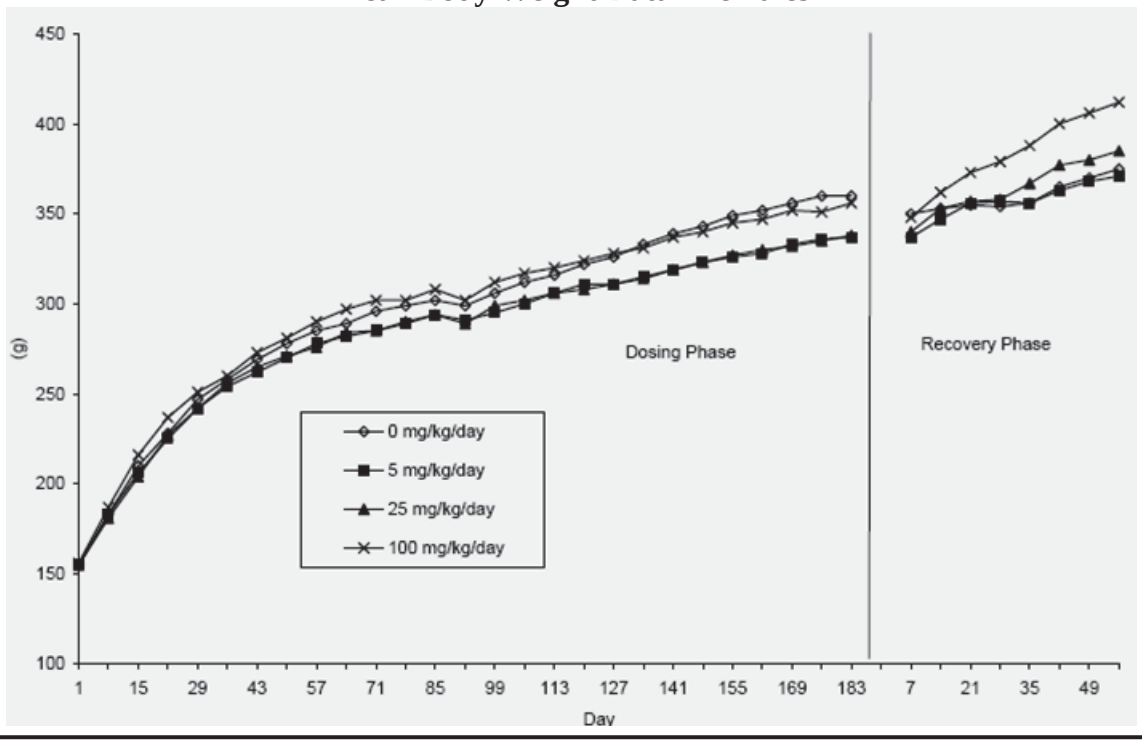
Males Body Weight (Dosing)				
Sex	Dose, mg/kg	Starting BW, g	Final BW, g	BW % control
Males	0	205	679	100 %
	5	206	635	93%
	25	206	646	95 %
	100	207	600	88 %*
	(*) $p \leq 0.05$			

Females Body Weight (Dosing)				
Sex	Dose, mg/kg	Starting BW, g	Final BW, g	BW % control
Females	0	155	360	100 %
	5	155	337	94%
	25	155	338	94 %
	100	156	356	99 %
	(*) $p \leq 0.05$			

Mean Body Weight Data - Males

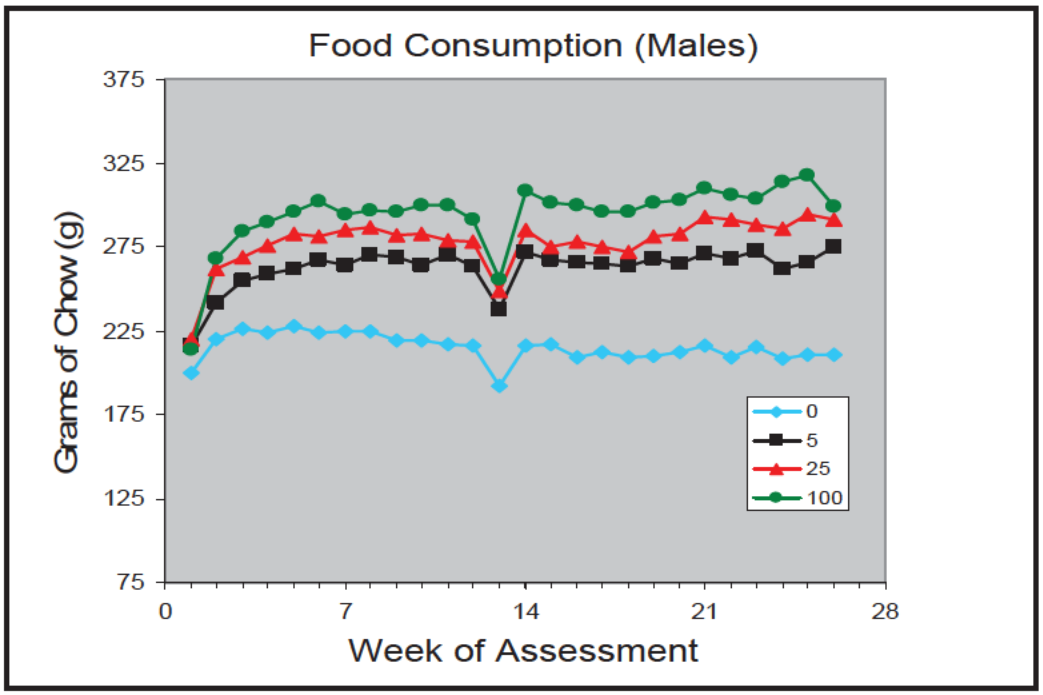


Mean Body Weight Data - Females

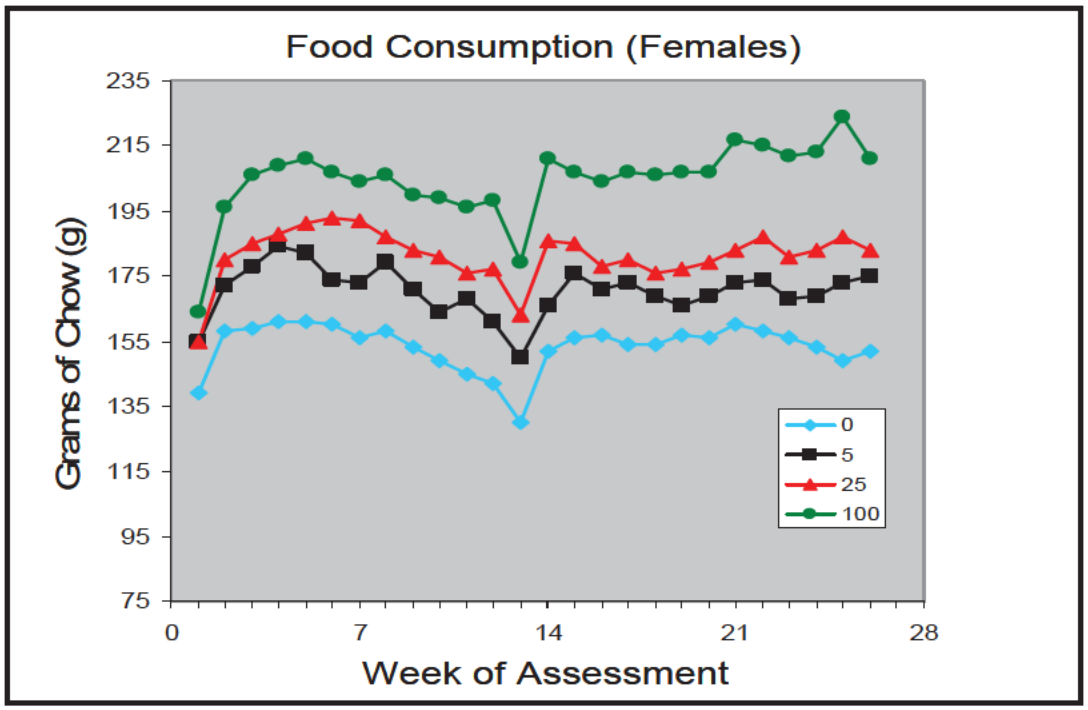


Food Consumption

A significant and dose-related increase in food consumption occurred in all male dosing groups beginning at Week 1 of the administration phase (See reviewer's figure below).



A dose-related increase in food consumption occurred in females beginning at Week 1 of the administration phase. The difference was statistically significant during all weeks in females administered ≥ 25 mg/kg/day and during most weeks for female dosed at 5 mg/kg.



At the end of the recovery phase, food consumption in males was not notably different from controls.

At the end of the recovery phase, food consumption in females dosed at 25 or 100 mg/kg/day was slightly higher, albeit not significantly different from controls.

Changes in food consumption were not associated with adverse clinical signs and are consistent with the intended pharmacology (SGLT2 inhibition resulting in marked glucosuria, weight loss, and compensatory increased food consumption).

Ophthalmoscopy

No visible lesions were noted at the examinations during Weeks 13 and 26 of the dosing phase.

Hematology

(**Erythrocytic Parameters**)

Small magnitudes of change occurred in erythrocytic parameters. Due to the limited number of recovery animals (five/sex/group), reversibility of these toxicities was difficult to assess. These changes are consistent with mild dehydration resulting from polyuria.

Dose dependent decreases in RBC counts were observed in males (↓1% to ↓4% - week 13 and week 26*) and females (↓3% to ↓4%* - week 13).

Decreases in reticulocyte counts were observed in males dosed at 100 mg/kg during week 13 (↓16 %*) and at the end of the administration phase (↓19 %). Reticulocyte counts tended to remain lower in males previously dosed at 100 mg/kg following the recovery period. Significant changes in reticulocyte counts were not observed in females at any time during this study.

Changes in RBC counts were accompanied by decreases in hemoglobin (↓2% to ↓4%) and hemocrit (↓2% to ↓5%) in females during week 13 and week 26. These parameters tended to remain lower in dosed animals following the 8 week recovery period.

Mean corpuscular volumes were elevated in dosed males during week 13 (↑1% to ↑2%) and in females at the end of the administration phase (↑1%). Mean corpuscular hemoglobin (↑1% to ↑5%) and mean corpuscular hemoglobin concentration (↑2% to ↑4%) increased significantly during weeks 13 and/or week 26 in males only. These parameters all tended to recover following dosing cessation for 8 weeks.

Red cell distribution width declined significantly in males during week 13 (↓5% to ↓9%) and at the end of dosing (↓5% to ↓9%). This trend was apparent in females at the end of dosing (↓1% to ↓3%). This parameter tended to remain lower in previously dosed males following the recovery period.

(**Leukocytic Parameters**)

Dose-related changes in leukocytic parameters were consistent with a stress response (endogenous corticosteroid release) and were associated with correlative microscopic findings in the adrenal gland (minimal hypertrophy and vacuolation) and lymphoid tissues (C-cell hyperplasia thyroid and hyperplasia of the thymus epithelium).

Platelet counts tended to decline with dose in males at the end of dosing (↓1% to ↓7%) and recovery males continued to have depressed platelet counts at the 100 mg/kg dose (↓14 %). No consistent trend was observed in female platelet counts.

WBC counts tended to decline in males (↓8% to ↓29 %*) and females (↓2% to ↓21 %*) follow 13 weeks and 26 weeks (M - ↓7% to ↓26 %* and F - ↓1% to ↓26 %*) of dosing. This trend was not apparent in recovery animals.

Lymphocyte counts declined with dose in both genders following 13 weeks (M - ↓12% to ↓37 %* and F - ↓2% to ↓34 %*) and 26 weeks (M - ↓15% to ↓31 %* and F - ↓26 %* to ↓27 %*) of dosing. Animals dosed at 100 mg/kg tended to have lower lymphocyte counts at the end of recovery period.

Eosinophil counts tended to decline with dose in both genders at the end of the administration period (M - ↓8% to ↓31% and F - ↓33 %).

Basophil counts were lower in dosed males and females during week 13 (M - ↓20% to ↓60 %* and F - ↓33% to ↓67 %*) and week 26 (M - ↓33 %* and F - ↓50 %*).

A significant decline (↓33 %*) in leukocyte counts was observed in males only dosed at 100 mg/kg during week 13.

(Coagulation Parameters)

Prothrombin times tended to decrease (↓0.3 to ↓1.1 seconds) in recovery females previously exposed to PF04971729. This trend was not observed in females at the end of dosing or in males at any time during this study.

Clinical Chemistry

(Serum Glucose)

The most prominent serum clinical chemistry effect was lower serum glucose, consistent with the pharmacologic activity of PF04971729 (reduced renal tubular reabsorption of glucose from the glomerular filtrate). A significant decline in serum glucose levels was observed in both genders at all doses during week 13 (M - ↓31% to ↓49% and F - ↓9% to ↓42%) and at the end of dosing (M - ↓34% to ↓46% and F - ↓9% to ↓39%). Following the 8 week recovery period serum glucose levels remained lower in animals dosed at 100 mg/kg (M - ↓18 %* and F - ↓16%).

(BUN)

BUN levels increased significantly with dose in both genders at all doses during week 13 (M - ↑69% to ↑154% and F - ↑33% to ↑140%) and at the end of dosing (M - ↑69% to ↑146% and F - ↑33% to ↑120%). Even though elevated serum urea nitrogen levels suggest prerenal azotemia secondary to osmotic diuresis and dehydration, serum creatinine was actually lower (see below) for dosed animals and perhaps resulted from reduced muscle mass. In addition, some of the increase in serum urea nitrogen may have been due to increased protein catabolism.

(Serum Creatinine)

A significant decline in serum creatinine was observed in both genders at the 100 mg/kg dose during week 26 (M - ↓14 % and F - ↓25 %).

(Serum Total Protein)

A dose dependent decline in serum total protein was observed in both genders during week 13 (M - ↓1% to ↓4 %* and F - ↓1% to ↓5%) and at the end of dosing (M - ↓1% to ↓5 %* and F - ↓4% to ↓7 %*).

(Serum Cholesterol)

Serum cholesterol was significantly decreased in males administered 100 mg/kg for 13 weeks (↓22%) and 26 weeks (↓26%). Serum cholesterol remained lower in males previously dose at 100 mg/kg (↓29%) following the 8 week recovery period. Effects on serum cholesterol and total protein are consistent with secondary changes in lipid and protein metabolism.

(Serum Globulin)

Serum globulin declined with dose in males following 13 weeks (↓6% to ↓9 %*) and 26 weeks (↓4% to ↓11 %*) of dosing. A significant decline in serum globulin (week 26 - ↓7%) and serum albumin (week 13 - ↓8%) was observed in females dosed at 100 mg/kg. The albumin-globulin ratio was increased significantly in males administered 100 mg/kg for 13 weeks only (↑7%).

(Serum Bilirubin/AST)

Serum total bilirubin declined significantly in all dosed males and in females administered ≥ 25 mg/kg during week 13. A significant decline in total bilirubin was observed in females only at the end of dosing. Total bilirubin tended to remain lower in females dosed at ≥ 25 mg/kg following 8 weeks of recovery.

A significant increase in AST was observed at the 100 mg/kg dose during week 13 (↑24%) and at all doses during week 26 (↑24% to ↑39%) in males. Serum AST was increased significantly in females dosed at 100 mg/kg for 13 weeks only (↑26%).

(Serum ALT)

Serum ALT was increased dose dependently in males during week 13 (↑24 %* to ↑32 %*) and at the end of dosing (↑5% to ↑21%). Significant changes in serum ALT were not noted in females.

(Serum ALP)

Dose dependent increases in serum ALP were observed in males following 13 weeks of dosing (↑5% to ↑16%). Serum ALP was increased dose dependently in females following 13 weeks (↑2% to ↑14) and 26 weeks (↑8% to ↑20) of dosing.

Mechanisms for the effects on serum total bilirubin and aminotransferase activities were not apparent, although the increase in aminotransferase activities may have been associated with an increase in gluconeogenesis.

Several changes in serum clinical chemistry parameters may have been secondary to glucosuria-related osmotic diuresis. These include elevated serum urea nitrogen and inorganic phosphorus, consistent with relative dehydration, and lower serum calcium, sodium, potassium, and chloride, consistent with increased urinary excretion.

(Serum Calcium)

Serum calcium declined significantly with dose during week 13 (↓4% to ↓5%) and week 26 (↓4% to ↓6%) in males. A significant decline in serum calcium was observed during week 13 (↓3% to ↓4%) and week 26 (↓2% to ↓5%) in females, although it lacked clear dose dependency.

Males			Females		
	Ca mg/dL			Ca mg/dL	
DSNG 86	DSNG 184	RECO 57	DSNG 86	DSNG 184	RECO 57
11.4	11.2	10.9	11.8	11.4	11.1
0.50	0.36	0.37	0.47	0.44	0.43
20	20	5	20	20	5
10.9*	10.7*	10.9	11.4	11.2*	11.2
0.29	0.30	0.58	0.54	0.40	0.15
20	20	5	20	20	5
10.9*	10.5*	11.0	11.4*	11.1*	11.3
0.36	0.30	0.40	0.52	0.26	0.39
20	20	5	19	19	5
10.8*	10.5*	10.8	11.3*	10.8*	11.2
0.30	0.31	0.35	0.41	0.40	0.54
20	19	5	20	20	5
P	P	P	P	P	P

(Serum Phosphorous)

Serum phosphorous levels were significantly elevated in females dosed at 100 mg/kg during week 13 (↑12%) and in both genders at the end of the administration phase (M - ↑13% and F - ↑17%). Low levels of PTH observed at the 100 mg/kg dose could increase serum phosphorous concentrations.

(Serum Sodium)

Serum sodium declined in both genders although these changes never exceeded (↓2%).

(Serum Potassium)

A dose dependent decline in serum potassium was observed in males (↓2% to ↓13 %*) and females (↓2% to ↓15 %*) during week 13. Significant hypokalemia was present in males only at the end of dosing (↓7 %* to ↓13 %*) and absent in recovery animals regardless of gender.

(Serum Chloride)

Serum chloride tended to decline in males during week 13 (↓2 %* to ↓6% *) and week 26 (↓3 %* to ↓5 %*). A significant decline in serum chloride was observed in all dosed females during week 13 (↓2% to ↓5%) and at the 100 mg/kg dose during week 26 (↓1%).

(Parathyroid Hormone Analysis)

Mean serum parathyroid hormone levels were reduced in both genders administered 100 mg/kg PF04971729, although the significance of these changes is difficult to assess because of the variable range of measurements taken within each group. Parathyroid hormone declined in males (↓31%) and females (↓42%) at the end of the administration period. Low levels of PTH are known to lower serum calcium (activation of osteoblast and inhibition of osteoclast activity) and increase serum phosphorus concentrations. The decline PTH levels provides a mechanism of action for the increased trabecular bone (males) and bone hyperplasia (females) observed in rats dosed at 100 mg/kg.

Urine Chemistry and Urinalysis

The pharmacologic activity of PF04971729 (reduced renal tubular reabsorption of glucose from the glomerular filtrate) and subsequent osmotic diuresis and systemic metabolic changes were plausibly responsible for the majority of urinalysis and urine chemistry effects. In addition to the effect of osmotic diuresis, urine calcium excretion may have been further accentuated by glucose reabsorption in late proximal and/or distal tubules.

These effects correlated with pathological changes noted in the kidney (hypertrophy of proximal tubules, tubular mineralization, and tubular dilatation) and bone (increased trabecular bone/hyperplasia). Hypertrophy of adrenal gland zona glomerulosa may have been associated with increased aldosterone production in response to fluid and electrolyte losses.

(Urine Glucose)

Significant increases in urine glucose concentrations were observed at all doses in males (↑343-fold to ↑451-fold) and females (↑388-fold to ↑426-fold) at the end of the administration period.

(Urine Creatinine)

Urine creatinine levels decreased with dose in males (↓54 %* to ↓78 %*) and females (↓45% to ↓74 %*) and urine glucose: urine creatine ratios increased with dose in males (↑1257-fold to ↑1951-fold) and females (↑572-fold to ↑1101-fold) at the end of the administration period.

(Urine Protein)

Total urine protein concentrations declined with dose in males only (↓41% to ↓65 %*) at the end of the administration period. Urine protein: urine creatine ratios increased with dose in males (↑33% to ↑55%) and females (↑40% to ↑247%) at the end of the administration period. Urine protein: urine creatine ratios were notably elevated in males (↑293%) and females (↑964%) previously dose at 100 mg/kg following the 8 week recovery period.

(Urine Phosphorous)

Urine phosphorous concentrations decreased with dose in males (↓3% to ↓33 %*) at the end of dosing and increased with dose in females (↑32% to ↑75%) following the 8 week recovery period. Total phosphorous excretion increased with dose in males at the end of the administration period (↑154% to ↑270%). Total phosphorous excretion was significantly increased in all dosed females (↑81% to ↑786%).

(Urine Sodium)

Urine sodium concentrations decreased with dose in males only (↓11% to ↓30%) at the end of the administration period. Total sodium excretion increased with dose in males at the end of the administration period (↑124% to ↑288%). Total sodium excretion was significantly increased in all dosed females (↑53% to ↑253%).

(Urine Potassium)

Urine potassium concentrations declined with dose in males (↓32 %* to ↓56 %*) and females (↓33% to ↓44 %*) at the end of the administration period. Total potassium excretion increased with dose in males (↑65% to ↑127%) and females (↑57% to ↑248%) at the end of the administration period. A dose dependent increase in total potassium excretion was still observed in females following the 8 week recovery period (↑18% to ↑67%).

(Urine Chloride)

Urine chloride concentrations declined with dose in males (↓35% to ↓54 %) and females (↓22% to ↓42 %*) at the end of the administration period. Total chloride excretion increased with dose in males (↑95% to ↑195%) and females (↑57% to ↑179%) at the end of the administration period.

(Urine Calcium)

No clear change was noted in the concentration of urine calcium, although total calcium excretion increased with dose in both genders at the end of dosing (M - ↑152% to ↑674% and F - ↑104% to ↑322%).

Males

Group/ Sex		CaX mg DSNG 184	CaX mg RECO 57
1M	Mean	0.27	0.30
	SD	0.117	0.085
	N	20	5
2M	Mean	0.68*	0.54
	SD	0.407	0.265
	N	20	5
3M	Mean	0.79*	0.47
	SD	0.628	0.254
	N	19	5
4M	Mean	2.09*	0.33
	SD	1.526	0.187
	N	19	5

Females

Group/ Sex		CaX mg DSNG 184	CaX mg RECO 57
1F	Mean	0.73	0.81
	SD	0.701	0.843
	N	20	5
2F	Mean	1.49*	0.96
	SD	1.002	0.831
	N	20	5
3F	Mean	1.82*	0.97
	SD	1.271	0.349
	N	19	5
4F	Mean	3.08*	0.76
	SD	1.264	0.407
	N	20	5

(Urine Volume)

Urine volume was significantly increased with dose during week 13 (M - ↑25% to ↑123% and F - ↑24% to ↑149%) and at the end of the administration period (M - ↑72% to ↑233% and F - ↑83% to ↑374%) in both genders.

(Urine Specific Gravity and pH)

Significant increases in urine specific gravity were observed in females only during week 13 (↑2% to ↑3%) and at the end of dosing (↑1% to ↑2%).

Urine ph in both genders became more acidic with ascending doses of PF04971729 during week 13 (M – 6.7 to 6.1 and F – 6.5 to 6.0) and at the end of the administration period (M – 6.7 to 6.1 and F – 6.4 to 5.9).

Gross Pathology

Macroscopic observations of interest that were uniquely noted in dosed animals at the end of the administration period included: 3 males with large renal pelvises, 4 males and 1 female with distended urinary bladders, 1 male with fluid in the urinary bladder, and discolored stomachs in 7 males and 11 females. Changes in the kidneys and urinary bladder were consistent with the changes noted microscopically and those of higher urine volumes described above.

Twenty brown, black, red, and/or white discolored foci were noted in the glandular stomachs at the end of the administration phase in 18 dosed animals. Four brown, two black, and a single red foci corresponded to erosions/ulcers microscopically that were observed with an increased incidence in females at the 100 mg/kg dose. One brown and three red foci corresponded microscopically to congestion. The remaining foci had no corresponding microscopic lesions.

GROSS OBSERVATIONS – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	15
Kidney	Enlarged		0	0	2	1
Urinary Bladder	Distended		0	1	1	2
	Fluid		0	0	1	0
Stomach	Discolored		0	1	4	2
Jejunum	Discolored		0	0	1	0
Prostate	Raised Area		0	1	0	0
Joint	Enlarged		0	0	0	1

GROSS OBSERVATIONS – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	15
Urinary Bladder	Distended		0	1	0	0
Stomach	Discolored		0	0	5	6

An increased incidence of mammary cysts was observed grossly in recovery females previously dosed at ≥ 25 mg/kg (NOAEL, 60x MRHD, AUC basis). This observation correlated with an increased incidence of cystic dilatation and hyperplasia/hypertrophy of the breast glandular epithelium at 100 mg/kg.

GROSS OBSERVATIONS – FEMALES - RECOVERY						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	5	5	5	5
Mammary	Cyst		0	0	1	2

Organ Weights

Statistically significant and dose-related increases in absolute kidney weights were observed in males administered ≥ 5 mg/kg/day and females at 100 mg/kg/day.

KIDNEY WEIGHTS – TERMINAL SACRIFICE						
	Male			Female		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑18*	↑30*	↑19*	↑8	↑16*	↑9
25	↑22*	↑35*	↑27*	↑9	↑19*	↑8
100	↑31*	↑50*	↑33*	↑27*	↑34*	↑26*
(*) Significant Change						

Increased kidney weights observed at the end of the administration period in males were associated with the PF04971729-related microscopic finding of epithelial hypertrophy of proximal tubules at doses ≥ 25 mg/kg/day. The relatively larger weight changes in the kidney-to-terminal body weight were a reflection of the lower terminal body weights relative to control animals.

Statistically significant increases in the kidney weights of females were observed at the end of the dosing period. The presence of dilated tubules in the medulla and papilla may have resulted from increased diuresis and contributed to this weight increase.

The relative adrenal gland weights of males were significantly increased at 100 mg/kg/day.

ADRENAL WEIGHTS – TERMINAL SACRIFICE			
	Male		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑9	↑19	↑9
25	↑3	↑13	↑7
100	↑22	↑39*	↑24*
(*) Significant Change			

Statistically significant weight increases were present in the adrenal gland-to-terminal body weight and adrenal gland-to-brain weight of males administered 100 mg/kg/day. Although not statistically significant, the absolute adrenal gland weights of males administered 100 mg/kg/day were increased by (↑22%).

Hypertrophy and vacuolation of the adrenal gland zona glomerulosa noted microscopically plausibly contributed to the increased adrenal gland weights noted in males.

Dose related increases in liver weight relative to body weight were noted in females at the end of the administration period (↑1% to ↑13%) with statistical significance noted at 100 mg/kg.

Interestingly, a dose related decrease in absolute liver weight (↓6% to ↓8%) and increase liver weight relative to body weight (↑3% to ↑7%) was noted in males at the end of the administration period. These changes were not determined to be statistically significant.

Dose related increases in epididymal tissue weights were observed in males at the end of the administration period. These changes were not determined to be statistically significant.

EPIDIDYMIS WEIGHTS – TERMINAL SACRIFICE			
	Male		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑0.1	↑9	↑0.1
25	↑0.2	↑10	↑4
100	↑2	↑17	↑5
(*) Significant Change			

(Recovery)

Statistically significant increases in multiple organ-to-body weight ratios present in recovery males previously dosed at 100 mg/kg are plausibly attributed to lower body weights. A dose related increase in kidney weight relative to body weight was still apparent in males at the end of the recovery period (↑1% to ↑23%).

While no statistically significant organ weight changes were present in recovery females, kidney weight and spleen weight tended to increase with dose.

FEMALE ORGAN WEIGHTS – RECOVERY SACRIFICE						
	Kidney			Spleen		
Dose (mg/kg)	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ	Absolute %Δ	OW:BW %Δ	OW:BrW %Δ
5	↑4	↑6	-	↑3	↑6	-
25	↑11	↑9	↑8	↑15	↑13	↑12
100	↑31	↑20	↑28	↑28	↑19	↑25
(*) Significant Change						

Histopathology

Microscopic findings were noted in the kidneys, adrenal glands, pancreas, bone, and stomach of dosed animals. PF04971729-related findings with the exception of tubular mineralization in the kidney and increased trabecular bone in the sternum, resolved in recovery animals. Microscopic findings in the breast were noted only in high dose recovery animals.

The kidneys of males administered ≥ 25 mg/kg/day presented with dose-responsive hypertrophy of proximal tubule epithelium characterized by an increase in size and eosinophilia of the epithelium, with smaller nuclei more luminal in location.

A PF04971729 related increase in the incidence and severity of tubular mineralization was noted at the end of the administration phase in males. This change is consistent with increased calcium and phosphorus excretion. Tubular mineralization continued to be present with an increased incidence in recovery males administered 100 mg/kg.

RENAL HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Kidney	Hypertrophy, Epithelium, Proximal Tubule		0	0	9*	13 (10*/3@)
	Mineralization, Tubule		4*	5*	8*	13 (11*/2@)
	Hyperplasia, Tubular, Focal		0	0	0	1@
	Dilatation, Pelvis		0	0	2P	1P
(P) Present (*) Minimal (@) Slight ($) Moderate						

Slight mixed cell inflammation was present in the renal pelvis of one dosing phase female from each group administered 5 or 25 mg/kg/day, and moderate inflammation was present in one dosing phase female administered 100 mg/kg/day. Minimal to slight inflammation in the pelvis (some cases associated with transitional cell hyperplasia) was present in the kidneys of 3/5 recovery females administered 100 mg/kg/day and 1 recovery female dosed at 25 mg/kg/day.

RENAL HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Kidney	Inflammation, Pelvis, Mixed Cells		0	1@	1@	1$
	Hyperplasia, Transitional Cells		0	0	1*	1*
	Degeneration/Necrosis, Tubule		0	0	0	1*
	Dilatation, Tubule(s), Random		1*	0	1@	4 (1*/3@)
	Edema		0	0	0	1@
(P) Present (*) Minimal (@) Slight ($) Moderate						

RENAL HISTOPATHOLOGY – MALES – END OF RECOVERY							
Tissue	Finding	Main Study					
		dose	0	5	25	100	
		n	5	5	5	5	
Kidney	Mineralization, Tubule		1*	2*	5*	5*	
(P) Present (*) Minimal (@) Slight ($) Moderate							

RENAL HISTOPATHOLOGY – FEMALES – END OF RECOVERY							
Tissue	Finding	Main Study					
		dose	0	5	25	100	
		n	5	5	5	5	
Kidney	Mineralization, Tubule		1*	0	1*	4*	
	Inflammation, Pelvis, Mixed Cells		0	0	1*	3 (1*/2@)	
	Hyperplasia, Transitional Cells		0	0	0	2@	
(P) Present (*) Minimal (@) Slight ($) Moderate							

Inflammation was noted in the urinary bladder (with transitional cell hyperplasia) and ureter of one recovery female with inflammation in the renal pelvis. It is unclear if the increased incidence in recovery females previously administered 100 mg/kg/day is PF04971729-related since an increased incidence was not noted in females at the end of the dosing phase.

URINARY BLADDER HISTOPATHOLOGY – MALES – END OF DOSING							
Tissue	Finding	Main Study					
		dose	0	5	25	100	
		n	15	15	15	14	
Urinary Bladder	Inflammation		0	1@	1@	0	
	Hyperplasia, Transitional Cell		0	1@	1@	0	
(P) Present (*) Minimal (@) Slight ($) Moderate							

URINARY BLADDER/ URETER HISTOPATHOLOGY – FEMALES – END OF RECOVERY							
Tissue	Finding	Main Study					
		dose	0	5	25	100	
		n	5	5	5	5	
Urinary Bladder	Inflammation		0	0	0	1@	
	Hyperplasia, Transitional Cell		0	0	0	1@	
Ureter	Inflammation		0	0	0	1*	
(P) Present (*) Minimal (@) Slight ($) Moderate							

Minimal hypertrophy and vacuolation of the adrenal gland zona glomerulosa was present in dosing phase males and females administered ≥ 5 mg/kg/day. This finding is consistent with the secondary effects of fluid/electrolyte loss caused by the diuresis. Hypertrophy and vacuolation resolved in recovery animals although cystic degeneration was noted in one high dose animal from both genders.

ADRENAL GLAND HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	15	15	15	14
Adrenal Cortex	Hypertrophy/Vacuolation, Zona Glomerulosa		0	9*	12*	14*
(P) Present (*) Minimal (@) Slight ($) Moderate						

ADRENAL GLAND HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	15	15	14	15
Adrenal Cortex	Hypertrophy/Vacuolation, Zona Glomerulosa		0	7*	9*	14*
	Vacuolation, Zona Fasciculata		0	0	0	1*
(P) Present (*) Minimal (@) Slight ($) Moderate						

A decrease in pancreatic zymogen granules relative to control animals was present in a dose-responsive pattern in both genders at the end of the administration period. This finding is consistent with the secondary effects of increased food consumption.

PANCREAS HISTOPATHOLOGY – MALES – END OF DOSING						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	15	15	15	14
Pancreas	Decreased Zymogen Granules		2*	4*	6*	8*
(P) Present (*) Minimal (@) Slight ($) Moderate						

PANCREAS HISTOPATHOLOGY – FEMALES – END OF DOSING						
Tissue	Finding		Main Study			
		dose	0	5	25	100
		n	15	15	14	15
Pancreas	Decreased Zymogen Granules		0*	1*	5*	8*
(P) Present (*) Minimal (@) Slight ($) Moderate						

Erosions/ulcers corresponded to macroscopic findings of brown/black discoloration and were present microscopically in (1 to 2/15) animals in each male dosed group, (1/15) females at 25 mg/kg, and (5/15) females administered 100 mg/kg PF04971729.

Minimal hyperplasia of the glandular stomach foveolar layer was noted in one male and four females administered 100 mg/kg PF04971729. Minimal to slight degeneration of the crypts in the pylorus of the glandular stomach was noted with an increased incidence and severity at doses ≥ 25 mg/kg/day. All stomach findings resolved in recovery animals and were consistent with the secondary effects of the PF04971729 dosing; potentially resulting from chronically increased gastric secretion/distension related to increased food consumption and/or off target inhibition of SGLT1.

GASTRO-INTESTINAL HISTOPATHOLOGY – MALES – END OF DOSING

Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Stomach	Hyperplasia, Foveolar Layer		0	0	0	1*
	Degeneration, Crypts, Pylorus		1*	0	5 (4*/1@)	5*
	Erosion/Ulcer		0	1*	2 (1*/1@)	1*
	Inflammation		0	0	0	2
	Dilatation, Gland		1*	1*	2 (1*/1@)	2*
	Hemorrhage		0	0	0	1@

(P) Present (*) Minimal (@) Slight ($) Moderate

GASTRO-INTESTINAL HISTOPATHOLOGY – FEMALES – END OF DOSING

Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Stomach	Hyperplasia, Foveolar Layer		0	0	0	4*
	Degeneration, Crypts, Pylorus		2*	2*	3 (2*/1@)	8*
	Erosion/Ulcer		0	0	1@	5 (1*/4@)
	Dilatation, Gland		2*	0	2*	4*

(P) Present (*) Minimal (@) Slight ($) Moderate

An increased incidence of mammary cysts was observed grossly in recovery females previously dosed at ≥ 25 mg/kg (NOAEL, 60x MRHD, AUC basis). This observation correlated with an increased incidence of cystic dilatation and hyperplasia/hypertrophy of the breast glandular epithelium in high dose recovery females (100 mg/kg). It is interesting to note that this class of drug has recently been associated with an increased incidence of breast cancer in humans.

Microscopic findings noted in the sternal and femur bone included a minimal to slight increase in trabecular bone in males and hyperplasia of the physis in a single female dosed at 100 mg/kg. It has been hypothesized that off-target inhibition of SGLT1 may induce bone changes in rats by increasing calcium absorption in the gut. In addition, low levels of PTH are known to lower serum calcium levels through the activation of osteoblast and inhibition of osteoclast activity. A concurrent increase in serum phosphorus at the 100 mg/kg dose supports the concept that low PTH levels are affecting circulating cation concentrations. This change resolved in the femur and partially resolved in the sternum (2/5 animals affected) of recovery males.

BONE HISTOPATHOLOGY – MALES – END OF DOSING

Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	15	14
Bone Femur	Trabecular Bone, Increased		0	0	0	3 (2*/1@)
Bone Sternum	Trabecular Bone, Increased		1*	1*	1*	12 (11*/1@)

(P) Present (*) Minimal (@) Slight ($) Moderate

BONE HISTOPATHOLOGY – MALES – END OF RECOVERY

Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	5	5	5	5
Bone Sternum	Trabecular Bone, Increased		0	0	0	2*

(P) Present (*) Minimal (@) Slight ($) Moderate

BONE HISTOPATHOLOGY – FEMALES – END OF DOSING

Tissue	Finding	Main Study				
		dose	0	5	25	100
		n	15	15	14	15
Bone Femur	Hyperplasia, Physis		0	0	0	1*

(P) Present (*) Minimal (@) Slight ($) Moderate

C-cell hyperplasia was noted in a single female from the 100 mg/kg dosing group. C-cell hyperplasia was not observed in males or in recovery females

Hyperplasia of the thymus epithelium was increased in the 100 mg/kg group relative to controls in both genders at the end of dosing. The thymus epithelium was not assessed in low dose and mid dose animals. This trend was not observed in recovery animals of either gender.

Males administered 100 mg/kg presented with an increased incidence (3/15) of mononuclear infiltrates into the epididymal interstitium at the end of dosing. Cellular debris of the epididymal lumen and hypospermia were observed in a single high dose recovery male.

Toxicokinetics

While the sponsor indicated that there were no consistent gender-related differences in systemic exposure (as assessed by C_{max} and AUC_{0-24}) the table below illustrates that this was not the case. Exposures in females were consistently higher than males at all doses and on all study days.

Gender averaged t_{max} was variable and occurred from 1 to 7 hrs postdose. T_{max} was observed at 2.88, 5.50, and 5.88 hrs on study wk 1, at 1.75, 4.75, and 3.25 hrs on study wk 13, and at 1.38, 2.50 and 1.75 hrs on study wk 26 at doses of 5, 25, and 100 mg/kg/day, respectively.

Systemic exposure (assessed by C_{max} and AUC_{0-24}) increased with ascending dose. Gender averaged C_{max} and AUC_{0-24} increased by 17x and 17x, respectively, on study week 1, by 14x and 23x, respectively, on study week 13, and by 17x and 27x, respectively, on study week 26, with a 20x increase in dose (from 5 to 100 mg/kg/day).

Gender averaged systemic exposures increased from week 1 to week 26. On study week 26, C_{max} values were (1.6x, 1.5x and 1.7x) and AUC_{0-24} values were (1.0x, 1.1x and 1.5x) compared to study week 1 for the 5, 25, and 100 mg/kg dose groups, respectively. On study week 26, C_{max} values were (1.5x and 1.8x) and AUC_{0-24} values were (1.1x and 1.9x) compared to study week 1 for males and females, respectively.

Mean Toxicokinetic Parameters for PF04971729 in Rats after Oral Administration of PF04971729 on Study Weeks 1, 13 and 26

Dose (mg/kg/ day)	Study Week	Gender	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
5	1	Male	1.56	0.133	4	2.50	1.73	4	19.7	3.19	4
		Female	2.15	0.378	4	3.25	1.50	4	26.6	5.62	4
		Overall	1.86	0.410	8	2.88	1.55	8	23.2	5.61	8
	13	Male	1.70	0.294	4	2.50	1.73	4	17.4	4.79	4
		Female	3.66	0.791	4	1.00	0.00	4	25.3	6.06	4
		Overall	2.68	1.18	8	1.75	1.39	8	21.3	6.57	8
	26	Male	2.49	0.647	4	1.00	0.00	4	17.6	3.84	4
		Female	3.62	0.360	4	1.75	1.50	4	26.9	5.06	4
		Overall	3.06	0.776	8	1.38	1.06	8	22.3	6.46	8
25	1	Male	8.99	1.69	4	5.50	1.73	4	123	32.5	4
		Female	11.0	0.512	4	5.50	1.73	4	147	12.8	4
		Overall	10.0	1.59	8	5.50	1.60	8	135	26.2	8
	13	Male	8.61	1.32	4	4.75	2.87	4	120	19.4	4
		Female	11.3	2.04	4	4.75	1.50	4	147	19.4	4
		Overall	9.97	2.15	8	4.75	2.12	8	134	23.2	8
	26	Male	12.9	3.36	4	2.50	1.73	4	128	31.9	4
		Female	17.6	7.20	4	2.50	1.73	4	167	38.1	4
		Overall	15.2	5.77	8	2.50	1.60	8	148	38.4	8
100	1	Male	26.4	6.84	4	5.50	1.73	4	359	98.0	4
		Female	35.4	5.31	4	6.25	1.50	4	440	173	4
		Overall	30.9	7.41	8	5.88	1.55	8	400	137	8
	13	Male	29.9	5.83	4	3.25	1.50	4	372	24.2	4
		Female	47.7	7.40	4	3.25	2.87	4	612	16.2	4
		Overall	38.8	11.3	8	3.25	2.12	8	492	129	8
	26	Male	38.9	6.42	4	1.00	0.00	4	397	26.6	4
		Female	63.7	10.9	4	2.50	3.00	4	814	113	4
		Overall	51.3	15.6	8	1.75	2.12	8	605	235	8

Overall = Male plus Female combined

Stability and Homogeneity and Control Sample Analysis

Stability and homogeneity of prepared doses are reported to be within acceptable specifications. No quantifiable concentrations of PF04971729 were found in plasma samples collected from control animals on Study weeks 1, 13, and 26 with the exception of 1 sample (animal B69659), but this finding does not impact the interpretation of the toxicokinetic data or study integrity.

11 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Distribution

PF04971729 distributed preferentially into plasma over red blood cells in whole blood in rats and humans.

Tissue distribution of [^{14}C] PF04971729-derived radioactivity was widespread at 1 hour postdose, with highest concentrations in bile and urine in Long-Evans rats. The tissues that had the highest C_{max} values included the urinary bladder, liver, kidney medulla, and kidney. Tissues with the highest $AUC_{0-\alpha}$ values were the urinary bladder, kidney (including the cortex and medulla), liver, adrenal gland, Harderian gland, and pancreas.

The distribution of [^{14}C] PF04971729-derived radioactivity was mainly to tissues responsible for metabolism and elimination of PF04971729. The data over the course of the study showed that the elimination of PF04971729-derived radioactivity was virtually complete by 168 hours postdose, residual radioactivity was detected only in the kidney.

[^{14}C] PF04971729-derived radioactivity distributed across the blood-brain barrier, concentrations of drug-derived material in the non-circumventricular CNS tissues were at levels substantially lower (3-fold to 63-fold) than blood concentrations. Concentrations of radioactivity were also observed in two regions of the brain that are not protected by a blood-brain barrier (choroid plexus and pituitary gland). Concentrations of radioactivity in the choroid plexus and pituitary gland were similar to or were approximately 2-fold greater than blood concentrations, respectively. Concentrations of [^{14}C] PF04971729-derived radioactivity in all brain substructures were below the limit of quantitation or not detected by 24 hours postdose.

Metabolism

No unique human metabolites of PF04971729 have been identified. PF04971729 underwent minimal phase I metabolism in humans and the major metabolic pathway was glucuronidation. The glucuronidation occurred on the hydroxyl groups of the modified glucose moiety of PF04971729 and its des-ethyl metabolite, M2. Glucuronides were primarily excreted in urine. Isomeric glucuronides of PF04971729 (M5a, M5b, M5c), and those of M2, (M6a, and M6b) were the major radioactivity constituents in urine. Collectively they accounted for 43.9% of the administered dose, and 87.8% of radioactivity excreted in urine. Glucuronides (M5a, M5b, M5c, and M6a) were also the major circulating metabolites, representing 12.2%, 4.1%, 24.1% and 6.0% of total radioactivity in plasma. Collectively they accounted for 46.4% of circulating radioactivity. Unchanged PF04971729 accounted for 49.9% of total radioactivity in plasma and 1.5% of the radioactive dose in urine.

M5a (12.2%) and M5c (24.1%) exceed 10% of the total drug related exposure in human plasma and each represent only (0.5%) of the total drug related exposure in rat plasma.

M5a and M5c are O-glucuronides of the parent molecule and glutathione conjugates are normally of low toxicological concern, although this is certainly not the case for all glutathione conjugates.

In terms of absolute exposure, the highest dose tolerated in rats (250 mg/kg) exposed animals to (21-fold to 86-fold) the human M5c/M5a metabolite exposure expected from the sponsor's anticipated maximum clinical phase 3 dose (5 mg/kg – Therapeutic dose).

The proposed (10 mg/kg) high dose in the 2 year rat CARC study will expose animals to (1x to 4x) the human M5c/M5a metabolite exposure expected from the sponsor's anticipated maximum clinical phase 3 dose (5 mg/kg – Therapeutic dose).

Based on these exposure multiples the metabolites M5a and M5c have been adequately characterized and no special studies are required. Adequate exposure to these metabolites should be achieved at the proposed high dose (10 mg/kg) during the 2 year in the CARC study.

Excretion

The predominant route of elimination of [^{14}C] PF04971729-derived radioactivity was feces and bile in rats (> 47% in bile-duct cannulated rats and > 58% in rats). In humans, radioactivity in urine and feces accounted for 50.2% and 40.9% of the dose, respectively. Based on the summation of radioactivity in urine and bile in rat and urine alone in human, oral absorption in rat and human was estimated at greater than 78% and 50%, respectively.

General toxicology:

The current submission includes a 6-month study in Sprague-Dawley rats in support of a protocol for carcinogenicity studies.

In the pivotal 6 month study, oral administration of PF04971729 to rats was tolerated at doses up to 100 mg/kg, although significant decreases in body weight gain and final body weights were noted in males at 100 mg/kg (NOAEL, 284x MRHD, AUC basis) with significant increases in food consumption at doses ≥ 5 mg/kg in both genders. Serum glucose was decreased and urinary glucose increased markedly at all doses, consistent with the effects on body weight and the pharmacodynamic activity of PF04971729.

A significant reduction in RBC counts (both genders), reticulocyte counts (males) and red cell distribution widths (males) were observed at 100 mg/kg (NOAEL, 328x MRHD, AUC basis gender ave). Male absolute reticulocyte counts and red cell distribution widths did not resolve fully by the end of recovery.

Decreased serum parathyroid hormone (100 mg/kg) and adrenal hypertrophy/ vacuolation (≥ 5 mg/kg) associated with increased adrenal weights were observed in both genders. Increased trabecular bone (femur and sternum) was observed in males dosed at 100 mg/kg (NOAEL, 284x MRHD, AUC basis) indicating that decreased PTH levels may play a role in driving serum calcium levels down through the stimulation of osteoblast activity and inhibition of osteoclast activity in the bone. Hypertrophy of adrenal gland zona glomerulosa is consistent with increased aldosterone production in response to fluid and electrolyte losses. It is interesting to note that other SGLT2 inhibitors have been linked to adrenal tumor formation in the rat.

Increased BUN and inorganic phosphorus were consistent with relative dehydration and lower serum calcium, sodium, potassium, and chloride correlated with increased urinary excretion. The pharmacologic activity of PF04971729 (reduced renal tubular reabsorption of glucose from the glomerular filtrate) and subsequent osmotic diuresis and systemic metabolic changes were plausibly responsible for the majority of urinalysis and urine chemistry effects. These effects correlated with pathological changes in the kidneys that included: hypertrophy of proximal tubules (NOAEL, 49x MRHD, AUC basis), tubular mineralization (NOAEL, not established), and tubular dilatation (NOAEL, 49x MRHD, AUC basis) and pathological changes in the bone that included: increased trabecular bone (males) or hyperplasia of the physis (females) (NOAEL, 328x MRHD, AUC basis gender ave).

Discoloration of the stomach noted in both genders at the end of dosing corresponded to microscopic findings of erosions/ulcers (NOAEL, not established). An increased incidence of foveolar hyperplasia (100 mg/kg) and crypt degeneration in the pylorus (≥ 25 mg/kg) was present in both genders (NOAEL, 49x MRHD, AUC basis). A decrease in pancreatic zymogen granules was present at all dose levels. Findings in the stomach and pancreas resolved in recovery animals and were consistent with the secondary effects of increased food consumption and/or off target inhibition of SGLT1.

An increased incidence of mammary cysts was observed grossly in recovery females previously dosed at ≥ 25 mg/kg (NOAEL, 60x MRHD, AUC basis), no cysts were observed at the 5 mg/kg dose or in control animals. This observation correlated with an increased incidence of cystic dilatation and hyperplasia/hypertrophy of the breast glandular epithelium in high dose recovery females (100 mg/kg). While the relationship between these events and PF04971729 dosing is not definitive, it is interesting to note that this class of drug has recently been associated with an increased incidence of breast cancer in humans.

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

/s/

JEFFREY A QUINN
08/29/2011

TODD M BOURCIER
08/29/2011

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

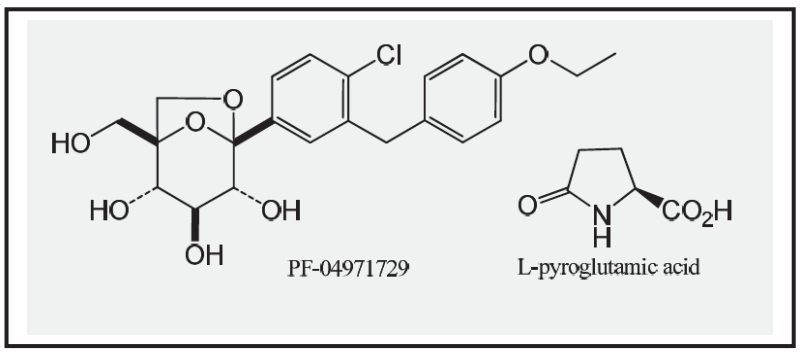
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 106447
Review number: 3
Sequence number/date/type of submission: SD 26 (SN 26) / September 27, 2010
SD 31 (SN 30) / December 10, 2010
Information to sponsor: No
Sponsor and/or agent: Pfizer Global Research and Development
Manufacturer for drug substance: Pfizer Global Research and Development
Reviewer name: Jeffrey Quinn, Ph.D.
Division name: Metabolic and Endocrine Products
HFD #: 510
Review completion date: May 23rd 2011

Drug:

Trade name:	N/A
Generic name:	N/A
Code name:	PF-04971729 or PF-04971729 (b) (4)
Chemical name:	(1*S*, 2*S*, 3*S*, 4*R*, 5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-1-hydroxymethyl-6, 8-dioxabicyclo [3.2.1] octane-2, 3, 4-triol
CAS registry number:	N/A
Molecular formula/molecular weight:	PF-04971729 (amorphous form) $C_{22}H_{25}ClO_7$ / 436.88 Daltons PF-04971729 (b) (4) (L-pyroglutamic acid co-crystal form) $C_{27}H_{32}ClNO_{10}$ / 566.00 Daltons

Structure: The pharmaceutical preparation of PF-04971729 is an (b) (4) of the L-pyroglutamic acid co-crystal form.



Relevant INDs/NDAs/DMFs:

(b) (4)

Drug class: PF-04971729 is a sodium glucose co-transporter 2 (SGLT2) inhibitor.

Intended clinical population: Type 2 diabetics

Clinical formulation: PF-04971729 will be supplied as 1 mg, 5 mg and 25 mg tablets as required by the dose administered.

Impurities: Impurity (b) (4) was found to be (b) (4)% in the nonclinical Lot GR02546 and was not detected in clinical Lot GR02694. There are no other identified organic impurities for PF-04971729 (b) (4) drug substance.

Organic:

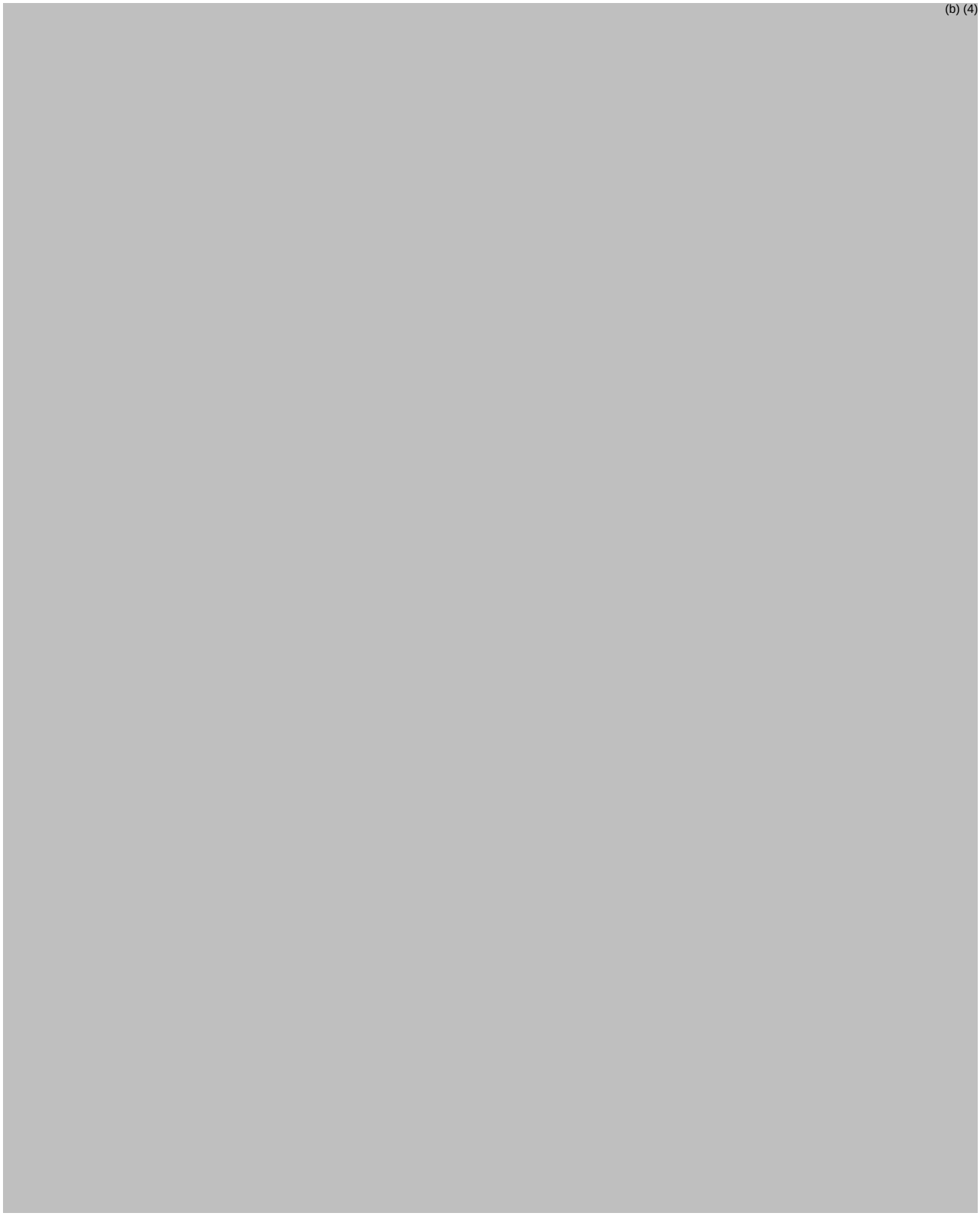
Impurity (chemical name or code #)	Structure	Source
(b) (4)		

Inorganic: (b) (4) compounds are used in the (b) (4) process and are expected to be (b) (4).

(b) (4)

Excipients:

(b) (4)



Route of administration: Oral

Proposed clinical protocols:

Study Title: A Phase 1, Randomized, Double Blind, Placebo-Controlled, Parallel Cohort, Single Dose Escalation and Multiple Dose Study in Japanese Healthy Subjects, and Open Label, Single Dose Escalation Study in Western Healthy Subjects to Investigate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of PF-04971729.

Study ID: (B1521009)

Doses: The single doses that will be administered in this study are 1 mg, 5 mg and 25 mg. The multiple dose level is 25 mg once daily for 7 days.

Trial Design

Cohort	Group	Population	Number of Subject	Period/Treatment		
				1	2	3
A	1	Japanese	6	1 mg	5 mg	25 mg
	2	Japanese	3	placebo	placebo	placebo
	3	Westerner	6	1 mg	5 mg	25 mg
B	1	Japanese	6	25 mg QD X 7 Days		
	2	Japanese	3	Placebo QD X 7 Days		

Study Participants: 9 Japanese healthy subjects and 6 western healthy subjects (BMI: 17.5-30.5 kg/m^2) otherwise healthy adult male and female (non-childbearing potential) subjects.

Protocol Summary: Cohort A will be a single dose Cohort in which 9 Japanese healthy subjects will be allocated to receive 3 ascending single doses (1 mg, 5 mg and 25 mg) of PF-04971729 or placebo through 3-dosing periods in a randomization ratio of 2:1. Six western healthy subjects will also be allocated to Cohort A to receive the 3 ascending single doses of PF-04971729. Study medication will be dosed on Day 1 of each Period under fasted condition.

Cohort B will be a multiple dose Cohort in which 9 Japanese healthy subjects will be allocated to receive once-daily 25 mg PF-04971729 or placebo for 7 days in a randomization ratio of 2:1. Study medication will be administered immediately (i.e., within 5 minutes) after a light breakfast (approximately 650 calories).

Primary Endpoints: Safety, tolerability, pharmacokinetics and pharmacodynamics of single and multiple doses of PF-04971729 in Japanese and Western healthy subjects. Compare the pharmacokinetics and pharmacodynamics of single doses of PF-04971729 in Japanese and Western healthy subjects.

Safety Measures: Physical examination, adverse event monitoring, 12-lead ECGs, vital signs, clinical safety laboratory measurements and fluid balance.

Previous Clinical Experience:

As of 22 March 2011, dosing with PF-04971729 has been completed in five Phase 1 clinical studies, and two Phase 2 studies are ongoing now. Among these completed Phase 1 studies, a total of 74 lean, overweight, or obese, otherwise healthy subjects and 52 T2DM patients have been exposed to at least a single, oral dose of PF-04971729. A total of 32 healthy subjects have been exposed to repeated, oral doses of PF-04971729 for a total duration of up to 14 days. Full clinical study reports have been submitted for **B1521001**, **B1521003** and **B1521007**.

B1521001 was a randomized, placebo-controlled, ascending single oral dose study in which subjects received placebo plus 2 active doses of PF-04971729 under fasted conditions.

Across the placebo and PF-04971729 dose groups together, there were no serious adverse events (SAEs) reported and a total of 19 adverse events (AEs) were reported in eight (8) subjects. Of these AEs, 16 AEs were reported in seven (7) subjects while receiving PF-04971729. All AEs were deemed to be mild in intensity except an episode of headache rated as 'moderate' starting at 23 hours post the 100 mg dose of PF-04971729, which lasted 4.75 hours and was managed with the administration of acetaminophen / paracetamol. Constipation was the only frequent AE (4 episodes reported in 2 subjects while receiving placebo, 0.5 mg, 10 mg, and 100 mg of PF-04971729). There was no apparent dose-related increase in frequency of AEs with increasing dose and none of the subjects were withdrawn as a result of AEs. There were no clinically significant changes in laboratory tests, vital signs, or 12-lead ECGs observed across the doses evaluated.

B1521002 was a randomized, placebo-controlled, parallel-group, ascending multi-dose study in which subjects received either placebo or 1 of 4 active doses of PF-04971729 administered with breakfast for 14 days.

Across the placebo and PF-04971729 dose groups together, there were no serious adverse events (SAEs) reported; a total of 35 AEs were reported in 22 subjects. Of these, 26 AEs were reported in 16 subjects receiving 1-mg, 5-mg, and 25-mg doses of PF-04971729. No AEs were reported in subjects receiving 100 mg PF-04971729 QD. While receiving PF-04971729, AEs reported by more than 1 subject (in all cases $\leq$2 subjects) were: constipation, diarrhea, discolored feces, and folliculitis. All AEs were deemed to be mild in intensity except a fall rated as 'moderate' that occurred 5 days following last day of dosing with 25 mg QD and was deemed unrelated to study treatment.

Adverse events requiring pharmacological management were limited to constipation; this AE was treated with bisacodyl in 2 subjects - both receiving 1 mg QD dose of PF-04971729. There was no apparent dose-related increase in frequency of AEs with increasing dose and none of the subjects were withdrawn as a result of AEs. There were no clinically significant changes in laboratory tests, vital signs, or 12-lead ECGs observed across the PF-04971729 doses evaluated.

B1521003 was a nonrandomized, open-label, single-period PK study with a single group assignment. One single dose of radiolabeled [^{14}C] PF-04971729 25 mg oral suspension (containing approximately 100 µCi of [^{14}C] PF-04971729) was administered to 6 healthy males to study the absorption, distribution, metabolism and elimination (ADME) of PF-04971729 for 7 days postdose.

There were no deaths, SAEs, or permanent withdrawals due to AEs during the study. Overall, 4 AEs were reported in 3 subjects, of which 3 AEs were deemed by the investigator as dosing-related. All AEs were mild in severity.

There were no clinically significant trends or clinically significant abnormalities in clinical laboratory tests, vital signs measurements, or ECG findings.

B1521007 was a randomized, double-blind, sponsor-open, 4-arm study using 2 cohorts and 2-way crossover.

There were no deaths or SAEs or AEs of severe intensity reported in this study. In addition, there was no need for dose reduction or temporary discontinuation of study medication as a result of AEs during this study. The number of AEs and the number of subjects with AEs was numerically higher in both groups receiving QD dosing (i.e., PF-04971729 2 mg QD and PF-04971729 4 mg QD) than for subjects receiving BID dosing (i.e., PF-04971729 1 mg BID and PF-04971729 2 mg BID). One subject was discontinued due to an AE, which was considered as not related to dosing by the investigator. Overall, 2 AEs of moderate severity were reported in each treatment group of Cohort 1 (i.e., PF-04971729 1 mg BID and PF-04971729 2 mg QD); each of the 4 moderate-severity AEs was considered by the investigator as related to treatment. The rest of the AEs were mild in severity.

The most common treatment-emergent AEs (TEAEs) were headache (9 all-causality events and 7 treatment-related events) and diarrhea (4 all-causality events and 4 treatment-related events).

Sponsor's Predicted Efficacious Human Dose: PF-04971729 doses selected for this study are based on observed UGE in the First-in-Human (FIH) study - B1521001 and confirmed based on observed UGE in the 2-week, multi-dose, dose-escalating study-B1521002. Sponsor modeling of the exposure-response data suggests a half-maximal effect dose (ED_{50}) for UGE at approximately 2.5 to 3 mg QD (C_{max} 42.8 ng/mL and $AUC_{0-\alpha}$ 231 ng.h/mL).

Sponsor's Maximum Recommended Human Dose: The maximal proposed dose of (b) (4)

THE MAXIMUM RECOMMENDED HUMAN DOSE FOR PF-04971729 IS: (b) (4)

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

Background: Pfizer submitted the original IND for PF-04971729 in September, 2009, for the treatment of type 2 diabetes mellitus. Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

2.6.2.1 Brief summary

PF-04971729 is a selective SGLT2 inhibitor that results acutely in a concentration-dependent glucosuria in rats. *In vitro*, PF-04971729 is a highly potent inhibitor of rat and human SGLT2 and possesses a high selectivity against glucose transport via human and rat SGLT1 and several other glucose transporters (GLUT1-4).

Studies on the secondary pharmacology evaluated *in vitro* binding activity of PF-04971729 against a broad panel of receptors, transporters, ion channels, and enzyme assays, and the results indicated no significant inhibition (>50%) of binding or enzyme activity.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: PF-04971729 is a selective SGLT2 inhibitor. Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

Drug activity related to proposed indication:

No New Studies

2.6.2.3 Secondary pharmacodynamics

No New Studies

2.6.2.4 Safety pharmacology

Brief Summary of PF-04971729 Safety Pharmacology Program

Study	Concentration or Dose[a]	Study Number
In Vitro Screening Studies		
hERG Patch-clamp Assay	10, 30, 100, or 300 µM	PF-04971729HERG
Neurofunctional – Rat	5, 25, 500 mg/kg	
Functional Observational Battery		09GR146
Locomotor Activity		
Body Temperature		
Pulmonary Function – Rat	5, 25, 500 mg/kg	09GR146
Respiratory Rate, Tidal and Minute Volumes		
Cardiovascular – Conscious Dog	1, 5, 50 mg/kg	09GR145
Blood Pressure, Heart Rate, and Electrocardiogram		

hERG = Human ether-à-go-go-related gene. 10 µM PF-04971729 = 4.3 µg/mL
[a] Single dose.

Neurological effects: Male SD rats dosed with 500 mg/kg of PF-04971729 (b) (4) had a 0.4°C decrease in average body temperature. At 500 mg/kg, PF-04971729 produced decreases in locomotor activity measurements (~ 30-40%).

Cardiovascular effects: PF-04971729 inhibited the hERG channel *in vitro* with an IC_{50} of >300 μM. Significant inhibition of hERG was observed at doses ≥ 30 μM. While the (average % inhibition) was 8.3% at 100 μM, the 6 individual data points used to calculate this mean had a broad range of values (2.9% - 18.1%).

Dosing with PF-04971729 (b) (4) at 50 mg/kg in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and heart rate (and associated RR interval shortening) as well as an increase in systolic blood pressure and lengthening of the PR interval.

Note: (*The NOAEL for this study is 5 mg/kg based on the shortening of the corrected QT interval (QTc), the increase in the PR interval, decrease in left ventricular +dP/dT, increase in systolic blood pressure, decrease in heart rate and the associated increase in RR interval.*

Pulmonary effects: PF-04971729 at 25 and 500 mg/kg produced significant increases in both respiratory rate and minute volume at the 101-120 minute interval. The 25 mg/kg dose, produced increases in respiratory rate of 33 b.p.m. (29% increased over control), and minute volume of 36 mL/min (25% increased over control). At the 500 mg/kg dose, a respiratory rate of 45 b.p.m. (40% increased over control) and a minute volume of 33 mL/min (23% increased over control) were seen.

Renal effects: No renal safety studies were performed although PF-04971729 causes increase urinary glucose excretion and kidney alterations in rats and dogs.

Gastrointestinal effects: No GI safety studies were performed although PF-04971729 causes changes in stool quality, vomiting and ulceration of the tongue.

2.6.2.5 Pharmacodynamic drug interactions:

Pharmacodynamic drug interaction studies with PF-04971729 have not been conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The table below was provided by the sponsor.

In Vitro

Type of Test	Test Cells/Tissues	Test Concentrations	Results	GLP Compliance	Study Number
Patch-clamp	HEK-293	10	NS	No	PF04971729HERG
	stably	30	2.9% inhibition		
	expressing	100	8.3% inhibition		
	hERG	300	33.5% inhibition		

[Click here to type]

GLP = Good Laboratory Practice; HEK = Human embryonic kidney; hERG = Human ether-a-go-go related gene; NS = not significant

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/ Sex/Group	Noteworthy Findings	GLP Compliance	Study Number
Pulmonary Respiratory Rate (RR) Tidal Volume (TV) Minute Volume (MV)	Rat/Sprague-Dawley	Oral gavage, 10 mL/kg	0, 5, 25, 500	6M	None	Yes	09GR146
Nervous Functional Observational Battery (FOB) Body Temperature (BT) Locomotor Activity (LA)	Rat/Sprague-Dawley	Oral gavage, 10 mL/kg	0, 5, 25, 500	6M	None	Yes	09GR146
Cardiovascular Blood Pressure Systolic (SBP) Heart Rate (HR) Electrocardiogram QT interval corrected for heart rate (QTc) PR interval (PR-I) Left Ventricular Pressure Rate of rise of LV pressure over time (+ dP/dT)	Dog/Beagle	Oral gavage, 5 mL/kg (co-crystal)	0, 1, 5, 50	4M	50 mg/kg: ↓QTc (6 msec) ↓HR (6 bpm) ↓+dP/dT (489 mmHg/sec) ↑SBP (6 mmHg) ↑PR-I (4 msec) 1 and 5 mg/kg: no effects	Yes	09GR145

Cardiovascular Study Toxicokinetic Parameters: include tmax = 3.5 hrs pd?

Dose (mg/kg)	0	1	5	50	50[b]
Cmax (ng/mL)	NA[c]	NA[c]	NA[c]	NA[c]	44.7
AUC(0-24) (ng•h/mL)	NA[c]	NA[c]	NA[c]	NA[c]	530
Plasma Concentration (ug/mL) (7 hours postdose)	NA[d]	0.414	1.94	22.8	29.1

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/ Sex/Group	Noteworthy Findings	GLP Compliance	Study Number

GLP = Good Laboratory Practice; M = Male; Cmax = Maximum (peak) observed drug concentration; NA = Not applicable; AUC(0-24) = Area under concentration-time curve from 0 to 24 hours postdose; [List additional abbreviations in the order in which they appear in the table].

[a] Single dose unless specified otherwise.

[b] Plasma samples obtained for toxicokinetic analyses during the toxicokinetic leg of the study.

[c] Samples not collected for toxicokinetic leg of the study.

[d] All samples below limit of quantitation (0.050 ug/mL).

2.6.4 PHARMACOKINETICS/ADME/TOXICOKINETICS

2.6.4.1 Brief summary

Adsorption: Nonclinical *in vitro* and *in vivo* data indicate PF-04971729 is well-absorbed from the gastrointestinal tract and oral bioavailability of the amorphous form following a 2 mg/kg dose in rats and dogs is 67% and 97%, respectively. Oral bioavailability of the co-crystal form following a 5 mg/kg dose in rats or 2 mg/kg dose in dogs was similar at 69% and 94%, respectively.

Distribution: The pharmacokinetics of PF-04971729, following intravenous (IV) administration to rats and dogs, is characterized by low clearance (rat: 4.04 mL/min/kg; dog: 1.64 mL/min/kg), a moderate volume of distribution at steady state (rat: 1.13 L/kg; dog: 0.828 L/kg) and a moderate-to-long half-life (rat: 4.08 hours; dog: 7.63 hours).

PF-04971729, at concentrations of 1 and 10μg/mL, is highly bound to rat, dog, and human plasma proteins, with mean unbound fractions (fu) ranging from 0.032 to 0.064, and binding appeared to be independent of concentration. The free fraction was slightly higher in human compared to the nonclinical species tested.

The sponsor feels that the pharmacological effect of PF-04971729 and possibly its toxicological effects are more closely related to the unbound fraction in plasma rather than the total plasma concentration. Therefore, species differences in unbound plasma fraction (at 1 μg/mL) were incorporated into the PK/PD modeling and safety margin calculations were based on that exposure by the sponsor. ***Pertaining to this review, safety margins will be based on total, not free, drug levels. The percent (%) plasma protein binding across species ranged from 96-98%, which for the purposes of this review will not be considered as a meaningful difference in protein binding.***

Metabolism: Metabolism of PF-04971729 may be catalyzed by multiple enzymes, including CYP3A4, CYP3A5, CYP2D6, UGT1A9, and UGT2B7. *In vitro* hepatocyte data suggests that the dog may model human metabolism of PF-04971729 more closely than the rat.

Excretion: Studies in rats and dogs indicate that renal and biliary excretions of unchanged PF-04971729 are not significant clearance pathways and that metabolism is expected to be the major clearance mechanism. *In vitro* metabolite profiles of PF-04971729 were qualitatively similar across nonclinical species and humans and no unique human metabolites are "anticipated" by the sponsor.

Pharmacokinetic Drug Interactions

At the predicted human efficacious dose, clinically significant drug-drug interactions resulting from PF-04971729-mediated CYP450 inhibition or induction are not "anticipated" by the sponsor.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: PF-04971729 (non-co-crystal form) was administered orally to rats and dogs in 1 month (GLP) studies. The no observed adverse effect levels (NOAELs) in these studies were 25 mg/kg (29x MRHD) and 1 mg/kg (3x MRHD) with an AUC_{0-24} of 81 µg.hr/mL and 8 µg.hr/mL for rats and dogs, respectively. PF-04971729 (b) (4) (Co-crystalline form) was administered orally to rats and dogs in 3 month (GLP) studies. The NOAELs were not determined (< 7x MRHD) and 10 mg/kg (33x MRHD) with an AUC_{0-24} of (< 19.9 µg.hr/mL) and (91.9 µg.hr/mL) for rats and dogs, respectively.

Target organs identified in these studies included kidney, gallbladder, G.I. tract, liver and lung. Rats were sensitive to kidney effects and developed progressive nephropathy at doses ≥ 250 mg/kg. The incidence of vacuolation of the gallbladder was increased at doses of drug ≥ 10 mg/kg in dogs. Perturbations of the lung were seen most frequently in dogs at the maximum dose of 150 mg/kg. G.I. effects were present at all doses in dogs but significant changes in both species were seen at the highest doses only. Liver cell necrosis was noted in female dogs after 3 months of dosing at 150 mg/kg.

Genetic toxicology: There were no treatment related reductions in the mean percent polychromatic erythrocytes (PCE) reported in rats, suggesting a low incidence of bone marrow toxicity. No statistically significant increases in the numbers of PCE with micronuclei suggests that PF-04971729 does not possess, under these conditions, the ability to induce chromosomal damage and thereby increase the frequency of micronucleated PCEs.

Exposure to PF-04971729 did not increase the mean number of revertants with any tester strain either in the presence or absence of S9.

PF-04971729 did not induce structural chromosome aberrations in human lymphocyte *in vitro* cultures.

Increases in polyploidy were seen in a 3 hr test with S9 at 173 µg/mL and 156 µg/mL. These doses demonstrated a marked mitotic suppression (55% and 43%, respectively).

While there were no increases in polyploidy or hyperploidy in a test with a longer exposure time, the 24-hour test was performed in the absence of metabolic activation (S9).

Reproductive and developmental toxicology:

PF-04971729 (b) (4) (Co-crystalline form) was administered orally to rats (GD-6 to GD-17) and rabbits (GD-7 to GD-19) in (GLP) embryo fetal development studies (Segment 2).

Maternal toxicity NOAELs were 100 mg/kg (163x MRHD) and not determined (< 74x MRHD) with an AUC_{0-24} of (457 µg.hr/mL) and (< 207 µg.hr/mL) for rats and rabbits, respectively. Decreases in body weight and body weight gain were noted in the rat at 250 mg/kg and at all doses in the rabbit. Decreased food consumption was noted in the rat at the start of dosing and in rabbits that aborted early. An increase in post implantation loss and reduced litter size were observed in the rat at 250 mg/kg and at all doses in the rabbit.

Developmental toxicity NOAELs were 100 mg/kg (163x MRHD) and 100 mg/kg (151x MRHD) with an AUC_{0-24} of (457 µg.hr/mL) and (424 µg.hr/mL) for rats and rabbits, respectively. External malformations (Trunk; Omphalocele, Forepaw; ectrodactyly and Tail; short) were noted only in the rat (two different animals from two different litters at 250 mg/kg). Ventricular septum defects and aortic arch malformations were observed in both species at the 250 mg/kg dose (348x and 411x MRHD, rat and rabbit respectively). A narrowed pulmonary trunk malformation was observed only in the rabbit at 250 mg/kg.

In the rat, visceral variations were noted at doses ≥ 100 mg/kg (absent Innominate artery) and at the 250 mg/kg dose (hemorrhagic adrenal). Gallbladder variations (absent gallbladder and small gallbladder) and retrocaval ureter variation (250 mg/kg) were observed in the rabbit.

Skeletal malformations that occurred at a higher incidence at the 250 mg/kg dose were absent metacarpal, fused sternebra and hemicentric thoracic centrum in the rat. In the rabbit, skeletal malformations (250 mg/kg) noted in the same fetus were: supernumerary cervical centrum and fused rib. Misshapen interparietal bone (250 mg/kg) was noted in a single fetus from a separate litter.

The skeletal variation (sternebra with an extra ossification site) was observed with an increased incidence at 250 mg/kg in both rats and rabbits.

A wide range of additional skeletal variations were observed in rats at all doses. These variations included: (250 mg/kg dose) Incomplete Ossification of Lumbar and Thoracic Centrum, Unossified or Misaligned Thoracic Centrum, Vertebrae 27th Presacral and Full Supernumerary Ribs; (≥ 100 mg/kg dose) Unossified 7th Cervical Centrum and Skull Extra Ossifications and (≥ 50 mg/kg dose) Unossified Metatarsal and Short Supernumerary Ribs.

2.6.6.2 Single-dose toxicity

No New Studies

2.6.6.3 Repeat-dose toxicity

No New Studies

2.6.6.4 Genetic toxicology

No New Studies

2.6.6.6 Reproductive and developmental toxicology

Embryofetal development

Oral Dose Range Finding Study of PF-04971729 in Pregnant Rats

Key study findings:

- This study should provide sufficient information to set the doses for the definitive embryo-fetal development study in the Sprague-Dawley rat.

Dams:

- Decreases in body weight and body weight change were not dose related.
- A dose-related decrease in food consumption was observed from GD 6 to 9.

Fetuses:

- There were no effects on fetal viability and fetal body weight.
- There were no external malformations related to dosing.

Study no.: 09GR435
Study report location: (SD26 - eCTD 4.2.3.5.2.1) (DARRTS SD26)
Conducting laboratory and location: Pfizer Global Research, Groton CT
Date of study initiation: January 7, 2010
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: PF-04971729 (b) (4), GR08247, 99.9%

Methods	
Doses:	0, 50, 100, and 250 mg/kg/day (D-6 to D-17 of Gestation)
Species/source	Rats/Crl:CD(SD)/Female (b) (4)
Age/Weight:	10 to 12 weeks / ~225 g
Dosing n/sex/group:	6/female/dose group
Route, formulation, dose volume	Oral dosing in 0.5% MC + 10% PEG400 – 10 ml/kg

Observation and Times of Dams	
Observations, Examinations and Mortality checks:	Non Treatment Period - Observations took place 2x/day Treatment Period - Observations took place 3x/day Day of C-Section - Observations took place 1x/day
Clinical Findings:	See Above
Body weights:	Once Daily - All animals – GD 6 to GD 21
Food consumption:	Once Daily - All animals – GD 6 to GD 21
Gross Pathology	D-21 of gestation (GD 21)
Post-Mortem Evaluations	
Macroscopic	D-21 of gestation (GD 21)
Uterine & Ovarian Exams	D-21 of gestation (GD 21)
Examination of embryos and fetuses	D-21 of gestation (GD 21)

Results

Mortality (dams):

There were no mortalities in the study, with all animals surviving to the scheduled euthanasia.

Clinical signs (dams):

There were no clinical signs and no abortions.

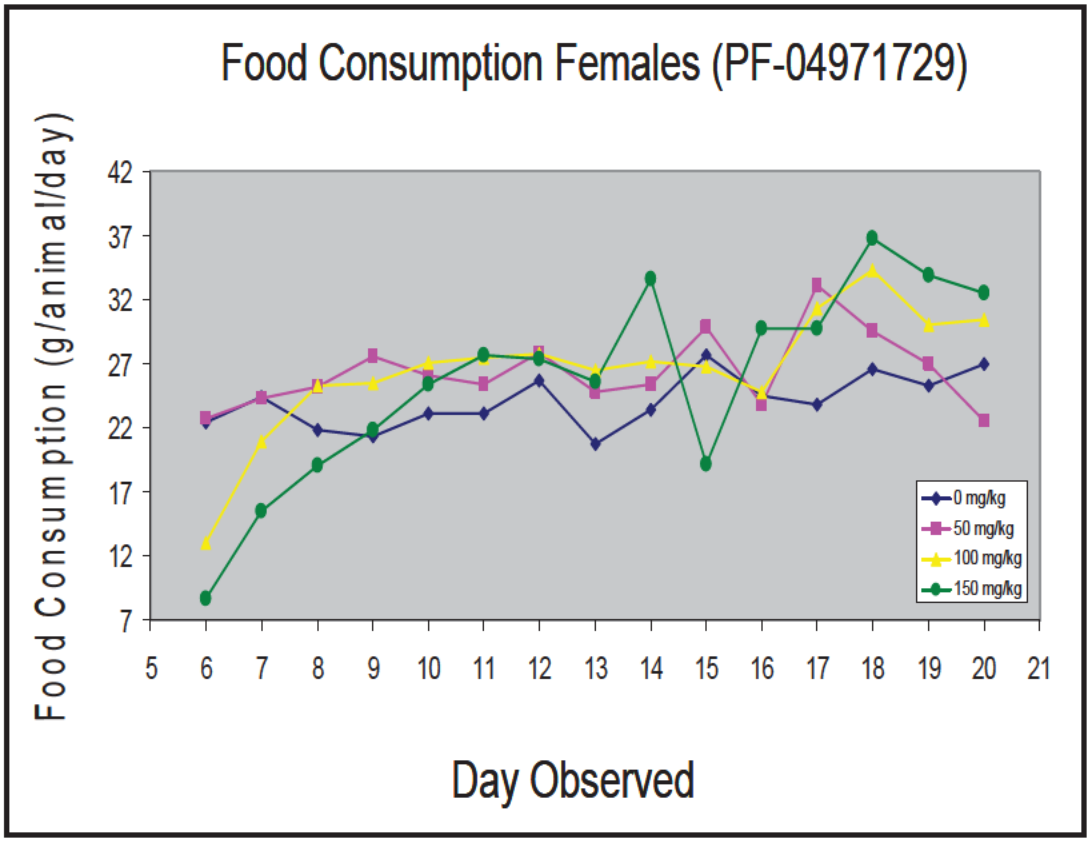
Body weight (dams):

Decreases in body weight and body weight change were observed at 50 and 250 mg/kg/day, with no effect on body weight or body weight gain at 100 mg/kg/day.

Females: Body Weight from GD6 to GD21				
Study Time	Dose, mg/kg	BW gain (g)	% Decrement	BW % control
Gestation (Day 6 to 21)	0	160.6	0%	100%
	50	138.1	↓14%	94%
	100	165.4	↑3%	101%
	150	139.8	↓13%	95%

Food consumption:

A dose-related decrease in food consumption was observed at 100 and 250 mg/kg/day from GD 6-9. After this initial decrease, food consumption was similar to control levels for the remainder of the study and the mean food consumption over the course of the study (GD 6-21) was similar to control (25.8 g at 250 mg/kg/day compared to 24.0 g for control animals). Food consumption at 50 mg/kg/day was similar to control animals.



Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	6	6	6	6
Corpora Lutea	Total	87	84	91	90
No. per animal	Mean	14.5	14.0	15.2	15.0
	S.d.	2.3	1.7	3.0	2.7
Implantation Sites	Total	82	73	87	81
No. per animal	Mean	13.7	12.2	14.5	13.5
	S.d.	1.4	3.1	2.1	1.5
Preimplantation Loss	Total	5	11	4	9
No. per animal	Mean	0.8	1.8	0.7	1.5
	S.d.	1.2	3.3	1.2	2.1
% per animal	Mean	5.0	12.5	3.5	8.8
	S.d.	6.4	23.0	6.1	11.5
Fetuses	Total	80	65	85	74
No. per animal	Mean	13.3	10.8	14.2	12.3
	S.d.	1.4	4.2	1.7	3.1
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	Total	80	65	85	74
No. per animal	Mean	13.3	10.8	14.2	12.3
	S.d.	1.4	4.2	1.7	3.1
Malformed Fetuses (External)	Total	0	0	1	1
No. per animal	Mean	0.0	0.0	0.2	0.2
	S.d.	0.0	0.0	0.4	0.4
Dead Fetuses	Total	0	0	0	0
No. per animal	Mean	0.0	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
% per animal	Mean	0.0	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Early Resorption	Total	2	8	2	6
No. per animal	Mean	0.3	1.3	0.3	1.0
	S.d.	0.5	3.3	0.8	1.7
% per animal	Mean	2.4	10.2	2.0	8.2
	S.d.	3.7	25.1	4.8	13.3
Late Resorption	Total	0	0	0	1
No. per animal	Mean	0.0	0.0	0.0	0.2
	S.d.	0.0	0.0	0.0	0.4
% per animal	Mean	0.0	0.0	0.0	1.5
	S.d.	0.0	0.0	0.0	3.7
Not Applicable for Pfizer DART Studies	Total	0	0	0	0
No. per animal	Mean	0.0	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Postimplantation Loss	Total	2	8	2	7
No. per animal	Mean	0.3	1.3	0.3	1.2
	S.d.	0.5	3.3	0.8	1.8
% per animal	Mean	2.4	10.2	2.0	9.7
	S.d.	3.7	25.1	4.8	15.0
Affected Implants	Total	2	8	3	8
No. per animal	Mean	0.3	1.3	0.5	1.3
	S.d.	0.5	3.3	0.8	1.8
% per animal	Mean	2.4	10.2	3.4	10.9
	S.d.	3.7	25.1	5.3	14.4

Uterine Weight:

Sex: **Female**

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Uterus weight (g)	Mean	103.09 n	87.38	109.45	95.78
	S.d.	12.37	28.77	13.01	21.61
	N	6	6	6	6

Fetal Sex and Body Weights:

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Litters, used for calculation	N	6	6	6	6
Males	Total	35	33	38	42
No. per animal	Mean	5.8	5.5	6.3	7.0
	S.d.	2.6	1.8	2.0	2.8
Females	Total	45	32	47	32
No. per animal	Mean	7.5	5.3	7.8	5.3
	S.d.	2.2	2.7	2.7	2.5
% of Males per animal	Mean	43.3	53.3	45.5	56.9
	S.d.	17.2	9.7	15.2	15.1
Fetus Weight	Mean	5.9	6.2	6.1	5.7
	S.d.	0.2	0.4	0.2	0.2
Fetus Weight of Male Fetuses	Mean	6.1	6.4	6.3	5.9
	S.d.	0.2	0.4	0.2	0.2
Fetus Weight of Female Fetuses	Mean	5.7	6.0	6.0	5.6
	S.d.	0.3	0.4	0.2	0.2

Offspring (malformations, variations, etc.):

One fetus at 100 mg/kg/day and 1 fetus at 250 mg/kg/day exhibited external malformations. At 100 mg/kg/day 1 incidence of omphalocele was noted, and at 250 mg/kg/day 1 incidence of a malrotated hind limb was noted. These findings are anomalies that were not considered to be related to dosing by the sponsor.

External malformations and variations:

			0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	80	65	85	74
Litters evaluated		N	6	6	6	6
Total CS External Observation	Litters Affected	N	0	0	1	1
		%	0	0	16.7	16.7
	Fetuses Affected	N	0	0	1	1
	% per Litter	Mean	0.0	0.0	1.4	1.2
Trunk *Omphalocele (M)*	Litters Affected	N	0	0	1	0
		%	0	0	16.7	0
	Fetuses Affected	N	0	0	1	0
	% per Litter	Mean	0.0	0.0	1.4	0.0
Hindlimb *Malrotated (M)*	Litters Affected	N	0	0	0	1
		%	0	0	0	16.7
	Fetuses Affected	N	0	0	0	1
	% per Litter	Mean	0.0	0.0	0.0	1.2

ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF PF-04971729 IN RATS

Key study findings:

Dams:

- Maternal systemic exposures at 50, 100 and 250 mg/kg on GD 17 were 15.4, 31.9 and 66.7 µg/mL (C_{max}) and 199, 457 and 975 µg.hr/mL (AUC_{0-24})
- Decreases in BW and BW gain were observed at 250 mg/kg.
- A decrease in food consumption (↓27%) was observed (GD 6-9) at 250 mg/kg.
- A dosing-related increase in early resorptions resulted in an increase in post implantation loss and reduced litter size at 250 mg/kg/day.

Fetuses:

- External malformations (Trunk; Omphalocele, Forepaw; ectrodactyly and Tail; short) were noted in two different animals from two different litters at 250 mg/kg.
- The incidence of the visceral malformation (membranous ventricular septum defect) and the visceral variation (absent Innominate artery) were increased relative to historical controls at the 250 mg/kg dose.
- Skeletal malformations that occurred at a higher incidence at the 250 mg/kg dose were absent metacarpal, fused sternebra and hemicentric thoracic centrum.
- Skeletal variations that were significantly increased relative to controls at the 250 mg/kg dose included: unossified 7th cervical centrum (84% Litters and 45% Fetuses - Affected), incomplete ossification of the thoracic centrum (74% Litters and 29% Fetuses - Affected), vertebrae 27th presacral (42% Litters and 18% Fetuses - Affected), full supernumerary ribs (26% Litters and 8% Fetuses - Affected), short supernumerary ribs (100% Litters and 68% Fetuses - Affected) and unossified metatarsal (26% Litters and 5% Fetuses - Affected).

Reviewer Comments:

Based on decreased body weight and increased incidence of early resorptions at 250 mg/kg/day, the reviewer agrees with the sponsor that the no observed adverse effect level (NOAEL) for maternal toxicity was 100 mg/kg/day.

External malformations, visceral malformations and skeletal malformations occurred in fetuses at 250 mg/kg/day. Based on this the NOAEL for developmental toxicity was 100 mg/kg/day. Maternal systemic exposure at 100 mg/kg/day on GD 17 was 31.9µg/mL C_{max} and 457µg.hr/mL (AUC_{0-24}).

Maternal Toxicity (Rat) (50, 100 and 250 mg/kg/day)	**NOAEL**	**Multiple of Starting Dose (1 mg) (0.09 µg.h/mL)**	**Multiple of Max Dose** (b) (4)
Decreased BW and BW Gain	100 mg/kg	5000X	163X
Decreased Food Consumption	100 mg/kg	5000X	163X
Increase Early Resorptions Increase Post Implantation Loss Reduced Litter Size	100 mg/kg	5000X	163X

Developmental Toxicity (Rat) (50, 100 and 250 mg/kg/day)	**NOAEL**	**Multiple of Starting Dose (1 mg) (0.09 µg.h/mL)**	**Multiple of Max Dose** (b) (4)
External Malformations (Trunk; Omphalocele, Forepaw; ectrodactyly and Tail; short)	100 mg/kg	5000X	163X
Visceral Malformations (Membranous Ventricular Septum Defect)	100 mg/kg	5000X	163X
Visceral Variations (Absent Innominate Artery)	50 mg/kg	2200X	71X
Skeletal Malformations (Absent Metacarpal, Fused Sternebra and Hemicentric Thoracic Centrum)	100 mg/kg	5000X	163X
Skeletal Variations (Incomplete Ossification of Lumbar and Thoracic Centrum, Unossified or Misaligned Thoracic Centrum Vertebrae 27th Presacral, Full Supernumerary Ribs, Sternebra Extra Ossification Sites)	100 mg/kg	5000X	163X
Skeletal Variations (Unossified 7th Cervical Centrum and Skull Extra Ossifications)	50 mg/kg	2200X	71X
Skeletal Variations (Unossified Metatarsal and Short Supernumerary Ribs)	-	ND	ND

Study no.: 10GR058
Study report location: (SN30 - eCTD 4.2.3.5.2.1) (DARRTS SD31)
Conducting laboratory and location: Pfizer Global Research, Groton CT
TK Analysis laboratory and location: (b) (4)
Date of study initiation: February 21, 2010
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: PF-04971729 (b) (4), GR02847, 99.9% (Active Moiety 75.6%)

Methods	
Doses:	Daily - 0, 50, 100, and 250 mg/kg/day (D-6 to D-17 of Gestation)
Species/source	Rats/Crl:CD(SD)/Female ((b) (4))
Age/Weight:	10 to 12 weeks/(D6 – 263.1g to 266.2g)
Dosing n/sex/group:	20/Timed pregnant females/dose group
TK n/sex/group:	3/Timed pregnant females/control group 5/Timed pregnant females/dose group
Route, formulation, dose volume	Oral dosing in 0.5% MC + 10% PEG400 – 10 ml/kg

Observation and Times of Dams	
Observations, Examinations and Mortality checks:	Non Treatment Period - Observations took place 2x/day Treatment Period - Observations took place 3x/day Day of C-Section - Observations took place 1x/day
Clinical Findings:	See Above
Body weights:	Once Daily - All animals – GD 3 and GD 5 to GD 21
Food consumption:	Once Daily - All animals – GD 6 to GD 21
Toxicokinetics	Group 6, 7, and 8 TK animals ~ 1, 4, 7, and 24 hours postdose on GD 17 Group 5 TK animals at ~ 1, 4, and 24 hours postdose on GD 17.
Gross Pathology	D-21 of gestation (GD 21)
Post-Mortem Evaluations	
Macroscopic	D-21 of gestation (GD 21)
Uterine & Ovarian Exams	D-21 of gestation (GD 21)
Examination of embryos and fetuses	D-21 of gestation (GD 21)

Variables Analyzed

Maternal and Cesarean-Section Data	
Maternal body weight, body weight gains	Dunnett's test (Dunnett, 1955, 1964)
Corrected maternal body weight gain[a]	
Maternal food consumption	
Gravid uterine weights	
Numbers of corpora lutea, implantations sites, total fetuses, live fetuses, dead fetuses, male fetuses, female fetuses, malformed fetuses (external), abortion sites, early resorptions, late resorptions, pre-implantation loss, post implantation loss, affected implants, males per animal, females per animal	Dunn's test (Dunn, 1964)
% live fetuses, % dead fetuses, % dead fetuses per animal, % early resorptions, % late resorptions, % per animal affected implants, % male fetuses per animal, Percentages of pre- and postimplantation loss[b,c]	
Fetal Data	
Combined fetal weights	Dunnett's test (Dunnett, 1955, 1964)
External, skeletal, and visceral observations: Incidences of specific observations expressed as a percentage of the litter affected	Dunn's test (Dunn, 1964)
Incidences of affected litters in each group	Fisher's Exact test (Fisher, 1950)

[a] Corrected maternal body weight gain = [(final gestation body weight - gravid uterine weight) - GD 6 body weight].
[b] Pre-implantation loss: 100 x [(No. of corpora lutea - No. of implantations)/No. of corpora lutea].
[c] Post-implantation loss: 100 x [(No. of implantations - No. of viable fetuses)/No. of implantations].

Results

Mortality (dams):

There were no dosing-related mortalities. A single control group animal was euthanized following an early delivery on GD 21. All other animals survived to the scheduled euthanasia.

Clinical signs (dams):

There were no dosing-related clinical signs.

Body weight (dams):

There were no adverse effects on body weight or body weight gain at 50 mg/kg/day.

Decreases in BW and BW gain were observed intermittently at 100 mg/kg/day.

Clear dosing-related decreases in body weight and body weight gain were noted at 250 mg/kg/day.

At 250 mg/kg/day, a body weight loss was noted over the first 3 days of treatment (GD 6-9 mean loss of 4.4g at 250 mg/kg/day compared to a mean gain of 13.4g for the control animals). Body weight gain was comparable to control from GD 9-15, but was slightly reduced from GD 15-18. The body weight loss and decreased gain was reflected in a consistent decrease in mean maternal body weight; the mean GD18 body weight was 5% less than control.

Body Weight – Pregnant Rats

Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	End BW % control
Females (GD6 – GD9)	0	13.4	0%	100%
	50	15.4	14.9%	101%
	100	9.8	-26.9%	98%
	250	-4.4**	-132.8%	94%**
Females (GD9 – GD12)	0	16.7	0%	100%
	50	23.2	39%	103%
	100	21.1	26.3%	100%
	250	20.8	24.6%	96%
Females (GD12 – GD15)	0	19.1	0%	100%
	50	16.5	-13.6%	102%
	100	16.1	-15.7%	99%
	250	19.7	3.1%	97%

($p < 0.01$**) (*** $p < 0.05$**)**

Body Weight – Pregnant Rats				
Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	End BW % control
Females (GD15 – GD18)	0	38	0%	100%
	50	33.3	-12.4%	100%
	100	32.8	-13.7%	98%
	250	29.9*	-21.3%	95%*
Females (GD6 – GD18)	0	87.3	0%	100%
	50	88.5	1.4%	100%
	100	79.8	-8.6%	98%
	250	66.1**	-24.3%	95%*
Females (GD18 – GD21)	0	48.2	0%	100%
	50	46.6	-3.3%	100%
	100	50	3.7%	99%
	250	47.7	-1.0%	96%
Females (GD6 – GD21)	0	133.7	0%	100%
	50	135	1%	100%
	100	129.8	-2.9%	99%
	250	113.8**	-14.9%	96%
(** p < 0.01) (* p < 0.05)				

Summary of Mean Adjusted Body Weight Change and Uterine Weight (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Uterus weight (g)	Mean	96.53 n	98.40	98.05	84.23
	S.d.	10.08	16.57	19.53	23.89
	N	19	20	20	20
Carcass Weight (g)	Mean	300.84 n	300.61	294.82	295.74
	S.d.	15.97	17.28	15.15	19.58
	N	19	20	20	20
Net weight change (g) From Gestation day 6	Mean	37.21 n	36.62	31.75	29.57
	S.d.	11.70	14.77	11.70	13.85
	N	19	20	20	20

Uterine weights were reduced in the 250 mg/kg group although this change was not found to be significant.

Food Consumption:

A dosing-related decrease in mean food consumption was noted at 250 mg/kg/day from GD 6-9 (27% less than control). Following that initial loss, food consumption at 250 mg/kg/day was similar to or greater than control. Similar increases in food consumption were noted at 100 and 50 mg/kg/day.

Summary of Mean Maternal Food Consumption (g/animal/day)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
d 20 -> 21	Mean [g]	25.4 n	28.6	30.4*	32.4**
	S.d.	6.2	5.2	5.5	5.8
	N	20	20	20	20
d 6 -> 9	Mean [g]	22.0 n	23.9	20.0	16.1**
	S.d.	2.5	3.3	2.9	2.8
	N	20	20	19	20
d 9 -> 12	Mean [g]	23.5 n	27.5**	26.2	25.2
	S.d.	2.9	2.6	3.8	4.7
	N	20	20	20	20
d 12 -> 15	Mean [g]	23.7 n	26.0	25.6	27.6**
	S.d.	3.5	4.4	3.6	3.9
	N	20	20	20	20
d 15 -> 18	Mean [g]	25.5 n	27.3	25.9	28.5*
	S.d.	3.0	3.9	4.2	3.2
	N	20	20	20	20
d 6 -> 18	Mean [g]	23.7 n	26.2**	24.3	24.3
	S.d.	2.2	2.5	2.9	2.9
	N	20	20	19	20
d 18 -> 21	Mean [g]	24.1 n	27.4*	30.6**	34.4**
	S.d.	4.2	4.1	3.8	3.8
	N	20	20	20	20
d 6 -> 21	Mean [g]	23.8 n	26.4**	25.6	26.3**
	S.d.	2.3	2.3	2.8	2.7
	N	20	20	19	20

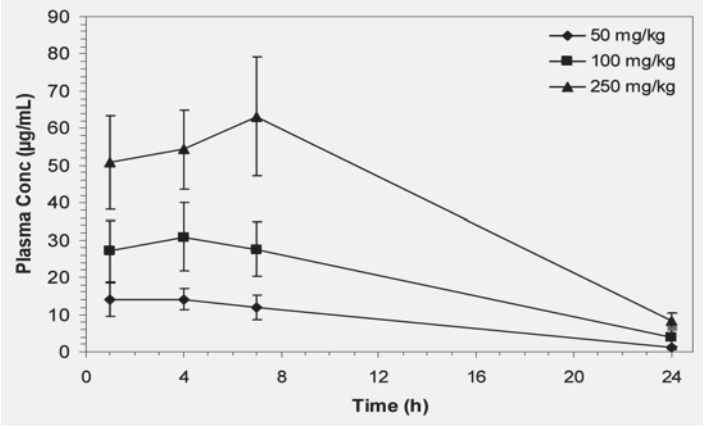
Toxicokinetics:

Following once daily oral administration of PF-04971729 to timed-pregnant Sprague-Dawley rats, t_{max} was variable and occurred from 1 to 7 hours postdose. Systemic exposure (assessed by C_{max} and AUC_{0-24}) increased with ascending dose. Mean C_{max} and AUC_{0-24} increased by (4.3X) and (4.9X) on Gestational Day 17 with a (5X) increase in dose (from 50 to 250 mg/kg/day).

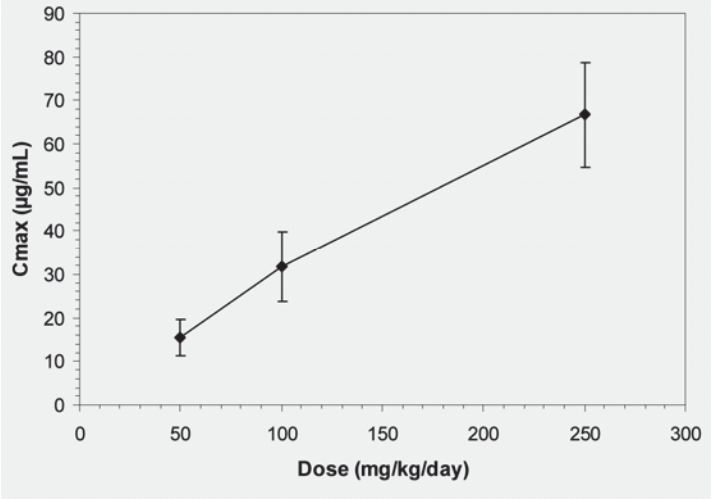
Mean Toxicokinetic Parameters for PF-04971729 in Timed-Pregnant Sprague-Dawley Rats after Oral Administration of PF-04971729 on Gestational Day 17

Dose (mg/kg/day)	Gestational Day	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
		Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
50	17	15.4	4.15	5	2.80	1.64	5	199	46.2	5
100		31.9	8.03	5	4.00	2.12	5	457	103	5
250		66.7	11.9	5	5.20	2.68	5	975	173	5

Mean (± SD) PF-04971729 Concentration in Timed-Pregnant Sprague-Dawley Rats versus Time after Oral Administration of PF-04971729 on Gestational Day 17



C_{max} versus Dose



Reproductive and Postmortem Examinations

Gestation Day 21 Cesarean Sections:

A dosing-related increase in post implantation loss was noted at 250 mg/kg/day (13.1% per litter compared to 4.0% per litter for the control group) secondary to an increase in early resorptions (0.6, 0.8, 0.6, 1.6 resorptions per litter at 0, 50, 100, and 250 mg/kg/day, respectively) and subsequent reduction in live litter size (12.7, 12.6, 12.8, and 11.6 fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively). There were no dosing-related effects on cesarean section parameters at 100 mg/kg/day and 50 mg/kg/day.

Gross Necropsy Observations:

Summary of Mean Cesarean Section Values

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	20	20	20
Corpora Lutea	Total	277	282	290	278
No. per animal	Mean	14.6s	14.1	14.5	13.9
	S.d.	2.4	2.2	1.7	2.4
Implantation Sites	Total	253	267	269	264
No. per animal	Mean	13.3s	13.3	13.4	13.2
	S.d.	1.9	1.7	1.9	2.4
Preimplantation Loss	Total	24	15	21	14
No. per animal	Mean	1.3s	0.8	1.1	0.7
	S.d.	1.9	1.2	2.1	0.7
% per animal	Mean	8.0s	4.8	6.5	4.9
	S.d.	9.5	6.7	12.2	5.2
Fetuses	Total	242	251	257	232
No. per animal	Mean	12.7s	12.6	12.8	11.6
	S.d.	1.5	2.5	2.5	3.6
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	Total	242	251	257	232
No. per animal	Mean	12.7s	12.6	12.8	11.6
	S.d.	1.5	2.5	2.5	3.6
Malformed Fetuses (External)	Total	0	0	0	2
No. per animal	Mean	0.0s	0.0	0.0	0.1
	S.d.	0.0	0.0	0.0	0.3
Dead Fetuses	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
% per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Early Resorption	Total	11	16	12	31
No. per animal	Mean	0.6s	0.8	0.6	1.6
	S.d.	0.8	1.5	1.1	2.5
% per animal	Mean	4.0s	6.4	5.3	12.8
	S.d.	5.2	12.4	11.7	22.3
Late Resorption	Total	0	0	0	1
No. per animal	Mean	0.0s	0.0	0.0	0.1
	S.d.	0.0	0.0	0.0	0.2
% per animal	Mean	0.0s	0.0	0.0	0.3
	S.d.	0.0	0.0	0.0	1.5
Not Applicable for Pfizer DART Studies	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Postimplantation Loss	Total	11	16	12	32
No. per animal	Mean	0.6s	0.8	0.6	1.6
	S.d.	0.8	1.5	1.1	2.5
% per animal	Mean	4.0s	6.4	5.3	13.1
	S.d.	5.2	12.4	11.7	22.3

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	20	20	20
Affected Implants	Total	11	16	12	34
No. per animal	Mean	0.6s	0.8	0.6	1.7
	S.d.	0.8	1.5	1.1	2.4
% per animal	Mean	4.0s	6.4	5.3	13.9
	S.d.	5.2	12.4	11.7	22.1

Fetal Observations/Measurements

Fetal Body Weights

There were no clear drug effects on fetal weights at necropsy, although the mean weight of fetuses derived from animals dosed at 250 mg/kg was the lowest of all groups.

Summary of Mean Fetal Body Weights (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Litters, used for calculation	N	19	20	20	19
Fetus Weight	Mean	5.7n	6.0	5.7	5.4
	S.d.	0.4	0.3	0.5	0.4

Fetal External Exams

External malformations (Trunk; Omphalocele, Forepaw; ectrodactyly and Tail; short) were noted in two different animals from two different litters at 250 mg/kg.

Summary of Fetal External Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	242		251	257	232
Litters evaluated		N	19		20	20	19
Total CS External Observation	Litters Affected	N	0	f	0	0	2
		%	0		0	0	10.5
	Fetuses Affected	N	0		0	0	2
	% per Litter	Mean	0.0	s	0.0	0.0	0.9
Trunk *Omphalocele (M)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.4
Forepaw *Ectrodactyly (M)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.5
Tail *Short (M)*	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.3
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.5

M = Malformation

Fetal Visceral Exams

Visceral malformations noted at doses ≥ 100 mg/kg were right sided aortic arch and membranous ventricular septum defect (mVSD). Malformations of the right sided aortic arch were noted at doses ≥ 100 mg/kg (0, 0, 0.8, and 0.9 % fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively). The increase mVSD at 250 mg/kg is considered to be related to dosing as the incidence was greater than in the control and lower dose groups (3.0, 1.7, 0.7, and 7.0 % fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively) and was higher than historical control data (max. incidence of 3.8% fetuses per litter).

Visceral variations noted at 250 mg/kg/day were hemorrhagic adrenal (noted for 0, 0, 0, and 0.8 % fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively) and absent Innominate artery (0, 0, 0.8, and 2.4 % fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively). Both of these variations were noted at higher incidence in the 250 mg/kg/day group than in the control or lower dose groups. Hemorrhagic adrenal was noted in a single fetus. The maximum historical control incidence of absent Innominate artery is 2.1 % fetuses per litter).

Summary of Fetal Visceral Evaluations

			0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	117	121	123	111
Litters evaluated		N	19	20	20	19
Total CS Visceral Observation	Litters Affected	N	2 f	3	3	6
		%	10.5	15.0	15.0	31.6
	Fetuses Affected	N	3	3	3	13
	% per Litter	Mean	3.0 s	2.4	2.3	10.1
Adrenal gland *Hemorrhagic (V)*	Litters Affected	N	0 f	0	0	1
		%	0	0	0	5.3
	Fetuses Affected	N	0	0	0	1
	% per Litter	Mean	0.0 s	0.0	0.0	0.8
Aortic arch *Right-sided (M)*	Litters Affected	N	0 f	0	1	1
		%	0	0	5.0	5.3
	Fetuses Affected	N	0	0	1	1
	% per Litter	Mean	0.0 s	0.0	0.8	0.9
Heart *Membranous ventricular septum defect (M)*	Litters Affected	N	2 f	2	1	4
		%	10.5	10.0	5.0	21.1
	Fetuses Affected	N	3	2	1	9
	% per Litter	Mean	3.0 s	1.7	0.7	7.0
Innominate *Absent (V)*	Litters Affected	N	0 f	0	1	3
		%	0	0	5.0	15.8
	Fetuses Affected	N	0	0	1	3
	% per Litter	Mean	0.0 s	0.0	0.8	2.4
Lung	Litters Affected	N	0 f	0	1	0
		%	0	0	5.0	0
	Fetuses Affected	N	0	0	1	0
	% per Litter	Mean	0.0 s	0.0	0.8	0.0
Absent lobe(s) (V)	Litters Affected	N	0 f	0	1	0
		%	0	0	5.0	0
	Fetuses Affected	N	0	0	1	0
	% per Litter	Mean	0.0 s	0.0	0.8	0.0
Absent post caval lobe (V)	Litters Affected	N	0 f	0	1	0
		%	0	0	5.0	0
	Fetuses Affected	N	0	0	1	0
	% per Litter	Mean	0.0 s	0.0	0.8	0.0

			0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	117	121	123	111
Litters evaluated		N	19	20	20	19
Thyroid gland *Hemorrhagic (V)*	Litters Affected	N	0 f	0	1	0
		%	0	0	5.0	0
	Fetuses Affected	N	0	0	1	0
	% per Litter	Mean	0.0 s	0.0	0.7	0.0
Ureter *Dilated (V)*	Litters Affected	N	0 f	1	0	0
		%	0	5.0	0	0
	Fetuses Affected	N	0	1	0	0
	% per Litter	Mean	0.0 s	0.7	0.0	0.0

V = Variation, M = Malformation

Fetal Skeletal Exams

Summary of Fetal Skeletal Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F	
Fetuses Examined		N	125		130	134	121	
Litters evaluated		N	19		20	20	19	
Total CS Skeletal Observation	Litters Affected	N	12	f	15	16	19	**
		%	63.2		75.0	80.0	100.0	
	Fetuses Affected	N	22		35	44	112	
	% per Litter	Mean	17.3	s	26.3	30.8	92.2	**
Cervical centrum *7th cervical centrum unossified (V)*	Litters Affected	N	1	f	1	3	16	**
		%	5.3		5.0	15.0	84.2	
	Fetuses Affected	N	1		1	3	58	
	% per Litter	Mean	0.8	s	0.8	2.1	44.6	**
Lumbar centrum *Incomplete ossification (V)*	Litters Affected	N	0	f	0	0	2	
		%	0		0	0	10.5	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.6	
Metacarpal *Absent (M)*	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	1	
	% per Litter	Mean	0.0	s	0.0	0.0	0.8	
Rib	Litters Affected	N	11	f	15	15	19	**
		%	57.9		75.0	75.0	100.0	
	Fetuses Affected	N	18		33	39	90	
	% per Litter	Mean	14.5	s	24.9	27.5	75.5	**
7th Cervical (V)	Litters Affected	N	1	f	2	1	1	
		%	5.3		10.0	5.0	5.3	
	Fetuses Affected	N	1		2	1	2	
	% per Litter	Mean	0.7	s	1.5	0.8	1.5	
Full supernumerary (V)	Litters Affected	N	0	f	0	0	5	*
		%	0		0	0	26.3	
	Fetuses Affected	N	0		0	0	10	
	% per Litter	Mean	0.0	s	0.0	0.0	8.0	**
Short (V)	Litters Affected	N	1	f	0	0	0	
		%	5.3		0	0	0	
	Fetuses Affected	N	1		0	0	0	
	% per Litter	Mean	0.7	s	0.0	0.0	0.0	
Short supernumerary (V)	Litters Affected	N	10	f	14	13	19	**
		%	52.6		70.0	65.0	100.0	
	Fetuses Affected	N	16		30	36	80	
	% per Litter	Mean	13.2	s	22.8	25.4	67.5	**
Wavy (V)	Litters Affected	N	0	f	1	2	0	
		%	0		5.0	10.0	0	
	Fetuses Affected	N	0		1	3	0	
	% per Litter	Mean	0.0	s	0.6	1.9	0.0	
Skull *Extra ossification site (V)*	Litters Affected	N	0	f	0	1	2	
		%	0		0	5.0	10.5	
	Fetuses Affected	N	0		0	1	2	
	% per Litter	Mean	0.0	s	0.0	0.6	2.2	
Sternebra	Litters Affected	N	0	f	0	1	2	
		%	0		0	5.0	10.5	
	Fetuses Affected	N	0		0	1	4	
	% per Litter	Mean	0.0	s	0.0	0.8	2.8	
Extra ossification site (V)	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.3	
Fused (M)	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	1	
	% per Litter	Mean	0.0	s	0.0	0.0	0.8	
Unossified #5 and/or #6 (V)	Litters Affected	N	0	f	0	1	1	
		%	0		0	5.0	5.3	
	Fetuses Affected	N	0		0	1	1	
	% per Litter	Mean	0.0	s	0.0	0.8	0.8	
Thoracic centrum	Litters Affected	N	3	f	0	2	14	**
		%	15.8		0	10.0	73.7	
	Fetuses Affected	N	3		0	3	37	
	% per Litter	Mean	2.1	s	0.0	2.0	28.9	**

V = Variation, M = Malformation

Summary of Fetal Skeletal Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F	
Fetuses Examined		N	125		130	134	121	
Litters evaluated		N	19		20	20	19	
Hemicentric (M)	Litters Affected	N	0	f	0	0	2	
		%	0		0	0	10.5	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.5	
Incomplete ossification (V)	Litters Affected	N	3	f	0	2	14	**
		%	15.8		0	10.0	73.7	
	Fetuses Affected	N	3		0	3	37	
	% per Litter	Mean	2.1	s	0.0	2.0	28.9	**
Misaligned (V)	Litters Affected	N	0	f	0	0	2	
		%	0		0	0	10.5	
	Fetuses Affected	N	0		0	0	2	
	% per Litter	Mean	0.0	s	0.0	0.0	1.5	
Unossified (V)	Litters Affected	N	0	f	0	0	1	
		%	0		0	0	5.3	
	Fetuses Affected	N	0		0	0	1	
	% per Litter	Mean	0.0	s	0.0	0.0	0.8	
Vertebra *27 Presacral (V)*	Litters Affected	N	0	f	0	0	8	**
		%	0		0	0	42.1	
	Fetuses Affected	N	0		0	0	23	
	% per Litter	Mean	0.0	s	0.0	0.0	17.8	**
Metatarsal	Litters Affected	N	1	f	1	1	5	
		%	5.3		5.0	5.0	26.3	
	Fetuses Affected	N	1		1	1	6	
	% per Litter	Mean	0.8	s	0.6	0.8	4.7	
Malpositioned (M)	Litters Affected	N	1	f	0	0	0	
		%	5.3		0	0	0	
	Fetuses Affected	N	1		0	0	0	
	% per Litter	Mean	0.8	s	0.0	0.0	0.0	
Unossified (V)	Litters Affected	N	0	f	1	1	5	*
		%	0		5.0	5.0	26.3	
	Fetuses Affected	N	0		1	1	6	
	% per Litter	Mean	0.0	s	0.6	0.8	4.7	*

V = Variation, M = Malformation

Skeletal malformations that occurred at a higher incidence at the 250 mg/kg dose were absent metacarpal (0, 0, 0, and 0.8% fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively), fused sternebra (noted for 0, 0, 0, and 0.8% fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively), and hemicentric thoracic centrum noted for 0, 0, 0, and 1.5% fetuses per litter at 0, 50, 100, and 250 mg/kg/day, respectively). The fused sternebra and a single incidence of the hemicentric thoracic centrum malformation occurred in unique litters. The absent metacarpal and the remaining incidence of hemicentric thoracic centrum malformations occurred in the same litter but in different fetuses. All of these malformations occurred in female pups.

Skeletal variations were noted at all doses. Skeletal variations that increased in incidence relative to controls were variations of the centrum (unossified 7th cervical centrum (≥ 100 mg/kg/day), incomplete ossification of lumbar centrum (250 mg/kg/day) and incomplete ossification (250 mg/kg/day), unossified (250 mg/kg/day), and misaligned thoracic centrum (250 mg/kg/day), vertebrae 27th presacral (250 mg/kg/day), unossified metatarsal (≥ 50 mg/kg/day), and ribs full (250 mg/kg/day) and short supernumerary ribs (≥ 50 mg/kg/day), skull extra ossifications (≥ 100 mg/kg/day) and sternebra (extra ossification site (250 mg/kg/day).

Oral Dose Range Finding Study of PF-04971729 in Pregnant Rabbits

Key study findings:

- Oral dose range-finding study of PF-04971729 in pregnant rabbits. To be issued.

Does:

Fetuses:

Study no.: 09GR436
Study report location:
Conducting laboratory and location: Pfizer Global Research, Groton CT
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: PF-04971729 (b) (4)

Summary (From Study Rationale Section of (10GR059)

This was a dose range-finding study in pregnant rabbits with doses of 25, 50, 100, and 250 mg/kg/day, administered by oral gavage on Gestation Days (GD) 7-19 (Study 09GR436). Because no overt toxicity was noted, the 25 mg/kg/day group was increased to 500 mg/kg/day beginning on GD 15. Immediately following the increase in dose level a reduction in food consumption and body weight loss was noted at 500 mg/kg/day. These adverse effects persisted after cessation of dosing and 1 doe died on GD 23 and 4 does aborted between GD 22 and 29; only 1 doe carried a litter to the scheduled necropsy. Therefore the dose of 500 mg/kg/day was precluded from use in the definitive embryo fetal development study. The high dose in the definitive study, 250 mg/kg/day, is expected to decrease maternal body weight gain and food consumption. The intermediate dose, 100 mg/kg/day, and the low dose, 50 mg/kg/day, will be used to determine dose response relationships and to determine a NOAEL.

ORAL EMBRYO-FETAL DEVELOPMENT STUDY OF PF-04971729 IN RABBITS

Key study findings:

Does:

- Maternal systemic exposures at 50, 100 and 250 mg/kg on GD 19 were 22.5, 46 and 115 µg/mL (C_{max}) and 207, 424 and 1150 µg.hr/mL (AUC_{0-24}). **Note**: Pre-dose drug concentrations were used in place of 24 hr post-dose drug time points.

- Three rabbits at the 250 mg/kg dose aborted early and were euthanized.

- Decreases in body weight and/or body weight gain were noted at all dose levels (GD 7-20 body weight gains were 52%, 48%, and 71% less than control at 50, 100, and 250 mg/kg/day, respectively).

- A significant increase in mean food consumption was observed at doses ≥ 100 mg/kg/day (Day 20 to Day 29 and Day 7 to Day 29). Decreases in food consumption were noted in the three rabbits that aborted early.

- All dosed groups presented with an increased incidence of post-implantation loss and decreased number of live fetuses relative to controls.

Fetuses:

- Visceral malformations were noted in one (250 mg/kg) fetus: (muscular ventricular septum defect, dilated aortic arch, narrowed pulmonary trunk).

- Visceral variations related to dosing were: gallbladder variations (absent gallbladder and small gallbladder) and retrocaval ureter variation (250 mg/kg).

- Skeletal malformations (250 mg/kg) noted in the same fetus were: supernumerary cervical centrum and fused rib. Misshapen interparietal bone (250 mg/kg) was noted in a single fetus from a separate litter.

- Skeletal variations related to dosing were: sternebra with an extra ossification site (250 mg/kg – 3 fetuses/2 litters).

Reviewer Comments:

Three rabbits at the 250 mg/kg/day dose aborted early. These abortions were likely attributed to severe body weight loss and secondary to maternal toxicity. Based on the reductions in maternal body weight and body weight gain, the increased incidence of post-implantation loss and the decrease in the total number of live fetuses at all dose levels; the reviewer agrees with the sponsor that a NOAEL for maternal toxicity could not be determined for this study (< 74x MRHD). The 50 mg/kg and 100 mg/kg dose levels did not illicit these toxicities in the rabbit dose range finding study (09GR436).

The NOAEL for developmental toxicity was 100 mg/kg/day. Maternal systemic exposure at 100 mg/kg/day on GD 19 was 46µg/mL C_{max} and 424µg.hr/mL (AUC_{0-24}). This was based on observations at the 250 mg/kg dose that included: visceral malformations (similar to rat findings at the same dose), visceral variations, skeletal malformations and skeletal variations (similar to rat findings at the same dose).

The sponsor concluded a NOAEL of 250 mg/kg, the highest dose in this study, stating that there were no dosing-related effects on fetal viability, growth, or morphological development. The reviewer does not concur.

Maternal Toxicity (Rabbit) (50, 100 and 250 mg/kg/day)	**NOAEL**	**Multiple of Starting Dose (1 mg) (0.09 µg.h/mL)**	**Multiple of Max Dose (25 mg) (2.8 µg.h/mL)**
Increased Incidence of Abortion	100 mg/kg	4700X	151X
Decreased BW and BW Gain	-	ND	ND
Increase Post Implantation Loss	-	ND	ND

Developmental Toxicity (Rabbit) (50, 100 and 250 mg/kg/day)	**NOAEL**	**Multiple of Starting Dose (1 mg) (0.09 µg.h/mL)**	**Multiple of Max Dose (25 mg) (2.8 µg.h/mL)**
Decrease in Total Live Fetuses	-	ND	ND
Visceral Malformations (Muscular Ventricular Septum Defect, Dilated Aortic Arch, Narrowed Pulmonary Trunk)	100 mg/kg	4700X	151X
Visceral Variations (Absent Gallbladder and Small Gallbladder)	100 mg/kg	4700X	151X
Skeletal Malformations (Supernumerary Cervical Centrum, Fused Rib and Misshapen Interparietal Bone)	100 mg/kg	4700X	151X
Skeletal Variations (Sternebra Extra Ossification Sites)	100 mg/kg	4700X	151X

Study no.: 10GR059
Study report location: (SN30 - eCTD 4.2.3.5.2.1) (DARRTS SD31)
Conducting laboratory and location: Pfizer Global Research, Groton CT
TK Analysis laboratory and location: (b) (4)
Date of study initiation: February 28, 2010
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: PF-04971729 (b) (4), GR02847, 99.9% (Active Moiety 75.6%)

Methods	
Doses:	Daily - 0, 50, 100, and 250 mg/kg/day (D-7 to D-19 of Gestation)
Species/source	New Zealand White Rabbits /Female (b) (4)
Age/Weight:	4.5-6.0 months/(2.7-3.5 kilograms (kg)
Dosing n/sex/group:	20/Timed pregnant females/dose group
TK n/sex/group:	3/Timed pregnant females/control group 5/Timed pregnant females/dose group
Route, formulation, dose volume	Oral dosing in 0.5% MC + 10% PEG400 – 2.5 ml/kg

<table>
<tr><th colspan="2">Observation and Times of Dams</th></tr>
<tr><td>Observations, Examinations and Mortality checks:</td><td>Non Treatment Period - Observations took place 2x/day
Treatment Period - Observations took place 3x/day
Day of C-Section (GD29) - Observations took place 1x/day</td></tr>
<tr><td>Clinical Findings:</td><td>See Above</td></tr>
<tr><td>Body weights:</td><td>Once Daily - All animals – Arrival, GD 4, GD 6 to GD 20 and GD 23, 26 and 29. Animals found dead or euthanized prior to scheduled necropsy were not weighed.</td></tr>
<tr><td>Food consumption:</td><td>Once Daily - All animals – GD 7 to GD 29. Food consumption was not recorded for animals found dead or euthanized prior to scheduled necropsy.</td></tr>
<tr><td>Toxicokinetics</td><td>Group 6, 7, and 8 TK animals 0, 0.5, 1, 2, 4, and 7 hours postdose on GD 19. Group 5 TK animals at 0, 0.5 and 4 hours postdose on GD 19. Note: Pre-dose samples were used as 24 hour post-dose time points.</td></tr>
<tr><td>Gross Pathology</td><td>D-29 of gestation (GD 29)</td></tr>
<tr><th colspan="2">Post-Mortem Evaluations</th></tr>
<tr><td>Macroscopic</td><td>D-29 of gestation (GD 29)</td></tr>
<tr><td>Uterine & Ovarian Exams</td><td>D-29 of gestation (GD 29)</td></tr>
<tr><td>Examination of embryos and fetuses</td><td>D-29 of gestation (GD 29)</td></tr>
<tr><td>Variables Analyzed</td><td>
<table>
<tr><th colspan="2">Maternal and Cesarean-Section Data</th></tr>
<tr><td>Maternal body weight, body weight gains</td><td rowspan="4">Dunnett's test (Dunnett, 1955, 1964)</td></tr>
<tr><td>Corrected maternal body weight gain[a]</td></tr>
<tr><td>Maternal food consumption</td></tr>
<tr><td>Gravid uterine weights</td></tr>
<tr><td>Numbers of corpora lutea, implantations sites, total fetuses, live fetuses, dead fetuses, male fetuses, female fetuses, malformed fetuses (external), abortion sites, early resorptions, late resorptions, pre-implantation loss, post implantation loss, affected implants, males per animal, females per animal</td><td rowspan="2">Dunn's test (Dunn, 1964)</td></tr>
<tr><td>% live fetuses, % dead fetuses, % dead fetuses per animal, % early resorptions, % late resorptions, % per animal affected implants, % male fetuses per animal, Percentages of pre- and postimplantation loss[b,c]</td></tr>
<tr><th colspan="2">Fetal Data</th></tr>
<tr><td>Combined fetal weights</td><td>Dunnett's test (Dunnett, 1955, 1964)</td></tr>
<tr><td>External, skeletal, and visceral observations: Incidences of specific observations expressed as a percentage of the litter affected</td><td>Dunn's test (Dunn, 1964)</td></tr>
<tr><td>Incidences of affected litters in each group</td><td>Fisher's Exact test (Fisher, 1950)</td></tr>
</table>
[a] Corrected maternal body weight gain = [(final gestation body weight - gravid uterine weight) - GD 6 body weight].
[b] Pre-implantation loss: 100 x [(No. of corpora lutea - No. of implantations)/No. of corpora lutea].
[c] Post-implantation loss: 100 x [(No. of implantations - No. of viable fetuses)/No. of implantations].
</td></tr>
</table>

Results

Mortality (Does):

Two does at 250 mg/kg/day that aborted (Does 63 and 65 on GD 19 and 21, respectively) were euthanized.

In addition doe 95 in the 250 mg/kg/day TK group was euthanized on GD 28, following completion of blood collection, due to clinical signs suggestive of abortion (blood clots and red fluid in the cage) although no aborted fetuses were noted.

All 3 of these animals had severe decreases in food consumption and body weight loss (prior to euthanasia body weights had decreased from the initiation of dosing by 22%, 13%, and 14% for does 63, 65, and 95, respectively); therefore, the abortions and euthanasia were considered secondary to maternal toxicity.

Clinical Signs (Does):

Summary of Clinical Signs

			0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
day 7 [AM Check] -> day 29 [Disposition]	Animals examined	N	20	20	20	20
	Animals with signs	N	1	2	2	4
	Normal *Normal*	N	20	20	20	19
	Animal Disposition List	N	20	20	20	20
	Scheduled Euthanasia	N	20	20	20	18
	Unscheduled Euthanasia	N	0	0	0	2
	Gastrointestinal Function *Feces Soft*	N	1	1	0	1
	General Appearance and Condition *Discolored Material In/Under Cage*	N	0	1	0	2
	Skin and Haircoat	N	0	1	2	2
	Abrasion	N	0	0	1	1
	Hair Loss	N	0	0	1	0
	Haircoat Stained	N	0	1	0	1

Body weight (Does):

Decreases in body weight and/or body weight gain were noted at all dose levels (GD 7-20 body weight gains were (↓52%), (↓48%), and (↓71%) less than control at 50, 100, and 250 mg/kg/day, respectively).

At 250 mg/kg/day, a body weight loss was noted from GD 7-10 and body weight gain was reduced for the remainder of the dosing period resulting in significantly decreased mean body weights from GD 17-20 (↓5-6% less than controls).

At 100 mg/kg, a body weight loss was noted from GD 7-10, body weight gain was reduced (↓22%) from GD 10-13, and there was a decrease in body weight from GD 13-20.

A similar pattern was noted at 50 mg/kg, although the body weight decrement for the GD 7-10 interval was not as severe at 50 mg/kg/day as at 100 mg/kg/day.

Body Weight – Pregnant Rabbits				
Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	End BW % control
Females (GD7 – GD10)	0	52	0%	100%
	50	1*	-98%	96%
	100	-7**	-113%	97%
	250	-10**	-119%	98%
Females (GD10 – GD13)	0	50	0%	100%
	50	40	-20%	96%
	100	39	-22%	97%
	250	17	-66%	97%
Females (GD13 – GD16)	0	55	0%	100%
	50	48	-12.7%	96%
	100	57	3.6%	97%
	250	16	-71%	95%
Females (GD16 – GD20)	0	79	0%	100%
	50	26**	-67%	95%*
	100	36**	-54.4%	96%
	250	14**	-82.3%	94%*
Females (GD7 – GD20)	0	237	0%	100%
	50	115**	-51.5%	95%*
	100	124**	-47.7%	96%
	250	69**	-70.9%	94%*
Females (GD20 – GD29)	0	190	0%	100%
	50	245	28.9%	96%
	100	229	20.5%	97%
	250	236	24.2%	97%
Females (GD7 – GD29)	0	427	0%	100%
	50	361	-15%	96%
	100	353	-17.3%	97%
	250	334	-21.8%	97%
(** p < 0.01) (* p < 0.05)				

Mean Uterine Weight (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Uterus weight (g)	Mean	542.15 n	497.93	475.62	487.90
	S.d.	80.55	143.21	79.68	92.65
	N	19	19	20	17

Food Consumption:

A significant increase in mean food consumption was observed in rabbits following dosing at 100 mg/kg/day and 250 mg/kg/day (Day 20 to Day 29 and Day 7 to Day 29). Decreases in food consumption were noted in the three rabbits that aborted early.

Summary of Mean Maternal Food Consumption (g/animal/day)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
d 7 -> 10	Mean [g]	144.7 s	135.0	134.0	137.4
	S.d.	9.2	16.7	23.2	15.8
	N	19	19	20	19
d 10 -> 13	Mean [g]	135.9 s	125.9	128.0	124.5
	S.d.	16.8	23.7	27.2	29.8
	N	19	19	20	19
d 13 -> 16	Mean [g]	123.8 s	134.6	134.2	124.1
	S.d.	35.2	22.6	24.2	45.1
	N	19	19	20	19

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
d 16 -> 20	Mean [g]	141.0 s	143.3	144.2	144.6
	S.d.	14.2	15.3	11.5	9.5
	N	19	19	20	16
d 7 -> 20	Mean [g]	136.7 s	135.3	135.8	139.3
	S.d.	15.6	15.3	19.0	10.6
	N	19	19	20	16
d 20 -> 29	Mean [g]	122.9 s	135.9	142.6 **	142.8 **
	S.d.	16.8	15.5	10.1	16.6
	N	19	19	20	17
d 7 -> 29	Mean [g]	131.1 s	135.6	138.6 *	140.7 *
	S.d.	11.1	14.0	13.2	8.9
	N	19	19	20	16

Toxicokinetics:

Note: Pre-dose samples (Time 0) were used in place of 24 hour post-dose time points.

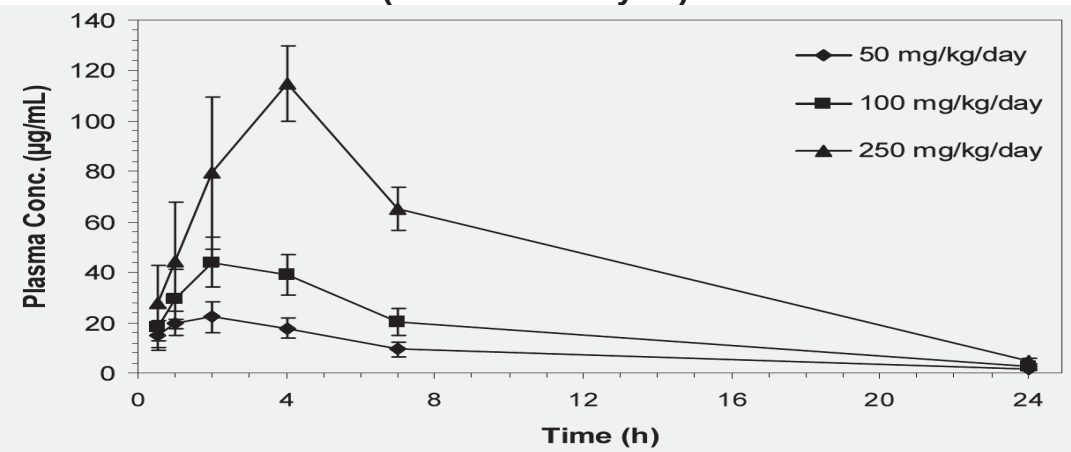
Following once daily oral administration of PF-04971729 to timed-pregnant New Zealand White rabbits, t_{max} was variable and occurred from 2 to 4 hours postdose on Gestational Day 19. Mean t_{max} was observed at 2.40, 2.40, and 4.00 hours postdose for 50, 100, and 250 mg/kg/day doses, respectively.

Systemic exposure (assessed by C_{max} and AUC_{0-24}) increased with ascending dose. Mean C_{max} and AUC_{0-24} increased by 5.1X and 5.6X, respectively, on Gestational Day 19 with a 5X increase in dose (from 50 mg/kg/day to 250 mg/kg/day).

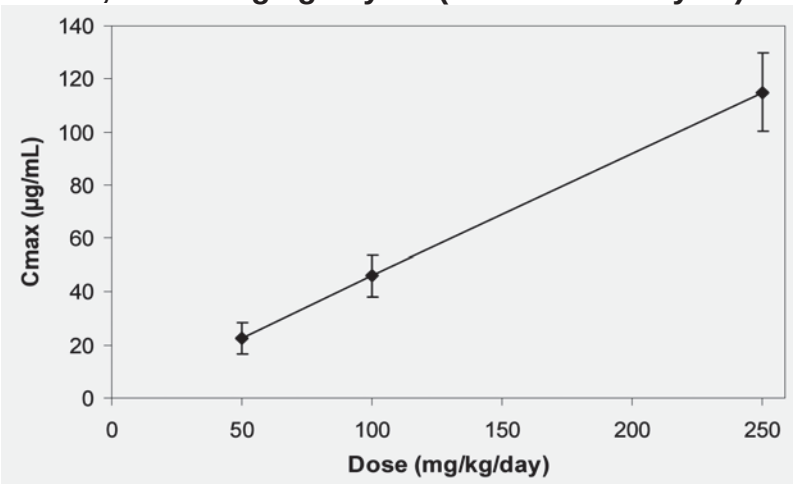
Mean Toxicokinetic Parameters for PF-04971729 in Timed-Pregnant New Zealand White Rabbits after Oral Administration of PF-04971729 on (Gestational Day 19)

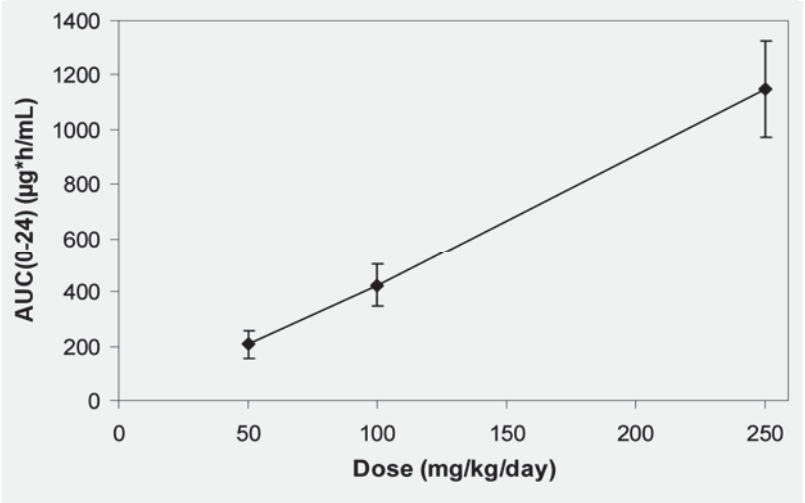
Dose (mg/kg/day)	Gestational Day	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
		Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
50	19	22.5	5.91	5	2.40	0.894	5	207	51.6	5
100		46.0	7.67	5	2.40	0.894	5	424	77.6	5
250		115	14.9	4	4.00	0	4	1150	177	4

Mean (± SD) PF-04971729 Concentration in Timed-Pregnant New Zealand White Rabbits versus Time after Oral Administration of PF-04971729 on (Gestational Day 19)



Mean PF-04971729 C_{max} and AUC_{0-24} versus Dose in Timed-Pregnant New Zealand White Rabbits after Oral Administration of PF-04971729 at 50, 100, or 250 mg/kg/day on (Gestational Day 19)





Reproductive and Postmortem Examinations

Gestation Day 29 Cesarean Sections

Mean Cesarean Section Values

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	19	20	17
Corpora Lutea	Total	188	190	190	164
No. per animal	Mean	9.9s	10.0	9.5	9.6
	S.d.	1.8	1.5	2.0	1.4
Implantation Sites	Total	180	181	164	152
No. per animal	Mean	9.5s	9.5	8.2	8.9
	S.d.	1.7	1.9	1.9	1.8
Preimplantation Loss	Total	8	9	26	12
No. per animal	Mean	0.4s	0.5	1.3	0.7
	S.d.	0.5	1.0	1.5	1.3
% per animal	Mean	4.1s	5.1	13.0	7.3
	S.d.	5.0	11.7	14.0	13.8
Fetuses	Total	179	167	160	143
No. per animal	Mean	9.4s	8.8	8.0	8.4
	S.d.	1.7	2.9	1.8	1.8
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	Total	179	167	160	143
No. per animal	Mean	9.4s	8.8	8.0	8.4
	S.d.	1.7	2.9	1.8	1.8
Malformed Fetuses (External)	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Dead Fetuses	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
% per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Early Resorption	Total	1	12	2	6
No. per animal	Mean	0.1s	0.6	0.1	0.4
	S.d.	0.2	2.3	0.3	0.8
% per animal	Mean	0.5s	6.4	1.2	3.7
	S.d.	2.3	22.9	3.8	8.6
Late Resorption	Total	0	2	2	3
No. per animal	Mean	0.0s	0.1	0.1	0.2
	S.d.	0.0	0.3	0.3	0.4
% per animal	Mean	0.0s	1.1	1.0	1.8
	S.d.	0.0	3.2	2.9	4.1
Not Applicable for Pfizer DART Studies	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Postimplantation Loss	Total	1	14	4	9
No. per animal	Mean	0.1s	0.7	0.2	0.5*
	S.d.	0.2	2.3	0.4	0.8
% per animal	Mean	0.5s	7.5	2.2	5.6*
	S.d.	2.3	22.8	4.5	8.7

Pre-implantation Loss = Corpora Lutea - Implantation Sites
Post-implantation Loss = Early/Late resorptions + Dead Fetuses

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Pregnant, used for calculation	N	19	19	20	17
Affected Implants	Total	1	14	4	9
No. per animal	Mean	0.1s	0.7	0.2	0.5*
	S.d.	0.2	2.3	0.4	0.8
% per animal	Mean	0.5s	7.5	2.2	5.6*
	S.d.	2.3	22.8	4.5	8.7

Affected Implants = Early/Late resorptions + Dead Fetuses + Malformed Fetuses (External)

A statistically significant increase in post-implantation loss was noted at 250 mg/kg/day (0.5, 7.5, 2.2, and 5.6% per animal at 0, 50, 100, and 250 mg/kg/day, respectively). The total number of live fetuses decreased with dose (179, 167, 160 and 143, respectively).

The historically maximum rate of post-implantation loss per animal in this laboratory was reported to be 8.09%. The increased loss observed at 250 mg/kg likely represents normal biological variation, although it should be noted that all dosed groups presented with an increased incidence of post-implantation loss relative to controls.

Fetal Observations/Measurements

Fetal Body Weights

There were no clear drug effects on fetal weights at necropsy, although fetuses obtained from does dosed at 250 mg/kg weighed the least of all groups.

Mean Fetal Body Weights (g)

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Litters, used for calculation	N	19	18	20	17
Fetus Weight	Mean	41.7n	41.4	43.2	41.0
	S.d.	4.3	5.1	4.1	3.7
	Deviation Vs Control		-0.6	3.7	-1.6

Fetal External Exams and Gender Ratios

The only external finding was noted in a single control fetus (Forelimb Hyperflexion).

Gender Ratios

		0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Litters, used for calculation	N	19	18	20	17
Males	Total	80	76	82	68
No. per animal	Mean	4.2s	4.0	4.1	4.0
	S.d.	1.8	2.0	1.6	2.4
Females	Total	99	91	78	75
No. per animal	Mean	5.2s	4.8	3.9*	4.4
	S.d.	1.6	2.2	1.4	2.0
% of Males per animal	Mean	44.5s	46.0	51.1	46.0
	S.d.	15.8	19.6	16.0	22.5

Fetal Visceral Exams

Fetal Visceral Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Total CS Visceral Observation	Litters Affected	N	11	f	8	8	14
		%	57.9		44.4	40.0	82.4
	Fetuses Affected	N	23		18	13	33
	% per Litter	Mean	13.0	s	11.8	7.0	21.8
Aortic arch	Litters Affected	N	0	f	1	0	1
		%	0		5.6	0	5.9
	Fetuses Affected	N	0		1	0	1
	% per Litter	Mean	0.0	s	0.6	0.0	0.7
Dilated (M)	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.9
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.7
Narrowed (M)	Litters Affected	N	0	f	1	0	0
		%	0		5.6	0	0
	Fetuses Affected	N	0		1	0	0
	% per Litter	Mean	0.0	s	0.6	0.0	0.0
Brain *Dilated cerebral ventricle (M)*	Litters Affected	N	0	f	1	0	0
		%	0		5.6	0	0
	Fetuses Affected	N	0		1	0	0
	% per Litter	Mean	0.0	s	0.6	0.0	0.0
Carotid artery *Left from innominate (V)*	Litters Affected	N	1	f	4	1	1
		%	5.3		22.2	5.0	5.9
	Fetuses Affected	N	1		4	2	1
	% per Litter	Mean	0.5	s	3.1	0.9	0.6
Gallbladder	Litters Affected	N	6	f	1	1 *	5
		%	31.6		5.6	5.0	29.4
	Fetuses Affected	N	7		2	2	15
	% per Litter	Mean	3.6	s	1.1	1.0	9.3
Absent (V)	Litters Affected	N	1	f	1	0	3
		%	5.3		5.6	0	17.6
	Fetuses Affected	N	1		1	0	5
	% per Litter	Mean	0.6	s	0.6	0.0	3.2

V = Variation, M = Malformation

Fetal Visceral Evaluations

			0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179	167	160	143
Litters evaluated		N	19	18	20	17
Small (V)	Litters Affected	N	5 f	1	1	5
		%	26.3	5.6	5.0	29.4
	Fetuses Affected	N	6	1	2	10
	% per Litter	Mean	3.0 s	0.6	1.0	6.2
Heart	Litters Affected	N	1 f	2	0	1
		%	5.3	11.1	0	5.9
	Fetuses Affected	N	1	4	0	1
	% per Litter	Mean	0.7 s	2.2	0.0	0.7
Hydropericardium (M)	Litters Affected	N	1 f	1	0	0
		%	5.3	5.6	0	0
	Fetuses Affected	N	1	3	0	0
	% per Litter	Mean	0.7 s	1.7	0.0	0.0
Membranous ventricular septum defect (M)	Litters Affected	N	0 f	1	0	0
		%	0	5.6	0	0
	Fetuses Affected	N	0	1	0	0
	% per Litter	Mean	0.0 s	0.6	0.0	0.0
Muscular ventricular septum defect (M)	Litters Affected	N	0 f	0	0	1
		%	0	0	0	5.9
	Fetuses Affected	N	0	0	0	1
	% per Litter	Mean	0.0 s	0.0	0.0	0.7
Innominate *Absent (V)*	Litters Affected	N	2 f	0	0	1
		%	10.5	0	0	5.9
	Fetuses Affected	N	2	0	0	1
	% per Litter	Mean	1.0 s	0.0	0.0	0.6
Kidney	Litters Affected	N	0 f	1	0	1
		%	0	5.6	0	5.9
	Fetuses Affected	N	0	1	0	1
	% per Litter	Mean	0.0 s	0.7	0.0	0.7
Dilated renal pelvis (V)	Litters Affected	N	0 f	0	0	1
		%	0	0	0	5.9
	Fetuses Affected	N	0	0	0	1
	% per Litter	Mean	0.0 s	0.0	0.0	0.7

			0 mg/kg F	50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179	167	160	143
Litters evaluated		N	19	18	20	17
Malpositioned (M)	Litters Affected	N	0 f	1	0	0
		%	0	5.6	0	0
	Fetuses Affected	N	0	1	0	0
	% per Litter	Mean	0.0 s	0.7	0.0	0.0
Small (M)	Litters Affected	N	0 f	1	0	0
		%	0	5.6	0	0
	Fetuses Affected	N	0	1	0	0
	% per Litter	Mean	0.0 s	0.7	0.0	0.0
Lung	Litters Affected	N	4 f	2	1	5
		%	21.1	11.1	5.0	29.4
	Fetuses Affected	N	5	5	3	7
	% per Litter	Mean	3.1 s	3.2	1.7	4.8
Small (M)	Litters Affected	N	0 f	1	0	0
		%	0	5.6	0	0
	Fetuses Affected	N	0	1	0	0
	% per Litter	Mean	0.0 s	0.7	0.0	0.0
Absent post caval lobe (V)	Litters Affected	N	4 f	1	1	5
		%	21.1	5.6	5.0	29.4
	Fetuses Affected	N	5	4	3	7
	% per Litter	Mean	3.1 s	2.5	1.7	4.8
Ovary *Hemorrhagic (V)*	Litters Affected	N	2 f	0	0	1
		%	10.5	0	0	5.9
	Fetuses Affected	N	3	0	0	1
	% per Litter	Mean	1.9 s	0.0	0.0	0.6
Pulmonary trunk	Litters Affected	N	0 f	1	0	1
		%	0	5.6	0	5.9
	Fetuses Affected	N	0	1	0	1
	% per Litter	Mean	0.0 s	0.6	0.0	0.7
Dilated (M)	Litters Affected	N	0 f	1	0	0
		%	0	5.6	0	0
	Fetuses Affected	N	0	1	0	0
	% per Litter	Mean	0.0 s	0.6	0.0	0.0

V = Variation, M = Malformation

Fetal Visceral Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Narrowed (M)	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.9
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.7
Subclavian artery *Retroesophageal right (V)*	Litters Affected	N	1	f	0	0	1
		%	5.3		0	0	5.9
	Fetuses Affected	N	1		0	0	1
	% per Litter	Mean	0.4	s	0.0	0.0	0.7
Thymus *Hemorrhagic (V)*	Litters Affected	N	1	f	0	1	0
		%	5.3		0	5.0	0
	Fetuses Affected	N	2		0	2	0
	% per Litter	Mean	0.9	s	0.0	1.2	0.0
Ureter *Retrocaval (V)*	Litters Affected	N	2	f	3	3	3
		%	10.5		16.7	15.0	17.6
	Fetuses Affected	N	2		3	3	7
	% per Litter	Mean	1.2	s	2.0	1.7	5.2
Veins *Supernumerary renal (V)*	Litters Affected	N	2	f	1	1	1
		%	10.5		5.6	5.0	5.9
	Fetuses Affected	N	2		1	1	1
	% per Litter	Mean	1.1	s	0.6	0.5	0.6

V = Variation, M = Malformation

One 250 mg/kg/day fetus (#79-8) presented with 3 related malformations (muscular ventricular septum defect, dilated aortic arch, narrowed pulmonary trunk). These observations are similar to the visceral malformations noted in the rat embryo fetal development study at the same dose.

Visceral variations noted to occur at a higher incidence at 250 mg/kg/day than in the control or lower dose groups were absent gallbladder (0.6, 0.6, 0, and 3.2% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) and small gallbladder (3.0, 0.6, 1.0, and 6.2% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively). The incidences of these variations in the gall bladder are above the sponsor's maximum historical control data and are therefore considered related to dosing by this reviewer. The sponsor feels that these changes are representative of normal biological variation and unrelated to dosing. **Note:** An increased incidence of gallbladder vacuolation was observed in the 1 month repeat dose dog toxicity study (09GR184) at doses ≥ 10 mg/kg/day (74.4 µg.hr/mL; AUC_{0-24} and 11.1 µg/mL; C_{max}).

A retrocaval ureter variation (1.2, 2.0, 1.7, and 5.2% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) occurred in 3 litters and 7 fetuses at the 250 mg/kg dose. The historical control incidence (maximum rate for retrocaval ureter) is 4.2% fetuses. This variation appears to be related to dosing. The sponsor feels that this incidence is comparable to the historical control value (5.2% vs. 4.2%) and unrelated to dosing.

Absent postcaval lung lobe (3.1, 2.5, 1.7, and 4.8% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) occurred in 5 litters and 7 fetuses. The historical control incidence (maximum rate for absent postcaval lung lobe) is 11.9% fetuses. This variation is likely related to normal biological variation.

Additional, visceral variations noted to occur at a higher incidence at 250 mg/kg/day in single fetuses were: dilated renal pelvis (0, 0, 0, and 0.7% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) and retroesophageal subclavian artery (0.4, 0, 0, and 0.7% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively).

Fetal Skeletal Exams

Fetal Skeletal Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Total CS Skeletal Observation	Litters Affected	N	17	f	17	19	16
		%	89.5		94.4	95.0	94.1
	Fetuses Affected	N	64		68	54	56
	% per Litter	Mean	35.3	n	39.5	33.9	39.5
Caudal centrum *Misaligned (V)*	Litters Affected	N	1	f	2	1	4
		%	5.3		11.1	5.0	23.5
	Fetuses Affected	N	2		3	1	4
	% per Litter	Mean	1.0	s	1.7	0.6	2.4
Cervical centrum *Supernumerary (M)*	Litters Affected	N	0	f	1	0	1
		%	0		5.6	0	5.9
	Fetuses Affected	N	0		1	0	1
	% per Litter	Mean	0.0	s	0.5	0.0	0.8
Forepaw phalanx *Unossified (V)*	Litters Affected	N	8	f	8	8	12
		%	42.1		44.4	40.0	70.6
	Fetuses Affected	N	17		17	15	31
	% per Litter	Mean	9.7	s	9.0	9.8	23.2
Hyoid *Bent arch (V)*	Litters Affected	N	3	f	3	7	4
		%	15.8		16.7	35.0	23.5
	Fetuses Affected	N	3		4	8	5
	% per Litter	Mean	1.6	s	2.8	6.1	3.2
Interparietal	Litters Affected	N	0	f	0	1	1
		%	0		0	5.0	5.9
	Fetuses Affected	N	0		0	1	1
	% per Litter	Mean	0.0	s	0.0	0.5	0.6
Absent (M)	Litters Affected	N	0	f	0	1	0
		%	0		0	5.0	0
	Fetuses Affected	N	0		0	1	0
	% per Litter	Mean	0.0	s	0.0	0.5	0.0

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Misshapen (M)	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.9
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.6
Lumbar centrum *Hemicentric (M)*	Litters Affected	N	0	f	0	1	0
		%	0		0	5.0	0
	Fetuses Affected	N	0		0	1	0
	% per Litter	Mean	0.0	s	0.0	0.8	0.0
Metacarpal *Unossified (V)*	Litters Affected	N	5	f	4	3	2
		%	26.3		22.2	15.0	11.8
	Fetuses Affected	N	9		7	4	4
	% per Litter	Mean	4.7	s	3.6	2.1	2.3
Rib	Litters Affected	N	4	f	5	2	1
		%	21.1		27.8	10.0	5.9
	Fetuses Affected	N	5		6	2	1
	% per Litter	Mean	2.7	s	4.2	1.5	0.8
7th Cervical (V)	Litters Affected	N	4	f	3	1	0
		%	21.1		16.7	5.0	0
	Fetuses Affected	N	5		4	1	0
	% per Litter	Mean	2.7	s	3.1	0.8	0.0
Detached (M)	Litters Affected	N	0	f	1	0	0
		%	0		5.6	0	0
	Fetuses Affected	N	0		1	0	0
	% per Litter	Mean	0.0	s	0.7	0.0	0.0

V = Variation, M = Malformation

Fetal Skeletal Evaluations

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Fused (M)	Litters Affected	N	0	f	1	1	1
		%	0		5.6	5.0	5.9
	Fetuses Affected	N	0		1	1	1
	% per Litter	Mean	0.0	s	0.5	0.6	0.8
Short (V)	Litters Affected	N	0	f	0	0	1
		%	0		0	0	5.9
	Fetuses Affected	N	0		0	0	1
	% per Litter	Mean	0.0	s	0.0	0.0	0.8
Skull *Extra ossification site (V)*	Litters Affected	N	1	f	4	4	1
		%	5.3		22.2	20.0	5.9
	Fetuses Affected	N	1		8	4	1
	% per Litter	Mean	0.5	s	4.9	2.6	0.7
Sternebra	Litters Affected	N	13	f	12	15	11
		%	68.4		66.7	75.0	64.7
	Fetuses Affected	N	37		33	31	21
	% per Litter	Mean	20.3	s	18.4	18.5	14.0
Extra ossification site (V)	Litters Affected	N	1	f	0	0	2
		%	5.3		0	0	11.8
	Fetuses Affected	N	1		0	0	3
	% per Litter	Mean	0.7	s	0.0	0.0	2.3
Fused (M)	Litters Affected	N	5	f	2	4	3
		%	26.3		11.1	20.0	17.6
	Fetuses Affected	N	11		2	9	3
	% per Litter	Mean	5.6	s	1.0	4.8	2.1

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Misaligned (V)	Litters Affected	N	1	f	1	1	1
		%	5.3		5.6	5.0	5.9
	Fetuses Affected	N	1		1	1	1
	% per Litter	Mean	0.6	s	0.6	0.6	0.7
Unossified #5 and/or #6 (V)	Litters Affected	N	10	f	11	12	7
		%	52.6		61.1	60.0	41.2
	Fetuses Affected	N	27		31	22	15
	% per Litter	Mean	15.3	s	17.4	13.8	9.7
Talus *Unossified (V)*	Litters Affected	N	1	f	0	0	0
		%	5.3		0	0	0
	Fetuses Affected	N	1		0	0	0
	% per Litter	Mean	0.4	s	0.0	0.0	0.0
Thoracic arch *Absent (M)*	Litters Affected	N	0	f	2	2	0
		%	0		11.1	10.0	0
	Fetuses Affected	N	0		2	2	0
	% per Litter	Mean	0.0	s	1.2	1.5	0.0
Thoracic centrum	Litters Affected	N	0	f	2	1	0
		%	0		11.1	5.0	0
	Fetuses Affected	N	0		2	1	0
	% per Litter	Mean	0.0	s	1.2	0.6	0.0
Absent (M)	Litters Affected	N	0	f	1	1	0
		%	0		5.6	5.0	0
	Fetuses Affected	N	0		1	1	0
	% per Litter	Mean	0.0	s	0.5	0.6	0.0

			0 mg/kg F		50 mg/kg F	100 mg/kg F	250 mg/kg F
Fetuses Examined		N	179		167	160	143
Litters evaluated		N	19		18	20	17
Hemicentric (M)	Litters Affected	N	0	f	1	0	0
		%	0		5.6	0	0
	Fetuses Affected	N	0		1	0	0
	% per Litter	Mean	0.0	s	0.7	0.0	0.0

V = Variation, M = Malformation

Skeletal malformations were noted in 11, 5, 12, and 5 fetuses at 0, 50, 100, and 250 mg/kg/day, respectively. Skeletal malformations that occurred at a higher incidence in the 250 mg/kg/day group than in the control and lower dose groups were supernumerary cervical centrum (0, 0.5, 0, and 0.8% fetuses/litter) and fused rib (0, 0.5, 0.6, and 0.8% fetuses/litter) at 0, 50, 100, and 250 mg/kg/day, respectively). These two malformations occurred in the same fetus. Misshapen interparietal bone (0, 0, 0, and 0.6% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) was noted in a single fetus from a separate litter. Based on the increased incidence of these malformations at the 250 mg/kg dose, the lack of comparative historical control data and the known effects of SGLT2 inhibitors on the bone these findings are considered dosing related.

The sponsor stated that these skeletal malformations were common to rabbits (no historical data was provided in this case). In addition, because these findings were noted only for single fetuses they can be attributed to normal biological variation.

Skeletal variations were noted for 57, 66, 44, and 55 fetuses in the 0, 50, 100, and 250 mg/kg/day groups, respectively.

Sternebra with an extra ossification site (0.7, 0, 0, and 2.3% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) was noted for 1 fetus in the control group and 3 fetuses in the 250 mg/kg/day group (2 separate litters). This variation has not been noted previously in the sponsor's historical control database for rabbits. Sternebra with an extra ossification site was noted in the rat embryo fetal development study at the same dose.

Based on the increased incidence of an extra ossification site of the sternebra at the 250 mg/kg dose, the absence of this finding in rabbit historical control data and the similarity to the findings in the rat reproductive toxicity study, this variation is considered related to dosing by the reviewer. The sponsor feels that because of the low number of fetuses with this finding, the lack of increase in related findings, and the lack of an increase in the overall number of fetuses with skeletal variations at 250 mg/kg/day, the increased number of fetuses with extra ossification site in the sternebra is considered to represent normal biological variation and not related to dosing.

Skeletal variations that occurred at a higher incidence at 250 mg/kg/day than in the control and lower dose were misaligned caudal centrum (1.0, 1.7, 0.6, and 2.4% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively); unossified forepaw phalanx (9.7, 9.0, 9.8, and 23.2% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively) short rib (0, 0, 0, and 0.8% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively); and misaligned sternebra (0.6, 0.6, 0.6, and 0.7% fetuses/litter at 0, 50, 100, and 250 mg/kg/day, respectively). Based on historical control data (4.0, 26.8, 1.2, and 1.3% fetuses for misaligned caudal centrum, unossified forepaw phalanx, short rib, and misaligned sternebra, respectively), it is likely that these variations are unrelated to dosing.

2.6.6.8 SPECIAL TOXICOLOGY STUDIES

No phototoxicity studies have been conducted as UV-visible spectral analysis of PF-04971729 indicated that the compound has a shoulder at 290 nM with a molar extinction coefficient (MEC) of less than 1000 L/mol.cm.

2.6.7 TOXICOLOGY TABULATED SUMMARY

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES (SEGMENT II)			
SPECIES/ STUDY (EXPOSURE)	NOAEL	MULTIPLE OF MRHD (AUC*)	BASIS
Rat GD-6 to GD-17 (GLP) Dose: 50, 100, 250 mg/kg Co-crystalline Drug Form Average exposure: 199, 457, 975 µg.hr mL	**Maternal** 100 mg/kg (457 µg.hr/mL) **Developmental** 100 mg/kg (457 µg.hr/mL)	**Maternal** **163x** **Developmental** **163x**	**Maternal** **250 mg/kg:** ↓BW and ↓BWG ↓FC, ↑ Early Resorptions ↑Post Implantation Loss and ↓ Litter Size **Developmental** **≥ 50 mg/kg:** Skeletal Variations **≥ 100 mg/kg:** Visceral Malformations Visceral Variations **250 mg/kg:** External Malformations Skeletal Malformations
Rabbit GD-7 to GD-19 (GLP) Dose: 50, 100, 250 mg/kg Co-crystalline Drug Form Average exposure: 207, 424, 1150 µg.hr mL	**Maternal** Not Determined **Developmental** 100 mg/kg (424 µg.hr/mL)	**Maternal** **< 74x** **Developmental** **151x**	**Maternal** **250 mg/kg:** ↓BW, ↓BWG and ↓FC ↑Post Implantation Loss ↑Aborted Pregnancy **Developmental** **≥ 50 mg/kg:** ↓ # of Live Fetuses (Maternal Toxicity) **250 mg/kg:** Visceral Malformations Visceral Variations Skeletal Malformations Skeletal Variations

*AUC in human: 2.8µg.hr/mL at 25 mg/kg/day – MRHD

(Previous Review) Toxicology Studies – 3 Month			
Species/ Study (Exposure)	**NOAEL**	**Multiple of MRHD (AUC*)**	**Basis**
Rat Duration: 3 Month (GLP) Dose: 5, 25, 250 mg/kg Co-crystalline Drug Form Average exposure: 19.9, 89.4, 738 µg.hr mL	**Not Determined**	LOAEL: 7x	**≥ 5 mg/kg:** Pelvic Tubule Dilatation Mineral Deposition M: GI Tract Dilatation ↑Adrenal Weight Adrenal Histopath **≥ 25 mg/kg:** ↓Prostate Weight Inflammation Stomach Erosion Ulcer M: Hyperostosis F: GI Tract Dilatation **250 mg/kg:** Pelvic/Bladder Hyperplasia ↑Severity CPN Heart Myonecrosis F: Hyperostosis
Dog Duration: 3 Month (GLP) Dose: 1, 10, 150 mg/kg Co-crystalline Drug Form Average exposure: 9.79, 91.9, 1100 µg.hr mL	**10 mg/kg** Ave: (91.9µg.hr/mL) M: 74.8µg.hr/mL F: 109µg.hr/mL	33x	**≥ 1 mg/kg:** Glycogen Depletion (Liver) **≥ 150 mg/kg:** Adverse GI (exceeding MTD) (Vomiting) (Diarrhea) (Liquid/Mucoid feces) (Lack of BW gain) Liver Cell Necrosis (Females)

*AUC in human: 2.8µg.hr/mL at 25 mg/kg/day – MRHD

(Previous Review) Toxicology Studies (1 Week and 4 Week)			
Species/ Study	**NOAEL**	**Multiple of MRHD (AUC)***	**Basis**
Rat 1 Week (non-GLP) 5, 50, 500 mg/kg M:17, 99, 1500 µg.hr/mL Amorphous Drug Form	50 mg/kg (99 µg.hr/mL)	(35x)	500 mg/kg: Loose Stool ↓BW ↓WBCs Histological changes Pancreas and Liver
4 Week (GLP) 5, 25, 500→(D11) 250 mg/kg M: 8, 69, 541 µg.hr/mL F: 15, 93, 718 µg.hr/mL Co-crystalline Drug Form	25 mg/kg (81µg.hr/mL) (M/F average)	(29x)	500→ (D11) 250 mg/kg: Mortality, ↑ severity CPN, stomach erosion / squamous hyperplasia.
Dog Single Dose (non-GLP) 5, 50, 500 mg/kg M: 27, 386, 138 µg.hr/mL F: 51, 474, 465 µg.hr/mL Amorphous Drug Form	M:LOAEL 500 mg/kg F:50 mg/kg (474 µg.hr/mL)	(169x)	500 mg/kg: Vomiting M/F 50 mg/kg: Salivation F
1 Week (non-GLP) 5, 50, 250→(D3) 150 mg/kg M: 55, 373, 1150 µg.hr/mL F: 63, 627, 789 µg.hr/mL Amorphous Drug Form	50 mg/kg (500µg.hr/mL) (M/F average)	(179x)	250→(D3) 150 mg/kg: Vomiting and soft mucoid and watery feces
1 Week (non-GLP) 5, 50, 150 mg/kg M: 77, 660, 679 µg.hr/mL F: 59, 834, 511 µg.hr/mL Co-crystalline Drug Form	50 mg/kg (750µg.hr/mL) (M/F average)	(268x)	150 mg/kg: Vomiting
4 Week (GLP) 1, 10, 150 mg/kg M: 7, 77, 1050 µg.hr/mL F: 8, 71, 1170 µg.hr/mL Co-crystalline Drug Form	1 mg/kg 8 µg.hr/mL) (M/F average)	(3x)	≥10 mg/kg: Gallbladder Vacuolation. 150 mg/kg: Renal tubular Degeneration, emesis, salivation, soft/watery feces.

*AUC in human: 2.8µg.hr/mL at 25 mg/kg/day – MRHD

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Reproductive and Developmental Toxicity:

The effects of PF-04971729 (b) (4) on embryo-fetal development were assessed in definitive segment II studies in rats and rabbits. An adequate number of timed pregnant females were dosed at 50, 100 and 250 mg/kg and were assessed for changes in clinical signs, body weight, food consumption and reproductive function. Minimal maternal toxicity was observed in the rat at 250 mg/kg. Maternal toxicity occurred at all doses in the rabbit. Necropsy evaluations satisfactorily addressed embryonic development/fetal death, altered fetal development/growth and structural changes incited by PF-04971729 (b) (4).

Teratogenic Findings

(External Malformations)

Two rat fetuses at the 250 mg/kg dose were noted with external malformations; 1 with omphalocele and one with ectrodactyly and short tail. Maternal toxicity at this dose was minimal as demonstrated by an increased weight loss at the initiation of dosing (likely do to in palatability of PF-04971729 (b) (4)) that was associated with a decrease in food consumption. Uterine and fetal weights were not significantly reduced at the 250 mg/kg dose, although an increase in post implantation loss secondary to an increase in early resorptions and subsequent reduction in live litter size was noted. While external malformations were limited to two animals, a drug related effect cannot be excluded, as these malformations were confined to the high dose group where only minimal maternal toxicity was observed.

(Visceral Malformations)

Visceral malformations noted in the rat at 250 mg/kg were right sided aortic arch and membranous ventricular septum defect. It is difficult to assess if the right sided aortic arch finding was related to PF-04971729 (b) (4) dosing as there was no increase in the incidence and only a single occurrence was present in the 100 mg/kg and 250 mg/kg groups. The incidence of the membranous ventricular septum defect at 250 mg/kg was increased with PF-04971729 (b) (4) dosing and occurred at a frequency above historical control data. The sponsor recognized the membranous ventricular septum defects as being related to PF-04971729 (b) (4) dosing.

One rabbit fetus at the 250 mg/kg dose presented with 3 related visceral malformations (muscular ventricular septum defect, dilated aortic arch, narrowed pulmonary trunk). These observations in the rabbit are similar to the visceral malformations noted in the rat embryo fetal development study at the same dose. Rabbit maternal toxicity at the 250 mg/kg dose was demonstrated by spontaneous abortions (3) with severe decreases in body weight and/or body weight gain and food consumption. Uterine and fetal weights were not significantly reduced at the 250 mg/kg dose, although an increase in post implantation loss and subsequent reduction in live litter size were noted.

(Skeletal Malformations)

Skeletal malformations that occurred in rats at a higher incidence at the 250 mg/kg dose were absent metacarpal, fused sternebra and hemicentric thoracic centrum. The fused sternebra and a single incidence of the hemicentric thoracic centrum malformation occurred in unique litters. The absent metacarpal and the remaining incidence of hemicentric thoracic centrum occurred in the same litter but in different fetuses. The absence of these skeletal malformations in control animals and the minimal maternal toxicity at this dose make it difficult to exclude these effects from being related to dosing.

Skeletal malformations that occurred in rabbits at a higher incidence in the 250 mg/kg group were supernumerary cervical centrum and fused rib (Same Fetus). Misshapen interparietal bone was noted in a single fetus from a separate litter. Malformations that occurred in these fetuses are likely a consequence of maternal toxicity.

Non-Teratogenic Findings

(Visceral Variations)

Visceral variations noted in rats at the 250 mg/kg dose were limited to hemorrhagic adrenal and absent innominate artery. The significance of the hemorrhagic adrenal variation is unclear because the finding was limited to a single fetus. The absent innominate artery variation occurred in a dose dependent manner, exceeded the historical control incidence at the 250 mg/kg dose and is likely related to PF-04971729 (b) (4) dosing.

In the rabbit, dosing related visceral variations noted to occur at a higher incidence in the 250 mg/kg group were absent gallbladder and small gallbladder. In addition, an increased incidence of retrocaval ureter variation occurred in 3 litters and 7 fetuses at the 250 mg/kg dose. While the noted variations are directly related to the administration of PF-04971729 (b) (4), severe maternal toxicity was observed at this dose.

(Skeletal Variations)

Skeletal variations that were significantly increased in rats at the 250 mg/kg dose included: unossified 7th cervical centrum, incomplete ossification of the thoracic centrum, vertebrae 27th presacral, full supernumerary ribs, short supernumerary ribs and unossified metatarsal. Most skeletal variations noted at doses < 250 mg/kg did not occur in a dose responsive manner and none were significantly different from concurrent controls.

The incidence of an extra ossification site of the sternebra increased at the 250 mg/kg dose and had not been noted previously in the sponsor's historical control database for rabbits. This skeletal variation occurred at the same dose in the rat embryo fetal development study in a single fetus and did not appear in control rats. While it is likely that this variation is the result of maternal toxicity in the rabbit, its presence in both non-clinical species at the same dose makes it worth noting here.

Safety Margins

(Rat)

Based on decreased body weight and increased incidence of early resorptions at 250 mg/kg/day the NOAEL for maternal toxicity was determined to be (100 mg/kg). External malformations, visceral malformations and skeletal malformations occurred in fetuses at 250 mg/kg/day. Based on these observations the NOAEL for developmental toxicity was determined to be (100 mg/kg - 457µg.hr/mL). This dose provides a safety margin (163X) to the maximum recommended human dose (b) (4)

(Rabbit)

Based on the increased incidence of spontaneous abortions (250 mg/kg), reductions in maternal body weight and body weight gain, the increased incidence of post-implantation loss and the decrease in the total number of live fetuses at all dose levels, a NOAEL for maternal toxicity was not identified in this study (< 74x MRHD). The NOAEL for developmental toxicity was determined to be (100 mg/kg - 424µg.hr/mL). This was based on observations at the 250 mg/kg dose that included significant increases in: visceral malformations, visceral variations, skeletal malformations and skeletal variations. This dose provides a safety margin (151X) to the maximum recommended human dose (b) (4)

Internal comments:

None

External comments (to sponsor):

None

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

/s/

JEFFREY A QUINN
05/23/2011

TODD M BOURCIER
05/23/2011

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

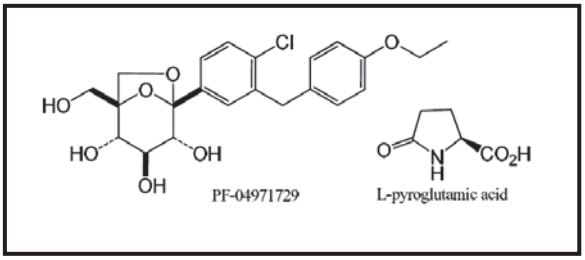
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 106447
Review number: 1
Sequence number/date/type of submission: SD1 / 29 September 2009 / Original IND
Information to sponsor: Yes (XX) No ( )
Sponsor and/or agent: Pfizer Global Research and Development
Manufacturer for drug substance: Pfizer Global Research and Development
Reviewer name: Jeffrey Quinn, Ph.D.
Division name: Metabolic and Endocrine Products
HFD #: 510
Review completion date: 22 October 2009

Drug:

Trade name:	N/A
Generic name:	N/A
Code name:	PF-04971729 or PF-04971729 (b) (4)
Chemical name:	(1*S*, 2*S*, 3*S*, 4*R*, 5*S*)-5-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-1-hydroxymethyl-6, 8-dioxabicyclo [3.2.1] octane-2, 3, 4-triol
CAS registry number:	N/A
Molecular formula/molecular weight:	PF-04971729 (amorphous form) $C_{22}H_{25}ClO_7$ / 436.88 Daltons PF-04971729 (b) (4) (L-pyroglutamic acid co-crystal form) $C_{27}H_{32}ClNO_{10}$ / 566.00 Daltons

Structure: The pharmaceutical preparation of PF-04971729 is an (b) (4) of the L-pyroglutamic acid co-crystal form.



Relevant INDs/NDAs/DMFs:

(b) (4)

Drug class: PF-04971729 is a sodium glucose co-transporter 2 (SGLT2) inhibitor.

Intended clinical population: Type 2 diabetics

Clinical formulation: The drug product is an (b) (4) oral solution/suspension accommodating administration of doses ranging from 0.3 to 300 mg active.

Impurities: Impurity (b) (4) was found to be (b) (4) % in the nonclinical Lot GR02546 and was not detected in clinical Lot GR02694. There are no other identified organic impurities for PF-04971729 (b) (4) drug substance.

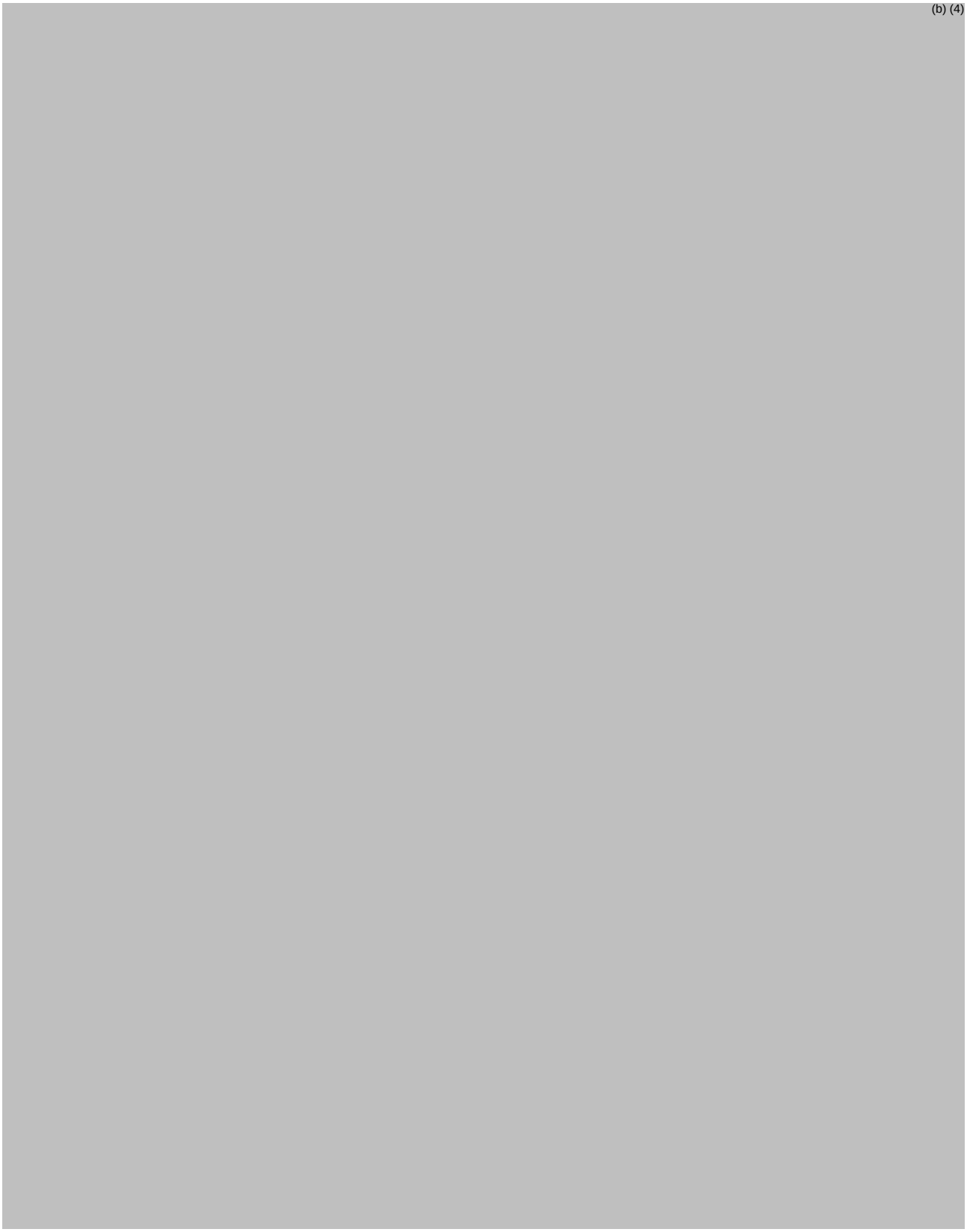
Organic:

Impurity (chemical name or code #)	Structure	Source
(b) (4)		

Inorganic: (b) (4) compounds are used in the (b) (4) process and are (b) (4).

(b) (4)

Excipients:



Route of administration: Oral

Proposed clinical protocols:

Study Title: A phase 1 placebo-controlled study to assess the safety, tolerability and pharmacokinetics of PF-04971729 after administration of single escalating oral doses under fed and fasted conditions in healthy volunteers.
Study ID: (B1521001)

Doses: A total of 6 dose levels (0.5, 2.5, 10, 30, 100 and 300 mg) plus placebo are planned along with addition of a cohort to study the effect of high-fat meal on the pharmacokinetics of PF-04971729.

Table 2. Dosing Schedule

			Week 1		Week 2		Week 3		Week 4
	Sequence	N	Period 1 (fasted)		Period 2 (fasted)		Period 3 (fasted)		Period 4 (fed)
Cohort 1	1	4	Placebo		10 mg		100 mg		100 mg
	2	4	0.5 mg		Placebo		100 mg		100 mg
	3	4	0.5 mg		10 mg		Placebo		100 mg
				Period 1 (fasted)		Period 2 (fasted)		Period 3 (fasted)	
Cohort 2	1	4		Placebo		30 mg		300 mg	
	2	4		2.5 mg		Placebo		300 mg	
	3	4		2.5 mg		30 mg		Placebo	

Study Participants: Lean and overweight (BMI: 18-30 kg/m2) otherwise healthy adult male and female subjects.
Protocol Summary: The study will be conducted over 4 treatment periods in Cohort 1 (n=12) and over 3 treatment periods in Cohort 2 (n=12). The 4th period of Cohort 1 will be dedicated to assess the effect of food on PF-04971729 pharmacokinetic parameters at one of the doses previously tested.
Primary Endpoints: Safety and tolerability of PF-04971729, PK, PD (Cumulative urinary glucose excretion over 24 hours).
Safety Measures: Adverse events, clinical chemistry, BP, PR, ECG, telemetry, urinalysis (drug and glucose), physical exam.

Previous Clinical Experience: None, this is a first-in-man trial

Proposed Clinical Studies in 1st Year Development: Following the completion of (B15210012) subsequent planned trials include:

1. Phase 1 Study (B1521002) - first multi-dose, dose-escalating trial in overweight and obese (BMI: 25-35 kg/m2) otherwise healthy adult subjects to assess the safety/tolerability, pharmacokinetics as well as effect on urinary glucose excretion (UGE) of a range of oral doses administered once daily for 14-days. A total of 4 dose levels plus placebo are planned based on results from B1521001.

2. Phase 1 Study (B1521003) - human ADME study to assess the absorption, distribution, metabolism and elimination characteristics of PF-04971729 using [^{14}C]-radiolabel. Healthy adult male subjects will be exposed to a single oral dose of [^{14}C] PF-04971729. The dose of PF-04971729 chosen will be dependent on the results from B1521001 and B1521002 and as such will be within the range of doses planned for Phase 2/3.

3. Phase 2 Study (B1521004) - 4-week study in subjects with T2DM, Stage 1-2 hypertension, creatinine clearance ≥ 50 mL/min and on either angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) to assess effect of PF-04971729 on blood pressure (assessed using ambulatory blood pressure monitoring) as well as renal function (assessed using CrCL, measurement of glomerular filtration rate ± exploratory markers of renal impairment). A total of 3 dose levels plus placebo are planned.

4. Phase 2 Study (B1521006) - 12-week study in subjects with T2DM not well controlled on a background of metformin; subjects will be randomized to receive 1 of 4 dosing regimens of PF-04971729 or placebo following a run-in period of 8-12 weeks during which time background agents for management of glycemic control as well as hypertension will be stabilized.

Sponsor's Predicted Efficacious Human Dose: Predicted efficacious steady state dose of 13 mg QD, the projected total C_{max} and AUC_{0-24} are 0.078µg/mL (0.005µg/mL free) and 1.13µg.h/mL (0.072µg.h/mL free), respectively.

Sponsor's Maximum Recommended Human Dose: The maximal proposed dose of 300 mg is 15-fold higher than the highest projected clinically efficacious dose (20 mg). The predicted human exposure at 300 mg is (C_{max} 1.390 µg/mL) and ($AUC_{0-\alpha}$ 27.2µg.h/mL).

The sponsor makes an additional note that the mean estimated total C_{max} and AUC_{0-24} within a cohort will not exceed 6.88µg/ml and 50.63µg.h/ml, respectively. These values will be utilized from here on as the MRHD.

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

TABLE OF CONTENTS

2.6.2 PHARMACOLOGY

Background: Pfizer submitted the original IND for PF-04971729 in September, 2009, for the treatment of type 2 diabetes mellitus. This will be the first time the drug has been given to humans. Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

2.6.2.1 Brief summary

PF-04971729 is a selective SGLT2 inhibitor that results acutely in a concentration-dependent glucosuria in rats. In vitro, PF-04971729 is a highly potent inhibitor of rat and human SGLT2 and possesses a high selectivity against glucose transport via human and rat SGLT1 and several other glucose transporters (GLUT1-4). Studies on the secondary pharmacology evaluated in vitro binding activity of PF-04971729 against a broad panel of receptors, transporters, ion channels, and enzyme assays, and the results indicated no significant inhibition (>50%) of binding or enzyme activity. Safety pharmacology studies that were conducted to assess potential pharmacodynamic effects of vital organ systems identified mild effects on the central nervous system, cardiovascular system and respiratory systems.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: PF-04971729 is a selective SGLT2 inhibitor. Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

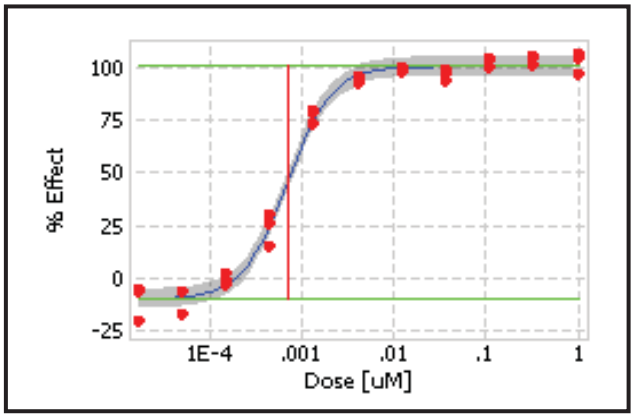
Drug activity related to proposed indication:

In vitro studies: A glucose transport functional assay was used to establish potency, selectivity and inhibitor classification of PF-04971729. CHO cells expressing human and rat isoforms of SGLT2 and SGLT1 were used in assays measuring the sodium dependent uptake of ^{14}C-labeled methyl-α-D glucopyranoside (AMG) a non-metabolizable form of glucose. Cells were treated with 3-fold incremental increasing concentrations of PF-04971729 prior to AMG addition to establish dose response inhibition curves for IC_{50} determination. PF-04971729 was identified to be a highly potent inhibitor of human SGLT2 mediated glucose transport with an IC_{50} of 0.877 ± 0.369 nM (n = 10). PF-04971729 showed very little inhibition of human SGLT1 mediated transport with an IC_{50} of 1960 ± 642 nM (n = 8). Similar potency and selectivity were seen in the rat SGLT assays (rat SGLT2: IC_{50} = 1.15 ± 0.289 nM, rat SGLT1: IC_{50} = 352 ± 54.2 nM, (n = 4).

See Sponsor's figures Below: PF-04971729 was determined to be a selective inhibitor of SGLT2 by displaying potent inhibition of AMG transport via SGLT2 and not SGLT1, and by competitive binding versus an established radio-labeled SGLT2 inhibitor. The treatment of CHO cells expressing hSGLT2 with an 11 point, three-fold incremental

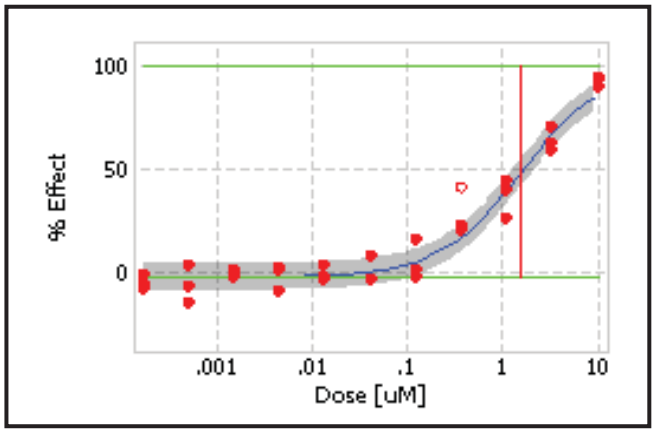
concentration curve for PF-04971729 resulted in a dose response inhibition of AMG transport with a maximum inhibition occurring around 10 nM.

Dose response inhibition of AMG transport in CHO cells expressing hSGLT2



Despite running a 10 fold higher concentration curve against the human SGLT1 expressing CHO cells, complete inhibition of AMG transport in these cells was only reached at 10 µM PF-04971729.

Dose response inhibition of AMG transport in CHO cells expressing hSGLT1



Calculating a geometric mean of the IC_{50}'s for 10 replications of the assay gave an IC_{50} value of 0.877 +/- 0.369 nM for inhibiting AMG transport via hSGLT2 and an IC_{50} of 1960 +/- 642 nM for hSGLT1.

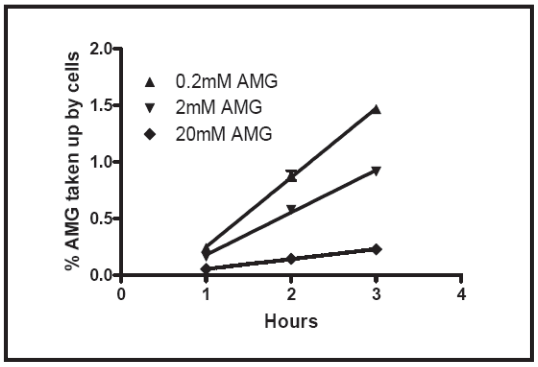
Summary of IC_{50} data for inhibition of AMG transport

AMG transport assay	IC_{50} Geometric Mean	95% Confidence Interval	IC_{50} Arithmetic Mean	Std. Dev.	Assay Replicates
Human SGLT2	0.877 nM	0.704 - 1.09 nM	0.927 nM	0.369 nM	10
Human SGLT1	1960 nM	1460 - 2620 nM	2050 nM	642 nM	8
Rat SGLT2	1.15 nM	0.757 - 1.74 nM	1.18 nM	0.289 nM	4
Rat SGLT1	352 nM	274 - 453	356 nM	54.2 nM	4

At physiological glucose concentrations, PF-04971729 maintained a high potency for inhibiting AMG transport in SGLT2 expressing cells with IC_{50} values of 1.20, 1.31, and 3.18 nM at AMG concentrations of 0.2, 2, and 20 mM respectively. The inhibition by PF-04971729 is competitive with glucose with an equilibrium dissociation constant (K_i) value of 1.42 ± 0.11 nM for human SGLT2 as determined by enzyme kinetics model comparison.

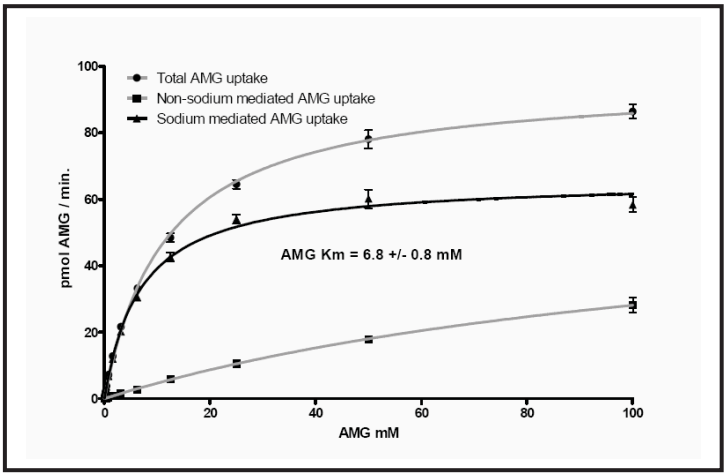
See Sponsor's figures Below: To determine the potency of PF-04971729 at higher concentrations of the AMG substrate and classify the mode of inhibition, a glucose transport function screening assay was performed. Measurements were being conducted in the linear range for AMG transport where the uptake of AMG into the cells stays linear from 1 to 3 hours of incubation at all the AMG concentrations tested.

Time course of AMG transport in SGLT2 expressing CHO cells



The 2 hour time point was selected for further experimentation. To determine the K_m for AMG, the AMG concentration was increased to 100 mM to ensure saturation of uptake. At this high concentration, transport via the endogenous transporter GLUT1 in the CHO cells would contribute to glucose uptake. To determine the rate of facilitative or GLUT1 mediated transport, a sodium free, choline-based buffer was used. By subtracting this background transport from the total AMG transport, a sodium-dependent AMG transport curve was established. The K_m value of AMG was determined to be 6.8 mM under these conditions.

AMG K_m determination for sodium-dependent transport



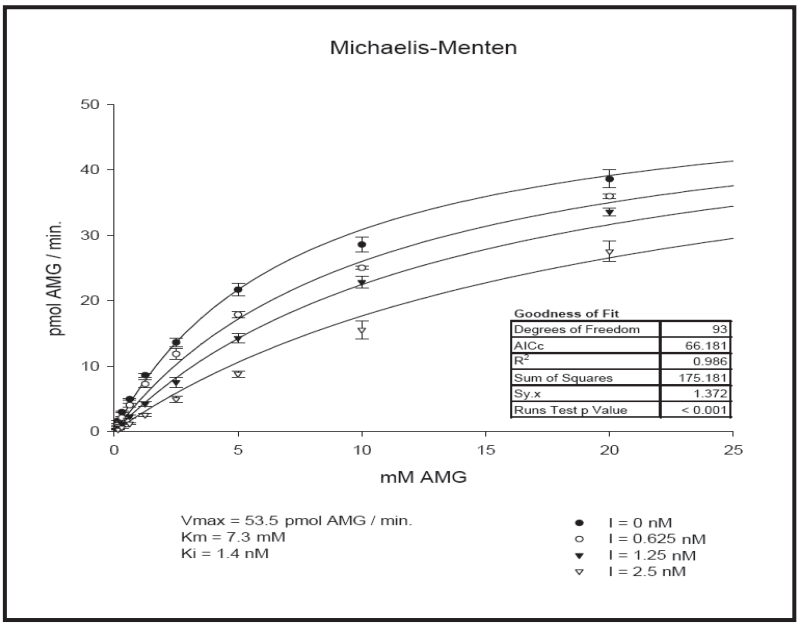
To test the potency of PF-04971729 at physiological glucose levels, the dose response for PF-04971729 was tested in the SGLT2 functional transport assay at 0.2, 2 and 20 mM AMG. PF-04971729 remained potent with average IC_{50} values of 1.20, 1.31 and 3.18 nM, respectively.

IC_{50} and fold change values for increasing AMG substrate concentrations

Exp. #	IC_{50} at 0.2 mM AMG	IC_{50} at 2 mM AMG	IC_{50} at 20 mM AMG
1	1.18 nM	1.30 nM	2.60 nM
2	1.25 nM	1.32 nM	2.74 nM
3	1.18 nM	not measured	4.21 nM
Average	1.20 nM	1.31 nM	3.18 nM
St. Dev.	0.04 nM	0.01 nM	0.89 nM

In order to determine the inhibition mode of PF-04971729, the AMG uptake was measured in the absence or in the presence of PF-04971729 at 0.625, 1.25, and 2.5 nM with varying concentrations of AMG between 0.16 and 20 mM. The resulting data were fit into Michaelis-Menton equations for different modes of inhibition, including competitive, uncompetitive, noncompetitive, and mixed modes, using Sigma Plot with the Enzyme Kinetics Module. The best fit of the data was obtained for a competitive mode of inhibition with a K_i value of 1.42 nM, indicating that PF-04971729 is a competitive inhibitor.

AMG dose response transport at 3 concentrations of PF-04971729 and goodness of fit to competitive inhibition model



Using the Cheng-Prusoff equation for a competitive inhibition: $IC_{50} = K_i (1+[S]/K_m)$, the K_i was calculated for the IC_{50}'s generated at the different AMG concentrations and the values were compared to the K_i calculated in the original screening assay run at 11 µM AMG. The K_i held constant at around 1 nM independent of AMG concentrations used.

Calculated K_i values for increasing AMG substrate concentrations

AMG (mM)	Mean IC50 (nM)	Calculated Ki (nM)	Replicate assays
0.011	0.877	0.876	10
0.2	1.20	1.17	3
2	1.31	1.01	2
20	3.18	0.808	3

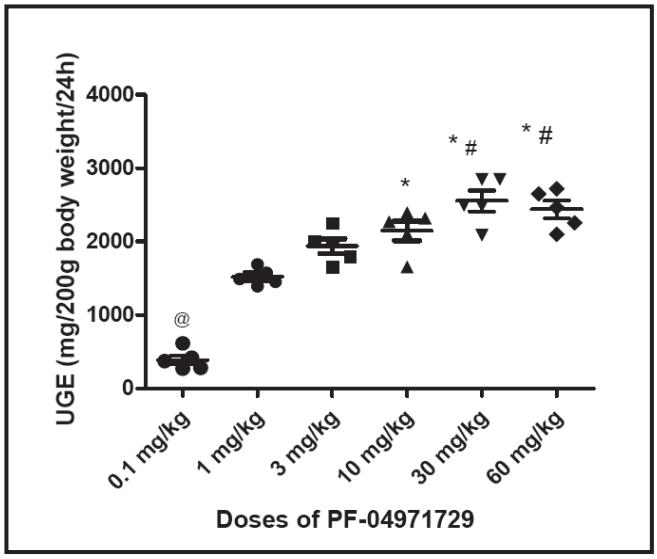
In vivo Studies:

Nonclinical efficacy in lowering plasma glucose levels was not evaluated in these studies. The *in vivo* pharmacology evaluation focused on the mechanism biomarker, urinary glucose excretion (UGE). The sponsor referenced others (Komoroski et al, 2009), who have demonstrated the mechanism biomarker relationship to the outcome biomarker for support of this methodology.

Studies in rats:

The *in vivo* efficacy of PF-04971729 was assessed by quantifying UGE in rats following oral administration. PF-04971729 administration resulted in a dose-dependent increase in urinary glucose excretion. Maximal urinary glucose excretion was reached at the dose of 30 mg/kg. The dose of 60 mg/kg did not result in a further increase in glucosuria. The vehicle group resulted in a urinary glucose excretion of 16.4 ± 4.69 mg/ 200g of body weight/24 h, which was found to be negligible compared to the PF-04971729 treated groups.

Effect of PF-04971729 on Urinary Glucose Excretion in Sprague Dawley Rats



Doses of 0.1, 1, 3, 10, 30 and 60 mg/kg PF-04971729 resulted in urinary glucose excretion of 389.0 ± 62.54, 1519 ± 52.02, 1937 ± 101.1, 2145 ± 132.3, 2554 ± 141.1, and 2437 ± 116.7 mg/ 200 g of body weight/ 24 h, respectively. PF-04971729 at a concentration of 1 µg/mL (equivalent to 2.3 µM) is highly bound (96%) to plasma proteins in rat with a free fraction (fu) of 0.040. Thus, the free AUC_{0-24} following doses of 0.1, 1, 3, 10, 30, and 60 mg/kg PF-04971729 are 0.00752, 0.0800, 0.283, 1.06, 4.28, 9.32 µg.h/mL, respectively.

Summary of Results from In Vivo Acute Studies with PF-04971729

Treatment group	Urinary glucose excretion (mg/ 200g of body weight/ 24h) ± SEM	Corresponding $AUC_{(0-24)}$ free (ng*h/mL)
0.1 mg/kg PO	389.0 ± 62.54	7.52
1 mg/kg PO	1519 ± 52.02	80.0
3 mg/kg PO	1937 ± 101.1	283
10 mg/kg PO	2145 ± 132.3	1056
30 mg/kg PO	2554 ± 141.1	4280
60 mg/kg PO	2437 ± 116.7	9320

SEM: Standard error of the mean

Mean Pharmacokinetic Parameters for PF-04971729 in Rats after Oral Administration of 0.1, 1, 3, 10, 30, and 60 mg/kg PF-04971729

PF-04971729 Dose (mg/kg)	(b) (4) Study ID	Total Cmax (ng/mL)	Tmax (h)	Total AUC(0-24) (ng*h/mL)	n
0.1	SGLT2-0051 rat PKPD	43.4	1.50	188	2
1	SGLT2-0035 PKPD	372	1.00	2000	2
3	SGLT2-0035 PKPD	1320	1.00	7080	2
10	SGLT2-0035 PKPD	3100	2.50	26400	2
30	SGLT2-0038 rat PKPD	10500	1.00	107000	2
60	SGLT2-0046 rat PKPD	25300	2.00	233000	2

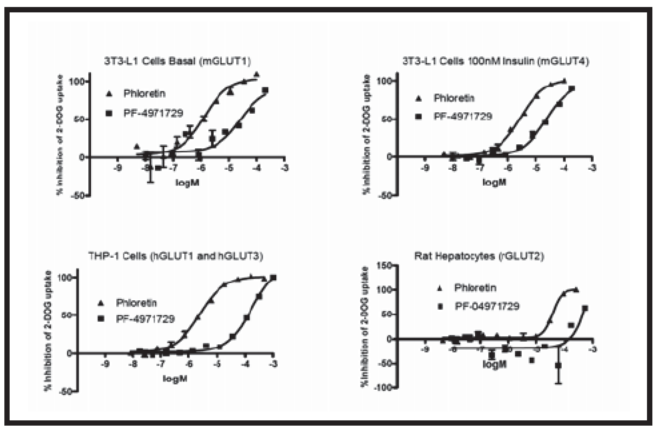
Mean Plasma Concentration of PF-04971729 in Rats after Oral Administration of 0.1, 1, 3, 10, 30, and 60 mg/kg PF-04971729

PF-04971729 Dose (mg/kg)	(b) (4) Study ID	PF-04971729 Total Plasma Concentrations (ng/mL) by Time (h)					n
		1	2	4	7	24	
0.1	SGLT2-0051 rat PKPD	41.3	37.7	28.0	13.1	0.00	2
1	SGLT2-0035 PKPD	372	218	170	87.7	0.00	2
3	SGLT2-0035 PKPD	1320	1020	582	278	0.00	2
10	SGLT2-0035 PKPD	3090	2750	2440	1270	36.7	2
30	SGLT2-0038 rat PKPD	10500	9940	7790	6110	75.8	2
60	SGLT2-0046 rat PKPD	24200	25300	17400	12500	297	2

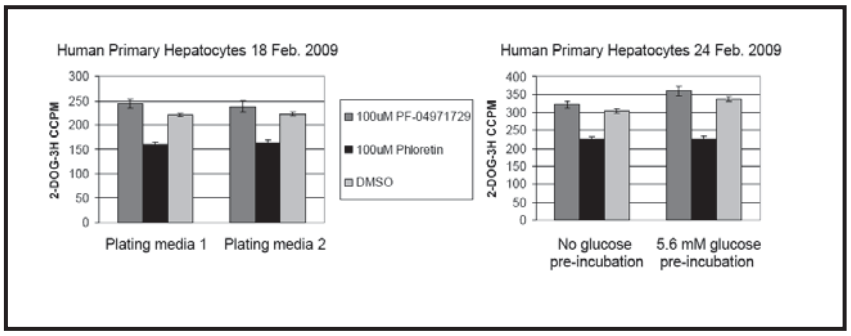
2.6.2.3 Secondary pharmacodynamics

PF-04971729 was tested in four different cell types for glucose uptake: THP-1 cells for human GLUT1 and GLUT3, differentiated 3T3-L1 cells for mouse GLUT1 and GLUT4, and rat and human primary hepatocytes for GLUT2-dependent glucose uptake. In the THP-1 and 3T3-L1 cell assays, phloretin (positive control) inhibited glucose transport with IC_{50} values ranging from 1.3 to 2.8 µM, while PF-04971729 was unable to reach a maximum inhibition plateau sufficient for an IC_{50} calculation. In rat and human hepatocytes, PF-04971729 up to 100 µM did not inhibit glucose uptake. These combined data indicate that PF-04971729 is highly selective for SGLT2 over the facilitative glucose transporters.

Inhibition S-curves for 2-DOG uptake by phloretin and not PF-04971729.



Inhibition of 2-DOG uptake by phloretin, and not PF-04971729 in human hepatocytes



PF-04971729 was profiled in vitro against a broad panel of receptors and ion channels in the CEREP Wide Ligand Profile screen (Study # 75701155) at a single concentration of 10 µM (4.3µg/mL). No significant inhibition (>50%) of binding or enzyme activity was observed at this concentration, which is 860-fold the projected efficacious C_{max} in human (free plasma concentration) of 0.0114 µM (0.005 µg/mL). Results of this study are summarized in the sponsor's tables below.

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
A_1 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	4
A_{2A} *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	0
α_1 (non-selective) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	1
α_{2A} *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	2
α_{2B} (h) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-11
β_1 *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	6
β_2 *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	1
AT_1 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-20
BZD (central) (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-1
CB_1 *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	9
CB_2 *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-5
CCK_1 (CCK_A) *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-14
CCK_2 (CCK_B) *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-11
D_1 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-18
D_{2S} *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-4
D_3 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-5
$GABA_A$ (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	2
$GABA_{B(1b)}$ *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-6
AMPA (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-22
kainate (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	13
NMDA (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-4
glycine (strychnine-insensitive) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	0
H_1 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-9
H_2 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	5
H_3 *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	3
MAO-A (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	7
M_1 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	6
M_2 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-1

Assay Cerep Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
M_3 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	5
N neuronal α-BGTX-insensitive (α4β2) (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	4
N muscle-type *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-10
δ_2 (DOP) *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-1
κ (KOP) (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	10
μ (MOP) *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	1
$PPAR\gamma$ *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	11
5-HT_{1A} *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-10
5-HT_{1B} (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-21
5-HT_{2A} *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-1
5-HT_{2B} *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	19
5-HT_{2C} *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-11
5-HT_3 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-1
5-HT_4 *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-8
5-HT_7 *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	2
GR *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-9
V_{1a} *(h)* (agonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	0
Ca^{2+} channel (L, dihydropyridine site) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-4
Ca^{2+} channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-7
Ca^{2+} channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-5
Ca^{2+} channel (N) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-8
Na^+ channel (site 2) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	6
Cl^- channel (GABA-gated) (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	12
norepinephrine transporter *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	5
dopamine transporter *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	11
GABA transporter (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	1
choline transporter (CHT1) *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	6
5-HT transporter *(h)* (antagonist radioligand)			
75701155-2	PF-04971729-00	1.0E-05	-10

2.6.2.4 Safety pharmacology

Brief Summary of PF-04971729 Safety Pharmacology Program

Study	Concentration or Dose[a]	Study Number
In Vitro Screening Studies		
hERG Patch-clamp Assay	10, 30, 100, or 300 µM	PF-04971729HERG
Neurofunctional – Rat	5, 25, 500 mg/kg	
Functional Observational Battery		09GR146
Locomotor Activity		
Body Temperature		
Pulmonary Function – Rat	5, 25, 500 mg/kg	09GR146
Respiratory Rate, Tidal and Minute Volumes		
Cardiovascular – Conscious Dog	1, 5, 50 mg/kg	09GR145
Blood Pressure, Heart Rate, and Electrocardiogram		

hERG = Human ether-à-go-go-related gene. 10 µM PF-04971729 = 4.3 µg/mL
[a] Single dose.

Neurological effects: Male SD rats dosed with 500 mg/kg of PF-04971729-GE had a 0.4°C decrease in average body temperature. At 500 mg/kg, PF-04971729 produced decreases in locomotor activity measurements (~ 30-40%).

Cardiovascular effects: PF-04971729 inhibited the hERG channel in vitro with an IC_{50} of >300 µM. Significant inhibition of hERG was observed at doses ≥ 30 µM. While the (average % inhibition) was 8.3% at 100 µM (below the 10% cut-off of "biologically relevance") the 6 individual data points used to calculate this mean had a broad range of values (2.9% - 18.1%).

Treatment with PF-04971729 (b) (4) at 50 mg/kg in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and heart rate (and associated RR interval shortening) as well as an increase in systolic blood pressure and lengthening of the PR interval. (*The NOAEL for this study is 5 mg/kg based on the shortening of the corrected QT interval (QTc), the increase in the PR interval, decrease in left ventricular +dP/dT, increase in systolic blood pressure, decrease in heart rate and the associated increase in RR interval.*

Pulmonary effects: PF-04971729 at 25 and 500 mg/kg produced significant increases in both respiratory rate and minute volume at the 101-120 minute interval. The 25 mg/kg dose, produced increases in respiratory rate of 33 b.p.m. (29% increased over control), and minute volume of 36 mL/min (25% increased over control). At the 500 mg/kg dose, a respiratory rate of 45 b.p.m. (40% increased over control) and a minute volume of 33 mL/min (23% increased over control) were seen.

Renal effects: No renal safety studies were performed although PF-04971729 causes increase urinary glucose excretion and kidney alterations in rats and dogs.

Gastrointestinal effects: No GI safety studies were performed although PF-04971729 causes changes in stool quality, vomiting and ulceration of the tongue.

Pivotal Studies:

Neurofunctional and Pulmonary Assessment of PF-04971729 (b) (4) in Male Rats (09GR146)

Key study findings from sponsor:

- Male SD rats dosed with 500 mg/kg of PF-04971729 (b) (4) had a 0.4°C decrease in average body temperature when compared to control animals. This value was found to be statistically significant and lower than the historical control data range by ~ 0.9%.

- A dose of 500 mg/kg, produced decreases in locomotor activity measurements (~ 30-40%) when compared to vehicle control rats.

- PF-04971729 at 25 and 500 mg/kg produced significant increases in both respiratory rate and minute volume at the 101-120 minute interval. The 25 mg/kg dose, produced increases in respiratory rate of 33 b.p.m. (29% increased over control), and minute volume of 36 mL/min (25% increased over control). At the 500 mg/kg dose, a respiratory rate of 45 b.p.m. (40% increased over control) and a minute volume of 33 mL/min (23% increased over control) were seen.

Reviewer Comments: Although the decreases in locomotor activity were not statistically significant when compared to control animals, there was a trend of decreasing activity with increased dose seen in this study. While the sponsor feels that the significantly increased pulmonary values are irrelevant based on the fact that these findings presented at one hour post T_{max}, the reviewer feels that these findings are drug related and should be recognized as such. Tissues involved in these findings may have very different T_{max} values than observed in the blood and signals leading to these indications may occur long after drug concentrations have begun to fall in the plasma. The NOAEL for this study is 5 mg/kg (AUC~0.5 μg.h/mL).

Rat	NOAEL
Neurological (↓Body Temp and ↓Locomotor function)	25 mg/kg
Pulmonary(↑Respiration rate and associated minute volume)	5 mg/kg

Study no.: 09GR146
Volume # and page #: EDR (4.2.1.3.1)
Conducting laboratory and location: Pfizer Global Research and Development New London, CT USA.
Date of study initiation: 12 May 2009

GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: PF-04971729 (b) (4) L-pyroglutamic acid (LPGA) co-crystal, Lot 00701380-094-01, Active Moiety – 75.8%

Methods: Four groups of 6 rats were dosed orally with vehicle or PF-04971729 (b) (4), at either 5, 25, 500 mg/kg. The FOB and body temperature assessments took place approximately 1 hour post dose, immediately after which the rats were placed into activity monitoring cages and their spontaneous locomotor activity recorded for 30 minutes. An additional 4 groups of 6 rats were dosed orally with vehicle or PF-04971729 (b) (4), at either 5, 25, 500 mg/kg, and pulmonary function measurements, consisting of respiratory rate, tidal volume, and minute volume were assessed using whole body plethysmography for 120 minutes.

Neurofunctional Results: PF-04971729 (b) (4), ≤500 mg/kg, had no effect in any of the Functional Observational Battery (FOB) measurements. PF-04971729 (b) (4), 500 mg/kg, produced a 0.4°C decrease in body temperature as shown in the sponsor's figure below.

PF-04971729 (b) (4)
FOB Continuous Responses
Summary Statistics

	Treatment											
	Vehicle			PF-04971729 (b) (4) 5 mg/kg			PF-04971729 (b) (4) 25 mg/kg			PF-04971729 (b) (4) 500 mg/kg		
Assessment	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Body temperature (°C)	6	37.3	0.2	6	37.4	0.4	6	37.1	0.2	6	36.9	0.4
Forelimb grip strength (kg)	6	1.21	0.07	6	1.20	0.11	6	1.23	0.13	6	1.19	0.06

PF-04971729 (b) (4) was administered at 0, 5, 25, or 500 by oral gavage to male rats ~ 1 hour prior to testing in the neurofunctional assessment. Shaded cells indicate significant differences from vehicle (0 mg/kg) using the appropriate statistical analysis as described in the protocol ($p < 0.05$).

PF-04971729 (b) (4), 500 mg/kg, produced a decrease in locomotor activity assessments (~ 30% for horizontal movements and ~ 40% for vertical movements) when compared to vehicle treated rats.

PF-04971729 (b) (4)
Locomotor Activity Responses
Summary Statistics

	Treatment											
	Vehicle			PF-04971729 (b) (4) 5 mg/kg			PF-04971729 (b) (4) 25 mg/kg			PF-04971729 (b) (4) 500 mg/kg		
Assessment	n	Geo. Mean	IQR	n	Geo. Mean	IQR	n	Geo. Mean	IQR	n	Geo. Mean	IQR
Horizontal movements (beam breaks)	6	962	1109	6	1199	529	6	950	220	6	679	617
Vertical movements (beam breaks)	6	62	64	6	87	52	6	53	17	6	37	40

PF-04971729 (b) (4) was administered at 0, 5, 25, or 500 mg/kg by oral gavage male rats ~ 1 hour prior to testing in the neurofunctional assessment. Shaded cells indicate significant differences from vehicle (0 mg/kg) using the appropriate statistical analysis as described in the protocol ($p < 0.05$).
Geo. Mean = Geometric Mean; IQR - Interquartile Range.

Pulmonary Results: PF-04971729 at 25 and 500 mg/kg produced significant increases in both respiratory rate and minute volume at the 101-120 minute interval. PF-04971729, 25 mg/kg, produced increases in respiratory rate of 33 b.p.m. (29% changed from control), and minute volume of 36 mL/min (25% changed from control). PF-04971729, 500 mg/kg, produced increases in respiratory rate of 45 b.p.m. (40% changed from control) and in minute volume of 33 mL/min (23% changed from control).

Compound PF-04971729
Statistical summary of Average Respiratory Rate (breaths/min)

	Treatment											
	Vehicle			5 mg/kg			25 mg/kg			500 mg/kg		
Time	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n
-20	117	9.7	6	121	18.2	6	141	44.5	6	122	8.7	6
20	168	30.1	6	170	31.5	6	191	38.0	6	164	33.5	6
40	128	24.0	6	130	41.0	6	120	14.2	6	150	75.3	6
60	118	11.6	6	136	41.6	6	121	18.0	6	124	22.0	6
80	121	20.4	6	123	24.5	6	157	76.3	6	151	53.6	6
100	118	17.8	6	119	16.5	6	134	33.5	6	116	13.5	6
120	113	13.3	6	126	28.4	6	146	54.8	6	158	43.5	6

Compound PF-04971729
Statistical summary of Average Minute Volume (mL/min)

	Treatment											
	Vehicle			5 mg/kg			25 mg/kg			500 mg/kg		
Time	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n	Mean	s.d.	n
-20	157	16.3	6	156	22.0	6	174	36.4	6	145	11.9	6
20	215	43.2	6	211	18.7	6	235	36.4	6	211	22.9	6
40	171	21.0	6	158	29.5	6	159	21.1	6	164	47.3	6
60	151	13.2	6	161	26.6	6	162	35.1	6	146	10.1	6
80	152	26.7	6	154	17.6	6	185	57.4	6	164	39.4	6
100	146	12.0	6	155	19.1	6	164	36.0	6	152	25.8	6
120	142	6.9	6	155	20.9	6	178	33.8	6	175	27.3	6

Effect of PF-04971729 on hERG Potassium Current Stably Expressed in HEK-293 cells (PF04971729HERG).

Key study findings from sponsor:

- PF-04971729 inhibited the hERG channel in vitro with an IC_{50} of >300 µM.
- Statistically significant inhibition of hERG was observed at doses ≥ 30 µM.

Reviewer Comments: An IC_{50} could not be determined from the doses used by the sponsor. Significant inhibition of the hERG current was seen at 100 µM PF-04971729. While the (average % inhibition) was 8.3% at this dose (below the 10% cut-off of "biologically relevance") the 6 individual data points used to calculate this mean had a broad range of values (2.9% - 18.1%). QT effects should be monitored in future toxicology studies.

Study no.:	PF04971729HERG
Volume # and page #:	EDR (4.2.1.3.1)
Conducting laboratory and location:	Pfizer Global Research and Development New London, CT USA.
Date of study initiation:	Not Stated
GLP compliance:	No
QA report:	None
Drug, lot #, and % purity:	PF-04971729-00-0004, not stated, not stated

Methods: HEK-293 cells stably transfected with the hERG channel ((b) (4)) were used to measure the ability of 10, 30, 100 and 300 µM (PF-04971729) to block hERG currents. Dofetilide (UK-068798) and Cisapride (067K4716) were used as a positive control. DMSO was used as the solvent for the drugs; the final concentration of DMSO was 0.3% (v/v) at the highest dose of drug (300 µM). The hERG current was activated with a voltage step to +30 mV for 1 second followed by a ramp to -80 mV at 0.55 V/s. Test pulses were delivered at 0.25 Hz to evaluate the stability of the peak hERG current over time, and to measure compound block. Four minute control recordings were made for estimation of current rundown prior to the first exposure of the cell to test compounds. Two-four compound concentrations were tested on each cell, and each concentration lasted for at least 1.5 min to reach apparent steady state and followed by addition of 10 µM dofetilide (IKr blocker) to inhibit any remaining hERG current at the end of the experiment. As a positive control, cisapride (10 nM, 30 nM, and 100 nM) was tested in a separate experiment. Peak tail current was recorded.

Results: Each cell was exposed to 2-4 concentrations of PF-04971729. Exposure at each concentration lasted 1.5-10.5 minutes, to achieve an apparent steady-state effect. Results obtained at each compound concentration are shown in the table below. The mean percent inhibition for 30, 100 and 300 µM (PF-04971729) was 2.9, 8.3 and 35.5% respectively.

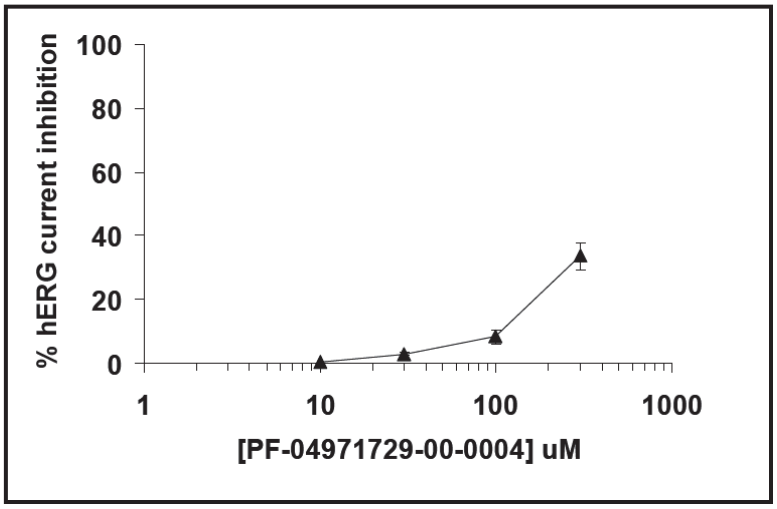
Inhibition of hERG Potassium Currents by PF-04971729

Cell Number	% Inhibition 10 μM	% Inhibition 30 μM	% Inhibition 100 μM	% Inhibition 300 μM
1	0.5	1.7	2.9	26.5
2	N/A	0.4	4.8	25.7
3	N/A	4.4	18.1	48.6
4	N/A	4.7	9.8	31.5
5	N/A	3.2	7.7	35.4
6	N/A	3.2	6.5	N/A
Mean ± SEM	N/A	2.9 ± 0.7 p <0.05	8.3 ± 2.2 p <0.05	33.5 ± 4.2 p <0.05

N/A = Not tested.

A plot of the concentration-response relationship is shown in the sponsors figure below. The IC_{50} for hERG current inhibition by PF-04971729 was >300 μM. Statistically significant inhibition of hERG was observed at 30 and 100 μM as well.

PF-04971729 concentration-response curve (Data expressed as the mean ± SEM).



Cisapride (10 nM, 30 nM, and 100 nM) was tested on 4 separate cells as a positive control. An IC_{50} value of 26.9 ± 1.1 nM was generated.

Cardiovascular Assessment of Orally Administered PF-04971729 (b) (4) in Male Telemetry-Instrumented Beagle Dogs (09GR145)

Key study findings from sponsor:

- Treatment with PF-04971729 (b) (4) at 50 mg/kg in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and lengthening of the PR interval at 3-8h postdose, which is reportedly at the time of C_{max}. At 8-16h postdose, a reduced heart rate (associated RR interval shortening) as well as an increase in systolic blood pressure were observed.

- TK analysis demonstrated a 55-fold increase in plasma drug concentration (0.4→ 22.8 µg/mL) associated with a 50-fold increase in dose (1.0→ 50 mg/kg).

Reviewer Comments: TK measurements for doses < 50 mg/kg were limited to the 7 hour time point, so comparisons of significant changes in telemetered data to C_{max} or AUC_{0-24} on an individual basis is not possible at these lower doses. During the first treatment cycle, 1 animal (Dog 7, Dose 5 mg/kg) vomited immediately following dosing, lowering plasma concentrations of drug significantly in that dog. Plasma concentrations of PF-04971729 (treatment 5) at 7 hours postdose (50 mg/kg) were 25% greater when compared to those collected during treatments 1-4. This difference was attributed to a single animal (Dog 8, Dose 50 mg/kg) that demonstrated a lower exposure than cohorts at the 7 hour postdose time point within the cardiovascular leg of the study, but no explanation of why this animal had such a low drug profile was provided by the sponsor.

The NOAEL for this study is 5 mg/kg based on the shortening of the corrected QT interval (QTc), the increase in the PR interval, decrease in left ventricular +dP/dT, increase in systolic blood pressure, decrease in heart rate and the associated increase in RR interval.

Plasma drug concentration peaked at 43µg/mL approximately 4 hours after the 50mg/kg dose. Assuming linearity, the next lower dose of 5mg/kg resulted in ~4.3µg/mL. By comparison, the proposed clinical study is targeting a maximum drug level of 1.4 to 6.9µg/mL in human subjects.

Study no.:	09GR145
Volume # and page #:	EDR (4.2.1.3.1)
Conducting laboratory and location:	Pfizer Global Research and Development Groton, CT USA.
	(b) (4)
	(*Plasma Drug Conc. and Toxicokinetics*)
Date of study initiation:	21 May 2009
GLP compliance:	Yes
QA report:	Yes

Drug, lot #, and % purity: PF-04971729 (b) (4) L-pyroglutamic acid (LPGA) co-crystal, Lot 00701380-094-01, Active Moiety – 75.8%

Methods: Male beagle dogs (b) (4), n=4, 12-36 months old, 7-15 kg) instrumented with radio-telemetry transmitters were dosed orally with 0, 1, 5, and 50 mg/kg, followed by a 1 week washout period prior to subsequent dosing. A (5 mL/kg) solution of drug in (0.5% methylcellulose (w/v) with 10% PEG 400 (v/v)) was used. Following the washout period subsequent to treatment 4, all animals received a single oral dose of 50 mg/kg for toxicokinetic profiling. Telemetered electrocardiographic, hemodynamic, and activity data were acquired continuously beginning (~1 hour predose) through (~ 24 hours postdose) for treatments 1 through 4. No telemetry data was obtained during the toxicokinetic leg of the study (treatment 5). The treatment regimen is outlined in the table below. Note: Dogs were conscious for this study.

Treatment Regimen

Animal Number	Sex	CM ID	Treatment 1 21 May 09	Treatment 2 28 May 09	Treatment 3 04 Jun 09	Treatment 4 11 Jun 09	Treatment 5 (Toxicokinetic) 18 Jun 09
5	M	03-08-30210[a]	Vehicle	Low	High	Mid	High
6	M	03-07-30062[a]	Low	Mid	Vehicle	High	High
7	M	03-08-30208[a]	Mid	High	Low	Vehicle	High
8	M	03-08-30216[a]	High	Vehicle	Mid	Low	High

CM ID = Comparative Medicine (Animal) Identification Number.
[a]Animals were selected based on viable telemetry signals and the review of their most recent hematology and clinical chemistry data and were deemed to be acceptable.

Results: Telemetered data collected during treatments 1-4, were binned into 4 postdose periods for statistical analysis. These time periods were: Period 1= 0.5-3 hours, Period 2 = 3-6.75 hours, Period 3 = 8-16 hours, and Period 4 = 16-24 hours. Statistically significant effects by time period and parameter are shown in the sponsor's figure below.

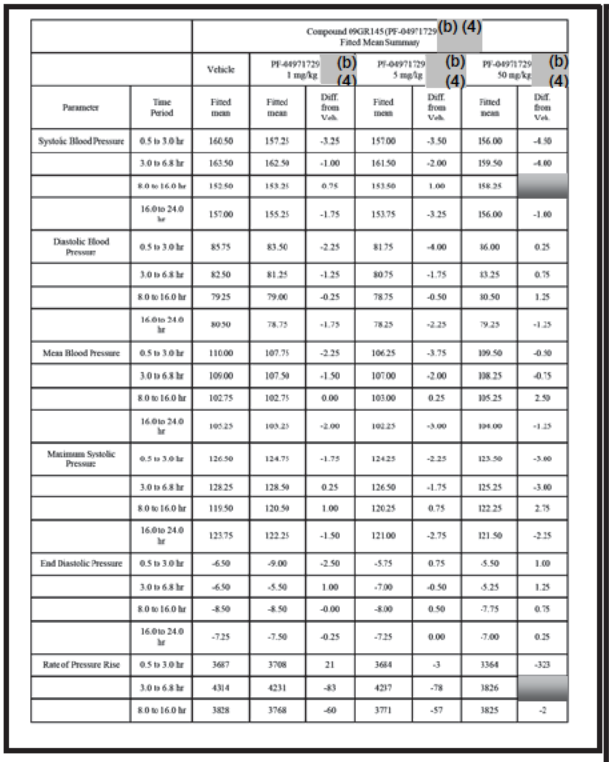
Statistically significant effects by time period and parameter

Parameter	Dose mg/kg	0.5–3.0 hours	3.0–6.8 hours	8.0–16.0 hours	16.0–24.0 hours
Activity (arbitrary units)	1				
	5				
	50				
Systolic blood pressure (mmHg)	1				
	5				-3
	50			+6	
Diastolic blood pressure (mmHg)	1		-1		
	5		-2		
	50				
Mean blood pressure (mmHg)	1				
	5		-2		
	50				
Heart Rate (beats per minute (bpm))	1				
	5				
	50			-6	
PR-Interval (msec)	1				
	5				
	50		+4		
QRS-Interval (msec)	1				
	5				
	50				
QT-Interval (msec)	1				
	5				
	50				
QTc-Interval (msec)	1				
	5				
	50		-6		
RR-Interval (msec)	1				+35
	5				
	50			+78	+40

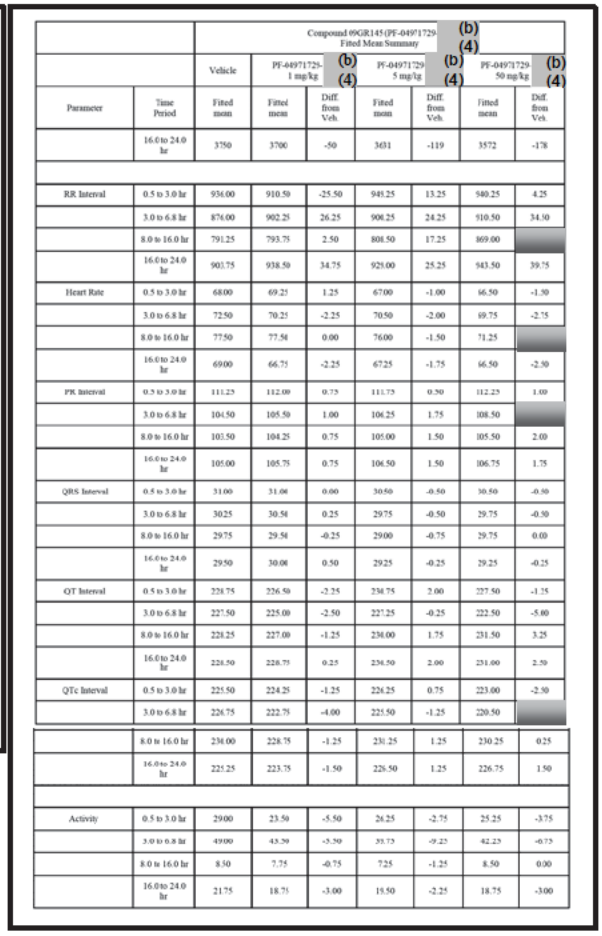
Parameter	Dose mg/kg	0.5–3.0 hours	3.0–6.8 hours	8.0–16.0 hours	16.0–24.0 hours
End Diastolic_LVP (mmHg)	1				
	5				
	50				
Max Systolic_LVP (mmHg)	1				
	5				
	50				
+dP/dT (mmHg/sec)	1				
	5				
	50	-323	-489		-178

Blank cells = No significant treatment effect between treated and control groups.

Statistically significant changes that were documented at the 50 mg/kg dose were: increased systolic blood pressure (0.5-3 hrs), decreased heart rate (8-16 hours), and increased PR-interval (3-6.8 hrs), decreased QTc-interval (3-6.8hrs), increased RR-interval (8-24 hrs) and a decrease in +dP/dT (0.5-6.8 and then returning at 16-24 hrs). Dosing at 5 mg/kg had statistically significant effects on: decreased systolic blood pressure (16-24 hrs), decreased diastolic blood pressure (3-6.8 hrs) and decreased mean blood pressure (3-6.8 hrs). At the lowest dose (1 mg /kg) animals presented with decreased diastolic blood pressure (3-6.8 hrs) and increased RR-interval (16-24 hrs). A summary of the mean data and the difference of that mean from the control are shown in the sponsor's tables below.



		Compound 09GR145 (PF-04971729) (b) (4) Fitted Mean Summary					
		Vehicle	PF-04971729 1 mg/kg (b) (4)		PF-04971729 5 mg/kg (b) (4)	PF-04971729 50 mg/kg (b) (4)	
Parameter	Time Period	Fitted mean	Fitted mean	Diff. from Veh.	Fitted mean	Diff. from Veh.	Fitted mean	Diff. from Veh.
Systolic Blood Pressure	0.5 to 3.0 hr	160.50	157.25	-3.25	157.00	-3.50	156.00	-4.50
	3.0 to 6.8 hr	163.50	162.50	-1.00	161.50	-2.00	159.50	-4.00
	8.0 to 16.0 hr	152.50	153.25	0.75	153.50	1.00	158.25	
	16.0 to 24.0 hr	157.00	155.25	-1.75	153.75	-3.25	156.00	-1.00
Diastolic Blood Pressure	0.5 to 3.0 hr	85.75	83.50	-2.25	81.75	-4.00	86.00	0.25
	3.0 to 6.8 hr	82.50	81.25	-1.25	80.75	-1.75	83.25	0.75
	8.0 to 16.0 hr	79.25	79.00	-0.25	78.75	-0.50	80.50	1.25
	16.0 to 24.0 hr	80.50	78.75	-1.75	78.25	-2.25	79.25	-1.25
Mean Blood Pressure	0.5 to 3.0 hr	110.00	107.75	-2.25	106.25	-3.75	109.50	-0.50
	3.0 to 6.8 hr	109.00	107.50	-1.50	107.00	-2.00	108.25	-0.75
	8.0 to 16.0 hr	102.75	102.75	0.00	103.00	0.25	105.25	2.50
	16.0 to 24.0 hr	105.25	103.25	-2.00	102.25	-3.00	104.00	-1.25
Maximum Systolic Pressure	0.5 to 3.0 hr	126.50	124.75	-1.75	124.25	-2.25	123.50	-3.00
	3.0 to 6.8 hr	128.25	128.50	0.25	126.50	-1.75	125.25	-3.00
	8.0 to 16.0 hr	119.50	120.50	1.00	120.25	0.75	122.25	2.75
	16.0 to 24.0 hr	123.75	122.25	-1.50	121.00	-2.75	121.50	-2.25
End Diastolic Pressure	0.5 to 3.0 hr	-6.50	-9.00	-2.50	-5.75	0.75	-5.50	1.00
	3.0 to 6.8 hr	-6.50	-5.50	1.00	-7.00	-0.50	-5.25	1.25
	8.0 to 16.0 hr	-8.50	-8.50	-0.00	-8.00	0.50	-7.75	0.75
	16.0 to 24.0 hr	-7.25	-7.50	-0.25	-7.25	0.00	-7.00	0.25
Rate of Pressure Rise	0.5 to 3.0 hr	3687	3708	21	3684	-3	3364	-323
	3.0 to 6.8 hr	4314	4231	-83	4237	-78	3826	
	8.0 to 16.0 hr	3828	3768	-60	3771	-57	3825	-2



		Compound 09GR145 (PF-04971729) (b) (4) Fitted Mean Summary					
		Vehicle	PF-04971729 1 mg/kg (b) (4)		PF-04971729 5 mg/kg (b) (4)	PF-04971729 50 mg/kg (b) (4)	
Parameter	Time Period	Fitted mean	Fitted mean	Diff. from Veh.	Fitted mean	Diff. from Veh.	Fitted mean	Diff. from Veh.
	16.0 to 24.0 hr	3750	3700	-50	3631	-119	3572	-178
RR Interval	0.5 to 3.0 hr	936.00	910.50	-25.50	949.25	13.25	940.25	4.25
	3.0 to 6.8 hr	876.00	902.25	26.25	900.25	24.25	910.50	34.50
	8.0 to 16.0 hr	791.25	793.75	2.50	808.50	17.25	869.00	
	16.0 to 24.0 hr	903.75	938.50	34.75	929.00	25.25	943.50	39.75
Heart Rate	0.5 to 3.0 hr	68.00	69.25	1.25	67.00	-1.00	66.50	-1.50
	3.0 to 6.8 hr	72.50	70.25	-2.25	70.50	-2.00	69.75	-2.75
	8.0 to 16.0 hr	77.50	77.50	0.00	76.00	-1.50	71.25	
	16.0 to 24.0 hr	69.00	66.75	-2.25	67.25	-1.75	66.50	-2.50
PR Interval	0.5 to 3.0 hr	111.25	112.00	0.75	111.75	0.50	112.25	1.00
	3.0 to 6.8 hr	104.50	105.50	1.00	106.25	1.75	108.50	
	8.0 to 16.0 hr	103.50	104.25	0.75	105.00	1.50	105.50	2.00
	16.0 to 24.0 hr	105.00	105.75	0.75	106.50	1.50	106.75	1.75
QRS Interval	0.5 to 3.0 hr	31.00	31.00	0.00	30.50	-0.50	30.50	-0.50
	3.0 to 6.8 hr	30.25	30.50	0.25	29.75	-0.50	29.75	-0.50
	8.0 to 16.0 hr	29.75	29.50	-0.25	29.00	-0.75	29.75	0.00
	16.0 to 24.0 hr	29.50	30.00	0.50	29.25	-0.25	29.25	-0.25
QT Interval	0.5 to 3.0 hr	228.75	226.50	-2.25	230.75	2.00	227.50	-1.25
	3.0 to 6.8 hr	227.50	225.00	-2.50	227.25	-0.25	222.50	-5.00
	8.0 to 16.0 hr	228.25	227.00	-1.25	230.00	1.75	231.50	3.25
	16.0 to 24.0 hr	228.50	228.75	0.25	230.50	2.00	231.00	2.50
QTc Interval	0.5 to 3.0 hr	225.50	224.25	-1.25	226.25	0.75	223.00	-2.50
	3.0 to 6.8 hr	226.75	222.75	-4.00	225.50	-1.25	220.50	
	8.0 to 16.0 hr	230.00	228.75	-1.25	231.25	1.25	230.25	0.25
	16.0 to 24.0 hr	225.25	223.75	-1.50	226.50	1.25	226.75	1.50
Activity	0.5 to 3.0 hr	29.00	23.50	-5.50	26.25	-2.75	25.25	-3.75
	3.0 to 6.8 hr	49.00	43.50	-5.50	39.75	-9.25	42.25	-6.75
	8.0 to 16.0 hr	8.50	7.75	-0.75	7.25	-1.25	8.50	0.00
	16.0 to 24.0 hr	21.75	18.75	-3.00	19.50	-2.25	18.75	-3.00

At the 50 mg/kg dose, changes that would be considered treatment related and biologically relevant (*indicated by grey boxes above*) include: the 6 msec shortening of the corrected QT interval (QTc), the 4 msec increase in the PR interval, and a 489 mmHg /sec decrease in left ventricular +dP/dT at 3-7 hours postdose. In addition, the increase in systolic blood pressure (6 mmHg), decrease in heart rate (6 b.p.m.), and associated increase in RR interval (78 msec) between 8 and 16 hours postdose are considered to be biologically-relevant. Statistically significant changes did present at the 1 and 5 mg/kg dose levels, although they were smaller in nature and occurred transiently. A comparison between these telemetered events at the lower doses (1 and 5 mg/kg) and the PK values at those time points would have been helpful in defining their biological relevancy and drug relatedness.

Plasma Drug Concentrations: For treatments 1-4, blood samples were collected from each of the telemetry-instrumented animals at pre-dose and 7 hours post-dose. Following an additional one week washout period after administration of treatment 4, all dogs received a single dose of 50 mg/kg PF-04971729 (b) (4) (treatment 5). Serial blood samples were collected from individual animals at the following time points: predose, 0.5, 1, 2, 4, 7, and 24 hours after administration. The mean plasma concentrations of PF-04971729 at 7 hours postdose rose with increasing dose (treatments 1-4) and resulted in mean (± SD) plasma concentrations of 0.41 ± 0.1, 1.9 ± 1.1, and 22.8 ± 12.5 µg/mL, for the 1, 5, and 50 mg/kg dose groups, respectively.

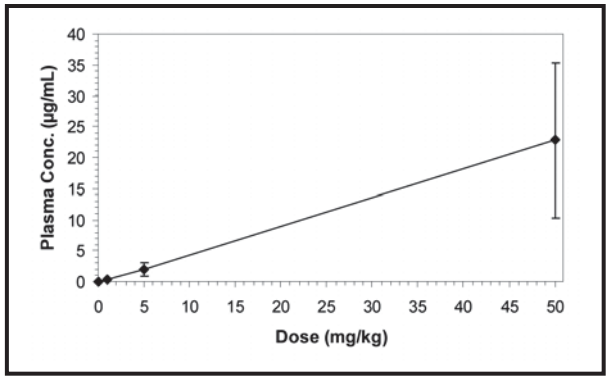
Mean Plasma Concentration Data (µg/mL) for PF-04971729 in Male Dogs after Oral Administration of PF-04971729

Dose (mg/kg)	Gender	Mean Plasma Concentration (µg/mL) by Time (h)																				
		0			0.5			1			2			4			7			24		
		Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
0 (Vehicle)	Male	0.00	0.00	4	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.00	4	-	-	-
1	Male	0.00	0.00	4	-	-	-	-	-	-	-	-	-	-	-	-	0.414	0.0756	4	-	-	-
5	Male	0.00	0.00	4	-	-	-	-	-	-	-	-	-	-	-	-	1.94	1.10	4	-	-	-
50	Male	0.00	0.00	4	-	-	-	-	-	-	-	-	-	-	-	-	22.8	12.5	4	-	-	-
50 (Treatment 5)	Male	0.00	0.00	4	17.1	7.13	4	24.3	3.92	4	38.3	9.60	4	43.4	6.19	4	29.1	1.26	4	5.42	1.14	4

- = No sample per study protocol or amendment
Vehicle: 90% 0.5% Methylcellulose (w/v) with 10% PEG400 (v/v)

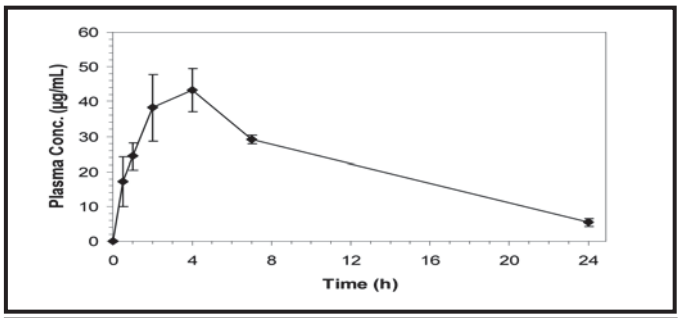
These values represent a ~55-fold increase in plasma concentration associated with a 50-fold increase in dose (1→ 50 mg/kg).

Mean (± SD) Drug Concentrations at 7 Hours Post-Dose in Male Dogs versus Dose after Oral Administration of PF-04971729 (Treatment 1 through Treatment 4)



For treatment 5, the toxicokinetic leg of the study, T_{max} (50 mg/kg) occurred at 3.5 hours postdose; C_{max} and AUC_{0-24} were 44.7µg/mL and 530µg.h/mL, respectively. The mean (± SD) plasma concentrations of PF-04971729 (treatment 5) at 7 hours postdose (50 mg/kg) were 25% greater (29.1 ± 1.3 µg/mL) compared to those collected during treatments 1-4 (22.8 ± 12.5 µg/mL). The difference can be attributed to a single high dose animal (Dog 8) that demonstrated a lower exposure than cohorts (4.1 µg/mL) at the 7 hour postdose time point from the cardiovascular leg of the study.

Mean (± SD) Drug Concentration versus Time and Mean Toxicokinetic Parameters in Male Dogs after Oral Administration of 50 mg/kg PF-04971729 (Treatment 5)



Dose (mg/kg)	Gender	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
		Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
50 (Treatment 5)	Male	44.7	6.94	4	3.50	1.00	4	530	48.1	4

Cardiovascular Assessment of PF-04971729 in Telemetry-Implanted Male Rats (08GR483)

Key study findings from sponsor:

- Treatment of male Sprague Dawley rats with 25 mg/kg (PF-04971729) resulted in a mean T_{max} of 3.33 hrs, a C_{max} of 7.3µg/mL, and an AUC_{0-24} of 98.3µg.h/mL.

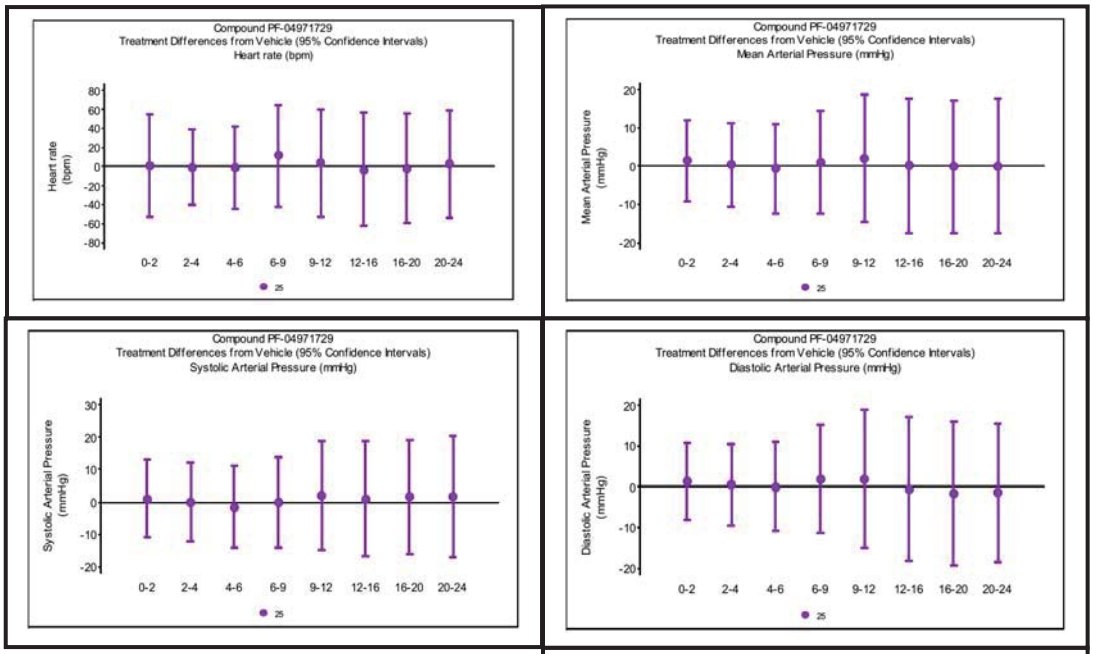
- Changes in cardiovascular assessment parameters following a 25 mg/kg dose of (PF-04971729) were not found to be significant when evaluated by sponsor's method of analysis.

Reviewer Comments: Raw data was not provided by the sponsor; therefore trends within the data set that may have been missed are impossible to identify. The corrected QT interval (QTc) was not chosen as one of cardiovascular parameters to be analyzed in this study. Based on the results from the dog (09GR145) and the hERG (PF04971729HERG) studies, QTc data would have bridged the nonclinical cardiovascular data sets significantly. Since no date of study initiation was found in the documents submitted by sponsor, understanding how this study relates to the other cardiovascular studies submitted in time and/or in purpose is difficult.

Study no.:	08GR483
Volume # and page #:	EDR (4.2.1.3.1)
Conducting laboratory and location:	Pfizer Global Research and Development Groton, CT USA.
Date of study initiation:	Not Stated
GLP compliance:	No
QA report:	None
Drug, lot #, and % purity:	PF-04971729, PF-04971729-00-0008, Activity – 99.7%

Methods: Male Sprague Dawley ([redacted (b) (4)]), n=4, rats previously implanted with a blood pressure transmitter (TA11PA-C40 or C50-PXT, [redacted (b) (4)] were dosed orally with 0 or 25 mg/kg (PF-04971729), followed by a 48-hour washout period prior to subsequent dosing in a cross-over design. (10 mL/kg) solution of drug in (24% HPBCD (w/v) with 20% PEG 400 (v/v)) was used. Clinical signs were recorded once daily prior to treatment and 3 times on dosing days. Body weights were recorded on dosing days for the calculation of dose volume but were not reported. Heart rate (HR), mean arterial pressure (MAP), systolic, and diastolic pressure, and relative activity (dependent on the distance of the rat from the receiver as well as the speed of its movement) were recorded for 1 hour predose and 24 hours postdose. Pharmacokinetic samples were collected from a satellite group of animals (n = 3) at the time points of 0.5, 1, 2, 4, 6, and 24 hours postdose.

Results: Data recorded via transmitters (HR, MAP, systolic and diastolic pressure and activity) were not provided in raw form. Data were collected every 60 seconds and averaged into 15-minute mean values relative to the point of dosing. These data were then split into time bins as follows: 0-2, 2-4, 4-6, 6-9, 9-12, 12-16, 16-20, and 20-24 hours. Predose baselines were used to calculate the deltas from the treated group. The deltas were then used to normalize data and statistical analysis compared the effects of data for PF-04971729 (25 mg/kg) and control groups at each time band using ANOVA.



While the sponsor relayed that initial statistical analysis of raw data demonstrated a significant "separation" between treated and control animals immediately post dose, this analysis depicts the data obtained from the treated group as variable and insignificantly different from control animals.

Pharmacokinetics: The analysis of PK samples at the 25 mg/kg dose was: mean T_{max} of 3.33 hours, a C_{max} of 7.3µg/mL, and an AUC of 98.3µg.h/mL.

1.2 Mean Plasma Concentrations

Parameter	**Cmax**	**tmax**	**AUC**	AUC Interval
Units	ng/mL	Hours	ng*Hours/mL	
Subject Rat 01	7770	2.00	92100	(0-24 Hours)
Subject Rat 02	6490	6.00	112000	(0-24 Hours)
Subject Rat 03	7640	2.00	90800	(0-24 Hours)
Mean	**7300**	**3.33**	**98300**	
S.D.	704	2.31	11900	
%CV	9.7	69.3	12.1	
n	3	3	3	

2.6.2.5 Pharmacodynamic drug interactions:

Pharmacodynamic drug interaction studies with PF-04971729 have not been conducted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The table below was provided by the sponsor.

In Vitro

Type of Test	Test Cells/Tissues	Test Concentrations	Results	GLP Compliance	Study Number
Patch-clamp	HEK-293 stably expressing hERG	10	NS	No	PF04971729HERG
		30	2.9% inhibition		
		100	8.3% inhibition		
		300	33.5% inhibition		

[Click here to type]

GLP = Good Laboratory Practice; HEK = Human embryonic kidney; hERG = Human ether-a-go-go related gene; NS = not significant

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/ Sex/Group	Noteworthy Findings	GLP Compliance	Study Number
Pulmonary Respiratory Rate (RR) Tidal Volume (TV) Minute Volume (MV)	Rat/Sprague-Dawley	Oral gavage, 10 mL/kg	0, 5, 25, 500	6M	None	Yes	09GR146
Nervous Functional Observational Battery (FOB) Body Temperature (BT) Locomotor Activity (LA)	Rat/Sprague-Dawley	Oral gavage, 10 mL/kg	0, 5, 25, 500	6M	None	Yes	09GR146
Cardiovascular Blood Pressure Systolic (SBP) Heart Rate (HR) Electrocardiogram QT interval corrected for heart rate (QTc) PR interval (PR-I) Left Ventricular Pressure Rate of rise of LV pressure over time (+ dP/dT)	Dog/Beagle	Oral gavage, 5 mL/kg (co-crystal)	0, 1, 5, 50	4M	50 mg/kg: ↓ QTc (6 msec) ↓HR (6 bpm) ↓+dP/dT (489 mmHg/sec) ↑SBP (6 mmHg) ↑PR-I (4 msec) 1 and 5 mg/kg: no effects	Yes	09GR145

Cardiovascular Study Toxicokinetic Parameters: include tmax = 3.5 hrs pd?

Dose (mg/kg)	0	1	5	50	50[b]
Cmax (ng/mL)	NA[c]	NA[c]	NA[c]	NA[c]	44.7
AUC(0-24) (ng•h/mL)	NA[c]	NA[c]	NA[c]	NA[c]	530
Plasma Concentration (ug/mL) (7 hours postdose)	NA[d]	0.414	1.94	22.8	29.1

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/ Sex/Group	Noteworthy Findings	GLP Compliance	Study Number

GLP = Good Laboratory Practice; M = Male; Cmax = Maximum (peak) observed drug concentration; NA = Not applicable; AUC(0-24) = Area under concentration-time curve from 0 to 24 hours postdose; [List additional abbreviations in the order in which they appear in the table].

[a] Single dose unless specified otherwise.

[b] Plasma samples obtained for toxicokinetic analyses during the toxicokinetic leg of the study.

[c] Samples not collected for toxicokinetic leg of the study.

[d] All samples below limit of quantitation (0.050 ug/mL).

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Nonclinical in vitro and in vivo data indicate PF-04971729 is well-absorbed from the gastrointestinal tract and oral bioavailability of the amorphous form following a 2 mg/kg dose in rats and dogs is 67% and 97%, respectively. Oral bioavailability of the co-crystal form following a 5 mg/kg dose in rats or 2 mg/kg dose in dogs was similar at 69% and 94%, respectively.

The pharmacokinetics of PF-04971729, following intravenous (IV) administration to rats and dogs, is characterized by low clearance (rat: 4.04 mL/min/kg; dog: 1.64 mL/min/kg), a moderate volume of distribution at steady state (rat: 1.13 L/kg; dog: 0.828 L/kg) and a moderate-to-long half-life (rat: 4.08 hours; dog: 7.63 hours).

PF-04971729, at concentrations of 1 and 10µg/mL, is highly bound to rat, dog, and human plasma proteins, with mean unbound fractions (fu) ranging from 0.032 to 0.064, and binding appeared to be independent of concentration. The free fraction was slightly higher in human compared to the nonclinical species tested. The sponsor feels that the pharmacological effect of PF-04971729 and possibly its toxicological effects are more closely related to the unbound fraction in plasma rather than the total plasma concentration. Therefore, species differences in unbound plasma fraction (at 1 µg/mL) were incorporated into the PK/PD modeling and safety margin calculations were based on that exposure by the sponsor. Pertaining to this review, safety margins will be based on total, not free, drug levels. The percent (%) plasma protein binding across species ranged from 96-98%, which for the purposes of this review will not be considered as a meaningful difference in protein binding.

Studies in rats and dogs indicate that renal and biliary excretions of unchanged PF-04971729 are not significant clearance pathways and that metabolism is expected to be the major clearance mechanism. In vitro metabolite profiles of PF-04971729 were qualitatively similar across nonclinical species and humans and no unique human metabolites are anticipated by the sponsor.

Metabolism of PF-04971729 may be catalyzed by multiple enzymes, including CYP3A4, CYP3A5, CYP2D6, UGT1A9, and UGT2B7. In vitro hepatocyte data suggests that the dog may model human metabolism of PF-04971729 more closely than the rat. At the predicted human efficacious dose, clinically significant drug-drug interactions resulting from PF-04971729-mediated CYP450 inhibition or induction are not anticipated by the sponsor.

The nonclinical pharmacokinetic/ pharmacodynamic (PK/PD) relationship between PF-04971729 exposure following single oral doses of 0.1 to 60 mg/kg and urinary glucose excretion (UGE), the mechanism biomarker, was characterized in male Sprague-Dawley rats. The IC_{50} obtained in rats (0.022 µg/mL) was scaled by the sponsor to a

human IC_{50} (0.011 µg/mL) by accounting for species differences in SGLT2 potency reflected in a CHO cell assay and in unbound plasma fraction. The projected CL, Vss, t½, absorption rate, and bioavailability in humans based on single species allometric scaling of rat PK data were calculated to be 1.7 mL/min/kg, 1.8 L/kg, 12 hours, 1.9 h^{-1} and 65%, respectively. Based on the PK/PD and predicted human pharmacokinetics of PF-04971729, a total of 75 grams of urinary glucose excretion (UGE) over 24 hours can be achieved either through a single dose of 17 mg, or 13 mg QD at steady state. (**At this predicted efficacious dose, the projected total AUC_{0-24} and C_{max} are 1.13 µg.h/mL (0.072 µg.h/mL free) and 0.078 µg/mL (0.005 µg/mL free; 0.0114 µM free), respectively.)**

2.6.4.2 Methods of Analysis

Liquid chromatography/mass spectrometry (LC-MS/MS) was used to determine PF-04971729 concentrations in plasma samples from pharmacokinetic and exploratory toxicokinetic studies performed in rat and dog. The lower limit of quantitation in these various assays was 0.00500µg/mL using a 20 µL plasma sample.

For the 1-month rat and dog toxicokinetic studies, validated LC-MS/MS assays were developed to measure plasma concentrations of PF-04971729. These assays were validated over a concentration range of 0.00500 to 5.00µg/mL and 0.0500 to 50.0µg/mL for rat and dog, respectively, using a 20 µL plasma sample.

2.6.4.3 Absorption

Single Dose Pharmacokinetics and Oral Bioavailability of PF-04971729 in Rats Following Intravenous or Oral Administration (134900)

Key study findings from sponsor:

- Oral dosing of (2 mg/kg) in male rats gave an (AUC_{0-20}) of 5.49 µg.hr/mL, T_{max} value of 1.33 and a C_{max} of 772.0 ng/mL.
- Intravenous dosing resulted in a 1.5-fold increase in AUC_{0-20} over the orally dosed animals (8.2µg.hr/mL).
- Following i.v. administration (2 mg/kg), PF-04971729 exhibited a mean systemic clearance of 4.04 mL/min/kg and a volume of distribution of 1.13 L/kg resulting in a terminal elimination half-life of 4.1 hours.
- Following the 2 mg/kg PO dose, oral bioavailability of PF-04971729 was ~67%.
- The urinary excretion of PF-04971729 following a 2 mg/kg i.v. dose was determined to be 27.5% in rats.

Study no.: 134900
Volume # and page #: EDR (4.2.2.2.1)
Conducting laboratory and location: Pfizer Global Research and Development Groton, CT USA.
Date of study initiation: Not Stated
GLP compliance: No
QA report: None
Drug, lot #, and % purity: PF-04971729
PF-04971729-00-0001 (Intravenous)
PF-04971729-00-0008 (Oral)
Activity – 99.7%

Methods: Male Sprague-Dawley rats (N=2 Intravenous, 314-361 g and N=3 Oral, 315-356 g) were dosed once i.v. with PF-04971729 in 2 mL/kg – DMSO / PEG400 / 30% SBECD and once orally with PF-04971729 in 2 mL/kg - 0.5% MC/Tween-80 solution. All animals received 2 mg/kg of drug. For oral dosing, animals were fasted. Blood samples were taken after i.v. dosing at 0.0833, 0.25, 0.5, 1, 2, 4, 6, 7, 8, and 20 h post dose. After oral dosing, samples were collected at 30 minutes and 1, 2, 4, 7, 12, and 20 h post dose.

Results:
Mean and individual plasma concentration-time data, urinary excretion data and PK parameters for PF-04971729 in rats following a single i.v. or P.O. dose of 2 mg/kg are shown in the sponsor's tables below. The mean T_{max} value for the orally dosed rats was 1.33 h. Intravenous dosing resulted in a 1.5-fold increase in AUC_{0-20} over the orally dosed animals (8.2 compared to 5.49µg.hr/mL). The C_{max} in orally dosed rats was determined to be 772.0ng/mL. Following i.v. administration (2 mg/kg), PF-04971729 exhibited a mean systemic clearance of 4.04 mL/min/kg and a volume of distribution of 1.13 L/kg resulting in a half-life of 4.1 hours. Following the 2 mg/kg PO dose, oral bioavailability of PF-04971729 was ~67%. The urinary excretion of PF-04971729 following a 2 mg/kg i.v. dose was determined to be 27.5% in rats.

Species	Rat			Rat		
Gender (M/F)/Number of Animals	M/2			M/3		
Feeding Condition	Fed			Fasted		
Compound Form	Amorphous			Amorphous		
Vehicle/Formulation	DMSO/PEG400/30% SBECD (10/30/60) (v/v/v)			0.5% MC/Tween 80 (99.9/0.1) (v/v)		
Method of Administration	Intravenous			Oral		
Dose (mg/kg)	2.0			2.0		
Sample	Plasma			Plasma		
Analyte	PF-04971729			PF-04971729		
Assay	LC-MS/MS			LC-MS/MS		
PK Parameters:	Mean	SD	n	Mean	SD	n
Cmax (µg/mL)	--	--	--	0.772	0.0476	3
Tmax (h)	--	--	--	1.33	0.577	3
$AUC_{(0-20)}$ (µg·h/mL)	8.20	NC	2	5.49	0.599	3
$AUC_{(0-\infty)}$ (µg·h/mL)	8.48	NC	2	5.65	0.758	3
Clearance (mL/min/kg)	4.04	NC	2	--	--	--
Volume of Distribution (L/kg)	1.13	NC	2	--	--	--
Half-life (h)	4.08	NC	2	3.65	0.878	3
Bioavailability (%)[a]	--	--	--	66.6	8.96	3
C(0)(µg/mL)	3.40	NC	2	--	--	--
% PF-04971729 excreted (unchanged) in urine	27.5	NC	2	--	--	--

-- = Data not available or not applicable; DMSO = Dimethyl Sulfoxide; PEG = Polyethylene Glycol; SBECD = Sulfobutyl Ether Beta-Cyclodextrin; MC = Methyl Cellulose; PK = Pharmacokinetics; C(0) = Concentration at time zero extrapolated by linear regression from the apparent distribution phase following IV administration; Cmax = Maximum observed plasma concentration; Tmax = Time to reach Cmax; $AUC_{(0-20)}$ = Area under the plasma concentration-time curve from time 0 to 20 hours postdose; $AUC_{(0-\infty)}$ = Area under the plasma concentration-time curve from time 0 to infinite time: NC = Not calculated, n<3
a = (Dose,iv/Dose,po)*($AUC_{(0-\infty),po}$/$AUC_{(0-\infty),iv}$)

	Plasma Concentration (µg/mL)				
Time (h)	Rat 1 IV (2 mg/kg)	Rat 2 IV (2 mg/kg)	Rat 1 PO (2 mg/kg)	Rat 2 PO (2 mg/kg)	Rat 3 PO (2 mg/kg)
0.0833	2.84	3.06	--	--	--
0.25	2.15	2.30	--	--	--
0.5	1.62	1.68	0.369	0.742	0.722
1	1.64	1.10	0.516	0.804	0.794
2	0.904	0.889	0.717	0.667	0.633
4	0.626	0.538	0.542	0.444	0.518
6	0.519	0.316	--	--	--
7	--	--	0.355	0.232	0.248
8	0.361	0.205	--	--	--
20	0.0681	0.0167	0.0507	0.0136	0.0141
0-20 h urine concentration (µg/mL)	5.14	4.17	--	--	--
0-20 h urine volume (mL)	37.5	42.0	--	--	--
PF-04971729 in 0-20 h urine (µg)	192.5	175.1	--	--	--
PF-04971729 dose (mg)	0.628	0.722	--	--	--
Cmax (µg/mL)	--	--	0.717	0.804	0.794
Tmax (h)	--	--	2.0	1.0	1.0
$AUC_{(0-20)}$ (µg·h/mL)	9.36	7.04	6.17	5.03	5.28
$AUC_{(0-\infty)}$ (µg·h/mL)	9.83	7.12	6.51	5.09	5.34
Half-life (h)	4.85	3.31	4.66	3.18	3.1
Clearance (mL/min/kg)	3.39	4.68	--	--	--
Volume of Distribution (L/kg)	1.18	1.08	--	--	--
Bioavailability (%)[a]	--	--	76.8	60.0	63.0
C(0) (µg/mL)	3.26	3.53	--	--	--
% PF-04971729 excreted (unchanged) in urine	30.7	24.3	--	--	--

-- = Data not available or not applicable; C(0) = Concentration at time zero extrapolated by linear regression from the apparent distribution phase following IV administration; Cmax = Maximum observed plasma concentration; Tmax = Time to reach Cmax; $AUC_{(0-\infty)}$= Area under the plasma concentration-time curve from time 0 to infinite time; $AUC_{(0-20)}$ = Area under the plasma concentration-time curve from time 0 to 20 hours postdose
a = (Dose,iv/Dose,po)*($AUC_{(0-\infty),po}$/$AUC_{(0-\infty),iv}$)

Single Dose Pharmacokinetics Bridging study of PF-04971729 in Rats Following Oral Administration (115138)

Key study findings from sponsor:

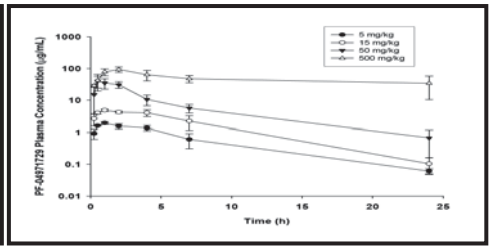
- Oral dosing of 5, 15, 50 and 500 mg/kg in male rats gave an AUC_{0-20} of 14.7, 45.3,180 and 1150 µg.hr/mL.
- Average T_{max} was 1.25h.
- Mean C_{max} values of 1.94, 4.88, 40.5 and 98.9 µg/mL were achieved at doses of 5, 15, 50 and 500 mg/kg respectively.
- This study indicates that the two formulations of PF0491729 provide similar pharmacokinetics in rats.

Study no.:	115138
Volume # and page #:	EDR (4.2.2.2.1)
Conducting laboratory and location:	Pfizer Global Research and Development Groton, CT USA.
Date of study initiation:	Not Stated
GLP compliance:	No
QA report:	None
Drug, lot #, and % purity:	PF-04971729, 00701380-070-01 (Co-crystal form)

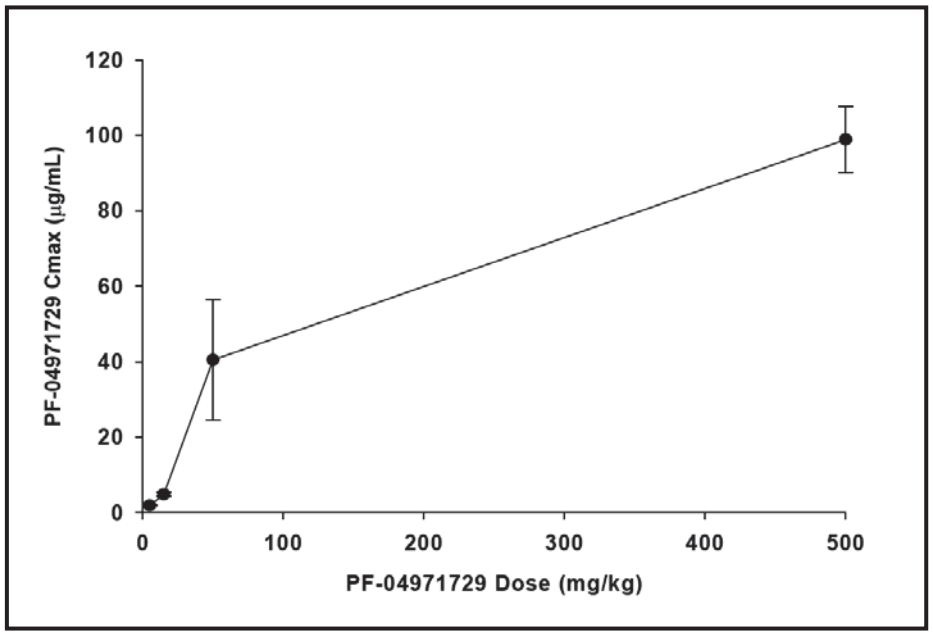
Methods: Fasted male Sprague-Dawley rats (N=12, 286-350 g) were dosed once orally with PF-04971729 in 10 mL/kg - 0.5% MC/10% PEG400 suspension. Animals received 5, 15, 50, and 500 mg/kg of drug. After oral dosing, samples were collected at 0.25, 0.5, 1, 2, 4, 7, 12, and 24 h post dose.

Results: Mean pharmacokinetic parameter values following single dose oral administration of 5, 15, 50, and 500 mg/kg PF-04971729 to fasted male rats are shown in the sponsor's table below (left). Systemic exposure (as assessed by C_{max} and AUC_{0-24}) increased with dose. Mean C_{max} and AUC_{0-24} increased by 2.5X and 3.1X, respectively, with a 3X increase in dose (from 5 to 15 mg/kg). An additional 3.3X increase in dose (from 15 to 50 mg/kg) resulted in an 8.3X and 4.0X increase in mean C_{max} and AUC_{0-24}, respectively. A further 10x increase in dose (from 50 to 500 mg/kg) resulted in a 2.4x and 6.4x increase in mean C_{max} and AUC_{0-24}, respectively. The mean (± SD) plasma drug concentration versus time in rats after a single oral dose of PF-04971729 is shown in the sponsor's figure below (right).

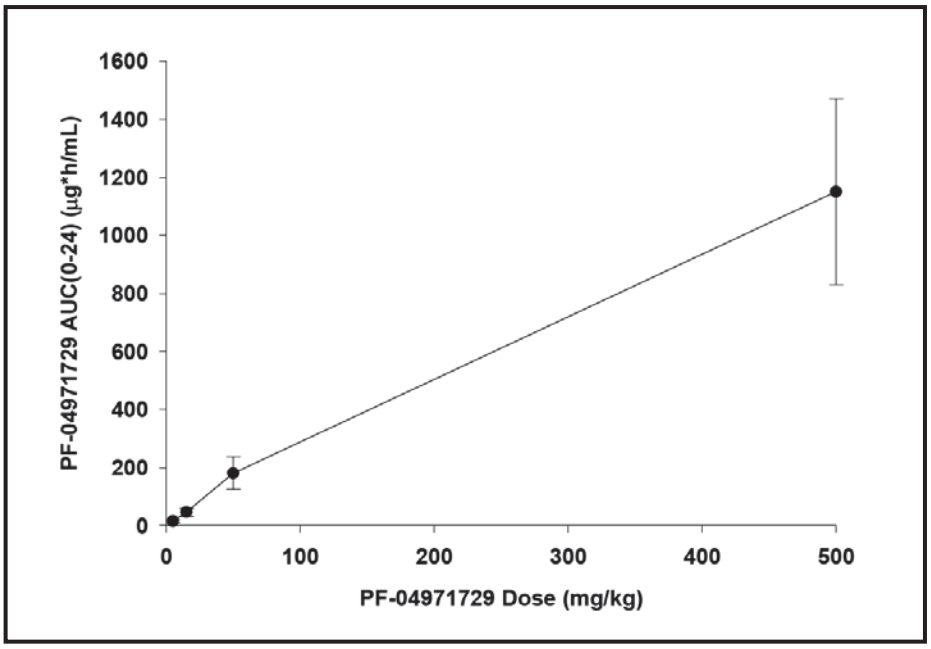
PF-04971729 Dose (mg/kg)	Cmax (µg/mL)		Tmax (h)		AUC(0-24) (µg*h/mL)		n
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
5	1.94	0.185	1.00	0.00	14.7	4.32	3
15	4.88	0.450	1.00	0.00	45.3	14.1	3
50	40.5	15.9	0.667	0.289	180	56.5	3
500	98.9	8.70	2.33	1.53	1150	320	3



Mean (± SD) PF-04971729 C_{max} versus Dose in Rats after Single Dose Oral Administration of 5, 15, 50, and 500 mg/kg PF-04971729



Mean (± SD) PF-04971729 AUC_{0-24} versus Dose in Rats after Single Dose Oral Administration of 5, 15, 50, and 500 mg/kg PF-04971729



Single dose Pharmacokinetics and oral bioavailability of PF-04971729 in Dogs following Intravenous or Oral Administration (134221)

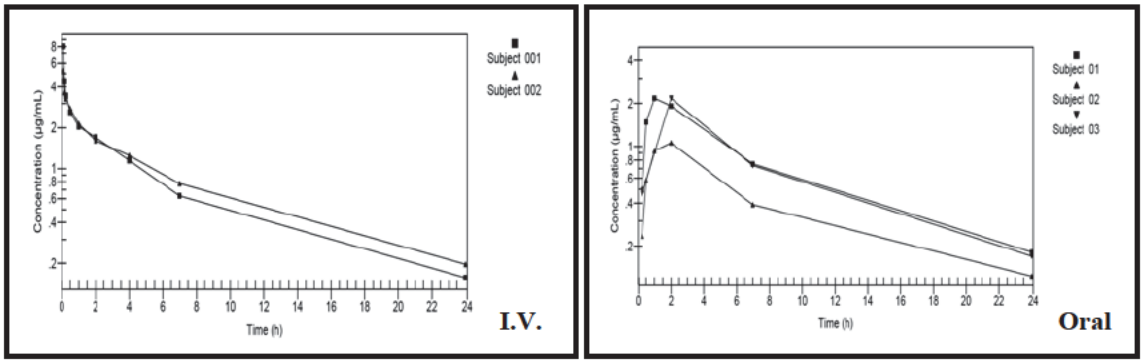
Key study findings:

- Oral dosing of 2 mg/kg gave an exposure of ~17.5 µg.h/mL in male dogs regardless of drug form used. I.V. dosing gave an exposure of 18.5 µg.h/mL.
- Bioavailability was ~95% with an average T_{max} of 1.2h and an average $t_{1/2}$ of 7.8h.
- The mean C_{max} value following an oral dose of 2 mg/kg was 2.18µg/mL (amorphous) and 2.5µg/mL (Co-crystal).
- Amorphous and the co-crystal forms were evaluated; each provided similar PK in the dog.

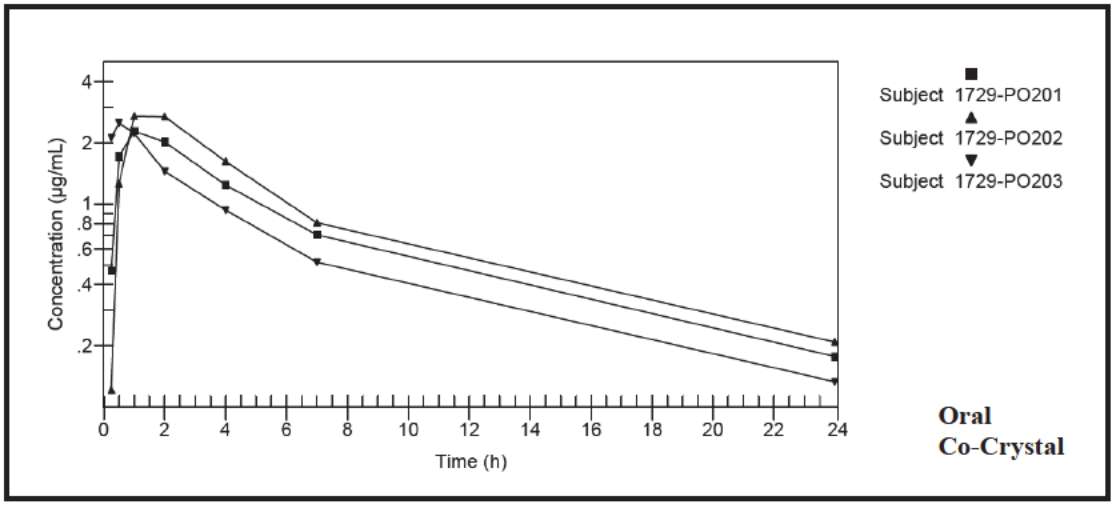
Study no.:	134221
Volume # and page #:	EDR (4.2.2.2.1)
Conducting laboratory and location:	Pfizer Global Research and Development Groton, CT USA.
Date of study initiation:	Not Stated
GLP compliance:	No
QA report:	None
Drug, lot #, and % purity:	PF-04971729 *(Amorphous forms)* Intravenous - PF-04971729-00-0004 Oral - PF-04971729-00-0006 *(Co-crystal form)* - PF-04971729/00710380-070-01

Methods: Fasted male beagle dogs were dosed once orally (N=3, 11.1-12.8kg) with 2 mg/kg PF-04971729 (*Amorphous*) in 2 mL/kg of 0.5% MC/0.1% Tween-80 solution and once orally (N=3, 9.4-10.8kg) with 2 mg/kg PF-04971729 (*co-crystal*) in 2 mL/kg of 0.5% MC/0.1% PEG400 solution. Fed male beagle dogs (N=2, 10.5-11.6kg) were dosed once (i.v.) with 2 mg/kg PF-04971729 (*Amorphous*) in 0.5 mL/kg 5% PEG400 in 23% hydroxypropyl-β-cyclodextrin (HPCD). Blood samples were taken after oral dosing at 0, 0.25, 0.5, 1, 2, 4, 7, and 24h post dose. After i.v. dosing, blood samples were collected at 5, 10, 15, and 30 minutes and 1, 2, 4, 7 and 24h post dose. Urine sample time points were 0-7 and 7-24 hr post dose.

Results:
As shown in the sponsor's figures below, elimination time courses were similar after oral and i.v. dosing of amorphous PF-04971729, with mean ($t_{1/2}$) values being 8.16 and 7.63h, respectively. Bioavailability is (~95%) under these conditions, which is higher than the observations made in the rat (67%). The V.O.D. (0.83 L/kg) suggests distribution to total body water. This value is lower than what was seen in the rat (1.13 L/kg).



The elimination time course after oral dosing with the co-crystal form of PF-04971729 was similar to the results seen with the amorphous form, with the ($t_{1/2}$) value being 7.48h.



The mean urinary excretion of PF-04971729 following a 2 mg/kg (i.v.) dose was determined to be ~ 2.0%.

	IV (2 mg/kg)					
	Dog 1			Dog 2		
PF-04971729 dose (mg)	21.0			23.2		
	0-7 h urine	7-24 h urine	Total (0-24 h urine)	0-7 h urine	7-24 h urine	Total (0-24 h urine)
PF-04971729 concentration (µg/mL)	3.33	0.841	--	1.57	1.21	--
Urine volume (mL)	85	265	--	65	215	--
PF 04971729 in urine (µg)	283	223	--	102	260	
% PF-04971729 excreted (unchanged) in urine	1.35	1.06	2.41	0.44	1.12	1.56

IV = Intravenous; -- = Data not available or not applicable.

A summary of mean PK parameters and urinary excretion data of PF-04971729 is shown in the sponsor's table below.

Species	Dog			Dog			Dog		
Gender (M/F)/Number of Animals	M/2			M/3[a]			M/3		
Feeding Condition	Fed			Fasted			Fasted		
Compound Form	Amorphous			Amorphous			Co-crystal		
Vehicle/Formulation	5% PEG400 in 23% HPBCD			99.9% 0.5%MC/0.1% Tween 80			0.5% MC/PEG 400 (90/10)		
Method of Administration	Intravenous			Oral			Oral		
Dose (mg/kg)	2.0			2.0			2.0		
Sample	Plasma			Plasma			Plasma		
Analyte	PF-04971729			PF-04971729			PF-04971729		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS		
PK Parameters:	Mean	SD	n	Mean	SD	n	Mean	SD	n
C_{max} (µg/mL)	--	--	--	2.18	NC	2	2.50	0.215	3
Tmax (h)	--	--	--	1.50	NC	2	0.833	0.289	3
$AUC_{(0-24)}$ (µg·h/mL)	18.5	NC	2	17.7	NC	2	17.2	3.25	3
$AUC_{(0-\infty)}$ (µg·h/mL)	20.4	NC	2	19.7	NC	2	19.1	3.65	3
Half-life (h)	7.63	NC	2	8.16	NC	2	7.48	0.134	3
Clearance (mL/min/kg)	1.64	NC	2	--	--	--	--	--	--
Volume of Distribution (L/kg)	0.828	NC	2	--	--	--	--	--	--
Bioavailability (%)	--	--	--	96.6[b]	NC	2	93.6[c]	--	--
C(0) (µg/mL)	11.1	NC	2	--	--	--	--	--	--
% PF-04971729 excreted (unchanged) in urine	1.99	NC	2	--	--	--	--	--	--

HPBCD = Hydroxypropyl-Beta-Cyclodextrin; MC = Methyl Cellulose; PEG = Polyethylene Glycol;
-- = Data not available or not applicable; NC = Not calculated, n<3; PK = Pharmacokinetics;
C(0) = Concentration at time zero extrapolated by linear regression from the apparent distribution phase following IV administration; C_{max} = Maximum observed plasma concentration; Tmax = Time to reach C_{max}; $AUC_{(0-24)}$ = Area under the plasma concentration-time curve from time 0 to 24 h postdose; $AUC_{(0-\infty)}$ = Area under the plasma concentration-time curve from time 0 to infinite time
a = Data for Dog 2 was excluded from calculations as the dog did not receive the full dose of PF-04971729
b = (Dose,iv/Dose,po)*(AUC(0-∞),po/AUC(0-∞),iv)
c = (Dose,iv/Dose,po)*(Mean AUC(0-∞),po/Mean AUC(0-∞),iv)

2.6.4.4 Distribution

Plasma Protein Binding of PF-04971729 in Rat, Dog and Human (102049)

Key study findings:

- Mean plasma protein binding of PF-04971729 was very similar between species: Dogs (97%), Humans (94%), and Mice (96%).

Study no.: 102049
Volume # and page #: EDR (4.2.2.3.1)
Conducting laboratory and location: Pfizer Global Research and Development Groton, CT USA.
Date of study initiation: Not Stated
GLP compliance: No
QA report: None
Drug, lot #, and % purity: PF-04971729, PF-04971729-00-0001

Methods: This report describes the extent of in vitro binding of PF-04971729 at concentrations of 1 and 10 µg/mL (equivalent to 2.3 and 23 µM) to plasma proteins from male rat, dog, and human measured by equilibrium dialysis.

Results: The measured mean plasma protein binding values of PF-04971729 in male rat, dog and human plasma are shown in the sponsor's figure below. Plasma binding was measured in several species by adding test compound at 1 or 10 µM to the protein. Aliquots (n=3;150 µl) of each matrix were dialyzed against an equal volume of physiological saline for 6 hrs at 37°C on an oscillating platform in a 5% CO_2 atmosphere. There was little variation in the fraction of protein bound, with dogs at (97%), humans at (94%), and mice at (96%). The sponsor reported a mean percent recovery range of 89.1% to 102%.

Species	Gender	Samples (n=)	Fraction Unbound (Mean ± SD)		Percent Bound (Mean ± SD)	
			1000 ng/mL (2.3 µM)	10,000 ng/mL (23 µM)	1000 ng/mL (2.3 µM)	10,000 ng/mL (23 µM)
Rat	Male	3	0.040 ± 0.0040	0.036 ± 0.05	96.0 ± 0.4	96.4 ± 0.5
Dog	Male	3	0.032 ± 0.0024	0.032 ± 0.001	96.8 ± 0.02	96.8 ± 0.1
Human	Male	3	0.064* ± NC	0.053 ± 0.003	93.6* ± NC	94.7 ± 0.3
Footnotes: NC= standard deviation (SD) not calculated as n<3. Molecular weight (MW) of PF-04971729 =436.888. * = n of 2						

2.6.4.5 Metabolism

Identification of Metabolites of PF-04971729 in Rat Urine, Bile, Plasma, Liver Microsomes and Hepatocytes from Human, Dog and Rat, and from Recombinant Human CYP and UGT Isoforms (162240)

Key study findings:

- A total of 12 metabolites of PF-04971729 were identified (M1-M4, M5{a-c}, M6{a-c}, M7 and M8.
- No human unique metabolites were detected in this study, although M7 and M8 appear to be rat specific.
- CYP3A4 and 3A5 are responsible for the formation of the major oxidative metabolites (M1) and O-desethyl product (M2).
- No novel metabolites were identified during *in vivo* studies in rats when compared to the *in vitro* examinations.

Reviewer Comments: Interesting to note is that the two rat specific metabolites (M7 and M8) that were identified by the in vitro assay utilizing rat hepatocytes were absent in experiments involving whole animals. In addition, M4, M6b and M6c where not identified by these same in vivo studies, while they were commonly detected across all species observed by in vitro studies. Also of note, quantitation of parent and metabolites present in the plasma, bile, and urine from rats was not provided; only HPLC chromatograms were presented.

Study no.:	162240
Volume # and page #:	EDR (4.2.2.4.1)
Conducting laboratory and location:	Pfizer Global Research and Development Groton, CT USA.
Date of study initiation:	Not Stated
GLP compliance:	No
QA report:	None
Drug, lot #, and % purity:	PF-04971729, PF-04971729-00-0001 and PF-04971729-00-0006

Methods:

Microsomal Incubation of PF-04971729: PF-04971729 (10 μM) was incubated with liver microsomes (P450 concentration: 0.5μM) from human, dog and rat for 60 min. All incubations were carried out in 0.1 mM potassium phosphate buffer (pH=7.4) in the presence (1mM) and absence of NADPH at 37ºC in a shaking water bath. The reaction was initiated by the addition of substrate to a pre-incubated microsomal preparation and was stopped by adding acetonitrile to the incubation mixture at the ratio of 1:4.

Incubation of PF-04971729 with human recombinant CYPs: PF-04971729 (20 μM) was incubated with 7 human recombinant CYP isoforms in 0.1 mM potassium phosphate buffer (pH=7.4). The incubation was carried out at 50 pmol P450 per mL in the presence of NADPH (1mM) for 60 min. The incubation was stopped by adding acetonitrile to the incubation mixture at the ratio of 1:4.

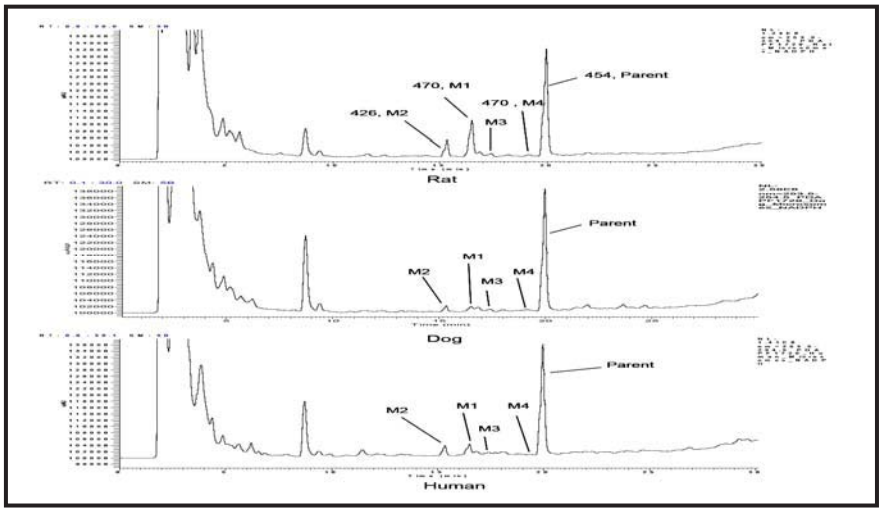
Incubation of PF-04971729 with human recombinant UGTs: PF-04971729 (20 μM) was incubated with 12 human recombinant UGT isoforms for 40 min. The incubation was carried out at protein concentration of 0.5 mg/mL. The reaction mixture contained UDPGA (1mM), Magnesium chloride (10 mM), and Alamethicin (25 μg/mL). The incubation was stopped by adding acetonitrile to the incubation mixture at the ratio of 1:4.

Incubation of PF-04971729 with hepatocytes: PF-04971729 (10 μM) was incubated with human, dog and rat hepatocytes at 37ºC in a shaking water bath for 4 hours. Hepatocytes were suspended in Williams E media (WEM) with 10% fetal bovine serum at a concentration of 2.0×10^6 cells/mL. After 4 hours, the incubation was stopped by adding 10 mL of acetonitrile to the incubation mixture (2 mL) and then sonicated with a probe-type sonicater for 5 min before further extraction.

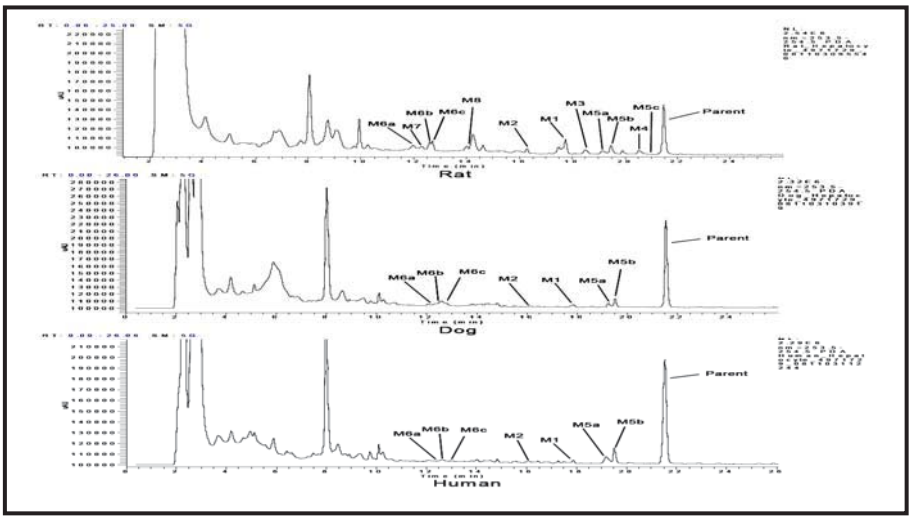
Animal dosing and sample collection: Two male bile-duct cannulated SD rats received a 2 mg/kg (i.v.) bolus dose, bile samples were collected at the timed intervals of 0-8h and 8-24h. Another two male SD rats received a 250 mg/kg oral dose, urine samples were collected for 0-20 hours. Plasma samples were collected for IVT study where a group of 6 male rats received a 500 mg/kg oral dose, plasma samples were collected at 1, 4, 7, 24 and 145 hours post dose.

Results: A total of 12 metabolites of PF-04971729 were identified (M1-M4, M5{a-c}, M6{a-c}, M7 and M8). The *in vitro* metabolic profiles of PF-04971729 were similar across preclinical species and human. No human unique metabolites were detected in this study, although M7 and M8 appear to be rat specific. While the production of the M5c metabolite appears to be rat liver specific, human recombinant UGTs were capable of glucuronidating PF-04971729 to this metabolite.

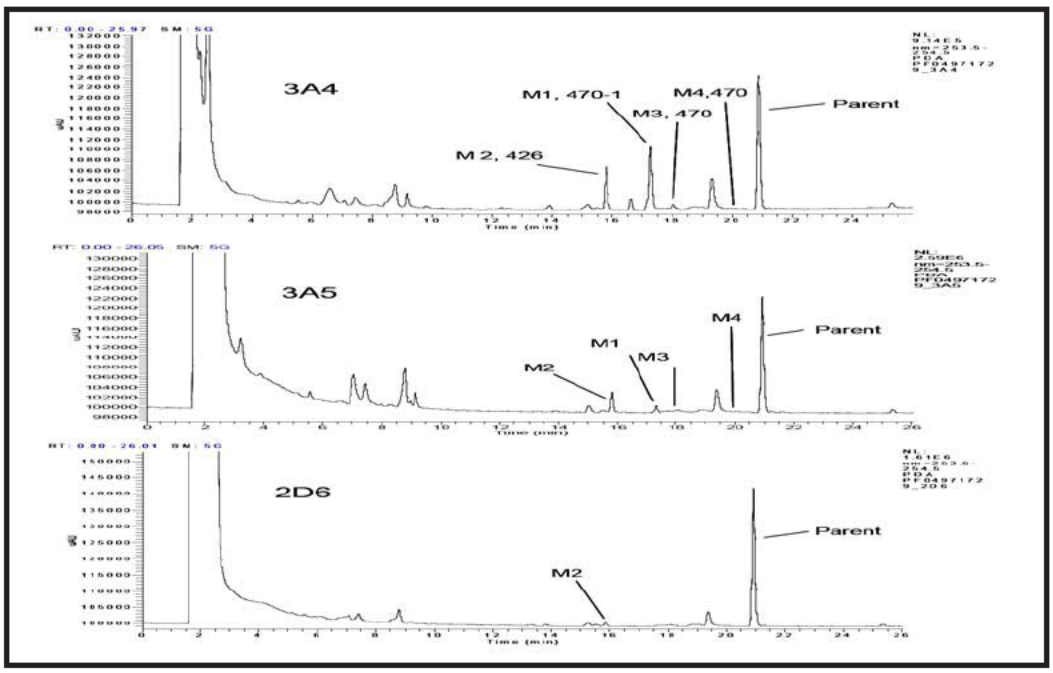
In liver microsomes fortified with NADPH, a total of 4 phase I metabolites were identified. Shown in the sponsor's figure below, metabolites, M1 and M2 were the major metabolites.



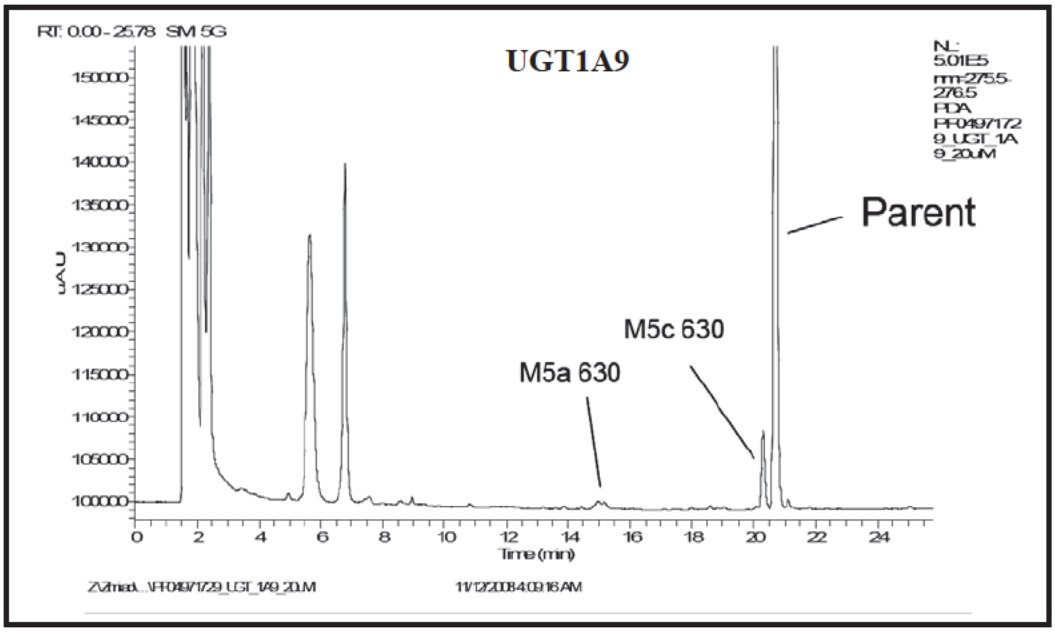
In hepatocytes, in addition to major oxidative metabolites (M1 and M2), two groups of isomeric glucuronides were detected, the first group included M5a, M5b and M5c derived directly from parent, and the second group included M6a, M6b and M6c resulting from glucuronidation of O-desethyl product (M2). Note that human and dog hepatocytes yield the same metabolites in vitro, whereas metabolism by rat hepatocytes was more extensive. This suggests that dogs may model human metabolism of PF-04971729 more closely than rats. Based on the mass spectrometry data, the sites of glucuronidation were identified on the glucose moiety.



PF-04971729 was also incubated with a battery of recombinant human CYP isoforms including: 3A4, 3A5, 2D6, 2C9, 2C19, 1A2, 2E1, 1A9, 1A10, 2B4, 2B7, 2B15, 1A1, 1A3, 1A4, 1A6 and 1A8. Results from these experiments suggested a role of CYP3A4 and 3A5 in the formation of the major oxidative metabolite (M1) and O-desethyl product (M2). Incubation with CYP2D6 also led to the formation of minor quantities of the desethyl metabolite M2. CYP1A9 and 2B7 demonstrated the ability to form small amounts of the M5a and M5c metabolites. No other tested CYP isoforms appeared to be involved in the metabolism of PF-0491729.



Preliminary assessments were also made to characterize the UGT isozymes responsible for glucuronidation of PF-04971729. Amongst a panel of recombinant UGT isoforms tested, only UGT1A9 (shown below) and UGT2B7 were capable of glucuronidating PF-04971729 to metabolites M5a and M5c.



A summary of the metabolites of PF-04971729 identified by the *in vitro* assays is shown in the sponsor's table below.

			Microsomes			rCYPs			Hepatocytes			rUGTs	
Metabolite	*m/z* (NH4+)	Rt (min)*	Rat	Dog	Human	3A4	3A5	2D6	Rat	Dog	Human	1A9	2B7
M6a	602	11.6							x	x	x		
M7	442	11.9							x				
M6b	602	12.2							x	x	x		
M6c	602	12.3							x	x	x		
M8	646	13.9							x				
M2	426	15.9	x	x	x	x	x	x	x	x	x		
M1	470	17.3	x	x	x	x	x		x	x	x		
M3	470	18.1	x	x	x	x	x		x				
M5a	630	18.7							x	x	x	x	x
M5b	630	19.1							x	x	x		
M4	470	20.1	x	x	x	x	x		x				
M5c	630	20.6							x			x	x
PF-04971729	454	21.1	x	x	x	x	x	x	x	x	x	x	x

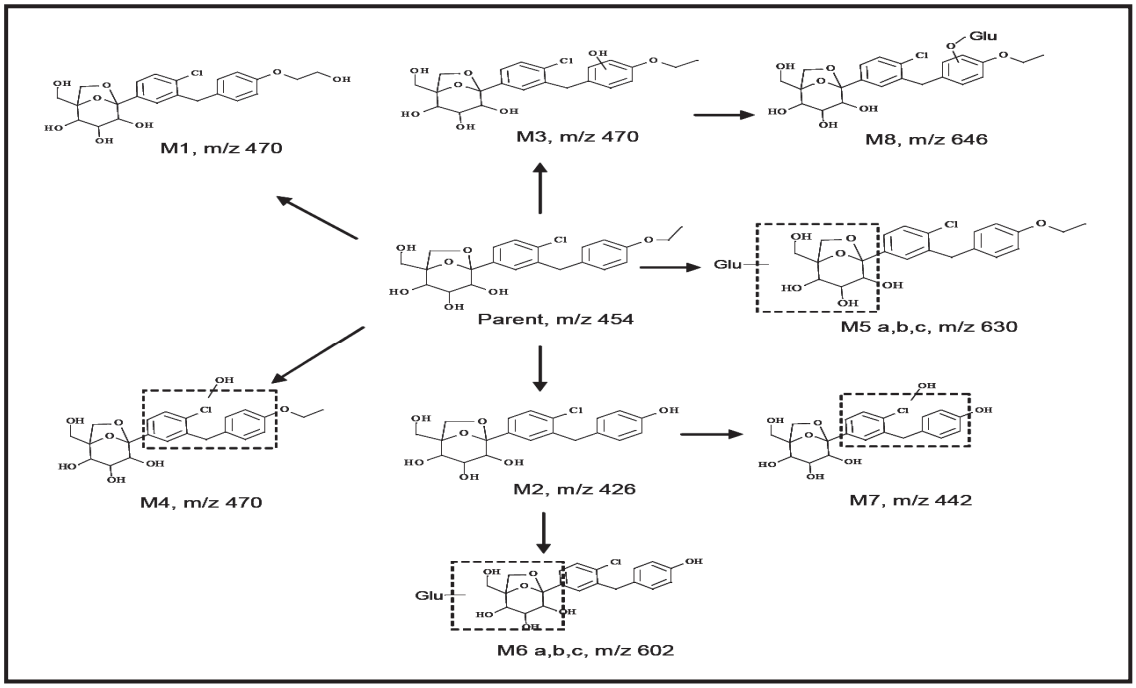
x: detected by Mass spectrometry
* based on the rat hepatocytes

When *in vivo* metabolism of PF-04971729 was examined in rats the metabolites detected in urine, bile and plasma were the same as those identified by the *in vitro* systems of analysis utilized above (*see sponsor's table below*). The HPLC chromatograms suggest that PF-04971729 presented at a higher level than metabolites in urine and plasma, and with lower levels of metabolites in bile; however, the sponsor failed to provide quantitative data. The major identified circulating metabolites were M1, M2.

			Rat		
	m/z (NH4+)	Rt (min)*	Urine	Bile	Plasma
M6a	602	23.8	x		x
M2	426	29.8	x	x	x
M5a	630	31.6	x	x	x
M1	470	32.2	x	x	x
M3	470	32.7	x	x	x
M5b	630	33.5			x
M5c	630	37.0			x
PF-04971729	454	39.1	x		x

x: detected by Mass spectrometry

The predicted structures and proposed metabolic pathways of PF-04971729 are shown in the sponsor's figure below.



2.6.4.6 Excretion

See above studies - (Absorption: 134900 and 134221) and (Metabolism: 162240).

2.6.4.7 Pharmacokinetic drug interactions

See above study - (Metabolism: 162240).

2.6.4.8 Other Pharmacokinetic Studies

Estimation of Human Pharmacokinetics and Pharmacodynamics and Projection of Human Efficacious Dose for PF04971729 (164231)

Reviewer Comments: The objective of this report was to describe the preclinical estimation of pharmacokinetics and pharmacodynamics of PF-04971729 and the projection of its efficacious dose in man.

Study no.:	164231
Volume # and page #:	EDR (4.2.2.7.1)
Conducting laboratory and location:	Pfizer Global Research and Development Groton, CT USA.
Date of study initiation:	Not Stated
GLP compliance:	No
QA report:	None
Drug, lot #, and % purity:	PF-04971729

Methods:

PK/PD Model

PF-04971729 plasma exposures measured in a cohort of Sprague-Dawley rats were modeled using a one compartment pharmacokinetic model with first order absorption and elimination. Estimated pharmacokinetic parameters were fixed in order to characterize the PK/PD relationship with respect to urinary glucose excretion.

Projection of Human Efficacious Dose

Rat PK/PD modeling results were used to project the human dose necessary to achieve 24 hour urinary glucose excretion of 25, 50 and 75 grams. The sponsor's predictions employed the same structural model used to characterize the PK/PD with respect to UGE in rats. In these predictions, IC_{50} estimated in rats was scaled to humans based on known differences in unbound plasma fraction and intrinsic potency between rats and humans.

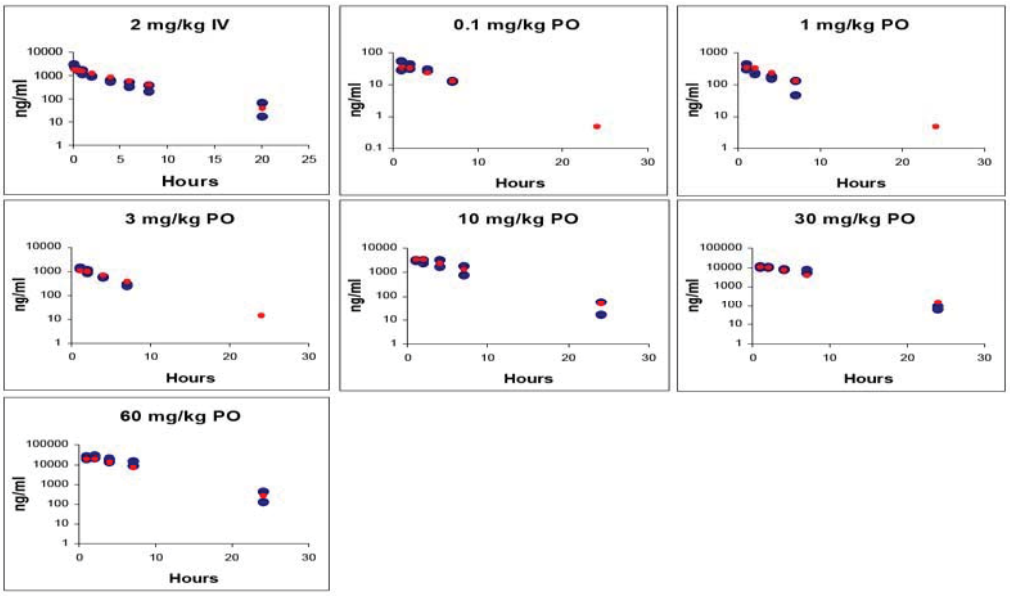
Results:

Projection of Human Pharmacokinetic Parameters

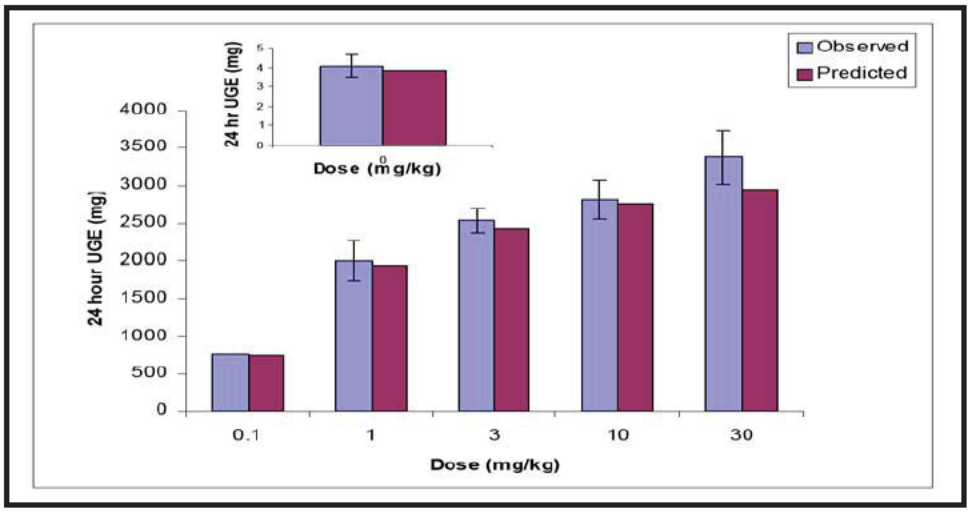
Mean rat pharmacokinetic parameters and plasma protein binding values are summarized in the sponsor's table below and have been previously reported in study 102049 and 134900. The human PK predictions were based on allometric scaling from rats after correction for protein binding in each species. Single-species allometric scaling of rat clearance, which employed an exponent of 0.75, provided a human clearance prediction of 1.7 mL/min/kg. Single species scaling of rat steady-state volume of distribution provided a human volume prediction of 1.8 L/kg. Based on these projected CL and Vdss values, the projected human t½ value was calculated to be 12 hours. Oral bioavailability was predicted from the rat pharmacokinetic data to be and 65%.

Species:	Rat	Human
Body Weight (kg):	0.338 (0.314, 0.361)	70
Free Fraction Plasma (Fu):	0.040	0.064
Blood to Plasma Ratio (B/P):	0.66	0.66
Experimental CLp (mL/min/kg):	4.04 (3.39, 4.68)	1.70
Experimental Vdss, plasma (L/kg):	1.13 (1.18, 1.08)	1.80
Oral Bioavailability F (%)	67	65

Plasma PF-04971729 exposure in rats and associated model predictions are depicted in the sponsor's figure below. Red symbols represent the model prediction, blue symbols represent exposure data.



Urinary glucose excretion in rats and associated model predictions are depicted in the sponsor's figure below.



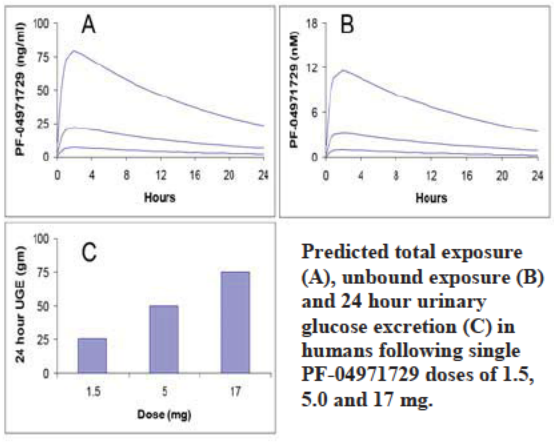
These figures are used to demonstrate that the proposed PK and PK/PD models provide a usable characterization of the observed data. Pharmacokinetic and pharmacodynamic parameter estimates associated with the modeling are reported in the sponsor's table below.

Parameter	Estimate	Standard Error
F (%)	50	5.0
Ka (hr-1)	1.9	0.6
Kel (hr-1)	0.193	0.010
Vd (L/kg)	1.10	0.10
f_{reabs} (no units)	0.9994	0.0093
I_{max} (no units)	0.43	0.02
IC_{50} (ng/ml)	22	6.0

Human Exposure and Response Projections

Using the projected human pharmacokinetic and pharmacodynamic parameter values listed in the table below (left), single PF-04971729 doses of 1.5, 5.0 and 17 mg were predicted to provide 24 hour urinary glucose excretions of 25, 50 and 75 mg, respectively (see sponsor's figure below, right).

Parameter	Value
Body Weight (kg)	70
F (%)	65
Ka (hr-1)	1.9
CL (ml/min/kg)	1.70
Vd (L/kg)	1.80
f_{reabs} (no units)	0.9994
I_{max} (no units)	0.37
IC_{50} (ng/ml)	11
GFR (ml/min)	115
PG (mg/dl)	154



Predicted total exposure (A), unbound exposure (B) and 24 hour urinary glucose excretion (C) in humans following single PF-04971729 doses of 1.5, 5.0 and 17 mg.

The same exposures and responses are predicted following once daily PF-04971729 doses of 1.1, 3.5 and 13 at steady-state. A summary of the predicted exposures and the urinary glucose excretions associated with these doses is provided in the table below.

Projected Human Dose and Associated Summary Pharmacodynamic and Pharmacokinetic Values

Single Dose (mg/70 kg)	Steady-State dose (mg / 70 kg QD)	UGE (mg / 24 hours)	Total PF-04971729 Plasma Exposure Summary Values						
				Cmax	Cavg	Cmin	AUC24 h, total (ng•h/mL)	Tmax (h)	t1/2 (h)
1.5	1.1	25	ng/mL	7.0	4.2	2.0	101	1.9	12
			nM	16	9.6	4.6			
5	3.5	50	ng/mL	22	13	6.4	312	1.9	12
			nM	50	30	15			
17	13	75	ng/mL	78	47	23	1128	1.9	12
			nM	179	108	53			

Single Dose (mg/70 kg)	Steady-State dose (mg / 70 kg QD)	UGE (mg / 24 hours)	Unbound PF-04971729 Plasma Exposure Summary Values						
				Cmax	Cavg	Cmin	AUC24 h, total (ng•h/mL)	Tmax (h)	t1/2 (h)
1.5	1.1	25	ng/mL	0.45	0.27	0.13	6.5	1.9	12
			nM	1.0	0.61	0.29			
5	3.5	50	ng/mL	1.4	0.83	0.41	20	1.9	12
			nM	3.2	1.9	0.94			
17	13	75	ng/mL	5.0	3.0	1.5	72	1.9	12
			nM	11	6.9	3.4			

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: PF-04971729 was administered orally to rats and dogs in 1 month (GLP) studies. The no observed adverse effect levels (NOAELs) in these studies were 25 mg/kg (1X MRHD) and 1 mg/kg (< 1XMRHD) with an AUC_{0-24} of 81 µg.hr/mL and 8 µg.hr/mL for rats and dogs, respectively.

Target organs identified in these studies included kidney, gallbladder, G.I. tract and lung. Rats were sensitive to kidney effects and developed progressive nephropathy at doses ≥ 250 mg/kg. The incidence of vacuolation of the gallbladder was increased at doses of drug ≥ 10 mg/kg in dogs. Perturbations of the lung were seen most frequently in dogs at the maximum dose of 150 mg/kg. G.I. effects were present at all doses in dogs but significant changes in both species were seen at the highest doses only.

Genetic toxicology: There were no treatment related reductions in the mean percent polychromatic erythrocytes (PCE) reported in rats, suggesting a low incidence of bone marrow toxicity. No statistically significant increases in the numbers of PCE with micronuclei suggests that PF-04971729 does not possess, under these conditions, the ability to induce chromosomal damage and thereby increase the frequency of micronucleated PCEs.

Exposure to PF-04971729 did not increase the mean number of revertants with any tester strain either in the presence or absence of S9.

PF-04971729 did not induce structural chromosome aberrations in human lymphocyte *in vitro* cultures.

Increases in polyploidy were seen in a 3 hr test with S9 at 173 µg/mL and 156 µg/mL. These doses demonstrated a marked mitotic suppression (55% and 43%, respectively).

While there were no increases in polyploidy or hyperploidy in a test with a longer exposure time, the 24-hour test was performed in the absence of metabolic activation (S9).

Carcinogenicity & Reproductive toxicology: No studies have been submitted.

2.6.6.2 Single-dose toxicity

Escalating Dose Oral Toxicity Study of PF-04971729 in Dogs (08GR497), Non-GLP

Key study findings:

- Single doses of 5 and 50 mg/kg (<1x and ~9X MRHD). Exposures (AUC_{0-24}) were non-linear in males (26.5, 386, and 138 µg.h/mL, males) and females (51.0, 474, and 465 µg.h/mL) between the two top doses (50 and 500mg/kg).*

- Results from HD (500 mg/kg) males and females are most likely invalid due to vomiting of drug within the first hour. The non-linearity in dose mentioned above was most likely caused by this emesis as well.

Dog, Single dose	NOAEL (AUC_{0-24} µg.hr/mL)	multiple of starting dose (0.045 µg.hr/mL)	multiple of max dose (50.63 µg.hr/mL)
Vomiting	M/F: 50 mg/kg (AUC_{0-24} ~430)	9500X	~8X
Salivation	F: 50 mg/kg (AUC_{0-24} ~474)	10500X	9X

Study no.: 08GR497
Volume #, and page #: EDR (4.2.3.1.1)
Conducting laboratory and location: Pfizer Global Research and Development Groton, CT USA.
Date of study initiation: 12 January 2009
GLP compliance: No
QA report: No
Drug, lot #, and % purity: PF-04971729, 00701380-030-01, 99%

Methods	
Doses	*Active Moiety* - 5, 50, 500 mg/kg (single dose)
Species/source	Beagle dog / (b) (4)
Age / Weight	>8 months / M:6.5-11.1kg F: 7.7-9.3kg
n/sex/group (main study)	1/sex/group
TK groups	D-1 (0.5, 1, 4, 7 and 24 hours postdose)
Recovery groups	none
Route, formulation, dose volume	Oral gavage in 5 mL/kg - 0.5% methylcellulose with 10% polyethylene glycol 400 (PEG 400)

Observations and Times	
Mortality checks	4 times – (predose and 1, 3 and 6 hours post dose)
Clinical Findings	4 times – (predose and 1, 3 and 6 hours post dose)
Body weights	Pretreatment and prior to dosing D1

Food consumption	Measured daily
Hematology& Coagulation	Fasted animals pretreatment and D-2 (Standard Battery)
Clinical chemistry	Fasted animals pretreatment and D-2 (Standard Battery)
Urinalysis	Not measured
Gross pathology	All main study animals
Organ weights	Not measured
Histopathology	Not measured

Results:

Mortality: There were no deaths during the study.

Clinical Signs: Treatment-related clinical signs included white foamy vomit on D-1 at 500 mg/kg at approximately 0.5 hours postdose for 1 male (Animal 3, 500 mg/kg) and at approximately 1 hour postdose for both male (Animal 3, 500 mg/kg) and female (Animal 6, 500 mg/kg). Salivation was also noted on Day 1 for HD female (Animal 6).

Food Consumption: There were no treatment-related effects on food consumption.

Hematology: Neutrophil and monocyte numbers tended to decline with dose in male dogs.

Hematology and Coagulation

08GR497: ESCALATING DOSE ORAL TOXICITY STUDY OF PF-04971729 IN DOGS

Dose (mg/kg)	Animal Number	Day	NEUT (10e3/uL)	NEUT P (%)	LYM (10e3/uL)	LYM P (%)	MONO (10e3/uL)	MONO P (%)	EO (10e3/uL)	EO P (%)	BASO (10e3/uL)	BASO P (%)
						MALE						
5	1	-7	3.54	54.2	2.39	36.6	0.40	6.1	0.15	2.3	0.05	0.7
		2	6.36	73.8	1.66	19.3	0.46	5.3	0.11	1.3	0.02	0.3
50	2	-7	3.41	66.5	1.27	24.7	0.16	3.2	0.26	5.0	0.02	0.4
		2	5.31	72.6	1.34	18.4	0.33	4.5	0.30	4.1	0.02	0.2
500	3	-7	4.30	67.7	1.71	27.0	0.16	2.5	0.13	2.1	0.03	0.5
		2	4.23	65.4	1.81	27.9	0.22	3.4	0.17	2.6	0.03	0.5

- Value not applicable or not available.
HPD = Hours Postdose.
a = Unscheduled value will not be included in the calculation of mean and standard deviation.

In female dogs: RBC, mean cell hemoglobin count (MCHC) and red cell distribution width (RDW) demonstrated dose dependent decreases.

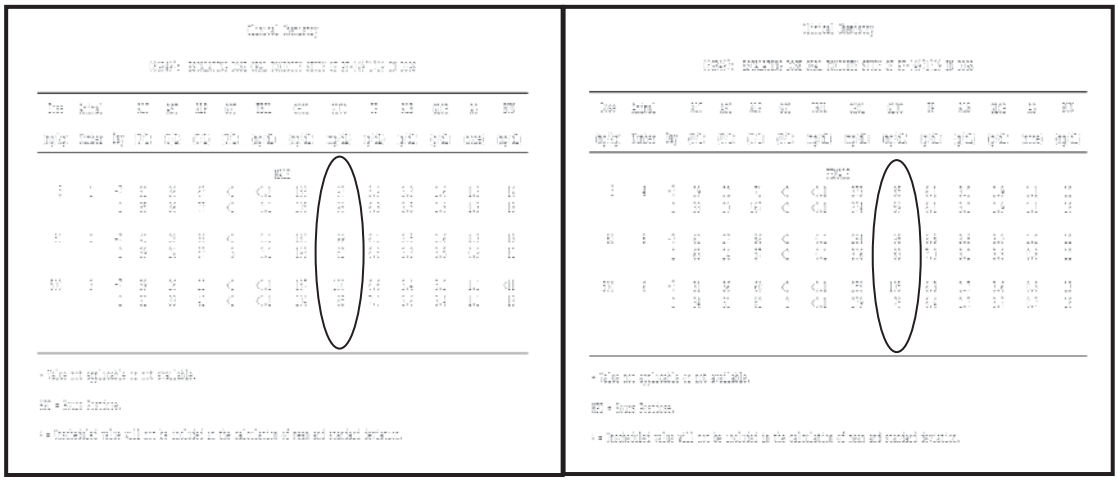
Hematology and Coagulation

08GR497: ESCALATING DOSE ORAL TOXICITY STUDY OF PF-04971729 IN DOGS

Dose (mg/kg)	Animal Number	Day	RBC (10e6/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	RETIC P (%)	RETIC (10e3/uL)	PLT (10e3/uL)	WBC (10e3/uL)
							FEMALE						
5	4	-7	6.60	14.9	44.5	67.4	22.6	33.5	13.0	0.5	33	445	8.6
		2	7.37	16.6	48.2	65.4	22.5	34.5	13.3	0.8	59	417	12.1
50	5	-7	7.07	16.4	50.6	71.5	23.1	32.3	12.8	0.4	28	389	5.0
		2	7.29	16.9	49.7	68.2	23.3	34.1	12.3	0.2	15	338	4.6
500	6	-7	6.95	15.9	47.9	68.9	22.8	33.2	11.7	1.0	70	533	7.4
		2	6.88	15.6	46.5	67.6	22.7	33.6	11.7	0.6	41	521	10.3

- Value not applicable or not available.
HPD = Hours Postdose.
a = Unscheduled value will not be included in the calculation of mean and standard deviation.

Clinical Chemistry: Treatment-related effects in clinical chemistry parameters included a decrease (15%-17% from pretreatment value) in glucose in males at 50 and 500 mg/kg (table below, left). In female dogs all dosed animals saw a reduction in glucose levels although the decreases were not relevant to the dose used: (5 mg/kg, 31%), (50 mg/kg, 13%) and (500 mg/kg, 26%) from pretreatment value (table below, right). *Glucose levels may appear artificially elevated in the HD groups due to the fact that animals vomited drug at this dose within 1 hour.*



Toxicokinetics: The observed T_{max} for the 5 and 50 mg/kg/day doses was ~0.6h, while the T_{max} was much higher for the 500 mg/kg/day dose (4 hours). In males and females, C_{max} and AUC_{0-24} increased approximately dose-dependently between 5 and 50 mg/kg. At 500 mg/kg, C_{max} and AUC_{0-24} do not increase further, in fact values tended to drop at this dose level in both sexes. *The decreased values at 500 mg/kg are most likely due to the fact that animals at this dose level vomited within an hour of dosing.*

PF-04971729 Dose (mg/kg/day)	Study Day	Gender	Cmax (µg/mL)	tmax (h)	AUC(0-24) (µg*h/mL)	n
5	1	Male	3.45	0.500	26.5	1
		Female	6.37	0.500	51.0	1
		Overall	4.91	0.500	38.8	2
50	1	Male	34.1	0.500	386	1
		Female	58.4	1.00	474	1
		Overall	46.3	0.750	430	2
500	1	Male	12.2	4.00	138	1
		Female	38.6	4.00	465	1
		Overall	25.4	4.00	302	2
Overall = Male and Female Combined						

Plasma concentrations of PF-04971729 in dogs after oral administration of drug on study day 1 are shown in the sponsor's table below.

PF-04971729 Dose (mg/kg/day)	Study Day	Gender	PF-04971729 Plasma Concentrations (µg/mL) by Time (h)					n
			0.5	1	4	7	24	
5	1	Male	3.45	3.32	1.85	1.13	0.254	1
		Female	6.37	6.07	3.47	2.06	0.725	1
		Overall	4.91	4.70	2.66	1.60	0.490	2
50	1	Male	34.1	34.0	26.2	18.9	4.88	1
		Female	49.2	58.4	30.3	22.1	4.21	1
		Overall	41.7	46.2	28.3	20.5	4.55	2
500	1	Male	5.43	11.7	12.2	6.33	1.76	1
		Female	20.4	35.9	38.6	23.9	4.41	1
		Overall	12.9	23.8	25.4	15.1	3.09	2
Overall = Male and Female Combined								

2.6.6.3 Repeat-dose toxicity

<table>
<tr><th colspan="2">IN VIVO TOLERATION STUDY OF PF-04971729 IN RATS (08GR396, NON-GLP)</th></tr>
<tr><td>SPECIES
DOSES AND ADMINISTRATION
ANIMALS</td><td>NOAEL= 50 MG/KG (~2X MRHD, µg.h/mL Basis)</td></tr>
<tr><td>Sprague-Dawley Rats

0, 5, 50, 500 mg/kg/d p.o. in 20% PEG/24% HPBCD for 7 days. QD

N=5/males/group</td><td>
<table>
<tr><th></th><th>AUC, µg.h/ml</th></tr>
<tr><th></th><th>Males</th></tr>
<tr><th>Dose, mg/kg</th><th>Day 1</th></tr>
<tr><td>5</td><td>16.8</td></tr>
<tr><td>50</td><td>98.8</td></tr>
<tr><td>500</td><td>1500</td></tr>
</table>
</td></tr>
<tr><td colspan="2"><u>Mortality:</u> There were no unscheduled deaths during this study.</td></tr>
<tr><td colspan="2"><u>Clinical Signs</u>: At 500 mg/kg loose stools were reported throughout the study.</td></tr>
<tr><td colspan="2"><u>Body Weight</u>: At 500 mg/kg there was a decrease in body weight and food consumption and decreased body weight gain in both the 50 and 500 mg/kg/day groups through the course of the study. The decrease in bodyweight was (92% to 94% of the control mean) on Days 3 to 7 and in food consumption (54% to 95% of control mean) on Days 3 and 5 in the 500 mg/kg/day group.</td></tr>
<tr><td colspan="2"><u>Hematology</u>: The hematology changes included decreases in the WBC (45% of the control mean), LCT (40% of the control mean), EOCT (15% of the control mean), BCT (38% of the control mean), and PLT (67% of the control mean) in the 500 mg/kg/day animals and decreases in MOCT (53 and 35% of the control mean) in the 50 and 500 mg/kg/day animals, respectively. A slight elevation in HCT (1.06x control mean) in the 500 mg/kg/day animals was observed as well.</td></tr>
<tr><td colspan="2"><u>Clinical Chemistry</u>: Treatment-related serum clinical chemistry parameter changes in ALT and BUN occurred at 50 and 500 mg/kg/day animals; a treatment-related change in the GLU in the 50 mg/kg/day dose group; and a treatment-related change in the AST and CHOL in the 500 mg/kg dose group. The serum clinical chemistry changes included increases in the ALT (1.76 – 3.16x control mean) and (BUN (1.77 – 3.12x control mean) in the 50 and 500 mg/kg/day animals; and increases in AST (3.49x control mean) and CHOL (1.72x control mean) in the 500 mg/kg/day animals. There was a significant decrease in Glucose (69% of the control mean) in 50 mg/kg/day animals although a very minimal reduction in the 5 and 500 mg/kg/day groups.</td></tr>
<tr><td colspan="2"><u>Urinalysis:</u> Treatment-related urinalysis parameter changes included: changes in urine volume and in the urine clinical chemistry parameters including UGLU, UCREA and the UGLU/UCREA ratio. These urinalysis parameter changes were an increase in the urine volume of (2.3, 2.9 and 2.6x control mean) and UGLU (321, 326 and 390x control mean); a decrease in UCREA (60, 49 and 53% of the control mean); and concomitant increase in the UGLU/UCREA ratio (527, 660 and 738x control mean) in the 5, 50 and 500 mg/kg/day animals, respectively.</td></tr>
</table>

Organ Weights: A dose dependent drop in liver weight is seen with PF-04971729. Mean liver weights for 0, 5, 50 and 500 mg/kg dosed animals are: 10.2, 9.6, 8.8 and 8.5g respectively.

Histopathology: There were dose-dependent histomorphological changes in the 50 and 500 mg/kg/day dose groups. These changes included lipid depletion of the mesenteric fat and depletion of the zymogen granules in the pancreas. The lipid depletion of the mesenteric fat ranged from minimal (1/5) in the 50 mg/kg/day group and moderate to more severe (5/5) in the 500 mg/kg/day group. The lipid depletion of fat is characterized by a collapse of the fibrovascular stroma with a concomitant decrease in adipocyte size and the presence of adipocytes with multiple small to single variable-sized intracytoplasmic vacuoles. The depletion of pancreatic zymogen granules ranged from minimal to mild in the 50 (3/5) and 500 (2/5) mg/kg groups. One (500 mg/kg) dosed male presented with vacuolation and regional inflammation of the liver.

Other Findings: The oral administration of PF-04971729 to rats did not induce micronuclei in the polychromatic bone marrow erythrocytes at ≤500 mg/kg.

Male Sprague-Dawley Rats
TK:

Dose (mg/kg)	Sex	Animal number	**Cmax** (µg/mL)	**Tmax** (hr)	**AUC_{0-24}** (µg*Hours/mL)
5	M	6	0.996	4.0	14.3
	M	7	0.834	1.0	9.08
	M	8	1.66	4.0	23.3
	M	9	1.67	4.0	27.3
	M	10	0.783	1.0	10.0
		Mean	**1.19**	**2.8**	**16.8**
		S.D.	0.442	1.64	8.13
		%CV	37.2	58.7	48.4
50	M	11	8.43	4.0	98.6
	M	12	7.21	1.0	101
	M	13	7.35	4.0	95.3
	M	14	8.31	1.0	105
	M	15	6.93	4.0	94.0
		Mean	**7.65**	**2.8**	**98.8**
		S.D.	0.679	1.64	4.43
		%CV	8.9	58.7	4.50
500	M	16	91.6	7.0	1440
	M	17	101	4.0	1450
	M	18	76.6	4.0	1350
	M	19	109	7.0	1950
	M	20	72.8	1.0	1330
		Mean	**90.2**	**4.6**	**1500**
		S.D.	15.5	2.51	255
		%CV	17.2	54.6	16.9

1-Month Oral Toxicity Study and Micronucleus Assessment of PF-04971729 in Rats (09GR185) (b) (4)

Key study findings from sponsor:

- After one month of dosing, female rats given 5, 25, and 500 → (D11)250 mg/kg/day had exposures of 16.2, 93.0 and 718 µg.h/mL (<1X, ~2X and ~14X MRHD, µg/h/mL basis) compared to males 5, 25, and 500 → (D11)250 mg/kg/day had exposures of 8.4, 69.3 and 541 µg.h/mL (<1X, ~1X and ~11X MRHD, µg/h/mL basis).
- Significant clinical signs in HD animals included: (Abdomen distended, reduction activity, anogenital staining, fecal discoloration and/or softness and changes in hair quality. Several animals at the original HD of 500 mg/kg/day were euthanized in moribund condition or found dead within the first 3 weeks. There were no significant clinical signs ≤ 25 mg/kg.
- Treated males given ≤ 25 mg/kg lost 4% more weight than controls, at ≥ 250 mg/kg males lost 28%. Females lost on the average 16% more weight than controls across all dosing levels. Weight loss occurred despite substantial increases in food consumption.
- Decreases (2.3% to 6.0% vs. control) in group mean red blood cell count (RBC), hematocrit (HCT), and/or hemoglobin (HGB) were observed in all rats at 500/250 mg/kg.
- By the completion of the study, serum glucose levels had decreased by (16.6% to 39.7% vs. control) in all treated rats.
- Increased BUN levels were accompanied by decreased creatinine levels (14%-18% vs. control) in male and (6%-10% vs. control) female rats.
- There were dose-dependent increases in group mean absolute and relative kidney weights (1.1-1.4x control).
- In the kidney, treatment related dilatation of renal tubules (mainly distal nephron segments) was noted in the cortex and medulla of males treated at ≥5 mg/kg and females treated at ≥25 mg/kg.
- AST and ALT increased 2-3 fold at 250mg/kg, with smaller increases occurring at 5 and 25mg/kg. No change was observed in total bilirubin or in liver histology.
- There were no treatment related reductions in polychromatic erythrocytes (PCE), suggesting a low incidence of bone marrow toxicity. No significant change in the numbers of PCE with micronuclei suggests that PF-04971729 does not possess, under these conditions, the ability to induce chromosomal damage.

Reviewer Comments: Throughout the study, females had higher exposures relative to males, which may have contributed to the more severe clinical signs and increased mortality. Most of the findings in this study could reasonably be attributed to drug induced glucosuria, osmotic diuresis and a catabolic state. The relatively modest (≤ 3 fold) increase in ALT and AST were not accompanied by bilirubin elevation or adverse liver histology, but should nevertheless be monitored in clinical studies. Of note, all doses were associated with adrenal hypertrophy (males), pancreatic zymogen depletion, and atrophy of adipose tissue. Dilatation of renal tubules also

occurred at all doses in males and ≥ 25mg/kg in females. These findings were not considered as a basis for a NOAEL as they reasonably reflect a non-toxic compensatory response to glucosuria. Rather, the NOAEL is based on the clearly evident toxic effects of moribundity at 500mg/kg and increased severity of chronic progressive nephropathy and stomach erosion/squamous hyperplasia in females at 250mg/kg.

PF-04971729 (b) (4) did not alter bone in the stifle joint or the sternum, unlike many other SGLT2 inhibitors that cause hyperostosis of bone.

Rat, 1 month	NOAEL	multiple of starting dose (0.045µg h/mL)	multiple of max dose (50.63µg h/mL)
Mortality	250 mg/kg (611 µg.h/mL)	13500X	12X
Increased Chronic Nephropathy	25 mg/kg (81 µg.h/mL)	1800X	~1X

Study no.: 09GR185
Volume # and page #: EDR (4.2.3.2.1)
Conducting laboratory and location: Pfizer Global Research and Development Groton, CT USA.
Date of study initiation: 28 May 2009
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: PF-04971729 (b) (4), GR02546, 99.5% Active Moiety: 76%

Methods	
Doses	0, 5, 25, 500→(D11)250 mg/kg/d for one month
Species/source	Sprague Dawley (Crl:CD®[SD])/ (b) (4)
Age / Weight	6-8 weeks / 264.3-328.6g (M) 190.4-234g (F)
n/sex/group (main study)	10/sex/group
TK groups	6/sex/dose (satellite) D1 and D28 – 1 animal/time point - 0.5, 1, 2, 4, 7, 24
Micronucleus Positive Control Animals	5/sex/group
Route, formulation, dose volume	Oral dosing in 0.5% (w/v) MC + 10% (v/v) PEG-400 ~ 10 mLs/kg

Observations and Times	
Mortality checks	Pretreatment/Predose: once per day in the morning Dosing: twice per day (2 hours postdose and in the afternoon).
Clinical Findings	Pretreatment: once per day in the morning Dosing: twice per day (2 hours postdose and in the afternoon). Day of Necropsy: Once Micronucleus Positive control animals: Once a day.
Body weights	Pretreatment, prior to dosing on days: 1, 7, 14, 21 and 28.

	A fasted weight was taken just before necropsy. Body weights of micronucleus positive control rats were determined once pretreatment and on the first day of dosing with cyclophosphamide.
Food consumption	Treatment period: weekly
Ophthalmology	Pretreatment and on Day 23(males) and Day 24(females)
Hematology	All main study animals tested at autopsy: RBC, Hb, Hct, MCV, MCH, MCHC, RCDW, Ret, PLT, MPV, WBC, WCD and fibrinogen
Clinical chemistry	Day 8, D29(M) and D30(F): Standard Battery
Urinalysis	Day 28(males) and Day 29(females) following parameters: Clarity, color, bilirubin, occult blood, pH, protein, specific gravity, urobilinogen, glucose, ketones and total volume. Urine sediment: Urinary Glucose and Urinary Creatinine
Gross pathology	All animals
Organ weights	Adrenals, Brain, Epididymis, Heart, Kidney, Liver, Ovary, Prostate, Spleen, Testis and Thymus.
Histopathology	All control and HD animals. LD and MD animals selected exams

Organs Collected	Organs Weighed	Tissues Examined Microscopically Dose Groups (mg/kg/day) 0	5	25	500/250 ‡
Adrenal	X	X	X	X	X
Aorta		X			X
Bone Marrow		X			X
Brain	X	X			X
Cecum		X			X
Cervix		X			X
Colon		X			X
Duodenum		X			X
Epididymis	X	X			X
Esophagus		X			X
Eye		X			X
GALT		X			X
Harderian Gland		X	X	X	X
Heart	X	X			X
Ileum		X			X
Inguinofemoral Lymph Node		X			X
Jejunum		X			X
Joint		X			X
Kidney	X	X	X	X	X

Organs Collected	Organs Weighed	Tissues Examined Microscopically Dose Groups (mg/kg/day) 0	5	25	500/250 ‡
Larynx					
Liver †	X	X			X
Lung		X			X
Mammary Gland		X	X *	X *	X
Mesenteric Lymph Node		X			X
Mesentery		X	X	X	X
Optic Nerve		X			X
Ovary	X	X	X	X	X
Oviduct		X			X
Pancreas		X	X	X	X
Parathyroid		X			X
Peripheral Nerve		X			X
Pituitary		X			X
Prostate	X	X	X	X	X
Salivary Gland **		X	X	X	X
Seminal Vesicle		X			X
Skeletal Muscle		X			X
Skin and Adnexa		X			X
Spinal Cord		X			X
Spleen	X	X			X
Sternum		X			X
Stomach		X			X
Testis	X	X			X
Thymus	X	X	X ***	X ***	X
Thyroid		X			X
Tongue		X			X
Trachea		X			X
Ureter		X			X
Urinary Bladder		X			X
Uterus		X			X
Vagina		X	X	X	X

Adequate Battery: yes (X), no ()

Peer review: yes (X), no ()

Results:

Mortality: Five animals in the 500→250 mg/kg dose group (Main Study and TK Study) were euthanized in moribund condition or found dead during Weeks 1-3. Animal 78F was euthanized in moribund condition on Day 7. Animal 75F was found dead following scheduled blood collection on Day 8. Animal 546F was found dead on Day 11. Animal 37M was euthanized in moribund condition on Day 12.

In light of the treatment related mortality and morbidity, the high dose level was reduced by the sponsor to 250 mg/kg from 500 mg/kg on Day 11. Subsequent to the dose reduction, Animal 32M in the 500/250 mg/kg dose group was found dead on Day 20.

Two females (61F, 79F) from the 25 and 500/250 mg/kg dose group (Main Study) were found dead following schedule blood collection on the day of necropsy (Day 30). The mortality noted in the single rat at 25 mg/kg and 500/250 mg/kg on Day 30 was considered incidental by the sponsor and related to blood collection as these animals did

not present with any remarkable in life or pathological findings. Death of Animal 79F appears to be absent from sponsor's data summary table.

MORTALITY								
	MALES				FEMALES			
DOSE	***0***	***5***	***25***	***500/250***	***0***	***5***	***25***	***500/250***
Died	0	0	0	1/10	0	0	1/10	3/10
Unscheduled Euthanasia	0	0	0	1/10	0	0	0	1/10

Clinical signs:

Treatment related clinical signs noted at 500/250 mg/kg included: anogenital staining, discolored (pale, brown) and soft feces, distended abdomen, and/or stained hair coat.

Additional clinical signs that were observed in a limited number of high dose animals (35M, 37M, 71F, 72F, and 78F) also included: decreased skin turgor, hunched posture, decreased activity / ataxia, noisy respirations, cool to the touch, and/or rough and stained hair coat. Of these animals, high dose animal 37M and 78F were euthanized in moribund condition on Day 12.

Clinical signs noted in TK study animals at 500/250 mg/kg were limited to anogenital staining and discolored (pale, brown) and soft feces, with the exception of high dose animal 546F which also exhibited decreased activity and skin turgor, and was found dead on Day 11.

Subsequent to reducing the high dose level to 250 mg/kg on Day 11, animals at the 500/250 mg/kg dose level were reported to show significant improvement in their clinical condition. Clinical signs for these animals had returned to "normal" over Days 11-16, with the exception of high dose animal 35M which continued to show a rough hair coat, and high dose animal 79F which continued to show a distended abdomen through Day 27. High dose animal 32M was reported with decreased activity, hunched posture, and partially closed eyes on Day 19 and was subsequently found dead on Day 20.

There were no remarkable treatment related clinical signs noted at 5 and 25 mg/kg except a single animal at 5 mg/kg (52F) exhibited decreased activity (Days 24-25), stained/rough fur coat (Days 23-30), and/or piloerection (Days 23-28). There were no remarkable changes in body weight observed in this animal. The observations in this animal correlated with moderate chronic inflammation in the spleen (see histopathology section) accompanied by secondary clinical laboratory findings consistent with inflammation. The observations in this animal were considered unrelated to treatment by the sponsor, due to their low incidence, the absence of similar changes in other treated animals, and/or the absence of a dose relationship.

Incidental findings, not attributed to treatment, were noted sporadically in a limited number of animals and included: hair loss (limb), abrasion (cervical), and/or skin ulcerations (scrotum, tail).

CLINICAL SIGNS								
Finding	MALES							
	0		5		25		500→(250)	
	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals
Abdomen Distended	0	0	0	0	0	0	5/10	11
Activity Decreased	0	0	0	0	1/10	1	3/10	5
Anogenital Staining	0	0	0	0	0	0	9/10	42
Hypothermia	0	0	0	0	0	0	1/10	1
Decreased Skin Turgor	0	0	0	0	0	0	2/10	11
Eye Partially Closed (Unilateral)	0	0	0	0	0	0	1/10	1
Feces Discolored, Pale	0	0	0	0	0	0	10/10	70
Feces Soft	0	0	0	0	0	0	10/10	91
Hair Loss Limb/Paw	0	0	0	0	2/10	11	0	0
Hair Coat Rough	0	0	0	0	0	0	3/10	20
Hair Coat Stained	0	0	0	0	0	0	1/10	9
Posture Hunched	0	0	0	0	0	0	3/10	10
Ulceration Scrotum	0	0	0	0	0	0	1/10	2
Ulceration Tail	0	0	0	0	0	0	1/10	8

CLINICAL SIGNS								
Finding	FEMALES							
	0		5		25		500→(250)	
	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals
Abdomen Distended	0	0	0	0	0	0	6/10	36
Activity Decreased	0	0	1/10	2	0	0	2/10	3
Anogenital Staining	0	0	0	0	0	0	9/10	34
Ataxia	0	0	0	0	0	0	1/10	1
Hypothermia	0	0	0	0	0	0	1/10	1
Decreased Skin Turgor	0	0	0	0	0	0	2/10	6
Eye Partially Closed (Bilateral)	0	0	0	0	0	0	1/10	5
Feces Discolored, Pale	0	0	0	0	0	0	10/10	59
Feces Soft	0	0	0	0	0	0	10/10	72
Hair Loss Limb/Paw	1/10	14	0	0	2/10	22	1/10	23
Hair Coat Rough	0	0	1/10	8	0	0	0	0
Hair Coat Stained	0	0	1/10	6	0	0	2/10	3
Noisy Respiration	0	0	0	0	0	0	1/10	1
Piloerection	0	0	1/10	6	0	0	0	0
Posture Hunched	0	0	0	0	0	0	2/10	10

Body weights:

Mean body weights (vs. control) were decreased -9.7% to -13.0% for males at 500/250 mg/kg on Days 7, 14, 21, and 28. The decreases in mean body weights in males at 500/250 mg/kg correlated with marked decreases in mean weekly body weight gains (vs. control) of -98.3% and -34.3% over Days 1-7 and 8-14, respectively. Subsequent to the dose reduction for the high dose group, mean weekly body weight gains (vs. control) for this dose group had recovered by Days 15-21 (+2.6%) and Days 22-28 (+26.4%) and were comparable to that noted in controls. Body weight changes noted in 500/250 mg/kg males (TK Study) were reported as comparable to that of those observed in the main study males by the sponsor.

Males Body Weight (Weekly) High Dose 500→(250 mg/kg) Group Only					
Sex	Dose, mg/kg	Days	BW gain (g) over dosing	% Change in Gain vs. Control	Mean Body Weight vs. control
Males	0	1-7	36.11	0%	0%
		8-14	34.10	0%	0%
		15-21	34.81	0%	0%
		22-28	32.6	0%	0%
	500→(250)	1-7	0.6	-98.3%	-10.6%
		8-14	22.4	-34.3%	-13.0%
		15-21	35.7	2.6%	-12.5%
		22-28	41.2	26.4%	-9.7%

Male body weight gain, % change in gain and body weight percent of control are reported for the entire study below.

Males Body Weight (Entire Study)				
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Males	0	138	0%	100%
	5	132	-4%	99.5%
	25	132	-4%	99.6%
	500→(250)	100	-28%	90.3%

In high dose females, mean body weights (vs. control) were decreased by -2.7% on Day 7. Subsequent to the dose reduction, mean body weights remained on par with control groups during days 8-14 and 15-21. By day 28 female mean body weight had dropped again by -4.0%. Mean weekly body weight gains for females over Days 1-7 were decreased -31.6% (vs. control) which was attributed to changes noted in two animals (75F and 78F) that exhibited marked loss in body weight over this interval (-11.3% and -29.9%, respectively). Mean weekly body weight gains for this dose group were increased +15.4% (vs. control) on Days 8-14, decreased -8.9% (vs. control) on Days 15-21, and decreased -81.6% (vs. control) on Days 22-28. The sponsor stated that no remarkable changes in mean body weight parameters were noted in 500/250 mg/kg females (TK Study).

Females Body Weight (Weekly) High Dose 500→(250 mg/kg) Group Only

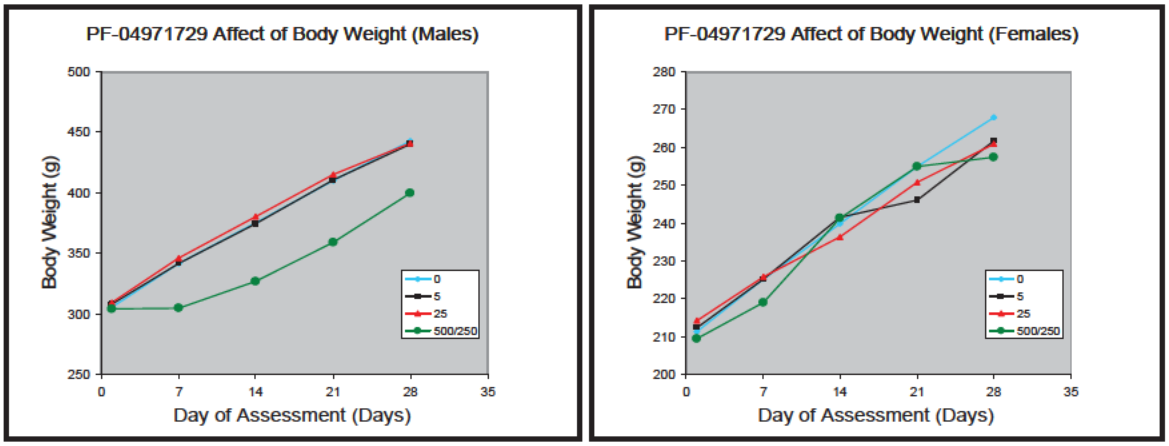
Sex	Dose, mg/kg	Days	BW gain (g) over dosing	% Change in Gain vs. Control	Mean Body Weight vs. control
Females	0	1-7	13.9	0%	0%
		8-14	14.6	0%	0%
		15-21	15.1	0%	0%
		22-28	13.1	0%	0%
	500→(250)	1-7	9.5	-31.6%	-2.7%
		8-14	16.9	15.4%	0.6%
		15-21	13.8	-8.9%	0%
		22-28	2.4	-81.6%	-4.0%

Female body weight gain, % change in gain and body weight percent of control are reported for the entire study below.

Females Body Weight (Entire Study)

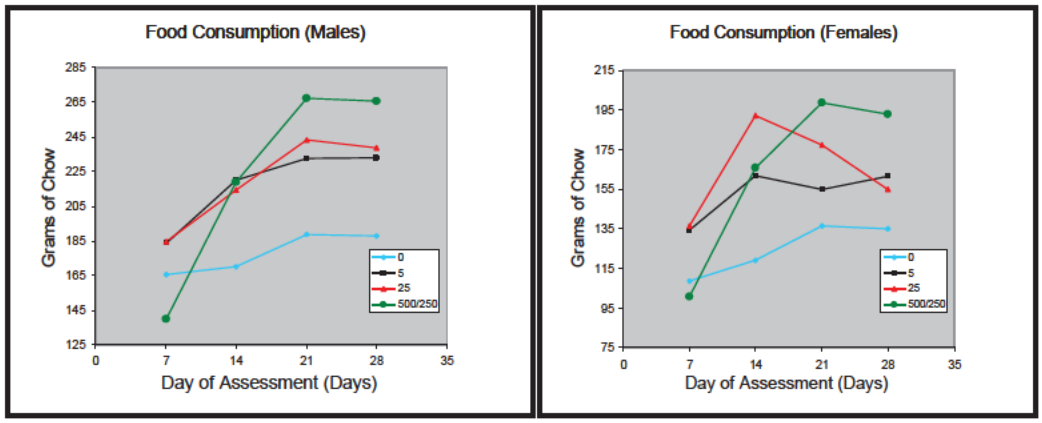
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Females	0	56.7	0%	100%
	5	49.5	-12.7%	101%
	25	46.7	-17.6%	100%
	500→(250)	46.6	-17.8%	96.0%

A marked decrease in mean weekly body weight gain of -70.3% (vs. control) was noted for females at 5 mg/kg over Days 15-21. These animals exhibited a +13.5% increase (see below) in weekly mean food consumption vs. control for this study interval. Mean weekly body weight gain for this dose group was comparable to controls for all earlier and later study intervals. This single observation was considered incidental by the sponsor and not related to treatment due to its transient occurrence and lack of a dose relationship. Graphical depiction of sponsor's data is shown in reviewer's figures below.



Food consumption:

Treatment related increases in weekly mean food consumption (vs. control) were observed at 5 and 25 mg/kg ranging from +11.0% to +29.3% in males and ranging from +13.5% to +61.4% in females. Graphical depiction of sponsor's data is shown in reviewer's figures below.



Treatment related changes in weekly mean food consumption (vs. control) at 500/250 mg/kg demonstrated a decrease ranging from -7.3% (females) to -15.6% (males) during days 1-7 which recovered following the dose reduction. Treatment related changes in weekly mean food consumption (vs. control) at 500/250 mg/kg demonstrated an increase ranging from +28.6% to +41.7% (males) and ranging from +39.0% to +45.6% (females) over Days 8-14, 15-21, and 22-28.

An increase in weekly mean food consumption was noted for subsequent weekly intervals when compared to consumption on days 1-7 ranging from ~ +15% to +40% at 5 and 25 mg/kg and ~ +60% to +100% at 500/250 mg/kg. Changes in weekly mean food consumption in control animals over the same intervals ranged from ~ +3% to +26%. This time dependent increase in weekly food consumption seen across all dose levels is likely a compensatory response to the increased urinary excretion of glucose.

Hematology:

Decreases (2.3% to 6.0% vs. control) in group mean red blood cell count (RBC), hematocrit (HCT), and/or hemoglobin (HGB) were observed in all treated female and male rats at 500/250 mg/kg. In both sexes at 500/250 mg/kg, these changes were accompanied by increases (1.03-1.07x control) in red cell distribution width (RDW), and in females mean corpuscular volume (MCV) was slightly increased (1.03x control) while reticulocytes were decreased (34.8% vs. control).

Increases in group mean platelet volume (1.06-1.19x control) were noted in all treated female and male rats at ≥25 mg/kg, while platelet number seemed relatively unchanged under these conditions. Group mean white blood cell counts were decreased (16.7% to 41.4% vs. control) in all treated female and male rats at ≥25 mg/kg. These decreases were largely attributable to lower lymphocyte counts (18.4% to 46.4% vs. control), and to a lesser extent, monocyte counts (22.4% to 42.7% vs. control). The sponsor references that these findings were similar to effects noted in rats following 2 weeks of feed restriction (Levin, 1993). Based on this data the sponsor purposed that these effects were secondary to the temporary or sustained energy deficits resulting from pharmacologically induced glucosuria rather than direct adverse effects of PF-04971729.

HEMATOLOGY

Males

Group No:		1	2	3	4
	Day	0 mg/kg	5 mg/kg	25 mg/kg	500/250 mg/kg
		MALE			
RBC (10e6/uL)	29 (10)	8.517± 0.4100	(10) 8.415± 0.3367	(10) 8.221± 0.3754	(8) 8.035± 0.1749‡
HGB (g/dL)	29 (10)	15.86± 0.450	(10) 15.82± 0.286	(10) 15.78± 0.575	(8) 15.36± 0.537
HCT (%)	29 (10)	46.37± 1.301	(10) 46.13± 1.229	(10) 46.09± 2.104	(8) 45.08± 1.202
MCV (fL)	29 (10)	54.51± 2.113	(10) 54.86± 1.813	(10) 56.07± 1.457	(8) 56.06± 1.434
MCH (pg)	29 (10)	18.66± 0.802	(10) 18.82± 0.711	(10) 19.20± 0.392	(8) 19.11± 0.681
MCHC (g/dL)	29 (10)	34.22± 0.361	(10) 34.31± 0.553	(10) 34.23± 0.495	(8) 34.06± 0.441
RDW (%)	29 (10)	11.29± 0.325	(10) 10.92± 0.297	(10) 10.87± 0.170	(8) 11.68± 0.198§
RETIC (10e3/uL)	29 (10)	184.3± 33.15	(10) 171.3± 30.34	(10) 180.2± 20.69	(8) 201.4± 56.69
PLT (10e3/uL)	29 (10)	1168.0± 153.06	(10) 1198.0± 102.94	(10) 1145.5± 131.22	(8) 1171.8± 177.13
MPV (fL)	29 (10)	6.99± 0.451	(10) 6.93± 0.177	(10) 7.52± 0.355§	(8) 8.08± 0.406§
WBC (10e3/uL)	29 (10)	13.05± 1.954	(10) 13.65± 4.227	(10) 10.51± 3.189	(8) 7.65± 2.923‡
NEUT (10e3/uL)	29 (10)	1.227± 0.2459	(10) 1.191± 0.5170	(10) 1.035± 0.3263	(8) 1.253± 0.6796
LYM (10e3/uL)	29 (10)	11.245± 1.9093	(10) 11.959± 3.8690	(10) 9.018± 3.0963	(8) 6.028± 2.1976§
MONO (10e3/uL)	29 (10)	0.300± 0.0826	(10) 0.272± 0.0985	(10) 0.216± 0.1264*	(8) 0.180± 0.0917*
EO (10e3/uL)	29 (10)	0.118± 0.0312	(10) 0.105± 0.0583	(10) 0.112± 0.0575	(8) 0.109± 0.0772
BASO (10e3/uL)	29 (10)	0.062± 0.0193	(10) 0.052± 0.0240	(10) 0.055± 0.0232	(8) 0.034± 0.0192*
LUC	29 (10)	0.084± 0.0310	(9) 0.081± 0.0420	(10) 0.069± 0.0341	(8) 0.036± 0.0192

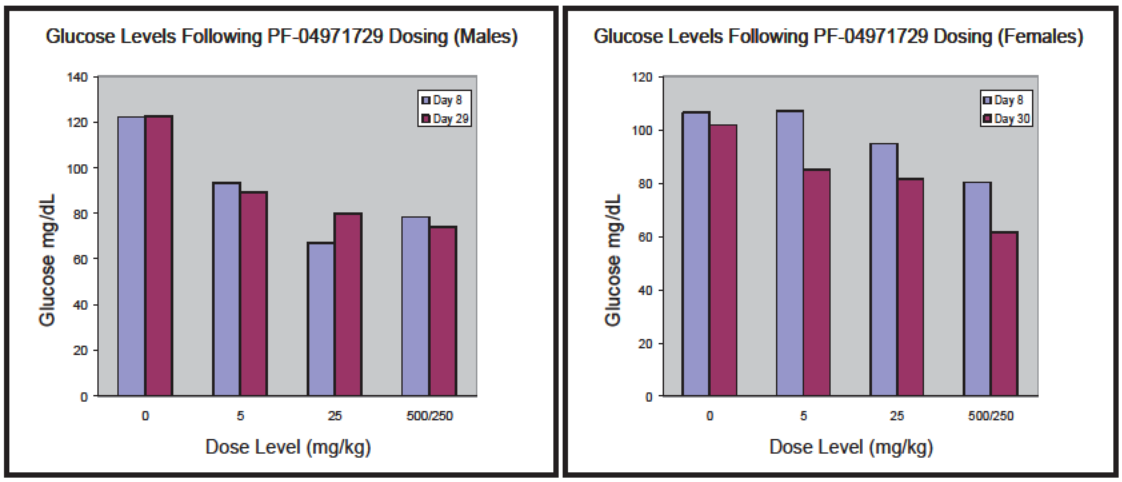
HEMATOLOGY

Females

Group No:		1	2	3	4
	Day	0 mg/kg	5 mg/kg	25 mg/kg	500/250 mg/kg
		FEMALE			
RBC (10e6/uL)	30 (10)	8.272± 0.3100	(10) 7.777± 0.6958*	(10) 8.014± 0.2485*	(8) 7.820± 0.3653*
HGB (g/dL)	30 (10)	15.49± 0.431	(10) 14.73± 1.548	(10) 15.14± 0.498	(8) 15.01± 0.473
HCT (%)	30 (10)	44.44± 1.242	(10) 42.10± 4.061	(10) 42.96± 1.053*	(8) 43.38± 1.311*
MCV (fL)	30 (10)	53.74± 1.285	(10) 54.09± 1.298	(10) 53.60± 0.968	(8) 55.53± 1.301*
MCH (pg)	30 (10)	18.75± 0.597	(10) 18.90± 0.594	(10) 18.90± 0.340	(8) 19.20± 0.460
MCHC (g/dL)	30 (10)	34.89± 0.390	(10) 34.94± 0.572	(10) 35.26± 0.389	(8) 34.60± 0.513
RDW (%)	30 (10)	10.56± 0.372	(10) 10.89± 0.726	(10) 10.64± 0.378	(8) 11.25± 0.411*
RETIC (10e3/uL)	30 (10)	161.9± 24.79	(10) 166.6± 61.88	(10) 140.3± 29.82	(8) 99.1± 33.40†
PLT (10e3/uL)	30 (7)	1214.9± 95.80	(9) 1366.6± 373.10	(10) 1326.3± 139.17	(8) 1169.8± 109.20
MPV (fL)	30 (7)	7.56± 0.321	(9) 8.04± 0.336†	(10) 8.55± 0.620§	(8) 8.96± 0.503§
WBC (10e3/uL)	30 (10)	10.65± 4.930	(10) 9.57± 4.123	(10) 8.87± 2.124	(8) 7.10± 2.718*
NEUT (10e3/uL)	30 (10)	0.704± 0.2914	(10) 2.103± 3.8976	(10) 0.833± 0.4473	(8) 1.753± 1.9681
LYM (10e3/uL)	30 (10)	9.403± 4.6909	(10) 7.093± 0.8483*	(10) 7.674± 1.9819*	(8) 5.040± 1.4354§
MONO (10e3/uL)	30 (10)	0.246± 0.1383	(10) 0.191± 0.0845	(10) 0.169± 0.0555	(8) 0.141± 0.1068*
EO (10e3/uL)	30 (10)	0.114± 0.0540	(10) 0.103± 0.0450	(10) 0.100± 0.0437	(8) 0.081± 0.0236
BASO (10e3/uL)	30 (10)	0.029± 0.0166	(10) 0.025± 0.0143	(10) 0.035± 0.0071	(8) 0.034± 0.0106
LUC	30 (9)	0.072± 0.0211	(8) 0.054± 0.0169	(10) 0.053± 0.0231	(8) 0.041± 0.0099

Clinical chemistry:

Serum glucose levels decreased on day 8 (24.7% to 44.9% vs. control) in all treated male and in female rats at 500/250 mg/kg. By the completion of the study, serum glucose levels had decreased by (16.6% to 39.7% vs. control) in all treated rats (see reviewer figure below). These changes in serum glucose were attributed directly to the pharmacology of PF04971729. Glucose assessment was performed in fasted animals and the effects of treatment on glucose in non-fasted animals were not ascertained by the sponsor.



Liver enzymes were increased by day 8 in all treated rats, (1.1-3.2x control) in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Elevation of these enzyme levels persisted through the entire study (with the exception of AST in female rats at 5 mg/kg) although the magnitude of change (1.2-1.6x control) was somewhat lower by the end of the study. The changes in liver enzyme levels were accompanied by decreases in alkaline phosphatase (21.9% to 24.4% vs. control) in animals treated at 500/250 mg/kg. The sponsor noted no histopathological changes in the liver (see histopathology section below), suggesting that these changes are most likely an adaptive response (gluconeogenesis) to glucosuria.

When observing kidney function, BUN was increased (1.2-2.5x control) at all dose levels and values for this parameter were similar (within dose group and gender) on days 8 and at the completion of the study. Increased BUN levels were accompanied by decreased creatinine levels (14%-18% vs. control) in male and (6%-10% vs. control) female rats. These results are likely due to dehydration related to osmotic diuresis and/or protein catabolism as a compensatory mechanism for glucosuria and associated kidney impairment.

Decreased sodium (0.8% to 1.7% vs. control) was noted in all treated female rats on day 8 and in male rats at 500/250 mg/kg on day 29. Serum chloride was decreased (1.2% to 4.6% vs. control) at 500/250 mg/kg in female rats at 500/250 mg/kg on day 8 and in all treated rats at the completion of the study. Serum potassium was decreased in male rats (8.0% to 11.3% vs. control) on day 8 at 500/250 mg/kg and on day 29 at ≥25 mg/kg.

Serum inorganic phosphate was decreased (2.6% to 8.3% vs. control) in all treated male rats on day 8 and day 29 suggesting changes in the adrenal cortex. An increase (1.1x control) in inorganic phosphate, noted by the sponsor, in female rats treated at 500/250 mg/kg on day 8 was likely adaptive in nature as it did not persist or worsen by the end of the study.

Additional changes in serum chemistry values, that were considered to be secondary to changes in energy balance resulting from glucose loss and/or osmotic diuresis, often observed with feed restriction were decreases (3.3% to 9.7% vs. control) in total serum protein and albumin in all treated male rats on day 8 and at the completion of the study and decreases (6.1% to 10.2% vs. control) in globulin in male rats at ≥25 mg/kg and female rats at 500/250 mg/kg at the completion of the study. The sponsor suggests that, in male rats at 500/250 mg/kg, the decrease observed in serum calcium (6.0% vs. control) on day 29 is secondary to the decrease in albumin and the resulting loss of a portion of the protein bound fraction. Serum cholesterol was decreased (12.5% to 29.6% vs. control) in all treated female rats throughout the study.

CLINICAL CHEMISTRY

Males

Group No:		1		2		3		4	
	Day	0 mg/kg		5 mg/kg		25 mg/kg		500/250 mg/kg	
				MALE					
ALT	8	(10) 50.9±	7.20	(10) 70.3±	11.81$	(10) 86.4±	20.08$	(10) 121.7±	35.91$
(U/L)	29	(10) 43.8±	4.89	(10) 52.1±	8.12†	(10) 60.8±	13.41$	(8) 71.6±	13.70$
AST	8	(10) 80.4±	8.17	(10) 98.7±	16.82‡	(10) 107.4±	10.34$	(10) 186.0±	122.88$
(U/L)	29	(10) 70.0±	4.03	(10) 87.0±	14.95‡	(10) 89.5±	13.00$	(8) 102.5±	12.88$
ALP	8	(10) 336.1±	98.30	(10) 355.7±	100.56	(10) 296.1±	43.93	(10) 254.1±	39.45‡
(U/L)	29	(10) 236.1±	71.71	(10) 217.4±	63.17	(10) 186.0±	25.22	(8) 220.3±	55.49
GGT	8	(10) <2.0±	>0.00	(10) <2.0±	≥0.00	(10) <2.0±	>0.00	(10) <2.0±	>0.00
(U/L)	29	(10) <2.0±	≥0.00	(10) <2.0±	>0.00	(10) <2.0±	≥0.00	(8) <2.0±	>0.00
TBIL	8	(10) <0.10±	>0.000	(10) <0.10±	>0.000	(10) <0.10±	>0.000	(10) <0.10±	>0.000
(mg/dL)	29	(10) <0.10±	>0.000	(10) <0.10±	>0.000	(10) <0.10±	>0.000	(8) <0.10±	≥0.000
CHOL	8	(10) 56.8±	9.46	(10) 52.1±	6.45	(10) 54.1±	8.75	(10) 50.7±	11.72
(mg/dL)	29	(10) 56.1±	7.96	(10) 48.9±	8.92	(10) 52.3±	8.56	(8) 56.6±	8.65
GLUC	8	(10) 122.3±	9.52	(10) 93.3±	13.89$	(10) 67.4±	11.41$	(10) 78.5±	16.07$
(mg/dL)	29	(10) 122.7±	7.30	(10) 89.3±	8.97$	(10) 80.1±	11.63$	(8) 74.1±	10.13$
TP	8	(10) 6.42±	0.297	(10) 6.10±	0.325	(10) 6.07±	0.275*	(10) 5.97±	0.267†
(g/dL)	29	(10) 6.39±	0.213	(10) 6.18±	0.266*	(10) 6.04±	0.255‡	(8) 5.90±	0.185$
ALB	8	(10) 4.05±	0.135	(10) 3.87±	0.157†	(10) 3.84±	0.126‡	(10) 3.68±	0.193$
(g/dL)	29	(10) 3.93±	0.142	(10) 3.79±	0.160*	(10) 3.73±	0.134†	(8) 3.69±	0.146†
GLOB	8	(10) 2.37±	0.216	(10) 2.32±	0.187	(10) 2.23±	0.195	(10) 2.29±	0.110
(g/dL)	29	(10) 2.46±	0.135	(10) 2.39±	0.129	(10) 2.31±	0.137*	(8) 2.21±	0.064$
AG	8	(10) 1.73±	0.164	(10) 1.68±	0.092	(10) 1.75±	0.172	(10) 1.61±	0.074*
(none)	29	(10) 1.58±	0.103	(10) 1.59±	0.057	(10) 1.63±	0.067	(8) 1.68±	0.089*
BUN	8	(10) 17.5±	3.50	(10) 20.9±	3.03*	(10) 25.5±	3.69$	(10) 37.9±	9.37$
(mg/dL)	29	(10) <13.8±	>2.20	(10) 19.3±	2.75$	(10) 25.5±	4.25$	(8) 30.3±	4.83$
CREA	8	(10) 0.28±	0.042	(10) 0.28±	0.042	(10) 0.24±	0.052	(10) 0.23±	0.082
(mg/dL)	29	(10) 0.28±	0.042	(10) 0.28±	0.063	(10) 0.26±	0.052	(8) 0.23±	0.071
PHOS	8	(10) 9.04±	0.481	(10) 8.47±	0.479*	(10) 8.29±	0.621*	(10) 8.70±	0.510*
(mg/dL)	29	(10) 7.70±	0.245	(10) 7.11±	0.412‡	(10) 7.09±	0.387‡	(8) 7.50±	0.493‡
CA	8	(10) 11.16±	0.334	(10) 10.90±	0.467	(10) 10.91±	0.401	(10) 10.60±	0.497*
(mg/dL)	29	(10) 10.48±	0.249	(10) 10.05±	0.327‡	(10) 10.06±	0.357‡	(8) 9.85±	0.251$
NA	8	(10) 143.3±	0.95	(10) 142.8±	0.79	(10) 142.0±	1.15	(10) 143.2±	1.14
(mmol/L)	29	(10) 144.4±	1.07	(10) 143.8±	0.92	(10) 143.4±	1.51	(8) 142.0±	0.53$
K	8	(10) 4.79±	0.223	(10) 4.73±	0.200	(10) 4.55±	0.288	(10) 4.37±	0.395*

Group No:		1		2		3		4	
	Day	0 mg/kg		5 mg/kg		25 mg/kg		500/250 mg/kg	
				MALE					
(mmol/L)	29	(10) 4.60±	0.226	(10) 4.42±	0.385	(10) 4.23±	0.356*	(8) 4.08±	0.427†
CL	8	(10) 99.9±	1.52	(10) 99.9±	0.99	(10) 98.1±	1.37	(10) 100.4±	2.17
(mmol/L)	29	(10) 102.8±	1.23	(10) 101.4±	1.84*	(10) 101.6±	1.17*	(8) 98.1±	1.13$

Note: Values expressed as (n) mean ± standard deviation.

- Value not applicable or not available.

HPD = Hours Postdose.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001

CLINICAL CHEMISTRY

Females

Group No:		1			2			3			4	
	Day	0 mg/kg			5 mg/kg			25 mg/kg			500/250 mg/kg	
						FEMALE						
ALT	8	(10)	46.5±	6.77	(10)	64.5±	18.52‡	(10)	67.5±	15.84$	(9)	149.4± 48.76$
(U/L)	30	(10)	40.4±	8.33	(10)	49.9±	12.10	(10)	56.5±	16.98†	(8)	63.3± 13.22$
AST	8	(10)	94.7±	13.86	(10)	107.0±	18.29*	(10)	114.7±	12.24†	(9)	247.6± 102.24$
(U/L)	30	(10)	100.8±	12.80	(10)	104.6±	19.94	(10)	122.3±	25.34*	(8)	129.3± 21.78‡
ALP	8	(10)	188.1±	47.29	(10)	210.0±	58.88	(10)	207.7±	30.55	(9)	146.9± 50.38*
(U/L)	30	(10)	136.1±	34.09	(10)	135.7±	29.03	(10)	121.0±	22.19	(8)	110.3± 26.64
GGT	8	(10)	<2.1±	>0.32	(10)	<2.1±	>0.32	(10)	<2.0±	≥0.00	(9)	<2.2± >0.67
(U/L)	30	(10)	<2.0±	≥0.00	(10)	<2.0±	≥0.00	(10)	<2.0±	≥0.00	(8)	<2.0± ≥0.00
TBIL	8	(10)	<0.10±	>0.000	(10)	<0.10±	>0.000	(10)	<0.10±	>0.000	(9)	<0.10± >0.000
(mg/dL)	30	(10)	<0.10±	>0.000	(10)	<0.10±	>0.000	(10)	<0.10±	>0.000	(8)	<0.10± ≥0.000
CHOL	8	(10)	73.0±	5.83	(10)	57.2±	13.42‡	(10)	51.4±	10.20$	(9)	54.1± 8.07$
(mg/dL)	30	(10)	75.5±	10.44	(10)	59.9±	11.37*	(10)	57.5±	13.23*	(8)	66.1± 9.19*
GLUC	8	(10)	106.5±	10.53	(10)	106.9±	29.04	(10)	95.0±	25.85	(9)	80.2± 12.46$
(mg/dL)	30	(10)	101.8±	6.66	(10)	84.9±	6.52$	(10)	81.7±	12.28$	(8)	61.4± 9.88$
TP	8	(10)	6.28±	0.434	(10)	6.15±	0.268	(10)	6.25±	0.237	(9)	6.10± 0.283
(g/dL)	30	(10)	6.81±	0.381	(10)	6.40±	0.271*	(10)	6.57±	0.353*	(8)	6.20± 0.239‡
ALB	8	(10)	4.04±	0.165	(10)	3.90±	0.141	(10)	3.94±	0.165	(9)	3.81± 0.252
(g/dL)	30	(10)	4.21±	0.292	(10)	3.89±	0.173*	(10)	4.07±	0.221*	(8)	3.80± 0.141‡
GLOB	8	(10)	2.24±	0.401	(10)	2.25±	0.165	(10)	2.31±	0.129	(9)	2.29± 0.105
(g/dL)	30	(10)	2.60±	0.156	(10)	2.51±	0.223	(10)	2.50±	0.194	(8)	2.40± 0.151*
AG	8	(10)	1.90±	0.462	(10)	1.74±	0.117	(10)	1.72±	0.103	(9)	1.68± 0.139
(none)	30	(10)	1.62±	0.123	(10)	1.57±	0.149	(10)	1.64±	0.126	(8)	1.59± 0.083
BUN	8	(10)	15.9±	1.60	(10)	20.2±	4.34†	(10)	25.3±	3.23$	(9)	39.0± 7.18$
(mg/dL)	30	(10)	14.8±	1.14	(10)	18.5±	2.27‡	(10)	22.5±	3.03$	(8)	32.6± 5.58$
CREA	8	(10)	0.30±	0.000	(10)	0.27±	0.048	(10)	0.28±	0.042	(9)	0.28± 0.044
(mg/dL)	30	(10)	0.33±	0.048	(10)	0.28±	0.042	(10)	0.31±	0.032	(8)	0.28± 0.046
PHOS	8	(10)	7.74±	0.412	(10)	7.01±	0.515	(10)	7.24±	0.690	(9)	8.56± 0.735‡
(mg/dL)	30	(10)	6.34±	0.506	(10)	6.30±	0.544	(10)	6.37±	0.353	(8)	6.81± 0.464
CA	8	(10)	10.90±	0.275	(10)	10.55±	0.344	(10)	10.62±	0.405	(9)	10.84± 0.255
(mg/dL)	30	(10)	10.19±	0.378	(10)	9.91±	0.318	(10)	10.01±	0.273	(8)	9.88± 0.139
NA	8	(10)	143.3±	0.82	(10)	141.6±	1.07‡	(10)	142.1±	1.29‡	(9)	141.3± 1.12$
(mmol/L)	30	(10)	142.7±	0.82	(10)	141.6±	0.84	(10)	142.3±	1.42	(8)	141.9± 1.55
K	8	(10)	4.25±	0.372	(10)	4.32±	0.301	(10)	4.37±	0.414	(9)	4.22± 0.303

Group No:		1			2			3			4	
	Day	0 mg/kg			5 mg/kg			25 mg/kg			500/250 mg/kg	
						FEMALE						
(mmol/L)	30	(10)	4.23±	0.211	(10)	4.34±	0.353	(10)	4.33±	0.263	(8)	4.05± 0.378
CL	8	(10)	102.2±	1.62	(10)	101.5±	2.32	(10)	101.1±	1.60	(9)	98.3± 2.60†
(mmol/L)	30	(10)	102.2±	1.55	(10)	100.7±	0.95*	(10)	100.9±	0.99*	(8)	97.6± 1.60$

Note: Values expressed as (n) mean ± standard deviation.

\- Value not applicable or not available.

HPD = Hours Postdose.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001

Urinalysis:

Urine glucose and the ratio of glucose to creatinine in the urine were both elevated (594-714x control and 1196-2179x control, respectively) at all dose levels, consistent with pharmacological inhibition of glucose reuptake in the renal tubules.

At all dose levels, glucosuria resulted in increased urine specific gravity (1.004-1.014x control) and volume (2.1-4.1x control), with correspondingly decreased urine creatinine concentration (45.0% to 67.9% vs. control). Decreased urine pH (7.7% to 19.2% vs. control) was also noted at all dose levels. The increases in urine volume were dose related (up to ~ 4x control) indicating that the total amount of glucose excreted increased with dose.

Urinalysis (Males)

Group No:		1		2		3		4	
	Day	0 mg/kg		5 mg/kg		25 mg/kg		500/250 mg/kg	
				MALE					
SG (none)	28 (10)	1.0329±	0.00833	(10) 1.0425±	0.00870*	(10) 1.0425±	0.00558†	(8) 1.0458±	0.00590‡
VOLUME (mL)	28 (10)	5.20±	1.229	(10) 12.00±	2.582$	(10) 15.70±	3.368$	(8) 21.38±	5.502$
pH (none)	28 (10)	8.35±	0.474	(10) 7.50±	0.624‡	(10) 7.10±	0.699$	(8) 6.75±	0.598$
U CREA (mg/dL)	28 (10)	54.51±	11.890	(10) 27.90±	6.482$	(10) 21.49±	3.526$	(8) 18.38±	2.298$
U GLUC (mg/dL)	28 (10)	9.9±	2.33	(10) 5883.7±	1355.69$	(10) 5992.5±	1019.22$	(8) 6432.1±	977.67$
U GLUC R (mg/mg)	28 (10)	0.183±	0.0157	(10) 218.911±	62.3348$	(10) 282.946±	55.3655$	(8) 350.643±	35.2932$

Note: Values expressed as (n) mean ± standard deviation.

- Value not applicable or not available.

HPD = Hours Postdose.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001

Urinalysis (Females)

Group No:		1		2		3		4	
	Day	0 mg/kg		5 mg/kg		25 mg/kg		500/250 mg/kg	
				FEMALE					
SG (none)	29 (10)	1.0340±	0.01178	(10) 1.0481±	0.01052*	(10) 1.0385±	0.00620*	(8) 1.0420±	0.00316*
VOLUME (mL)	29 (10)	3.00±	1.054	(10) 6.30±	2.584‡	(10) 9.70±	2.312$	(8) 12.38±	2.264$
pH (none)	29 (10)	>7.80±	>0.856	(10) 7.15±	0.747*	(10) 7.20±	0.675*	(8) 6.56±	0.563†
U CREA (mg/dL)	29 (10)	57.00±	18.944	(10) 31.33±	8.992$	(10) 20.10±	2.966$	(8) 18.30±	1.930$
U GLUC (mg/dL)	29 (10)	10.1±	3.07	(10) 7213.3±	1623.18$	(10) 6249.3±	1265.18$	(8) 7172.5±	709.69$
U GLUC R (mg/mg)	29 (10)	0.181±	0.0307	(10) 237.493±	52.9623$	(10) 309.933±	32.5462$	(8) 394.451±	48.2268$

Note: Values expressed as (n) mean ± standard deviation.

- Value not applicable or not available.

HPD = Hours Postdose.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001

Micronucleus Assessment:

There were no statistically significant, treatment related reductions in the mean percent polychromatic erythrocytes (PCE) reported, suggesting a low incidence of bone marrow toxicity. The fact that, no statistically significant increases in the number of PCEs with micronuclei was reported, suggests that PF-04971729 does not possess, under these conditions, the ability to induce chromosomal damage. Results of the male and female rat bone marrow micronucleus assays are shown in the sponsor's tables below.

In Vivo Micronucleus Assay of PF-04971729

Male Flow Cytometric Data Summary

0.5% Methycellulose / 10% PEG 400

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
1	19948	36	36426	6	35.4	0.18	0.02
2	19925	39	44497	13	31.0	0.20	0.03
3	19937	34	38032	11	34.4	0.17	0.03
4	19935	46	46105	16	30.2	0.23	0.03
5	19929	44	58202	17	25.5	0.22	0.03
			Mean		**31.3**	**0.20**	0.03
			St Dev		**3.9**	**0.03**	0.01

Cyclophosphamide 10 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
601	19766	170	86936	89	18.6	0.85	0.10
602	19711	241	84415	118	19.1	1.21	0.14
603	19406	554	100895	189	16.5	2.78	0.19
604	19749	220	99856	74	16.7	1.10	0.07
605	19731	224	107890	88	15.6	1.12	0.08
			Mean		**17.3**	**1.41**	0.12
			St Dev		**1.5**	**0.77**	0.05

PF-04971729 5 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
11	19917	19	46078	14	30.2	0.10	0.03
12	19936	38	58392	12	25.5	0.19	0.02
13	19948	39	26404	15	43.1	0.20	0.06
14	19956	25	29559	6	40.3	0.13	0.02
15	19928	49	25103	19	44.3	0.25	0.08
			Mean		**36.7**	**0.17**	0.04
			St Dev		**8.4**	**0.06**	0.02

PF-04971729 25 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
21	19940	27	26811	16	42.7	0.14	0.06
22	19929	45	47519	16	29.6	0.23	0.03
23	19894	46	37129	12	34.9	0.23	0.03
24	19952	34	46367	11	30.1	0.17	0.02
25	19952	27	48772	13	29.1	0.14	0.03
			Mean		**33.3**	**0.18**	0.04
			St Dev		**5.8**	**0.05**	0.01

PF-04971729 250 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
31	19967	20	29030	5	40.8	0.10	0.02
33	19939	24	21127	14	48.6	0.12	0.07
34	19920	51	46636	17	30.0	0.26	0.04
35	19963	29	17282	1	53.6	0.15	0.01
36	19942	33	39930	17	33.3	0.17	0.04
			Mean		**41.3**	**0.16**	0.03
			St Dev		**10.0**	**0.06**	0.02

In Vivo Micronucleus Assay of PF-04971729

Female Flow Cytometric Data Summary

0.5% Methycellulose / 10% PEG 400

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
41	19926	30	70036	23	22.2	0.15	0.03
42	19961	27	23540	10	45.9	0.14	0.04
43	19922	31	44621	10	30.9	0.16	0.02
44	19929	42	73106	18	21.5	0.21	0.02
45	19956	35	25195	8	44.2	0.18	0.03
			Mean		**32.9**	**0.17**	0.03
			St Dev		**11.7**	**0.03**	0.01

Cyclophosphamide 10 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
606	19426	520	89287	296	18.2	2.61	0.33
607	19549	396	145089	194	12.1	1.99	0.13
608	19578	349	83027	281	19.3	1.75	0.34
609	19520	399	190533	146	9.5	2.00	0.08
610	19709	252	66510	126	23.1	1.26	0.19
			Mean		**16.4**	**1.92**	0.21
			St Dev		**5.5**	**0.49**	0.12

PF-04971729 5 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
51	19942	39	15157	7	56.9	0.20	0.05
52	19950	42	21285	10	48.4	0.21	0.05
53	19926	34	22980	5	46.5	0.17	0.02
54	19893	63	30371	25	39.6	0.32	0.08
55	19942	20	39876	13	33.4	0.10	0.03
			Mean		**44.9**	**0.20**	0.05
			St Dev		**8.9**	**0.08**	0.02

PF-04971729 25 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
62	19928	42	48353	22	29.2	0.21	0.05
63	19948	30	107214	37	15.7	0.15	0.03
64	19938	37	33099	13	37.6	0.19	0.04
65	19924	29	44236	11	31.1	0.15	0.02
66	19940	26	68514	13	22.6	0.13	0.02
			Mean		**27.2**	**0.16**	0.03
			St Dev		**8.4**	**0.03**	0.01

PF-04971729 250 mg/kg

Animal No.	No. PCE	No. MN-PCE	No. NCE	No. MN-NCE	%PCE	%MN-PCE	%MN-NCE
71	19954	26	20514	24	49.3	0.13	0.12
72	19946	21	46420	15	30.1	0.11	0.03
73	19938	22	32109	18	38.3	0.11	0.06
74	19912	34	72650	27	21.5	0.17	0.04
76	19952	28	27985	9	41.6	0.14	0.03
			Mean		**36.2**	**0.13**	0.05
			St Dev		**10.7**	**0.03**	0.04

Toxicokinetics:

While the sponsor indicated that there were no apparent gender-related differences in systemic exposure (as assessed by C_{max} and AUC_{0-24}) the sponsor's own table below indicates that this is not the case. Exposures in females were higher than males at all doses and on all study days.

Dose (mg/kg/day)	Study Day	Gender	Cmax (µg/mL)	tmax (h)	AUC(0-24) (µg*h/mL)
5	1	Male	1.52	2.00	12.9
		Female	1.93	0.500	19.5
		Overall	1.53	0.500	16.2
	28	Male	1.29	2.00	8.40
		Female	2.70	0.500	14.9
		Overall	1.94	0.500	11.6
25	1	Male	8.23	4.00	103
		Female	9.37	4.00	108
		Overall	8.80	4.00	106
	28	Male	8.98	0.500	69.3
		Female	12.7	0.500	93.0
		Overall	10.9	0.500	81.2
500/250*	1	Male	73.3	7.00	1440
		Female	76.7	4.00	1480
		Overall	74.3	4.00	1460
	28	Male	42.4	4.00	541
		Female	47.6	7.00	718
		Overall	44.4	4.00	611

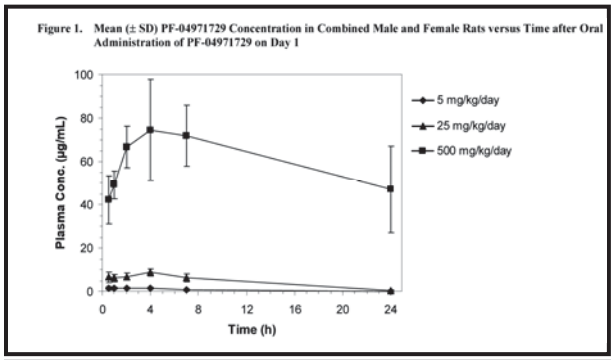
Overall = Male Plus Female Combined
* = Per study protocol amendment # 2, on Day 11 the high dose was reduced to 250 mg/kg/day

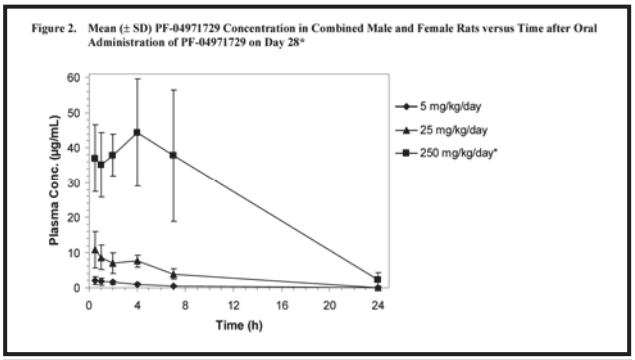
Values for female C_{max} were increased over males by (27%-109% ~ 5 mg/kg/day), (14%-41% ~ 25 mg/kg/day) and (5%-12% ~ 500/250 mg/kg/day). Female rats AUC_{0-24} were increased over males by (51%-77% ~ 5 mg/kg/day), (5%-34% ~ 25 mg/kg/day) and (3%-33% ~ 500/250 mg/kg/day).

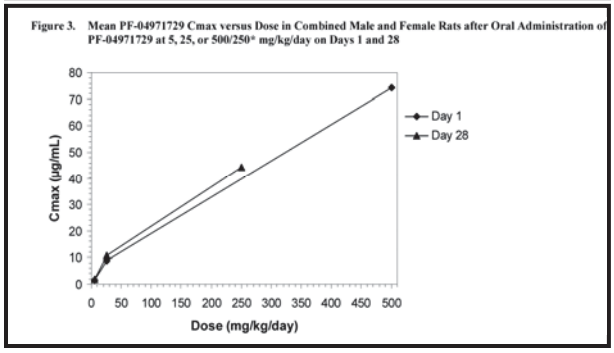
Differences between systemic exposures on day 1 and day 28 tended to decrease over the course of the study in males when comparing C_{max} and AUC_{0-24} between those two sampling days. Strangely, the female exposure values tended to increase over the course of the study when comparing C_{max} but decrease when comparing AUC_{0-24} between the same sampling days. While this data supports the idea that there is minimal plasma drug accumulation after 28 days of daily oral dosing of PF04971729, the inconsistencies in C_{max} and AUC_{0-24} values in between and within dosing groups fail to provide a clear picture of what occurred within this study. Increases in exposure were greater than dose-proportional when comparing 5 mg/kg/day to 25 mg/kg/day directly.

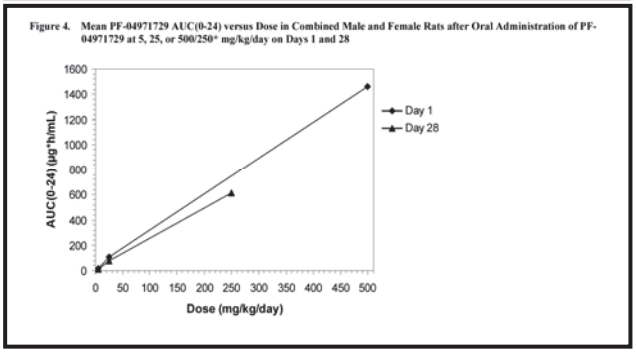
Mean t_{max} on day 1 and day 28 was observed between 0.5 and 4 hours postdose, although at the high dose (500/250 mg/kg/day) t_{max} was reported as high as 7 hours.

The sponsors chose to report and discuss all toxicokinetic data as combined male and female data sets. As reported by the sponsor, on Day 28, mean combined C_{max} and AUC_{0-24} values for PF04971729 at 5, 25, and 250 mg/kg were 1.94, 10.9, and 44.4 µg/mL, respectively, for C_{max}, and 11.6, 81.2, and 611 µg.hr/mL, respectively, for AUC_{0-24}. The mean combined PF-04971729 concentration versus time on day 1 and day 28, C_{max} vs. dose and AUC_{0-24} vs. dose are shown in the sponsor's figures below.





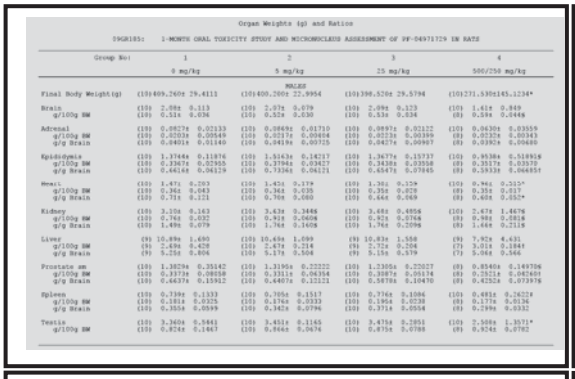




Organ weights:

As shown in the tables below, there were dose-dependent increases in group mean absolute and relative kidney weights (1.1-1.4x control). These increases in kidney size can be attributed to the pharmacological effects of treatment and increased fluid load within the nephron.

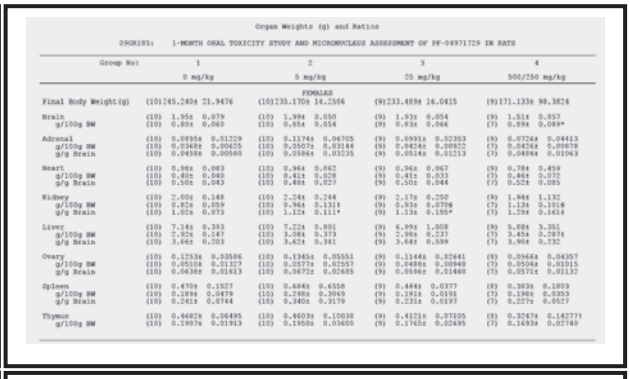
BEST AVAILABLE COPY



Organ Weights (g) and Ratios

09GR185: 1-MONTH ORAL TOXICITY STUDY AND MICRONUCLEUS ASSESSMENT OF PF-04971729 IN RATS

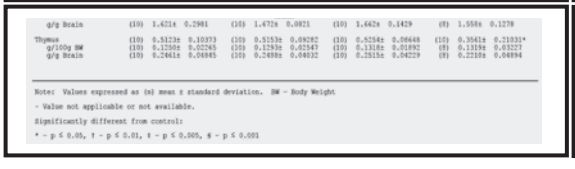
Group No:	1	2	3	4
	0 mg/kg	5 mg/kg	25 mg/kg	500/250 mg/kg
MALES				
Final Body Weight (g)	(10)409.260± 29.4111	(10)400.200± 22.9954	(10)398.520± 29.5794	(10)271.530±145.1234*
Brain	(10) 2.08± 0.113	(10) 2.07± 0.079	(10) 2.09± 0.123	(10) 1.41± 0.849
g/100g BW	(10) 0.51± 0.036	(10) 0.52± 0.030	(10) 0.53± 0.034	(8) 0.59± 0.0448
Adrenal	(10) 0.0827± 0.02133	(10) 0.0869± 0.01710	(10) 0.0897± 0.01122	(10) 0.0630± 0.03559
g/100g BW	(10) 0.0203± 0.00549	(10) 0.0217± 0.00404	(10) 0.0223± 0.00399	(8) 0.0232± 0.00343
g/g Brain	(10) 0.0401± 0.01140	(10) 0.0419± 0.00725	(10) 0.0427± 0.00907	(8) 0.0392± 0.00680
Epididymis	(10) 1.3744± 0.11876	(10) 1.5163± 0.14217	(10) 1.3677± 0.15737	(10) 0.9538± 0.518918
g/100g BW	(10) 0.3367± 0.03565	(10) 0.3794± 0.03427	(10) 0.3438± 0.03558	(8) 0.3517± 0.03570
g/g Brain	(10) 0.6616± 0.06129	(10) 0.7336± 0.06121	(10) 0.6547± 0.07845	(8) 0.5833± 0.066857
Heart	(10) 1.47± 0.203	(10) 1.45± 0.179	(10) 1.36± 0.15>	(10) 0.96± 0.315*
g/100g BW	(10) 0.36± 0.043	(10) 0.36± 0.035	(10) 0.35± 0.028	(8) 0.35± 0.017
g/g Brain	(10) 0.71± 0.121	(10) 0.70± 0.080	(10) 0.64± 0.069	(8) 0.60± 0.050*
Kidney	(10) 3.10± 0.163	(10) 3.63± 0.3446	(10) 3.68± 0.4856	(10) 2.67± 1.4676
g/100g BW	(10) 0.76± 0.032	(10) 0.91± 0.0604	(10) 0.92± 0.0766	(8) 0.98± 0.0818
g/g Brain	(10) 1.49± 0.079	(10) 1.76± 0.1408	(10) 1.76± 0.2096	(8) 1.66± 0.2118
Liver	(9) 10.89± 1.490	(10) 10.49± 1.099	(9) 10.83± 1.588	(9) 7.92± 4.631
g/100g BW	(9) 2.69± 0.428	(10) 2.67± 0.214	(9) 2.72± 0.204	(7) 3.01± 0.1845
g/g Brain	(9) 5.25± 0.804	(10) 5.17± 0.504	(9) 5.15± 0.579	(7) 5.06± 0.566
Prostate am	(10) 1.3829± 0.35142	(10) 1.3196± 0.22222	(10) 1.2305± 0.22027	(8) 0.8540± 0.149706
g/100g BW	(10) 0.3372± 0.08558	(10) 0.3311± 0.06354	(10) 0.3087± 0.05174	(8) 0.3521± 0.042405
g/g Brain	(10) 0.6637± 0.15912	(10) 0.6407± 0.12121	(10) 0.5878± 0.10470	(8) 0.6252± 0.073976
Spleen	(10) 0.739± 0.1333	(10) 0.705± 0.1517	(10) 0.776± 0.1086	(10) 0.491± 0.2622*
g/100g BW	(10) 0.181± 0.0325	(10) 0.176± 0.0333	(10) 0.196± 0.0238	(8) 0.177± 0.0136
g/g Brain	(10) 0.355± 0.0599	(10) 0.342± 0.0796	(10) 0.371± 0.0554	(8) 0.299± 0.0332
Testis	(10) 3.340± 0.5441	(10) 3.451± 0.1165	(10) 3.475± 0.2851	(10) 2.508± 1.3571*
g/100g BW	(10) 0.814± 0.1467	(10) 0.866± 0.0674	(10) 0.875± 0.0788	(8) 0.914± 0.0782



Organ Weights (g) and Ratios

09GR185: 1-MONTH ORAL TOXICITY STUDY AND MICRONUCLEUS ASSESSMENT OF PF-04971729 IN RATS

Group No:	1	2	3	4
	0 mg/kg	5 mg/kg	25 mg/kg	500/250 mg/kg
FEMALES				
Final Body Weight (g)	(10)245.240± 21.9476	(10)235.170± 14.2586	(9)233.489± 16.0415	(9)171.133± 98.3824
Brain	(10) 1.95± 0.079	(10) 1.99± 0.050	(9) 1.93± 0.054	(9) 1.51± 0.857
g/100g BW	(10) 0.80± 0.060	(10) 0.85± 0.054	(9) 0.83± 0.066	(7) 0.89± 0.089*
Adrenal	(10) 0.0855± 0.01229	(10) 0.1174± 0.06705	(9) 0.0991± 0.02353	(7) 0.0726± 0.04413
g/100g BW	(10) 0.0348± 0.00425	(10) 0.0507± 0.03144	(9) 0.0424± 0.00922	(7) 0.0426± 0.00878
g/g Brain	(10) 0.0458± 0.00580	(10) 0.0586± 0.03235	(9) 0.0514± 0.01213	(7) 0.0404± 0.01063
Heart	(10) 0.98± 0.083	(10) 0.96± 0.062	(9) 0.96± 0.067	(9) 0.78± 0.459
g/100g BW	(10) 0.40± 0.040	(10) 0.41± 0.028	(9) 0.41± 0.033	(7) 0.46± 0.072
g/g Brain	(10) 0.50± 0.043	(10) 0.48± 0.027	(9) 0.50± 0.044	(7) 0.52± 0.085
Kidney	(10) 2.00± 0.148	(10) 2.24± 0.244	(9) 2.17± 0.250	(9) 1.94± 1.132
g/100g BW	(10) 0.82± 0.059	(10) 0.96± 0.1314	(9) 0.93± 0.0706	(7) 1.13± 1.1816
g/g Brain	(10) 1.02± 0.073	(10) 1.12± 0.111*	(9) 1.13± 0.155*	(7) 1.29± 0.1418
Liver	(10) 7.14± 0.393	(10) 7.22± 0.801	(9) 6.99± 1.008	(9) 5.08± 3.351
g/100g BW	(10) 2.92± 0.147	(10) 3.08± 0.373	(9) 2.98± 0.237	(7) 3.45± 0.2878
g/g Brain	(10) 3.66± 0.203	(10) 3.62± 0.341	(9) 3.64± 0.599	(7) 3.90± 0.232
Ovary	(10) 0.1253± 0.03586	(10) 0.1345± 0.05651	(9) 0.1144± 0.02641	(8) 0.0964± 0.04357
g/100g BW	(10) 0.0510± 0.01327	(10) 0.0577± 0.02557	(9) 0.0488± 0.00949	(7) 0.0504± 0.01015
g/g Brain	(10) 0.0638± 0.01413	(10) 0.0672± 0.02685	(9) 0.0596± 0.01460	(7) 0.0571± 0.01132
Spleen	(10) 0.470± 0.1527	(10) 0.484± 0.4556	(9) 0.444± 0.0377	(8) 0.383± 0.1803
g/100g BW	(10) 0.189± 0.0479	(10) 0.198± 0.3049	(9) 0.191± 0.0191	(7) 0.196± 0.0353
g/g Brain	(10) 0.241± 0.0744	(10) 0.240± 0.3179	(9) 0.231± 0.0197	(7) 0.227± 0.0527
Thymus	(10) 0.4482± 0.06495	(10) 0.4603± 0.10038	(9) 0.4121± 0.07105	(8) 0.3247± 0.14277*
g/100g BW	(10) 0.1907± 0.01913	(10) 0.1958± 0.03600	(9) 0.1765± 0.02695	(7) 0.1693± 0.02740



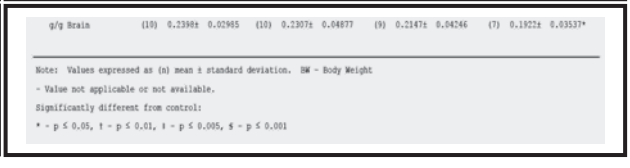
g/g Brain	(10) 1.421± 0.2981	(10) 1.472± 0.0821	(10) 1.442± 0.1429	(8) 1.558± 0.1278
Thymus	(10) 0.5123± 0.10373	(10) 0.5153± 0.09282	(10) 0.5254± 0.06648	(10) 0.3561± 0.21533*
g/100g BW	(10) 0.1350± 0.02265	(10) 0.1393± 0.02547	(10) 0.1318± 0.01892	(8) 0.1319± 0.03227
g/g Brain	(10) 0.2461± 0.04885	(10) 0.2488± 0.04832	(10) 0.2515± 0.04229	(8) 0.2210± 0.04894

Note: Values expressed as (n) mean ± standard deviation. BW = Body Weight

- Value not applicable or not available.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001



g/g Brain	(10) 0.2398± 0.02985	(10) 0.2307± 0.04877	(9) 0.2147± 0.04246	(7) 0.1922± 0.03537*

Note: Values expressed as (n) mean ± standard deviation. BW = Body Weight

- Value not applicable or not available.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001

Group mean absolute and relative prostate weights were decreased in males (25.3% to 38.2% vs. control) at 500/250 mg/kg.

Group mean absolute and relative thymus weights were decreased in females (11.2% to 20.7% vs. control) at 500/250 mg/kg.

The lower prostate, but not thymus, weights were accompanied by histological findings (see histopathology section below). The sponsor attributed these changes in organ weight to alterations in energy balance related to glucosuria and/or stress.

Other reported statistically significant differences in organ weights included: epididymis, heart, spleen, liver and brain, but were considered unrelated to treatment by the sponsor, due to their small scale or the incongruence among changes in absolute and relative weights.

Gross pathology:

Gross observations were noted in the stomach, adrenal gland, and/or thymus in animals that died or were euthanized before the end of the study. These included erosion/ulcer of the non-glandular region of the stomach and enlargement of the adrenal gland (Animals 75F and 78F) and small thymus (Animals 32M and 37M). See sponsor's table below.

Gross Observations

09GR185: 1-MONTH ORAL TOXICITY STUDY AND MICRONUCLEUS ASSESSMENT OF PF-04971729 IN RATS

Sex:	Male				Female			
Group No:	1	2	3	4	1	2	3	4
Dose (mg/kg):	0	5	25	500/250	0	5	25	500/250
Animals on Study:	10	10	10	10	10	10	10	10
Adrenal								
Animals Examined:	10	10	10	10	10	10	10	10
Unremarkable	10	10	10	10	10	10	10	8
Remarkable Observations	-	-	-	-	-	-	-	2
Enlarged	-	-	-	-	-	-	-	2
Kidney								
Animals Examined:	10	10	10	10	10	10	10	10
Unremarkable	10	10	8	10	10	10	10	10
Remarkable Observations	-	-	2	-	-	-	-	-
Dilatation	-	-	2	-	-	-	-	-
Liver								
Animals Examined:	10	10	10	10	10	10	10	10
Unremarkable	10	10	10	9	10	10	10	10
Remarkable Observations	-	-	-	1	-	-	-	-
Abnormal color	-	-	-	1	-	-	-	-
Spleen								
Animals Examined:	10	10	10	10	10	10	10	10
Unremarkable	10	10	10	10	10	9	10	10
Remarkable Observations	-	-	-	-	-	1	-	-
Abnormal surface	-	-	-	-	-	1	-	-
Stomach								
Animals Examined:	10	10	10	10	10	10	10	10
Unremarkable	10	10	10	10	10	10	10	8
Remarkable Observations	-	-	-	-	-	-	-	2
Abnormal surface	-	-	-	-	-	-	-	1
Erosion/ulcer	-	-	-	-	-	-	-	1
Thymus								
Animals Examined:	10	10	10	10	10	10	10	10
Unremarkable	10	10	10	8	10	9	9	10
Remarkable Observations	-	-	-	2	-	1	1	-
Abnormal color	-	-	-	-	-	-	1	-

Most of these observations were associated with microscopic changes (see histopathology section below) and attributed to stress and/or the moribund condition of the animals.

Other gross observations noted in the table above were considered unrelated to treatment due to their sporadic occurrence, lack of relationship with dose, and/or association with procedural complications.

Histopathology:

Treatment related microscopic findings were noted in the kidney, adrenal gland, mandibular salivary gland, pancreas, mesentery, prostate, Harderian gland, and tissues of the female reproductive tract. The sponsor stated that the microscopic findings in the animals found dead or euthanized in moribund condition at 500/250 mg/kg were similar to those surviving to the end of the study and the specific cause for debilitation and mortality in these animals was not determined.

In the kidney, treatment related dilatation of renal tubules (mainly distal nephron segments) was noted in the cortex and medulla of males treated at ≥5 mg/kg and females treated at ≥25 mg/kg. In the cortex, epithelial cells were normal to slightly attenuated, while those in affected medullary segments were sometimes slightly hypertrophied with ample clear cytoplasm. The incidence of dilatation was similar across dose levels in males but increased from 25 to 500/250 mg/kg in females while severity tended to increase at ≥25 mg/kg in both sexes. Minimal, focal, chronic progressive nephropathy (CPN) was observed in most treated and control groups with similar incidence. However, in females treated at 500/250 mg/kg, CPN was more often graded as mild based upon a multifocal distribution within the affected kidneys and this increase in severity was considered treatment related by the sponsor. Additionally, treatment related deposition of mineral was noted within or adherent to the papillary epithelium at the pelvic fornix or in cortical tubules of a few male and female rats treated at 500/250 mg/kg.

Treatment related hypertrophy of cells of the zona glomerulosa in the adrenal gland was noted in male rats at all dose levels and in female rats at 500/250 mg/kg. Morphologically, cells of the glomerulosa were slightly increased in size and often pale due to an increase in cytoplasmic vacuolation.

Cells of the zona glomerulosa produce and secrete aldosterone, particularly in response to sodium depletion. Glucosuria induces an osmotic diuresis which, in turn, leads to increased natriuresis hypertrophy of the zona glomerulosa; this was considered by the sponsor to be an adaptive response by the adrenal gland to increased renal tubular sodium uptake.

Minimal to mild hypertrophy of mandibular salivary gland mucous cells was noted in some females and males at 500/250 mg/kg. Histologically, mucous cells diffusely in the gland were enlarged with increased, pale staining cytoplasm and serous cells were decreased in prominence. In animals that were euthanized early or that died, hypertrophy tended to be mild and was accompanied by single cell necrosis and/or atrophy of the granular ducts, likely related to moribundity and decreased food intake.

Atrophy of (white) adipose tissue in the mesentery was noted in treated animals at all doses. The incidence and severity of this finding tended to increase with dose. The sponsor considered this finding an adaptive response to compensate for energy depletion during periods where increased food intake was not matched to renal energy losses.

Zymogen granules were decreased (in association with increased cytoplasmic basophilia) in exocrine cells of the pancreas in treated animals at all doses. It was purposed by the sponsor that this finding may have reflected increased exocrine discharge of zymogen granules in response to increased food intake.

Increased pigment was noted within the acini and ducts of the Harderian gland of some rats treated at 500/250 mg/kg. This finding was attributed by the sponsor to high water loss related to diuresis, although other factors were considered, including electrolyte changes, stress, and/or moribundity.

In the prostate, decreased secretory material was noted in male rats treated at 500/250 mg/kg, corresponding with decreases in group mean prostatic weight (see above). The sponsor attributed this finding to stress and/or negative energy balance caused by glucosuria.

Asynchrony of the estrous cycle was noted in 3 female rats treated at 500/250 mg/kg. The sponsor noted that, characteristic histological changes occur in tissues of the female reproductive tract at specific stages of the rodent estrous cycle. Asynchrony was characterized by discordance of these morphological features in the ovary, uterus, and/or vagina and sometimes included the presence of large (preovulatory) atretic ovarian follicles. The female reproductive system is sensitive to environmental and nutritional stresses which are known to adversely impact the rodent estrous cycle. The sponsor attributed this asynchrony to nonspecific stress at a non-tolerated dose rather than a direct effect of the drug.

The sponsor noted that several findings were observed only in animals that were found dead or euthanized early. These findings were consistent with changes typically observed with severe stress and/or moribundity and were not attributed directly to treatment by the sponsor. These findings included erosion/ulceration and squamous hyperplasia (observed grossly as erosion/ulceration) of the non-glandular stomach, lymphocytolysis and/or decreased cellularity of lymphoid tissues including the GALT (gut associated lymphoid tissue), thymus (corresponding with small thymus), spleen (including periarteriolar sheath or PALS), and inguinofemoral lymph node, hypertrophy of the zona fasciculata of the adrenal cortex (corresponding with gross enlargement), focal infarction of the adrenal cortex, and atrophy of the granular ducts of the submandibular salivary gland.

A number of solitary microscopic findings of note were observed in treated animals in this study. At 500/250 mg/kg, these findings included moderate chronic inflammation of the caudate lobe in the liver (Animal 35M) and mammary duct epithelial hyperplasia (Animal 36M). At 5 mg/kg, moderate chronic inflammation was noted in the spleen of (Animal 52F) accompanied by secondary clinical signs and laboratory findings consistent with the inflammatory process. The sponsor considered the findings in all of these animals unrelated to treatment due to their low incidence, their isolation within the affected tissue, the absence of similar changes in other treated animals, and/or the absence of a dose relationship.

HISTOPATHOLOGY - MALES							
Tissue	Finding	Main Study					
		dose	0	5	25	500/250	
		n	10	10	10	10	
Adrenal	Hypertrophy (zona glomerulosa)		0	3*	6*	8*	
Gut-Associated Lymphoid Tissue	Mineralization		1*	0	1	3*	
Harderian gland	Pigmentation		0	0	0	5*	←
Heart	Inflammation (epicardium)		0	0	0	1*	
Kidney	Chronic Progressive Nephropathy		8*	6*	9*	8*	
	Mineralization		0	0	0	1*	
	Dilatation tubule		0	6*	4^	7^	
Liver	Inflammation (chronic)		0	0	0	1^	
Lung	Congestion		0	0	0	2*	
Mammary gland	Hyperplasia		0	0	0	1*	
Mandibular gland	Hypertrophy / Necrosis		0	0	0	2*	
Mesentery	Atrophy (adipose tissue)		0	3*	5*	7*	
Pancreas	Depletion (zymogen)		0	6*	6*	10*	←
Prostate	Decreased secretory content		0	0	0	4*	
Spleen	Decreased Cellularity (Lymphocyte)		0	0	0	1*	
Testis	Degeneration (Sem. Tubule)		1*	0	0	2*	
Thymus	Lymphocytolysis		0	0	0	1*	
			(*) Minimal (^) Mild (¥) Severe (U) Unilateral (B) Bilateral				

SEVERITY

HISTOPATHOLOGY - FEMALES							
Tissue	Finding	Main Study					
		dose	0	5	25	500/250	
		n	10	10	10	10	
Adrenal	Hypertrophy (zona glomerulosa)		1*	1*	0	7*	
	Vacuolation / Mineralization		0	0	0	1*	
Gut-Associated Lymphoid Tissue	Lymphocytolysis		0	0	1	1*	
Harderian gland	Pigmentation		0	0	0	3*	
Heart	Infiltration (mononuclear cell)		0	0	0	1*	
Kidney	Chronic Progressive Nephropathy		7*	6*	8*	10^	←
	Mineralization		0	0	0	5*	
	Dilatation tubule		0	0	2^	7^	
Liver	Congestion		0	0	0	2*	
Lung	Inflammation acute (alveolus)		0	0	0	2*	
Lymph Node	Lymphocytosis		0	0	0	1*	
Mandibular gland	Atrophy, granular duct		0	0	0	2*	
	Hypertrophy		0	0	0	6*	
Mesentery	Atrophy (adipose tissue)		1*	2*	3*	9*	
Ovary	↑Atresia (large antral follicles)		0	0	0	2*	
Pancreas	Depletion (zymogen)		0	7*	9*	9*	←
Spleen	Decreased Cellularity (PALS)		0	0	0	2*	
Stomach	Erosion (ulcer)		0	0	0	1*	
	Hyperplasia (squamous cell)		0	0	0	2*	
Thymus	Lymphocytolysis		0	0	0	1*	
	Increase Macrophages		0	0	0	3*	
Vagina	Asynchrony (estrous cycle)		0	1*	0	3*	
			(*) Minimal (^) Mild (¥) Severe (U) Unilateral (B) Bilateral				

SEVERITY

Serum and Liver Biomarker Analysis:

Treatment with PF04971729 resulted in increases in serum and liver ALT isoenzymes. On Day 8, increases in serum ALT1 (1.4-3.4x control) were noted in rats at all dose levels. By Day 29/30, elevations in ALT1 persisted though tended to decrease in the 25 mg/kg and 500/250 mg/kg dose groups (1.4-1.7x control). On Day 8, serum ALT2 was increased in males at 500/250 mg/kg (4x - control) with a similar trend in females at 500/250 mg/kg. By Day 29, elevations in ALT2 in males remained elevated (3.2x control) at 500/250 mg/kg.

Liver total ALT activity was increased at all dose levels in males (1.2-2.0x control) and in females at 500/250 mg/kg (1.7x control). Liver ALT1 activity was similarly increased in the same dose groups in males (1.3-2.2x control) and females (1.8x control). The two ALT isoenzymes in rats differ in their tissue distribution and subcellular location, ALT1 is predominantly cytosolic and ALT2 mitochondrial in location and both are present in the liver and other tissues. ALT isoenzymes are both important in gluconeogenesis and amino acid metabolism, with ALT2 favoring gluconeogenesis.

The sponsor stated that, the increase in serum total ALT and ALT1 at Day 8 and Day 29/30 correlated with the increase in total liver ALT and ALT1 at the end of the study. Due to the limited amount of histological changes identified in the liver and the decrease in serum ALT despite continual treatment with PF04971729, the sponsor felt that this reflected an adaptive gluconeogenic response.

In addition to the changes in ALT1, serum ALT2 was increased in the males and females at 500/250 mg/kg on Day 8 and males on Day 29. These increases again likely reflect the need for increases in gluconeogenesis with the greater demand in male rats due to the sustained negative energy balance as demonstrated by the weight loss in this group. Although ALT2 was not found to be increased in the liver, it may be due to increases in gluconeogenesis in other tissues. Monitoring of transaminase levels in the clinic, followed by (if required) mechanistic studies in animals will help to define these drug related changes. The sponsor's summary tables of this data are shown below.

Treatment Group Summary - Serum ALT isoenzymes; Day 8

Dose Grp	Sex	Compound	Dose (mg/kg)	Study Day	Serum ALT1 (U/L) Mean	Serum ALT1 (U/L) SD	Serum ALT1 FoldChange or Percent vs Vehicle	Serum ALT1 t-test: p Value vs Vehicle	Serum ALT2 (U/L) Mean	Serum ALT2 (U/L) SD	Serum ALT2 FoldChange or Percent vs Vehicle	Serum ALT2 t-test: p Value vs Vehicle
1	Male	Vehicle	0	8	43.2	7.1			7.7	6.6		
2	Male	PF-04971729	5	8	61.4	9.1	1.4	p <0.01	8.9	8.6	1.2	-
3	Male	PF-04971729	25	8	75.4	20.9	1.7	p <0.01	11.0	8.3	1.4	-
4	Male	PF-04971729	500/250	8	86.8	32.6	2.0	p <0.01	30.5	14.5	4.0	p <0.01
1	Female	Vehicle	0	8	37.9	6.5			8.9	9.2		
2	Female	PF-04971729	5	8	52.4	14.4	1.4	p <0.01	12.1	8.9	1.3	-
3	Female	PF-04971729	25	8	60.5	14.9	1.6	p <0.01	7.9	8.2	88%	-
4	Female	PF-04971729	500/250	8	127.4	35.2	3.4	p <0.01	22.8	19.1	2.6	-

Treatment Group Summary - Serum ALT isoenzymes; Day 29 (males)/30 (females)

Dose Grp	Sex	Compound	Dose (mg/kg)	Study Day	Serum ALT1 (U/L) Mean	Serum ALT1 (U/L) SD	Serum ALT1 FoldChange or Percent vs Vehicle	Serum ALT1 t-test: p Value vs Vehicle	Serum ALT2 (U/L) Mean	Serum ALT2 (U/L) SD	Serum ALT2 FoldChange or Percent vs Vehicle	Serum ALT2 t-test: p Value vs Vehicle
1	Male	Vehicle	0	29	36.6	4.8			7.2	4.2		
2	Male	PF-04971729	5	29	41.6	6.9	1.1	-	10.5	5.0	1.5	-
3	Male	PF-04971729	25	29	50.3	13.9	1.4	p <0.01	10.5	7.7	1.5	-
4	Male	PF-04971729	500/250	29	54.2	22.0	1.5	p <0.05	23.0	14.6	3.2	p <0.01
1	Female	Vehicle	0	30	35.3	7.2			6.3	7.5		
2	Female	PF-04971729	5	30	42.1	11.4	1.2	-	8.1	5.7	1.3	-
3	Female	PF-04971729	25	30	53.5	16.5	1.5	p <0.01	4.2	4.5	66%	-
4	Female	PF-04971729	500/250	30	59.2	10.6	1.7	p <0.01	4.0	4.1	63%	-

Treatment Group Summary - Liver ALT isoenzymes

Dose Grp	Sex	Compound	Dose (mg/kg)	Study Day	Liver Total ALT (U/mg protein) Mean	Liver Total ALT (U/mg protein) SD	Liver Total ALT FoldChange or Percent vs Vehicle	Liver Total ALT t-test: p Value vs Vehicle	Liver ALT1 (U/mg protein) Mean	Liver ALT1 (U/mg protein) SD	Liver ALT1 FoldChange or Percent vs Vehicle	Liver ALT1 t-test: p Value vs Vehicle	Liver ALT2 (U/mg protein) Mean	Liver ALT2 (U/mg protein) SD	Liver ALT2 FoldChange or Percent vs Vehicle	Liver ALT2 t-test: p Value vs Vehicle
1	Male	Vehicle	0	29	482.6	122.5			430.8	119.2			51.7	16.2		
2	Male	PF-04971729	5	29	600.4	124.8	1.2	p <0.05	554.6	112.8	1.3	p <0.05	45.9	22.0	89%	-
3	Male	PF-04971729	25	29	694.2	122.4	1.4	p <0.01	658.6	123.3	1.5	p <0.01	39.6	18.8	77%	-
4	Male	PF-04971729	500/250	29	977.6	270.3	2.0	p <0.01	929.5	269.2	2.2	p <0.01	48.1	8.8	93%	-
1	Female	Vehicle	0	30	330.0	111.2			304.6	107.3			25.6	16.6		
2	Female	PF-04971729	5	30	449.0	164.6	1.4	-	415.8	158.5	1.4	-	33.1	14.1	1.3	-
3	Female	PF-04971729	25	30	433.6	147.5	1.3	-	403.7	129.1	1.3	-	29.9	21.8	1.2	-
4	Female	PF-04971729	500/250	30	576.2	159.3	1.7	p <0.01	549.8	165.0	1.8	p <0.01	26.4	11.5	1.0	-

' - ' indicate p values >0.05

7-DAY ORAL *IN VIVO* TOLERATION STUDY OF PF-0491729 IN DOGS (09GR008, NON-GLP)

SPECIES **DOSES AND ADMINISTRATION** **# ANIMALS**	**NOAEL= 50 MG/KG (~10X MRHD, µg.h/mL Basis)**

Beagle Dogs

0, 5, 50, 250→150 mg/kg/day p.o. in 5% MC + 10% PEG 400. Once Daily – **7 days**

N=1/sex/dose/group

Amorphous Drug Form

	AUC, µg.h/ml					
	Males			Females		
Dose, mg/kg	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
5	45	-	55.1	49.1	-	62.9
50	322	-	373	468	-	627
250→150	1320	1240	1150	418	746	789

Mortality: There were no unscheduled deaths during this study.

Clinical Signs: White foamy emesis at 250→150 mg/kg dose – 0.5-4 hours after dosing. Soft mucoid feces were noted at ≥ 50 mg/kg sporadically and watery feces 250→150 mg/kg dose.

Body Weight: Treatment-related changes in a single female at the 250→150 mg/kg dose included a decrease in body weight (6% of pretreatment value) with a corresponding decrease in body weight change. No significant changes in food consumption were reported.

Electrocardiogram: The QRS interval in male dogs declined slightly with dose. Many of the ECG parameters tended to change in a dose dependent fashion, although untreated animals were consistently lower than treated animals so a dose response was impossible to determine. Due to the small number of animals these results should remain inconclusive.

Hematology: Many of the hematology parameters tended to decrease in a dose dependent fashion, although untreated animals were consistently lower than treated animals so a dose response was impossible to determine. Due to the small number of animals these results should remain inconclusive.

Clinical Chemistry: ALT and AST numbers increased with dose in males, while in females this was limited to AST. A dose dependent decrease in total protein was seen in both sexes.

Histopathology: Minimal degeneration and regeneration of the kidney tubular epithelium was seen in 75% of males and females regardless of treatment. 100% of males presented with one or more testicular abnormalities regardless of dose. Lung aggregates where present in MD (50 mg/mL) male dogs or in control and LD (5 mg/mL) female dogs. The HD male (250→150 mg/kg) presented with inflammation of the liver and depletion of glycogen. In females, depletion of glycogen occurred much earlier in dosing (5 mg/kg and 50 mg/kg) but was not observed in the HD animal. However, the HD female did present with inflammation of the liver as seen in male dogs at the same dose. Congestion of the urinary bladder presented in LD and HD females, but was absent in all males and MD females.

Combined Male and Female Beagle Dogs
TK:

PF-04971729 Dose (mg/kg/day)	Study Day	Subject	Gender	Cmax (µg/mL)	tmax (h)	AUC(0-24) (µg*h/mL)	n
5	1	0002M	Male	6.71	1.00	45.0	1
		0006F	Female	6.59	1.00	49.1	1
			Overall	6.65	1.00	47.1	2
	7	0002M	Male	7.22	1.00	55.1	1
		0006F	Female	8.13	0.500	62.9	1
			Overall	7.68	0.750	59.0	2
50	1	0003M	Male	45.8	1.00	322	1
		0007F	Female	48.1	1.00	468	1
			Overall	47.0	1.00	395	2
	7	0003M	Male	51.1	1.00	373	1
		0007F	Female	54.7	0.500	627	1
			Overall	52.9	0.750	500	2
250	1	0004M	Male	89.0	4.00	1320	1
		0008F	Female	31.9	1.00	418	1
			Overall	60.5	2.50	869	2
250→150*	3	0004M	Male	87.8	4.00	1240	1
		0008F	Female	72.3	1.00	746	1
			Overall	80.1	2.50	993	2
	7	0004M	Male	94.8	1.00	1150	1
		0008F	Female	67.0	4.00	789	1
			Overall	80.9	2.50	970	2

* = On Day 3, the dose was reduced from 250 mg/kg/day to 150 mg/kg/day due to clinical signs observed at the 250 mg/kg/day dose
Overall = Male and female combined

Combined Male and Female Beagle Dogs
Plasma Concentration Data:

PF-04971729 Dose (mg/kg/day)	Study Day	Subject ID	Gender	PF-04971729 Plasma Concentration (µg/mL) by Time (h)						n
				0	0.5	1	4	7	24	
5	1	0002M	Male	NA	5.34	6.71	3.36	1.65	0.468	1
		0006F	Female	NA	5.11	6.59	3.25	1.97	0.660	1
			Overall	NA	5.23	6.65	3.31	1.81	0.564	2
	7	0002M	Male	NA	6.48	7.22	3.79	2.35	0.494	1
		0006F	Female	NA	8.13	7.68	4.08	2.71	0.689	1
			Overall	NA	7.31	7.45	3.94	2.53	0.592	2
50	1	0003M	Male	NA	35.3	45.8	25.3	13.1	2.04	1
		0007F	Female	NA	27.0	48.1	35.9	18.7	8.85	1
			Overall	NA	31.2	47.0	30.6	15.9	5.45	2
	7	0003M	Male	NA	43.6	51.1	29.3	16.0	1.61	1
		0007F	Female	NA	54.7	54.2	43.1	23.2	16.4	1
			Overall	NA	49.2	52.7	36.2	19.6	9.01	2
250	1	0004M	Male	NA	31.3	45.6	89.0	80.4	17.5	1
		0008F	Female	NA	31.3	31.9	31.7	17.8	8.63	1
			Overall	NA	31.3	38.8	60.4	49.1	13.1	2
250→150*	3	0004M	Male	22.3	59.9	61.6	87.8	70.6	14.6	1
		0008F	Female	8.11	59.6	72.3	60.6	33.9	7.88	1
			Overall	15.2	59.8	67.0	74.2	52.3	11.2	2
	7	0004M	Male	NA	69.9	94.8	82.6	56.2	15.7	1
		0008F	Female	NA	44.6	55.8	67.0	41.7	5.79	1
			Overall	NA	57.3	75.3	74.8	49.0	10.7	2

* = On Day 3, the dose was reduced from 250 mg/kg/day to 150 mg/kg/day due to clinical signs observed at the 250 mg/kg/day dose
Overall = Male and female combined
NA = Not specified in protocol, by Study Director, or by Amendment

7-DAY ORAL DOSE RANGE-FINDING STUDY OF PF-0491729 IN DOGS (09GR105, NON-GLP)

SPECIES
DOSES AND ADMINISTRATION
 # ANIMALS

NOAEL= 50 MG/KG (~15X MRHD, µg.h/mL Basis)

Beagle Dogs

0, 5, 50, 150 mg/kg/day
p.o. in 5% MC + 10% PEG 400.
Once Daily – 7 days

N=1/sex/dose/group

Co-crystalline Drug Form

	AUC, µg.h/ml			
	Males		Females	
Dose, mg/kg	Day 1	Day 7	Day 1	Day 7
5	61.2	76.7	44.4	58.8
50	486	660	494	834
150	182	679	538	511

Mortality: There were no unscheduled deaths during this study.

Clinical Signs: Emesis at 150 mg/kg dose – 0 to 4 hours post dosing (~1 hr). HD male food emesis and soft feces. MD (50 mg/kg) female presented with emesis on Day 1 – (0.5 hrs) post dose. Emesis associated salivation occurred in HD and MD animals with that complication.

Body Weight: Treatment-related decreases in total body weight occurred in a dose dependent fashion in both sexes. Body weight changes over the study declined in all dosed males, but remained unchanged or declined in females regardless of dose. The sponsor reported that there were no treatment-related changes in food consumption (data not shown).

Hematology: Male reticulocyte numbers rose slightly with dose. Female monocyte numbers tended to decrease, while female eosinophil number rose in a dose-dependent manner.

Clinical Chemistry: Dose-dependent reduction in serum glucose was clear in male animals; while numbers from females tended to decline, they were less consistent with dose. The decrease was (13%-34% from the pretreatment value) in serum glucose at ≥ 50 mg/kg of drug. Small treatment-related increases in BUN numbers were seen in males as well, while female data was again inconsistent.

Toxicokinetics: Mean T_{max} values ranged from 1.25-2 hours. The flat or inverse in exposure levels between 50 and 150 mg/kg is probably due to emesis. The comparability of exposure values between days 1 and 7 suggests that there is some accumulation of PF-04971729 under these conditions, although with unknown amounts of drug being lost (emesis) it is hard to make any solid conclusions about anything related to drug doses above 50 mg/kg.

Combined Male and Female Beagle Dogs
TK :

PF-04971729 Dose (mg/kg/day)	Study Day	Gender	Cmax (µg/mL)	tmax (h)	AUC(0-24) (µg*h/mL)	n
5	1	Male	6.53	1.00	61.2	1
		Female	4.63	2.00	44.4	1
		Overall	5.58	1.50	52.8	2
	7	Male	8.30	1.00	76.7	1
		Female	6.35	2.00	58.8	1
		Overall	7.33	1.50	67.8	2
50	1	Male	35.0	2.00	486	1
		Female	48.2	2.00	494	1
		Overall	41.6	2.00	490	2
	7	Male	55.6	2.00	660	1
		Female	82.6	1.00	834	1
		Overall	69.1	1.50	747	2
150	1	Male	18.8	2.00	182	1
		Female	52.9	1.00	538	1
		Overall	35.9	1.50	360	2
	7	Male	71.7	2.00	679	1
		Female	69.7	0.500	511	1
		Overall	70.7	1.25	595	2

Overall = Male and Female Combined

Combined Male and Female Beagle Dogs
Plasma Concentration Data:

PF-04971729 Dose (mg/kg/day)	Study Day	Gender	PF-4971729 Plasma Concentrations (µg/mL) by Time (h)						n
			0.5	1	2	4	7	24	
5	1	Male	5.61	6.53	5.29	3.94	2.70	1.02	1
		Female	2.33	4.49	4.63	3.34	1.99	0.547	1
		Overall	3.97	5.51	4.96	3.64	2.35	0.784	2
	7	Male	6.95	8.30	6.95	4.93	3.51	1.04	1
		Female	3.83	6.23	6.35	4.25	2.58	0.712	1
		Overall	5.39	7.27	6.65	4.59	3.05	0.876	2
50	1	Male	20.2	33.0	35.0	34.0	24.6	7.99	1
		Female	38.7	46.2	48.2	35.3	22.9	5.91	1
		Overall	29.5	39.6	41.6	34.7	23.8	6.95	2
	7	Male	35.8	51.8	55.6	45.5	32.6	9.13	1
		Female	40.3	82.6	79.1	59.6	38.8	11.0	1
		Overall	38.1	67.2	67.4	52.6	35.7	10.1	2
150	1	Male	18.1	15.3	18.8	13.3	8.38	1.93	1
		Female	39.2	52.9	51.2	44.8	25.5	4.07	1
		Overall	28.7	34.1	35.0	29.1	16.9	3.00	2
	7	Male	32.1	39.1	71.7	50.5	32.4	8.71	1
		Female	69.7	57.8	53.3	41.0	21.8	3.64	1
		Overall	50.9	48.5	62.5	45.8	27.1	6.18	2

Overall = Male and Female Combined

1-Month Oral Toxicity Study of PF-04971729 in Dogs (09GR184)

Key study findings from sponsor:

- After one month of dosing, dogs given 1, 10, and 150 mg/kg/day had average exposures of 7.35, 74.4 and 1080 µg.h/mL (<1X, ~2X and ~21X MRHD, µg.h/mL basis).
- Significant clinical signs in all treated animals included soft feces, which progressed to watery feces and at high incidence in the high dose group. This may be related to off-target inhibition of SGLT1 in the GI tract.
- Vomiting (white foamy or yellow) and/or salivation occurred in HD animals.
- Treated males given ≥ 1 mg/kg did not gain weight throughout the study. The percent change in weight gain ranged from (-276% to -335%). Treated females given ≥ 1 mg/kg gained little weight as well (-89% to -126%) percent change in weight gain. At ≥ 150 mg/kg male and female weights were at 89% to 90% of control animal weights.
- ECG analysis suggests a possible lengthening of the PR interval in HD males and reduced HR in HD females after dosing.
- Males presented with slight decreases in platelet numbers and female WBC, NEU, EOS, and Monocyte numbers declined slightly with dose as well.
- Serum glucose levels did not decrease in male dogs and the reduction in females was extremely limited. AP levels declined similarly to the rat study (09GR185).
- Urinary glucose increased in dosed animals (219x-644x) over pretreatment means.
- There were dose-dependent decreases in group mean absolute and relative male adrenal and thymus weights. Treated females tended to have smaller adrenals, spleens and thymus weights.
- Vacuolation of the gallbladder occurred more frequently at doses ≥ 10 mg/kg.
- Kidney epithelial degeneration was increased in HD treated animals.
- Decreased glycogen content in the liver was observed at all levels of drug treatment and was absent in all control animals.

Reviewer Comments: Two HD females were reported to have been improperly dosed during this study leading to their eventual deaths. Unfortunately in a study that consists of animal numbers so low (n=3), the loss of 66% of the high dose data weakens the study immensely. ECG readings were taken for HD animals at 60 min post dose while C_{max} in these animals was not reached until 2-3 hours post dose.

Vacuolation of the gall bladder at ≥10mg/kg in both sexes was an unexpected finding, and is considered potentially adverse (NOAEL 1mg/kg). The contents of the vacuoles were not described. No further injury was reported in the surrounding liver or bile ducts; thus, the toxicological relevance of this finding is uncertain but should be clarified in 3 and 9m studies in dogs. Presently this finding would not impact the proposed clinical study.

The sponsor concluded a NOAEL of 150mg/kg, the high dose in this study, citing the myriad findings of changes in body weight and stools as being secondary to glucosuria. Based on very frequent clinical signs of soft and watery feces at 150mg/kg, the tolerability of this dose for durations beyond 1 month is questionable. Moreover, there was a slightly higher incidence of renal tubular degeneration/regeneration in HD females. Thus, the reviewer concludes that the NOAEL relevant for risk assessment for the proposed clinical study equals the mid-dose of 10 mg/kg (~2x MRHD).

Dog, 1 month	NOAEL	multiple of starting dose (0.045µg.h/mL)	multiple of max dose (50.63µg h/mL)
Soft/Watery Feces	10 mg/kg (74.4 µg h/mL)	1650X	~2X
Vomiting / Salivation	10 mg/kg (74.4 µg h/mL)	1650X	~2X
Vacuolation Gallbladder	1 mg/kg (7.4 µg.h/mL)	164x	<1X
Kidney Degeneration	10 mg/kg (74.4 µg h/mL)	1650X	~2X

Study no.: 09GR184
Volume # and page #: EDR (4.2.3.2.1)
Conducting laboratory and location: Pfizer Global Research and Development Groton, CT USA.
Date of study initiation: 26 May 2009
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: PF-04971729 (b) (4) (co-crystalline form) GR02546, 99.5% Active Moiety: 76%

Methods	
Doses	0, 1, 10, 150 mg/kg/day
Species/source	Dog Beagle / (b) (4)
Age / Weight	>8 months / 7.3-8.4kg (M) 6.7-8.5kg (F)
n/sex/group (main study)	3/sex/group
TK groups	3/sex/dose D1 and D28 - 0.5, 1, 2, 4, 7, 24 hours post dose
Route, formulation, dose volume	Oral dosing in 0.5% (w/v) MC + 10% (v/v) PEG-400 ~ 10 mLs/kg

Observations and Times	
Mortality checks	Pretreatment/Predose: once per day in the morning Dosing: 3 times per day (predose, 1 hour postdose and in the afternoon).
Clinical Findings	Pretreatment: once per day in the morning Dosing: 3 times per day (predose, 1 hour postdose and in the afternoon). Day of Necropsy: Once
Body weights	Pretreat, prior to dosing on days: 1, 8, 15, 22 and prior to necropsy Day 29.

Food consumption	1 week pretreatment and treatment period: Daily
Ophthalmology	Pretreatment and on Day 27
Electrocardiograms	Once pretreatment and predose and 1-2 hrs postdose and D23 all animals
Hematology	Fasted Pretreatment and all main study animals fasted o/n D28 - tested at autopsy: RBC, Hb, Hct, MCV, MCH, MCHC, RCDW, Ret, PLT, WBC, WCD
Clinical chemistry	Fasted Pretreatment and Day 28, D3(22F) and D24(23F): Standard Battery
Urinalysis	Pretreatment and Day 27 - following parameters: Clarity, color, bilirubin, occult blood, pH, protein, specific gravity, urobilinogen, glucose, ketones and total volume. Urine sediment: Urinary Glucose and Urinary Creatinine (24F – Retested D 28 ~ contamination
Gross pathology	All animals
Organ weights	Adrenals, Brain, Heart, Kidney, Liver, Spleen, Testis and Thymus.
Histopathology	All control and HD animals. Larynx Animal (23F)

Organs Collected	Organs Weighed	Tissues Examined Microscopically Dose Groups (mg/kg/day)			
		0	1	10	150
Colon		X	X	X	X
Duodenum		X	X	X	X
Epididymis		X	X	X	X
Esophagus		X	X	X	X
Eye		X	X	X	X
Gallbladder		X	X	X	X
GALT		X	X	X	X
Heart	X	X	X	X	X
Ileum		X	X	X	X
Jejunum		X	X	X	X
Joint					
Kidney	X	X	X	X	X
Larynx					X †
Liver	X	X	X	X	X
Lung		X	X	X	X
Mammary Gland		X	X	X	X
Mesenteric Lymph Node		X	X	X	X
Optic Nerve		X	X	X	X
Ovary		X	X	X	X
Oviduct		X	X	X	X
Pancreas		X	X	X	X
Parathyroid		X	X	X	X
Peripheral Nerve		X	X	X	X
Pituitary		X	X	X	X
Popliteal Lymph Node		X	X	X	X
Prostate		X	X	X	X
Salivary Gland		X	X	X	X
Skeletal Muscle		X	X	X	X
Skin and Adnexa		X	X	X	X
Spinal Cord		X	X	X	X
Spleen	X	X	X	X	X

Organs Collected	Organs Weighed	Tissues Examined Microscopically Dose Groups (mg/kg/day)			
		0	1	10	150
Adrenal	X	X	X	X	X
Aorta		X	X	X	X
Bone Marrow		X	X	X	X
Brain	X	X	X	X	X
Cecum		X	X	X	X
Cervix		X	X	X	X

Organs Collected	Organs Weighed	Tissues Examined Microscopically Dose Groups (mg/kg/day)			
		0	1	10	150
Sternum		X	X	X	X
Stomach		X	X	X	X
Testis	X	X	X	X	X
Thymus	X	X	X	X	X
Thyroid		X	X	X	X
Tongue		X	X	X	X
Trachea		X	X	X	X
Ureter		X	X	X	X
Urinary Bladder		X	X	X	X
Uterus		X	X	X	X
Vagina		X	X	X	X

Adequate Battery: yes (X), no ()

Peer review: yes (X), no ()

Results:

Mortality: Two females in the 150 mg/kg group were euthanized in moribund condition during the study. On Day 3, Animal (22F) presented with red colored emesis ~1 hour post dose. This was accompanied by decrease activity and labored breathing. On Day 24, Animal (23F) presented with decreased activity and labored breathing patterns shortly after dosing. Necropsy was performed on both animals. The sponsor noted that gross and histomorphological findings in these animals indicated a dosing error and was considered the cause of their condition. The adverse lung, trachea, and larynx findings identified in these animals (histopathology section) appear to be related to failed gavage. The timing of deaths is consistent with this type of technical error as well. No animals died in the prior 7 day study at the same dose supporting this concept further.

MORTALITY								
	MALES				FEMALES			
DOSE	***0***	***1***	***10***	***150***	***0***	***1***	***10***	***150***
Unscheduled Euthanasia	0	0	0	0	0	0	0	2/10

Clinical signs:

Treatment related clinical signs that were noted at all dose levels were: soft and/or watery feces (greatest incidence at 150 mg/kg) and emesis (white foamy or yellow) or salivation 1 hour post dose in the 150 mg/kg group.

Ophthalmological findings are limited based on the fact that observations in these animals were made only during pretreatment and the day before sacrifice. Documentation of when an ocular finding first presented and how it progressed would provide insight into these data.

Findings considered to be incidental and not attributed to dosing were: vomiting of food prior to dosing, hind paw edema and associated limping, interdigital cyst and umbilical hernia.

CLINICAL SIGNS								
Finding	MALES							
	0		1		10		150	
	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals
Cyst Interdigital	0	0	0	0	0	0	1/3	22
Emesis (White) Post Dose	0	0	0	0	0	0	1/3	3
Emesis (Food) Pre Dose	0	0	1/3	1	0	0	0	0
Feces (Soft)	0	0	1/3	4	2/3	2	3/3	18
Feces (Watery)	0	0	0	0	0	0	3/3	51
Fundus Hypopigment Moderate	0	0	2/3	2	0	0	1/3	1
Lens Opacity Multifocal	0	0	0	0	0	0	1/3	1
Abdomen Raised Area	1/3	29	0	0	0	0	0	0

CLINICAL SIGNS								
Finding	FEMALES							
	0		1		10		150	
	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals	Number Animals with Finding	Daily Occurrences Totals
Activity Decreased	0	0	0	0	0	0	2/3	2
Breathing Labored	0	0	0	0	0	0	2/3	2
Cyst Hind Paw	0	0	1/3	8	0	0	0	0
Edema Hind Paw	0	0	1/3	3	0	0	0	0
Emesis (White) Post Dose	0	0	0	0	0	0	3/3	9
Emesis (Yellow) Post Dose	0	0	0	0	0	0	1/3	1
Emesis (Red) Post Dose	0	0	0	0	0	0	1/3	1
Feces (Soft)	1/3	1	1/3	4	1/3	1	2/3	4
Feces (Watery)	0	0	1/3	1	0	0	3/3	23
Fundus Hypopigment Moderate	0	0	1/3	1	1/3	1	0	0
Lens Opacity	0	0	1/3	1	0	0	0	0
Salivation	0	0	0	0	0	0	1/3	4
Vitreous Hyaloid Remnant	0	0	1/3	1	3/3	3	0	0

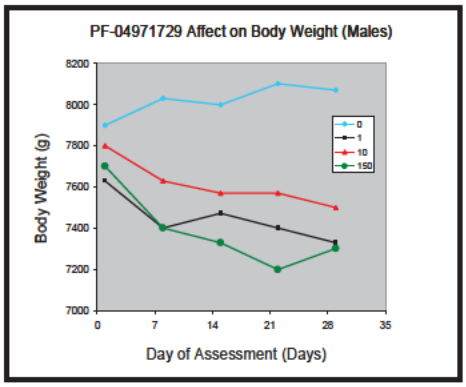
Body weights:

Treatment-related changes in body weight and body weight gains were more pronounced in males and demonstrated a clear dose relationship.

The sponsor reported that changes in individual body weights in dosed male dogs ranged from (-2.7% to -11%) when Day 29 values were compared to Day 1. Body weights changes in concurrent male control animals ranged from (0% to +3.8%). Changes in individual body weights in dosed female dogs ranged from (0% to -4%) when Day 29 values were compared to Day 1. Body weights changes in concurrent female control animals ranged from (+2.8% to +4.2%).

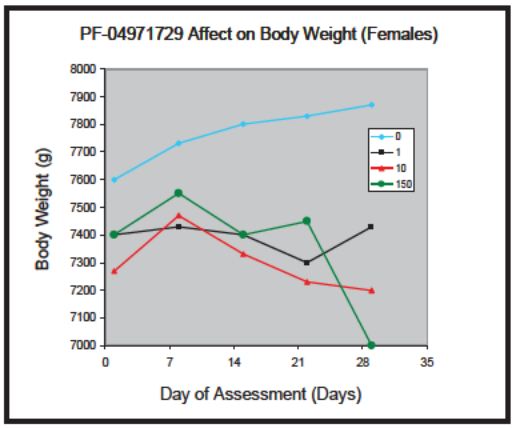
Body weight changes over dosing, % change in gain and body weight percent of control are reported for the entire study below.

Males Body Weight				
Sex	Dose, mg/kg	BW change (g) over dosing	% Change in Gain	BW % control
Males	0	170	0%	100%
	1	-300	-276%	91.0%
	10	-300	-276%	93.0%
	150	-400	-335%	90.5%



*HD female data is difficult to interpret because of the number of animals remaining at the end of the study at this dose was 1.

Females Body Weight				
Sex	Dose, mg/kg	BW gain (g) over dosing	% Change in Gain	BW % control
Females	0	270	0%	100%
	1	30	-89.0%	94.4%
	10	-70	-126.0%	91.5%
	150	0*	-100.0%	88.9%



Food consumption:

Food consumption data was determined qualitatively and not reported. The sponsor stated that there were no treatment-related effects on food consumption.

Ophthalmology:

There were no treatment-related ophthalmologic findings. Final ophthalmic examinations were not conducted in two HD (150 mg/kg) females because they were euthanized before the scheduled study completion date.

Electrocardiogram Analysis:

Treatment-related changes in ECG parameters were:

In male animals ECG parameters tended to increase with dose with the greatest change occurring in the PR interval. When comparing pre- to post dose values in MD and HD animals, the PR interval appears prolonged with the greatest difference occurring in HD males. Untreated male animals tended to have higher values than the LD group but the trend was still towards an increase when comparing between dosed animals only.

In females, animals heart rates tended to drop at the HD, although other parameters observed seemed to change inconsistently. At least one of the HD females being observed in this study was reported by the sponsor to have received improper doses of drug 24 hours prior to these ECG measurements. Based on this knowledge, it is difficult to have confidence in this data and it weakens this study immensely.

In addition to the problems presented by procedural errors, the ECG readings at all dosing levels were taken at 60 min, while this was adequate for the LD and MD groups that achieved C_{max} at approximately 1 hour; the C_{max} for the HD groups was not reached until 2 hrs or longer on the average. The reviewer's table below summarizes the sponsor's average ECG data.

Electrocardiogram									
		MALES				FEMALES			
Reading	***Time***	***0***	***1***	***10***	***150***	***0***	***1***	***10***	***150***
Heart Rate	Pre	103.7	89.2	102.3	88.9	96.8	105.2	87.4	118.8
Heart Rate	1 Hr	94.3	91.8	96.8	97.0	104.6	110.6	85.3	87.8
PR	Pre	89.7	94.7	101.9	79.4	99.4	96.6	100.5	96.4
PR	1 Hr	98.3	93.0	109.2	117.4	110.7	102.1	110.3	101.9
QRS	Pre	39.9	40.1	40.5	41.8	41.0	38.6	38.9	39.6
QRS	1 Hr	39.2	38.8	40.3	40.6	40.8	38.8	42.4	40.3
QT	Pre	211.2	214.0	206.1	223.0	215.3	208.4	219.4	209.5
QT	1 Hr	219.1	211.2	214.0	214.6	214.9	210.5	220.5	221.1
Canine QT	Pre	213.2	208.2	207.3	217.1	213.6	211.2	212.7	219.6
Canine QT	1 Hr	216.0	206.8	212.3	213.0	217.4	216.1	212.6	214.6

Hematology:

No significant treatment-related findings were identified in any group. Male platelet number did decrease with dose but the change was not remarkable. Female WBC, neutrophil, eosinophil and monocyte counts declined slightly with dose as well. Changes in female hematology are complicated again by errors in dosing and lack of numbers due to animal mortality.

Clinical chemistry:

Interestingly, serum glucose levels did not decrease in male dogs; in fact HD males (150 mg/kg) had elevated (99.3 mg/dL) serum glucose levels on Day 28 when compared to controls (94.3 mg/dL). Female data was again limited by lack of numbers but the trend in glucose reduction (as seen in rats 09GR185) was apparent, albeit small.

Increases in liver enzyme levels (ALT/AST), although present, were not as dramatic in dogs as they were in rats. On the other hand, male dogs presented with a similar dose dependent decline in alkaline phosphatase levels as seen in rats (09GR185), but this trend was absent in female dogs.

Changes in other clinical chemistry parameters were consistently small in nature in males and inconsistent in females across all dosing levels.

Urinalysis:

In dogs, treatment with PF-04971729 resulted in increased urinary glucose (219x-644x fold increase over the pretreatment mean), similar to the changes seen in rats. This was accompanied by a decrease in urinary creatinine (68%-90%) across all dosing levels, but not a significant loss of glucose from the serum (see clinical chemistry above), this suggest that gluconeogenesis can effectively counter the glucosuria in normal dogs.

Urine volumes were variable and not available for all animals due to sponsor methodology. Specific gravity and pH remained unchanged in males, while females tended to have these values decline slightly with increased dose of drug. Significance of the female data is again limited by numbers.

Urinalysis (Males)

Group No:		1	2	3	4
	Day	0 mg/kg	1 mg/kg	10 mg/kg	150 mg/kg
			MALE		
SG	-13 (3)	1.0410± 0.00529	(3) 1.0460± 0.00500	(3) 1.0533± 0.00751	(3)>1.0560±>0.01229
(none)	27 (3)	1.0457± 0.00896	(3) 1.0550± 0.00693	(3) 1.0550± 0.00794	(3) 1.0550± 0.00872
VOLUME	-13	-	(2) 10.50± 10.607	-	(2) 25.50± 34.648
(mL)	27 (2)	61.00± 26.870	(3) 40.00± 10.000	(2) 61.00± 26.870	(3) 52.67± 34.078
pH	-13 (3)	6.00± 0.500	(3) 6.00± 0.000	(3) 6.00± 0.500	(3) 6.00± 0.500
(none)	27 (3)	5.83± 0.289	(3) 6.33± 0.577	(3) 5.67± 0.289	(3) 5.83± 0.289

Group No:		1	2	3	4
	Day	0 mg/kg	1 mg/kg	10 mg/kg	150 mg/kg
			MALE		
U CREA	-13 (3)	236.27± 84.093	(3) 244.83± 131.496	(3) 263.37± 31.510	(3) 312.40± 186.313
(mg/dL)	27 (3)	150.40± 14.148	(3) 79.30± 16.488*	(3) 73.07± 9.929*	(3) 82.67± 35.089*
U GLUC	-13 (3)	11.3± 1.53	(3) 12.3± 5.13	(3) 13.7± 1.53	(3) 20.0± 14.80
(mg/dL)	27 (3)	12.0± 5.00	(3) 7922.3± 1382.29*	(3) 8378.7± 545.57*	(3) 9224.0± 1584.09*
U GLUC R	-13 (3)	0.050± 0.0100	(3) 0.053± 0.0058	(3) 0.050± 0.0100	(3) 0.060± 0.0100
(mg/mg)	27 (3)	0.080± 0.0361	(3) 100.630± 10.3763*	(3) 115.543± 10.7869*	(3) 118.757± 25.6050*

Note: Values expressed as (n) mean ± standard deviation.

- Value not applicable or not available.

Urinalysis (Females)

Group No:		1	2	3	4
	Day	0 mg/kg	1 mg/kg	10 mg/kg	150 mg/kg
			FEMALE		
SG	-13 (3)	1.0520± 0.00985	(3) 1.0437± 0.00777	(3)>1.0533±>0.01443	(3) 1.0517± 0.00503
(none)	27 (3)	1.0530± 0.01000	(3) 1.0473± 0.01601	(3) 1.0493± 0.01301	-
	28	-	-	-	(1) 1.0170± .
VOLUME	-13 (3)	18.33± 12.583	(1) 45.00± .	(2) 13.50± 16.263	(2) 49.00± 1.414
(mL)	27 (2)	60.00± 28.284	(3) 83.33± 55.076	(2) 60.00± 28.284	-
pH	-13 (3)	5.67± 0.289	(3) 5.83± 0.289	(3) 6.00± 0.500	(3) 5.67± 0.289
(none)	27 (3)	6.00± 0.500	(3) 5.83± 0.289	(3) 5.67± 0.289	-
	28	-	-	-	(1) 6.00± .

Group No:		1	2	3	4
	Day	0 mg/kg	1 mg/kg	10 mg/kg	150 mg/kg
			FEMALE		
U CREA	-13 (3)	274.90± 62.091	(3) 232.60± 68.500	(3) 286.97± 40.023	(3) 255.53± 46.139
(mg/dL)	27 (3)	135.50± 11.406	(3) 62.33± 27.419*	(3) 61.10± 20.004*	-
	28	-	-	-	(1) 32.40± .
U GLUC	-13 (3)	15.0± 1.73	(3) 12.7± 3.06	(3) 14.7± 1.53	(3) 13.7± 2.08
(mg/dL)	27 (3)	11.3± 5.77	(3) 7345.7± 2295.03*	(3) 8315.7± 1300.97*	-
	28	-	-	-	(1) 3505.0± .
U GLUC R	-13 (3)	0.053± 0.0058	(3) 0.053± 0.0058	(3) 0.050± 0.0100	(3) 0.053± 0.0058
(mg/mg)	27 (3)	0.080± 0.0346	(3) 122.180± 18.6157*	(3) 141.960± 26.8613*	-
	28	-	-	-	(1) 108.180± .

Note: Values expressed as (n) mean ± standard deviation.

- Value not applicable or not available.

HPD = Hours Postdose.

Significantly different from control:

* - p ≤ 0.05, † - p ≤ 0.01, ‡ - p ≤ 0.005, $ - p ≤ 0.001

Toxicokinetics:

The gender differences in exposure seen in the rat study (09GR185) were not seen in the dog. Two of the high dose females (22F and 24F) on Day 1 vomited following treatment, which explains the decline in C_{max} and AUC_{0-24} for those individuals. Mean t_{max} were between (0.75 and 2.2 hrs) on Day 1 and (0.92 and 2 hrs) on Day 28. Systemic exposure increased with dose on Day 1 and Day 28, values for C_{max} and AUC_{0-24} on these days were comparable indicating that drug accumulation after 28 days of oral dosing of PF-04971729 was minimal.

Dose (mg/kg/day)	Study Day	Gender	Cmax (µg/mL)			tmax (h)			AUC(0-24) (µg*h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
1	1	Male	0.981	0.148	3	0.667	0.289	3	6.18	0.430	3
		Female	1.10	0.0500	3	0.833	0.289	3	6.50	0.541	3
		Overall	1.04	0.118	6	0.750	0.274	6	6.34	0.472	6
	28	Male	1.15	0.170	3	0.500	0.00	3	6.66	1.16	3
		Female	1.04	0.0524	3	1.33	0.577	3	8.03	2.57	3
		Overall	1.09	0.129	6	0.917	0.585	6	7.35	1.94	6
10	1	Male	11.2	1.27	3	0.833	0.289	3	70.4	9.10	3
		Female	9.04	0.251	3	0.833	0.289	3	54.3	4.55	3
		Overall	10.1	1.43	6	0.833	0.258	6	62.4	10.9	6
	28	Male	11.2	1.52	3	1.00	0.866	3	77.4	13.1	3
		Female	11.0	0.361	3	0.833	0.289	3	71.4	9.08	3
		Overall	11.1	0.997	6	0.917	0.585	6	74.4	10.6	6
150	1	Male	63.9	19.4	3	3.33	1.16	3	702	334	3
		Female	34.0	12.4*	3	1.00	0.866	3	277*	136	3
		Overall	49.0	21.9	6	2.17	1.57	6	489	326	6
	28	Male	98.5	14.5	3	2.00	0.00	3	1050	162	3
		Female	103	NC	1	2.00	NC	1	1170	NC	1
		Overall	99.6	12.1	4	2.00	0.00	4	1080	146	4

Overall = Male Plus Female Combined; NC = Not Calculated, n < 3
* = Lower observed exposure possibly due to emesis in animals 22 and 24 (females) following dosing on Day 1

As reported by the sponsor, on Day 28, mean combined C_{max} and AUC_{0-24} values for PF04971729 at 1, 10, and 150 mg/kg were 1.09, 11.1, and 99.6 µg/mL, respectively, for C_{max}, and 7.35, 74.4, and 1080 µg.hr/mL, respectively, for AUC_{0-24}.

The mean combined PF-04971729 concentration versus time on day 1 and day 28, C_{max} vs. dose and AUC_{0-24} vs. dose are shown in the sponsor's figures below.

Figure 1. Mean (± SD) PF-04971729 Concentration in Combined Male and Female Dogs versus Time after Oral Administration of PF-04971729 on Day 1

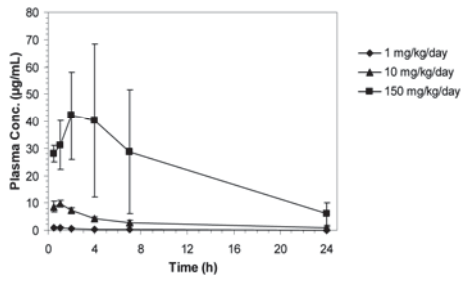


Figure 2. Mean (± SD) PF-04971729 Concentration in Combined Male and Female Dogs versus Time after Oral Administration of PF-04971729 on Day 28

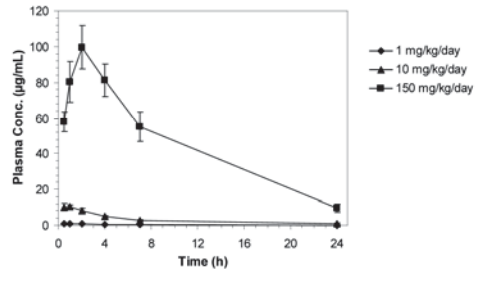


Figure 3. Mean (±SD) PF-04971729 Cmax versus Dose in Combined Male and Female Dogs after Oral Administration of PF-04971729 at 1, 10, or 150 mg/kg/day on Days 1 and 28

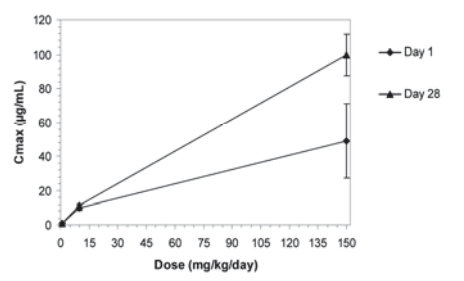
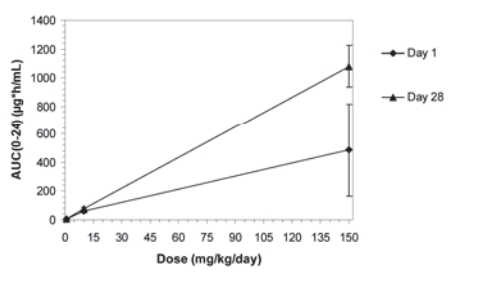
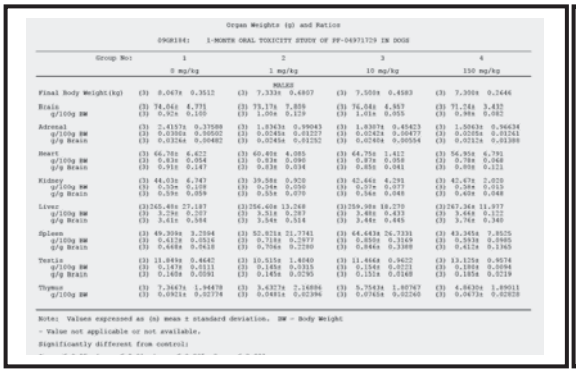
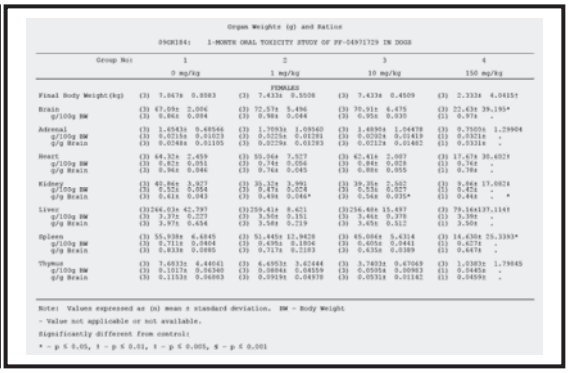


Figure 4. Mean (±SD) PF-04971729 AUC(0-24) versus Dose in Combined Male and Female Dogs after Oral Administration of PF-04971729 at 1, 10, or 150 mg/kg/day on Days 1 and 28



As shown in the tables below, there were dose-dependent decreases in group mean absolute and relative male adrenal weights. Male thymus absolute and relative weights were decreased across all dose levels. In females the data presented for the HD animals was difficult to interpret for the same reasons discussed earlier. When comparing the LD and MD females to the controls, adrenal, spleen and thymus absolute and relative weights tended to decrease with dose.





BEST AVAILABLE COPY

Gross pathology:

Treatment-related findings in the gross necropsy observations were isolated to HD female animals (150 mg/kg). These included abnormal color in the larynx and trachea from a single animal (23F) that died on Day 24. In addition, HD females presented with discoloration of the liver and lung, not seen at any other dose or within the control group. Dose-related findings from gross observations within the males were absent. See sponsor's table below.

Gross Observations

09GR184: 1-MONTH ORAL TOXICITY STUDY OF PF-04971729 IN DOGS

Sex:	Male				Female			
Group No:	1	2	3	4	1	2	3	4
Dose (mg/kg):	0	1	10	150	0	1	10	150
Animals on Study:	3	3	3	3	3	3	3	3
Abdominal cavity								
Animals Examined:	1	–	–	–	–	–	–	–
Remarkable Observations	1	–	–	–	–	–	–	–
Hernia	1	–	–	–	–	–	–	–
Larynx								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	3	3	3	2
Remarkable Observations	–	–	–	–	–	–	–	1
Abnormal color	–	–	–	–	–	–	–	1
Liver								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	3	3	3	2
Remarkable Observations	–	–	–	–	–	–	–	1
1 discoloration	–	–	–	–	–	–	–	1
Lung								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	3	3	3	1
Remarkable Observations	–	–	–	–	–	–	–	2
1 Discoloration	–	–	–	–	–	–	–	1
Abnormal color	–	–	–	–	–	–	–	1
Mammary gland								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	3	3	2	3
Remarkable Observations	–	–	–	–	–	–	1	–
Abnormal color	–	–	–	–	–	–	1	–
Spleen								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	3	2	3	3
Remarkable Observations	–	–	–	–	–	1	–	–
Abnormal surface	–	–	–	–	–	1	–	–

Gross Observations

09GR184: 1-MONTH ORAL TOXICITY STUDY OF PF-04971729 IN DOGS

Sex:	Male				Female			
Group No:	1	2	3	4	1	2	3	4
Dose (mg/kg):	0	1	10	150	0	1	10	150
Trachea								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	3	3	3	2
Remarkable Observations	-	-	-	-	-	-	-	1
Abnormal color	-	-	-	-	-	-	-	1
Urinary bladder								
Animals Examined:	3	3	3	3	3	3	3	3
Unremarkable	3	3	3	3	2	3	3	3
Remarkable Observations	-	-	-	-	1	-	-	-
Abnormal color	-	-	-	-	1	-	-	-
The following tissues were unremarkable:								
Adrenal	3	3	3	3	3	3	3	3
Aorta	3	3	3	3	3	3	3	3
Bone marrow	3	3	3	3	3	3	3	3
Brain	3	3	3	3	3	3	3	3
Cecum	3	3	3	3	3	3	3	3
Cervix	-	-	-	-	3	3	3	3
Colon	3	3	3	3	3	3	3	3
Duodenum	3	3	3	3	3	3	3	3
Epididymis lg	3	3	3	3	-	-	-	-
Esophagus	3	3	3	3	3	3	3	3
Eye (left)	3	3	3	3	3	3	3	3
Eye (right)	3	3	3	3	3	3	3	3
Gallbladder	3	3	3	3	3	3	3	3
Gut-associated lymphoid tissue	3	3	3	3	3	3	3	3
Heart	3	3	3	3	3	3	3	3
Ileum	3	3	3	3	3	3	3	3
Jejunum	3	3	3	3	3	3	3	3
Kidney	3	3	3	3	3	3	3	3
Mesenteric lymph node	3	3	3	3	3	3	3	3
Optic nerve	3	3	3	3	3	3	3	3
Ovary	-	-	-	-	3	3	3	3
Oviduct	-	-	-	-	3	3	3	3
Pancreas	3	3	3	3	3	3	3	3
Parathyroid	3	3	3	3	3	3	3	3
Peripheral nerve	3	3	3	3	3	3	3	3

Gross Observations

09GR184: 1-MONTH ORAL TOXICITY STUDY OF PF-04971729 IN DOGS

Sex:	Male				Female			
Group No:	1	2	3	4	1	2	3	4
Dose (mg/kg):	0	1	10	150	0	1	10	150
Pituitary	3	3	3	3	3	3	3	3
Popliteal lymph node	3	3	3	3	3	3	3	3
Prostate lg	3	3	3	3	-	-	-	-
Salivary gland'	3	3	3	3	3	3	3	3
Skeletal muscle	3	3	3	3	3	3	3	3
Skin and adnexa	3	3	3	3	3	3	3	3
Spinal cord	3	3	3	3	3	3	3	3
Sternum	3	3	3	3	3	3	3	3
Stifle joint'	3	3	3	3	3	3	3	3
Stomach	3	3	3	3	3	3	3	3
Testis	3	3	3	3	-	-	-	-
Thymus	3	3	3	3	3	3	3	3
Thyroid	3	3	3	3	3	3	3	3
Tongue	3	3	3	3	3	3	3	3
Ureter	3	3	3	3	3	3	3	3
Uterus	-	-	-	-	3	3	3	3
Vagina	-	-	-	-	3	3	3	3

Histopathology:

The sponsor stated that there were no treatment-related findings and that all histomorphological findings, except for those related to gavage errors, were typical of the age, sex and breed of the dog used in this study. The reviewer does not agree with this statement.

In addition, the sponsor included euthanized females #22 and 23 in the histological evaluation. Female #22 was sacrificed on day 3 and should not have been included in the histological evaluation (other than to identify cause of death) given the short exposure to drug. Female #23 was sacrificed on day 24 and is reasonably acceptable for analysis. Thus, only 2 high dose females are considered valid for histological analysis.

Treatment related microscopic findings were noted in the duodenum, gall bladder, kidney, larynx, liver, lung, thyroid, tongue and trachea.

The most notable histological finding is vacuolation of the gallbladder epithelium that occurred more frequently at doses ≥ 10 mg/kg in males and females. Though not recognized as drug-related by the sponsor, this finding is considered potentially adverse. The relevance of this finding will be clearer from the 3 and 9 month dog studies. Note that gallbladder vacuolation was observed in female #22 that was sacrificed on day 3. This supports Pfizer's view that this finding is unrelated to drug treatment, but it does not dismiss the increased incidence in treated males.

In the duodenum, only one HD female presented with inflammation.

Degeneration and subsequent regeneration of the kidney epithelium was seen in male dogs at the MD and HD only, while in females it was not dose responsive but did occur with the greatest frequency at the HD (150 mg/kg).

The single HD female presented with acidophilic material in her larynx and trachea (gavage error).

Decreased glycogen content in the liver was seen in the female dogs at the MD and HD only, while in male dogs only LD and MD animals presented with this finding, though it was absent from all control animal.

In the lung most of the dose-dependent findings were limited to the female dogs that were subjected to improper gavage. These included congestion (2HD), disposition of acidophilic material (1MD/1HD), edema (2HD) and necrosis (2HD). The only dose-dependent finding in males was hyperplasia of the mesothelial cell layer in one HD animal.

1 HD male presented with erosions and ulcerations of the tongue, while a single HD female had inflamed tongue tissue.

HISTOPATHOLOGY - MALES							
Tissue	Finding		Main Study				
		dose	0	1	10	150	
		n	3	3	3	3	
Gallbladder	Vacuolation: epithelium		1*	1*	3*	3*	← SEVERITY ←
Kidney	Cast		0	0	0	1*	
	Degeneration/Regeneration Tubular Epithelium		0	0	1*	1*	
Liver	Decreased Glycogen Content		0	1*	1*	0	
Lung	Hyperplasia of mesothelial cells		0	0	0	1*	
Tongue	Erosion / Ulceration		0	0	0	1*	
			(*) Minimal (^) Mild (¥) Severe (U) Unilateral (B) Bilateral				

HISTOPATHOLOGY - FEMALES							
Tissue	Finding		Main Study				
		dose	0	1	10	150	
		n	3	3	3	3	
Duodenum	Inflammation		0	0	0	1*	
Gallbladder	Vacuolation: epithelium		1*	1*	3*	2*	
Kidney	Degeneration/Regeneration Tubular Epithelium		1*	1*	0	2*	← SEVERITY ←
Larynx	Disposition of Acidophilic material		0	0	0	1*	
Liver	Decreased Glycogen Content		0	0	1*	1*	
Lung	Congestion		0	0	0	2*	
	Disposition of Acidophilic material		0	0	1*	1*	
	Edema		0	0	0	2*	
	Necrosis		0	0	0	2*	
Thyroid	Edema		0	0	0	1*	
Tongue	Inflammation		0	0	0	1*	
Trachea	Disposition of Acidophilic material		0	0	0	1*	
			(*) Minimal (^) Mild (¥) Severe (U) Unilateral (B) Bilateral				

Phototoxicity Studies

No phototoxicity studies have been conducted as UV-visible spectral analysis of PF-04971729 indicated that the compound has a shoulder at 290 nM with a molar extinction coefficient (MEC) of less than 1000 L/mol.cm.

APPEARS THIS WAY ON ORIGINAL

2.6.6.4 Genetic toxicology

Bacterial Reverse Mutation Assay with a Confirmatory Assay (09GR181)

Key findings: Exposure to PF-04971729 did not increase the mean number of revertants per plate with any tester strain either in the presence or absence of microsomal enzyme prepared S9.

Study:	09GR181
Volume and page:	EDR 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4 June 2009
GLP compliance:	Yes
QA reports:	Yes
Drug, lot #, and % purity:	PF-04971729, Lot GR02546, 99.5%

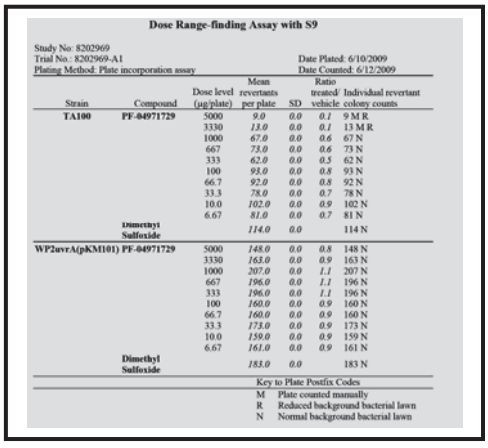
Methods

Strains/species/cell line:

Tester Strain	*his*/*trp* Mutation	Additional Mutations: Repair	Additional Mutations: LPS	Plasmid
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
WP2*uvr*A(pKM101)	*trp*	*uvr*A	–	pKM101

Doses used in definitive study: 78.0, 156, 313, 625, 1250, 2500, and 5000 µg/plate.

Basis of dose selection: Normal growth was observed in the tester strain WP2uvrA with and without S9. Inhibited growth was observed in the tester strain TA100 at doses ≥ 3300 µg/plate with S9 and at doses ≥ 1000 µg/plate without S9 (sees results below). 5000µg/plate was set as the high dose, in accordance with the appropriate guidelines, and a common factor of two was used to set the lower doses. In addition, the test article was found to be freely soluble at all doses evaluated with and without S9.

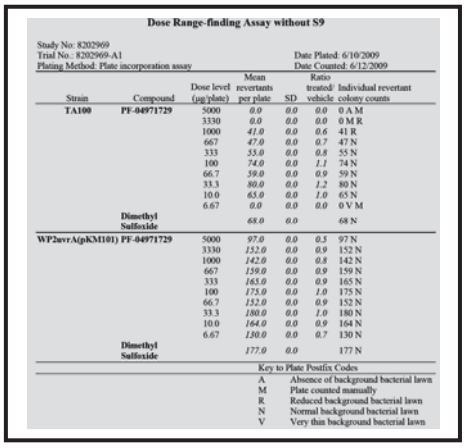


Dose Range-finding Assay with S9

Study No: 8202969
Trial No.: 8202969-A1
Plating Method: Plate incorporation assay
Date Plated: 6/10/2009
Date Counted: 6/12/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA100	PF-04971729	5000	9.0	0.0	0.1	9 M R
		3330	13.0	0.0	0.1	13 M R
		1000	67.0	0.0	0.6	67 N
		667	73.0	0.0	0.6	73 N
		333	62.0	0.0	0.5	62 N
		100	93.0	0.0	0.8	93 N
		66.7	92.0	0.0	0.8	92 N
		33.3	78.0	0.0	0.7	78 N
		10.0	102.0	0.0	0.9	102 N
		6.67	81.0	0.0	0.7	81 N
	Dimethyl Sulfoxide		114.0	0.0		114 N
WP2uvrA(pKM101)	PF-04971729	5000	148.0	0.0	0.8	148 N
		3330	163.0	0.0	0.9	163 N
		1000	207.0	0.0	1.1	207 N
		667	196.0	0.0	1.1	196 N
		333	196.0	0.0	1.1	196 N
		100	160.0	0.0	0.9	160 N
		66.7	160.0	0.0	0.9	160 N
		33.3	173.0	0.0	0.9	173 N
		10.0	159.0	0.0	0.9	159 N
		6.67	161.0	0.0	0.9	161 N
	Dimethyl Sulfoxide		183.0	0.0		183 N

Key to Plate Postfix Codes
M Plate counted manually
R Reduced background bacterial lawn
N Normal background bacterial lawn



Dose Range-finding Assay without S9

Study No: 8202969
Trial No.: 8202969-A1
Plating Method: Plate incorporation assay
Date Plated: 6/10/2009
Date Counted: 6/12/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA100	PF-04971729	5000	0.0	0.0	0.0	0 A M
		3330	0.0	0.0	0.0	0 M R
		1000	41.0	0.0	0.6	41 R
		667	47.0	0.0	0.7	47 N
		333	55.0	0.0	0.8	55 N
		100	74.0	0.0	1.1	74 N
		66.7	59.0	0.0	0.9	59 N
		33.3	80.0	0.0	1.2	80 N
		10.0	65.0	0.0	1.0	65 N
		6.67	0.0	0.0	0.0	0 V M
	Dimethyl Sulfoxide		68.0	0.0		68 N
WP2uvrA(pKM101)	PF-04971729	5000	97.0	0.0	0.5	97 N
		3330	152.0	0.0	0.9	152 N
		1000	142.0	0.0	0.8	142 N
		667	159.0	0.0	0.9	159 N
		333	165.0	0.0	0.9	165 N
		100	175.0	0.0	1.0	175 N
		66.7	152.0	0.0	0.9	152 N
		33.3	180.0	0.0	1.0	180 N
		10.0	164.0	0.0	0.9	164 N
		6.67	130.0	0.0	0.7	130 N
	Dimethyl Sulfoxide		177.0	0.0		177 N

Key to Plate Postfix Codes
A Absence of background bacterial lawn
M Plate counted manually
R Reduced background bacterial lawn
N Normal background bacterial lawn
V Very thin background bacterial lawn

Negative controls: DMSO

Positive controls: Positive controls are shown in the table below.

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	CAS No.	Lot No.
TA98	–	2-nitrofluorene	1.0	607-57-8	01508BE
TA100, TA1535	–	sodium azide	2.0	26628-22-8	017K0136
TA1537	–	ICR-191	2.0	17070-45-0	115K1328
					116K1026
WP2*uvr*A (pKM101)	–	4-nitroquinoline-N-oxide	2.0	56-57-5	117K1485
TA98	+	benzo[a]pyrene	2.5	50-32-8	017K1044
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	12317CE
WP2*uvr*A (pKM101)	+	2-aminoanthracene	5.0	613-13-8	12317CE

Incubation and sampling times: Treatments were performed by adding 100 mL tester strain and 50 µL of test or control article to 2.5 mL of molten selective top agar (maintained at 45 ± 2°C). After the required components had been added, the mixture was vortexed and overlaid onto the surface of 25 mL minimal bottom agar in a 15 x 100 mm Petri dish. After the overlay solidified, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2°C. Cultures were treated in the presence of S9 in an identical manner, except using 2.0 mL undiluted molten selective top agar and adding 500 µL S9 mix.

Results

Study validity: The positive controls used in this assay were adequate and the description of the S9 characterization was complete. Acceptance criteria for valid studies were given in the protocol and met by the results.

Study outcome:
Preliminary Plate Incorporation: PF-04971729 was evaluated in the dose range-finding assay in tester strains TA100 and WP2*uvr*A(pKM101). Ten doses of test article, from 6.67 to 5000 µg/plate, were evaluated with and without S9 (one plate per dose; Trial 8202969-A1, see tables below). There was no evidence of significant dose-related increase in the mean number of revertants with any of the strains tested in either the presence or absence of S9.

Dose Range-finding Assay with S9

Study No: 8202969
Trial No.: 8202969-A1 Date Plated: 6/10/2009
Plating Method: Plate incorporation assay Date Counted: 6/12/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA100	**PF-04971729**	5000	*9.0*	*0.0*	*0.1*	9 M R
		3330	*13.0*	*0.0*	*0.1*	13 M R
		1000	*67.0*	*0.0*	*0.6*	67 N
		667	*73.0*	*0.0*	*0.6*	73 N
		333	*62.0*	*0.0*	*0.5*	62 N
		100	*93.0*	*0.0*	*0.8*	93 N
		66.7	*92.0*	*0.0*	*0.8*	92 N
		33.3	*78.0*	*0.0*	*0.7*	78 N
		10.0	*102.0*	*0.0*	*0.9*	102 N
		6.67	*81.0*	*0.0*	*0.7*	81 N
	Dimethyl Sulfoxide		*114.0*	*0.0*		114 N
WP2uvrA(pKM101)	**PF-04971729**	5000	*148.0*	*0.0*	*0.8*	148 N
		3330	*163.0*	*0.0*	*0.9*	163 N
		1000	*207.0*	*0.0*	*1.1*	207 N
		667	*196.0*	*0.0*	*1.1*	196 N
		333	*196.0*	*0.0*	*1.1*	196 N
		100	*160.0*	*0.0*	*0.9*	160 N
		66.7	*160.0*	*0.0*	*0.9*	160 N
		33.3	*173.0*	*0.0*	*0.9*	173 N
		10.0	*159.0*	*0.0*	*0.9*	159 N
		6.67	*161.0*	*0.0*	*0.9*	161 N
	Dimethyl Sulfoxide		*183.0*	*0.0*		183 N

Key to Plate Postfix Codes
M Plate counted manually
R Reduced background bacterial lawn
N Normal background bacterial lawn

Dose Range-finding Assay without S9

Study No: 8202969
Trial No.: 8202969-A1 Date Plated: 6/10/2009
Plating Method: Plate incorporation assay Date Counted: 6/12/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA100	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 A M
		3330	*0.0*	*0.0*	*0.0*	0 M R
		1000	*41.0*	*0.0*	*0.6*	41 R
		667	*47.0*	*0.0*	*0.7*	47 N
		333	*55.0*	*0.0*	*0.8*	55 N
		100	*74.0*	*0.0*	*1.1*	74 N
		66.7	*59.0*	*0.0*	*0.9*	59 N
		33.3	*80.0*	*0.0*	*1.2*	80 N
		10.0	*65.0*	*0.0*	*1.0*	65 N
		6.67	*0.0*	*0.0*	*0.0*	0 V M
	Dimethyl Sulfoxide		*68.0*	*0.0*		68 N
WP2uvrA(pKM101)	**PF-04971729**	5000	*97.0*	*0.0*	*0.5*	97 N
		3330	*152.0*	*0.0*	*0.9*	152 N
		1000	*142.0*	*0.0*	*0.8*	142 N
		667	*159.0*	*0.0*	*0.9*	159 N
		333	*165.0*	*0.0*	*0.9*	165 N
		100	*175.0*	*0.0*	*1.0*	175 N
		66.7	*152.0*	*0.0*	*0.9*	152 N
		33.3	*180.0*	*0.0*	*1.0*	180 N
		10.0	*164.0*	*0.0*	*0.9*	164 N
		6.67	*130.0*	*0.0*	*0.7*	130 N
	Dimethyl Sulfoxide		*177.0*	*0.0*		177 N

Key to Plate Postfix Codes
A Absence of background bacterial lawn
M Plate counted manually
R Reduced background bacterial lawn
N Normal background bacterial lawn
V Very thin background bacterial lawn

Main Plate Incorporation: Based upon the results of the dose range-finding assay, PF-04971729 was evaluated in the initial mutagenicity assay, in tester strains TA98, TA100, TA1535, and TA1537 at doses of 78.0, 156, 313, 625, 1250, 2500, and 5000 µg/plate and in tester strain WP2*uvr*A(pKM101) at doses of 156, 313, 625, 1250, 2500, and 5000 µg/plate with and without S9 (Trial 8202969-B1, see tables below).

All doses of the test article, as well as the concurrent positive and vehicle controls were evaluated in triplicate plates.

Inhibited growth was observed in tester strain TA1537 without S9 at doses ≥1250 µg/plate, in tester strain TA1537 with S9 at doses ≥2500 µg/plate, and in tester strains TA98, TA100, and TA1535 with and without S9 at doses ≥2500 µg/plate.

There was no evidence of significant dose-related increase in the mean number of revertants with any of the strains tested in either the presence or absence of S9. All data were acceptable and no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

Initial Mutagenicity Assay Results with S9

Study No: 8202969
Trial No.: 8202969-B1 Date Plated: 6/23/2009
Plating Method: Plate incorporation assay Date Counted: 6/25/2009 to 6/26/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**PF-04971729**	5000	*11.0*	*3.5*	*0.5*	13 M R, 13 M R, 7 M R
		2500	*18.3*	*5.5*	*0.8*	24 R, 13 R, 18 R
		1250	*20.3*	*2.1*	*0.9*	18 N, 21 N, 22 N
		625	*23.7*	*1.5*	*1.1*	22 N, 25 N, 24 N
		313	*19.0*	*6.1*	*0.9*	22 N, 12 N, 23 N
		156	*25.0*	*4.6*	*1.1*	24 N, 30 N, 21 N
		78.0	*16.7*	*4.7*	*0.8*	22 N, 15 N, 13 N
	Dimethyl Sulfoxide		*22.0*	*1.0*		23 N, 22 N, 21 N
TA100	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 R M, 0 R M, 0 R M
		2500	*37.0*	*2.6*	*0.3*	35 R M, 36 R M, 40 R M
		1250	*71.0*	*9.6*	*0.6*	64 N, 82 N, 67 N
		625	*88.3*	*11.7*	*0.8*	86 N, 101 N, 78 N
		313	*78.3*	*4.9*	*0.7*	75 N, 76 N, 84 N
		156	*96.0*	*9.8*	*0.9*	85 N, 99 N, 104 N
		78.0	*103.3*	*5.9*	*0.9*	110 N, 99 N, 101 N
	Dimethyl Sulfoxide		*109.7*	*8.5*		101 N, 118 N, 110 N
TA1535	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 R M, 0 M R, 0 R M
		2500	*3.3*	*0.6*	*0.3*	4 M R, 3 M R, 3 R
		1250	*6.7*	*1.2*	*0.6*	8 M N, 6 N, 6 N
		625	*16.7*	*7.8*	*1.5*	8 N, 19 N, 23 N
		313	*10.0*	*2.0*	*0.9*	12 M N, 8 N, 10 N
		156	*9.7*	*5.7*	*0.9*	5 N, 8 N, 16 N
		78.0	*13.7*	*4.7*	*1.2*	10 N, 19 N, 12 N
	Dimethyl Sulfoxide		*11.3*	*6.1*		18 N, 10 N, 6 N
TA1537	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 M R, 0 M R, 0 M R
		2500	*2.7*	*2.1*	*0.4*	1 M R, 5 M R, 2 M R
		1250	*5.0*	*1.0*	*0.8*	4 N, 6 M N, 5 N
		625	*9.0*	*2.6*	*1.5*	10 N, 6 N, 11 N
		313	*9.0*	*1.7*	*1.5*	10 N, 10 N, 7 N
		156	*7.0*	*1.7*	*1.2*	8 N, 8 N, 5 N
		78.0	*6.0*	*1.7*	*1.0*	7 N, 7 N, 4 N
	Dimethyl Sulfoxide		*6.0*	*1.7*		7 N, 7 N, 4 N

Key to Plate Postfix Codes
R Reduced background bacterial lawn
M Plate counted manually
N Normal background bacterial lawn

Initial Mutagenicity Assay Results without S9

Study No: 8202969
Trial No.: 8202969-B1 Date Plated: 6/23/2009
Plating Method: Plate incorporation assay Date Counted: 6/25/2009 to 6/26/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**PF-04971729**	5000	*1.0*	*1.0*	*0.1*	0 M R, 2 M R, 1 M R
		2500	*2.7*	*2.1*	*0.2*	1 M R, 5 M R, 2 M R
		1250	*14.7*	*5.5*	*1.0*	11 N, 21 N, 12 N
		625	*13.7*	*2.9*	*0.9*	12 N, 17 N, 12 N
		313	*14.0*	*5.2*	*0.9*	17 N, 8 N, 17 N
		156	*14.7*	*5.7*	*1.0*	21 N, 10 N, 13 N
		78.0	*16.3*	*5.0*	*1.1*	11 N, 17 N, 21 N
	Dimethyl Sulfoxide		*15.3*	*7.5*		8 N, 23 N, 15 N
TA100	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 M R, 0 M R, 0 M R
		2500	*5.3*	*6.1*	*0.1*	0 M R, 4 M R, 12 M R
		1250	*59.7*	*9.3*	*0.7*	52 N, 70 N, 57 N
		625	*76.7*	*6.8*	*1.0*	79 N, 82 N, 69 N
		313	*81.3*	*10.1*	*1.0*	76 N, 93 N, 75 N
		156	*87.0*	*16.5*	*1.1*	76 N, 79 N, 106 N
		78.0	*82.0*	*10.8*	*1.0*	85 N, 91 N, 70 N
	Dimethyl Sulfoxide		*80.7*	*4.5*		81 N, 85 N, 76 N
TA1535	**PF-04971729**	5000	*0.3*	*0.6*	*0.0*	0 M R, 1 M R, 0 M R
		2500	*0.7*	*0.6*	*0.1*	1 M R, 0 M R, 1 M R
		1250	*8.0*	*2.6*	*0.7*	11 M N, 7 N, 6 N
		625	*12.0*	*4.0*	*1.0*	8 N, 16 N, 12 N
		313	*10.7*	*0.6*	*0.9*	11 N, 10 N, 11 N
		156	*9.0*	*1.7*	*0.8*	8 N, 8 N, 11 N
		78.0	*11.7*	*4.2*	*1.0*	15 N, 7 N, 13 N
	Dimethyl Sulfoxide		*11.7*	*1.5*		12 N, 10 N, 13 N
TA1537	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 M R, 0 M R, 0 M R
		2500	*0.3*	*0.6*	*0.1*	0 M R, 1 M R, 0 M R
		1250	*5.3*	*1.2*	*1.0*	6 R, 4 R, 6 R
		625	*7.3*	*3.2*	*1.4*	5 N, 6 N, 11 N
		313	*4.0*	*1.7*	*0.8*	5 N, 5 N, 2 N
		156	*5.7*	*1.2*	*1.1*	5 N, 5 N, 7 N
		78.0	*6.0*	*1.0*	*1.1*	6 N, 5 N, 7 N
	Dimethyl Sulfoxide		*5.3*	*2.1*		6 N, 7 N, 3 M N

Key to Plate Postfix Codes
M Plate counted manually
R Reduced background bacterial lawn
N Normal background bacterial lawn

Initial Mutagenicity Assay Results with S9

Study No: 8202969
Trial No.: 8202969-B1 Date Plated: 6/23/2009
Plating Method: Plate incorporation assay Date Counted: 6/25/2009 to 6/26/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2uvrA(pKM101)	**PF-04971729**	5000	*203.0*	*15.7*	*1.0*	196 N, 192 N, 221 N
		2500	*222.0*	*15.9*	*1.1*	210 N, 216 N, 240 N
		1250	*213.7*	*17.6*	*1.0*	195 N, 216 N, 230 N
		625	*237.3*	*11.9*	*1.1*	241 N, 224 N, 247 N
		313	*221.0*	*20.5*	*1.1*	222 N, 241 N, 200 N
		156	*192.0*	*11.1*	*0.9*	182 N, 190 N, 204 N
	Dimethyl Sulfoxide		*209.3*	*16.8*		190 N, 218 N, 220 N
TA98	**BP**	2.5	*421.3*	*6.1*	*19.2*	428 N, 420 N, 416 N
TA100	**2AA**	2.5	*1277.7*	*92.1*	*11.7*	1378 N, 1258 N, 1197 N
TA1535	**2AA**	2.5	*188.0*	*65.2*	*16.6*	262 N, 163 N, 139 N
TA1537	**2AA**	2.5	*102.3*	*10.1*	*17.1*	96 N, 114 N, 97 N
WP2uvrA(pKM101)	**2AA**	5.0	*1313.7*	*68.9*	*6.3*	1235 N, 1363 N, 1343 N

Key to Positive Controls
BP Benzo [a] pyrene
2AA 2-aminoanthracene

Key to Plate Postfix Codes
R Reduced background bacterial lawn
M Plate counted manually
N Normal background bacterial lawn

Initial Mutagenicity Assay Results without S9

Study No: 8202969
Trial No.: 8202969-B1 Date Plated: 6/23/2009
Plating Method: Plate incorporation assay Date Counted: 6/25/2009 to 6/26/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2uvrA(pKM101)	**PF-04971729**	5000	*103.7*	*17.6*	*0.6*	102 N, 122 N, 87 N
		2500	*155.0*	*25.5*	*0.9*	154 N, 181 N, 130 N
		1250	*177.3*	*5.9*	*1.0*	184 N, 175 N, 173 N
		625	*189.3*	*18.4*	*1.1*	205 N, 194 N, 169 N
		313	*187.3*	*21.4*	*1.1*	212 N, 175 N, 175 N
		156	*177.3*	*12.7*	*1.0*	169 N, 192 N, 171 N
	Dimethyl Sulfoxide		*174.0*	*21.9*		198 N, 169 N, 155 N
TA98	**2NF**	1.0	*206.0*	*21.0*	*13.4*	183 N, 224 N, 211 N
TA100	**SA**	2.0	*1213.3*	*29.8*	*15.0*	1229 N, 1232 N, 1179 N
TA1535	**SA**	2.0	*818.7*	*93.9*	*70.2*	923 N, 741 N, 792 N
TA1537	**ICR-191**	2.0	*376.3*	*24.7*	*70.6*	383 N, 397 N, 349 N
WP2uvrA(pKM101)	**4NQO**	2.0	*2524.3*	*263.6*	*14.5*	2391 N, 2828 N, 2354 N

Key to Positive Controls
2NF 2-nitrofluorene
SA Sodium azide
ICR-191 ICR-191
4NQO 4-nitroquinoline-N-oxide

Key to Plate Postfix Codes
M Plate counted manually
R Reduced background bacterial lawn
N Normal background bacterial lawn

Confirmatory Assay: PF-04971729 was re-evaluated in the confirmatory assay in tester strains TA98, TA100, TA1535, and TA1537 at doses of 78.0, 156, 313, 625, 1250, 2500, and 5000 µg/plate and in tester strain WP2*uvr*A(pKM101) at doses of 313, 625, 1250, 2500, and 5000 µg/plate with and without S9 and similar results were observed (Trial 8202969-C1, see figures below).

Inhibited growth was observed in tester strain TA1537 without S9 at doses ≥1250 µg/plate, in tester strain TA1537 with S9 at doses ≥2500 µg/plate, and in tester strains TA98, TA100, and TA1535 with and without S9 at doses ≥2500 µg/plate.

There was no evidence of significant dose-related increase in the mean number of revertants with any of the strains tested in either the presence or absence of S9. All data were acceptable and no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix. All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

Confirmatory Mutagenicity Assay Results with S9

Study No: 8202969
Trial No.: 8202969-C1 Date Plated: 7/10/2009
Plating Method: Plate incorporation assay Date Counted: 7/13/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**PF-04971729**	5000	*1.3*	*0.6*	*0.1*	2 M R, 1 M R, 1 M R
		2500	*15.3*	*4.7*	*0.9*	10 R, 19 R, 17 R
		1250	*24.0*	*7.8*	*1.4*	28 N, 29 N, 15 N
		625	*20.0*	*3.6*	*1.1*	19 N, 17 N, 24 N
		313	*20.0*	*2.6*	*1.1*	22 N, 21 N, 17 N
		156	*19.7*	*6.8*	*1.1*	25 N, 12 N, 22 N
		78.0	*17.7*	*3.1*	*1.0*	17 N, 15 N, 21 N
	Dimethyl Sulfoxide		*17.7*	*3.1*		21 N, 17 N, 15 N
TA100	**PF-04971729**	5000	*0.3*	*0.6*	*0.0*	1 R M, 0 R M, 0 R M
		2500	*36.7*	*9.6*	*0.4*	28 R, 35 R, 47 R
		1250	*80.0*	*12.0*	*0.8*	92 N, 68 N, 80 N
		625	*98.3*	*10.0*	*1.0*	106 N, 102 N, 87 N
		313	*103.3*	*9.8*	*1.0*	109 N, 92 N, 109 N
		156	*93.7*	*7.1*	*0.9*	95 N, 100 N, 86 N
		78.0	*105.7*	*13.3*	*1.0*	109 N, 91 N, 117 N
	Dimethyl Sulfoxide		*102.3*	*16.1*		84 N, 114 N, 109 N
TA1535	**PF-04971729**	5000	*1.3*	*1.5*	*0.1*	0 M R, 3 M R, 1 M R
		2500	*2.7*	*1.2*	*0.2*	4 M R, 2 M R, 2 M R
		1250	*10.0*	*2.0*	*0.8*	8 N, 10 N, 12 N
		625	*12.0*	*5.0*	*0.9*	12 N, 7 N, 17 N
		313	*11.3*	*6.0*	*0.9*	12 N, 17 N, 5 N
		156	*13.0*	*0.0*	*1.0*	13 N, 13 N, 13 N
		78.0	*13.0*	*1.7*	*1.0*	12 N, 12 N, 15 N
	Dimethyl Sulfoxide		*12.7*	*2.5*		15 N, 13 N, 10 N
TA1537	**PF-04971729**	5000	*0.3*	*0.6*	*0.0*	0 R M, 0 R M, 1 R M
		2500	*4.0*	*1.0*	*0.5*	4 R M, 3 R M, 5 R M
		1250	*5.7*	*4.7*	*0.7*	2 N, 4 N, 11 N
		625	*9.7*	*2.5*	*1.1*	12 N, 7 N, 10 N
		313	*8.0*	*4.4*	*0.9*	13 N, 6 N, 5 N
		156	*8.3*	*3.8*	*1.0*	4 N, 10 N, 11 N
		78.0	*8.3*	*2.5*	*1.0*	8 N, 6 N, 11 N
	Dimethyl Sulfoxide		*8.7*	*4.0*		5 N, 8 N, 13 N

Key to Plate Postfix Codes

R	Reduced background bacterial lawn
M	Plate counted manually
N	Normal background bacterial lawn

Confirmatory Mutagenicity Assay Results without S9

Study No: 8202969
Trial No.: 8202969-C1 Date Plated: 7/10/2009
Plating Method: Plate incorporation assay Date Counted: 7/13/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	**PF-04971729**	5000	*0.7*	*1.2*	*0.1*	0 M R, 2 M R, 0 M R
		2500	*9.3*	*3.2*	*0.9*	7 R, 8 R, 13 R
		1250	*10.0*	*3.0*	*1.0*	13 N, 10 N, 7 N
		625	*9.7*	*5.5*	*1.0*	15 N, 10 N, 4 N
		313	*10.0*	*2.0*	*1.0*	8 N, 10 N, 12 N
		156	*12.3*	*4.5*	*1.2*	17 N, 8 N, 12 N
		78.0	*14.0*	*4.6*	*1.4*	10 N, 19 N, 13 N
	Dimethyl Sulfoxide		*10.0*	*2.0*		12 N, 10 N, 8 N
TA100	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 R M, 0 R M, 0 R M
		2500	*8.7*	*5.5*	*0.1*	15 R M, 6 R M, 5 R M
		1250	*61.3*	*12.5*	*0.8*	70 N, 47 N, 67 N
		625	*83.7*	*6.0*	*1.1*	83 N, 90 N, 78 N
		313	*79.0*	*13.5*	*1.0*	90 N, 83 N, 64 N
		156	*76.3*	*15.0*	*1.0*	64 N, 72 N, 93 N
		78.0	*77.3*	*4.9*	*1.0*	74 N, 83 N, 75 N
	Dimethyl Sulfoxide		*75.7*	*2.9*		79 N, 74 N, 74 N
TA1535	**PF-04971729**	5000				A, A, A
		2500	*1.3*	*1.5*	*0.1*	1 R M, 3 R M, 0 R M
		1250	*8.0*	*2.0*	*0.6*	6 N, 10 N, 8 N
		625	*15.7*	*5.0*	*1.1*	11 N, 21 N, 15 N
		313	*13.7*	*2.1*	*1.0*	16 N, 12 N, 13 N
		156	*11.7*	*5.1*	*0.9*	13 N, 16 N, 6 N
		78.0	*11.7*	*1.5*	*0.9*	10 N, 12 N, 13 N
	Dimethyl Sulfoxide		*13.7*	*3.1*		13 N, 11 N, 17 N
TA1537	**PF-04971729**	5000	*0.0*	*0.0*	*0.0*	0 R M, 0 R M, 0 R M
		2500	*1.0*	*1.7*	*0.2*	0 R M, 0 R M, 3 R M
		1250	*7.0*	*1.0*	*1.4*	7 R, 8 R, 6 R
		625	*2.0*	*1.7*	*0.4*	4 N, 1 N, 1 N
		313	*3.0*	*2.6*	*0.6*	6 N, 2 N, 1 N
		156	*4.0*	*1.7*	*0.8*	5 N, 5 N, 2 N
		78.0	*5.3*	*3.1*	*1.1*	6 N, 2 N, 8 N
	Dimethyl Sulfoxide		*5.0*	*3.6*		8 N, 6 N, 1 N

Key to Plate Postfix Codes

R	Reduced background bacterial lawn
M	Plate counted manually
N	Normal background bacterial lawn
A	Absence of background bacterial lawn

Confirmatory Mutagenicity Assay Results with S9

Study No: 8202969
Trial No.: 8202969-C1 Date Plated: 7/10/2009
Plating Method: Plate incorporation assay Date Counted: 7/13/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2uvrA(pKM101)	**PF-04971729**	5000	*144.7*	*11.8*	*0.7*	151 N, 152 N, 131 N
		2500	*156.3*	*12.0*	*0.8*	144 N, 157 N, 168 N
		1250	*200.7*	*26.5*	*1.0*	209 N, 222 N, 171 N
		625	*186.0*	*15.5*	*1.0*	171 N, 185 N, 202 N
		313	*188.0*	*14.0*	*1.0*	172 N, 194 N, 198 N
	Dimethyl Sulfoxide		*193.0*	*14.1*		208 N, 191 N, 180 N
TA98	**BP**	2.5	*460.3*	*28.0*	*26.1*	450 N, 439 N, 492 N
TA100	**2AA**	2.5	*2287.0*	*61.7*	*22.3*	2351 N, 2282 N, 2228 N
TA1535	**2AA**	2.5	*257.0*	*3.6*	*20.3*	261 N, 254 N, 256 N
TA1537	**2AA**	2.5	*149.3*	*15.8*	*17.2*	163 N, 132 N, 153 N
WP2uvrA(pKM101)	**2AA**	5.0	*1326.3*	*29.0*	*6.9*	1358 N, 1301 N, 1320 N

Key to Positive Controls

BP	Benzo[a]pyrene
2AA	2-aminoanthracene

Key to Plate Postfix Codes

R	Reduced background bacterial lawn
M	Plate counted manually
N	Normal background bacterial lawn

Confirmatory Mutagenicity Assay Results without S9

Study No: 8202969
Trial No.: 8202969-C1 Date Plated: 7/10/2009
Plating Method: Plate incorporation assay Date Counted: 7/13/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2uvrA(pKM101)	**PF-04971729**	5000	*122.3*	*16.2*	*0.7*	113 N, 113 N, 141 N
		2500	*152.3*	*15.0*	*0.9*	161 N, 135 N, 161 N
		1250	*150.0*	*28.2*	*0.9*	180 N, 146 N, 124 N
		625	*150.3*	*8.3*	*0.9*	153 N, 141 N, 157 N
		313	*179.0*	*10.1*	*1.0*	181 N, 188 N, 168 N
	Dimethyl Sulfoxide		*172.7*	*11.4*		176 N, 182 N, 160 N
TA98	**2NF**	1.0	*276.7*	*67.7*	*27.7*	308 N, 199 N, 323 N
TA100	**SA**	2.0	*988.0*	*9.5*	*13.1*	977 N, 994 N, 993 N
TA1535	**SA**	2.0	*894.0*	*13.7*	*65.4*	897 N, 879 N, 906 N
TA1537	**ICR**	2.0	*438.0*	*53.1*	*87.6*	495 N, 390 N, 429 N
WP2uvrA(pKM101)	**4NQO**	2.0	*1810.3*	*235.1*	*10.5*	1617 N, 1742 N, 2072 N

Key to Positive Controls

2NF	2-nitrofluorene
SA	Sodium azide
ICR	ICR-191
4NQO	4-nitroquinoline-N-oxide

Key to Plate Postfix Codes

R	Reduced background bacterial lawn
M	Plate counted manually
N	Normal background bacterial lawn
A	Absence of background bacterial lawn

GENETIC TOXICOLOGY HUMAN LYMPHOCYTE ASSAY OF PF-04971729 (09GR182)

Key findings: PF-04971729 did not induce structural chromosome aberrations in human lymphocyte in vitro cultures, under these test conditions, when tested up to concentrations that produce ~50% mitotic suppression (46-58% mitotic expression).

Increases in polyploidy were seen in the 3 hr test with S9 at 173 µg/mL and 156 µg/mL. These doses demonstrated marked mitotic suppression (55% and 43%, respectively). There were no increases in polyploidy or hyperploidy in the 24-hour test, although this test was performed in the absence of metabolic activation (without S9).

Study:	09GR182
Volume and page:	EDR 4.2.3.3.1
Conducting laboratory and location:	Pfizer Global Research, Groton, CT, USA *(Slide Analysis)* (b) (4)
Date of study initiation:	11 May 2009
GLP compliance:	Yes
QA reports:	Yes
Drug, lot #, and % purity:	PF-04971729, 00701380-70-1 (Preliminary) PF-04971729, GRO 2546 (Definitive Tests) 99.76%

Methods

Strains/species/cell line: Purified human peripheral lymphocytes were obtained from whole venous blood collected from healthy donors.

Doses used in definitive study:
3 hrs with S9: 102- 400 µg/mL
3 hrs without S9: 102-400 µg/mL
24 hrs without S9: 38.4-213 µg/mL

Basis of dose selection: Dose selection was based on preliminary cytotoxicity tests. In the 3-hour test without metabolic activation, cultures treated with ≥375 µg/mL were toxic and the next lower concentration (188µg/mL) produced (11%) mitotic suppression. In the 3-hour test with metabolic activation, cultures treated with (188 µg/mL) produced 50% mitotic suppression and the next lower concentration (94.0µg/mL) produced only 1% mitotic suppression. In the 24-hour test without metabolic activation, cultures treated with 94.0 µg/mL produced 62% mitotic suppression and the next lower concentration of 47.0 µg/mL produced minimal (8%) mitotic suppression. There was no evidence of test article insolubility in any of the 100x stock solutions prepared in DMSO. There was evidence of compound insolubility in cultures treated with PF-04971729 at concentrations ≥1500 µg/mL in all tests (see table below).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: FLOW CYTOMETRIC ANALYSIS
Preliminary Cytotoxicity Test PF-04971729 Test 1,2,3

Treatment	3 HOUR TREATMENT				24 HOUR TREATMENT	
	Without Activation		With Activation		Without Activation	
	(%) Mitotic Index[a]	Mean (%) Mitotic Suppression[b]	(%) Mitotic Index[a]	Mean (%) Mitotic Suppression[b]	(%) Mitotic Index[a]	Mean (%) Mitotic Suppression[b]
Negative Control: DMSO						
1.0%	12.16	0	11.08	0	9.59	0
Test Article: PF-04971729 (µg/ml)						
5.90	12.28	0	13.05	0	9.61	0
11.8	12.55	0	11.49	0	9.04	6
23.5	11.22	8	11.69	0	9.35	3
47.0	11.38	6	11.95	0	8.78	8
94.0	11.23	8	10.93	1	3.65	62
188	10.88	11	5.53	50	0.60 ^	94
375	T	--	T	--	T	--
750	T	--	T	--	T	--
1500+	T	--	T	--	T	--
3000+	T	--	T	--	T	--

[a] (%) Mitotic Index (MI) is calculated by dividing the R2 (mitotic) events by the R1 (total events) then multiplying by 100 to express as a percent.

[b]Mean (%) Mitotic Suppression =
(One minus the quotient [Mean Test Article Mitotic Index/Mean Negative Control Mitotic Index]) (x) 100.

Abbreviations:
DMSO = Dimethylsulfoxide.
-- = Dashes indicate data not available or determined.
^ = Acquisition of mitotic events was limited due to low numbers of mitotic cells.
T = Toxic; sample was not acquired and/or value was not calculated due to toxicity.
+ = Precipitate observed in cultures.

Negative controls: DMSO

Positive controls: Mitomycin C was used in the absence of S9 at 0.4 and 0.05 µg/mL for the 3 and 24h treatments, respectively. Cyclophosphamide (10 µg/mL) was used for the 3h treatment in the presence of metabolic activation.

Incubation and sampling times: The sponsor performed 3h incubations with or without metabolic activation followed by 21-hour in fresh media. 24-hr incubations were performed in the absence of S9 and treated continuously. Colcemid solution was added 3h before cell harvest (0.075 µg/ml). Chromosome specimens were prepared and examined under microscope for structural aberrations and polyploid cells. Approximately 2 mLs of cell suspension was removed from each culture and harvested for the flow cytometric mitotic index assessment.

Results:

Study validity: The criteria for a valid test were stated in the report and met by the study results.

Dose selection: 3 hr test (No S9): There test concentrations (173, 192, and 237 µg/mL), producing 11 to 46% mitotic suppression, were selected to evaluate chromosome damage (see table below).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: Cytotoxicity by Flow Cytometry
3-Hour Treatment Without Metabolic Activation PF-04971729 Test 7

Treatment	Mean (%) Mitotic Index	Mean (%) Mitotic Suppression	Mean (%) Polyploidy
Negative Control: DMSO 1%	17.72*	0	0.00*
Dose (µg/ml) PF-04971729			
102	16.12	9	0.00
113	15.47	13	0.00
126	15.96	10	0.05
140	16.35	8	0.05
156	13.94	21	0.00
173	13.67	23	0.00
192	15.80	11	0.10
213	9.94	44	0.10
237	9.59	46	0.30
263	4.84	73	--
292	T	--	--
324	T	--	--
360	T	--	--
400	T	--	--
Positive Control: Mitomycin-C 0.40 µg/ml	6.59	63	--

DMSO = Dimethylsulfoxide.
* = Information based on one culture.
-- = Dashes indicate data not available or determined.
T = Toxic; sample was not acquired and/or value was not calculated due to toxicity.

There was no significant increase in the number of abnormal cells (see table below) compared to the concurrent negative control (DMSO) at any of the concentrations evaluated. There were no dose-related increases in polyploidy observed in the PF-04971729 treated cultures and the frequencies were within the expected historical control values (see table above).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: SUMMARY OF TEST
3-HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment[1]	Note	Total Cells Analyzed	Mean (%) Abnormal Cells[2]	Mean p-Value[3]
Negative Control				
Negative Control: DMSO 1.0%	--	200	0.0	--
Test Article: PF-04971729 (µg/ml)				
173	--	200	0.0	>0.500
192	--	200	0.0	>0.500
237	--	200	0.5	0.500
Positive Control: Mitomycin-C 0.40 µg/ml	*	100	23.0	<0.001

[1] Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage, unless ≥10 aberrations were noted in 50 cells per culture.
[2] Mean (%) Abnormal Cells = Sum of (%) Abnormal Cells for duplicate cultures divided by 2.
[3] Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.

Abbreviations:
-- = Dashes indicate data not available or determined.
* = Some cells contain more than one aberration.

Day 0 = 17 JUN 09 Test 7 09GR182

Dose selection: 3 hr test (+ S9): There test concentrations (102, 140, and 173 µg/mL), producing 14 to 55% mitotic suppression, were selected to evaluate chromosome damage (see table below).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: Cytotoxicity by Flow Cytometry
3-Hour Treatment With Metabolic Activation PF-04971729 Test 8

Treatment	Mean (%) Mitotic Index	Mean (%) Mitotic Suppression	Mean (%) Polyploidy
Negative Control:			
DMSO 1.0 %	8.51	0	0.20
Dose: (µg/ml) PF-04971729 (µg/ml)			
102	7.32	14	0.45
113	6.84	20	0.25
126	6.77	20	0.10
140	5.94	30	0.45
156	4.81	43	0.75
173	3.85	55	1.50
192	3.81	55	--
213	1.20	86	--
237	T	--	--
263	T	--	--
292	T	--	--
324	T	--	--
360	T	--	--
400	T	--	--
Positive Control: Cyclophosphamide			
10 µg/ml	4.17	51	--

DMSO = Dimethylsulfoxide.
-- = Dashes indicate data not available or determined.
T = Toxic; sample was not acquired and/or value was not calculated due to toxicity.

There was no significant increase in the number of abnormal cells compared to the concurrent negative control (DMSO) at any of the concentrations evaluated (see table below). There was an increase in polyploidy observed in the PF-04971729 treated cultures (see table above) at the highest dose analyzed for chromosome aberrations (1.50% mean polyploidy frequency at 173 µg/mL) and at 156 µg/mL (0.75% mean polyploidy frequency), which was not analyzed for chromosome aberrations (see below).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: SUMMARY OF TEST
3-HOUR TREATMENT
WITH METABOLIC ACTIVATION

Treatment[1]	Note	Total Cells Analyzed	Mean (%) Abnormal Cells[2]	Mean p-Value[3]
Negative Control: DMSO 1.0%	--	200	0.0	--
Test Article: PF-04971729 (µg/ml)				
102	--	200	0.5	0.500
140	--	200	0.0	>0.500
173	--	200	0.0	>0.500
Positive Control: Cyclophosphamide 10.0 µg/ml	*	100	37.0	<0.001

[1] Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage, unless ≥10 aberrations were noted in 50 cells per culture.
[2] Mean (%) Abnormal Cells = Sum of (%) Abnormal Cells for duplicate cultures divided by 2.
[3] Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.

Abbreviations:
-- = Dashes indicate data not available or determined.
* = Some cells contain more than one aberration.

Day 0 = 17 JUN 09 Test 8 09GR182

Dose selection: 24 hr test (No S9): There test concentrations (38.4, 53.2, and 73.7 µg/mL), producing 12 to 58% mitotic suppression, were selected to evaluate chromosome damage (see table below).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: Cytotoxicity by Flow Cytometry
24-Hour Treatment Without Metabolic Activation PF-04971729 Test 9

Treatment	Mean (%) Mitotic Index	Mean (%) Mitotic Suppression	Mean (%) Polyploidy	Mean (%) Hyperdiploidy
Negative Control:				
DMSO 1.0 %	9.06	0	0.08	0.63
Dose: (µg/ml) PF-04971729				
(µg/ml)				
38.4	7.94	12	0.18	0.93
45.2	6.98	23	0.10	0.88
53.2	6.00	34	0.13	0.53
62.6	5.56	39	0.08	1.00
73.7	3.77	58	0.35	0.63
86.7	2.12	77	--	--
102	1.87	79	--	--
113	1.50	83	--	--
126	1.32	85	--	--
140	1.05	88	--	--
156	T	--	--	--
173	T	--	--	--
192	T	--	--	--
213	T	--	--	--
Noscapine HCl Control:				
25.0 µg/ml	7.47	18	0.43	6.98
Positive Control: Mitomycin-C				
0.05 µg/ml	6.28	31	--	--

DMSO = Dimethylsulfoxide.
-- = Dashes indicate data not available or determined.
T = Toxic; sample was not acquired and/or value was not calculated due to toxicity.

There was no significant increase in the number of abnormal cells compared to the concurrent negative control (DMSO) at any of the concentrations evaluated (see table below). There were no dose-related increases in numerical chromosome changes (polyploidy and hyperdiploidy) observed in the PF-04971729 treated cultures and the frequencies were within the expected historical control values (see table above).

HUMAN LYMPHOCYTE CYTOGENETICS ASSAY: SUMMARY OF TEST
24-HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment[1]	Note	Total Cells Analyzed	Mean (%) Abnormal Cells[2]	Mean p-Value[3]
Negative Control: DMSO 1.0%	--	200	0.0	--
Test Article: PF-04971729 (µg/ml)				
38.4	--	200	0.0	>0.500
53.2	--	200	0.5	0.500
73.7	--	200	0.0	>0.500
Positive Control: Mitomycin-C 0.05 µg/ml	*	200	8.0	<0.001

[1] Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage, unless ≥10 aberrations were noted in 50 cells per culture.
[2] Mean (%) Abnormal Cells = Sum of (%) Abnormal Cells for duplicate cultures divided by 2.
[3] Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.

Abbreviations:
-- = Dashes indicate data not available or determined.
* = Some cells contain more than one aberration.

Day 0 = 17 JUN 09 Test 9 09GR182

2.6.7 TOXICOLOGY TABULATED SUMMARY

Sponsor's Overview of Toxicology Program

Study[a]	Study Number	Concentration or Dose	Tabulated Summary
Single-Dose Toxicity			
Dose Escalation Dog	08GR497	5, 50, 500 mg/kg	2.6.7.5
Repeat-Dose Toxicity			
Nonpivotal Studies			
7-Day Rat + Micronucleus	08GR396	5, 50, 500 mg/kg	2.6.7.6
7-Day Dog	09GR008	5, 50, 250→150 mg/kg[b]	2.6.7.6
7-Day Dog Bridging	09GR105	5, 50, 150 mg/kg	2.6.7.6
Pivotal Studies			
4-Week Rat + Micronucleus	09GR185	5, 25, 500→250 mg/kg[c]	2.6.7.7A
4-Week Dog	09GR184	1, 10, 150 mg/kg	2.6.7.7B
Genotoxicity			
In Vitro Studies			
Bacterial Mutation (AMES)	09GR181	Up to 5000 µg/plate	2.6.7.8A
In vitro cytogenetics	09GR182		2.6.7.8B
In Vivo Studies			
Micronucleus	09GR185	5, 25, 500→250 mg/kg[c]	2.6.7.9
Carcinogenicity			
Not conducted			
Reproductive and Developmental Toxicity			
Not Conducted			
Local Tolerance			
Not conducted			
Other Toxicity Studies			
Not conducted			

[a] All in vivo studies were conducted with males and female animals, except Study 08GR396 (males only).
[b] Dose reduction 250→150 mg/kg on Day 3 due to clinical signs.
[c] Dose reduction 500→250 mg/kg on Day 11 due to unscheduled deaths.

SPECIES TOXICOLOGY STUDIES			
SPECIES/ STUDY	**NOAEL**	**MULTIPLE OF MRHD (µg.hr/mL)**	**BASIS**
Rat 1 Week (non-GLP) 5, 50, 500 mg/kg M:17, 99, 1500 µg.hr/mL	50 mg/kg (99 µg.hr/mL)	2X	500 mg/kg: Loose Stool ↓BW ↓WBCs Histological changes Pancreas and Liver
4 Week (GLP) 5, 25, 500→(D11) 250 mg/kg M: 8, 69, 541 µg.hr/mL F: 15, 93, 718 µg.hr/mL	25 mg/kg (81 µg.hr/mL) (M/F average)	1X	500→ (D11) 250 mg/kg: Mortality, ↑ severity CPN, stomach erosion/squamous hyperplasia.
Dog Single Dose (non-GLP) 5, 50, 500 mg/kg M: 27, 386, 138 µg.hr/mL F: 51, 474, 465 µg.hr/mL	M:LOAEL 500 mg/kg F:50 mg/kg (474 µg.hr/mL)	9X	500 mg/kg: Vomiting M/F 50 mg/kg: Salivation F
1 Week (non-GLP) 5, 50, 250→(D3) 150 mg/kg M: 55, 373, 1150 µg.hr/mL F: 63, 627, 789 µg.hr/mL Amorphous Drug Form	50 mg/kg (500 µg.hr/mL) (M/F average)	10X	250→(D3) 150 mg/kg: Vomiting and soft mucoid and watery feces
1 Week (non-GLP) 5, 50, 150 mg/kg M: 77, 660, 679 µg.hr/mL F: 59, 834, 511 µg.hr/mL Co-crystalline Drug Form	50 mg/kg (750 µg.hr/mL) (M/F average)	15X	150 mg/kg: Vomiting
4 Week (GLP) 1, 10, 150 mg/kg M: 7, 77, 1050 µg.hr/mL F: 8, 71, 1170 µg.hr/mL	1 mg/kg (8 µg.hr/mL) (M/F average)	< 1X	≥10 mg/kg: Gallbladder Vacuolation. 150 mg/kg: Renal tubular Degeneration, emesis, salivation, soft/watery feces.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Pharmacology: PF-04971729 is a selective SGLT2 inhibitor. Inhibitors of SGLT2 block the transport of glucose from the pro-urine across the apical membrane of the proximal epithelial cells, thereby resulting in significant glucosuria.

PF-04971729 was identified to be a highly potent inhibitor of human SGLT2 mediated glucose transport with an IC_{50} of 0.877 ± 0.369 nM (n = 10). PF-04971729 showed very little inhibition of human SGLT1 mediated transport with an IC_{50} of 1960 ± 642 nM (n = 8). Similar potency and selectivity were seen in the rat SGLT assays (rat SGLT2: IC_{50} = 1.15 ± 0.289 nM, rat SGLT1: IC_{50} = 352 ± 54.2 nM, (n = 4)

At physiological glucose concentrations, PF-04971729 maintained a high potency for inhibiting AMG transport in SGLT2 expressing cells with IC_{50} values of 1.20, 1.31, and 3.18 nM at AMG concentrations of 0.2, 2, and 20 mM respectively. The inhibition by PF-04971729 is competitive with glucose with an equilibrium dissociation constant (Ki) value of 1.42 ± 0.11 nM for human SGLT2 as determined by enzyme kinetics model comparison.

Doses of 0.1, 1, 3, 10, 30 and 60 mg/kg PF-04971729 resulted in urinary glucose excretion of 389.0 ± 62.54, 1519 ± 52.02, 1937 ± 101.1, 2145 ± 132.3, 2554 ± 141.1, and 2437 ± 116.7 mg/ 200 g of body weight/ 24 h, respectively. PF-04971729 at a concentration of 1 µg/mL (equivalent to 2.3 µM) is highly bound (96%) to plasma proteins in rat with a free fraction (fu) of 0.040. Thus, the free AUC_{0-24} following doses of 0.1, 1, 3, 10, 30, and 60 mg/kg PF-04971729 are 0.00752, 0.0800, 0.283, 1.06, 4.28, 9.32 µg.h/mL, respectively.

Safety Pharmacology: In the neurological assessment, rats dosed with 500 mg/kg of PF-04971729-GE had a 0.4°C decrease in average body temperature. At this same dose, PF-04971729 produced decreases in locomotor activity measurements (~ 30-40%). PF-04971729 inhibited the hERG channel in vitro with an IC_{50} of >300 µM. Significant inhibition of the hERG current was seen at 100 µM PF-04971729. While the (average % inhibition) was 8.3% at this dose (below the 10% cut-off of "biologically relevance") the 6 individual data points used to calculate this mean had a broad range of values (2.9% - 18.1%). However, the modest effect on hERG current at relatively high drug concentrations suggests a minimal risk of QT prolongation in vivo. Treatment with PF-04971729 (b) (4) at 50 mg/kg in Beagle dogs produced a moderate decrease in the QTc interval, cardiac contractility, and heart rate (and associated RR interval shortening) as well as an increase in systolic blood pressure and lengthening of the PR interval, yielding a NOAEL of 5 mg/kg. Plasma drug concentration peaked at 43µg/mL approximately 4 hours after the 50mg/kg dose (LOAEL). Assuming linearity, the next lower dose of 5mg/kg resulted in ~4.3µg/mL. By comparison, the proposed clinical study is targeting a maximum drug level of 1.4 to 6.9µg/mL in human subjects. Therefore these cardiovascular findings have limited relevance to the clinical study. Regardless these parameters should be monitored in the clinic.

PF-04971729 at 25 and 500 mg/kg produced significant increases in both respiratory rate and minute volume at the 101-120 minute interval. The 25 mg/kg dose, produced increases in respiratory rate of 33 b.p.m. (29% increase over control), and minute volume of 36 mL/min (25% increase over control). At the 500 mg/kg dose, a respiratory rate of 45 b.p.m. (40% increased over control) and a minute volume of 33 mL/min (23% increased over control) were seen. No renal safety studies were performed although PF-04971729 causes increase urinary glucose excretion in rats and dogs. In addition, no GI safety studies were performed although PF-04971729 causes changes in stool quality, vomiting and ulceration of the tongue.

Pharmacokinetics: Nonclinical in vitro and in vivo data indicate PF-04971729 is well-absorbed from the gastrointestinal tract and oral bioavailability of the amorphous form following a 2 mg/kg dose in rats and dogs is 67% and 97%, respectively. Oral bioavailability of the co-crystal form following a 5 mg/kg dose in rats or 2 mg/kg dose in dogs was similar at 69% and 94%, respectively. Therefore, the toxicological profiles of the amorphous and co-crystal forms are reasonably expected to be identical. The pharmacokinetics of PF-04971729, following intravenous (IV) administration to rats and dogs, is characterized by low clearance (rat: 4.04 mL/min/kg; dog: 1.64 mL/min/kg), a moderate volume of distribution at steady state (rat: 1.13 L/kg; dog: 0.828 L/kg) and a moderate-to-long half-life (rat: 4.08 hours; dog: 7.63 hours). PF-04971729, at concentrations of 1 and 10µg/mL, is highly bound to rat, dog, and human plasma proteins, with mean unbound fractions (fu) ranging from 0.032 to 0.064, and binding appeared to be independent of concentration. The free fraction was slightly higher in human compared to the nonclinical species tested. However, given the minimal variability of protein binding between species (94%-97%), all safety margin calculations will be based on total drug exposure, not on the fraction of free drug. In vitro metabolite profiles of PF-04971729 from incubations with liver microsomes and hepatocytes were qualitatively similar across nonclinical species and humans and no unique human metabolites are anticipated by the sponsor. Metabolism of PF-04971729 may be catalyzed by multiple enzymes, including CYP3A4, CYP3A5, CYP2D6, UGT1A9, and UGT2B7. At the predicted human efficacious dose, clinically significant drug-drug interactions resulting from PF-04971729-mediated CYP450 inhibition or induction are not anticipated by the sponsor. The IC_{50} obtained in rats (0.022 µg/mL) was scaled by the sponsor to a human IC_{50} (0.011 µg/mL) by accounting for species differences in SGLT2 potency reflected in a CHO cell assay and in unbound plasma fraction. The projected CL, Vss, t½, absorption rate, and bioavailability in humans based on single species allometric scaling of rat PK data were calculated to be 1.7 mL/min/kg, 1.8 L/kg, 12 hours, 1.9 h^{-1} and 65%, respectively. Based on the PK/PD and predicted human pharmacokinetics of PF-04971729, a total of 75 grams of urinary glucose excretion (UGE) over 24 hours can be achieved either through a single dose of 17 mg, or 13 mg QD at steady state. At this predicted efficacious dose, the projected total AUC_{0-24} and C_{max} are 1.13 µg.h/mL (0.072 µg.h/mL free) and 0.078 µg/mL (0.005 µg/mL free; 0.0114 µM free), respectively. The accuracy of this prediction will be tested in clinical studies.

General toxicology: PF-04971729 was administered orally to rats and dogs in 1 month (GLP) studies. The no observed adverse effect levels (NOAELs) in these studies were 25 mg/kg (1X MRHD) and 1 mg/kg (<1X MRHD) with an AUC_{0-24} of 81 µg.hr/mL and 8 µg.hr/mL for rats and dogs, respectively. The NOAEL in rats is based on morbidity and mortality at 500mg/kg and on increased severity of chronic progressive nephropathy and stomach erosion/squamous hyperplasia at 250mg/kg (14x MRHD). The NOAEL in dogs is based on uncharacterized vacuolation of the gall bladder at ≥ 10mg/kg (~2x MRHD). The toxicological relevance of the gall bladder finding in dogs is uncertain and will require clarification in longer term dog studies. The toxicity in dogs considered relevant for human risk consisted of very frequent soft and watery feces (i.e., GI intolerance) and a higher incidence of renal tubular degeneration/regeneration at 150mg/kg (21x MRHD). The NOAEL for these findings in dogs is 10mg/kg, or ~2x the clinical dose based on AUC exposure.

In rats, the pivotal one month study dosed female animals with 5, 25, and 500→(D11)250 mg/kg/day had average exposures of 16.2, 93.0 and 718 µg.h/mL (<1X, ~2X and ~14X MRHD, µg/h/mL basis) compared to males 5, 25, and 500→(D11)250 mg/kg/day had exposures of 8.4, 69.3 and 541 µg.h/mL (<1X, ~1X and ~11X MRHD, µg/h/mL basis). Several animals at the original HD of 500 mg/kg/day were euthanized in moribund condition or found dead within the first 3 weeks with clinical signs of distended abdomen, reduced activity, anogenital staining, fecal discoloration and/or softness and changes in hair quality. There were no significant clinical signs ≤ 25 mg/kg. Body weight decreased in all treatment groups despite a marked increase in food consumption, indicating that food intake and increased gluconeogenesis did not fully compensate for the caloric loss in the form of severe glucosuria. By the completion of the study, the glucosuria effectively decreased serum glucose 16.6% to 39.7% vs. control in all treated rats. Increased BUN levels were not accompanied elevated creatinine, and likely reflect increased catabolism of protein rather than renal dysfunction. Liver transaminases ALT and AST were modestly increased (<3 fold) in the absence of bilirubin elevation and adverse liver pathology. The sponsor suspects the increased transaminases are secondary to increased gluconeogenesis. Regardless of mechanism, liver transaminases will be monitored clinically. Kidney weight increased across the dose range as did dilatation of renal tubules, with the highest dose (250mg/kg, 14x) associated with an increased severity of chronic progressive nephropathy in females. Erosion and squamous hyperplasia of the stomach was also observed at 250mg/kg. Other changes reported in rats were reasonably considered secondary to severe glucosuria and osmotic diuresis (e.g., adrenal hypertrophy, pancreatic zymogen depletion, adipose atrophy).

In the pivotal dog study, animals were dosed with 1, 10, and 150 mg/kg/day had average exposures of 7.35, 74.4 and 1080 µg.h/mL (<1X, ~2X and ~21X MRHD, µg.h/mL basis). Significant clinical signs in all treated animals included soft feces that progressed to very frequent watery feces at the high dose. The relative GI intolerance at the high dose may reflect off-target inhibition of SGLT1, which is associated with duodenal absorption of glucose. Vomiting (white foamy or yellow) and/or salivation occurred in some HD animals. *Serum glucose levels did not decrease in male dogs and the reduction in females was extremely limited* despite substantial elimination of glucose in the urine (219x-644x).

Food consumption was reportedly not changed with treatment, suggesting that compensatory gluconeogenesis was sufficient to offset glucose loss in the urine. Compared to pre-study values, the treated animals either failed to gain weight (females) or lost some body weight (males). Presumably the effect on weight is secondary to increased energy consumption to compensate for the caloric loss via glucosuria. ECG analysis suggests a possible lengthening of the PR interval in HD males and reduced HR in HD females after dosing. Exposure at this dose corresponds to ~21x the MRHD and is therefore of limited importance to the clinical study. Monitoring of blood pressure, pulse rate, and ECG are part of the proposed clinical study protocol. The finding of increased gallbladder vacuolation at doses ≥ 10 mg/kg is of uncertain toxicological relevance and may or may not be related to drug treatment. If related to treatment, this finding would be considered adverse. The 3 month dog study will clarify the drug-relatedness of this finding, at which time the need for regulatory action will be assessed. Kidney epithelial degeneration was increased in HD treated animals (21x MRHD). Experience with other SGLT2 inhibitors suggests that this adverse renal finding will resolve and not progress with increased duration of treatment.

Genotoxicity: There were no PF-04971729 treatment related reductions in polychromatic erythrocytes (PCE) reported in rats, suggesting a low incidence of bone marrow toxicity. There were no significant increases in the numbers of PCE with micronuclei reported, suggesting that PF-04971729 does not possess the ability to induce chromosomal damage. Exposure to PF-04971729 was negative for genotoxicity in an Ames test using several tester strains either in the presence or absence of S9. PF-04971729 did not induce structural chromosome aberrations in human lymphocyte *in vitro* cultures.

PF-04971729 did increase polyploidy in a 3 hr test with metabolic activation (S9) at 173µg/mL and 156µg/mL. While no increases in polyploidy or hyperploidy were observed in a 24-hour test, this experiment was performed in the absence of metabolic activation (No S9).

Summary: Based on the MRHD being limited to exposure at the rat NOAEL and on the proposed clinical monitoring for cardiovascular, liver, and renal toxicities, the reviewer agrees that this study is reasonably safe to proceed.

Internal comments: The NOAELs considered relevant for the proposed clinical study provide ~1-2x safety margin to the stated maximum clinical exposure of 50.6 µg.h/mL AUC and 6.88 µg/mL C_{max}. The safety margin increases to 2-4x if the sponsor's predicted exposure at 300mg is accurate (C_{max} 1.39 mg/mL and an AUC of 27.2 µg.h/mL).

The NOAEL in rats (1x) is based on morbidity and mortality at 500mg/kg (est. 28x) and on increased severity of chronic progressive nephropathy and stomach erosion/squamous hyperplasia at 250mg/kg (14x MRHD). The NOAEL in dogs is based on very frequent soft and watery feces (i.e., GI intolerance) and on a higher incidence of renal tubular degeneration/regeneration at 150mg/kg (21x MRHD).

The cause of the adverse findings in rats is unclear, but the clinical dose is limited to exposure at the rat NOAEL and there is an adequate margin (11-14x) to the LOAEL.

The GI intolerance in dogs is arguably related to off-target inhibition of SGLT1. Similar though less severe GI intolerance has been observed in clinical studies with an SGLT1/2 mixed inhibitor (IND (b) (4)), and is easily monitored.

Other adverse findings in rats and dogs of potential relevance to the proposed clinical study include modest increases in liver transaminases ALT and AST and slight prolongation of PR interval. Clinical monitoring appears to be in place for both of these issues.

Based on the MRHD being limited to exposure at the rat NOAEL and on the proposed clinical monitoring for cardiovascular, liver, and renal toxicities, the reviewer agrees that this study is reasonably safe to proceed.

External comments (to sponsor):

1. The one month dog toxicology study reported an increased incidence of gall bladder vacuolation at 10 and 150mg/kg in males and females. A conclusive relationship to drug treatment is uncertain, but it also cannot be dismissed. If a similar increased incidence is observed in the 3 month dog study, we recommend that you provide a characterization of the vacuoles and how they compare to gall bladder vacuoles in the control animals (if present).

2. The tabulated histopathology table for the 1 month dog study apparently included female #22 in the main group (n=3 in high dose group). Because this female was sacrificed on day 3 of the study, histopathology data for this animal is best presented separately from the others that survived to or near to scheduled termination. We recommend that under similar circumstances, the tabulated histopathology table include columns for 'Died on Study' and 'Survived to Termination' for the dose groups affected by early deaths/euthanasia. This approach is also relevant for other pivotal toxicity endpoints in the study.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-106447	ORIG-1	PFIZER GLOBAL RESEARCH DEVELOPMENT	PF-04971729 ORAL

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

/s/

JEFFREY A QUINN
10/30/2009

TODD M BOURCIER
10/30/2009
I concur