CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204671Orig1s000

PHARMACOLOGY REVIEW(S)

Memo to File

Date: November 20, 2013
NDA#: 204,671
Applicant: Gilead Sciences, Inc.
Drug name: Sofosbuvir (GS-7977)
Reviewer: Christopher Ellis, Ph.D.

Summary: Subsequent to completion of the pharmacology/toxicology review for this NDA (signed off on September 6th), the applicant submitted additional data/information to the Application. The data/information provided was reviewed and no pharmacology/ toxicology issues were identified. Therefore, we reiterate our original conclusion that the nonclinical safety information supports marketing approval for sofosbuvir. However, given the longer treatment duration now recommended in the product label for some HCV infected populations (*e.g*. 24-weeks in genotype 3), we are requesting that the applicant submit final study reports for the 2 year carcinogenicity studies as a post-marketing requirement.

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/s/

CHRISTOPHER E ELLIS
11/20/2013

HANAN N GHANTOUS
11/20/2013

Memo to File

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NDA#: 204,671
Applicant: Gilead Sciences, Inc.
Drug name: Sofosbuvir (GS-7977)
Reviewer: Christopher Ellis, Ph.D.

Summary: This supporting document (SD#57), received on November 18th, provides Gilead's follow-up response to the Division's recommendation regarding a short duration rat study (see excerpt below). Gilead has agreed to conduct the study and plans on submitting the draft protocol to IND-106,739. Gilead's plan is acceptable.

Division's Recommendation (sent to applicant on September 12th):

"…We also recommend that you consider conducting a short duration rat toxicology study with sofosbuvir at dose levels up to 2000 mg/kg to determine its contribution to the heart degeneration and inflammation observed with GS-9851. This study could not only more clearly define drug-related exposure multiples (based on heart findings)but, if indeed contributing to heart toxicity, further characterize this toxicity by including treatment-free groups (to evaluate reversibility)and additional study endpoints (e.g., circulating biomarkers of cardiac toxicity, heart specific sofosbuvir and related metabolite concentrations)."

Gilead's Response:

As described in Gilead's previous response to this comment in Sequence No. 0028, dated 18 September 2013 (page 7), we have considered conducting a short duration rat study with sofosbuvir up to 2000 mg/kg/day and agree to conduct this study. We will submit the protocol describing study details to IND 106,739 under separate cover.

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/s/

CHRISTOPHER E ELLIS
11/20/2013

HANAN N GHANTOUS
11/20/2013

Comments on N204671 sofosbuvir

From A. Jacobs, AD

9/6/13

1. I concur that there are no approvability issues and the pregnancy labeling section

2. I concur that some follow-up studies on the cardiac effects in rats could be considered.

3. I have discussed some editorial comments with the reviewer, and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
09/06/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	204,671
Supporting document/s:	001
Applicant's letter date:	April 6, 2013
CDER stamp date:	April 8, 2013
Product:	Sofosbuvir (GS-7977)
Indication:	Treatment of chronic hepatitis C in combination with peginterferon alpha and ribavirin for 12 weeks (in treatment naive HCV genotype 1, 4, 5 or 6 adult patients) and in combination with ribavirin for 12 (genotype 2) or 16 (genotype 3) weeks
Applicant:	Gilead Sciences
Review Division:	Division of Antiviral Products
Reviewer:	Christopher Ellis, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, M.D.
Project Manager:	Linda Onaga, M.P.H.

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA #204,671 are owned by Gilead Sciences or are data for which Gilead Sciences has obtained a written right of reference.
Any information or data necessary for approval of NDA #204,671 that Gilead Sciences 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 #204,671.

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1 Executive Summary

1.1 Introduction

Sofosbuvir (SOF, GS-7977, PSI-7977) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted intracellularly to the active uridine triphosphate (GS-461203) within tissues. GS-461203 is a specific inhibitor of nonstructural protein 5B (NS5B) of hepatitis C virus (HCV) that has displayed potent inhibition of HCV replicon ribonucleic acid (RNA) replication *in vitro*. Sofosbuvir is one of two diastereoisomers in a mixture designated GS-9851, which was utilized in early nonclinical safety studies. The ratio of stereoisomers GS-7977 and GS-491241 in GS-9851 is approximately 50:50, with chemical interconversion of the two stereoisomers appearing unlikely. These stereoisomers are converted to either stereoisomeric metabolites, such as GS-566500 and GS-461203, or an identical metabolite (GS-331007), so that equimolar doses of the mixture (GS-9851) or the single isomer (GS-7977) yield similar metabolite exposures. This identical metabolite (GS-331007) is also the main circulating metabolite in both animals and humans, accounting for the majority of total drug-related exposure.

The nonclinical safety profile of sofosbuvir has been evaluated in: safety pharmacology studies in rats and dogs with GS-9851; single- and repeat-dose toxicology studies in mice, rats and dogs with GS-9851 and/or sofosbuvir alone for up to 3, 6 and 9 months duration, respectively; up to 1-month repeat-dose toxicology studies with sofosbuvir to qualify impurities; fertility and pre- and post-natal developmental studies in rats and embryo-fetal developmental studies in rats and rabbits with sofosbuvir; and genetic toxicology studies (Ames, *in vitro* chromosomal aberration and *in vivo* mouse micronucleus assays) with GS-9851. In addition, numerous *in vitro* and *in vivo* nonclinical pharmacokinetic studies, evaluating the absorption, distribution, metabolism and excretion of sofosbuvir, have been conducted.

1.2 Brief Discussion of Nonclinical Findings

Myocardial inflammation and degeneration occurred in rats administered oral GS-9851 doses of 2000 mg/kg/day (AUC_{last}~206 μg.h/ml for GS-331007) in a 7-day toxicology study. The estimated AUC exposure for sofosbuvir-derived GS-331007 is ~14-fold that in humans at the recommended sofosbuvir dose, since GS-9851 consists of 50% sofosbuvir. Six rats found dead following 5 days of dosing had myofiber degeneration with inflammatory infiltration in the heart. Although unclear if findings were sufficient to have caused death, one additional female had acute myocardial inflammation that was considered the probable cause of death. In addition to myofiber degeneration, one male had epicardial inflammation. Myofiber degeneration was also observed in one female, who survived until scheduled termination, and in 2 of 3 females following a 17 day treatment-free ("recovery") period. Although these surviving females gained weight during the study, severe body weight loss in rats found dead was considered a likely contributor to these early mortalities. Heart toxicity was not observed in rats administered oral doses of sofosbuvir up to 500 mg/kg/day (AUC_{last}~66 μg.h/ml for GS-331007) for 6 months, or in dogs and mice administered sofosbuvir at up to 500

and 1000 mg/kg/day (AUC_{last}~195 and 293 μg.h/ml for GS-331007), the highest doses examined in 9 and 3 month studies, respectively, corresponding to AUC exposures ~9 (rat), 27 (dog) and 41 (mouse)-fold that in humans at the recommended sofosbuvir dose. No clear cardiovascular safety signals have been observed in clinical trials.

Gastrointestinal (GI) hemorrhage occurred in male dogs administered oral sofosbuvir doses of 500 mg/kg/day (AUC_{last}~209 to 278 μg.h/ml for GS-331007 at 6 & 3 months, respectively), corresponding to AUC exposures ~29 to 39-fold that in humans at the recommended sofosbuvir dose. Hemorrhage occurred in the lamina propria of the pyloric stomach (1 of 4 males at 3 months) or jejunum (1 of 8 males prior to 6 months) resulting in moribund condition and euthanasia of the latter. Increased frequency and incidence of emesis and diarrhea also occurred at this dose level. These GI-related toxicities are dose-dependent and so most likely sofosbuvir-related; however, they also appear consistent with idiopathic hemorrhagic gastroenteritis of spontaneous origin. The NOEL for GI toxicity is 100 mg/kg/day (AUC_{last}~90 μg.h/ml for GS-331007) in dogs administered oral doses of sofosbuvir for up to 9 months, corresponding to AUC exposure ~13-fold that in humans at the recommended sofosbuvir dose. GI hemorrhage has not been observed in rats, mice or in clinical trials.

1.3 Recommendations

1.3.1 Approvability

Yes, the sponsor provided sufficient nonclinical safety information on sofosbuvir in support of approval for marketing in the U.S.

1.3.2 Additional Non Clinical Recommendations

We recommend that the sponsor consider conducting a short duration rat toxicology study with sofosbuvir at dose levels up to 2000 mg/kg to determine its contribution to the heart degeneration and inflammation observed with GS-9851. This study could not only more clearly define drug-related exposure multiples (based on heart findings) but, if indeed contributing to heart toxicity, further characterize this toxicity by including treatment-free groups (to evaluate reversibility) and additional study endpoints (*e.g.*, circulating biomarkers of cardiac toxicity, heart specific sofosbuvir and related metabolite concentrations).

1.3.3 Labeling

The Pharmacology/Toxicology portion of the sponsor's draft product label with reviewer suggested rearrangements (8.1 only) and/or modifications (designated by ~~strikethrough~~ or ***bold italics)*** is included below.

8.1 Pregnancy

Pregnancy Category X**:* (b) (4) ***Use with Peginterferon Alfa (b) (4) ***Ribavirin***

Extreme caution must be taken to avoid pregnancy in female patients and female partners of male patients while taking this combination. Women of childbearing potential

and their male partners should not receive ribavirin unless they are using two forms of effective contraception during treatment with ribavirin and for 6 months after treatment has concluded. There are no data on the effectiveness of systemic hormonal contraceptives in women taking [TRADENAME]. Therefore, two effective non-hormonal methods of contraception should be used during treatment with [TRADENAME] and concomitant ribavirin *[See Warnings and Precautions (5.1)]*.

In case of exposure during pregnancy, a Ribavirin Pregnancy Registry has been established to monitor maternal-fetal outcomes of pregnancies in female patients and female partners of male patients exposed to ribavirin during treatment and for 6 months following cessation of treatment. (b) (4) and patients are encouraged to report such cases by calling Ribavirin Pregnancy Registry at 1-800-593-2214.

Animal Data

Significant teratogenic and/or embryocidal effects have been demonstrated in all animal species exposed to ribavirin; and therefore ribavirin is contraindicated in women who are pregnant and in the male partners of women who are pregnant *[See Contraindications (4), Warnings and Precautions (5.1) and ribavirin Package Insert]*. Interferons have abortifacient effects in animals and should be assumed to have abortifacient potential in humans *[See peginterferon alfa Package Insert]*.

Pregnancy Category B: [TRADENAME]

There are no adequate and well-controlled studies with [TRADENAME] in pregnant women.

Animal Data

No effects on fetal development have been observed in rats and rabbits at the highest doses tested. In the rat and rabbit, ***AUC*** exposure to the predominant circulating metabolite GS-331007 (b) (4) ***increased over the course of gestation from*** approximately ***5 to*** 10-fold and ***12 to*** 28-fold the exposure in humans at the recommended clinical dose, respectively.

8.3 Nursing Mothers

It is not known whether sofosbuvir and its metabolites are excreted into human breast milk. The predominant circulating metabolite GS-331007 was the primary component observed in the milk of lactating rats (b) (4) Because of the potential for adverse reactions from the drug in nursing infants, (b) (4) See also the prescribing information for ribavirin.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

Use with Ribavirin and/or Peginterferon alfa: Ribavirin was shown to be genotoxic in several in vitro and in vivo assays. Ribavirin was not oncogenic in a 6-month p53+/- transgenic mouse study or a 2-year carcinogenicity study in rats. See the prescribing information for ribavirin.

Carcinogenicity studies of sofosbuvir in mice and rats are ongoing.

Sofosbuvir was not genotoxic in a battery of in vitro or in vivo assays, including bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes and in vivo mouse micronucleus assays.

Impairment of Fertility

Use with Ribavirin and/or Peginterferon alfa: In fertility studies in male animals, ribavirin induced reversible testicular toxicity; while peginterferon alfa may impair fertility in females. Refer to prescribing information for ribavirin and peginterferon alfa for additional information.

Sofosbuvir had no effects on embryo-fetal viability or on fertility when evaluated in rats. At the highest dose tested, ***AUC*** exposure to the predominant circulating metabolite GS-331007 was approximately 8-fold the exposure in humans at the recommended clinical dose.

(b) (4)

13.2 Animal Toxicology and/or Pharmacology

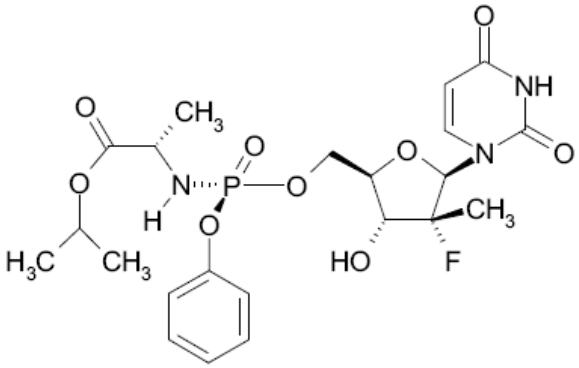
Heart degeneration and inflammation were observed in rats following GS-9851 (a stereoisomeric mixture containing approximately 50% sofosbuvir) doses of 2000 mg/kg/day for up to 5 days. At this dose, (b) (4) ***AUC exposure to the*** (b) (4) ***predominant*** (b) (4) ***metabolite GS-331007*** (b) (4)

No heart degeneration or inflammation was observed in rats following sofosbuvir doses of up to 500 mg/kg/day for 6 months. (b) (4)

In dogs and mice, heart degeneration and inflammation (b) (4) ***observed following sofosbuvir doses of up to 500 and 1000 mg/kg/day for 9 and 3 months, respectively, the highest doses tested. At these doses, AUC exposures*** (b) (4) ***approximately 27- and 41-fold the exposure in humans at the recommended clinical dose, respectively.***

2 Drug Information

2.1 Drug

CAS Registry Number	1190307-88-0
Generic Name	Sofosbuvir
Code Name	GS-7977 (PSI-7977)
Chemical Name	(S)-Isopropyl 2-((S)-(((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy) phosphorylamino) propanoate (IUPAC)
Molecular Formula/Molecular Weight	$C_{22}H_{29}FN_3O_9P$/529.4 (b) (4) g/mol
Structure	
Pharmacologic Class	Nucleoside inhibitor of HCV NS5B polymerase

2.2 Relevant INDs, NDAs, BLAs and DMFs

(b) (4) and IND-106,739

2.3 Drug Formulation

Sofosbuvir 400 mg tablets (~20 mm x 9 mm) are yellow, film-coated, capsule-shaped tablets (refer to Sponsor table below).

Table 1: Composition of sofosbuvir tablets

Component	Quality Standard	Composition		Function
		% w/w	mg/unit	
(b) (4)				
Sofosbuvir	In-house	(b) (4)	400.0[a]	Active Ingredient
Mannitol	USP, Ph. Eur.		(b) (4)	(b) (4)
Microcrystalline Cellulose	NF, Ph. Eur.			
Croscarmellose Sodium	NF, Ph. Eur.			
Colloidal Silicon Dioxide	NF, Ph. Eur.			
Magnesium Stearate	NF, Ph. Eur.			
(b) (4)				
Microcrystalline Cellulose	NF, Ph. Eur.			(b) (4)
Croscarmellose Sodium	NF, Ph. Eur.			
Colloidal Silicon Dioxide	NF, Ph. Eur.			
Magnesium Stearate	NF, Ph. Eur.			
Total		100	1200	
(b) (4)				
	In-house			(b) (4)
	USP, Ph. Eur.			
(b) (4)				

2.4 Comments on Novel Excipients

Not applicable. All excipients are compendial.

2.5 Comments on Impurities/Degradants of Concern

Qualification assessment of residual solvent, elemental and other sofosbuvir manufacturing process-related impurities was undertaken. The acceptability of proposed NMT limits for residual solvent and metal impurities was determined utilizing information from various sources including ICH Q3C. Numerous sofosbuvir impurities (specified and unspecified process intermediates, starting materials, degradants etc.) were evaluated by (Q)SAR analyses and are expected to be non-mutagenic (refer to Appendix 1). The Sponsor appears to be treating (b) (4) as a potentially mutagenic impurity, based on an equivocal prediction by Derek Nexus. In addition, repeat-dose

toxicology studies in rats were performed using sofosbuvir batches containing higher levels of total or specific impurities. No indication that the impurities present in these batches altered the toxicity profile of sofosbuvir was noted. Given this overall assessment, the seriousness of the disease to be treated, the fact that sofosbuvir will be given in combination with ribavirin (and peginterferon-alpha) and since no positive structural alerts were identified, both the nonclinical evaluation of impurities/degradants and the drug substance and product specifications (with regard to impurity content) appear acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Sofosbuvir is to be indicated for the treatment (400 mg tablet once a day) of adult patients infected with chronic hepatitis C in combination with peginterferon alpha and ribavirin for 12 weeks (in treatment naive HCV genotype 1, 4, 5 or 6 adult patients) or in combination with ribavirin for 12 (genotype 2) or 16 (genotype 3) weeks.

2.7 Regulatory Background

(b) (4) while IND-106,739 for PSI-7977 (GS-7977) was opened on December 13, 2009.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study #
Safety Pharmacology	
Effect of PSI-7851 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	SA-PSI-7851-08-009 (080521.QJB)
Effect of PSI-352707 on Cloned hERG Potassium Channels Expressed in Mammalian Cells	SA-PSI-7851-08-0023
Effect of PSI-7411 on Cloned hERG Potassium Channels Expressed in Mammalian Cells	SA-PSI-7851-08-0028
Effect of PSI-6206 on Cloned hERG Potassium Channels Expressed in Mammalian Cells	SA-PSI-7851-09-0001
ASSESSMENT OF ORALLY ADMINISTERED PSI-7851 IN THE IRWIN TEST IN MALE AND FEMALE SPRAGUE DAWLEY RATS	SA-PSI-7851-08-006 (b) (4) 693001)
CARDIOVASCULAR ASSESSMENT OF ORALLY ADMINISTERED PSI-7851 IN CONSCIOUS RADIOTELEMETRY-IMPLANTED NAÏVE MALE AND FEMALE BEAGLE DOGS	SA-PSI-7851-08-007 (b) (4) -693002)
RESPIRATORY ASSESSMENT OF ORALLY ADMINISTERED PSI-7851 IN PLETHYSMOGRAPH-RESTRAINED MALE AND	SA-PSI-7851-08-008 (b) (4) -693003)

FEMALE SPRAGUE DAWLEY RATS	
Analytical Method Validation	
Equivalency Test between PSI-7977 and PSI-7851 in Neat Solution and Rat Plasma by LC-MS/MS	BA-334-2001
Equivalency Test between PSI-7977 and PSI-7851 in Dog Plasma by LC-MS/MS	BA-334-2002
Validation of a method for the determination of PSI-352707, PSI-7411 and PSI-6206 in rat plasma by LC/MS/MS	PSI-7851-09-0004
Validation of a method for the determination of PSI-7851, PSI-352707, PSI-7411 and PSI-6206 in dog plasma by LC/MS/MS	PSI-7851-09-0005
Validation of a Method for the Determination of PSI-7851, PSI-352707, PSI-7411, and PSI-6206 in Rat Plasma by LC-MS/MS	PSI-7851-09-0007
ADME/Pharmacokinetics	
Determination of the effect of Concentration on the Bidirectional Permeability of GS-7977 through Monolayers of Caco-2 Cells	AD-334-2003
Plasma PK of GS-7977 in Portal Vein Cannulated Dogs Based on Portal and Jugular Sampling	AD-334-2011
Pharmacokinetics of GS-7977 and Its Metabolites in Plasma and Liver of Pentagastrin Pretreated Male Beagle Dogs	AD-334-2012
PSI-7851 stability in simulated gastric fluid or simulated intestinal fluid	PSI-7851-08-0012
Plasma and liver PK of PSI-7851 following single dose administration in SD rats	PSI-7851-08-0017
Plasma and liver PK of PSI-7851 in the beagle dog and cynomolgus monkey following multiple daily oral dose administration	PSI-7851-08-0018
Plasma and liver PK of PSI-7851 following single dose administration in CD-1 mice	PSI-7851-08-0019
Single dose oral PK study of PSI-7851 in beagle dogs	PSI-7851-09-0005
Oral crossover PK study of three formulations of PSI-7977 in dogs	PSI-7977-11-0004
In-vitro protein binding of PSI-7977 and PSI-6206 in mouse, rat, rabbit, dog and human plasma	PSI-7977-11-0001
Quantitative Tissue Distribution of Drug-Related Material Using Whole-Body Autoradiography Following a Single 20 mg/kg Oral Dose of [14C]PSI-7977 to Male Long-Evans and Sprague Dawley Rats	PSI-7977-09-0005
Determination of Placental and Milk Transfer in Female Sprague Dawley Rats Following a Single Oral Gavage Administration of [14C]PSI-7977 at 20 mg/kg	PSI-7977-11-0008
Test to Monitor Conversion from PSI-7977 to PSI-7976 in Rat,	AD-334-2014

Dog and Human Plasma and Human Urine by LC-MS/MS	
Identification of PSI-7851 metabolites in rat plasma and liver dosed with 1800 mg/kg PSI-7851 PO	PSI-7851-08-0001
Metabolite Profiling and Identification of [14C]PSI-7977 in Plasma, Urine, and Feces of Male Beagle Dogs	PSI-7977-09-0001
Metabolite Identification and Profiling of [14C]PSI-7977 in Plasma, Urine, Bile, Liver, and Feces of Male Sprague Dawley Rats	PSI-7977-09-0002
PSI-7409 (PSI-6206 5'-triphosphate) formation in primary rat, dog, monkey and human hepatocytes after treatment with 100 microM PSI-7851	PSI-7851-08-0011
Stability study of PSI-7851 in the whole blood of rat	PSI-7851-08-0015
Stability study of PSI-7851 in the whole blood of human, cynomolgus monkey, beagle dog and CD-1 mouse	PSI-7851-08-0016
Metabolite Identification and Profiling of [14C]PSI-7977 in Plasma, Urine, and Feces of CD-1 Mice	PSI-7977-11-0008
Identification and Profiling of [14C]PSI-7977 and Metabolites in Plasma and Milk of Female Sprague Dawley Rats	PSI-7977-11-0009
Mass Balance and Pharmacokinetics of Radioactivity in Male Rats Following a Single Oral Dose of [14C]PSI-7977	PSI-7977-09-0003
EXCRETION OF RADIOACTIVITY IN MALE BEAGLE DOGS ADMINISTERED A SINGLE ORAL DOSE OF 14C-PSI-7977	PSI-7977-10-0002
Excretion Mass Balance and Pharmacokinetics of Radioactivity in Male CD-1 Mice Following a Single Oral Dose of [14C]PSI-7977	PSI-7977-11-0007
General Toxicology	
Acute Oral Pilot Toxicity Study of PSI-7851 in Rats	SA-PSI-7851-09-0001 (0515-08127)
FOURTEEN-DAY ORAL GAVAGE STUDY OF PSI-7977 IN THE MOUSE	0515-09260
THREE-MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN THE MOUSE	SA-PSI-7851-09-0008 (0515-09261)
SEVEN DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN RATS	SA-PSI-7851-08-001 (0515-08275)
A 28-DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN SPRAGUE DAWLEY RATS	SA-PSI-7851-09-0003 (0515-09011)
A 90-DAY ORAL GLP TOXICITY STUDY OF PSI-7977 IN SPRAGUE DAWLEY RATS, WITH A 4-WEEK RECOVERY PERIOD	SA-PSI-7977-09-0007 (0515-09234)

A SIX-MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN RATS, WITH A 4-WEEK RECOVERY PERIOD	SA-PSI-7977-10-0004 (0515-10012)
SEVEN DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN BEAGLE DOGS	SA-PSI-7851-08-002 (0515-08274)
A 28-DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN BEAGLE DOGS	SA-PSI-7851-09-0002 (0515-09012)
A 90-DAY ORAL GLP TOXICITY STUDY OF PSI-7977 IN BEAGLE DOGS, WITH A 4-WEEK RECOVERY PERIOD	SA-PSI-7977-09-0006 (0515-09236)
A NINE MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN BEAGLE DOGS WITH A SIX-MONTH INTERIM ANALYSIS AND RECOVERY PERIODS	SA-PSI-7977-10-0003 (0515-10062)
<u>Genetic Toxicology</u>	
BACTERIAL REVERSE MUTATION ASSAY	SA-PSI-7851-08-003 (AC15DS.503.BTL)
In Vitro Mammalian Chromosome Aberration Test	SA-PSI-7851-08-004 (AC16DS.341.BTL)
Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of PSI-7851	SA-PSI-7851-08-005 (AC16DS.123.BTL)
<u>Reproductive and Developmental Toxicology</u>	
AN ORAL GLP FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION STUDY OF PSI-7977 IN RATS	SA-PSI-7977-10-0005 (0515-10239)
AN ORAL GLP STUDY OF THE EFFECTS OF PSI-7977 ON EMBRYO/FETAL DEVELOPMENT IN RATS	SA-PSI-7977-10-0008 (0515-10240)
AN ORAL RANGE-FINDING STUDY OF PSI-7977 IN PREGNANT RABBITS	SA-PSI-7977-11-0005 (0515-11177)
AN ORAL GLP STUDY OF THE EFFECTS OF PSI-7977 ON EMBRYO/FETAL DEVELOPMENT IN RABBITS	SA-PSI-7977-11-0006 (0515-10241)
An Oral (Gavage) Study of the Effects of GS-7977 on Pre- and Postnatal Development, Including Maternal Function, in Rats	TX-334-2003 (b) (4)-604017)
<u>Toxicology Studies (other)</u>	
A 7-DAY ORAL RANGE-FINDING STUDY OF PSI-7977 IN NONPREGNANT FEMALE RABBITS	SA-PSI-7977-11-002 (0515-10238)

A 14-DAY ORAL GLP BRIDGING TOXICITY STUDY COMPARING PSI-7851 TO THE SINGLE ISOMER PSI-7977 IN SPRAGUE DAWLEY RATS	SA-PSI-7977-09-0001 (0515-09233)
A 14-DAY ORAL GLP TOXICITY STUDY IN RATS TO QUALIFY THE SAFETY OF PSI-7977 LOT #40410003	SA-PSI-7977-11-0003 (0515-11126)
A 28-DAY ORAL GAVAGE QUALIFICATION TOXICITY GLP STUDY OF GS-7977 IN RATS	TX-334-2007 (0517-12180)
A 14-DAY ORAL GLP BRIDGING TOXICITY STUDY COMPARING PSI-7851 TO THE SINGLE ISOMER PSI-7977 IN BEAGLE DOGS	SA-PSI-7977-09-0002 (0515-09235)
The Bovine Corneal Opacity and Permeability Assay (BCOP)	TX-334-2008 (EUN0009)
GS-7977: Skin Irritation to the Rabbit	TX-334-2009 (EUN0011)
GS-7977: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)	TX-334-2010 (EUN0010)

3.2 Studies Not Reviewed

Study Title	Study #
Partial Validation of a Method for the Determination of PSI-7977, PSI-352707, and PSI-6206 in K2 EDTA Mouse Plasma by LC-MS/MS	BA-334-2003
Partial Validation of a Method for the Determination of PSI-7977 in Dog Plasma by LC-MS/MS	BA-334-2004
Partial Validation of a Method for the Determination of PSI-7977 in K_2 EDTA Rat Plasma by LC-MS/MS	BA-334-2005
LC/MS/MS assay for the quantitation of PSI-7851 in K2EDTA mouse plasma	PSI-7851-08-0002
Partial Validation of a Method for the Determination of PSI-7977 in Dose Solution (100% PEG 400) by HPLC-UV	PSI-7977-11-0001
Partial Validation of a Method for the Determination of PSI-7977, PSI-352707, and PSI-6206 in Rabbit Plasma by LC-MS/MS	PSI-7977-10-0001

3.3 Previous Reviews Referenced

Some GS-7977 (PSI-7977) and GS-9851 (PSI-7851) nonclinical safety studies, including safety pharmacology, ADME, repeat-dose toxicology and genetic toxicology studies have been reviewed by Dr. Verma to support the NDA, and will be summarized

in the appropriate sections of this review, with complete reviews of pivotal studies included within the review text.

4 Pharmacology

4.1 Primary Pharmacology

Sofosbuvir (GS-7977) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted intracellularly to the active uridine triphosphate (GS-461203) within tissues. GS-461203 is a specific inhibitor of nonstructural protein 5B (NS5B) of hepatitis C virus (HCV) that has displayed potent inhibition of HCV replicon ribonucleic acid (RNA) replication *in vitro*. Refer to the clinical virology review for a more detailed description and review of the primary pharmacology data of sofosbuvir submitted with this NDA.

Table 2: Sofosbuvir, diastereomers and metabolite terminology and descriptions

Gilead No.	Pharmasset No. (Previously Used)	Description
Sofosbuvir, SOF, GS-7977	PSI-7977	Nucleotide prodrug; S-diastereomer at phosphorous
GS-9851	PSI-7851	Nucleotide prodrug; isomeric mixture at phosphorous containing GS-7977 and GS-491241
GS-491241	PSI-7976	Nucleotide prodrug; R-diastereomer at phosphorous
GS-566500	PSI-352707	Nucleoside analog monophosphate alanine
GS-331007	PSI-6206	Nucleoside analog
GS-606965	PSI-7411	Nucleoside analog monophosphate
GS-607596	PSI-7410	Nucleoside analog diphosphate
GS-461203	PSI-7409	Nucleoside analog triphosphate; pharmacologically active metabolite

Table from sponsor

4.2 Secondary Pharmacology

Sofosbuvir and GS-9851 appear to have a low potential for mitochondrial-related cytotoxicity, since they did not affect mitochondrial deoxyribonucleic acid (mtDNA) synthesis or deplete cytochrome c oxidase in human cells lines at the top concentrations tested. In addition, the active GS-7977 triphosphate metabolite (GS-461203) did not inhibit human DNA polymerases α, β, and γ, or RNA polymerase II (IC50 > 200 μM). GS-461203 was also shown to be a weak substrate for mitochondrial RNA polymerase compared to some other HCV nucleotide inhibitors [Arnold, J.J. *et al*, (2012), PLOS Pathogens 8(11)]. GS-9851 also did not demonstrate induction or inhibition potential against a panel of 171 receptors, enzymes, and ion channels at 10 μM (5.3 μg/mL).

4.3 Safety Pharmacology

Note: The following study reviews were taken directly from the original (b) (4) review by Dr. Pritam Verma.

Brief summary

Single doses of PSI-7851 (GS-9851) did not adversely affect the function of the nervous, cardiovascular or respiratory systems or increase the risk of cardiac arrhythmia. PSI-7851 (parent) and its metabolites PSI-352707, PSI-6206, and PSI-7411 did not meaningfully inhibit hERG current *in vitro* at up to 159,123, 26 and 340 µg/mL, respectively, which were the highest concentrations tested. Repeated daily oral doses of PSI-7851 might lead to increases in electrocardiographic QT interval, based on the observation of a 15% prolongation of the QT interval in male dogs given PSI-7851 at 1500 mg/kg/day for 6 days. The NOEL for this effect on QT interval was 150 mg/kg/day.

Safety pharmacology studies

1. In Vitro hERG Assay with PSI-7851: Parent (SA-PSI-7851-08-009): PSI-7851 was evaluated at test concentrations of approximately 5.3 and 159 µg/mL (10 and 300 µM, respectively) for its ability to inhibit hERG-mediated potassium current in HEK293 cells expressing cloned hERG channels. PSI-7851 (parent) did not inhibit hERG current at 5.3 µg/mL but did inhibit hERG current by approximately 13% at 159 µg/mL. Based on these results, PSI-7851 was considered not to meaningfully affect hERG-mediated current.

2. In Vitro hERG Assay with PSI-352707: Metabolite (PC-PSI-7851-08-0023): PSI-352707 was evaluated at test concentrations of approximately 4.1, 41, and 123 µg/mL (10, 100, and 300 µM, respectively) for its ability to inhibit hERG-mediated potassium current in HEK293 cells expressing cloned hERG channels. PSI-352707 inhibited hERG current by approximately 5% at 123 µg/mL. Based on these results, PSI-352707 was considered not to meaningfully affect hERG-mediated current.

3. In Vitro hERG Assay with PSI-6206: Metabolite (PC-PSI-7851-09-0001): PSI-6206 was evaluated at test concentrations of approximately 2.6 and 26 µg/mL (10 and 100 µM, respectively) for its ability to inhibit hERG-mediated potassium current in HEK293 cells expressing cloned hERG channels. PSI-6206 did not inhibit hERG current at either test concentration. Based on these results, PSI-7411 was considered not to meaningfully affect hERG-mediated current.

4. In Vitro hERG Assay with PSI-7411: Metabolite (PC-PSI-7851-08-0028): PSI-7411 was evaluated at test concentrations of approximately 10, 34, and 340 µg/mL (3, 10, and 100 µM, respectively) for its ability to inhibit hERG-mediated potassium current in HEK293 cells expressing cloned hERG channels. PSI-7411 inhibited hERG current by approximately 4% at 340 µg/mL. Based on these results, PSI-7411 was considered not to meaningfully affect hERG-mediated current.

5. Single-Dose Oral CV Safety Pharmacology Study in Telemetry-Monitored Dogs (SA-PSI-7851-08-007; (b) (4) 6930002) The objective of this study was to evaluate the effects of orally administered PSI-7851 (parent) on CV function in Beagle dogs. To accomplish this, a single group of 6 dogs (3/sex) were given empty gelatin capsules or PSI-7851 at 100, 300, and 1000 mg/kg in a Latin square cross-over design with approximately 7 days between doses. Dogs were observed for clinical signs of toxicity twice daily and at 4 and 24 hours after each dose. Heart rate, arterial pressure (systolic, diastolic and mean), pulse pressure, ECG waveforms, and body temperature were recorded for 30-second intervals every 10 minutes from approximately 1 hour before through approximately 24 hours after each dose. The only clinical sign related to PSI-7851 was emesis at ≥300 mg/kg. PSI-7851 did not adversely affect heart rate, blood pressure (systolic, diastolic and mean), pulse pressure, body temperature, or ECG parameters (QT and heart-rate corrected QT intervals) at any dose level. Based on these results, the NOAEL was considered to be 1000 mg/kg, the highest dose administered.

6. ECGs in Restrained Dogs in a 7-Day Oral Toxicity Study with a 14-Day Recovery Period (SA-PSI-7851-08-002; BASi 0515-08274) Four groups of 8 dogs (4/sex) were given empty gelatin capsules or PSI-7851 twice daily (approximately 6 hours apart) for 7 days at dosages of 30, 150, and 1500 mg/kg/day. Among the data collected were ECGs recorded from restrained dogs before the dosing phase began, approximately four hours after the first dose on Day 6, and near the end of the recovery period. Blood samples were taken shortly before and at intervals after the first daily dose on Days 1 and 7 and analyzed to measure concentrations of PSI-7851(parent) and its metabolites PSI-6206, PSI-7411 and PSI-352707. Dogs tolerated twice daily oral doses of PSI-7851 for seven days at up to 1500 mg/kg/day, and no deaths occurred during the study. The only change in ECG parameters potentially related to PSI-7851 was an increased mean QT interval in males at 1500 mg/kg/day. Mean QT interval was 33 msec (15%) longer on Day 6 than before dosing began. Compared to the mean QT interval in the control group on Day 6, mean QT interval was 60 msec (33%) longer in high-dose males. This difference was not a consequence of differences in heart rate between groups, as the QTc interval was 41 msec (19%) longer in high-dose males than in control males. These data suggest that PSI-7851 may have affected cardiac repolarization in high-dose males. The lengthening of QT interval in high-dose males was reversible when dosing stopped, as QT and QTc intervals returned to baseline in the one male that entered the recovery period. Based on these results, the NOAEL for effects on CV function was considered to be 150 mg/kg/day. Neither PSI-7851 nor its metabolites PSI-6206, PSI-7411 and PSI-352707 produced meaningful effects on hERG-mediated current in vitro. Table 1 below compares the mean C_{max} values in male dogs on Days 1 and 7 to the highest test concentration in the hERG assay.

Table 3: Comparison of C_{max} values in male dogs with concentrations evaluated in the *in vitro* hERG assay.

	Mean C_{max} in Male Dogs (µg/mL)		Max. hERG Assay Test
Analyte	Day 1	Day 7	Conc (µg/mL)
PSI-7851	65	75	159
PSI-352707	10	10	123
PSI-6206	23	54	26
PSI-7411	2	2	340

These results suggest that QT prolongation in male dogs after 6 days of twice-daily dosing with PSI-7851 was probably not a consequence of hERG current inhibition by PSI-7851 itself or the metabolites PSI-352707 or PSI-7411. The possibility of hERG current inhibition by PSI-6206 cannot be ruled out because the mean peak plasma concentration of this metabolite on Day 7 (but not Day 1) exceeded the maximum concentration tested in the hERG assay *in vitro*. QT prolongation also may have been due to a mechanism other than hERG current inhibition.

7. Single-Dose Oral Respiratory Safety Pharmacology Study in Rats (SA-PSI-7851-08-008): The objective of this study was to assess the effects of orally administered PSI-7851 (parent) on respiratory function in rats. To accomplish this, groups of 10 rats (5/sex) were given single, oral doses of vehicle or PSI-7851 in vehicle at dose levels of 100, 300 and 1000 mg/kg. Rats were observed twice daily for mortality and morbidity, and clinical signs were recorded. For measurement of respiratory function parameters, each rat was placed in a head-out, neck-sealed plethysmograph, where respiratory frequency and tidal volume were measured for at least 5 hours post-dosing, and these data were used to calculate minute volume. PSI-7851 did not produce clinical signs considered to be adverse or affect respiratory frequency, tidal volume, or minute volume at any dose level. Based on these results, the NOAEL was considered to be 1000 mg/kg, the highest dose administered.

8. Single-Dose Oral Irwin Test in Rats (SA-PSI-7851-08-006): The objective of this study was to assess the effects of orally administered PSI-7851 (parent) on the gross behavior and physiological and neurological state of rats using a modification of a primary observation test, specifically the Irwin test. To accomplish this, groups of 10 rats (5/sex) were given single oral doses of vehicle or PSI-7851 in vehicle at dose levels of 100, 300 and 1000 mg/kg. For each rat, the parameters outlined in the Irwin test were evaluated shortly before and at 0.5, 1.5, 2.5, and 5 hours post dose and body temperature was recorded at the end of each observation period. PSI-7851 did not produce clinical signs of toxicity or affect survival, body temperature, gross behavior, or the physiological or neurological state of rats at any dose level. Based on these results, the no observed events level (NOAEL) was considered to be 1000 mg/kg, the highest dose administered.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption, distribution, metabolism and excretion of sofosbuvir (GS-7977, PSI-7977) have been studied in numerous *in vitro* and *in vivo* studies. A brief summary of the significant findings from studies in mice, rats, dogs and monkeys is presented below.

Absorption:

The oral bioavailability and fraction of sofosbuvir absorbed were estimated to be ~10 and 40%, respectively, in male dogs following administration by gavage in vehicle [5% ethanol, 55% PEG400 and 40% citrate buffer (pH 3.5)] (study #AD-334-2011). This difference between the bioavailability and the fraction absorbed is due to the high hepatic extraction of sofosbuvir (~75%). Although absolute oral bioavailability was not determined in other species (intravenous dosing not conducted), systemic exposures to sofosbuvir and related metabolites were robust suggesting that absorption of sofosbuvir was sufficient to achieve adequate circulating exposure levels in the toxicology studies submitted in these species. Oral T_{max} values for sofosbuvir in the dog varied from approximately 0.3 to 3 hours (depending on vehicle/formulation and dose level) but could not be measured in mice and rats due to blood instability of sofosbuvir (see below).

Metabolism:

GS-9851 (PSI-7851) was stable in simulated gastric and intestinal fluid, whole blood from dogs and monkeys, whole blood and plasma from humans but was unstable ($t_{½}$ <0.25 h) in rat and mouse blood and in human liver S9 fractions ($t_{½}$ <0.4 h) (study #PSI-7851-08-0013, 0015, 0016). The instability in rodent blood is thought to be due to high esterase activity which also occurs *in vivo* accounting for low systemic exposures to GS-9851 (and GS-7977) in rodents compared with dogs. Importantly, isomeric conversion between sofosbuvir (GS-7977) and GS-491241 was not detected in rat, dog, and human plasma and urine (study #AD-334-2014). Sofosbuvir is metabolized via sequential hydrolytic steps [ester cleavage by carboxylesterase (CES)1 or cathepsin A (CatA) and phosphoramidate cleavage by histidine triad nucleotide binding protein 1 (HINT1)] followed by consecutive phosphorylation steps via the pyrimidine nucleotide biosynthesis pathway (UMP-CMP and NDP kinases) (refer to Figure below).

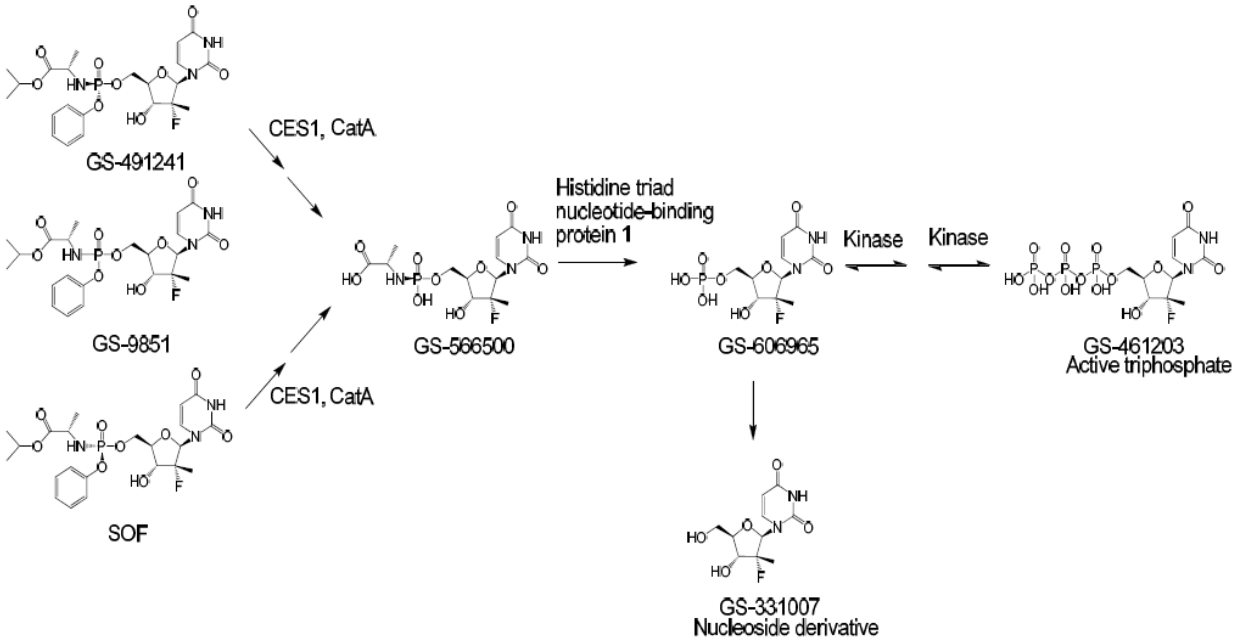


Figure 1: Intracellular metabolic pathway of GS-9851 [GS-7977 (SOF) and GS-491241] (from sponsor)

Note: CES1=carboxylesterase 1; CatA=cathepsin A

Dephosphorylation of GS-606965 in tissues (may also occur in blood since minor amounts of GS-606965 detected in plasma and/or urine in humans, dogs and rats) results in production of GS-331007, which can be released into circulation. Although re-phosphorylation of GS-331007 is thought to be inefficient, since GS-331007 lacks activity (EC_{90} > 78 μM) in *in vitro* HCV replicon assays (study #PC-334-2010), it is unclear whether some cell types could phosphorylate GS-331007 more efficiently and whether *in vivo* studies to confirm this inefficiency have been attempted. It does appear likely that tissue uptake of GS-331007 can occur at least to some extent *in vivo,* based on fetal distribution studies (study #PSI-7977-11-0008), but the presence of phosphorylated metabolites was not examined. Of note, GS-566500 also lacks activity (EC_{90} > 100 μM) in an *in vitro* HCV replicon assay (study #PSI-7851-08-0025). This lack of activity was attributed to its highly polar structure that was thought to prevent it from crossing the cell membrane. Tissue distribution and the potential for subsequent tissue metabolism of circulating sofosbuvir metabolites (GS-331007 and GS-566500) is an important consideration for assessing clinical risk in various special populations [*e.g*. pregnancy, nursing mothers, patients with severe renal impairment or end stage renal disease (ESRD)].

GS-331007 (PSI-6206) is the most abundant circulating metabolite in humans and animals, based on metabolic profiling studies with ^{14}C-sofosbuvir (study #PSI-7977-09-0001, 09-0002, 11-0008 and 11-0009), generally accounting for >75% of circulating plasma radioactivity. In humans and animals, GS-331007 is also the most abundant metabolite in urine and feces. Importantly, sufficient systemic exposure to the main circulating metabolites (GS-331007 and GS-566500) was demonstrated in animals. In addition, adequate systemic exposure to sofosbuvir was achieved in both rabbits and

dogs (but not rodents). Of note, the metabolite profile of sofosbuvir in plasma did not differ substantially between non-pregnant, pregnant and postpartum female rats (study #PSI-7977-11-0009).

Rats, mice, dogs and cynomolgus monkeys achieved measurable liver exposures to sofosbuvir and related metabolites also found in plasma (GS-331007 and GS-566500). Although human primary hepatocytes appear more efficient at producing the active tri-phosphorylated form (GS-461203) than species utilized for toxicology studies (refer to Figure below), rats and dogs achieved robust liver exposure to GS-461203 when administered as a single 50 mg/kg dose of GS-9851 (refer to Tables below). At this dose level, mice and cynomolgus monkeys failed to produce measurable amounts of GS-461203, despite adequate systemic and liver exposure to GS-9851 and related metabolites, suggesting that metabolic conversion to the active metabolite is inefficient in these species *in vivo*. These studies yielded GS-461203 liver concentrations of ~4 and 20 μM in rats and dogs, respectively, but <0.1 μM in mice and monkeys. Although much less efficient than rats, mice produced GS-461203 when administered higher GS-9851 dose levels (≥500 mg/kg). The relative percent of phosphorylated metabolites (GS-461203, GS-607596 & GS-606965 as compared to total GS-9851-related metabolites) increased in the liver with increasing GS-9851 dose levels. Although the relative percent of GS-461203 (versus total GS-9851-related phosphorylated metabolites) also increased in the liver with increasing GS-9851 dose levels, GS-461203 represented less than 5 and 1.5% of phosphorylated and total GS-9851-related metabolites, respectively, at all evaluated time-points. Despite this inefficiency, GS-461203 liver concentrations of ~28 μM were achieved in mice administered single 2000 mg/kg doses of GS-9851. By comparison, male rats achieved GS-461203 liver concentrations of almost 200 μM following administration of single 1800 mg/kg doses of GS-9851. Of note, the *in vitro* IC_{50} of GS-461203 is ~0.7 to 2.6 μM (study #PSI-7851-09-0007 and 09-0009). Thus, these high rodent dose levels achieved GS-461203 liver concentrations that exceeded the *in vitro* IC_{50} levels by ~10 to 100-fold.

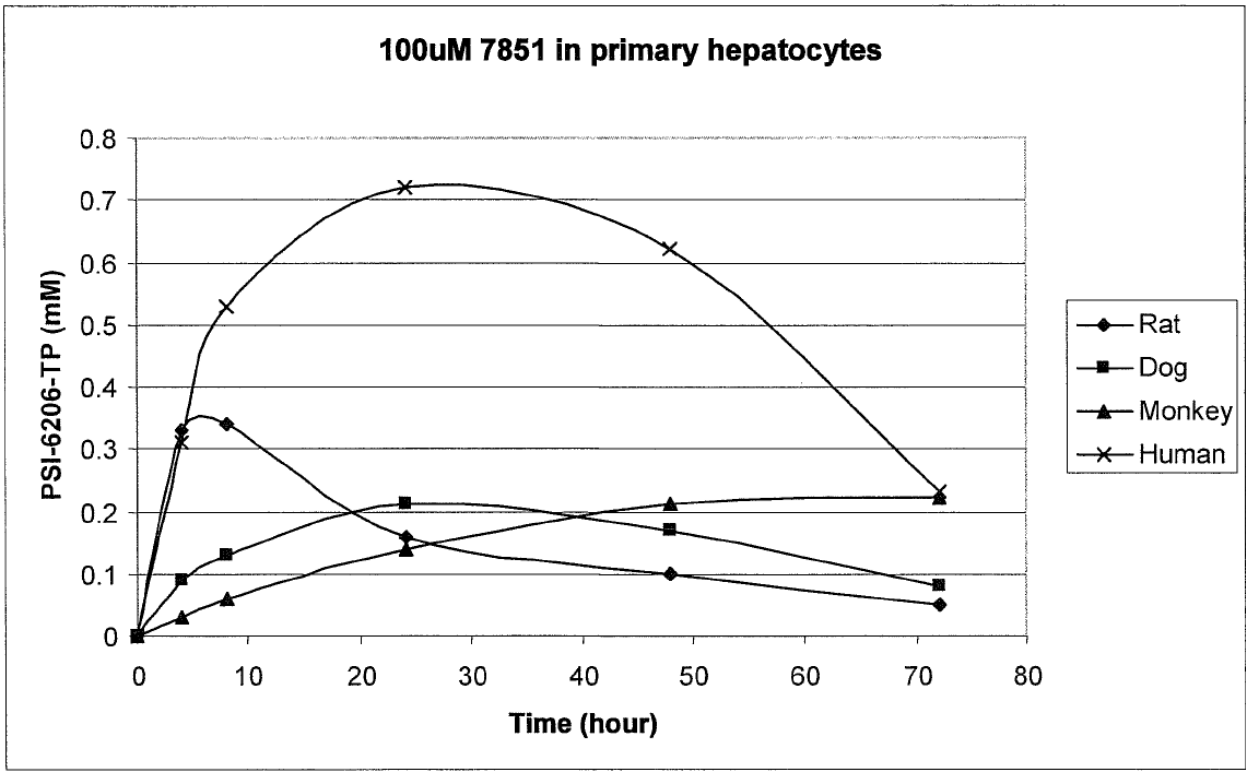


Figure 2: Time-dependent formation of GS-461203 (PSI-7409, also PSI-6206-TP) in rat, dog, monkey and human primary hepatocytes following incubation with GS-9851 (PSI-7851) (from sponsor)

Table 4: Mean systemic and liver exposure to selected GS-9851 related metabolites following a single dose of 50 mg/kg GS-9851 in mice (M) and rats (R)

	Plasma		Liver	
	GS-9851 (PSI-7851)	GS-331007 (PSI-6206)	GS-331007 (PSI-6206)	GS-461203 (PSI-7409)
C_{max} (μg/ml or μg/g)	NC	2.8 (M) 0.4 (R)	1.7 (M) 1.8 (R)	<0.04 (M) 1.9 (R)
T_{max} (hr)	NC	1 (M) 4 (R)	1 (M), 1 (R),	NC (M) 4 (R)
AUC_{inf} (μg·h/ml or μg·h/g)	NC	24.5 (M) 3.1 (R)	12.3 (M) 13.8 (R)	NC (M) 18.1 (R)
$T_{1/2}$ (hr)	NC	2.3 (M) 3 (R)	2.8 (M) 3.7 (R)	NC (M) 5.7 (R)

NC=not calculated (below level of quantitation);
Note: Data obtained from study #PSI-7851-08-0019 & 08-0017

Table 5: Mean systemic and liver exposure to GS-9851 and selected metabolites following daily doses of 50 mg/kg GS-9851 in dogs (D) and cynomolgus monkeys (CM) for 4 days

	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-461203 (PSI-7409)
Plasma:				
C_{max} (μg/ml)	6.2 (D) 0.03 (CM)	0.9 (D) 0.5 (CM)	2.6 (D) 0.4 (CM)	NC
T_{max} (hr)	0.5 (D) 1 (CM)	1 (D) 6 (CM)	6 (D) 2 (CM)	NC
AUC_{inf} (μg·h/ml)	6.9 (D) 0.17 (CM)	4.5 (D) 5.0 (CM)	34.8 (D) 80.4 (CM)	NC
$T_{1/2}$ (hr)	0.6 (D) NC (CM)	4.5 (D) 5.2 (CM)	6.2 (D) >24 (CM)	NC
$C_{4\ hrs\ post\text{-}dose\ on\ Day\ 4}$ (μg/ml)	0.3 (D) 0.05 (CM)	0.5 (D) 0.6 (CM)	1.6 (D) 0.2 (CM)	<0.05 (D) <0.05 (CM)
Liver:				
$C_{4\ hrs\ post\text{-}dose\ on\ Day\ 4}$ (μg/g)	0.6 (D) 0.2 (CM)	18.6 (D) 10.7 (CM)	2.6 (D) 1.4 (CM)	10.6 (D) <0.05 (CM)

NC=not calculated; Note: Data obtained from study #PSI-7851-08-0018

Table 6: Systemic and liver exposure to GS-9851 and selected metabolites relative to GS-9851 dose level following a single dose of 1800 mg/kg in rats (R) and 1000 or 2000 mg/kg in mice (M)

	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-461203 (PSI-7409)
Plasma:				
C_{max} (ng/ml)/ Dose level	NC	13.1 (R) 24.0 (MM) 36.2 (MH)	18.4 (R) 17.4 (MM) 10.8 (MH)	NC
$AUC_{0\text{-}24hr}$ (ng·h/ml)/ Dose level	NC	72.7 (R) 68.3 (MM) 82.2 (MH)	106.0 (R) 104.9(MM) 62.0 (MH)	NC
Liver:				
$C_{1.5\text{-}2\ hrs\ post\text{-}dose}$ (ng/g)/Dose level	0.9 (R) 49.1 (MM) 60.9 (MH)	556.1 (R) 1157 (MM) 1254 (MH)	61.7 (R) 69.8 (MM) 41.4 (MH)	28.9 (R) 2.1 (MM) 5.0 (MH)
$C_{6\ hrs\ post\text{-}dose}$ (ng/g)/Dose level	1.7 (R) 18.1 (MM) 30.2 (MH)	456.4 (R) 861 (MM) 670 (MH)	94.4 (R) 92.9 (MM) 77.8 (MH)	47.8 (R) 2.9 (MM) 6.9 (MH)

NC=not calculated; MM & MH= mouse medium & high dose level;
Note: Data obtained from study #PSI-7851-09-0001 & 7851-08-005

Table 7: Relative percent of total phosphorylated metabolites (GS-461203, GS-607596 & GS-606965) to total GS-9851-related metabolites in liver at various post-dose time-points in mice following a single GS-9851 dose of 1000 or 2000 mg/kg

Post-dose (hr)	Mean liver concentration (μg/g) of total metabolites	Mean liver concentration (μg/g) of phospho-metabolites	% phosphorylated metabolites
0.5	1,202 (M), 2,381 (H)	42 (M), 116 (H)	3.5 (M), 4.9 (H)
2	1,385 (M), 2,954 (H)	109 (M), 243 (H)	7.9 (M), 8.2 (H)
4	1,430 (M), 2,380 (H)	172 (M), 440 (H)	12.0 (M), 18.5 (H)
8	847 (M), 1,646 (H)	161 (M), 475 (H)	19.0 (M), 28.9 (H)

M=medium dose level, H=high dose level;
Note: Data obtained from study #PSI-7851-08-005

Table 8: Relative percent of each phosphorylated metabolite (GS-461203, GS-607596 & GS-606965) to total phosphorylated GS-9851-related metabolites in liver at various post-dose time-points in mice following a single GS-9851 dose of 1000 or 2000 mg/kg

Post-dose (hr)	GS-606965 (PSI-7411)	GS-607596 (PSI-7410)	GS-461203 (PSI-7409)
0.5	83.8 (M), 63.2 (H)	15.1 (M), 32.1 (H)	1.1 (M), 4.7 (H)
2	75.7 (M), 54.5 (H)	22.4 (M), 41.4 (H)	1.9 (M), 4.1 (H)
4	73.6 (M), 60.0 (H)	24.9 (M), 38.0 (H)	1.5 (M), 2.0 (H)
8	72.4 (M), 47.2 (H)	25.6 (M), 48.8 (H)	2.0 (M), 4.0 (H)

M=medium dose level, H=high dose level;
Note: Data obtained from study #PSI-7851-08-005

Phenol is formed during the initial esterase-mediated metabolic step that converts sofosbuvir to GS-566500. Since esterification of sofosbuvir is expected to be nearly complete (>99% in rodents & >95% in humans & dogs) and phenol accounts for ~18% of the molecular weight of sofosbuvir, there is a potential for substantial systemic exposure [*e.g.* up to 72 mg phenol/day in 400 mg sofosbuvir/day human dose (assuming complete sofosbuvir absorption)]. Therefore, it is important to consider the potential toxicological implications of phenol formed during sofosbuvir metabolism (refer to Appendix 2 for phenol toxicity summary).

Species differences in the location of phenol formation appear likely, since plasma esterase activity is higher in rodents compared to dogs and humans, and is an important consideration in evaluating potential risk of phenol to humans. Due to moderate absorption (~40%) and high hepatic extraction (~75%), the oral bioavailability of sofosbuvir was estimated to be ~10% in dogs; however, this information is not available for rodents. Phenol formation occurring prior to (in plasma or GI tract) or in zone 1 of the liver (periportal region) is thought to pose less risk, since it is more likely to undergo phase 2 conjugation, while phenol formed after zone 1 is less likely to be conjugated and more likely to undergo CYP450-mediated metabolism to more reactive metabolic species (refer to Appendix 2). However, the proportion of conjugated to unconjugated phenol is not known and cannot be estimated with any certainty based on existing data in these species. Although phenol was likely present at significant

concentrations in all non-clinical safety studies conducted, no clear phenol-related toxicities were observed in repeat-dose or reproductive toxicology studies, with safety margins (>15-fold) most likely existing at the 400 mg/day human sofosbuvir dose, based on human equivalent dose calculations made by estimating phenol exposure at the NOAEL in these studies.

The results of genotoxicity assays have been mixed, with phenol appearing to have some degree of genotoxic potential including positive results in *in vivo* assessments of micronuclei formation (refer to Appendix 2). However, the mouse micronucleus assay with GS-9851, which likely had significant amounts of phenol present, was negative (study #PSI-7851-08-005). Potential toxicities associated with sofosbuvir-derived phenol, as examined in non-clinical studies and clinical trials with sofosbuvir, appear to have been evaluated adequately. Although the localization, extent of phenol formation and proportion of conjugated to unconjugated phenol is unknown (not measured in clinical or nonclinical studies), phenol was likely present at significant concentrations in *in vivo* and *in vitro* non-clinical studies conducted, without safety issues identified. Overall, the available data do not suggest a substantial clinical risk associated with sofosbuvir-derived phenol.

Distribution:

Tissue distribution of sofosbuvir and related metabolites was examined using quantitative whole body autoradiography in male pigmented (Long-Evans) and non-pigmented (Sprague-Dawley) rats following a single 20 mg/kg dose of ^{14}C-sofosbuvir (study #PSI-7977-09-0005). No remarkable differences between pigmented and non-pigmented rats were identified. In Sprague-Dawley rats (measured at 1, 8, 24, 48 and 144 hours), concentrations of sofosbuvir and related metabolites in liver were ~13 and 15 times that in plasma 1 and 8 hours post-dose, at 24 hours were less than 3% those at 1 hour and were below the level of detection by 144 hours. Concentrations of sofosbuvir and related metabolites in heart (myocardium) were ~3 and 4 times that in plasma 1 and 8 hours post-dose and were below the level of detection by 24 hours. In addition to liver, the highest concentrations were measured in the alimentary canal, lymphatic and excretory systems (refer to Table below). Sofosbuvir and related metabolite concentrations were less than 50% of plasma (at all time-points) in only bone, eye lens and seminal vesicles. At 48 hours post-dose, sofosbuvir and related metabolites were almost completely absent from tissues and could only be detected in significant amounts in the alimentary canal and urinary bladder.

Table 9: Selected tissue to plasma ratios (those >2-fold) of sofosbuvir and related metabolites from male Sprague-Dawley rats administered a single 20 mg/kg dose of ^{14}C-sofosbuvir

Tissue	1 hr post-dose	8 hrs post-dose	24 hrs post-dose
Cecum	37.3	260.8	59.4
Large Intestine	3.5	222.6	12.5
Small Intestine	219.8	13.1	142.2
Stomach (gastric mucosa)	43.3	33.2	ND
Colon	3.0	14.0	8.0

Esophagus	4.0	4.3	ND
Liver	13.1	14.5	27.5
Spleen	5.7	20.5	8.9
Thymus	2.6	17.1	9.9
Lymph Node	5.0	14.3	10.2
Urinary Bladder	2.8	2.9	116.0
Renal Cortex	5.1	10.4	8.7
Renal Medulla	4.9	5.8	ND
Lung	5.2	6.5	3.4
Thyroid Gland	2.8	5.9	ND
Heart (myocardium)	2.8	4.4	ND
Adipose (brown)	NA	6.1	ND
Bone Marrow	NA	5.5	ND
Skin	NA	3.9	ND
Adrenal Gland	NA	3.3	ND
Salivary Gland	NA	3.0	ND
Pancreas	NA	2.6	ND
Skeletal Muscle	NA	2.2	ND
Pituitary Gland	NA	2.2	ND
Epididymis	NA	2.1	ND

ND=not determined due to insufficient data; NA=not applicable since less than 2-fold

Placental transfer and tissue distribution of sofosbuvir (GS-7977) and related metabolites was assessed using quantitative whole body autoradiography in pregnant and/or non-pregnant female Sprague-Dawley rats administered a single 20 mg/kg oral dose of ^{14}C-sofosbuvir (on gestation day 13 in pregnant females) and sacrificed at various post-dose time-points (study #PSI-7977-11-0008). The only significant maternal tissue distribution difference identified in non-pregnant versus pregnant females was distribution to the uterus in non-pregnant females (tissue to plasma ratio ~16). However, no remarkable tissue distribution differences were identified in females compared to male rats. In pregnant females (measured at 1, 4, 8, 24, 48, 72, 96 and 120 hours), concentrations of sofosbuvir and related metabolites in liver were ~13 to 51 times that in plasma from 1 to 8 hours post-dose and at 24 hours post-dose were less than 5% those at 1 hour post-dose. Concentrations of sofosbuvir and related metabolites in heart were less than that in plasma at all time-points, while concentrations in female reproductive organs were similar to or less than that in plasma. Similar to male rats, the highest concentrations were measured in the alimentary canal, lymphatic and excretory systems with the lowest concentrations observed in CNS, bone, eye lens and adipose (white) tissue. Sofosbuvir-derived radioactivity was detected in amniotic fluid, placenta, fetal blood, brain and liver with maximal concentrations observed 4 hours post-dose. Fetal blood and brain levels were higher than those observed in pregnant dams, while fetal liver tissue levels were only ~1/10th those observed in livers from dams (refer to Table below).

Table 10: Fetal tissue to maternal plasma ratios of sofosbuvir and related metabolites from Sprague-Dawley rats administered a single 20 mg/kg dose of ^{14}C-sofosbuvir

Fetal Tissue	1 hr post-dose	4 hrs post-dose	8 hrs post-dose	24 hrs post-dose
Amnion	0.7	5.1	2.2	ND
Placenta	0.8	2.1	4.1	3.4
Blood	0.3	1.8	2.6	10.3
Brain	0.5	2.9	4.0	5.0
Liver	0.3	1.9	3.3	3.3
Kidney	ND	ND	ND	ND

ND=not determined due to insufficient data (below detection)

Plasma protein binding of sofosbuvir was determined in mouse, rat, rabbit, dog and human plasma (study #PSI-7977-11-0001). The percent binding to plasma protein was less than 70% in dog and human, when sofosbuvir was incubated in plasma at 1 to 20 μg/ml concentrations. Protein binding could not be assessed in rat, mouse and rabbit due to sofosbuvir instability. In addition, protein binding of GS-331007 was quite low (less than 10%) in all of these species.

Excretion:

Mass balance and excretion were assessed in male mice, rats, dogs and in bile duct cannulated male rats administered a single 20 mg/kg oral dose of ^{14}C-sofosbuvir (study# PSI-7977-09-0003, 10-0002 and 11-0007). Total recovery of radioactivity ranged from ~85% in mice to 97% in dogs. Elimination of sofosbuvir-related metabolites occurs primarily through renal excretion (of GS-331007) in urine with mean urine recovery of ~72, 63, 66 and 81% in intact and cannulated rats, mice and dogs, respectively. In addition, ~18, 14 and 2% of sofosbuvir-related material was recovered in feces in rats, mice and dogs, respectively, with cannulated rats eliminating 6% of the total sofosbuvir dose in bile.

Transfer of sofosbuvir and related metabolites into milk was assessed in lactating Sprague-Dawley rats administered a single 20 mg/kg oral dose of ^{14}C-sofosbuvir on post-partum Day 2 (measured at 1, 6, 24 and 72 hours) (study #PSI-7977-11-0008). Milk to plasma ratios for sofosbuvir-related material collected from the dam were 0.1 and 0.8 at 1 and 24 hours post-dose, respectively. Concentration of sofosbuvir-related material in milk peaked at 1 hour post-dose, at 24 hours was ~6% that at 1 hour and was below detection by 72 hours. Sofosbuvir-related material was quantifiable in pups 24 hrs post-dose in liver (tissue to maternal plasma ratio ~0.3) and from 6 to 96 hours post-dose in GI tract (tissue to maternal plasma ratio ~4.5 at 24 hrs) but not in kidney or lungs. The maximal concentration of sofosbuvir-related material in the pup GI tract (at 48 hrs post-dose) was roughly equivalent to the maximal maternal plasma level. Of note, the majority of sofosbuvir-related material administered to pups in milk is GS-331007 and 2 sulfated conjugates of GS-331007, with only a minor amount of GS-566500 present (study #PSI-7977-11-0009).

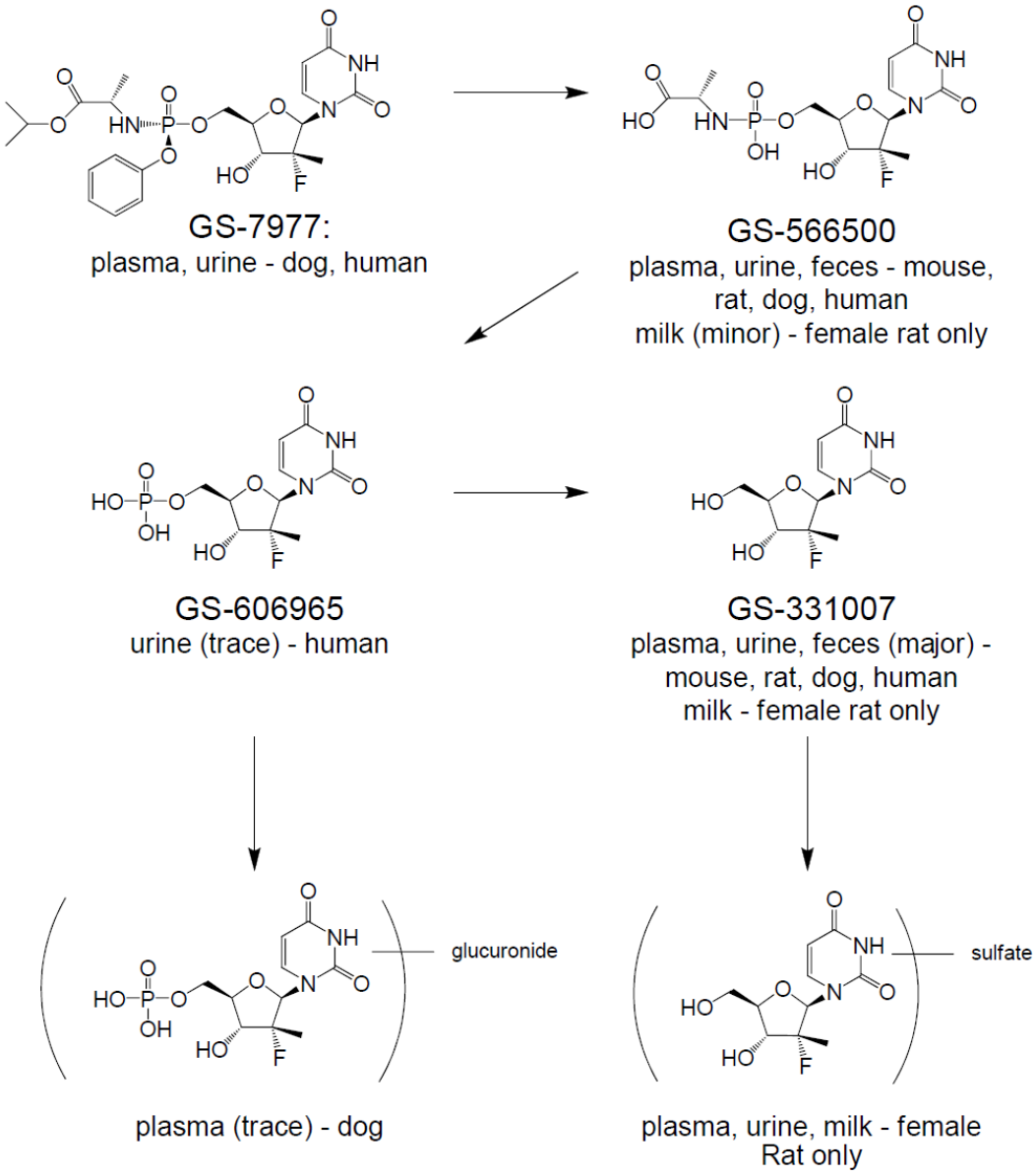


Figure 3: Summary of proposed biotransformation pathways for sofosbuvir (GS-7977) (from sponsor)

PK Drug Interactions:

The intracellular activation of sofosbuvir is mediated by low affinity and high capacity hydrolase (*e.g.*, CES1, CatA, HINT-1) and nucleotide phosphorylation (*e.g.*, UMP-CMP kinase, NDP kinase) pathways that are not thought to be readily inhibited by drugs that may be co-administered to HCV patients. Sofosbuvir does not appear to be a meaningful substrate, inhibitor, or inducer of CYP450 enzymes but is a substrate (but not an inhibitor) of Pgp and BCRP. Thus, absorption of sofosbuvir may be affected by co-administration of inducers or inhibitors of these transporters. The predominant circulating metabolite, GS-331007, was not an inhibitor or substrate of metabolizing

enzymes and transporters. Refer to the clinical pharmacology review for a detailed description and review of the PK drug interaction-related data for sofosbuvir.

5.2 Toxicokinetics

Refer to Section 6.

6 General Toxicology

6.1 Single-Dose Toxicity

A single-dose pilot (non-GLP) oral toxicity study was conducted in rats (study #PSI-7851-09-0001) administered PSI-7851 doses of 50, 300, or 1800 mg/kg or vehicle control (30% PEG 400, 30% Tween 20, 20% corn oil & 20% water) (3/sex/group) and observed for 14 days. PSI-7851 did not produce mortality, clinical signs of toxicity, body weight gain changes, macroscopic pathologic findings or organ weight changes (liver, kidney) at any dose level. Based on these limited endpoints, the NOAEL was considered to be 1800 mg/kg. Systemic and liver exposures to PSI-7851 and metabolites were evaluated in TK animals (10/sex/group) while only liver exposures were determined in main study animals, as summarized in the table below.

Table 11: Mean systemic and liver exposure to GS-9851 (PSI-7851) and selected metabolites in rats following a single GS-9851 dose of 50, 300 or 1800 mg/kg

	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-461203 (PSI-7409)
Plasma:				
C_{max} (μg/ml)	NC	0.7 (L) 2.9 (M) 23.6 (H)	0.7 (L) 4.9 (M) 15.1 (H)	NC
$AUC_{0\text{-}24hr}$ (μg·h/ml)	NC	2.8 (L) 11.6 (M) 130.9 (H)	5.9 (L) 43.3 (M) 190.8 (H)	NC
Liver:				
$C_{1.5\ hrs\ post\text{-}dose}$ (μg/g)	0.4 (L) <0.2 (M) 1.7 (H)	9.3 (L) 69.8 (M) 1,001 (H)	2.7 (L) 12.7 (M) 111 (H)	0.6 (L) 3.0 (M) 51.8 (H)
$C_{6\ hrs\ post\text{-}dose}$ (μg/g)	<0.2 (L) 0.7 (M) 3.0 (H)	9.2 (L) 106.8 (M) 822 (H)	2.4 (L) 38.9 (M) 170 (H)	2.5 (L) 21.4 (M) 85.8 (H)
$C_{24\ hrs\ post\text{-}dose}$ (μg/g)	<0.2 (L) <0.2 (M) <0.2 (H)	<0.02 (L) <0.02 (M) 1.2 (H)	0.2 (L) 0.7 (M) 1.9 (H)	<0.04 (L) 0.7 (M) 4.1 (H)

NC=not calculated; L=low, M=medium, H=high dose level

A single-dose oral tolerance/PK study (non-GLP) was conducted in female dogs (study #PSI-7851-09-0005) administered a PSI-7851 dose of 1000 mg/kg either in

capsules (n=3) or formulated in 30% PEG 400, 30% Tween 20, 20% corn oil, and 20% water (n=3). Dogs were observed for 24 hours post-dose for clinical signs of toxicity and then returned to the stock colony. All dogs survived with clinical signs of local GI tract irritation that included increased salivation, soft or mucoid feces and emesis in 2, 3 and 5 dogs, respectively. Refer to table below for plasma C_{max} and AUC values.

Table 12: Summary of plasma PK values in female dogs administered 1000 mg/kg GS-9851 (PSI-7851)

	Capsule			Liquid formulation		
	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)
C_{max} (μg/ml)	102.0	7.74	45.1	235.7	15.1	14.1
T_{max} (hr)	3.0	4.7	4.0	0.5	0.7	4.0
AUC (μg·h/ml)	556.1	58.0	423.7	319.2	30.9	117.2
$T_{1/2}$ (hr)	1.2	3.2	6.1	2.0	1.7	6.2

6.2 Repeat-Dose Toxicity

6.2.1 Studies with GS-9851 (PSI-7851)

Note: The following study reviews were summarized primarily from original (b) (4) IND-106,739 reviews by Dr. Pritam Verma.

Study title: 7-Day Oral Toxicity Study of PSI-7851 in Rats

Study no.: 0515-08275
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: August 13, 2008
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7851, 38508001 (1169-78-1) and 98.5%

Key study findings: A 7-day oral toxicity study with a 14-day recovery period (3/sex/group) was conducted in rats (10/sex/group) given twice-daily oral doses of vehicle (30% PEG 400, 30% Tween 20, 20% corn oil, and 20% water) or PSI-7851 at 30, 250, or 2000 mg/kg/day. The NOAEL was considered to be 250 mg/kg/day (AUC_{last}~31 μg.h/ml for PSI-6206). At 2000 mg/kg/day, rats began dying by Day 3, and dosing was stopped on Day 4.5. Nine (of 26) rats (6 females and 3 males) receiving 2000 mg/kg were found dead on Days 3, 5 or 6 (see pathologist's table below).

Histopathologic findings related to PSI-7851 administration were observed in heart and lymphoid tissues, with lymphoid effects considered to be stress-related.

From the pathologist's report for animals found dead: "In the heart, six rats had minimal to slight/mild myofiber degeneration, usually located at the apex of the heart and accompanied by variable amounts of basophilic mucinous interstitial stoma. The degeneration consisted of single myofiber hypereosinophilia or pyknosis with variable numbers of infiltrating inflammatory cells. Additionally, one female had slight/mild acute inflammation throughout the myocardium. The acute inflammation, comprised predominantly of neutrophils, surrounded mineralized myofibers, and this change was considered a more advanced change than myofiber degeneration." The heart lesion was considered by the pathologist to be the probable cause of death for the female with myocarditis. Regarding the cause of death in rats with myofiber degeneration the pathologist states: "It was not certain if the minimal to slight/mild degenerative changes seen in the heart of the six rats were sufficient to have caused death". Of note, the male found dead on Day 6 had epicardial inflammation in addition to myofiber degeneration. Minimal myofiber degeneration in the heart was also observed in one female who survived until scheduled termination (3 days following last dose) and in 2 of 3 females following the 14 day "recovery" period (17 days following last dose). It should also be noted that these two "recovery" females both gained weight during both the dosing and recovery phase.

In addition to the heart effects noted at 2000 mg/kg/day, effects related to PSI-7851 administration were limited to clinical signs in both sexes including body weight loss (~9% loss by Day 4), reduced food consumption, soft feces/watery diarrhea, anorexia, and respiratory crackles/rales (refer to Table below). All clinical signs subsided following cessation of dosing. Although most clinical signs were not more severe in rats found dead (compared to survivors), the exception was body weight loss, which was more severe in animals found dead (≥19% loss), and most likely contributed to these early mortalities.

Table 13: Pathologic findings and cause of death in rats administered 2000 mg/kg/day of GS-9851 (PSI-7851)

Animal No.	Day Found	Noteworthy Pathologic Findings	Cause of Death
CWM4F95	5	Minimal myocardial degeneration, cystic renal tubules, minimal to slight/mild increased individual lymphocyte necrosis in mesenteric lymph node and thymus, slight/mild lymphocyte depletion in spleen, moderate increased tingible body macrophages in the thymus.	Undetermined
CWM4F96	5	Moderate lymphocyte depletion in spleen and thymus, minimal increased individual lymphocyte necrosis in Peyer's patches.	Undetermined
CWM4F97	5	Slight/mild acute myocardial inflammation with myofiber mineralization, minimal renal tubular vacuolation, minimal lung mineralization, slight/mild increased tingible body macrophages in mesenteric lymph node, moderate to moderately severe lymphocyte depletion in spleen and thymus.	Heart-related
CWM4F100	5	Slight/mild myocardial degeneration, slight/mild unilateral acute inflammation of the renal pelvis and minimal acute inflammation of the urinary bladder, minimal focal necrosis and granulomatous inflammation in mesenteric lymph node, slight/mild increased tingible body macrophages in cervical lymph nodes, moderate to moderately severe lymphocyte depletion in spleen and thymus.	Undetermined
CWM4F102	5	Slight/mild myocardial degeneration, slight/mild renal mineralization, moderate increased tingible body macrophages in lymph nodes, moderately severe lymphocyte depletion in spleen and thymus.	Undetermined
CWM4F104	3	Congestion in multiple tissues.	Undetermined
CWM4M43	5	Slight/mild myocardial degeneration, moderate to moderately severe lymphocyte depletion in spleen and thymus, moderate thymic hemorrhage, slight/mild lung congestion.	Undetermined
CWM4M46	5	Minimal myocardial degeneration, slight/mild increased tingible body macrophages in lymph nodes, slight/mild to moderately severe lymphocyte depletion in spleen and thymus, slight/mild lung congestion, moderate increased necrosis of individual lymphocytes in Peyer's patch.	Undetermined
CWM4M49	6	Minimal myocardial degeneration and epicardial inflammation, minimal to moderately severe lymphocyte depletion in spleen and thymus, minimal focal granulomatous inflammation in mesenteric lymph node, moderate increased tingible body macrophages in lymph nodes.	Undetermined

Table from sponsor

Table 14: Number of rats observed with clinical signs during 7-days of GS-9851 (PSI-7851) dosing

	Males				Females			
Dosage (mg/kg/day) =	0	30	250	2000	0	30	250 *	2000
Wet body surface	1	4	9	10	1	7	9	10
Staining around anus	0	1	2	2	0	3	3	6
Unkempt appearance	0	1	0	5	1	5	3	8
Staining around nose	0	0	3	11	0	0	6	12
Staining around mouth	0	0	6	8	0	1	3	0
Appears dehydrated	0	3	2	6	3	1	1	7
Soft feces	0	1	1	5	1	2	1	3
Watery diarrhea	0	1	0	13	2	1	0	12
Anorexia	0	0	0	3	0	0	0	4
Respiration—crackles/rales	0	0	0	3	0	0	0	1

*Data from one female in the mid-dose group was not included (CWM3F80)

Table from sponsor

Table 15: Mean plasma TK parameters for predominant GS-9851 (PSI-7851) metabolites (PSI-352707, PSI-6206 and PSI-7411) in rats following single and multiple (up to 7-days) GS-9851 administration

	Males			Females		
PSI-7851 Dose (mg/kg/day) =	30	250	2000	30	250	2000
PSI-352707 [a]						
C_{max} (µg/mL)						
Day 1	0.5	4.8	29.8	0.3	8.4	14.2
Day 7	0.6	2.4	---	0.4	3.1	---
AUC_{last} (h*µg/mL)						
Day 1	4.4	40.8	284.3	4.5	32.4	119.7
Day 7	3.4	30.3	---	1.8	17.1	---
PSI-6206 [a]						
C_{max} (µg/mL)						
Day 1	0.5	4.2	14.2	0.3	11.5	13.5
Day 7	0.3	2.7	---	0.3	1.3	---
AUC_{last} (h*µg/mL)						
Day 1	4.1	56.4	219.1	4.2	53.1	192.9
Day 7	5.1	41.4	---	2.9	20.9	---
PSI-7411 [b]						
C_{max} (µg/mL)						
Day 1	0.03	0.94	4.51	0.03	2.46	2.24
Day 7	0.04	0.54	---	0.02	0.65	---
AUC_{last} (h*µg/mL)						
Day 1	0.07	10.66	44.25	0.12	8.62	21.85
Day 7	0.07	4.59	---	0.04	3.92	---

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL

[b] Values rounded to the nearest 0.01 µg/mL or h* µg/mL

--- = no data available because dosing was stopped early, before Day 7.

Table from sponsor

Study title: A 28-DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN SPRAGUE DAWLEY RATS

Study no.:	0515-09011
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 11, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7851, 38509001 and 99.6%

Key study findings: A 28-day oral toxicity study with a 14-day recovery period (5/sex/group) was conducted in rats (10/sex/group) administered daily doses of vehicle (95% PEG 400, 5% Tween 80) or PSI-7851 at 20, 100 or 500 mg/kg. The NOAEL was considered to be 500 mg/kg/day (AUC_{last}~57 μg.h/ml for PSI-6206 at 24 days). Non-adverse PSI-7851-related effects (partially vehicle-related) at 500 mg/kg were limited to clinical signs (increased incidence of staining around the mouth and/or nose, unkempt appearance, soft feces, and watery diarrhea in one or both sexes) that resolved following the dosing period. At 500 mg/kg/day, females had a slight increase (~40%) in CYP3A1/2 activity (compared to controls) that was associated with slightly greater mean liver weight. In males, CYP2A1 activity was increased (~90%) while NADPH-cytochrome *C* reduction and CYP2C11 activities were slightly lower (~20 to 30%) compared to controls.

Table 16: Mean plasma TK parameters for GS-9851 (PSI-7851) and metabolites (PSI-352707, PSI-6206 and PSI-7411) in rats following single and multiple (Day 24) GS-9851 administration

PSI-7851 Doses (mg/kg/day)	Analyte	Day 1 AUC_{last} (ng·h/mL)		Day 1 Ratio (Male / Female)	Day 24 AUC_{last} (ng·h/mL)		Day 24 Ratio (Male / Female)
		Male	Female		Male	Female	
20	PSI-7851	NA	NA	NA	NA	NA	NA
	PSI-352707	1994	1477	1.35	1619	1401	1.16
	PSI-6206	3692	1300	2.84	3515	1465	2.40
	PSI-7411	NA	NA	NA	NA	NA	NA
100	PSI-7851	NA	NA	NA	NA	NA	NA
	PSI-352707	10692	5535	1.93	8948	4498	1.99
	PSI-6206	16192	5904	2.74	16310	8063	2.02
	PSI-7411	NA	NA	NA	NA	NA	NA
500	PSI-7851	12.9	50.6	0.25	7.12	68.1	0.10
	PSI-352707	52784	26460	1.99	42469	43238	0.98
	PSI-6206	52173	22585	2.31	54973	59315	0.93
	PSI-7411	961	317	3.03	752	261	2.88

PSI-7851 plasma concentrations were BQL (< 5 ng/mL) at the 20 and 100 mg/kg/day doses. PSI-7411 plasma concentrations were BQL (< 50 ng/mL) at the 20 and 100 mg/kg/day doses.
NA: Not Applicable

Table from sponsor

Study title: SEVEN DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN BEAGLE DOGS

Study no.: 0515-08274
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: August 13, 2008
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7851, 38508001 and 99.5%

Key study findings: A 7-day oral toxicity study with a 14-day recovery period (1/sex/group) was conducted in dogs (3/sex/group) administered empty gelatin capsules or capsules containing PSI-7851 twice daily at dosages of 30, 150, and 1500 mg/kg/day. The NOAEL was considered to be 150 mg/kg/day (AUC_{last}~105 μg.h/ml for PSI-6206 at 7 days). Adverse effects at 1500 mg/kg/day included body weight loss (13% males and 8% in females) associated with emesis, diarrhea and reduced food consumption, increase (up to ~10-fold) in large unstained cells, slight increase (up to ~3-fold) in serum bilirubin, ALT, AST, and ALP, increased urine bilirubin and urobilinogen, APTT prolongation (~22% longer than controls), hepatobiliary changes including hypertrophy associated with increased liver weight, glycogen depletion, microvesiculation, apoptosis, pigment deposition in Kupffer cells and mononuclear-cell

infiltrates in the gallbladder, increased mucous secretion in the stomach, adrenal cortical hypertrophy and thymic atrophy/involution (probably due to generalized stress response). Males at 1500 mg/kg/day also exhibited depressed behavior, were “cool to touch”, had a prolonged QTc interval (mean 41 msec, 19% longer than controls) and increases in neutrophils and monocytes. This slight QTc prolongation is not of significant clinical concern since it occurred at very high drug exposures and was associated with significant adverse clinical signs. With the possible exception of APTT prolongation, toxic effects were reversible.

Table 17: Mean plasma TK parameters for GS-9851 (PSI-7851) and predominant metabolites (PSI-352707, PSI-6206 and PSI-7411) in dogs following single and multiple (Day 7) GS-9851 administration

	Males			Females		
PSI-7851 Dose (mg/kg/day) =	**30**	**150**	**1500**	**30**	**150**	**1500**
PSI-7851 [a]						
C_{max} (µg/mL)						
Day 1	0.4	2.3	65.3	0.2	5.3	64.3
Day 7	0.3	4.6	75.1	0.7	5.0	105.3
AUC_{last} (h*µg/mL)						
Day 1	0.8	6.4	437.1	0.5	13.7	543.8
Day 7	0.6	9.9	814.6	1.3	13.7	865.0
PSI-352707 [a]						
C_{max} (µg/mL)						
Day 1	0.3	1.2	9.7	0.4	1.5	10.2
Day 7	0.2	1.3	10.4	0.3	1.3	13.3
AUC_{last} (h*µg/mL)						
Day 1	1.1	7.2	103.0	1.1	7.7	119.4
Day 7	0.7	7.7	132.6	1.0	7.0	137.3
PSI-6206 [a]						
C_{max} (µg/mL)						
Day 1	1.6	6.0	22.9	1.8	6.4	22.7
Day 7	1.6	9.0	53.8	1.9	5.8	51.1
AUC_{last} (h*µg/mL)						
Day 1	19.4	73.8	335.6	25.0	89.7	368.4
Day 7	20.1	120.3	870.8	26.7	91.6	892.7
PSI-7411 [b]						
C_{max} (µg/mL)						
Day 1	0.08	0.23	2.25	0.13	0.32	3.02
Day 7	0.06	0.21	2.40	0.10	0.23	3.82
AUC_{last} (h*µg/mL)						
Day 1	0.04	1.16	19.41	0.07	1.74	32.35
Day 7	0.03	1.12	29.49	0.14	1.01	39.48

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL

[b] Values rounded to the nearest 0.01 µg/mL or h* µg/mL

Table from sponsor

Study title: A 28-DAY ORAL GLP TOXICITY STUDY OF PSI-7851 IN BEAGLE DOGS

Study no.:	0515-09012
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 11, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7851, 38509001 and 99.6%

Key study findings: A 28-day oral toxicity study with a 14-day recovery period (2/sex/group) was conducted in dogs (3/sex/group) administered empty gelatin capsules or capsules containing PSI-7851 at dosages of 20, 100 and 500 mg/kg/day once daily. The NOAEL was considered to be 100 mg/kg/day (AUC_{last}~55 μg.h/ml for PSI-6206 at 24 days). Potentially adverse PSI-7851-related effects at 500 mg/kg/day included slight body weight loss (~2 and 5% in males and females, respectively) associated with an increased incidence of soft feces and emesis, a slight decrease (10%) in mean erythron mass associated with minimal alterations in erythroid precursors (low total erythroid percent, high myeloid/erythroid ratio, high erythroid maturation index and/or decrease in late erythroid precursors or decreased polychromatophilic cells) (males) and slight increases in serum ALP (males) and liver weight (females). PSI-7851-related effects at 100 mg/kg/day were limited to a very slight increase in serum ALP (males). PSI-7851-related findings were generally absent following the “recovery” period.

Table 18: Mean plasma TK parameters for GS-9851 (PSI-7851) and predominant metabolites (PSI-352707, PSI-6206 and PSI-7411) in dogs following single and multiple (Day 24) GS-9851 administration

	Males			Females		
Dose (mg/kg/day) =	**20**	**100**	**500**	**20**	**100**	**500**
PSI-7851 [a]						
C_{max} (µg/mL)						
Day 1	0.3	7.7	26.6	1.2	7.1	34.8
Day 24	1.9	4.5	49.6	2.0	10.4	39.8
AUC_{last} (h*µg/mL)						
Day 1	0.8	14.4	81.0	1.7	13.1	106.9
Day24	2.6	14.0	124.0	2.4	22.4	113.4
PSI-6206 [a]						
C_{max} (µg/mL)						
Day 1	2.3	4.4	21.9	2.3	4.9	18.9
Day 24	1.2	3.5	26.5	1.5	6.0	20.2
AUC_{last} (h*µg/mL)						
Day 1	13.8	39.1	210.5	18.3	60.9	245.4
Day 24	14.4	39.7	291.0	13.9	68.6	266.0
PSI-352707 [a]						
C_{max} (µg/mL)						
Day 1	0.4	1.9	6.6	0.7	2.5	7.1
Day 24	0.4	1.4	6.4	0.4	2.1	6.1
AUC_{last} (h*µg/mL)						
Day 1	1.2	6.8	36.5	1.9	9.0	41.6
Day24	1.1	5.2	36.0	1.3	8.7	33.5
PSI-7411 [a]						
C_{max} (µg/mL)						
Day 1	NA	0.1	1.4	<0.1	0.2	0.7
Day 24	NA	0.1	1.5	NA	0.1	0.6
AUC_{last} (h*µg/mL)						
Day 1	NA	0.3	6.9	NA	0.3	3.3
Day 24	NA	0.2	7.2	NA	0.4	2.4

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL
NA = Not Applicable

Table from sponsor

6.2.2 Bridging Studies with GS-9851 (PSI-7851) and GS-7977 (PSI-7977)

Note: The following study reviews were summarized from the original IND-106,739 review by Dr. Pritam Verma.

Study title: 14-Day Oral Bridging Toxicity Study Comparing PSI-7851 To The Single Isomer PSI-7977 in Rats

Study no.:	0515-09233
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	August 11, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7851, 38509001 and 99.6% and PSI-7977, 40409001 and 99.6%

Key study findings: A 14-day oral bridging toxicity study was conducted in rats (10/sex/group) to compare the toxicity and TK of PSI-7851 and PSI-7977 when administered as daily oral doses of 500 mg/kg/day or vehicle (95% PEG 400, 5% Tween 80). The toxicity and systemic exposure profiles (AUC_{last}, T_{max}, and C_{max}) for the common PSI-7851 and PSI-7977 metabolites (PSI-352707 and PSI-6206) were generally similar. Although partially vehicle-related, effects attributed to PSI-7851 and PSI-7977 were limited to clinical signs (increased incidence of staining around nose, unkempt appearance, watery diarrhea, wet and stained body surface) in both sexes, slight organ weight changes in the absence of histopathological effects and a greater incidence of the uterus being in the metestrus/diestrus stage in both treatment groups versus control (70-80% versus 40%). The only findings that potentially differed between PSI-7851 and PSI-7977 were a greater mean thyroid weight in females administered PSI-7977 and minimal myofiber degeneration in the apex of the heart in one male given PSI-7851. However, neither of these findings could be definitely attributed to drug exposure.

Table 19: Mean plasma TK parameters for GS-331007 (PSI-6206) in rats following 14 days of GS-9851 (PSI-7851) or GS-7977 (PSI-7977) administration at 500 mg/kg

Dose (500mg/kg/day)	Sex[a]	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0\text{-last})}$ (µg·h/mL)
PSI-7851	F	4	3.32	35.42
PSI-7977	F	4	4.36	44.43
PSI-7851	M	6	4.84	58.71
PSI-7977	M	8	5.47	65.51

[a]F= Female; M= Male

Table from sponsor

Table 20: Mean plasma TK parameters for GS-566500 (PSI-352707) in rats following 14 days of GS-9851 (PSI-7851) or GS-7977 (PSI-7977) administration at 500 mg/kg

Dose (500mg/kg/day)	Sex[a]	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0\text{-last})}$ (µg·h/mL)
PSI-7851	F	1	2.75	12.18
PSI-7977	F	1	2.95	13.68
PSI-7851	M	4	6.46	37.19
PSI-7977	M	4	5.74	38.48

[a]F= Female; M= Male

Table from sponsor

Study title: A 14-Day Oral Bridging Toxicity Study Comparing PSI-7851 To The Single Isomer PSI-7977 in Beagle Dogs

Study no.: 0515-09235
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: August 11, 2009
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7851, 38509001 and 99.6% and PSI-7977, 40409001 and 99.6%

Key study findings: A 14-day oral bridging toxicity study was conducted in dogs (2/sex/group) to compare the toxicity and TK of PSI-7851 and PSI-7977 when administered as daily oral doses of empty gelatin capsules or capsules containing PSI-7851 or PSI-7977 at 500 mg/kg/day. The toxicity and systemic exposure profiles (AUC_{last}, T_{max}, and C_{max}) for PSI-7851 and PSI-7977 and their common metabolites (PSI-352707 and PSI-6206) were generally similar. Neither PSI-7851 nor PSI-7977 caused adverse effects with a slight increase in mean serum ALP activity in females being the only effect observed with both compounds.

Table 21: Mean plasma TK parameters for GS-9851 (PSI-7851) or GS-7977 (PSI-7977) in dogs following 14 days of GS-9851 or GS-7977 administration at 500 mg/kg

Dose (500mg/kg/day)	Sex[a]	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0\text{-last})}$ (µg·h/mL)
PSI-7851	F	3	80.82	307.44
PSI-7977	F	2	100.10	294.37
PSI-7851	M	4	80.11	263.18
PSI-7977	M	3	96.07	239.83

[a]F= Female; M= Male

Table from sponsor

Table 22: Mean plasma TK parameters for GS-331007 (PSI-6206) in dogs following 14 days of GS-9851 (PSI-7851) or GS-7977 (PSI-7977) administration at 500 mg/kg

Dose (500mg/kg/day)	Sex[a]	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0\text{-last})}$ (µg·h/mL)
PSI-7851	F	5	19.79	278.53
PSI-7977	F	3	29.32	264.86
PSI-7851	M	4	47.35	627.62
PSI-7977	M	4	30.72	289.52

[a]F= Female; M= Male

Table from sponsor

Table 23: Mean plasma TK parameters for GS-566500 (PSI-352707) in dogs following 14 days of GS-9851 (PSI-7851) or GS-7977 (PSI-7977) administration at 500 mg/kg

Dose (500mg/kg/day)	Sex[a]	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0\text{-last})}$ (µg·h/mL)
PSI-7851	F	4	8.31	44.60.
PSI-7977	F	2	9.95	40.63
PSI-7851	M	4	9.25	41.92
PSI-7977	M	3	11.69	44.48

[a]F= Female; M= Male

Table from sponsor

6.2.3 Studies with GS-7977 (PSI-7977)

Study title: FOURTEEN-DAY ORAL GAVAGE STUDY OF PSI-7977 IN THE MOUSE

Study no.:	0515-09260
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 8, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977, 40409001 and 99.2%

Key study findings: CD-1 mice (5/sex/dose) were administered PSI-7977 (0, 50, 150, 500, 1500 mg/kg) daily and evaluated for toxicity (mortality, clinical observations, body weight, clinical and gross pathology) in order to choose appropriate doses for the definitive 3-month study (used to identify an MTD for 2 year carcinogenicity study dose selection-see below). In addition to one unscheduled death (found dead on study day 10-cause of death undetermined), reduced body weight (↓8%) was observed in high-dose males at 14 days as compared to pre-dose levels, while control animals gained weight (↑4%). No other toxicologically significant findings were noted in this study.

Study title: THREE-MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN THE MOUSE

Study no.:	0515-09261
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 4, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977, 40409003 and 99.2%

Key study findings: Study was performed to identify an MTD appropriate for 2 year carcinogenicity study dose selection. Reduced body weight gain (≥30%) in mid and high-dose males and high dose females was associated with a reduction (10-20%) in food consumption in high dose animals only, indicating that these dose levels exceeded the MTD. No other toxicologically significant findings were noted in this study. The cause of death/morbidity in 17 animals [0, 8, 5, 4 (of 124) from 0, 100, 300 or 1000 mg/kg groups] was undetermined. The only consistent observation was gaseous distention of the intestines and/or stomach in 10 of these animals that were either found dead or sacrificed moribund. Thus, mortality is not dose dependent and is not likely of clinical significance, given the lack of significant toxicological findings in surviving animals.

Methods

Doses:	0, 100, 300, 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	PSI-7977 in 95% PEG 400, 5% Tween 80
Species/Strain:	Mouse/CD-1®
Number/Sex/Group:	20 (main), 42 (TK)
Age:	6 to 7 wks
Weight:	24 to 35 g
Satellite groups:	TK
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None due to test-article. In addition to the 21 unscheduled deaths, 6 animals died due to confirmed gavage error (torn/perforated esophagus). Of the 21, 4 may have been due to aspiration of formulation (including the one control animal in the table below), while the cause of death/morbidity in the remaining 17 animals was undetermined (refer to pathologist's summary tables below).

- The sponsor suggested that inadvertent aspiration due to reflux of small amounts of dosing solution could explain the deaths (without histopathologic evidence). Since a full stomach could contribute to this putative cause of death, mice were fasted for 2-4 hours prior to dosing to attempt to reduce the likelihood of this occurring (beginning on day 32) without clear benefit.
- Gaseous distention of the intestines and/or stomach was observed in 10/17 animals that were either found dead or sacrificed moribund (not related to gavage error).
- The mortality observed appears to be test-article related but not dose dependent and is of unknown significance.

Table 24: Summary of mortality in 3 month mouse toxicology study

Day	Number of Deaths (Toxicology and TK Animals Combined)*				
	Control	100 mg/kg	300 mg/kg	1000 mg/kg	Total
1-31	1	8	1	1	11
32-61	0	2	4	3	9
62-93	0	1	0	0	1
Total	**1/38 (2.6%)**	**11/81 (13.5%)**	**5/80 (6.3%)**	**4/79 (5.1%)**	**21/278 (7.6%)**

*Known gavage errors excluded and Day 1 TK animals excluded

Table from sponsor

Vehicle control group

Death Day	Animal # (DAE___)	Death Type	Noteworthy Gross Pathologic Findings	Noteworthy Histopathologic Findings	Suspected Cause Morbidity/Death
16	1M07	SM	No findings	Mild subacute lung inflammation	Possible aspiration
25	1M12	FD	Lungs: All lobes dark Thoracic cavity contains fluid	Marked acute periesophageal inflammation with foreign matter	Gavage error, torn esophagus

FD = found dead
SM = sacrificed moribund

PSI-7977 group (100 mg/kg/day)

Death Day	Animal # (DAE___)	Death Type	Noteworthy Gross Pathologic Findings	Noteworthy Histopathologic Findings	Suspected Cause Morbidity/Death
5	2F109	FD	Autolysis	None	-----
9	2M40	FD	Cornea, right: Opaque	Minimal acute bronchitis and/or bronchiolitis, with foreign material	Aspiration, likely due to reflux
14	2M37	FD	Stomach and small intestine: Distended with gas Lungs: Right median lobe dark; all lobes mottled	Moderate inflammation, erosion, and epithelial hyperplasia in bronchi and/or bronchioles Mild alveolar macrophages	Aspiration of dose solution
20	5F325	FD	No evidence of gavage error	Not examined – TK Animal	-----
23	2M24	FD	Changes consistent with autolysis	Mild alveolar macrophages	-----
27	5F321	FD	Not examined	Not examined – TK Animal	-----
27	2M30	SM	Stomach and small intestine: Distended with gas Stomach also has green fluid	None	-----
31	5M200	FD	No evidence of gavage error	Not examined—TK Animal	-----
36	5F319	FD	No evidence of gavage error	Not examined—TK Animal	-----
52	2F104	FD	Caudal lung lobes failed to inflate at necropsy Lung: Caudal; discolored; mottled	Minimal acute bronchitis and/or bronchiolitis Marked tracheal ulceration, fibroproliferation, and epithelial hyperplasia	Aspiration of dose solution; gavage error
67	2M22	SM	Stomach, small intestine, and cecum: Distended with gas	None	-----

FD = found dead
SM = sacrificed moribund

PSI-7977 group (300 mg/kg/day)

Death Day	Animal # (DAE___)	Death Type	Noteworthy Gross Pathologic Findings	Noteworthy Histopathologic Findings	Suspected Cause Morbidity/Death
20	3M47	FD	Brain: Autolysis Stomach and small intestine: Distended	Mild acute esophageal ulcer and inflammation with bacteria	Gavage error, torn esophagus
20	3M59	FD	Stomach and intestine: Distended Stomach also had black fluid	None	-----
39	6M229	SM	No evidence of gavage error	Not examined – TK Animal	-----
49	3M42	FD	Entire GI tract: Distended with gas Liver: Pale	Mild hepatocellular vacuolation	-----
56	6M230	FD	Stomach: Distended, no evidence of dosing error	Not examined—TK Animal	-----
57	3F135	SM	Stomach and small intestine: Distended with gas	None	-----
70	3M41	FD	Stomach and small intestine: Distended with gas	Massive acute esophageal ulcer, and inflammation with bacteria	Gavage error, torn esophagus

FD = found dead
SM = sacrificed moribund

PSI-7977 group (1000 mg/kg/day)

Death Day	Animal # (DAE___)	Death Type	Noteworthy Gross Pathologic Findings	Noteworthy Histopathologic Findings	Suspected Cause Morbidity/Death
28	7M277	FD	Necropsy not performed	Not examined – TK Animal	-------
28	4F156	SM	Esophageal perforation	Mild acute periesophageal inflammation Mild acute tracheal hemorrhage and peritracheal inflammation	Gavage error, torn esophagus
37	7M267	FD	Distended abdomen noted in the morning. Found dead in the afternoon. No evidence of gavage error. Stomach very distended with gas.	Not examined – TK Animal	--------
42	7M276	SM	No evidence of gavage error	Not examined – TK Animal	--------
47	7M281	FD	No evidence of gavage error	Not examined – TK Animal	--------
49	4F145	SM	Esophageal perforation	Moderate acute periesophageal inflammation.	Gavage error, torn esophagus
49	4F158	FD	Esophageal perforation	Marked acute periesophageal and perithymic inflammation	Gavage error, torn esophagus

FD = found dead
SM = sacrificed moribund

Clinical Signs: No test-article related changes noted.

Body Weights (1x/wk):

Table 25: Body weight change in mice following daily (up to 90-days) GS-7977 (PSI-7977) administration

	Males				Females			
Dose (mg/kg/day) =	**0**	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
Body weight (g)[a]								
Day –1	39.4	39.0	39.3	38.9	31.7	30.7	31.2	30.9
Day 28	41.3	41.1	41.0	39.0	33.4	32.9	33.9	31.6
Day 56	43.2	41.8	42.8	39.6	33.8	34.5	34.8	32.9
Day 91	44.6	44.0	42.9	40.9	35.7	35.6	35.9	34.2
Difference from control	---	–1%	***–4%***	***–8%***	---	±0%	+1%	***–4%***
Body weight change[b]								
In grams	+5.3	+5.2	***+3.7***	***+2.0***	+4.0	+4.6	+4.8	***+2.8***
Difference from control	---	–2%	***–30%***	***–62%***	---	+15%	+20%	***–30%***

[a] Means derived only from mice for which data were available on all days.
[b] Change over entire dosing period (Days –1 to 91).

Table from sponsor

Feed Consumption (1x/wk): ↓~10-15 & ~15-20% (♂&♀ HD) from week ~3-12

Ophthalmoscopy: No test-article related changes noted.

Hematology: No test-article related changes noted.

Clinical Chemistry: No test-article related changes noted.

Urinalysis: Not performed.

Gross Pathology: No test-article related changes noted.

Bone marrow cytology/morphology: No test-article related changes noted.

Organ Weights: adrenal gland ↑24(abs)/32(rel-BW)% (HD ♂)

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings: No test-article related changes noted.

Special Evaluation: None

Toxicokinetics: Note: Blood samples collected pre-dose, 1, 2, 4, 8, 12, and 24 hours post-dose on Day 1 and Day 87 from 3 mice/sex.

Table 26: Mean plasma TK parameters for predominant GS-7977 (PSI-7977) metabolites (PSI-352707 and PSI-6206) in mice following single and multiple (up to 90-days) GS-7977 administration

	Males				Females			
Dose (mg/kg/day) =	**0**	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
PSI-6206								
C_{max} (µg/mL)								
1st dose	---	4.4	9.8	13.7	---	5.8	15.6	23.4
87th dose	---	3.6	15.0	21.9	---	9.9	16.1	31.9
AUC_{last} (h*µg/mL)								
1st dose	---	24.7	71.3	136.1	---	64.6	145.4	249.9
87th dose	---	23.7	81.5	224.3	---	84.9	161.1	361.2
PSI-352707								
C_{max} (µg/mL)								
1st dose	---	1.8	5.2	11.2	---	1.5	5.2	11.8
87th dose	---	1.2	2.8	9.1	---	2.9	5.6	21.7
AUC_{last} (h*µg/mL)								
1st dose	---	8.5	24.6	66.6	---	6.3	20.4	65.4
87th dose	---	4.3	11.9	48.1	---	6.8	21.0	67.1

Table from sponsor

Dosing Solution Analysis: All dosing formulations (0, 20, 60 and 200 mg/ml) were analyzed for uniformity and concentration. All assay results were within an acceptable

concentration range (±13%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

Study title: A 90-DAY ORAL GLP TOXICITY STUDY OF PSI-7977 IN SPRAGUE DAWLEY RATS, WITH A 4-WEEK RECOVERY PERIOD

Study no.:	0515-09234
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 7, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977, 40409003 and 99.2%

Key Study Findings: Toxicity of PSI-7977 was evaluated in rats (15/sex/group) administered oral (gavage) doses of 20, 100, 500 mg/kg or vehicle (95% PEG 400, 5% Tween 80) for 3 months followed by a 4 week treatment-free "recovery" period (5/sex/group). All standard toxicity endpoints were evaluated in this study including clinical signs, body weights, food consumption, ophthalmology, clinical pathology and gross and histopathology. The NOAEL was 500 mg/kg/day (AUC_{last}~68 μg.h/ml for PSI-6206 at 91 days) since no adverse test-article related findings were noted.

Increased incidence of clinical signs (soft feces/diarrhea, wet and/or stained mouth, nares, body surface and/or anogenital region) and body weight gain (males ↑6, 10 & 20% & females ↑11, 13 & 22% compared to controls) was noted at all or most dose levels. Additionally, unilateral inguinal masses were observed at all dose levels in recovery group animals (7 of 15 males) and were due to abscess, minimal to mild inflammation and/or moderately severe dilation of ducts of preputial gland. Although considered somewhat common in rats, this finding was not observed in concurrent controls and occurred at a higher incidence than in historical controls. Thus, this finding appears to be test-article related; however, the clinical significance is unclear.

Table 27: Mean plasma TK parameters for predominant GS-7977 (PSI-7977) metabolites (PSI-352707 and PSI-6206) in rats following single and multiple (up to 90-days) GS-7977 administration

Analyte	PSI-7977 Dose (mg/kg/day)		Day	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	$t_{1/2}$ (h)	C_{max}/Dose (ng/mL/ mg/kg/day)	AUC_{last}/Dose (ng·h/mL/ mg/kg/day)	AUC_{last} Ratio
PSI-352707	20	M	1	536	2	12	2449	1.8	26.8	122.5	NA
			45	293	2	8	1434	1.5	14.7	71.7	0.6
			91	391	2	8	1544	1.6	19.5	77.2	0.6
		F	1	186	1	8	936	ND	9.3	46.8	NA
			45	199	1	6	701	ND	10.0	35.1	0.7
			91	232	1	8	748	1.3	11.6	37.4	0.8
	100	M	1	1710	2	12	10286	1.1	17.1	102.9	NA
			45	1768	4	12	9229	1.1	17.7	92.3	0.9
			91	1728	2	8	7206	3.5	17.3	72.1	0.7
		F	1	950	2	12	4211	1.9	9.5	42.1	NA
			45	1271	1	12	3932	1.7	12.7	39.3	0.9
			91	1336	1	12	5239	2.3	13.4	52.4	1.2
	500	M	1	11171	2	12	55966	1.6	22.3	111.9	NA
			45	8572	2	24	39293	3.5	17.1	78.6	0.7
			91	7405	2	24	37610	3.0	14.8	75.2	0.7
		F	1	3874	2	12	20998	1.5	7.7	42.0	NA
			45	3044	1	12	13084	2.4	6.1	26.2	0.6
			91	4348	2	12	18223	2.0	8.7	36.4	0.9
PSI-6206	20	M	1	408	6	24	4499	6.0	20.4	225.0	NA
			45	274	2	24	4200	15.9	13.7	210.0	0.9
			91	350	2	24	4046	7.7	17.5	202.3	0.9
		F	1	152	1	24	1631	ND	7.6	81.6	NA
			45	294	1	24	2635	34.7	14.7	131.8	1.6
			91	321	2	24	2598	15.6	16.0	129.9	1.6
	100	M	1	1798	6	24	17934	ND	18.0	179.3	NA
			45	2011	4	24	20506	ND	20.1	205.1	1.1
			91	1444	2	24	17857	ND	14.4	178.6	1.0
		F	1	927	2	24	8852	ND	9.3	88.5	NA
			45	1558	2	24	16695	ND	15.6	167.0	1.9
			91	1391	2	24	19160	6.0	13.9	191.6	2.2
	500	M	1	5642	6	24	78360	10.1	11.3	156.7	NA
			45	5414	6	24	79115	ND	10.8	158.2	1.0
			91	4304	6	24	74127	55.8	8.6	148.3	0.9
		F	1	4662	6	24	50625	5.4	9.3	101.3	NA
			45	3811	2	24	55731	ND	7.6	111.5	1.1
			91	5053	6	24	62037	8.1	10.1	124.1	1.2

NA: Not Applicable
ND: Not Determined ($r^2 < 0.85$ or insufficient data)
AUC_{last} Ratio = Day 45 or 91 AUC_{last} / Day 1 AUC_{last}

Table from sponsor

Study title: A SIX-MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN RATS, WITH A 4-WEEK RECOVERY PERIOD

Study no.:	0515-10012
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 18, 2010
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977, 1169-181-1 and 99.78%

Key Study Findings: Toxicity of PSI-7977 was evaluated in rats (15/sex/group) administered oral (gavage) doses of 20, 100, 500 mg/kg or vehicle (95% PEG 400, 5% Tween 80) for 6 months followed by a 4 week treatment-free "recovery" period (5/sex/group). The NOAEL was 500 mg/kg/day (AUC_{last}~66 μg.h/ml for PSI-6206 at 178 days) since no adverse test-article related findings were noted. Increased incidence of various clinical signs including soft feces/diarrhea, wet and/or stained mouth, nares, body surface and/or anogenital region, unkempt appearance and crackles was noted at all or most dose levels.

Methods

Doses:	0, 20, 100, 500 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	PSI-7977 in 95% PEG 400, 5% Tween 80
Species/Strain:	Rat/Hsd: Sprague Dawley®TM SD®TM
Number/Sex/Group:	15 (main), 9 (TK), 5 (recovery)
Age:	~8 wks
Weight:	235±20 (♂) & 181±11 (♀) g
Satellite groups:	Treatment-free (recovery) and TK groups
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None due to test-article. Eight animals died or were euthanized early (refer to Sponsor table below).

Table 28: Noteworthy findings in rats found dead or euthanized moribund

Animal No.	Study week	Status	Noteworthy In-Life and Postmortem Findings	Contributing Cause of Death
1M06	3	FD	Macroscopic: lung lobes discolored dark Microscopic: congestion; hemorrhage - alveoli	Likely accidental aspiration
3F130	8	FD	Macroscopic: lung lobes discolored dark Microscopic: congestion	Likely accidental aspiration
3M53	9	FD	Macroscopic: lung lobes discolored dark, outflow from bronchi, colorless fluid Microscopic hemorrhage - alveoli	Likely accidental aspiration
4M67	10	FD	Macroscopic: lung lobes discolored dark, outflow from bronchi, not collapsed; colorless fluid in thoracic cavity Microscopic: congestion	Likely inadvertent lung intubation
2F104	10	SM	Macroscopic: bladder; many calculi Microscopic: dilatation, inflammation	Urinary tract infection
1M13	11	FD	Macroscopic: esophageal perforation Microscopic: esophageal perforation	Esophageal perforation
2F112	13	FD	Macroscopic: lungs; dark red; Mass, neck region Microscopic: congestion; hemorrhage – alveoli; subcutaneous abscess containing foreign material	Likely esophageal perforation
3M42	18	FD	Macroscopic: lung lobes discolored dark Microscopic: congestion	Likely accidental aspiration

FD = Found Dead
SM = Sacrificed Moribund

Clinical Signs: Increased incidence of numerous clinical signs (refer to Sponsor table below).

Table 29: Incidence (percent affected) for selected clinical signs in rats administered GS-7977 (PSI-7977) or vehicle control.

	Males Affected				Females Affected			
Dosage (mg/kg/day) =	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
Dosing Period								
Nose/nares staining	40	**75**	**90**	**95**	35	**40**	**45**	**95**
Soft feces or diarrhea	15	**45**	**25**	**45**	35	40	30	**50**
Stained around mouth	15	**30**	**75**	**75**	10	5	**20**	**60**
Wet around mouth	0	**15**	**45**	**50**	0	**15**	0	**30**
Unkempt appearance	5	5	**25**	**30**	15	5	5	**40**
Wet body surface	5	10	**30**	**35**	5	20	10	5
Stained anogenital area	55	50	50	**90**	5	15	5	15
Crackles	45	35	45	**90**	20	50	15	65

Body Weights (1x/wk): No test-article related changes noted.

Feed Consumption (1x/wk): No test-article related changes noted.

Ophthalmoscopy: Note: Evaluated pre-dose and at day 182 and 210 (in recovery animals). No test-article related changes noted.

Hematology: No test-article related changes noted.

Clinical Chemistry: Note: Thyroid panel (T3, T4 and TSH) measured. No adverse test-article related changes noted (refer to Sponsor table below).

- Glucose concentration in high-dose females within historical control range.
- TSH differences in treated males not associated with other potentially related findings.

Table 30: Summary of clinical chemistry findings in rats following 6 months of GS-7977 (PSI-7977) administration

	Males				Females			
Dosage (mg/kg/day) =	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
Glucose (mg/dL)								
End of Dosing	202	225	208	215	141	137	138	**175**
End of Recovery period	177	211	202	234	141	137	146	132
TSH (ng/mL)								
End of Dosing	18.7	**15.8**	**14.0**	**13.5**	10.7	12.9	8.9	10.8
End of Recovery period	35.6	31.6	18.8	32.8	18.6	16.1	17.3	24.3

Urinalysis:

- ↑ amount of urobilinogen present (4 mg/dl) in 1 of 5 (HD ♂)
- ↑ amount of protein present (500 mg/dl) in 1 of 5 (HD ♀)
- Various "recovery" animals had similar urobilinogen and protein values (including controls) so findings are not adverse and not clearly drug-related.

Gross Pathology: No test-article related changes noted.

Organ Weights: No test-article related changes noted.

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings: No test-article related changes noted.

Special Evaluation: None

Toxicokinetics: Note: Blood samples collected pre-dose, 1, 2, 4, 6, 8, 12, and 24 hours post-dose on Day 1 and Day 178 from 3 rats/sex.

Table 31: Mean plasma GS-331007 (PSI-6206) TK parameters in rats following single and multiple (for 26-weeks) GS-7977 (PSI-7977) administration

	Males			Females		
Dose (mg/kg/day) =	**20**	**100**	**500**	**20**	**100**	**500**
PSI-6206 [a]						
C_{max} (µg/mL)						
Day 1	0.3	1.4	5.3	0.2	0.7	4.5
Day 178	0.3	1.2	3.8	0.4	1.2	4.7
AUC_{24h} (h*µg/mL)						
Day 1	3.7	15.4	58.9	1.9	7.4	37.2
Day 178	3.9	18.7	66.5	3.5	13.1	65.5

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL

Table from sponsor

Dosing Solution Analysis: All dosing formulations (0, 4, 20 and 100 mg/ml) were analyzed for uniformity and concentration. All assay results were within an acceptable concentration range (±16%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

Study title: A 90-DAY ORAL GLP TOXICITY STUDY OF PSI-7977 IN BEAGLE DOGS, WITH A 4-WEEK RECOVERY PERIOD

Study no.:	0515-09236
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	October 7, 2009
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977, 40410001 & 40410002 and 99.78%

Key Study Findings: Toxicity of PSI-7977 was evaluated in Beagle dogs (4/sex/group) administered oral doses of 20, 100, 500 mg/kg or control (empty gelatin capsule) for 3 months followed by a 4 week treatment-free "recovery" period (2/sex/group). All standard toxicity endpoints were evaluated in this study including clinical signs, body weights, food consumption, ophthalmology, ECG parameters, clinical pathology and gross and histopathology. The NOAEL was considered to be 100 mg/kg/day (AUC_{last}~89 µg.h/ml for PSI-6206 at 90 days) since adverse GI-related findings consisting of black foci on stomach mucosa corresponding to hemorrhage of the lamina propria of the pyloric stomach were noted in one male at 500 mg/kg/day.

A slight decrease in circulating erythron mass (up to 12% decrease in RBC's, Hct & Hb), the apparent result of suppression of erythropoiesis in bone marrow (reductions

in number of erythroid cells and precursors, mean erythroid maturation index and erythroid/myeloid ratio) and increased thyroid weight (males) were also observed at 500 mg/kg/day. In addition, dose-dependent increases in the frequency and incidence of emesis and soft stool/diarrhea, associated with very slight reductions in body weight gain, were observed at ≥100 mg/kg/day. These toxicity findings were absent in "recovery" animals.

Table 32: Mean plasma TK parameters for GS-7977 (PSI-7977) and predominant metabolites (PSI-352707 and PSI-6206) in dogs following single and multiple (up to 90-days) GS-7977 administration

	Males			Females		
Dose (mg/kg/day) =	**20**	**100**	**500**	**20**	**100**	**500**
PSI-7977 [a]						
C_{max} (µg/mL)						
Day 1	1.0	10.2	28.5	0.5	5.9	20.0
Day 43	0.9	14.3	47.8	0.6	14.0	40.1
Day 90	0.6	12.3	34.5	0.5	9.0	43.0
AUC_{last} (h*µg/mL)						
Day 1	1.8	19.7	87.1	0.7	11.2	60.6
Day 43	1.2	24.2	147.2	1.0	27.6	109.0
Day90	0.9	24.4	99.7	1.2	19.9	153.8
PSI-6206 [a]						
C_{max} (µg/mL)						
Day 1	4.1	9.1	28.2	4.1	12.5	23.6
Day 43	2.6	7.2	33.2	2.1	8.3	27.9
Day 90	1.8	6.8	23.2	2.3	8.3	29.3
AUC_{last} (h*µg/mL)						
Day 1	24.4	86.3	342.9	23.5	99.1	251.4
Day 43	17.4	94.2	394.4	18.3	104.9	313.5
Day90	17.1	86.8	278.1	20.8	90.2	349.2
PSI-352707 [a]						
C_{max} (µg/mL)						
Day 1	0.6	1.9	6.5	0.4	2.7	4.0
Day 43	0.6	2.5	7.7	0.5	2.8	5.6
Day 90	0.3	2.2	5.6	0.4	2.0	6.7
AUC_{last} (h*µg/mL)						
Day 1	1.4	6.9	34.3	1.0	7.3	23.4
Day 43	1.4	8.5	38.5	1.3	9.3	27.0
Day 90	1.1	7.7	29.1	1.3	7.6	33.6

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL

Table from sponsor

Study title(s): THE SIX MONTH INTERIM FINAL REPORT FOR: A NINE MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN BEAGLE DOGS WITH A SIX-MONTH INTERIM ANALYSIS AND RECOVERY PERIODS and A NINE MONTH ORAL GLP TOXICITY STUDY OF PSI-7977 IN BEAGLE DOGS WITH A SIX-MONTH INTERIM ANALYSIS AND RECOVERY PERIODS (NINE-MONTH SACRIFICE)

Study no.:	0515-10062
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 13, 2010
GLP compliance:	Yes
QA statement:	Yes (final reports)
Drug, lot #, and % purity:	PSI-7977, 40410001 & 40410002 and 99.2 & 99.7%

Key Study Findings: Toxicity of PSI-7977 was evaluated in Beagle dogs (4/sex/group) administered oral doses of 100, 500 mg/kg or control (empty gelatin capsule) for 6 months or 20, 100, 500 mg/kg or control for 9 months, each followed by 4 week treatment-free "recovery" periods (2/sex/group). The NOAEL was considered to be 100 mg/kg/day ($AUC_{0\text{-}24hr}$~90 μg.h/ml for PSI-6206 at 273 days) since adverse GI-related findings were observed at 500 mg/kg including increased salivation, emesis, diarrhea and intestinal hemorrhage likely originating from the upper GI tract (hemorrhage within the lamina propria of the jejunum mucosa observed) resulting in moribund condition and euthanasia of one male prior to 6 months.

A slight decrease in circulating erythron mass (up to 9% decrease in RBC's, Hct & Hb compared to controls) was observed at 9 months, the possible result of effects on erythropoiesis in bone marrow at 6 months, which included higher relative percents of erythroid precursors, increased erythrophagocytosis, presence of large erythroid precursors and/or atypical erythroid maturation. Additional findings included slight increases in serum ALP (females) and APTT at 500 mg/kg at 6 and 9 months, the presence of blood, bilirubin and urobilinogen in urine at 100 and 500 mg/kg at 6 months only and decreased thymus weight at 500 mg/kg/day at 9 months.

Methods

Doses:	0, 20, 100, 500 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	NA
Formulation/Vehicle:	PSI-7977 in gelatin capsule (size 11)
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4 (main), 2 (recovery)-refer to sponsor Table below
Age:	6-7 months
Weight:	5.6-8.6 kg
Satellite groups:	Treatment-free (recovery)
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Table 33: Study design summary for 9 month dog toxicology study

Group	Dosage mg/kg	Six Month Terminal Sacrifice		Six Month Recovery Sacrifice		Nine Month Terminal Sacrifice		Nine Month Recovery Sacrifice		Animal No.	
		M	F	M	F	M	F	M	F	M	F
DBM1	0 (vehicle)	4	4	2	2	4	4	2	2	1-12	41-52
DBM2	20	-	-	-	-	4	4	2	2	13-18	53-58
DBM3	100	4	4	-	-	4	4	2	2	19-28	59-68
DBM4	500	4	4	2	2	4	4	2	2	29-40	69-80

Observations and Results

Mortality: A single animal (HD ♂) was euthanized in moribund condition on Day 172 after a sudden onset of clinical signs including the presence of a large amount of melena, melena stained perianal region, emesis, generalized paresis and depression, hypothermia (BT= 97.0˚), mucous membranes pale, delayed capillary refill time ≤4 sec (normal ≤2 sec), tachycardia (HR= 126 beats/min) and tachypnea (RR= 60 breaths/min). Note: Melena defined as an abnormal black tarry stool containing digested blood (with a distinctive odor). It often results from bleeding in the upper GI tract and may be a sign of peptic ulcer or small bowel disease.

A large amount of red fluid passed from the dog's rectum at euthanasia. Necropsy findings consisted of reddened intestinal mucosa, gelatinous red intestinal contents from the jejunum through the rectum, a small spleen and large mesenteric lymph nodes. The exact source of bleeding was not identified despite careful examination. Microscopic examination revealed hemorrhage within the lamina propria of the jejunum mucosa and coagulated blood on the mucosal surfaces of the distal

intestinal tract. This animal also had moderate vacuolation of the myocardium (possibly the result of hypotension, tachycardia and hypovolemia). Moderate to moderately severe lymphoid depletion of the thymus, spleen, and lymph nodes were also present (likely stress response). Although considered potentially related to PSI-7977, findings also appear consistent with idiopathic hemorrhagic gastroenteritis of spontaneous origin.

Clinical Signs: Increased incidence and/or frequency of several clinical signs were observed (refer to Sponsor table below). These signs were absent during the recovery periods (both post-6-month and 9-month). Incidence and frequency of these findings did not increase with the additional 3-months of administration (6-month findings similar to those present during 9-months of dosing).

Table 34: Selected clinical signs in dogs administered GS-7977 (PSI-7977) for 6 months

	Males Affected				Females Affected			
Dosage (mg/kg/day) =	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
Soft feces or diarrhea								
Total dogs affected	6/12	4/6	7/10	**11/12**	7/12	4/6	7/10	8/12
% dogs affected	50	67	70	**92**	58	67	70	67
Frequency								
Instances/total dog-weeks*	23/324	14/162	35/270	**61/324**	25/324	6/162	24/270	22/324
% of instances	7.1	8.6	13.0	**18.8**	7.7	3.7	8.9	6.8
Emesis (food, frothy, fluid, material)								
Total dogs affected	3/12	1/6	5/10	**12/12**	4/12	2/6	**4/10**	**12/12**
% dogs affected	25	17	50	**100**	33	33	**40**	**100**
Frequency								
Instances/total dog-weeks*	3/234	2/162	11/270	**77/324**	6/324	2/162	**5/270**	**112/324**
% of instances	0.9	1.2	4.1	**23.8**	1.9	1.2	1.9	**34.6**
Increased salivation								
Incidence								
Dogs affected/total dogs	1/12	0/6	0/10	**6/12**	0/12	1/6	1/10	**8/12**
% affected	8	-	-	**50**	-	17	10	**67**

*Dogs-weeks = Number of dogs in the group x 27 weeks (number of weeks that clinical signs were recorded.

Body Weights (1x/wk): No test-article related changes noted.

Feed Consumption (1x/day):

- Numerous daily occurrences of statistically significant reduced consumption predominantly between 1 & 6 months (HD ♀).

Ophthalmoscopy: Note: Evaluated pre-dose and during weeks 26 and 30 (6-month interval) or 39 and 43 (9-month interval). No test-article related changes noted.

Hematology: Note: Measured pre-test and at weeks 26 and 30 (6-month interval) or 39 and 43 (9-month interval). No test-article related changes noted following 6-months of PSI-7977 administration.

- RBC's ↓8-9% versus control (HD ♂ & ♀) (actually increased versus pre-test)- similar to Hb results below

Table 35: Selected hematology values in dogs administered GS-7977 (PSI-7977) for 9 months

	Males Affected				Females Affected			
Dosage (mg/kg/day) =	**0**	**20**	**100**	**500**	**0**	**20**	**100**	**500**
Hemoglobin (g/dL)								
Dosing phase								
Pretest	14.2	14.7	13.4	13.5	14.2	14.6	13.9	14.6
Day 269	17.9	17.5	18.1	**16.5**	17.4	17.9	18.3	**16.6**
Vs. pretest	+3.7	+2.8	+4.7	+3.0	+3.2	+3.3	+4.4	**+2.0**
Recovery phase*								
Day 269	18.3	17.1	17.9	16.7	16.0	17.5	17.7	16.0
Day 301	16.1	17.7	16.1	15.7	15.8	16.7	17.3	16.1
Vs. Day 269	–2.2	+0.6	–1.8	–1.0	–0.2	–0.8	–0.4	+0.1
MCV (fL)								
Dosing phase								
Pretest	64.5	65.4	64.7	63.8	64.2	63.7	63.7	64.0
Day 269	66.9	67.6	**68.5**	**68.7**	66.7	**67.2**	**67.7**	**68.7**
Vs. pretest	+2.4	+2.2	**+3.8**	**+4.9**	+2.5	**+3.5**	**+4.0**	**+4.7**
Recovery phase*								
Day 269	68.4	67.5	69.8	70.4	66.0	65.4	65.4	68.9
Day 301	67.9	66.9	69.6	69.1	65.1	65.6	65.4	67.4
Vs. Day 269	–0.5	–0.6	–0.2	–1.3	–0.9	+0.2	±0	–1.5

*Means derived only from dogs for which data were available on both days.

Coagulation: Note: Measured pre-test and at weeks 26 and 30 (6-month interval) or 39 and 43 (9-month interval).

- APTT ↑10% (HD ♀) versus pre-test at 6 months (25.3 versus 23.0 sec) (controls= 22.3 versus 21.0 pre-test)
- APTT ↑19% (HD ♀) versus pre-test at 9 months in 1 of 6 animals (25.8 versus 21.6 sec).
- APTT ↑19% (HD ♂) versus pre-test at 6 months (24.4 versus 20.5 sec) (controls= 20.8 versus 20.9 pre-test)
- APTT ↑6% (HD ♂) versus pre-test at 9 months (23.0 versus 21.6 sec) (controls= 19.5 versus 20.0 pre-test). Effect due primarily to one male with APTT ↑29% versus pre-test (28.4 versus 22.0 sec).
- APTT in treated animals was similar to control values after "recovery" period.
- These minor APTT increases appear test-article related, but do not progress with increasing duration and are not considered adverse.

- Of note, the animal euthanized prior to 6-months had APTT values of 19.8 and 24.1 sec pre-test and immediately prior to euthanasia, respectively. Although feasible that the APTT increase in this animal contributed to intestinal hemorrhaging, the magnitude of the increase appears to be too small to be the cause of bleeding.

Clinical Chemistry: Note: Measured pre-test and at weeks 26 and 30 (6-month interval) or 39 and 43 (9-month interval).

- ALP ↑ 58 & 23% versus controls and pre-test values, respectively, at 6 months & ↑ 83 & 27% versus controls and pre-test values, respectively, at 9 months (HD ♀). One animal had ALP ↑173% (versus pre-test values) (biggest 9 month increase).
- Values were reduced to levels similar to control after "recovery" period.
- ALP increases are of minimal toxicological significance.

Urinalysis: Note: Measured pre-test and at weeks 26 and 30 (6-month interval) or 39 and 43 (9-month interval) (urine samples collected directly from bladder at euthanasia). See Sponsor table below. No test-article related changes noted following 9-months of PSI-7977 administration.

- Bilirubin present at 1,1,3 (♂ controls) and 1,3,3,3 mg/dl (MD & HD ♂) at 6 months
- Blood present at 50 to 250 ry/μ in 2 of 4 (MD & HD ♂) at 6 months
- Bilirubin present at 1,1,1 (♀ controls), 1,1,3,3 (MD ♀) and 1,1,1,3 mg/dl (HD ♀) at 6 months

Table 36: Selected urinalysis values in dogs administered GS-7977 (PSI-7977) for 6 months

	Males			Females		
Dosage (mg/kg/day) =	**0**	**100**	**500**	**0**	**100**	**500**
Urobilinogen (mg/dL)						
Dosing phase*						
Pretest	1,4,4	1,1,1,4	0,1,1,1	0,0,0,0	0,0,1,1	0,1,1,1
Day 183	1,1,4	**4,4,4,8**	**4,4,8,8**	1,1,4,4	**4,4,4,4**	**4,4,4,4**
Recovery Phase*						
Pretest	1,1	-	1,1	1,1	-	0,1
Day 211	4,4	-	**8,8**	1,4	-	0,4
Granular casts in urine sediment*						
Pretest	0/2	0/2	0/2	0/1	0/3	0/1
Day 183	NE	0/2	**1/2**	0/2	NE	0/1
Day 211	NE	-	NE	0/1	-	0/1

*Information only from dogs for which data were available on all days.
Urobilinogen, 0=Normal
NE = None Evaluated

ECG: Note: Measured at least twice pre-test and 4 & 8 hours post-dose at weeks 26 (6-month interval) and 39 (9-month interval) and after "recovery" period at weeks 30 and 43. No test-article related changes noted.

Gross Pathology: No test-article related changes noted.

Organ Weights:

- ↑ spleen weight (see Sponsor table below) due primarily to one male [absolute: 102 g, relative (% BW): 1.2]. This male had no correlative findings reported.

Table 37: Selected mean organ weights in dogs administered GS-7977 (PSI-7977) for 6 months

	Males			Females		
Dosage (mg/kg/day)	**0**	**100**	**500**	**0**	**100**	**500**
Livers						
Absolute (g)						
End of Dosing (n=4/group)	250	**209**	**218**	241	**218**	230
End of Recovery (n=2/group)	234	-	**219**	194	-	199
Relative (% body wt)						
End of Dosing (n=4/group)	2.91	**2.34**	**2.65**	3.05	**2.82**	3.02
End of Recovery (n=2/group)	2.42	-	**2.27**	2.33	-	2.66
Lungs						
Absolute (g)						
End of Dosing (n=4/group)	76	84	86	87	95	**112**
End of Recovery (n=2/group)	80	-	85	101	-	**129**
Relative (% body wt)						
End of Dosing (n=4/group)	0.88	0.94	1.04	1.13	**1.22**	**1.47**
End of Recovery (n=2/group)	0.82	-	0.88	1.21	-	**1.75**
Spleen						
Absolute (g)						
End of Dosing (n=4/group)	51	62	**78**	60	52	47
End of Recovery (n=2/group)	90	-	77	61	-	40
Relative (% body wt)						
End of Dosing (n=4/group)	0.57	0.69	**0.94**	0.74	0.67	0.59
End of Recovery (n=2/group)	0.92	-	0.80	0.73	-	0.53

Table 38: Selected mean organ weights in dogs administered GS-7977 (PSI-7977) for 9 months

	Males					Females			
Dosage (mg/kg/day)	**0**	**20**	**100**	**500**		**0**	**20**	**100**	**500**
Thymus									
Absolute (g)									
End of Dosing (n=4/group)*	19.5	12.2	**9.1**	**9.0**		10.5	8.9	**8.0**	**8.3**
End of Recovery (n=2/group)	13.6	11.0	9.3	11.2		8.2	7.3	8.7	23.7
Relative (% body wt)									
End of Dosing (n=4/group)*	0.22	0.13	**0.09**	**0.10**		0.12	0.11	**0.09**	**`0.10**
End of Recovery (n=2/group)	0.13	0.11	0.10	0.10		0.10	0.08	0.10	0.26

*n=3 in the male, 500 mg/kg/day group.

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings: No test-article related changes noted following 9-months of PSI-7977 administration.

Bone marrow findings from cytology report in animals sacrificed at 6-months included:

- Limited erythroid alterations including higher group mean basophilic rubricyte and erythroid maturation index values (HD ♀) and higher relative percents of erythroid precursors (prorubricyte, basophilic rubricyte, polychromatophilic rubricyte, and/or total erythroid percents) (HD ♂ & ♀).
- minimal (1 of 4) to mild (2 of 4) ↑ erythrophagocytosis (HD ♀)
- minimal to mild presence of large erythroid precursors and/or atypical erythroid maturation (HD ♂ & ♀).
- These minor cytology findings were not associated with changes in hematology parameters (erythroid mass or reticulocyte counts) or histopathologic findings in bone marrow or sites of potential extramedullary hematopoiesis in animals sacrificed at 6 months.

Special Evaluation: None

Toxicokinetics

Table 39: Mean plasma GS-331007 (PSI-6206) TK parameters in dogs following single and multiple (for 26-weeks) GS-7977 (PSI-7977) administration

	Males		Females	
Dose (mg/kg/day) =	**100**	**500**	**100**	**500**
PSI-6206 [a]				
C_{max} (µg/mL)				
Day 1	9.4	20.1	10.2	20.4
Day 182	5.3	16.2	8.3	23.8
AUC_{24h} (h*µg/mL)				
Day 1	94.2	193.3	104.0	215.5
Day 182	72.3	209.1	106.5	247.4

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL

Table 40: Mean plasma GS-331007 (PSI-6206) TK parameters in dogs following single and multiple (for 39-weeks) GS-7977 (PSI-7977) administration

	Males			Females		
Dose (mg/kg/day) =	**20**	**100**	**500**	**20**	**100**	**500**
PSI-6206 [a]						
C_{max} (µg/mL)						
Day 1	2.7	10.2	28.2	2.7	11.9	20.0
Day 273	3.8	6.6	15.0	2.7	9.9	20.1
AUC_{24}(h*µg/mL)						
Day 1	26.9	98.2	322.9	23.0	103.9	188.4
Day 273	26.6	76.3	175.4	27.0	103.7	215.2

[a] Values rounded to the nearest 0.1 µg/mL or h*µg/mL

Table from sponsor

Dosing Solution Analysis: Not applicable

7 Genetic Toxicology

Note: The following study reviews were taken directly from the original (b) (4) review by Dr. Pritam Verma.

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

Study title: PSI-7851: Ames reverse mutation study in Salmonella and Escherichia coli

Study no.:	SA-PSI-7851-08-003
Study report location:	4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 9, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PSI-7851, 1169-030-7 and 97.1%

Key Study Findings: PSI-7851 was tested for its ability to induce reverse mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* tester strain WP2*uvr*A, either in the presence or absence of mammalian metabolic activation system (liver S9). PSI- 7851 is rapidly metabolized by liver S9. The study was conducted in two phases: a Cytotoxicity Phase to identify the maximum tolerated test concentration and screen for mutagenic activity and a Definitive Phase to fully evaluate mutagenic potential. In both phases, each tester strain was exposed to PSI-7851 at up to 5000 µg/plate in the presence or absence of S9. No cytotoxicity was observed at any concentration in either phase. In both phases, PSI-7851 did not increase the number of mutant colonies at any concentration in any tester strain. Based on these results, PSI-7851 and its metabolites were negative for mutagenic potential *in vitro*.

Methods: PSI-7851 was tested for its ability to induce reverse mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* tester strain WP2*uvr*A, either in the presence or absence of mammalian metabolic activation system (liver S9).

Strains/species/cell line: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* tester strain WP2*uvr*A, either in the presence or absence of mammalian metabolic activation system (liver S9).

Mixed function oxidase: crude rat liver extract (S-9) provided the mixed function oxidase metabolic activation system. The extract was obtained from male Sprague-Dawley rats which were stressed with a single intraperitoneal injection of Aroclor 1250 (500 mg/kg) 5 days prior to sacrifice.

Doses used in definitive study: 0 to 5000 µg/plate

Basis of dose selection: a non-GLP exploratory study.

Negative controls: DMSO

Positive controls: Sodium azide (NaAz), 9-Aminoacridine (9AC), 2-Nitrofluorene (2NF), 2-aminoanthracene and methyl methanesulfonate

Incubation and sampling times: after solidification of the agar overlay, all plates were incubated aerobically at 37 degree C in darkness for 46-48 hr.

Results

No meaningful increases in colony counts were obtained with any strain following exposure to the test article in either the plate incorporation or pre-incubation assay in the presence or absence of S9 mix. All criteria for a valid study were met. It is concluded that PSI-7851 did not show any evidence of genotoxic activity in this *in vitro* mutagenicity assay.

Study validity: valid

Study outcome: PSI-7851 was not mutagenic in the Ames assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: PSI-7851 Chromosome Aberration Study in Human Lymphocytes

Study no.:	SA-PSI-7851-08-004
Study report location:	4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 16, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PSI-7851, 1169-030-7 and 97.1%

Key Study Findings: PSI-7851 was tested for clastogenic potential by evaluating its ability to induce chromosomal aberrations in human peripheral blood lymphocytes in the presence and absence of the mammalian metabolic activation system (liver S9). PSI-7851 is rapidly metabolized by human lymphocytes and liver S9. The study was conducted in two phases: a Cytotoxicity Phase to identify the maximum tolerated test concentrations for each test condition and a Definitive Phase to evaluate clastogenic potential. In both phases, cells were exposed at up to 5000 µg/mL for 4 hours with and without S9 and for 20 hours without S9. In the Cytotoxicity Phase, toxicity was observed at 5000 µg/mL in cells exposed for 4 hours without S9, at ≥1500 µg/mL in cells exposed for 20 hours without S9, but no toxicity was observed at any test concentration in cells exposed for 4 hours with S9. In the Definitive Phase, PSI-7851 did not produce a significant increase in the percentage of structural or numerical chromosome aberrations under any of the test conditions. Based on the results, PSI-7851 and its metabolites were negative for clastogenic potential *in vitro*.

Methods

The study was conducted in two phases: a Cytotoxicity Phase to identify the maximum tolerated test concentrations for each test condition and a Definitive Phase to evaluate clastogenic potential. In both phases, cells were exposed at up to 5000 µg/mL for 4 hours with and without S9 and for 20 hours without S9. In the Cytotoxicity Phase, toxicity was observed at 5000 µg/mL in cells exposed for 4 hours without S9, at ≥1500 µg/mL in cells exposed for 20 hours without S9.

Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive study: 1500-5000 µg/ml

Basis of dose selection: dose range finding study.

Negative controls: DMSO

Positive controls: Mitomycin C (MMC) and Cyclophosphamide monohydrate (cyclophosphamide, CP)

Incubation and sampling times: See table below.

Results: In the Cytotoxicity Phase, toxicity was observed at 5000 µg/mL in cells exposed for 4 hours without S9, at ≥1500 µg/mL in cells exposed for 20 hours without S9, but no toxicity was observed at any test concentration in cells exposed for 4 hours with S9. In the Definitive Phase, PSI-7851 did not produce a significant increase in the percentage of structural or numerical chromosome aberrations under any of the test conditions.

Table 41: Summary of Chromosome Aberration Study in Human Lymphocytes with GS-9851 (PSI-7851)

Treatment Time (hours)	Recovery Time (hours)	Harvest Time (hours)	S9	Mitotic Index Reduction[1] at highest dose scored (µg/mL)	LED[2] for Structural Aberrations (µg/mL)	LED[2] for Numerical Aberrations (µg/mL)
4	16	20	-	65% at 5000	None	None
20	0	20	-	53% at 1500	None	None
4	16	20	+	56% at 5000	None	None

[1] relative to the solvent control at high dose evaluated for chromosome aberrations
[2] LED = lowest effective dose level

Based on the results, PSI-7851 and its metabolites were negative for clastogenic potential *in vitro*.

Study validity: valid

Study outcome: PSI-7851 did not show any evidence of genotoxic activity in this *in vitro* test for induction of chromosome damage.

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

Study title: Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of PSI-7851

Study no:	SA-PSI-7851-08-005
Study report location:	4.2.3.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 21, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PSI-7851, 1169-030-7 and 97.1%

Key Study Findings: This study was conducted to evaluate the clastogenic (genotoxic) potential of PSI-7851 in vivo, as measured by its ability to induce micronucleated polychromatic erythrocytes (MPCEs) in mouse bone marrow. PSI-7851 did not produce mortality or clinical signs of toxicity at any dose level. Based on bone marrow analysis, the following were observed: PSI-7851 did not affect the ratio of polychromatic erythrocytes to total erythrocytes at any dose level, suggesting that it did not inhibit erythropoiesis; PSI-7851 did not significantly increase the incidence of MPCEs at any dose level in either sex at either bone marrow collection time; the incidence of MPCEs in the vehicle control and positive control groups was as expected and consistent with the historical control data. Based on these results, PSI-7851 and its metabolites PSI-352707, PSI-6206, and PSI-7411 were negative for clastogenic potential *in vivo*.

Methods

The study was conducted in two phases: a Micronucleus Phase and a Toxicokinetic Phase. In the Micronucleus Phase, groups of 10 mice (5/sex) were given single oral doses of vehicle, positive control article, or PSI-7851 at 500, 1000, or 2000 mg/kg and then were euthanized 24 hours later to harvest bone marrow. Two additional groups of 10 mice (5/sex) were given single oral doses of vehicle or PSI-7851 at 2000 mg/kg and then were euthanized 48 hours later to harvest bone marrow. In addition, a separate group of 10 mice (5/sex) were given single oral doses of PSI-7851 at 2000 mg/kg to be used as replacement group in the event of mortality at high dose. These mice were euthanized at 48 hours but no bone marrow was collected. In life, mice were observed for clinical signs of toxicity. Bone marrow was examined microscopically to determine the PCE/EC ratio as a measure of bone marrow toxicity and MPCE/PCE ratio as a measure of clastogenic potential. In the Toxicokinetic Phase, groups of 24 mice (12/sex) were given single oral doses of PSI-7851 at 500, 1000, or 2000 mg/kg. In each group, 6 mice (3/sex) were euthanized at 0.5, 2, 4, and 8 hours postdose, and plasma and liver samples were collected. A single group of 6 mice (3/sex) were given single oral doses

of vehicle and then euthanized at 2 hours postdose, and plasma and liver samples were collected.

Vehicle: The vehicle was 30% PEG400, 30% Tween 20, 20% Corn oil, and 20% Water.

Doses used in definitive study: 500, 1000, or 2000 mg/kg

Basis of dose selection: dose range finding study.

Positive control: Cyclophosphamide monohydrate

Results: The following results were generated: PSI-7851, given orally, did not produce mortality or clinical signs of toxicity at any dose level. All animals appeared normal following the treatment. PSI-7851 did not affect the ratio of polychromatic erythrocytes to total erythrocytes at any dose level, suggesting that it did not inhibit erythropoiesis. PSI-7851 did not significantly increase the incidence of MPCEs at any dose level in either sex at either bone marrow collection time. The incidence of MPCEs in the vehicle control and positive control groups was as expected and consistent with the historical control data.

Plasma analysis results indicate that the exposure of animals to PSI-7851 was limited presumably due to the instability of PSI-7851 in mouse blood. PSI-352707 (the intermediate form between prodrug and parent) and PSI-6206 (the uridine metabolite) were readily detectable at all dose levels in both sexes. Liver exposure to PSI-7851 reached peak concentrations at 0.5 hours post-dose. The PSI- 352707 reached peak concentrations at 2-4 hours post-dose. The PSI-6206 was readily detectable across all animals reaching peak concentrations at 4 to 8 hours post-dose. The monophosphate form, PSI-7411, was present in the highest concentrations followed by the diphosphate, PSI-7410, and then the tri-phosphate, PSI-7409.

Table 42: Mean Systemic Exposures to GS-9851 and Metabolites in Mice administered 1000 mg/kg of GS-9851

	GS-9851 (PSI-7851)		GS-566500 (PSI-352707)		GS-331007 (PSI-6206)		GS-606965 (PSI-7411)	
	male	female	male	female	male	female	male	female
C_{max} (μg/ml)	0.18	0.39	24.4	23.5	22.8	11.9	0.09	0.10
AUC_{0-24} (μg·h/ml)	NA	0.92	68.6	67.9	138	71.7	0.45	0.42

Table 43: Mean Systemic Exposures to GS-9851 and Metabolites in Mice administered 2000 mg/kg of GS-9851

	GS-9851 (PSI-7851)		GS-566500 (PSI-352707)		GS-331007 (PSI-6206)		GS-606965 (PSI-7411)	
	male	female	male	female	male	female	male	female
C_{max} (μg/ml)	0.41	1.0	98.0	46.6	22.3	20.7	0.23	0.15
AUC_{0-24} (μg·h/ml)	0.72	3.6	194.4	134.2	132.9	115.1	1.1	0.59

Table 44: Mean Liver Concentration (μg/g) of GS-9851 and Metabolites in Male Mice administered 1000 mg/kg of GS-9851

post-dose	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-606965 (PSI-7411)	GS-607596 (PSI-7410)	GS-461203 (PSI-7409)
0.5 hr	193.9*	1,241	67.4	45.1	7.6	0.4
2 hr	57.1	1,175	71.0	105.4	28.2	1.6
4 hr	29.4	1,245	81.5	130.8	44.4	2.4
8 hr	21.7	790	116.6	172.3	56.4	4.0
*high mean value due to one animal (464.3)						

Table 45: Mean Liver Concentration (μg/g) of GS-9851 and Metabolites in Female Mice administered 1000 mg/kg of GS-9851

post-dose	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-606965 (PSI-7411)	GS-607596 (PSI-7410)	GS-461203 (PSI-7409)
0.5 hr	58.3	708	50.9	25.3	5.1	0.5
2 hr	41.0	1,139	68.5	59.5	20.7	2.5
4 hr	14.3	1,041	103.5	122.6	41.5	2.6
8 hr	7.0	368	69.8	60.6	25.8	2.4

Table 46: Mean Liver Concentration (μg/g) of GS-9851 and Metabolites in Male Mice administered 2000 mg/kg of GS-9851

post-dose	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-606965 (PSI-7411)	GS-607596 (PSI-7410)	GS-461203 (PSI-7409)
0.5 hr	209.6	2,297	71.2	86.4	43.4	9.0
2 hr	139.9	2,586	96.5	155.2	121.5	13.7
4 hr	106.3	1,882	195.3	297.1	187.3	8.9
8 hr	78.1	1,242	152.8	270.9	238.3	19.1

Table 47: Mean Liver Concentration (μg/g) of GS-9851 and Metabolites in Female Mice administered 2000 mg/kg of GS-9851

post-dose	GS-9851 (PSI-7851)	GS-566500 (PSI-352707)	GS-331007 (PSI-6206)	GS-606965 (PSI-7411)	GS-607596 (PSI-7410)	GS-461203 (PSI-7409)
0.5 hr	231.0	1,654	68.7	59.5	30.8	1.9
2 hr	103.6	2,428	69.0	109.8	79.3	6.1
4 hr	46.8	1,508	142.1	231.4	146.9	8.3
8 hr	10.1	727	132.5	177.4	225.4	18.8

Study validity: valid

Study outcome: Based on these results, PSI-7851 and its metabolites PSI-352707, PSI-6206, and PSI-7411 were negative for clastogenic potential *in vivo*.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

None submitted. Note: Rat and mouse 2-year studies with GS-7977 are currently in progress.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: AN ORAL GLP FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION STUDY OF PSI-7977 IN RATS

Study no.:	0515-10239
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 3, 2010
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	PSI-7977, 40410001 & 40410002 and 99.2 & 99.7%

Key Study Findings: Potential PSI-7977 related effects on fertility and early embryonic development (EED) were evaluated in rats (22/sex/group) administered oral doses of 20, 100, 500 mg/kg or vehicle (95% PEG 400, 5% Tween 80) or water controls for 28 (males) or 14 (females) days prior to cohabitation and during (up to) 15 days of cohabitation, with females dosed through GD7. The NOEL for fertility and EED was

considered to be 500 mg/kg/day since no significant effects were observed. Increased incidence of various clinical signs including soft feces/diarrhea and wet and/or stained mouth, nares, body surface and/or anogenital region was noted in males and females at all or most dose levels. In addition, an increased incidence of unkempt and dehydrated appearance was described in females at 500 mg/kg/day.

Methods

Doses:	0 (vehicle), 0 (water), 20, 100, 500 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Oral gavage
Route of administration:	5 ml/kg
Formulation/Vehicle:	PSI-7977 in 95% PEG 400, 5% Tween 80
Species/Strain:	Rat/Hsd: Sprague Dawley®TM SD®TM
Number/Sex/Group:	22
Satellite groups:	None
Study design:	PSI-7977 administered for 28 (♂) or 14 days (♀) prior to cohabitation, during up to 15 days of cohabitation w/ females dosed through GD7 and males dosed through the day prior to necropsy (day after final female necropsy). Females sacrificed on GD15.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None due to test-article. Refer to Sponsor table below.

Table 48: Findings in rats either found dead or sacrificed moribund

Animal No.	Study Day	Status	Noteworthy In-Life and Postmortem Findings	Likely Contributing Cause of Death
4F189	7	SM	Mottled lung lobes and gas-filled stomach and cecum found at necropsy	Accidental aspiration
4M82	37	SM	Red lung lobes, colorless fluid in trachea, and perforated esophagus found at necropsy	Accidental aspiration and/ or esophageal perforation
5F208	2	FD	Dark lung lobes found at necropsy	Accidental aspiration
5M97	58	FD	Red lung lobes, colorless fluid in trachea, red fluid in thoracic cavity, and perforated esophagus found at necropsy	Accidental aspiration and/or esophageal perforation

FD = Found Dead
SM = Sacrificed Moribund

Clinical Signs

Table 49: Incidence of selected clinical signs in GS-7977 (PSI-7977) treated rats

	Males					Females				
Test Material =	**H_2O**	**Veh**	**PSI-7977**			**H_2O**	**Veh**	**PSI-7977**		
Dosage (mg/kg/day) =	**0**	**0**	**20**	**100**	**500**	**0***	**0***	**20**	**100**	**500**
Staining around nose/nares	0	14	**19**	**22**	**22**	0	2	**11**	**10**	**21**
Crackles	0	12	11	12	14	0	5	4	5	6
Soft feces or diarrhea	0	1	5	3	3	0	2	0	2	4
Staining around mouth	0	3	**18**	**19**	**22**	0	0	2	**8**	**16**
Staining around urogenital	0	1	**3**	**2**	**6**	0	0	1	1	**11**
Unkempt Appearance	0	2	3	5	3	0	0	0	2	**11**
Wet around mouth	0	0	**8**	**7**	**18**	0	0	1	1	**8**
Wet/stained body surface	0	0	**4**	**1**	**7**	0	0	0	**2**	**5**
Material around eyes	1	0	0	**4**	1	0	0	0	**3**	**3**
Appears Dehydrated	0	1	1	0	1	0	1	0	1	**5**

Body Weight: No test-article related changes noted.

- Mean BW gain decreased up to 35% in males that was attributed to the vehicle, since observed in the vehicle control and at all PSI-7977 dose levels (compared to those receiving the water control).

Feed Consumption: No test-article related changes noted.

- Mean consumption slightly higher in water controls compared to other groups.

Toxicokinetics: Not performed

Dosing Solution Analysis: All dosing formulations (0, 4, 20 and 100 mg/ml) were analyzed for uniformity and concentration. All assay results were within an acceptable concentration range (±11%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

Necropsy: No test-article related changes noted.

Estrous Cycling: No test-article related changes noted.

Fertility Parameters (mating/fertility index, corpora lutea, early/late resorption, pre-/post-implantation loss, etc.):

- Slight decrease in mean pre-coital interval was observed (refer to Sponsor table below). Note: This effect was not significant statistically in the HD group compared to vehicle controls.
- No significant effect was observed on any other parameter.

Table 50: Summary of mean pre-coital interval following GS-7977 (PSI-7977) administration in males and females

Test Material =	H_2O	Veh	PSI-7977		
Dosage (mg/kg/day) =	**0**	**0**	**20**	**100**	**500**
Pre-coital interval					
Mean (days)	4.62	3.38	3.50	2.90	2.58
Vs. water control (days)	---	–1.24	–1.12	**–1.72**	**–2.04**

Table 51: Summary of selected fertility and early embryonic development parameters in rats administered GS-7977 (PSI-7977) or vehicle.

	0 (vehicle)	20 mg/kg	100 mg/kg	500 mg/kg
# of females paired w/ a male:	22	22	21	22
# of females inseminated:	22	22	21	21
# of females pregnant:	19	22	18	21
Total # of litters:	19	22	18	21
# of litters w/ early resorption:	10	6	7	11
# of litters w/ late resorption:	0	0	0	0
# of litters w/ any dead fetus:	0	0	0	0
Corpora lutea/litter:	16.0	15.0	14.4	15.4
Implantations/litter:	15.3	13.9	13.4	14.4
Early resorptions/litter:	0.6	0.5	0.8	0.8
Total implantation loss/litter:	1.3	1.6	1.8	1.8

9.2 Embryonic Fetal Development

Study title: AN ORAL GLP STUDY OF THE EFFECTS OF PSI-7977 ON EMBRYO/FETAL DEVELOPMENT IN RATS

Study no.: 0515-10240
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: November 18, 2010
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7977, 40410002 and 99.7%

Key Study Findings: Potential PSI-7977 related effects on embryo-fetal development (EFD) were evaluated in pregnant rats (24/sex/group) administered oral doses of 20, 100, 500 mg/kg or vehicle (95% PEG 400, 5% Tween 80) or water controls from GD6 to 18. The NOAEL for maternal toxicity and the NOEL for EFD was considered to be 500 mg/kg/day ($AUC_{0\text{-}24hr}$~34 & 72 μg.h/ml for PSI-6206 at GD6 & 18, respectively) since no significant EFD or adverse maternal effects were observed. Dose-dependent increases in the incidence of various clinical signs including crackles and wet and/or stained mouth, nares and/or body surface were noted in pregnant females at all or most dose levels.

Methods

Doses: 0 (vehicle), 0 (water), 20, 100, 500 mg/kg/day
Frequency of dosing: Once daily
Dose volume: Oral gavage
Route of administration: 5 ml/kg

Formulation/Vehicle:	PSI-7977 in 95% PEG 400, 5% Tween 80
Species/Strain:	Rat/Hsd: Sprague Dawley®TM SD®TM
Number/Sex/Group:	24 ♀ (main), 9 (TK)
Satellite groups:	TK
Study design:	PSI-7977 administered to pregnant females GD 6-18 and sacrificed on GD20.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None due to test-article. Refer to Sponsor table below.

Table 52: Findings in female rats either found dead or sacrificed moribund

Animal No.	Study Day	Status	Noteworthy In-Life and Postmortem Findings	Likely Contributing Cause of Death
1F11	8	SM	Edema, ventral thorax clinically Esophageal perforation found at necropsy	Esophageal perforation
4F85	11	FD	Dark lungs found at necropsy. Animal was not pregnant	Accidental aspiration
5F120	15	FD	Red lungs found at necropsy	Accidental aspiration

FD = Found Dead
SM = Sacrificed Moribund

Clinical Signs

Table 53: Incidence of selected clinical signs in GS-7977 (PSI-7977) treated pregnant rats

	Females				
Test Material =	**Water**	**Vehicle**	**PSI-7977**		
Dosage (mg/kg/day) =	**0**	**0**	**20**	**100**	**500**
Staining around nose/nares	0	0	**2**	**6**	**20**
Crackles	0	0	**1**	**4**	**2**
Staining around mouth	0	0	0	0	**2**
Body surface stained	0	0	0	0	**1**
Body surface wet	0	0	0	0	**2**

Body Weight: Note: Measured pre-dose and on GD 6, 9, 12, 15, 17 & 20. No test-article related changes noted.

Feed Consumption: Note: Measured pre-dose and on GD 6, 9, 12, 15, 17 & 20. No test-article related changes noted.

Toxicokinetics: Note: Sampling times were pre-dose, 1, 2, 4, 6, 8, 12 & 24 hrs post-dose on GD 6 and 18 (using up to 3 rats/sex/group for each timepoint).

Table 54: Mean plasma GS-331007 (PSI-6206) and GS-566500 (PSI-352707) TK parameters in pregnant rats following GS-7977 (PSI-7977) administration

	Females		
Dose (mg/kg/day) =	**20**	**100**	**500**
PSI-352707 [a]			
C_{max} (µg/mL)			
GD 6	0.2	0.9	4.0
GD 18	0.3	1.1	5.0
AUC_{24h} (h*µg/mL)			
GD 6	1.0	4.7	18.5
GD 18	3.5	7.6	47.1
PSI-6206 [a]			
C_{max} (µg/mL)			
GD 6	0.1	0.7	4.1
GD 18	0.2	0.7	3.5
AUC_{24h} (h*µg/mL)			
GD 6	1.3	5.8	33.7
GD 18	3.3	9.6	72.1

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL
GD = Gestation Day

Table from sponsor

Dosing Solution Analysis: All dosing formulations (0, 4, 20 and 100 mg/ml) were analyzed for uniformity and concentration. All assay results were within an acceptable concentration range (±5%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

Necropsy: No test-article related changes noted in the dams on GD20.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.) No test-article related changes noted.

Table 55: Summary of selected cesarean section data from rats administered GS-7977 (PSI-7977) or controls

	0 (vehicle)	0 (water)	20 mg/kg	100 mg/kg	500 mg/kg
# of gravid females:	21	22	22	19	23

Viable fetuses/litter:	13.6	12.9	12.9	14.1	12.9
Dead fetuses/litter:	0	0	0	0	0
Early resorptions/litter:	0.3	0.8	0.2	0.2	0.7
Late resorptions/litter:	0	0	0	0	0
Corpora lutea/litter:	16.4	15.7	15.5	16.0	15.8
Implantation sites/litter:	13.9	13.7	13.0	14.3	13.6
Pre-implantation loss/litter:	2.5	2.0	2.4	1.9	2.4
Post-implantation loss/litter:	0.3	0.8	0.2	0.2	0.7

Offspring (Malformations, Variations, etc.) No test-article related changes noted.

The following sporadic malformations were observed:

- Vertebral agenesis in 1 of 297 fetuses examined (HD)
- Microphthalmia in 1 of 135 fetuses examined (LD)

The following sporadic visceral variations were observed:

- Major blood vessel variation in 2 of 138 (vehicle) and 1 of 140 (HD) fetuses examined

Table 56: Summary of selected offspring data from gravid rats administered GS-7977 (PSI-7977) or controls

	0 (vehicle)	0 (water)	20 mg/kg	100 mg/kg	500 mg/kg
# of litters examined:	21	22	22	19	23
Mean fetal weight (sexes combined):	3.6 g	3.5 g	3.7 g	3.6 g	3.7 g
% male:	48.0	53.6	48.7	50.4	50.5
# of fetuses examined externally:	285	283	283	267	297
# of fetuses examined viscerally:	138	137	135	129	140
# of fetuses examined skeletally:	147	146	148	138	157
% fetuses w/ external malformation:	0.0	0.0	0.0	0.0	0.3
% fetuses w/ visceral malformation:	0.0	0.0	0.6	0.0	0.0
% fetuses w/ skeletal malformation:	0.0	0.0	0.0	0.0	0.0
% fetuses w/ external variation:	0.0	0.0	0.0	0.0	0.0
% fetuses w/ visceral variation:	1.4	0.0	0.0	0.0	0.5
% fetuses w/ skeletal variation:	56.7	67.5	66.6	71.0	74.0

Table 57: Skeletal variations observed in offspring of rats administered GS-7977 (PSI-7977) or controls (% fetuses with variation)

	0 (vehicle)	0 (water)	20 mg/kg	100 mg/kg	500 mg/kg
Cervical centrum #1 ossified:	52.5	58.0	63.5	66.6	69.2
14^{th} rudimentary rib(s):	12.5	15.8	8.5	21.8	19.6
Sternebra(e) unossified (5 &/or 6):	0.7	0.6	0.8	0.0	0.0
14^{th} full rib(s):	0.0	0.0	0.6	0.8	0.6

Sternebra(e) malaligned:	0.7	1.3	0.8	0.0	2.1
Sternebra unossified (1,2,3 &/or 4):	0.0	1.5	0.0	0.7	0.0
Ossification of 13th rib reduced	0.0	0.0	0.0	0.0	0.5

Study title: AN ORAL RANGE-FINDING STUDY OF PSI-7977 IN PREGNANT RABBITS

Study no.: 0515-11177
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: April 28, 2011
GLP compliance: No
QA statement: No (final report)
Drug, lot #, and % purity: PSI-7977, 40410002 and 99.7%

Key Study Findings: This range-finding study evaluated PSI-7977 in pregnant rabbits (5/main group, 3/TK group) administered oral doses of 9, 30, 90, 300 mg/kg or vehicle (PEG 400) or water control from GD 6 to 19 followed by a 10 day treatment-free period, with animals sacrificed at GD 29. No significant test-article related maternal effects on morbidity/mortality, clinical signs, body weight, food consumption or gross pathology were observed. In addition, neither PSI-7977 nor vehicle produced external fetal malformations or variations.

Table 58: Mean plasma GS-7977 (PSI-7977), GS-331007 (PSI-6206) and GS-566500 (PSI-352707) TK parameters in pregnant rabbits following GS-7977 administration

	Females			
Dose (mg/kg/day) =	**9**	**30**	**90**	**300**
PSI-7977				
C_{max} (ng/mL)				
GD 6	6.96	48.9	214	611
GD 19	19.5	183	469	1971
AUC_{24h} (ng*h/mL)				
GD 6	65.7	97.4	451	1694
GD 19	87.0	506	1417	7258
PSI-352707				
C_{max} (ng/mL)				
GD 6	293	1650	5861	12507
GD 19	272	1296	2983	11954
AUC_{24h} (ng*h/mL)				
GD 6	1553	4661	14919	45375
GD 19	1299	4200	11199	45559
PSI-6206				
C_{max} (ng/mL)				
GD 6	186	647	2567	5435
GD 19	277	757	3208	9947
AUC_{24h} (ng*h/mL)				
GD 6	2749	6649	21891	85933
GD 19	4426	8717	47434	119964

GD = Gestation Day

Table from sponsor

Study title: AN ORAL GLP STUDY OF THE EFFECTS OF PSI-7977 ON EMBRYO/FETAL DEVELOPMENT IN RABBITS

Study no.: 0515-10241
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4) [redacted]
Date of study initiation: May 31, 2011
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7977, 40410002 and 99.7%

Key Study Findings: Potential PSI-7977 related effects on embryo-fetal development (EFD) were evaluated in pregnant rabbits (20/sex/group) administered oral (gavage) doses of 30, 90, 300 mg/kg or vehicle (PEG 400) or water controls from GD6 to 19 and sacrificed on GD29. The NOEL for EFD and maternal toxicity were considered to be

300 mg/kg/day ($AUC_{0\text{-}24hr}$~86 & 200 μg.h/ml for PSI-6206 at GD6 & 19, respectively) since no significant PSI-7977-related EFD or maternal effects were observed.

The high-dose level (300 mg/kg) was selected based on results of a pilot study in which this dose level did not produce maternal or fetal toxicity but "achieved acceptable systemic exposure". Although this justification does not appear adequate [based on ICH S5(R2) criteria] and the 300 mg/kg dose level seems suboptimal (given the lack of clear test-article related effects in the dams), the vehicle formulation appears to be dose limiting. Vehicle-related maternal toxicity was observed, including diarrhea/soft feces, decreased body weight gain and green material in the GI tract, despite being administered at relatively low amounts (1 ml/kg). Ideally, the sponsor should have examined additional formulations containing other vehicles in order to identify/select a more optimal PSI-7977 high dose [considering criteria in ICH S5(R2)] to examine in their pivotal study. However, it is unclear if the sponsor considered other vehicle formulations. In addition, as the result of vehicle/gavage related maternal deaths, the recommended 16 litters [refer to ICH S5(R2), section 4.1.3] were not examined in all groups, but nonetheless appear sufficient to fulfill the study objective, since only the vehicle control and low dose groups failed to achieve the recommended minimal number of litters.

Methods

Doses:	0 (vehicle), 0 (water), 30, 90, 300 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Oral gavage
Route of administration:	1 ml/kg
Formulation/Vehicle:	PSI-7977 in PEG 400
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	20 (main), 3 (TK)
Satellite groups:	TK
Study design:	PSI-7977 administered to pregnant females GD 6-19 and sacrificed on GD29.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None due to test-article. 11 animals were found dead or died during the study on GD 15-25 as follows: 4, 3, 1 and 2 females in the vehicle, 30, 90 and 300 mg/kg groups. Also 1 TK vehicle control animal found dead on GD17. Deaths all attributed to the vehicle and associated with green material in the GI tract with at least 8 due likely to aspiration.

Clinical Signs: No test-article related changes noted.

- Increased incidence of watery diarrhea and/or soft feces observed in vehicle and all PSI-7977 dose levels compared to the water control.

Body Weight: Note: Measured pre-dose and on GD 6, 9, 12, 15, 18, 21, 25 & 29. No test-article related changes noted.

- Reduced body weight gain in vehicle and PSI-7977 treated groups compared to the water control.

Feed Consumption: No test-article related changes noted.

Toxicokinetics: Note: Sampling times were pre-dose, 1, 2, 4, 6, 8, 12 & 24 hrs post-dose on GD 6 and 19.

Table 59: Mean plasma GS-7977 (PSI-7977), GS-331007 (PSI-6206) and GS-566500 (PSI-352707) TK parameters in pregnant rabbits following GS-7977 administration

	Females		
Dose (mg/kg/day) =	**30**	**90**	**300**
PSI-7977 [a]			
C_{max} (µg/mL)			
GD 6	0.1	0.3	0.9
GD 19	0.1	0.8	1.9
AUC_{24h} (h*µg/mL)			
GD 6	0.1	0.6	2.6
GD 19	0.6	2.1	8.7
PSI-352707 [a]			
C_{max} (µg/mL)			
GD 6	1.7	5.8	16.5
GD 19	0.8	6.0	11.3
AUC_{24h} (h*µg/mL)			
GD 6	6.6	20.5	58.9
GD 19	3.7	24.7	68.6
PSI-6206 [a]			
C_{max} (µg/mL)			
GD 6	0.7	2.5	6.6
GD 19	0.7	3.9	22.2
AUC_{24h} (h*µg/mL)			
GD 6	9.7	28.4	85.9
GD 19	8.1	54.5	200.0

[a] Values rounded to the nearest 0.1 µg/mL or h* µg/mL
GD = Gestation Day

Dosing Solution Analysis: All dosing formulations (0, 30, 90 and 300 mg/ml) were analyzed for uniformity and concentration. All assay results were within an acceptable concentration range (±12.2%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study.

Necropsy: No test-article related changes noted.

Abortions: Occurred in 1, 2 and 2 in the vehicle, 30 and 90 mg/kg groups from GD20-27.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): No test-article related changes noted.

- Early resorptions/litter were 1.5, 2.1 and 1.6% for 30, 90 and 300 mg/kg groups but were not observed in controls. These values were within the historical control range (0.0 to 8.7%).

Table 60: Summary of selected cesarean section data from rabbits administered PSI-7977 or controls.

	0 (vehicle)	0 (water)	30 mg/kg	90 mg/kg	300 mg/kg
# of females:	20	20	20	20	20
# of females found dead or died:	4	0	3	1	2
# of females that aborted:	1	0	2	2	0
# of surviving females nongravid:	2	3	5	0	0
# of gravid females:	13	17	10	17	18
Viable fetuses/litter:	9.1	9.5	7.5	7.9	8.8
Dead fetuses/litter:	0.0	0.0	0.1	0.0	0.1
Early resorptions/litter:	0.0	0.0	0.1	0.2	0.2
Late resorptions/litter:	0.6	0.5	0.6	1.1	0.6
Corpora lutea/litter:	10.7	11.5	12.0	10.6	10.9
Implantation sites/litter:	9.7	9.9	8.3	9.2	9.6
Pre-implantation loss/litter:	1.0	1.5	3.7	1.4	1.2
Post-implantation loss/litter:	0.6	0.5	0.8	1.3	0.8

Offspring (Malformations, Variations, etc.): No clear test-article related changes noted.

- Spina bifida observed in 2 of 158 fetuses (1 of 18 litters) examined (HD)-not seen in any other group. This external malformation was not considered test-article related since observed in a single litter and the mean litter percentage (1.1%) was within the historical control range (0.0 to 1.1%).
- Accessory lobule(s) of liver observed in 5 of 158 fetuses (2 of 18 litters) examined (HD)-not seen in any other group. This minor visceral variation was not considered test-article related since the mean litter percentage (2.3%) was within the historical control range (0.0 to 2.5%).
- Sternebra(e) malaligned (slight to moderate) in 1 of 118 fetuses (1 of 13 litters) in vehicle control, 1 of 161 fetuses (1 of 17 litters) in water control, 3 of 135 fetuses (2 of 17 litters) (MD) and 4 of 158 fetuses (3 of 18 litters) (HD). The test-article relationship of this minor skeletal variation appears unlikely. The low incidence of this variation and the lack of a potential test-article relationship with other

sternebra and skeletal variations suggest it is most likely sporadic in nature and not of significant concern.

Table 61: Summary of selected offspring data from gravid rabbits administered PSI-7977 or controls.

	0 (vehicle)	0 (water)	30 mg/kg	90 mg/kg	300 mg/kg
# of litters examined:	13	17	10	17	18
Mean fetal weight (sexes combined):	39.6 g	43.8 g	41.4 g	44.5 g	41.1 g
% male:	52.6	45.1	36.2	42.7	52.4
# of fetuses examined:	118	161	75	135	158
% fetuses w/ external malformation:	0.0	0.0	2.7	0.0	1.3
% fetuses w/ visceral malformation:	1.7	0.0	1.3	0.7	0.0
% fetuses w/ skeletal malformation:	3.4	2.5	5.3	2.2	3.8
% fetuses w/ external variation:	0.0	0.0	0.0	0.0	0.0
% fetuses w/ visceral variation:	13.8	24.7	22.4	18.5	18.4
% fetuses w/ skeletal variation:	77.5	68.9	56.0	83.1	82.6

9.3 Prenatal and Postnatal Development

Study title: An Oral (Gavage) Study of the Effects of GS-7977 on Pre- and Postnatal Development, Including Maternal Function, in Rats

Study no.: (b) (4)-604017
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: April 30, 2012
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: GS-7977, P-36-12001 and 99.8%

Key Study Findings: Potential GS-7977 related effects on pre- and post-natal development (PPND) were evaluated in pregnant rats (25/sex/group) administered oral doses of 50, 250, 500 mg/kg or vehicle (95% PEG 400, 5% Tween 80) or water controls from GD6 to lactation day 20 (LD20). The NOAEL for maternal toxicity and the NOEL for PPND was considered to be 500 mg/kg/day [maternal: $AUC_{0\text{-}24hr}$~40 & 83 μg.h/ml for GS-331007 at GD6 & LD10, respectively; F_1 rat pups: $AUC_{0\text{-}24hr}$~1.5 μg.h/ml for GS-331007 at PND10 (LD10)] since no significant PPND or adverse maternal effects were observed. Dose-dependent increases in the incidence of clear, red and/or white material around the mouth were observed at all GS-7977 dose levels 1-hour post-dose compared to the vehicle control.

Methods

Doses:	0, 50, 250, 500 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Oral gavage
Route of administration:	5 ml/kg
Formulation/Vehicle:	GS-7977 in 95% PEG 400, 5% Tween 80
Species/Strain:	Rat/Crl:CD(SD) Sprague Dawley
Number/Sex/Group:	25 ♀ (main), 8 (TK), 2 pups (F_1)/sex/litter (25 pups/sex/group for both subsets A & B)
Satellite groups:	TK
Study design:	• GS-7977 administered to pregnant females from gestation day (GD) 6 to lactation day (LD) 20 and sacrificed on LD21. F_1 pups not selected for F_1 generation sacrificed on PND21. • F_1 generation split into subsets A or B (see Sponsor table below). F_1 pups not selected for breeding phase sacrificed after attainment of developmental landmarks. • Laparohysterectomies performed on GD 20 (F_2 generation morphological assessment) for F_1 animals selected for breeding phase (reproductive assessment). F_1 males sacrificed.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Table 62: Offspring allocation of F1 generation

No. Selected	Age	Evaluation
1/pup/sex/litter/group (A)[a]	PND 20 and 60	Auditory startle
1/pup/sex/litter/group (A)[a]	PND 21 and 61	Locomotor Activity
1/pup/sex/litter/group (B)	PND 22	Learning and Memory [b]
1/pup/sex/litter/group (A)[a]	PND 62	Learning and Memory [b]
1/pup/sex/litter/group (A)[a]	Minimum of 85 days	Breeding

[a] = The same pup subset was used for auditory startle, locomotor activity, PND 62 learning and memory, and breeding.
[b] = Different pups were evaluated on PND 22 and 62.

Note: Biel maze swimming trial conducted to assess learning and memory (measured escape time and errors).

Observations and Results

F_0 Dams

Survival:	One low-dose animal euthanized *in extremis* (w/ rales & red material around mouth) on GD19. One mid-dose animal sacrificed on post-mating day 25 (failed to delivery litter consisting of one dead fetus) and one high-dose animal sacrificed following total litter loss on LD2.
Clinical signs:	Increased incidence of clear, red and/or white material around the mouth observed at all GS-7977 dose levels 1-hour post-dose compared to the vehicle control.
Body weight (2x/wk):	No test-article related changes noted during gestation or lactation.
Feed consumption (2x/wk):	No test-article related changes noted during gestation or lactation.
Gestation length and Parturition:	No test-article related changes noted. Mean gestation length was 21.8, 21.7 and 21.7 days in the 50, 250 and 500 mg/kg groups versus 21.8 days in the vehicle control.
Uterine content:	See table below
Necropsy observation:	No test-article related changes noted.
Toxicokinetics:	See Sponsor table below.
Dosing Solution Analysis	See below

Table 63: Summary of uterine content in F_0 dams administered GS-7977

	0 (vehicle)	50 mg/kg	250 mg/kg	500 mg/kg
# of dams that delivered:	25	25	24	25
Implantation sites/litter:	15.7	16.3	16.3	15.0
# pups born/litter:	14.6	15.9	15.5	14.3
Live litter size (PND 0)/litter:	14.5	15.7	15.2	14.0
% male:	50.7	47.5	49.6	49.9

Toxicokinetics: Note: Sampling times were pre-dose, 1, 2, 4, 8 & 24 hrs post-dose on GD 6 and LD10.

Table 64: Mean plasma GS-7977, GS-566500 and GS-331007 TK parameters in pregnant and lactating rats following GS-7977 administration

	Gestation Day 6			Lactation Day 10		
GS-7977 Dosage (mg/kg/day)	**50**	**250**	**500**	**50**	**250**	**500**
Parameter (Units)	GS-7977					
AUC_{last} (ng·h/mL)	NA	NR	NR	NA	NR	NR
C_{max} (ng/mL)	NA	NR	5.07	NA	NR	NR
T_{max} (h)	NA	NR	1	NA	NR	NR
$T_{1/2}$ (h)	NA	NR	NR	NA	NR	NR
	GS-566500					
AUC_{last} (ng·h/mL)	2608	13604	29920	6411	23826	58857
C_{max} (ng/mL)	829	3484	8810	2076	6078	10960
T_{max} (h)	1	1	1	1	1	2
$T_{1/2}$ (h)	1.4	1.6	1.4	1.3	1.8	2.0
	GS-331007					
AUC_{last} (ng·h/mL)	4479	16782	39862	6668	33828	83313
C_{max} (ng/mL)	573	2163	4750	842	4638	6732
T_{max} (h)	2	4	4	2	4	2
$T_{1/2}$ (h)	3.9	3.4	3.3	4.8	3.7	5.6

NA = Not applicable, all plasma concentrations were BLQ (5.00 ng/mL).
NR = Not reportable (insufficient data for calculation).

Table from sponsor

Dosing Solution Analysis: All dosing formulations (0, 10, 50 and 100 mg/ml) were analyzed for uniformity and concentration. Assay results were generally within an acceptable concentration range (±15%), while the vehicle and the compound in this vehicle were shown to be stable under the conditions of this study. One concentration measurement fell outside of the acceptable concentration range (differed by 21%, 79.1 versus 100 mg/ml). This sample was collected toward the end of the dosing period (after LD10). This result does not significantly affect the interpretation of the study.

F_1 Generation

Survival:	No test-article related changes noted. See table below
Clinical signs:	No test-article related changes noted.
Body weight:	No test-article related changes noted. See table below
Feed consumption:	No test-article related changes noted.
Physical development:	No test-article related changes noted.
Neurological assessment:	No test-article related changes on startle response (PND 20 or 60), motor activity (PND 21 or 61) or learning and memory (Biel maze at PND 22 or 62) noted.
Reproduction:	No test-article related changes noted. See Sponsor table below.
Toxicokinetics:	See Sponsor table below

Table 65: Summary of survival, body weight, clinical and selected developmental parameters in the F_1 generation

	0 (vehicle)	**50 mg/kg**	**250 mg/kg**	**500 mg/kg**
# of pups examined (clinical signs):	358	384	361	346
# of pups found dead:	11	13	11	15
Mean pup weight on PND 1 (♂):	7.0 g	6.8 g	7.0 g	6.9 g
Mean pup weight on PND 1 (♀):	6.6 g	6.5 g	6.5 g	6.5 g
PN survival (%)/litter (birth to PND 4):	96.8	95.9	94.9	**91.5***
PN survival (%)/litter (PND 4 to 21):	98.0	99.0	99.5	97.9
Balanopreputial separation (PND):	44.1	44.1	44.7	44.8
Mean BW at attainment age (♂):	247.1 g	234.6 g	246.3 g	244.0 g
Vaginal patency (PND):	32.8	31.9	32.2	32.8
Mean BW at attainment age (♀):	113.3 g	107.8 g	110.5 g	113.2 g

Note: PN=post-natal; BW=body weight; *due primarily to total litter loss in one animal on LD2

Table 66: Summary of F_1 reproductive performance

	Dosage Level (mg/kg/day)				WIL HC[a]
Parameter	0	50	250	500	Mean (Range)
Male Mating Index (%)	95.8	95.8	100.0	100.0	95.8 (84.0-100.0)
Female Mating Index (%)	95.8	100.0	100.0	100.0	98.2 (92.0-100.0)
Male Fertility Index (%)	91.7	95.8	100.0	95.8	90.0 (60.0-100.0)
Female Fertility Index (%)	91.7	100.0	100.0	95.8	92.9 (60.0-100.0)
Male Copulation Index (%)	95.7	100.0	100.0	95.8	93.2 (71.4-100.0)
Female Conception Index (%)	95.7	100.0	100.0	95.8	92.9 (65.2-100.0)
Estrous Cycle Length (days)	4.2	4.3	4.1	4.3	4.3 (4.0-5.0)
Pre-Coital Interval (days)	2.7	3.4	2.5	3.1	3.3 (2.4-4.8)

[a] = [redacted] historical control data

Table from sponsor

Table 67: Summary of selected laparohysterectomy data from F_1 animals.

	0 (vehicle)	20 mg/kg	100 mg/kg	500 mg/kg
# of gravid females:	22	24	24	23
Viable fetuses/litter:	13.7	15.1	14.5	13.6
Dead fetuses/litter:	0	0	0	0
Early resorptions/litter:	0.9	0.4	0.8	0.7
Late resorptions/litter:	0	0	0	0
Corpora lutea/litter:	17.0	17.3	17.0	16.3
Implantation sites/litter:	14.7	15.5	15.3	14.3
Pre-implantation loss/litter:	2.3	1.8	1.8	2.1
Post-implantation loss/litter:	1.0	0.4	0.8	0.7

Toxicokinetics: Note: Blood samples collected from 1 pup/sex/litter on PND 10 (LD 10).

Table 68: Mean plasma GS-7977, GS-566500 and GS-331007 TK parameters in F_1 rat pups on PND10

Gender	Males			Females		
GS-7977 Dosage (mg/kg/day)	50	250	500	50	250	500
Parameter (Units)	GS-7977					
AUC_{last} (ng·h/mL)	NA	NA	NA	NA	NA	NA
C_{max} (ng/mL)	NA	NA	NA	NA	NA	NA
T_{max} (h)	NA	NA	NA	NA	NA	NA
$T_{1/2}$ (h)	NA	NA	NA	NA	NA	NA
Pup/Maternal AUC_{last} Ratio	NA	NA	NA	NA	NA	NA
	GS-566500					
AUC_{last} (ng·h/mL)	NA	NR	NA	NA	NA	NA
C_{max} (ng/mL)	NA	NR	NA	NA	NA	NA
T_{max} (h)	NA	NR	NA	NA	NA	NA
$T_{1/2}$ (h)	NA	NR	NA	NA	NA	NA
Pup/Maternal AUC_{last} Ratio	NA	NR	NA	NA	NA	NA
	GS-331007					
AUC_{last} (ng·h/mL)	NA	784	1496	12.2	959	1483
C_{max} (ng/mL)	NA	63.1	90.4	6.08	74.4	85.8
T_{max} (h)	NA	4	8	8	4	8
$T_{1/2}$ (h)	NA	6.2	NR	NR	7.1	NR
Pup/Maternal AUC_{last} Ratio	NA	0.05	0.04	<0.01	0.06	0.04

NA = Not applicable, all plasma concentrations were BLQ.
NR = Not reportable (insufficient data for calculation or value is an approximation).

Table from sponsor

F_2 Generation

Survival: No test-article related changes noted.
Body weight: No test-article related changes noted.
External evaluation: No test-article related changes noted.
Male/Female ratio: See table below.

Table 69: Summary of fetal data for F2 generation

	0 (vehicle)	50 mg/kg	250 mg/kg	500 mg/kg
# of litters:	22	24	24	23
# of pups examined:	302	362	347	312
% male:	48.4	51.0	50.3	46.2
Mean fetal weight (sexes combined):	3.7 g	3.6 g	3.6 g	3.7 g
% fetuses w/ external malformation:	0.0	0.3	0.3	0.0
% fetuses w/ external variation:	0.0	0.0	0.0	0.0

10 Special Toxicology Studies

Study title: A 14-DAY ORAL GLP TOXICITY STUDY IN RATS TO QUALIFY THE SAFETY OF PSI-7977 LOT #40410003

Study no.: 0515-11126
Study report location: 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: April 4, 2011
GLP compliance: Yes
QA statement: Yes (final report)
Drug, lot #, and % purity: PSI-7977, 40410003 and 99.1%

Key study findings: A 14-day oral toxicity study was conducted in rats (10/sex/group) by administering daily PSI-7977 doses of 500 mg/kg/day or vehicle (95% PEG 400, 5% Tween 80). The purpose of this study was to qualify an impurity (b) (4)) that may not have been present in previous lots by comparing these results to those of previous toxicology studies with other PSI-7977 lots at the same dose level and duration. The lot administered in this study contains (b) (4) (b) (4) (equates to daily dose of (b) (4) mg/kg). Standard toxicity endpoints were evaluated including clinical signs, ophthalmic examinations, body weight, food consumption, hematology, coagulation, clinical chemistry, selected organ weights and gross and histopathology. The incidence of clinical signs of toxicity observed in this study was similar to that described previously.

Study title: A 28-DAY ORAL GAVAGE QUALIFICATION TOXICITY GLP STUDY OF GS-7977 IN RATS

Study no.:	0517-12180
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	June 27, 2012
GLP compliance:	Yes
QA statement:	Yes (final report)
Drug, lot #, and % purity:	GS-7977, 5364-84-9 & 40411001 and 94.8% & 99.6%

Key study findings: A 28-day oral toxicity and TK study was conducted in rats (10/sex/group) by administering daily GS-7977 doses of 100, 500 mg/kg/day (lot #5364-84-9), 500 mg/kg/day GS-7977 (lot #40411001) or vehicle (95% PEG 400, 5% Tween 80). The purpose of this study was to qualify potential impurities in GS-7977 lot #5364-84-9 by comparing toxicity and TK results directly to those using lot #40411001. Standard toxicity endpoints were evaluated including clinical signs, ophthalmic examinations, body weight, food consumption, hematology, coagulation, clinical chemistry, selected organ weights and gross and histopathology. Clinical signs of toxicity and TK parameters in animals administered GS-7977 (lot #5364-84-9) were similar to those for GS-7977 (lot #40411001). The NOAEL was considered to be 500 mg/kg (AUC_{last}~58 μg.h/ml for GS-331007) for both lots.

11 Integrated Summary and Safety Evaluation

Sofosbuvir (SOF, GS-7977, PSI-7977) is a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted intracellularly to the active uridine triphosphate (GS-461203) within tissues. GS-461203 is a specific inhibitor of nonstructural protein 5B (NS5B) of hepatitis C virus (HCV) that has displayed potent inhibition of HCV replicon ribonucleic acid (RNA) replication *in vitro*. Sofosbuvir is one of two diastereoisomers (at phosphorous) in a mixture designated GS-9851, which was utilized in early nonclinical safety studies. The ratio of stereoisomers GS-7977 and GS-491241 in GS-9851 is approximately 50:50, with chemical interconversion of the two stereoisomers appearing unlikely. These stereoisomers are converted to either stereoisomeric metabolites (GS-566500, GS-606965, GS-607596 and GS-461203) or an identical metabolite (GS-331007), so that equimolar doses of the mixture (GS-9851) or the single isomer (GS-7977) yield similar metabolite exposures. This identical metabolite (GS-331007) is also the main circulating metabolite in both animals and humans, accounting for the majority of total drug-related exposure.

Pharmacokinetics:

The oral bioavailability and fraction of sofosbuvir absorbed is ~10 and 40%, respectively, in male dogs, with this difference due to high hepatic extraction of sofosbuvir. GS-9851 is stable in blood from dogs and humans but unstable in rat and mouse blood due to high esterase activity that accounts for low systemic exposure to GS-9851 (and sofosbuvir) in rodents. Sofosbuvir is metabolized via sequential hydrolytic

steps followed by consecutive phosphorylation steps via the pyrimidine nucleotide biosynthesis pathway. Dephosphorylation of the mono-phosphorylated form (GS-606965) in tissues results in production of GS-331007, which is the most abundant metabolite in plasma (generally accounting for >75% of total drug-related exposure), urine and feces in humans and animals. Thus, elimination of sofosbuvir and related metabolites occurs primarily through renal excretion of GS-331007 with mean urine recovery ranging from 63 to 81% in rats, mice and dogs, with minor amounts eliminated in feces and bile. Of note, the highest concentrations of sofosbuvir and related metabolites in rats were measured in the alimentary canal, lymphatic and excretory systems. Importantly, sufficient systemic exposure to sofosbuvir (in rabbits and dogs) and its main circulating metabolites (GS-331007 and GS-566500) (in rats, mice, rabbits and dogs) is achieved.

Rats, mice, dogs and cynomolgus monkeys achieve measurable liver exposures to sofosbuvir and related metabolites also found in plasma (GS-331007 and GS-566500), with male and female rats having tissue to plasma ratios of 15 and 51 (8 hours post-dose), respectively. Although human primary hepatocytes appear more efficient at producing the active tri-phosphorylated form (GS-461203) than species utilized for toxicology studies, rats and dogs appear to efficiently produce GS-461203 *in vivo*, achieving robust liver exposures to GS-461203. Despite adequate systemic and liver exposure to GS-9851 and related metabolites, mice and cynomolgus monkeys are inefficient at producing GS-461203. For example, single (rats and mice) or multiple (dogs and monkeys) oral doses of GS-9851 (50 mg/kg) yielded GS-461203 liver concentrations of ~4 and 20 μM in rats and dogs, respectively, but <0.1 μM in mice and monkeys. Although much less efficient than rats, mice produced GS-461203 when administered higher GS-9851 dose levels (≥500 mg/kg), with GS-461203 never represented more than 1.5% of total GS-9851-related metabolite exposure in liver. Despite this inefficiency, GS-461203 liver concentrations of ~28 μM were achieved in mice administered single 2000 mg/kg doses of GS-9851. By comparison, male rats achieved GS-461203 liver concentrations of almost 200 μM following administration of single 1800 mg/kg doses of GS-9851. Of note, the *in vitro* IC_{50} of GS-461203 is ~0.7 to 2.6 μM. Thus, these high rodent dose levels achieved GS-461203 liver concentrations that exceeded the *in vitro* IC_{50} levels by ~10 to 100-fold.

Tissue distribution and the potential for subsequent tissue metabolism of circulating sofosbuvir metabolites (GS-331007 and GS-566500) is an important consideration for assessing clinical risk in various special populations [*e.g*. pregnancy, nursing mothers, patients with severe renal impairment or end stage renal disease (ESRD)]. Although tissue re-uptake and/or phosphorylation of GS-331007 are thought to be inefficient based on *in vitro* HCV replicon assays, this inefficiency has not been confirmed *in vivo*. In rats, exposure of the developing fetus and nursing pups to GS-331007 (and to a lesser extent GS-566500) occurs via maternal plasma and milk, respectively. This exposure apparently results in tissue uptake of GS-331007 *in vivo*, but the presence of phosphorylated metabolites was not evaluated. Although GS-566500 can be metabolized efficiently in tissues, it also lacks activity *in vitro*, which has been attributed to its highly polar structure thought to prevent it from crossing the cell membrane.

Phenol is formed during the initial esterase-mediated metabolic step that converts sofosbuvir to GS-566500. Since esterification of sofosbuvir is expected to be nearly complete and phenol accounts for ~18% of the molecular weight of sofosbuvir, there is a potential for substantial systemic exposure (*e.g*. up to 72 mg phenol/day in 400 mg sofosbuvir/day human dose). Although the localization, extent of phenol formation and proportion of conjugated to unconjugated phenol in animals (and humans) is unknown, phenol was likely present at significant concentrations in all non-clinical safety studies conducted. Despite this, no clear phenol-related toxicities were observed in repeat-dose or reproductive toxicology studies, with safety margins (>15-fold) most likely existing at the 400 mg/day human sofosbuvir dose, based on human equivalent dose calculations made by estimating phenol exposure at the NOAEL in these studies. In addition, although phenol appears to have some degree of genotoxic potential, genetic toxicology studies with GS-9851 were negative. Overall, the available data do not suggest a substantial clinical risk associated with sofosbuvir-derived phenol.

Safety Pharmacology:

No significant effects on neurologic (Irwin test) or respiratory parameters (respiratory rate, tidal or minute volumes) were observed in rats following single oral doses of GS-9851 up to 1000 mg/kg (estimated C_{max}>4.9 μg/ml for GS-331007), providing an >8-fold rat to human exposure multiple at the recommended sofosbuvir dose. This C_{max} estimate is based on data obtained from a single-dose toxicology study in rats (study #PSI-7851-09-0001). In addition, no significant cardiovascular effects on hemodynamic (HR and BP) or electrocardiographic parameters were noted for up to 24 hours post-dose in telemetry-monitored dogs given single oral doses of GS-9851 up to 1000 mg/kg [C_{max}~102(51) & 45 μg/ml for GS-9851(GS-7977) & GS-331007], providing ~85- & 77-fold dog to human exposure multiples for GS-7977 and GS-331007, respectively. These C_{max} estimates are based on data obtained from a non-GLP single dose PK study in female dogs (study #PSI-7851-09-0005). Although male dogs given repeat oral doses of GS-9851 at 1500 mg/kg/day for 6 days exhibited a prolonged QTc interval (19% longer than controls), this effect may be attributed to adverse toxicities at this dose level including mean body weight loss (13%) associated with emesis, diarrhea and reduced food consumption. These males also exhibited depressed behavior and were “cool to touch”. Thus, this slight QTc prolongation is not of significant clinical concern since it was associated with adverse clinical signs and occurred at high drug exposures [C_{max}~75(38) & 54 μg/ml for GS-9851(GS-7977) & GS-331007], providing ~62- & 93-fold dog to human exposure multiples at the recommended sofosbuvir dose for GS-7977 and GS-331007, respectively. GS-9851 and its metabolites GS-566500, GS-331007 and GS-606965 also did not significantly inhibit hERG current *in vitro* at up to the highest concentrations tested [159(300),123(300), 26(100) and 340(1000) μg/mL(μM), respectively]. No significant hemodynamic or electrocardiographic heart effects have been observed in clinical trials.

Repeat-Dose Toxicology Studies:

Heart toxicity:

Myocardial inflammation and degeneration occurred in rats administered oral GS-9851 doses of 2000 mg/kg/day (AUC_{last}~206 μg.h/ml for GS-331007) in a 7-day

toxicology study. The estimated AUC exposure for sofosbuvir-derived GS-331007 is ~14-fold that in humans at the recommended sofosbuvir dose, since GS-9851 consists of 50% sofosbuvir. Briefly, six of the nine rats found dead (beginning at Day 3) had minimal to slight/mild myofiber degeneration (usually located at apex of the heart) consisting of single myofiber hypereosinophilia or pyknosis with variable numbers of infiltrating inflammatory cells. Although it was not certain if the myofiber degeneration was sufficient to have caused death, one additional female had slight/mild acute inflammation throughout the myocardium that was considered to be the probable cause of death. In addition, one male had epicardial inflammation in addition to myofiber degeneration. Minimal myofiber degeneration was observed in one female who survived until scheduled termination and in 2 of 3 females following a 17 day treatment-free ("recovery") period. Although these "recovery" females gained weight during the dosing and recovery phase, body weight loss, which was severe in animals found dead, was a likely contributor to the early mortalities. Heart toxicity was not observed in rats administered oral doses of sofosbuvir up to 500 mg/kg/day (AUC_{last}~66 μg.h/ml for GS-331007) for 6 months, or in dogs and mice administered sofosbuvir at up to 500 and 1000 mg/kg/day (AUC_{last}~195 and 293 μg.h/ml for GS-331007), the highest doses examined in 9 and 3 month studies in dogs and mice, respectively, corresponding to AUC exposures ~9 (rat), 27 (dog) and 41 (mouse)-fold that in humans at the recommended sofosbuvir dose. No clear cardiovascular safety signals have been observed in clinical trials.

Gastrointestinal (GI) toxicity:

Gastrointestinal (GI) hemorrhage occurred in male dogs administered oral sofosbuvir doses of 500 mg/kg/day (AUC_{last}~209 to 278 μg.h/ml for GS-331007 at 6 & 3 months, respectively), corresponding to AUC exposures ~29 to 39-fold that in humans at the recommended sofosbuvir dose. Hemorrhage occurred in the lamina propria of the pyloric stomach (1 of 4 males at 3 months) or jejunum (1 of 8 males prior to 6 months) resulting in moribund condition and euthanasia of the latter. Of note, the euthanized animal had APTT values of 19.8 and 24.1 sec pre-test and immediately prior to euthanasia, respectively. Although feasible that this APTT increase contributed to intestinal hemorrhaging, the magnitude of the increase appears to be too small to be the cause of bleeding. Increased frequency and incidence of salivation, emesis and diarrhea also occurred at this dose level. These GI-related toxicities are dose-dependent and so most likely sofosbuvir-related; however, they also appear consistent with idiopathic hemorrhagic gastroenteritis of spontaneous origin. The NOEL for GI toxicity is 100 mg/kg/day (AUC_{last}~90 μg.h/ml for GS-331007) in dogs administered oral doses of sofosbuvir for up to 9 months, corresponding to AUC exposure ~13-fold that in humans at the recommended sofosbuvir dose. GI hemorrhage has not been observed in rats, mice or in clinical trials.

Hematological toxicity:

A slight decrease in circulating erythron mass (up to 12% decrease in RBC's, Hct & Hb), the apparent result of mild suppression of erythropoiesis in bone marrow (*e.g.*, reduced erythroid/myeloid ratio, higher relative percents of erythroid precursors, increased erythrophagocytosis, presence of large erythroid precursors and/or atypical

erythroid maturation) was observed in dogs administered 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$~195 μg·h/ml for GS-331007) of sofosbuvir for 3, 6 and/or 9 months, corresponding to AUC exposure ~27-fold that in humans at the recommended sofosbuvir dose. These minor effects did not progress with increasing duration of sofosbuvir administration and were not considered adverse. No clear exacerbation of hematological toxicities has been observed in humans when adding sofosbuvir to a regimen containing peginterferon-alpha and/or ribavirin.

Reproductive and Developmental Toxicology:

The NOEL for fertility and early embryonic development is 500 mg/kg/day (estimated AUC~57 μg.h/ml for GS-331007), the highest dose tested, in both male and female rats administered oral doses of sofosbuvir. The rat to human AUC exposure multiple is estimated to be ~8-fold at the recommended sofosbuvir dose. Since TK were not conducted in this study, exposures at the 500 mg/kg dose level were estimated based on data obtained elsewhere (study #0515-09011). In addition to increased incidence of soft feces/diarrhea and wet and/or stained mouth, nares, body surface and/or anogenital region in males and females at sofosbuvir doses ≥20 mg/kg, increased incidence of unkempt and dehydrated appearance was described in females at 500 mg/kg/day.

The NOAEL for maternal toxicity and the NOEL for embryo-fetal development (EFD) are 500 mg/kg/day ($AUC_{0\text{-}24hr}$~34 & 72 μg.h/ml for GS-331007 at GD6 & 18, respectively), the highest dose tested, in pregnant rats administered oral doses of sofosbuvir. Thus, no EFD or teratogenicity findings were observed in rats at GS-331007 AUC exposure multiples ~5- to 10-fold higher, than those in humans at the recommended sofosbuvir dose. Dose-dependent increases in the incidence of various clinical signs including crackles and wet and/or stained mouth, nares and/or body surface were noted in females at sofosbuvir doses ≥20 mg/kg. This lack of EFD findings occurred despite significant fetal exposure to sofosbuvir and related metabolites (predominately GS-331007), with distribution to amniotic fluid, placenta, fetal blood, brain and liver observed in a separate study.

The NOEL for EFD and maternal toxicity is 300 mg/kg/day ($AUC_{0\text{-}24hr}$~86 & 200 μg.h/ml for GS-331007 at GD6 & 19, respectively), the highest dose tested, in pregnant rabbits administered oral doses of sofosbuvir. Thus, no EFD or teratogenicity findings were observed in rabbits at GS-331007 AUC exposure multiples ~12- to 28-fold higher, than those in humans at the recommended sofosbuvir dose. This 300 mg/kg dose level was selected based on results of a pilot study in which this dose level failed to produce maternal toxicity but “achieved acceptable systemic exposure”. Although this justification does not appear adequate [based on ICH S5(R2) criteria] and the 300 mg/kg dose level seems suboptimal (given the lack of clear test-article related effects in the dams), the vehicle formulation appears to be dose limiting. Vehicle-related maternal toxicity was observed, including diarrhea/soft feces, decreased body weight gain and green material in the GI tract, despite being administered at relatively low amounts (1 ml/kg). Ideally, additional formulations prepared using other vehicles should have been examined in order to identify/select a more optimal sofosbuvir high dose to examine in the pivotal study. However, it should be noted that sofosbuvir will be administered in

combination with peginterferon-alpha, which is abortifacient, and/or ribavirin, which is teratogenic and embryocidal.

The NOAEL for maternal toxicity and the NOEL for pre- and post-natal development (PPND) is 500 mg/kg/day [maternal: $AUC_{0\text{-}24hr}$~40 & 83 μg.h/ml for GS-331007 at GD6 & LD10, respectively; F_1 rat pups: $AUC_{0\text{-}24hr}$~1.5 μg.h/ml for GS-331007 at PND10 (LD10)], the highest dose tested, in pregnant rats administered oral doses of sofosbuvir. Dose-dependent increases in the incidence of clear, red and/or white material around the mouth were observed 1-hour post-dose at sofosbuvir doses ≥20 mg/kg. Thus, no sofosbuvir-related effects on pregnancy, parturition and lactation of maternal animals (F0) or on the growth, viability, development or reproductive performance of the F1 generation, or survivability of the F2 generation were reported at GS-331007 AUC exposure multiples ~6- to 12-fold higher than those in humans at the recommended sofosbuvir dose. This lack of PPND findings occurred despite pup exposure to sofosbuvir and related metabolites during both fetal and lactation periods (milk concentrations peaking 1 hour post-dose at a milk to plasma ratio of 0.1). Of note, the majority of sofosbuvir-related material administered to pups in milk is GS-331007 and 2 sulfated conjugates of GS-331007, with only a minor amount of GS-566500 present. In pups exposed in milk only in a separate study, sofosbuvir and related metabolites were quantifiable in liver and GI tract but not in kidney or lungs.

Genetic Toxicology and Carcinogenicity:

Sofosbuvir was not mutagenic or clastogenic as tested with GS-9851 in the Ames assay, the *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes and the *in vivo* mouse micronucleus assay. In addition, two-year mouse and rat carcinogenicity studies with sofosbuvir are in progress.

Impurity/Degradant Qualification:

Qualification assessment of residual solvent, elemental and other sofosbuvir manufacturing process-related impurities was undertaken. Numerous sofosbuvir impurities were evaluated by (Q)SAR analyses and are expected to be non-mutagenic. In addition, repeat-dose toxicology studies in rats were performed using sofosbuvir batches containing higher levels of total or specific impurities without indication that impurities present altered the toxicity profile of sofosbuvir. Given this overall assessment, the seriousness of the disease to be treated, the fact that sofosbuvir will be given in combination with peginterferon-alpha and/or ribavirin and since no positive structural alerts were identified, the nonclinical evaluation of impurities/degradants appears acceptable.

Table 70: Summary of Systemic Exposure Multiples for Sofosbuvir Toxicology Studies

Species	Study Type/ Duration/Toxicity	Dose (mg/kg)	Exposure Multiple Based on GS-331007 AUC*	Exposure Multiple Based on GS-7977 AUC^
Mouse	Exceeded MTD 3-month RD	1000 (>MTD) 300 (>♂ MTD) 100	~41 ~17 ~8	NA
Rat	Lethality & heart toxicity 7-day RD	2000 GS-9851 (1000 GS-7977) 250 (NOAEL)	~29 (~14) 4.3	NA
	6-month RD	500 (NOAEL)	~9	NA
	Fertility & EED	500 (NOEL)	~8	NA
Rat (pregnant)	EFD	500 (NOEL)	4.7 @ GD6 & ~10 @ GD18	NA
	PPND	500 (NOEL)	5.6 @ GD6 & ~12 @ LD10	NA
Rabbit (pregnant)	EFD	300 (NOEL)	~12 @ GD6 & ~28 @ GD19	~3 @ GD6 & ~10 @ GD19
Dog	GI toxicity 3-month RD	500 100 (NOAEL)	~44 ~12	~147 ~26
	GI toxicity 9-month RD	500 100 (NOAEL)	~32 ~13	NA

*AUC in HCV-infected human subjects: 7,200 ng.hr/ml at 400 mg QD; ^AUC in HCV-infected human subjects: 860 ng.hr/ml at 400 mg QD; NA= not applicable (either not measured or below the level of detection); RD=repeat-dose toxicology study; EFD=embryo-fetal development study; PPND=pre- and post-natal development study; EED=early embryonic development; GD=gestational day; MTD=maximal tolerated dose; NOEL=no effect level; NOAEL=no adverse effect level

12 Appendix/Attachments

12.1 (Q)SAR Evaluation of GS-7977 Impurities

Title: ***(Q)SAR* Evaluation of GS-7977 Impurities**
Reviewer: Mark Powley, Ph.D.

Key Findings

- *With 1 exception, all GS-7977 impurities evaluated are expected to be non-mutagenic based on the initial (Q)SAR analyses and consideration of additional information. Although there*

were no positive predictions for [redacted] (b) (4) *, a weak alert was identified resulting in an equivocal prediction by Derek Nexus.*

Methods

	FDA Analysis
(Q)SAR System(s)	Derek Nexus (DX) v.3.0.1 Leadscope Model Applier (LMA) v.1.6.1 MultiCASE Ultra (CU) v1.4.6.0
Basis of Integrated (Q)SAR Prediction	A positive prediction in any single [redacted] (b) (4) was an overall positive.
Expert Knowledge	The following was considered where appropriate: • structural alert relevance based on similarity of the impurity with training set structures • empirical data for structural analogs

Results and Discussion

The structures of numerous GS-7977 impurities were submitted to the CDER Computational Toxicology Group for evaluation (Appendix 1). Results of the initial (Q)SAR analysis are summarized below.

Table 1. Results of CDER [redacted] (b) (4) analysis[1]

No.	Chemical	*Salmonella* Predictions			
		DX	LMA	CU	Overall
1	GS-7977	NSA	-	-	-
2	[redacted] (b) (4)	NSA	-	-	-
3		NSA	-	-	-
4		NSA	-	Eqv	-
5		NSA	-	-	-
6		NSA	-	-	-
7		NSA	-	-	-
8		NSA	-	-	-
9		NSA	-	-	-
10		NSA	-	-	-
11		NSA	-	-	-
12		NSA	-	-	-
13		NSA	-	-	-
14		NSA	-	-	-
15		Eqv	-	-	-
16		NSA	-	+	+
17		NSA	-	-	-
18		NSA	-	-	-
19		NSA	-	-	-

[1] + = positive; − = negative; Eqv = equivocal; NSA = no structural alerts are identified by DX (Derek Nexus cannot differentiate between a negative call and the inability to make a call because of no coverage); NC = test chemical features are not adequately represented in the model training data set, leading to a no call.

For most impurities, DX did not identify structural alerts and LMA and CU predictions were negative. Detailed discussion is provided for several impurities as the structures were associated with an equivocal prediction or were of concern based on the presence of potentially reactive functional groups.

[redacted] *Through visual inspection, this impurity was identified as potentially reactive due to the presence of* [redacted] *The analysis by DX did not identify any structural alerts and the impurity was predicted negative by LMA. CU identified the* [redacted] *as a positive alert but yielded an equivocal prediction due to the presence of* [redacted] *(see Table 2). In addition, the structural alert is in the* [redacted] *. Based on the totality of evidence,* [redacted] *is expected to be non-mutagenic.*

Table 2. Summary of [redacted] analysis

Software	Structural Feature	Confidence
[redacted]		

[redacted]*: Both statistically-based (Q)SAR systems yielded negative predictions; however, DX identified the* [redacted] *as an alert. Although the DX prediction was equivocal, the Sponsor appears to be treating* [redacted] *as a potentially mutagenic impurity.*

[redacted] *DX did not identify any structural alerts and the impurity was predicted negative by LMA. In contrast, the impurity was predicted positive by CU due to the* [redacted] *(see Table 3). A comparison of the impurity and the* [redacted] *suggests important structural differences. With 2 exceptions,* [redacted]

(b) (4)

In addition, the alert is in the (b) (4) *. Overall,* (b) (4) *is expected to be non-mutagenic.*

Table 3. Summary of (b) (4) analysis

Software	Structural Feature	Confidence
(b) (4)		

(b) (4) *Through visual inspection, these impurities were identified as potentially reactive* (b) (4) *However, no structural alerts were identified by DX and the structure was predicted negative by the statistically-based systems.* (b) (4)

that are likely responsible for the mutagenic response. Given the totality of information, both (b) (4) *are expected to be non-mutagenic.*

(b) (4)

(b) (4): *This impurity was identified as potentially problematic based on the presence of an* (b) (4) *However, it does not appear that the* (b) (4) *Therefore,* (b) (4) *is expected to be non-mutagenic.*

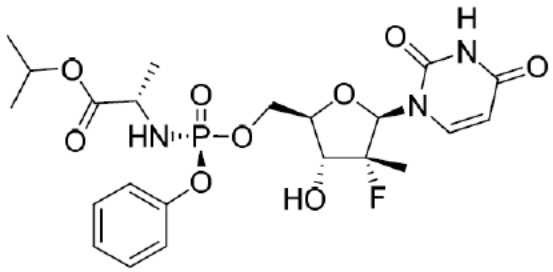
Conclusions

With 1 exception, all GS-7977 impurities evaluated are expected to be non-mutagenic based on the initial (Q)SAR analyses and consideration of additional details. Although there were no positive predictions for (b) (4)*, a possible alert was identified resulting in an equivocal prediction by DX. The Sponsor appears to be treating this as a potentially mutagenic impurity.*

Reference

(b) (4)

Appendix 1 - Structures Evaluated



GS-7977

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12.2 Brief Safety Summary of Phenol

Reviewer: Mark Powley, Ph.D.

General

The esterase-mediated metabolism of GS-7977 results in the release of phenol. Species differences in both the extent and location of phenol formation are possible as plasma esterase activity is higher in rodents vs. non-rodents and humans. Because phenol accounts for ~18% of the molecular weight of GS-7977 there is a potential for substantial exposure. Therefore, it is important to consider the potential toxicity associated with phenol formed during the metabolism of the GS-7977 pro-drug.

PK/ADME

Phenol is a metabolite of benzene, a known human carcinogen. Benzene absorbed from the GI undergoes CYP450-mediated metabolism to form oxidative metabolites. These metabolites, including phenol, are thought to play a role in the carcinogenic effects of benzene. In contrast, oral administration of phenol lacks clear carcinogenic activity (NTP, 1980). Medinsky *et al*. (1995) suggest that the differential effects are due to the location of hepatic enzymes involved in both oxidation and conjugation. For instance, ingested phenol is more likely to undergo conjugation in the gastrointestinal tract and Zone 1 of the liver. In contrast, phenol formed via benzene metabolism is more likely to escape initial conjugation and undergo further metabolism to potentially toxic species. Because the location of phenol formation is an important factor, it is unclear what extent the toxicity data obtained from oral administration of phenol is applicable to phenol released during GS-7977 metabolism.

Phenol is also present in humans as a result of environmental exposures, dietary consumption of phenol or phenol producing foods, consumer products, and endogenous protein catabolism (ATSDR, 2008). These exposures result in urinary excretion of up to ~10 mg phenol/day (Bone *et al*., 1976).

Toxicology

The following section summarizes key phenol-related toxicities as detailed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2008) and Environmental Protection Agency (EPA, 2002).

> General Toxicology: Acute oral administration (i.e., ≤ 14 days) of phenol resulted in respiratory, hematopoietic, renal, adrenal, immunological, and neurological effects. Phenol administration for > 14 to 364 days was associated with hematological, renal, hepatic, and neurological effects. Additional observations following acute and intermediate exposure included mortality and decreases in body weight parameters. Effects of chronic administration were limited to decreased body weight parameters.
>
> Developmental and Reproductive Toxicology: Adverse effects on embryofetal development included decreased number of liveborn pups, decreased fetal body weight, malformations, and development delays. In a 2 generation study, decreases in the % of live pups and decreased weights were observed in F_1 and F_2 pups. Sexual maturation was also delayed in F_1 pups.

Based on decreased maternal weight gain in an embryofetal development study (Argus, 1997), the EPA has calculated an oral reference dose [RfD; estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime) of 0.3 mg/kg/day (EPA, 2002].

Genetic Toxicology: Although results are mixed, phenol appears to have some degree of genotoxic potential based on studies most commonly conducted in support of drug development. Examples of positive *in vitro* studies include bacterial mutagenicity as well as mammalian cell assays designed to detect chromosomal aberrations and micronuclei. Positive results were also obtained from *in vivo* assessments of micronuclei formation. These studies are summarized in the table below.

Summary of genetic toxicology studies

Study	Results
in vitro	
- bacterial reverse mutation assay	Results appear to have been predominantly negative. There were reports of positive results in TA98 with metabolic activation and E. coli without activation; however, other studies in these strains were negative.
- chromosomal aberration assay	Chromosomal aberrations were detected in CHO cells with metabolic activation and SHE cells with and without metabolic activation. In contrast, another study conducted in CHO cells was negative without metabolic activation.
- micronucleus assay	Positive results were observed in CHO cells and HPBL with and without metabolic activation.
- mouse lymphoma assay	Results from a single study were deemed equivocal.
in vivo	
- micronucleus	There were multiple studies reporting both positive and negative results in bone marrow following i.p. dosing. Similar discordant results were obtained following oral dosing.

Carcinogenicity: Lifetime administration of phenol in drinking water was not carcinogenic in rodents (NTP, 1980).

References

ATSDR (2008) Toxicological profile for phenol.

Argus Research Laboratories, Inc. (1997 Oral (gavage) developmental toxicity study of phenol in rats. Horsham, PA. Protocol no. 916-011.

Bone, E., A. Tamm and M. Hill. 1976. The production of urinary phenols by gut bacteria and their possible role in the causation of large bowel cancer. *Am. J. Clin. Nutr.* 29:1448-1454.

EPA (2002) Toxicological review of phenol. EPA/635/R-02/006.

Medinsky M.A., Kenyon E.M., Schlosser P.M. (1995) Benzene: a case study in parent chemical and metabolite interactions. *Toxicology* 105:225-233.

NTP (1980) Bioassay of phenol for possible carcinogenicity. Technical Report Series No. 203; NTP No. 80-15.

12.3 Histopathology inventory

Study	0515-10012	0515-10062
Species	rat	dog
Adrenals	*, X	*, X
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	
Brain	*, X	*, X
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Gall bladder		X
Gross lesions	X	X
Harderian gland	X	
Heart	*, X	*, X
Ileum	X	X

Injection site		
Jejunum	X	X
Kidneys	*, X	*, X
Lachrymal gland	X	X
Larynx	X	X
Liver	*, X	*, X
Lungs	X	X
Lymph nodes, cervical	X	X
Lymph nodes mandibular		
Lymph nodes, mesenteric	X	X
Lymph nodes, popliteal		
Lymph nodes, unilateral		
Mammary Gland	X	X
Nasal cavity		
Optic nerves	X	X
Ovaries	*, X	*, X
Pancreas	X	X
Parathyroid	*, X	*, X
Peripheral nerve	X	X
Peyer's patches	X	X
Pharynx		
Pituitary	*, X	*, X
Prostate	*, X	*, X
Rectum	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	*, X	*, X
Sternum		
Stomach	X	X
Testes	*, X	*, X
Thymus	X	X
Thyroid	*, X	*, X
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X

Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed; *, organ weight obtained;
Note: some organs weighed together

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/s/

CHRISTOPHER E ELLIS
09/05/2013

HANAN N GHANTOUS
09/06/2013
I concur with Dr. Ellis's conclusion to approve sofosbuvir from a nonclinical perspective.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204,671 **Applicant:** Gilead Sciences **Stamp Date:** April 8, 2013

Drug Name: Sofosbuvir (GS-7977) **NDA Type:** Original

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Carcinogenicity studies in rats and mice are in progress. As agreed upon by the Agency, the Sponsor plans to submit study results post-approval.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Appropriateness of content will be determined upon review and discussed at labeling meetings.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Whether sponsor has addressed issues adequately is a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Not applicable

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Christopher Ellis, Ph.D.	May 8, 2013
Reviewing Pharmacologist	Date
Hanan Ghantous, Ph.D. DABT	May 8, 2013
Team Leader/Supervisor	Date

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

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/s/

CHRISTOPHER E ELLIS
05/09/2013

HANAN N GHANTOUS
05/09/2013