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RESEARCH**

APPLICATION NUMBER:

209195Orig1s000

NON-CLINICAL REVIEW(S)

Comments on N 209-195 fixed dose combination

From: A. Jacobs AD

Date: 4/28/17

1. I concur that there are no pharm-tox issues with approval of this NDA.
2. I have conveyed other comments to the reviewer and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
05/15/2017

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	209-195
Supporting document/s:	original
Applicant's letter date:	12/08/16
CDER stamp date:	12/08/16
Product:	Sofosbuvir/Velpatasvir/Voxilaprevir Fixed-Dose Combination (SOF/VEL/VOX FDC)
Indication:	Chronic Hepatitis C Virus (HCV) Infection
Applicant:	Gilead Sciences Inc.
Review Division:	Division of Antiviral Products
Reviewer:	Mark W. Powley, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
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1 Executive Summary

1.1 Introduction

Gilead Sciences Inc. has submitted a marketing application in support of a fixed-dose combination (FDC) therapy of sofosbuvir (SOF; GS-7977), velpatasvir (VEL; GS-5816), and voxilaprevir (VOX; GS-9857). The SOF/VEL/VOX FDC is indicated for the treatment of adult patients with chronic hepatitis C virus (HCV). SOF is an NS5B polymerase inhibitor previously approved as part of several combination therapies to treat HCV. VEL is an HCV NS5A inhibitor previously approved in combination with SOF to treat HCV. VOX, a new molecular entity, is an HCV NS3/4A protease inhibitor. The proposed clinical dosing regimen of SOF/VEL/VOX is 400/100/100 mg for 12 weeks.

1.2 Brief Discussion of Nonclinical Findings

VOX was not associated with clinically relevant adverse effects on cardiovascular, neurological, or respiratory endpoints evaluated in safety pharmacology studies. In addition, there were no clinically relevant adverse effects observed in pivotal repeat-dose general toxicology studies, a male and female fertility study, embryofetal development studies, or a pre/post-natal development study. VOX was not considered genotoxic based on negative results in the *in vitro* bacterial reverse mutation assay, *in vitro* mammalian chromosome aberration assay, and *in vivo* rat micronucleus assay. As VOX was not genotoxic and will be administered to humans for only 12 weeks, carcinogenicity studies were not required. Non-adverse increases in bilirubin parameters, consistent with expected organic anion transporting polypeptide (OATP) inhibition by VOX, were observed both nonclinically and clinically.

Neither SOF or VEL were associated with clinically relevant adverse effects. Because there were no specific safety concerns with the individual SOF/VEL/VOX components, nonclinical studies with the combination were not warranted.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data is sufficient to support marketing application approval.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

The sponsor's proposed product label is under review.

2 Drug Information

2.1 Drug

SOF

CAS Registry Number:
1190307-88-0

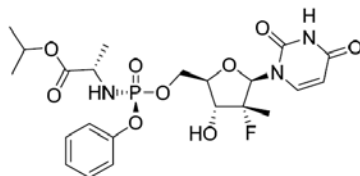
Generic Name:
Sofosbuvir

Code Name:
GS-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

Molecular Formula/Molecular Weight:
C₂₂H₂₉FN₃O₉P/529.45

Structure:



Pharmacologic Class:
HCV nucleotide analog NS5B polymerase inhibitor

VEL

CAS Registry Number:
1377049-84-7

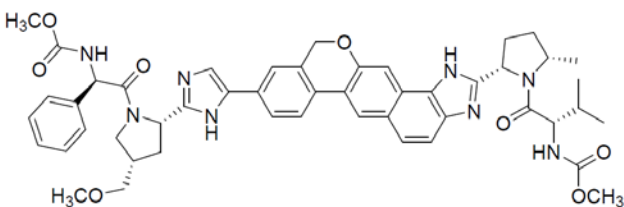
Generic Name:
Velpatasvir

Code Name:
GS-5816

Chemical Name:
Methyl {(1R)-2-[(2S,4S)-2-(5-{2-[(2S,5S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]-5-methylpyrrolidin-2-yl]-1,11-dihydro[2]benzopyrano[4',3':6,7]naphtho[1,2-d]imidazol-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl} carbamate

Molecular Formula/Molecular Weight:
C₄₉H₅₄N₈O₈/ 883.0

Structure:



Pharmacologic Class:
HCV NS5A inhibitor

VOX

CAS Registry Number:
1535212-07-7

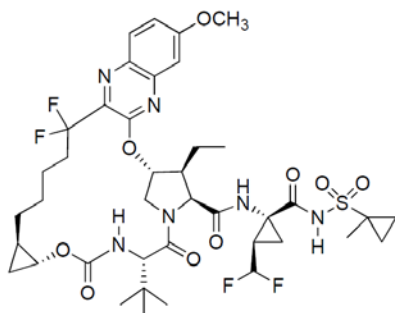
Generic Name:
Voxilaprevir

Code Name:
GS-9857

Chemical Name:
(1*aR*,5*S*,8*S*,9*S*,10*R*,22*aR*)-5-*tert*-butyl-*N*-{(1*R*,2*R*)-2-(difluoromethyl)-1-[(1-methylcyclopropane sulfonyl)carbamoyl]cyclopropyl}-9-ethyl-18,18-difluoro-14-methoxy-3,6-dioxo-,1*a*,3,4,5,6,9,10,18,19,20,21,22,22*a*-tetradecahydro-8*H*-7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-carboxamide

Molecular Formula/Molecular Weight:
C₄₀H₅₂F₄N₆O₉S/868.9

Structure:



Pharmacologic Class:
HCV NS3/4A protease inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND#119,926: VOX

IND#125,751: SOF/VEL/VOX FDC

NDA#204-671: Sovaldi™ (400 mg SOF + ribavirin with or without pegylated interferon α)

NDA#208-341: Epclusa® (SOF/VEL 400/100 mg FDC)

2.3 Drug Formulation

The components of the SOF/VEL/VOX FDC are described below (table taken from the sponsor submission). (b) (4)

Table 1. FDC formulation components

Component	Composition (% w/w)	Unit Formula (mg/tablet)	Reference Quality Standards	Function
(b) (4)				
Sofosbuvir ^a	(b) (4)	400.0	In-house	Active Ingredient
Velpatasvir ^{b,c}		100.0	In-house	Active Ingredient
Voxilaprevir ^{d,e,f}		100.0	In-house	Active Ingredient
Copovidone ^{b,c,e,f,g,h}		(b) (4)	NF, Ph. Eur.	(b) (4)
(b) (4)			USP, Ph. Eur.	
			USP, Ph. Eur.	
Lactose Monohydrate ^{a,e,f}			NF, Ph. Eur.	
Microcrystalline Cellulose			NF, Ph. Eur.	
Croscarmellose Sodium			NF, Ph. Eur.	
Colloidal Silicon Dioxide			NF, Ph. Eur.	
Magnesium Stearate			NF, Ph. Eur.	
(b) (4)				
			NF, Ph. Eur.	
			NF, Ph. Eur.	
			NF, Ph. Eur.	
			—	
			In-house	
			USP, Ph. Eur.	

(b) (4)

2.4 Comments on Novel Excipients

All excipient exposures fall below maximum potencies listed for approved products in the FDA Inactive Ingredient Database.

2.5 Comments on Impurities/Degradants of Concern

Proposed specifications, or lack of specifications, are considered acceptable from a pharmacology/toxicology perspective. See impurity review in the Appendix.

2.6 Comments on Clinical Metabolites of Concern

Based on a mass balance study using radiolabeled ^{14}C -VOX (Study no. GS-US-338-1124), unmetabolized VOX accounts for > 90% of systemic plasma exposure in healthy subjects. Therefore, there were no significant circulating clinical metabolites (e.g., >10% of total drug-related exposure) requiring nonclinical safety assessment.

2.7 Proposed Clinical Population and Dosing Regimen

The SOF/VEL/VOX FDC is indicated for the treatment of chronic HCV infection in adults. The proposed clinical dose of SOF/VEL/VOX is 400/100/100 mg for 12 weeks.

2.8 Regulatory Background

IND#119,926 (VOX) and IND#125,751 (SOF/VEL/VOX FDC) were originally submitted to DAVP on 1/9/14 and 4/22/15, respectively. NDA#209-195 (SOF/VEL/VOX) was submitted on 12/7/2016. The FDC was granted fast track designation on 6/12/15 and break through therapy designation on 2/19/16.

3 Studies Submitted to Support VOX Development

3.1 Studies Reviewed

Secondary Pharmacology

Study no. PC-230-2001 Lead Profiling Screen of GS-620857

Safety Pharmacology

Cardiovascular

Study no. PC-230-2005 Effect of GS-9857 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Study no. PC-230-2008 Cardiovascular Safety Pharmacology Evaluation of GS-9857 Administered by Oral Capsule to Male Telemetry-Instrumented Conscious Dogs

Neurological

Study no. PC-230-2007 Central Nervous System Safety Pharmacology Evaluation of GS-9857 following Oral Gavage Administration to Male Rats

Respiratory

Study no. PC-230-2006 Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of GS-9857 following Oral Gavage Administration to Male Rats

Pharmacokinetics/ADME**Absorption**

Study no. AD-338-2001 Single Dose Pharmacokinetic Study of GS-9857 in Male Sprague Dawley Rats

Study no. AD-338-2002 Single Dose Pharmacokinetic Study of GS-9857 in Male Beagle Dogs

Study no. AD-338-2014 Pharmacokinetics of GS-9857 Following Single Ascending Oral Doses in Beagle Dogs

Study no. AD-338-2017 Pharmacokinetics of GS-9857 Following Single Ascending Oral Doses in Male Sprague Dawley Rats

Study no. AD-338-2023 Pharmacokinetics of GS-9857 Following Single Oral Doses in Female New Zealand White Rabbits

Distribution

Study no. AD-338-2005 *In Vitro* Protein Binding Determination of GS-9857 by Equilibrium Dialysis

Study no. AD-338-2030 Pharmacokinetics, Absorption, and Excretion of ¹⁴C-GS-9857 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs

Study no. AD-338-2032 Pharmacokinetics, Absorption, Distribution, and Excretion of ¹⁴C-GS-9857 Following a Single Oral Administration to Rats

Metabolism

Study no. AD-338-2006 Cytochrome P450 Metabolic Reaction Phenotyping of GS-9857

Study no. AD-338-2018 *In Vitro* Metabolic Stability of GS-9857 in Hepatic Microsomal Fractions and Human Hepatocytes

Study no. AD-338-2031 Profiling and Identification of Metabolites of ¹⁴C-GS-9857 in Selected Plasma, Urine, Bile, and Feces Samples after a Single Oral Administration of ¹⁴C-GS-9857 to Dogs

Study no. AD-338-2033 Profiling and Identification of Metabolites of ¹⁴C-GS-9857 in Selected Plasma, Urine, Bile, and Feces Samples after a Single Oral Administration of ¹⁴C-GS-9857 to Rats

Drug Interactions

Study no. AD-230-2075 *In Vitro* Assessment of GS-9857 Inhibition of Human OATP1B1 and OATP1B3

General Toxicology

Study no. TX-230-2001 5-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Male Rats

Study no. TX-230-2006	2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Rats with a 2-Week Recovery Phase
Study no. TX-230-2007	2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Dogs with a 2-Week Recovery Phase
Study no. TX-338-2001	13-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Sprague Dawley Rats
Study no. TX-338-2002	13-Week Oral Capsule Toxicity and Toxicokinetic Study with GS-9857 in Dogs
Study no. TX-338-2006	A 26-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-9857 in Sprague Dawley Rats with a 4-Week Recovery Phase
Study no. TX-338-2007	39-Week Oral Capsule Toxicity and Toxicokinetic Study with GS-9857 in Beagle Dogs with a 4-Week Recovery Phase

Genetic Toxicology

Study no. TX-230-2003	Bacterial Reverse Mutation Assay Plate Incorporation Method with GS-9857
Study no. TX-230-2004	Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-9857
Study no. TX-230-2005	<i>In Vivo</i> Rat Bone Marrow Micronucleus Assay with GS-9857

Developmental and Reproductive Toxicology

Study no. TX-338-2004	Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetics Study with GS-9857 in Sprague Dawley Rats
Study no. TX-338-2005	Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetics Study with GS-9857 in Rabbits
Study no. TX-338-2008	Oral Gavage Embryo-Fetal Developmental Toxicity Study with GS-9857 in Rats
Study no. TX-338-2009	Oral Gavage Embryo-Fetal Developmental Toxicity and Toxicokinetic Study with GS-9857 in Rabbits
Study no. TX-338-2010	An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation of GS-9857 in Sprague Dawley Rats
Study no. TX-338-2011	An Oral (Gavage) Study of the Effects of GS-9857 on Pre- and Post-Natal Development, Including Maternal Function in Sprague Dawley Rats

Special Toxicology Studies

Study no. TX-338-2012	Neutral Red Uptake Phototoxicity Assay of GS-9857 in BALB/c 3T3 Mouse Fibroblasts
Study no. TX-338-2013	A Multiple Dose Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of GS-9857 on Eyes and Skin in Pigmented Rats
Study no. TX-338-2032	GS-9857: The Bovine Corneal Opacity and Permeability (BCOP) Assay
Study no. TX-338-2033	GS-9857: <i>In vitro</i> EPIDERM™ Skin Corrosion Test
Study no. TX-338-2034	GS-9857: Local Lymph Node Assay in the Mouse – Individual Method

Study no. TX-338-2037 GS-9857: Determination of Skin Irritation Potential using the EPISKINTM Reconstructed Human Epidermis Model

Impurities

Study no. TX-338-2026 2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-9857 in Male Sprague Dawley Rats

Study no. PDM-2593 (Q)SAR Evaluation of Voxilaprevir Potential and/or Actual Impurities for the Bacterial Revers Mutation Assay

3.2 Studies Not Reviewed

Studies considered irrelevant for the nonclinical safety assessment were not reviewed (e.g., primary pharmacology, PK comparison for different formulations, etc.).

3.3 Previous Reviews Referenced

NDA#204-671: SovaldiTM (400 mg SOF + ribavirin with or without pegylated interferon α)

NDA#208-341: Epclusa® (SOF/VEL 400/100 mg FDC)

4 Pharmacology**4.1 Primary Pharmacology**

See virology review.

4.2 Secondary Pharmacology

Title: Lead Profiling Screen of GS-620857 (Study no. PC-230-2001)

Summary – Radioligand binding was evaluated in a panel of receptors, ion channels, and transporters incubated with GS-9857. Significant response (i.e., $\geq 50\%$ inhibition or stimulation) was observed for tachykinin NK₁ (IC₅₀ = 0.74 μ M).

4.3 Safety Pharmacology**Cardiovascular**

Title: Effect of GS-9857 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study no. PC-230-2005)

Summary – hERG potassium channel tail current was evaluated in HEK293 cells (stably transfected with hERG cDNA) perfused with 10 or 30 μ M GS-9857. Positive (60 nM terfenadine) and negative controls (DMSO) induced the appropriate responses. Minimal inhibition of hERG tail current was observed (IC₅₀ > 30 μ M).

Title: Cardiovascular Safety Pharmacology Evaluation of GS-9857 Administered by Oral Capsule to Male Telemetry-Instrumented Conscious Dogs (Study no. PC-230-2008)

Summary – Mortality, clinical signs, body weight, food consumption, body temperature, hemodynamics (heart rate; systolic, diastolic, and mean arterial pressures; arterial pulse pressure), and ECG examinations (morphology; PR, QRS, QT, and QT_c intervals) were evaluated in telemetered conscious male Beagle dogs orally administered vehicle/control article (PEG 400), 3, 10, or 20 mg/kg GS-9857. Cardiovascular endpoints were assessed up to 49 hr post-dose. There were no drug-related effects.

Neurological

Title: Central Nervous System Safety Pharmacology Evaluation of GS-9857 following Oral Gavage Administration to Male Rats (Study no. PC-230-2007)

Summary – Mortality, clinical signs, and a modified Irwin battery of neurological assessments [home cage, hand-held, open-field, and elicited response (including body temperature) observations] were evaluated in male Sprague Dawley rats orally administered vehicle/control article (13.34 w/w ethanol, 8.88% w/w propylene glycol, 33.34% w/w Labrasol, and 44.44% Kolliphor HS-15), 10, 15, 30, or 100 mg/kg GS-9857. Neurological endpoints were assessed up to 48 hr post-dose. There were no drug-related effects.

Respiratory

Title: Respiratory Safety Pharmacology Evaluation Using Head-Out Plethysmography of GS-9857 following Oral Gavage Administration to Male Rats (Study no. PC-230-2006)

Summary – Mortality, clinical signs, and respiratory function (tidal volume, respiration rate, and minute volume) were evaluated in male Sprague Dawley rats orally administered vehicle/control article (13.34 w/w ethanol, 8.88% w/w propylene glycol, 33.34% w/w Labrasol, and 44.44% Kolliphor HS-15), 10, 15, 30, or 100 mg/kg GS-9857. Respiratory endpoints were assessed up to 48 hr post-dose. There were no drug-related effects.

5 Pharmacokinetics/ADME

Absorption

Title: Single Dose Pharmacokinetic Study of GS-9857 in Male Sprague Dawley Rats (Study no. AD-338-2001)

Summary – Pharmacokinetics were evaluated in male Sprague Dawley rats administered a single 2-hr iv infusion of 1 mg/kg GS-9857 or single oral dose of 3 mg/kg GS-9857. Clearance was less than hepatic blood flow suggesting the potential for accumulation. V_{ss} was greater than total body water indicating the likelihood of extravascular distribution. Absolute bioavailability of GS-9857 was 82.7%. The t_{1/2} was 1.65 and 1.59 hr following iv and oral dosing, respectively.

Title: Single Dose Pharmacokinetic Study of GS-9857 in Male Beagle Dogs (Study no. AD-338-2002)

Summary – Pharmacokinetics were evaluated in male Beagle dogs administered a single 2-hr iv infusion of 0.5 mg/kg GS-9857 or single oral dose of 1 mg/kg GS-9857. Clearance was less than hepatic blood flow suggesting the potential for accumulation. V_{ss} was greater than total body water indicating the likelihood of extravascular distribution. Absolute bioavailability of GS-9857 was 26.6%. The $t_{1/2}$ was 4.22 and 4.05 hr following iv and oral dosing, respectively.

Title: Pharmacokinetics of GS-9857 Following Single Ascending Oral Doses in Beagle Dogs (Study no. AD-338-2014)

Summary – Pharmacokinetics were evaluated in male Beagle dogs administered single oral doses of 5, 10, 20, or 40 mg/kg GS-9857. Increasing GS-9857 dose resulted in a greater than dose-proportional increase in C_{max} and $AUC_{0-24\text{ hr}}$ values from 5 to 20 mg/kg. Systemic exposures at 20 and 40 mg/kg were similar suggesting saturation occurred. Data is summarized below (table taken from the sponsor submission).

Table 2. Pharmacokinetics in male dogs

Dosage (mg/kg)	C_{max}		$AUC_{0-24\text{ h}}$	
	μM	$\mu\text{g/mL}$	$\mu\text{M}\cdot\text{h}$	$\mu\text{g}\cdot\text{h} / \text{mL}$
5	4.3 ± 2.2	3.7 ± 1.9	16.4 ± 6.6	14.3 ± 5.7
10	8.8 ± 3.3	7.6 ± 2.9	71.5 ± 23.0	62.1 ± 20.0
20	24.5 ± 8.1	21.3 ± 7.0	289 ± 113	251 ± 98
40	24.9 ± 18.5	21.6 ± 16.1	302 ± 279	262 ± 242

Title: Pharmacokinetics of GS-9857 Following Single Ascending Oral Doses in Male Sprague Dawley Rats (Study no. AD-338-2017)

Summary – Pharmacokinetics were evaluated in male Sprague Dawley rats administered single oral doses of 3, 10, 30, 100, or 300 mg/kg GS-9857. Increasing GS-9857 dose resulted in a greater than dose-proportional increase in C_{max} and $AUC_{0-24\text{ hr}}$ values from 3 to 100 mg/kg but less than dose-proportional increase from 100 to 300 mg/kg/day. Data is summarized below (table taken from the sponsor submission).

Table 3. Pharmacokinetics in male rats

Dosage (mg/kg)	C _{max}		AUC _{0-24h}	
	μM	μg/mL	μM•h	μg•h /mL
3	0.29 ± 0.06	0.25 ± 0.05	2.05 ± 0.18	1.78 ± 0.16
10	2.60 ± 0.54	2.26 ± 0.47	23.7 ± 5.59	20.6 ± 4.9
30	16.2 ± 4.1	14.1 ± 3.6	205 ± 33	178 ± 28.7
100	77.2 ± 17.6	67.1 ± 15.3	1250 ± 364	1086 ± 316
300	157 ± 27	136 ± 23.5	2230 ± 149	1938 ± 129

Title: Pharmacokinetics of GS-9857 Following Single Oral Doses in Female New Zealand White Rabbits (Study no. AD-338-2023)

Summary – Pharmacokinetics were evaluated in female New Zealand white rabbits administered single oral doses of 10, 30, 100, 300, or 1000 mg/kg GS-9857. Increasing GS-9857 dose resulted in a greater than dose-proportional increase in systemic exposures from 10 to 100 mg/kg and an approximately dose-proportional increase from 100 to 300 mg/kg. AUC_{0-24 hr} and C_{max} values at 1000 mg/kg were substantially less than 300 mg/kg. Vehicle selection had minimal impact on systemic exposures at 100 mg/kg. Data is summarized below (table taken from the sponsor submission).

Table 4. Pharmacokinetics in female rabbits

Parameter	Route of Administration			
	Oral	Oral	Oral	Oral
Dosage (mg/kg)	AUC _{0-24h} (ng•h/mL)	C _{max} (ng/mL)	T _{max} (h)	C _{24h} (ng/mL)
10 ^a	24.0 ± 14.9	10.6 ± 3.4	2.0 ± 0.0	BLQ
30 ^a	117 ± 31.5	41.3 ± 9.2	2.0 ± 0.0	BLQ
100 ^a	3750 ± 3480	1120 ± 1210	3.3 ± 1.2	9.7 ± 3.1
300 ^a	11000 ± 5640	4960 ± 2940	2.7 ± 1.2	10.5 ± 2.0
1000 ^a	1790 ± 733	562 ± 534	10.0 ± 12.2	76.0 ± 41.5
100 ^b	4550 ± 2220	1290 ± 602	4.0 ± 0.0	4.4 ± 1.2

a Formulation consisted of 0.5% Carboxymethylcellulose (CMC) Sodium, 0.5% Tween 20, 0.9% Benzyl Alcohol in 50 mM Phosphate Buffer (pH 7.5)

b Formulation consisted of 100% polyethylene glycol 400

Distribution

Title: *In Vitro* Protein Binding Determination of GS-9857 by Equilibrium Dialysis (Study no. AD-338-2005)

Summary – Protein binding was evaluated in plasma from Sprague Dawley rats, Beagle dogs, cynomolgus monkeys, rhesus monkeys, and humans incubated with GS-9857. A high degree of binding (> 99%) was observed in all species.

Title: Pharmacokinetics, Absorption, and Excretion of ^{14}C -GS-9857 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs (Study no. AD-338-2030)

Summary – Pharmacokinetics, absorption, and excretion were evaluated in intact and bile-duct cannulated male Beagle dogs administered a single oral dose of 10 mg/kg ^{14}C -GS-9857 (radiolabel incorporated into the carbamate moiety). The $t_{1/2}$ for total reactivity was 1.63 hr in blood and 1.57 hr in plasma. Blood:plasma was < 1 indicating limited distribution to blood cells. Results in bile-duct cannulated dogs indicated biliary excretion was the major route of elimination.

Title: Pharmacokinetics, Absorption, Distribution, and Excretion of ^{14}C -GS-9857 Following a Single Oral Administration to Rats (Study no. AD-338-2032)

Summary – Pharmacokinetics, absorption, distribution, and excretion were evaluated in intact and bile-duct cannulated male Sprague Dawley rats as well as intact male Long Evans rats administered a single oral dose of 30 mg/kg ^{14}C -GS-9857 (radiolabel incorporated into the carbamate moiety).

Sprague Dawley: Blood:plasma was < 1 indicating limited distribution to blood cells. Radioactivity was widely distributed with the highest C_{max} values occurring in liver, kidney cortex, kidneys, small intestine, kidney medulla, exorbital lacrimal gland, and brown fat. Low concentrations of radioactivity were detected in brain, testes, eye, and skin. Peak concentrations in tissues were generally reached by 8 hr and clearance achieved by 48 hr post-dose. Elimination $t_{1/2}$ ranged from 1.77 hr (blood) to 51.1 hr (stomach). Results in bile-duct cannulated rats indicated biliary excretion was a major route of elimination.

Long-Evans: Blood:plasma was < 1 indicating limited distribution to blood cells. Radioactivity was widely distributed with the highest C_{max} values occurring in the liver, small intestine, kidney cortex, kidneys, kidney medulla, intra-orbital lacrimal gland, and exorbital lacrimal gland. Low concentrations of radioactivity were detected in testes, eye, and skin. Peak concentrations in tissues were generally achieved by 8 hr and clearance achieved by 48 hr post-dose. Elimination $t_{1/2}$ ranged from 2.11 hr (blood) to 21.4 hr (thyroid).

Overall, radioactivity was widely distributed, primarily to organs of elimination. GS-9857 associated radioactivity did not readily cross the blood:brain and blood:testis barriers. The distribution patterns in uveal tract and skin suggested that melanin binding did not occur.

Metabolism

Title: Cytochrome P450 Metabolic Reaction Phenotyping of GS-9857 (Study no. AD-338-2006)

Summary – The metabolic capacity of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A was evaluated in bacterially expressed human CYP450 enzyme preparations incubated with GS-9857. Minimal metabolism was observed only in CYP2C8 and CYP3A4.

Title: *In Vitro* Metabolic Stability of GS-9857 in Hepatic Microsomal Fractions and Human Hepatocytes (Study no. AD-338-2018)

Summary – Metabolic stability was evaluated in hepatic microsomes from BALB/c mice, Sprague Dawley rats, Beagle dogs, cynomolgus monkey, and humans as well as human hepatocytes incubated with GS-9857. With the exception of cynomolgus monkey, GS-9857 was metabolically stable in all species.

Title: Profiling and Identification of Metabolites of ¹⁴C-GS-9857 in Selected Plasma, Urine, Bile, and Feces Samples after a Single Oral Administration of ¹⁴C-GS-9857 to Dogs (Study no. AD-338-2031)

Profiling and Identification of Metabolites of ¹⁴C-GS-9857 in Selected Plasma, Urine, Bile, and Feces Samples after a Single Oral Administration of ¹⁴C-GS-9857 to Rats (Study no. AD-338-2033)

A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism, and Excretion of GS-9857 (Study no. GS-US-338-1124)

Summary – Metabolite profiles were generated in samples from multiple species administered a single oral dose of ¹⁴C-GS-9857 (radiolabel incorporated into the carbamate moiety). Doses were 10 mg/kg in intact and bile-duct cannulated male dogs, 30 mg/kg in intact and bile-duct cannulated male Sprague Dawley rats, and 100 mg in healthy male clinical subjects. Data is summarized below (table and figure taken from the sponsor submission).

Table 5. Metabolite profile in rats, dogs, and humans

Analyte	Assignment	Rat	Dog	Human
VOX	Parent	i, p, u, f, b	i, p, u, f, b	i, p, f
M7	Oxy 1	i, u, f, b	-	i, f
M8	Dehydro 1	i, u, f, b	i, u, f, b	i, f
M9	Dehydro 2	i, u, f, b	i, u, f, b	i, f
M10	Desfluoro-oxy	u, f, b	i, f, b	f
M11	Oxy 3	i, f, b	i	i, f
M18	(Unknown)	-	b	-
M19	Hydrolyzed	f	f	f
M20	(Unknown)	-	b	-
M21	Hydrolyzed-oxy 1	f	-	f
M22	Hydrolyzed-dehydro	f	-	f
M23	Desmethyl	f	-	f
M25	Hydrolyzed-oxy 2	-	-	f
M26	Hydrolyzed-oxy 3	-	-	f

i: detected in vitro, p: detected in plasma, u: detected in urine, f: detected in feces, b: detected in bile (rat and dog only)

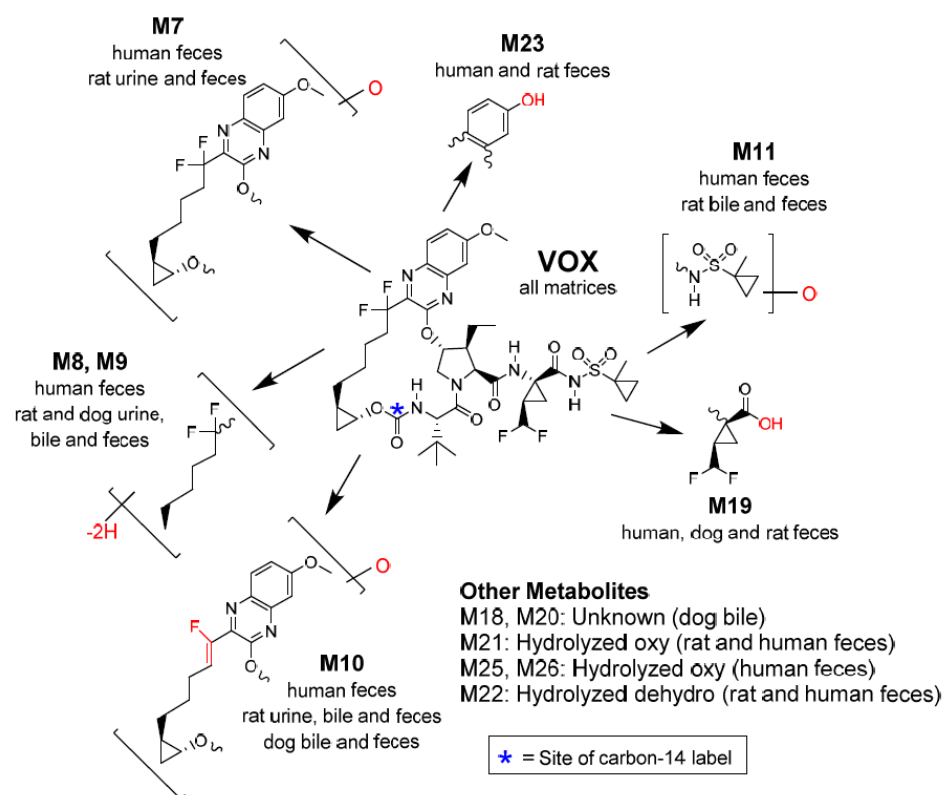


Figure 1. Proposed metabolic pathways in rats, dogs, and humans

Drug Interaction

Title: *In Vitro* Assessment of GS-9857 Inhibition of Human OATP1B1 and OATP1B3 (Study no. AD-230-2075)

Summary – Inhibition of solute carrier influx transporters was evaluated in wild-type Chinese Hamster Ovary cells or cells transfected with OATP1B1 or OATP1B3 incubated with 0.014 to 30 μ M GS-9857. Dose-dependent inhibition of both OATP1B1 (IC_{50} = 0.18 μ M) and OATP1B3 (IC_{50} = 0.70 μ M) was observed.

6 General Toxicology

Title: 5-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Male Rats (Study no. TX-230-2001)

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, hematology, coagulation, clinical chemistry, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in male Sprague Dawley rats administered oral doses of vehicle/control article (13.4% ethanol, 8.88% propylene glycol, 33.33% Labrasol, and 44.44% Kolliphor HS-15), 10, 25, or 100 mg/kg/day GS-9857 for 5 days. Decreased body weight gain and food consumption occurred. There were extensive drug-related changes in hematology (decreased RBC associated parameters and reticulocytes with increases in several WBC cell types) and clinical chemistry (decreased protein parameters, changes in electrolytes, as well as increases in total bilirubin). Gross pathological findings in the nonglandular stomach (discolored; raised area; rough surface) correlated with minimal to moderate microscopic lesions (erosion/ulcer; hemorrhage; hyperplasia/hyperkeratosis; inflammation, mixed cell). Several hematology and clinical chemistry changes were considered secondary to mild blood loss in nonglandular stomach. Increases in total bilirubin were likely associated with OATP inhibition. Drug-related effects were observed only in the high-dose group. Lesions in the nonglandular stomach were adverse; however, these findings were of limited clinical relevance and, therefore, not considered for the overall study NOAEL below. All other effects were deemed non-adverse.

Based on the absence of adverse effects at any dose, the NOAEL was 100 mg/kg/day ($AUC_{0-24\text{ hr}}$ = 670 μ g·hr/mL; C_{max} = 60.5 μ g/mL).

Title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Rats with a 2-Week Recovery Phase (Study no. TX-230-2006)

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, hematology, coagulation, clinical chemistry, urinalysis, d-dimer, gross pathology, organ weights, histopathology, hepatic microsomal activity, and toxicokinetics were evaluated in Sprague Dawley rats administered oral doses of vehicle/control article (13.34% ethanol, 8.88% propylene glycol, 33.34% Labrasol, and 44.44% Kolliphor HS-15), 10, 15, 30, or 100 mg/kg/day GS-9857 for 2 weeks followed by a 2 week recovery period. One low-dose female and one mid-dose female were found dead on Days 6 and 3, respectively. The cause of death was not determined but neither rat had clinical signs suggestive of drug-related toxicity.

These deaths were considered of uncertain relationship to treatment. There were extensive drug-related changes in hematology (decreased RBC associated parameters, increases in several WBC cell types, platelets, and reticulocytes), clinical chemistry (decreased protein parameters, changes in electrolytes, as well as increases in ALT, glucose, cholesterol, and bilirubin parameters), and urinalysis (decreased urine specific gravity with increased volume, pH, and urobilinogen). Organ weight increases were noted in the liver and kidney. Effects on the spleen included increased organ weight parameters and minimal to slight microscopic lesions (hematopoiesis, extramedullary, increased). Minimal microscopic lesions in bone marrow (cellularity, increased) were also observed. Effects on spleen and bone marrow were consistent with hematology and clinical chemistry changes suggesting potential mild blood loss and a compensatory response. These effects may have been secondary to minimal to slight microscopic lesions in nonglandular stomach (hyperplasia/hyperkeratosis, epithelium; inflammation, mixed cell/edema). Increases in bilirubin parameters were likely associated with OATP inhibition. An increase in CYP3A activity was observed in high-dose females. Drug-related effects were primarily observed in the high-dose group; however, the low/mid- and high/mid-dose groups were sometimes affected. All effects appeared reversible. Lesions in the nonglandular stomach were adverse; however, these findings were of limited clinical relevance and, therefore, not considered for the overall study NOAEL below. All other effects were deemed non-adverse.

Based on the absence of adverse effects at any dose, the NOAEL was 100 mg/kg/day ($AUC_{0-t} = 535 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 46.9 \mu\text{g/mL}$).

Title: 2-Week Oral Capsule Toxicity and Toxicokinetic Study with GS-9857 in Dogs with a 2-Week Recovery Phase (Study no. TX-230-2007)

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, ECG examinations, hematology, coagulation, clinical chemistry, urinalysis, D-dimer, gross pathology, organ weights, histopathology, hepatic microsomal activity, and toxicokinetics were evaluated in Beagle dogs administered oral doses of vehicle/control article (PEG 400), 3, 10, or 20 mg/kg/day GS-9857 for 2 weeks followed by a 2-week recovery period. Drug-related emesis, body weight loss, and decreased food consumption occurred. Clinical pathology changes included increased total bilirubin and increased urine urobilinogen. Increased total bilirubin was likely associated with OATP inhibition. Decreases in adrenal gland weight parameters as well as increases in pituitary gland and thyroid/parathyroid weights were observed. Minimal to slight microscopic lesions were noted in the gall bladder (hyperplasia, epithelium; vacuoles, epithelium increased; infiltrate, mononuclear cell). With the exception of microscopic vacuolation in the gall bladder, all effects appeared reversible. Drug-related effects were primarily observed in the high-dose group; however, the low- and mid-dose groups were sometimes affected. Emesis, body weight loss, and decreased food consumption were considered adverse at the high-dose. All other findings were considered non-adverse.

Based on the absence of adverse effects in the mid-dose group, the NOAEL was 10 mg/kg/day ($AUC_{0-t} = 47.5 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 5.59 \mu\text{g/mL}$).

Title: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9857 in Sprague Dawley Rats (Study no. TX-338-2001)

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Sprague Dawley rats administered oral doses of vehicle/control article (13.34% ethanol, 8.88% propylene glycol, 33.34% Labrasol, and 44.44% Kolliphor HS-15), 10, 30, or 100 mg/kg/day GS-9857 for 13 weeks. There were extensive drug-related changes in hematology (decreased RBC associated parameters with increases in several WBC types, platelets, and reticulocytes), clinical chemistry (decreased protein parameters, changes in electrolytes, as well as increases in ALT, cholesterol, triglycerides, and bilirubin parameters), and urinalysis (decreased urine specific gravity with increased volume and urobilinogen). Macroscopic lesions observed in liver (large) correlated with increased organ weight. Increased weight parameters were also noted in spleen, kidney, and heart. Minimal to slight microscopic lesions occurred in spleen (hematopoiesis) and bone marrow (increased cellularity). Effects in these tissues were consistent with hematology and clinical chemistry changes suggesting potential mild blood loss and a compensatory response. These effects may have been secondary to minimal to moderate microscopic lesions in nonglandular stomach (edema, mucosa; infiltrate, mixed cell; hyperplasia/hyperkeratosis; fibrosis, submucosa; neovascularization). Minimal to slight lesions also occurred in glandular stomach (edema, submucosa; infiltrate, mixed cell) while minimal lesions were observed in the liver (hyperplasia, bile duct). Increases in bilirubin parameters were likely associated with OATP inhibition. Drug-related effects primarily occurred in the high-dose group although low- and mid-dose animals were sometime affected. Lesions in the nonglandular stomach were adverse; however, these findings were of limited clinical relevance and, therefore, not considered for the overall study NOAEL below. All other effects were deemed non-adverse.

Based on the absence of adverse effects at any dose, the NOAEL was 100 mg/kg/day ($AUC_{0-t} = 558 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 45.8 \text{ ng/mL}$).

Title: 13-Week Oral Capsule Toxicity and Toxicokinetic Study with GS-9857 in Dogs (Study no. TX-338-2002)

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, ECG examinations, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Beagle dogs administered oral doses of vehicle/control article (PEG 400), 3, 10, or 15 mg/kg/day GS-9857 for 13 weeks. Drug-related effects included emesis as well as minimal to slight microscopic lesions in gall bladder (hyperplasia, epithelium; vacuoles, epithelium increased; infiltrate, mononuclear cell, increased), kidney (vacuolation, tubule epithelial cell, increased), cecum (infiltrate, neutrophils; hemorrhage), and rectum (infiltrate, neutrophils with erosion). Lesions in the gall bladder were primarily observed in the mid and high-dose groups; however, vacuoles were noted in a single low-dose male. Effects in other GI tissues were limited to the

high-dose group while the kidney lesion occurred in mid- and high-dose animals. All effects were considered non-adverse.

Based on the absence of adverse effects at any dose, the NOAEL was 15 mg/kg/day ($AUC_{0-t} = 246 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 18.6 \mu\text{g/mL}$).

Title: A 26-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-9857 in Sprague Dawley Rats with a 4-Week Recovery Phase (Study no. TX-338-2006)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	3/16/15
GLP Compliance:	yes, with exception of test article characterization and stability as well as minor documentation error regarding animal feed on day of arrival
QA Statement:	yes
Drug, Lot#, and Purity:	GS-9857, lot#PP-1027-5001, purity 99.5%

Key Findings

- Adverse effects were not observed.
- Non-adverse and/or clinically irrelevant effects were noted in the nonglandular stomach, glandular stomach, and liver. Additional effects consistent with a compensatory response to blood loss and OATP inhibition were also observed.
- NOAEL = 70 mg/kg/day

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Sprague Dawley rats administered oral doses of vehicle/control article (13.34% ethanol, 8.88% propylene glycol, 33.34% Labrasol, and 44.44% Kolliphor HS-15), 10, 30, or 70 mg/kg/day GS-9857 for 26 weeks followed by a 4-week recovery period. There were extensive drug-related changes in hematology (increases in several WBC types and reticulocytes), clinical chemistry (decreased protein parameters, changes in electrolytes, as well as increases in ALT, cholesterol, triglycerides, and bilirubin parameters), and urinalysis (decreased urine specific gravity with increased volume and urobilinogen). Increased liver weight parameters were observed. Minimal to mild microscopic lesions occurred in the nonglandular stomach (edema, mucosa; hyperplasia/hyperkeratosis; infiltrate, mixed cell). Minimal lesions were also noted in the glandular stomach (infiltrate, mixed cell) and liver (hyperplasia, bile duct). Hematology and clinical chemistry changes suggestive of potential mild blood loss with a compensatory response were considered secondary to lesions in the nonglandular stomach. Increases in bilirubin were likely associated with OATP inhibition. Although most effects appeared reversible, minor changes in individual clinical pathology parameters, liver weights, and bile duct hyperplasia were present following the recovery period. Drug-related effects primarily impacted the high-dose group although low- and mid-dose animals were sometime affected. Lesions in the nonglandular stomach were adverse;

however, these findings were of limited clinical relevance and, therefore, not considered for the overall study NOAEL below. All other effects were deemed non-adverse.

Based on the absence of adverse effects at any dose, the NOAEL was 70 mg/kg/day ($AUC_{0-24 \text{ hr}} = 631 \text{ } \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 56.1 \text{ } \mu\text{g/mL}$).

Methods

Doses:

Group	Subgroup	No. of Animals ^a		Dose Level (mg/kg/day)	Dose Concentration ^b (mg/mL)
		Male	Female		
1 (Control) ^c	1 (Toxicity)	15	15	0	0
	2 (Toxicokinetic)	3	3	0	0
2 (Low)	1 (Toxicity)	10	10	10	2
	2 (Toxicokinetic)	9	9	10	2
3 (Mid)	1 (Toxicity)	10	10	30	6
	2 (Toxicokinetic)	9	9	30	6
4 (High)	1 (Toxicity)	15	15	70	14
	2 (Toxicokinetic)	9	9	70	14

a Toxicity animals designated for recovery sacrifice (five animals/sex in Groups 1 and 4) underwent at least 4 weeks of recovery following dosing.

b Concentrations were based on the test article as supplied. No correction factor was used.

c Group 1 received vehicle control article only.

Frequency of Dosing:	daily
Route of Administration:	oral
Dose Volume:	5 mL/kg
Basis of Dose Selection:	High-dose selection was based on results of the 13-week study (TX-338-2001). The high-dose of 70 mg/kg/day selected for the 26-week study provided > 50x exposure margin vs. clinical exposures.
Vehicle/Control Article:	13.34% (w/w) ethanol, 8.88% (w/w) propylene glycol, 33.34% Labrasol, and 44.44% (w/w) Kolliphor HS-15
Species/Strain:	rat/Hsd:Sprague Dawley SD (b) (4)
Age at Study Start:	6 to 7 weeks
Weight at Study Start:	males = 175 to 233 g females = 152 to 192 g
Unique Study Design:	none
Protocol Deviations:	minor with no impact on study integrity

Observations and Results

Mortality

There were no drug-related effects. One high-dose female was found dead on Day 88 but the death was considered accidental based on timing (death occurred after blood collection). One low-dose female was sacrificed on Day 117 due to poor condition. The cause of the poor condition was not determined; however, this animal had clinical signs (head tilt and ataxia) not observed in other mid-dose animals or in the high-dose group. In addition, one mid-dose male designated for toxicokinetic analysis was found dead on Day 6. Although this animal had no clinical signs suggesting toxicity, a cause of death was not determined.

Clinical Signs/Physical Examinations

There were no drug-related effects.

Body Weights

There were no drug-related effects.

Food Consumption

There were no drug-related effects.

Ophthalmic Examinations (pre-test; Week 26)

There were no drug-related effects.

Hematology (unscheduled sacrifice; Weeks 4, 13, and scheduled necropsies)

There were no significant findings in the low-dose female sacrificed in poor condition. Drug-related effects in surviving animals were extensive. Increases in reticulocytes and red cell distribution width (RCDW) were observed at multiple sampling timepoints in the mid- and high-dose groups. These changes were consistent with a compensatory response to mild blood loss. Increases in white blood cell types were also noted at all sampling timepoints in all dose groups and were suggestive of mild chronic inflammation. The compensatory response and inflammation were potentially related to microscopic lesions in the stomach (see histopathology). Other effects included decreased MCHC with increased platelets and LUC. With the exception of increases in some white blood cell types for high-dose females, hematological changes appeared reversible. Overall, the effects were considered non-adverse. Data is summarized below.

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Table 6. Hematological changes in a 26-week rat study^a

	<u>Males</u>			<u>Females</u>		
	10	30	70	10	30	70
MCHC						
Week 4	-	-	-	-	-	-3.0%
Week 26	-	-	-	-	-	-1.6%
RCDW						
Week 4	-	+9.3%	+21%	-	+17%	+27%
Week 13	-	+4.0%	+8.7%	-	+6.1%	+9.7%
Week 26	-	-	+3.8%	-	+7.8%	+15%
reticulocytes						
Week 4	-	+24%	+41%	-	+52%	+85%
Week 13	-	+7.3%	+20%	-	-	+15%
Week 26	-	-	+11%	-	+26%	+40%
platelets						
Week 4	-	-	+13%	-	+16%	+17%
Week 13	-	-	+9.6%	-	-	-
Week 26	-	-	+22%	-	+12%	+22%
WBC						
Week 4	+14%	+11%	+52%	+8.5%	+17%	+36%
Week 13	+37%	+47%	+91%	+23%	+31%	+53%
Week 26	+15%	+20%	+65%	+16%	+48%	+100%
neutrophils						
Week 4	+28%	+16%	+35%	+11%	+35%	+37%
Week 13	+120%	+120%	+180%	+35%	+50%	+37%
Week 26	+30%	+9.1%	+41%	-	+52%	+68%
lymphocytes						
Week 4	+7.7%	+8.6%	+56%	+8.9%	+12%	+36%
Week 13	+16%	+30%	+70%	+20%	+27%	+54%
Week 26	+8.7%	+24%	+75%	+24%	+47%	+110%
monocytes						
Week 4	+92%	+54%	+42%	+13%	+60%	+53%
Week 13	+88%	+82%	+100%	+33%	+44%	+56%
Week 26	+26%	+16%	+74%	-	+50%	+75%
eosinophils						
Week 13	+86%	-	+86%	+100%	+60%	+80%
Week 26	-	+50%	+50%	-	+43%	+230%
basophils						
Week 13	-	-	+250%	-	-	-
Week 26	-	-	-	-	↑	↑
LUC						
Week 4	-	-	-	-	-	+100%
Week 13	-	-	+130%	-	-	+130%
Week 26	-	-	-	-	-	+100%

^a % change vs. concurrent controls**Coagulation** (unscheduled sacrifice; Weeks 4, 13, and scheduled necropsies)

There were no significant findings in the low-dose female sacrificed in poor condition. In animals that survived to the scheduled necropsy, drug-related decreases in fibrinogen ($\geq -10\%$ vs. concurrent controls) were observed in high-dose males at all sampling timepoints. The effect was reversible and considered non-adverse.

Clinical Chemistry (unscheduled sacrifice; Weeks 4, 13, and scheduled necropsies)

There were no significant findings in the low-dose female sacrificed in poor condition. Drug-related effects in surviving animals included decreases in protein parameters and chloride as well as increases in ALT, bilirubin (total, direct, and indirect), phosphorus, potassium, cholesterol, and triglycerides. Decreases in protein parameters correlated with hematological effects and were consistent with mild blood loss. Changes in electrolytes may have been related to decreased urine specific gravity and increased urine volume. Increases in bilirubin parameters were likely associated with inhibition of organic anion transporting polypeptide (OATP) by GS-9857. Increased ALT and ALP were possible related to increases in organ weight parameters and microscopic changes in liver (see histopathology section). Effects on clinical chemistry primarily affected the high-dose group with the mid-dose impacted to a lesser degree. With the exception of phosphorus in high-dose females, all clinical chemistry changes appeared reversible. Overall, changes in clinical chemistry parameters were considered non-adverse. Data is summarized below.

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Table 7. Clinical chemistry changes in a 26-week rat study^a

	<u>Males</u>			<u>Females</u>		
	10	30	70	10	30	70
total protein						
Week 13	-	-	-5.6%	-4.0%	-5.4%	-4.0%
Week 26	-	-	-7.0%	-	-	-11%
albumin						
Week 4	-	-	-	-	-	-6.4%
Week 13	-	-	-6.5%	-4.1%	-6.1%	-4.1%
Week 26	-	-	-4.4%	-	-3.9%	-9.6%
globulin						
Week 13	-	-3.7%	-7.4%	-	-	-
Week 26	-	-3.9%	-12%	-	-	-9.1%
cholesterol						
Week 4	-	+31%	+28%	-	-	+47%
Week 13	-	+20%	-	-	-	+32%
Week 26	-	-	-	-	-	+16%
triglycerides						
Week 4	-	-	+77%	-	-	+120%
Week 13	+39%	+48%	+63%	-	-	+120%
Week 26	-	+39%	+79%	-	-	+160%
total bilirubin						
Week 4	-	-	+200%	-	+100%	+200%
Week 13	-	+100%	+300%	-	+100%	+300%
Week 26	-	+100%	+300%	-	+100%	+500%
direct bilirubin						
Week 13	-	-	+200%	-	-	+200%
Week 26	-	-	+200%	-	-	+300%
indirect bilirubin						
Week 4	-	-	↑	-	↑	↑
Week 13	-	↑	↑	-	↑	↑
Week 26	-	↑	↑	-	↑	↑
ALT						
Week 4	-	-	+20%	-	-	+45%
Week 13	-	-	+28%	-	-	+53%
Week 26	-	-	+27%	-	-	+67%
ALP						
Week 4	-	-	+13%	-	+20%	+33%
Week 13	-	-	-	-	+18%	-
Week 26	-	-	+14%	+17%	+49%	+51%
phosphorus						
Week 4	-	-	+10%	-	-	+14%
Week 13	-	-	-	-	-	-
Week 26	-	-	+12%	-	+8.9%	+11%
potassium						
Week 4	-	+5.1%	+8.5%	-	+9.6%	+15%
Week 13	-	+9.1%	+7.3%	-	+10%	+14%
Week 26	-	-	+7.1%	-	+3.9%	-
chloride						
Week 4	-	-	-2.0%	-	-	-
Week 13	-	-	-2.0%	-	-	-
Week 26	-	-	-2.0%	-	-	-

^a % change vs. concurrent controls

Urinalysis (scheduled necropsies)

Drug-related effects included decreased urine specific gravity with increased urine volume and urobilinogen. The changes in urine specific gravity and volume had no correlating pathology suggesting kidney toxicity. Increases in urobilinogen were likely related to increased bilirubin. Effects were reversible and considered non-adverse. Data is summarized below.

Table 8. Urinalysis data in a 26-week rat study^a

	<u>Males</u>			<u>Females</u>		
	10	30	70	10	30	70
urine volume Week 26	-	+110%	+59%	+55%	+38%	+250%
specific gravity Week 26	-	-	-	-	-	-1.5%
urobilinogen Week 26	-	↑	↑	-	↑	↑

^a % change vs. concurrent controls

Gross Pathology (unscheduled and scheduled necropsies)

There were no drug-related effects.

Organ Weights (scheduled necropsies)

Drug-related increases in liver weight parameters were observed. The effect occurred in high-dose males ($\leq +18\%$ vs. concurrent controls) and females ($\leq +26\%$ vs. concurrent controls). Increase organ weight may have been partially attributed to microscopic lesions (see histopathology). Liver weight parameters remained slightly elevated following the recovery period. Overall, effects were considered non-adverse.

Histopathology (unscheduled and scheduled necropsies)

Adequate Battery – yes

Peer Review – yes

Histological Findings – Drug-related effects were observed in the nonglandular stomach (edema, submucosa; hyperplasia/hyperkeratosis; infiltrate, mixed cell), glandular stomach (infiltrate, mixed cell), and liver (hyperplasia, bile duct). The effects in nonglandular stomach were considered to be of limited clinical relevance as this organ is unique to rodents. Bile duct hyperplasia was consistent with increased liver weight parameters. Microscopic lesions occurred in the high-dose group. Nonglandular and glandular stomach lesions were not observed following the recovery period while bile duct hyperplasia persisted in high-dose females (i.e., minimal severity in 5/5 animals examined). With the exception of clinically irrelevant changes in the nonglandular stomach, the microscopic lesions were considered non-adverse. Data is summarized below (table taken from the sponsor submission).

Table 9. Histopathological lesions in a 26-week rat study

Tissue/finding	Sex	Males				Females			
GS-9857 Dose (mg/kg/day)		0	10	30	70	0	10	30	70
Stomach, nonglandular									
No. Examined:		10	10	10	10	10	9	10	9
Edema, submucosa									
Total number affected		1	0	0	3	0	0	0	1
Minimal		0	0	0	2	0	0	0	0
Slight		1	0	0	1	0	0	0	1
Hyperplasia/hyperkeratosis									
Total number affected		0	0	0	5	0	0	0	2
Minimal		0	0	0	4	0	0	0	2
Slight		0	0	0	1	0	0	0	0
Infiltrate, mixed cell									
Total number affected		0	0	0	4	0	0	0	1
Minimal		0	0	0	2	0	0	0	1
Slight		0	0	0	2	0	0	0	0
Stomach, glandular									
No. examined:		10	10	10	10	10	9	10	9
Infiltrate, mixed cell									
Total number affected		0	0	0	3	0	0	0	1
Minimal		0	0	0	3	0	0	0	1
Liver									
No. examined:		10	10	10	10	10	9	10	9
Hyperplasia, bile duct									
Total number affected		0	0	0	0	0	0	5	9
Minimal		0	0	0	0	0	0	5	9

Toxicokinetics (Days 1 and 26)

GS-9857 toxicokinetics were similar in both males and females. Repeat-dosing did not affect systemic exposure in the low- and mid-dose groups; however, systemic exposures were slightly higher in Week 26 vs. Day 1 for the high-dose group. This data suggests accumulation occurred at the highest dose tested. Increasing dose resulted in a greater than dose-proportional increase in C_{max} and $AUC_{0-24 \text{ hr}}$ values. Control contamination was not detected. Data is summarized below (table taken from the sponsor submission).

Table 10. Toxicokinetic data in a 26-week rat study

Interval	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
Day 1	2	10	M	1300	6.00	8230	8860	105	12.0
			F	622	6.00	4690	5180	81.6	12.0
			MF	961	6.00	6460	7020	93.2	12.0
	3	30	M	9810	6.00	91800	91800	30.9	24.0
			F	9880	6.00	78700	78700	46.3	24.0
			MF	9840	6.00	85300	85300	38.6	24.0
	4	70	M	20000	6.00	339000	339000	9300	24.0
			F	19800	6.00	235000	235000	5620	24.0
			MF	19900	6.00	287000	287000	7460	24.0
Week 26	2	10	M	642	6.00	4610	4610	6.89	24.0
			F	882	6.00	5990	6300	51.0	12.0
			MF	762	6.00	5480	5480	6.89	24.0
	3	30	M	18100	6.00	143000	143000	38.7	24.0
			F	14600	6.00	118000	118000	23.7	24.0
			MF	16400	6.00	130000	130000	32.7	24.0
	4	70	M	48500	6.00	582000	582000	2020	24.0
			F	63600	6.00	679000	679000	1870	24.0
			MF	56100	6.00	631000	631000	1940	24.0

F = Female; M = Male.

Note: MF data are based on the analysis of the combined concentration data for both males and females.

Dose Formulation Analysis (homogeneity: Day 1, Weeks 7 and 26; concentration: Day 1, Weeks 4, 13, and 26)

Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Title: 39-Week Oral Capsule Toxicity and Toxicokinetic Study with GS-9857 in Beagle Dogs with a 4-Week Recovery Phase (Study no. TX-338-2007)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	2/26/15
GLP Compliance:	yes, with exception of test article characterization and stability as well as minor bioanalytical issues
QA Statement:	yes
Drug, Lot#, and Purity:	GS-9857, lot#PP-1027-5001, purity 99.4%

Key Findings

- Adverse effects were not observed.
- Non-adverse effects were noted in the gall bladder.
- NOAEL = 15 mg/kg/day

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, ECG examinations, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Beagle dogs administered oral doses of vehicle/control article (PEG 400), 3, 10, or 15 mg/kg/day GS-9857 for 39 weeks followed by a 4-week recovery period. Drug-related effects included excess salivation, emesis, and red skin of the paw regions as well as decreased food consumption. Minimal to slight microscopic lesions were noted in the gall bladder (hyperplasia, epithelium; vacuoles, epithelium). With the exception of gall bladder lesions, changes appeared reversible. Effects were primarily observed in the mid and high-dose groups; however, the low-dose was sometimes affected. Overall, effects were considered non-adverse.

Based on the absence of adverse effects at any dose, the NOAEL was 15 mg/kg/day ($AUC_{0-24 \text{ hr}} = 253 \text{ } \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 20.7 \text{ } \mu\text{g/mL}$).

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Methods

Doses:

Group	No. of Animals ^a		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female		
1 (Control) ^b	6	6	0	0
2 (Low)	4	4	3	6
3 (Mid)	4	4	10	20
4 (High)	6	6	15	30

^a Animals designated for recovery sacrifice (two animals/sex in Groups 1 and 4) underwent at least 4 weeks of recovery following dosing.

^b Group 1 received vehicle control article only.

Frequency of Dosing:	daily
Route of Administration:	oral
Dose Volume:	0.5 mL/kg
Basis of Dose Selection:	High-dose selection was based on results of the 13-week study (TX-338-2002). The high-dose of 15 mg/kg/day selected for the 39-week study provided > 50x exposure margin vs. clinical exposures.
Vehicle/Control Article:	PEG 400
Species/Strain:	dog/Beagle (b) (4)
Age at Study Start:	9 to 10 months
Weight at Study Start:	males = 8.3 to 12.3 kg females = 5.9 to 9.9 kg
Unique Study Design:	none
Protocol Deviations:	minor with no impact on study integrity

Observations and Results

Mortality

There were no drug-related effects.

Clinical Signs/Physical Examinations

Drug-related effects included excess salivation, emesis, and red skin of the paw regions. Salivation was observed at all doses while the other clinical signs occurred in the mid- and high-dose groups. The effects were not observed during the recovery period. Overall, the effects were considered non-adverse.

Body Weights

There were no drug-related effects.

Food Consumption

Drug-related decreases in qualitative food consumption was observed in the high-dose group. The decrease did not affect body weight and, therefore, was considered non-adverse.

Ophthalmic Examinations (pre-test; Day 270)

There were no drug-related effects.

ECGs (pre-test; Week 39 4 hr post-dose)

There were no drug-related effects.

Hematology (pre-test; Days 86 and 274 of dosing phase; Day 29 of recovery phase)

There were no drug-related effects.

Coagulation (pre-test; Days 86 and 274 of dosing phase; Day 29 of recovery phase)

There were no drug-related effects.

Clinical Chemistry (pre-test; Days 86 and 274 of dosing phase; Day 29 of recovery phase)

There were no drug-related effects.

Urinalysis (pre-test; Days 86 and 274 of dosing phase; Day 29 of recovery phase)

There were no drug-related effects.

Gross Pathology (scheduled necropsies)

There were no drug-related effects.

Organ Weights (scheduled necropsies)

There were no drug-related effects.

Histopathology (scheduled necropsies)

Adequate Battery – yes

Peer Review – yes

Histological Findings – Drug-related effects occurred in the gall bladder (hyperplasia, epithelium; vacuoles, epithelium). These lesions were primarily observed in the mid and high-dose groups; however, vacuoles were also noted in a single low-dose male. Although less severe, both lesions were observed following the recovery period. All effects were considered non-adverse. Data is summarized below (table taken from the sponsor submission).

Table 11. Histopathological lesions in a 39-week dog study

Tissue/finding	Sex	Males				Females			
GS-9857 Dose (mg/kg/day)		0	3	10	15	0	3	10	15
Terminal Sacrifice									
No. Examined:		4	4	4	4	4	4	4	4
Gall Bladder									
Hyperplasia, epithelium									
Minimal		0	0	2	2	0	0	4	3
Slight		0	0	2	2	0	0	0	1
Vacuoles, epithelium									
Minimal		0	1	3	2	0	1	2	4
Slight		0	0	1	2	0	0	0	0
Recovery Sacrifice									
No. examined:		2	NA	NA	2	2	NA	NA	2
Gall Bladder									
Hyperplasia, epithelium									
Minimal		0	NA	NA	1	0	NA	NA	1
Vacuoles, epithelium									
Minimal		0	NA	NA	2	0	NA	NA	0

Toxicokinetics (Day 1, Weeks 13 and 39)

GS-9857 toxicokinetics were similar in both males and females. Repeat-dosing did not affect systemic exposure in the low- and mid-dose groups; however, systemic exposures were slightly higher following repeat-dosing in the high-dose group. This data suggests accumulation occurred at the highest dose tested. Increasing dose resulted in a greater than dose-proportional increase in C_{max} and $AUC_{0-24 \text{ hr}}$ values. Data is summarized below (table taken from the sponsor submission).

Table 12. Toxicokinetic data in a 39-week dog study

GS-9857 (mg/kg/day)	Sex	C_{max} (ng/mL)			AUC_{0-24} (ng·h/mL)		
		Day 1	Week 13	Week 39	Day 1	Week 13	Week 39
3	M	1120	797	775	5250	3870	2770
	F	602	1450	748	3370	5950	3520
	MF	859	1130	761	4310	4910	3150
10	M	6950	15100	10100	92300	164000	99600
	F	5610	6450	7070	49900	74200	56800
	MF	6280	10800	8610	71100	119000	78200
15	M	10100	24500	17200	129000	298000	191000
	F	11000	27500	23600	145000	392000	305000
	MF	10500	26000	20700	136000	345000	253000

Dosing Solution Analysis (homogeneity: Day 1; concentration: Day 1, Weeks 4, 13, and 39)

Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

7 Genetic Toxicology

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

Title: Bacterial Reverse Mutation Assay Plate Incorporation Method with GS-9857 (Study no. TX-230-2003)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	7/29/13
GLP compliance:	yes, with exception of test article characterization and stability
QA Statement:	included
Drug, Lot #, and Purity:	GS-9857, lot#5867-152-15, purity 98.2%

Key Findings

- GS-9857 was negative in the bacterial reverse mutation assay.

Summary - Mutagenicity was evaluated in *Salmonella* strains TA98, TA100, TA1535, and TA1537 as well as *E. coli* strain WP2uvrA at doses ≤ 5000 $\mu\text{g}/\text{plate}$ GS-9857 with or without metabolic activation. Positive (multiple compounds) and negative controls (DMSO) induced the appropriate responses. There were no drug-related increases in revertants/plate.

Methods

Strains:	TA98, TA100, TA1535, TA1537, and WP2uvrA
Concentrations:	5, 16, 50, 160, 500, 1600, and 5000 $\mu\text{g}/\text{plate}$
Basis of Concentration Selection:	limit dose of 5000 $\mu\text{g}/\text{plate}$
Negative Control:	DMSO
Positive Controls:	

Tester Strain(s)	S9	Positive Control	Dose ($\mu\text{g}/\text{plate}$)
TA98	–	2-nitrofluorene	1.0
TA100, TA1535	–	sodium azide	2.0
TA1537	–	ICR-191	2.0
WP2uvrA	–	4-nitroquinoline-N-oxide	1.0
TA98	+	benzo[a]pyrene	2.5
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5
WP2uvrA	+	2-aminoanthracene	25.0

Formulation/Vehicle:	DMSO
Incubation & Sampling Time:	Plates were incubated for 52 ± 4 hr at 37°C with or without metabolic activation (plate incorporation method). The metabolic activation system included 10% S9 fraction from Aroclor 1254 induced male Sprague Dawley rat liver.

Study Validity and Positive Response Criteria

- Tester Strain Integrity – Tester strains must exhibit the presence of appropriate modifications (i.e., *his*⁻, *trp*⁻, *rfa*, *uvrA* or *uvrB* mutations, and pKM101 plasmid).
- Spontaneous Revertants – Mean revertants/plate for the vehicle controls must be within acceptance limits based upon historical data and published reports.
- Tester Strain Culture Density – Each culture must reach a target of $\geq 10^9$ cells/mL.
- Positive Control Values – Positive control articles must induce a ≥ 3 -fold increase in mean revertants/plate vs. concurrent vehicle controls.
- Number of Dose Levels – A minimum of 3 non-toxic doses must be evaluated.
- Top Dose Level – The highest dose evaluated should be the limit dose of 5000 µg/plate, produce significant cytotoxicity, or exceed the limit of solubility (whichever is lowest).
- Positive Response – The test article must produce a dose-dependent increase in revertant frequency that is ≥ 2 -fold vehicle control values for TA98, TA100 and WP2uvrA, or ≥ 3 x vs. vehicle controls for TA1535 and TA1537.

Observations and Results

Mutagenicity Assay

There were no drug-related increases in revertants/plate. Precipitate was observed in all strains at doses ≥ 1600 µg/plate without metabolic activation and ≥ 500 µg/plate with metabolic activation. Toxicity (i.e., measured as reduction in revertants/plate) was noted in WP2uvrA at doses ≥ 500 µg/plate with and without metabolic activation as well as TA1535 and TA1537 at 5000 µg/plate without metabolic activation. The background lawn was normal in all experiments. Positive and negative controls induced the appropriate responses. Overall, the laboratory's criteria for a valid study were met.

Dose Formulation Analysis

Acceptance criteria were met for concentration. Test article was not detected in control formulations.

7.2 In Vitro Assays in Mammalian Cells

Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-9857 (Study no. TX-230-2004)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	7/29/13
GLP Compliance:	yes, with exception of test article characterization and stability
QA Statement:	included
Drug, Lot #, and Purity:	GS-9857, lot#5867-152-15, and purity 98.2%

Key Findings

- GS-9857 was negative in the *in vitro* chromosomal aberration assay.

Summary – Chromosomal aberrations were evaluated in human peripheral blood lymphocytes at doses ≤ 85.0 $\mu\text{g/mL}$ for 3 hr exposures without metabolic activation, ≤ 6.92 $\mu\text{g/mL}$ for 22 hr exposures without metabolic activation, and ≤ 58.8 $\mu\text{g/mL}$ for 3 hr exposures with metabolic activation. Positive (multiple compounds) and negative controls (DMSO) induced the appropriate responses. There were no drug-related increases in the number of cells with structural chromosomal aberrations, polyploidy, or endoreduplication.

Methods

Cell Line: human peripheral blood lymphocytes

Concentrations in Definitive Study:

S9	Time	Doses ^a
-	3 hr	3.39, 4.84, 6.92, 9.89, 14.1, 20.2, 28.8, <u>41.2</u> , <u>58.8</u> , <u>84.0</u> , 120, 172, 245, 350, and 500 $\mu\text{g/mL}$
	24 hr	<u>3.39</u> , <u>4.84</u> , <u>6.92</u> , 9.89, 14.1, 20.2, 28.8, 41.2, 58.8, 84.0, 120, 172, 245, 350, and 500 $\mu\text{g/mL}$
+	3 hr	3.39, 4.84, 6.92, 9.89, 14.1, 20.2, <u>28.8</u> , <u>41.2</u> , <u>58.8</u> , 84.0, 120, 172, 245, 350, and 500 $\mu\text{g/mL}$

^a underlined doses were scored for aberrations

Basis of Concentration Selection: The Sponsor evaluated the highest dose that resulted in ~50% cytotoxicity (i.e., measured as reduction in mitotic index).

Negative Control: DMSO

Positive Control:

S9	Control	Dose
-	MMC	3 hr = 1.0 $\mu\text{g/mL}$ 24 hr = 0.3 $\mu\text{g/mL}$
+	cyclophosphamide	3 hr = 15 and 25 $\mu\text{g/mL}$

Formulation/Vehicle: DMSO

Incubation & Sampling Time: Cells were exposed for 3 hr with and without metabolic activation or 24 hr without metabolic activation. All incubations were at 37 °C. Colcemid was present for the last 2 hr before harvest of mitotic cells at 24 hr. The metabolic activation system contained 1.5% S9 fraction from Aroclor 1254-induced male Sprague Dawley rat liver.

Study Validity and Positive Response Criteria

- Vehicle Controls – The vehicle controls must contain less than ~5% cells with aberrations.
- Positive Controls – The number of cells with aberrations must be significantly higher ($p \leq 0.01$) than vehicle controls.

- High Dose –If no cytotoxicity is observed the high-dose should reach the limit of solubility or ICH limit dose (i.e., lower of 1 mM or 0.5 mg/mL).
- Number of Doses – The study must include 3 analyzable concentrations.
- Positive Response – The test article must induce a significant increase ($p \leq 0.01$) in the number of cells with aberrations at 1 or more concentrations. A dose-response should be observed if a significant increase is observed at 1 or more concentrations.

Observations and Results

Chromosomal Aberration Assay

There were no drug-related increases in the number of cells with structural chromosomal aberrations, polyploidy, or endoreduplication. Cytotoxicity (i.e., measured as reduction in mitotic index) at the top doses were 59% for 3 hr exposure without metabolic activation, 53% for 24 hr exposure without metabolic activation, and 39% for 3 hr exposure with metabolic activation. Precipitate was observed at concentrations $\geq 120 \mu\text{g/mL}$. Positive and negative controls induced the appropriate responses. Overall, the laboratory's criteria for a valid study were met.

Dose Formulation Analysis

Acceptance criteria were met for concentration. Test article was not detected in control formulations.

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

Title: *In Vivo* Rat Bone Marrow Micronucleus Assay with GS-9857 (Study no. TX-230-2005)

Study Report Location:	EDR
Conducting Laboratory:	(b) (4)
Date of Study Initiation:	illegible (sponsor study monitor signed 7/29/13)
GLP Compliance:	yes, with exception of test article characterization and stability
QA Statement:	included
Drug, Lot #, and % Purity:	GS-9857, lot#5867-152-15, and purity 98.2%

Key Finding

- GS-9857 was negative in the *in vivo* micronucleus assay.

Summary – The induction of micronucleated polychromatic erythrocytes (PCE) was evaluated in bone marrow from male Sprague Dawley rats administered oral doses of vehicle/control article (13.34% ethanol, 8.88% propylene glycol, 33.34% Labrasol, and 44.44% Kolliphor HS-15), 500, 1000, or 2000 mg/kg/day GS-9857 for 2 days. One rat from each of the low- and mid-dose groups was found dead on Study Day 3. No cause of death was determined. Clinical signs included hypoactive behavior, discolored hair coat, and fecal changes (non-formed or liquid). There were no statistically significant increases in micronucleated PCE at any dose level. Bone

marrow toxicity was not observed. Positive (60 mg/kg cyclophosphamide) and negative controls (vehicle/control) induced the appropriate responses.

Methods

Doses in Definitive Study:

Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/24-Harvest Timepoint	Toxicokinetic Animals ^a
				Males	Males
PC, 60	6	10	Oral Gavage	5	-
VC, 0	0	10	Oral Gavage	5	3
GS-9857, 500	50	10	Oral Gavage	5	3
GS-9857, 1000	100	10	Oral Gavage	5	3
GS-9857, 2000	200	10	Oral Gavage	6	3

PC = Positive Control = Cyclophosphamide (cyclophosphamide monohydrate; CP)

VC = Vehicle Control = 13.34% (w/w) ethanol, 8.88% (w/w) propylene glycol, 33.34% (w/w) Labrasol, 44.44% (w/w) Kolliphor HS-15

a = On day 2 of dosing, three males per test article dose group were bled at approximately 0.5, 2, 6, 12, and 24 hours after dosing. Three additional males were dosed with the vehicle control and bled approximately 6 hours after dosing.

Frequency of Dosing: daily
Route of Administration: oral
Vehicle/Control: 13.34% (w/w) ethanol, 8.88% (w/w) propylene glycol, 33.34% (w/w) Labrasol, 44.44% (w/w) Kolliphor HS-15
Species/Strain: rat/ CrI:CD®(SD) Sprague Dawley (b) (4)
Basis of Dose Selection: Dose selection was based on results of a range-findings study (data not reviewed) where no effects were observed at doses of 1000 or 2000 mg/kg/day. As no toxicity was observed, the ICH S2(R1) limit dose of 2000 mg/kg/day was used as the high-dose in the definitive study. Also, there were no sex differences; therefore, males were used in the definitive study.
Positive Control: 60 mg/kg/day x single dose on Study Day 2
Micronucleus Analysis: Bone marrow was extracted ~24 hr following dosing on Study Day 2. For each sample, ≥ 2000 polychromatic erythrocytes (PCE) were analyzed to determine the frequency of normal and micronucleated PCE. Cytotoxicity was evaluated by determining the ratio of PCE and normochromatic erythrocytes (NCE) in 500 erythrocytes/animal.
Protocol Deviations: minor with no impact on study integrity

Study Validity and Positive Response Criteria

- Vehicle Control: Mean micronucleated PCEs must be within the historical control range and usually be < 0.4%.
- Positive Controls: Must induce a statistically significant increase in the mean micronucleated PCEs relative to the vehicle control group and be consistent with historical control data.
- High-Dose Level: Must be the limit dose of 2000 mg/kg, MTD (e.g., based on expected lethality), MFD, or saturation of exposure.
- Positive Response: Must exhibit a statistically significant increase in micronucleated PCEs for at least 1 dose level, and a statistically significant dose-related response.

Observations and Results

Mortality and Clinical Observations

One rat from each of the low- and mid-dose groups was found dead on Study Day 3. No cause of death was determined. Clinical signs included hypoactive behavior, discolored hair coat, and fecal changes (non-formed or liquid).

Micronucleus Assay

There were no statistically significant increases in micronucleated PCE at any dose level. Drug-related bone marrow toxicity was not observed. Overall, the laboratory's criteria for a valid test were met.

Toxicokinetics

Systemic exposures were similar across all dose levels. Control contamination did not occur. Data is summarized below (table taken from the sponsor submission).

Table 13. Toxicokinetic data in a rat micronucleus study

Dose Group	Dose Level (mg/kg)	Sex		C _{max} (ng/mL)	T _{max} (hr)	AUC _{0-t} (ng·hr/mL)	T _{last} (hr)	C _{last} (ng/mL)
3	500	M	Mean	147000	12.0	3070000	24.0	119000
			SD	NA	NA	NA	NA	NA
			N	2	2	2	2	2
4	1000	M	Mean	122000	12.0	2230000	24.0	57200
			SD	NA	NA	NA	NA	NA
			N	2	2	2	2	2
5	2000	M	Mean	137000	10.0	2750000	24.0	106000
			SD	33000	3.46	406000	0	16400
			N	3	3	3	3	3

Dose Formulation Analysis

Acceptance criteria were met for homogeneity and concentration. Test article was not detected in control formulations.

8 Reproductive and Developmental Toxicology

Abbreviations:

Gestation Day = GD
 Lactation Day = LD
 Post-Natal Day = PND
 Study Day = SD

8.1 Fertility and Early Embryonic Development

Title: An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation of GS-9857 in Sprague Dawley Rats (Study no. TX-338-2010)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	3/12/15
GLP Compliance:	yes, with exception of test article characterization and stability
QA Statement:	yes
Drug, Lot #, and % Purity:	GS-9857, lot PP-1027-5001, purity 99.5%

Key Findings

- No adverse effects were observed.
- Non-adverse effects included minor clinical signs.
- NOAEL for male fertility, female fertility and early embryonic development = 100 mg/kg/day

Summary - Mortality, clinical signs/physical examinations, body weight, food consumption, reproductive performance, gross pathology, organ weights, spermatogenic parameters, Cesarean-section parameters, and toxicokinetics were evaluated in Sprague Dawley rats (assigned 25/sex/group) administered oral doses of vehicle/control article [0.5% carboxymethylcellulose sodium (high viscosity), 0.5% Tween® 20, 0.9% benzyl alcohol, and 98.1% 50 mM phosphate buffer (pH 7.5 ± 0.2)], 10, 30, or 100 mg/kg/day GS-9857. Males were dosed for 28 days before cohabitation through mating until 1 day prior to scheduled sacrifice. Female rats were dosed 14 days before cohabitation until GD 7. Drug-related effects included non-adverse transient clinical signs in high-dose males.

Based on the absence of adverse effects at any dose, the NOAEL was 100 mg/kg/day for male fertility ($AUC_{0-24 \text{ hr}} = 333 \text{ } \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 28.3 \text{ } \mu\text{g/mL}$) as well as female fertility and early embryonic development ($AUC_{0-24 \text{ hr}} = 438 \text{ } \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 28.6 \text{ } \mu\text{g/mL}$).

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Methods

Doses:

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Rats/Sex	
				Main	TK
1	Vehicle control	0	5	25	4
2	GS-9857	10	5	25	8
3	GS-9857	30	5	25	8
4	GS-9857	100	5	25	8

Main = Main study phase

TK = Toxicokinetic phase

Frequency of Dosing: daily
 Route of Administration: oral
 Formulation/Vehicle: 0.5% (w/w) carboxymethylcellulose sodium (high viscosity), 0.5% (w/w) Tween® 20, 0.9% (w/w) benzyl alcohol, and 98.1% (w/w) 50 mM phosphate buffer, pH 7.5 ± 0.2
 Species/Strain: rat/Crl:CD(SD) Sprague Dawley (b) (4)
 Study Design: Main Study – Males were dosed 28 days before cohabitation through mating until 1 day prior to scheduled sacrifice (63-64 total doses). Females were dosed 14 days before cohabitation until GD 7 (22-35 total doses) and sacrificed on GD 15.

Toxicokinetic Study – Males were dosed from SD 0 to 28 (29 total doses). Females were dosed from SD 14 to 28 (15 total doses).

Dose Selection – Dose selection was based on results of the 13-week general toxicology study (TX-338-2001) and dose range-finding embryofetal development study (TX-338-2004).

Protocol Deviations: minor with no impact on study integrity

Observations and Results

Mortality

There were no drug-related effects in males or females. Deaths in one high-dose male (found dead on SD 44), one low-dose male (found dead on SD 63), and one control male (found dead on SD 36) were attributed to dosing errors or complications during dosing procedure.

Clinical Signs/Physical Examinations

Drug-related effects in males included red material around mouth/nose, clear material around mouth, and yellow material around the urogenital area at the high-dose. These minor effects were transient and considered non-adverse.

Body Weight

There were no drug-related effects in males or females during pre-mating or gestation.

Food Consumption

There were no drug-related effects in males or females during pre-mating or gestation.

Reproductive Performance – Male ($n \geq 23$ /group)

There were no drug-related effects on the following parameters:

- mating index
- fertility index
- copulation index

Reproductive Performance – Female ($n \geq 24$ /group)

There were no drug-related effects on the following parameters:

- mating index
- fertility index
- conception index
- estrus cycle length
- pre-coital interval

Terminal Procedures – Male ($n \geq 24$ /group)

There were no drug-related effects on the following parameters:

- gross pathology
- organ weights (brain, epididymides, pituitary gland, prostate gland, seminal vesicles, and testes)
- spermatogenic parameters (testicular sperm count, caudal epididymal sperm count, sperm production rate, motility, and morphology)

Terminal Procedures – Female ($n \geq 24$ /group)

There were no drug-related effects on the following parameters:

- gross pathology
- organ weights (brain, ovaries w/ oviducts, and pituitary gland)
- Caesarian-section parameters (corpora lutea, implantation sites, pre-implantation loss, early/late resorptions, live embryos, and post-implantation loss)

Toxicokinetic Analysis (Males: SD 0 and 28; Females: SD 14 and 28)

GS-9857 toxicokinetics were similar between male and female animals. Systemic exposures were consistent after single or multiple doses, suggesting little accumulation occurred. In general, increasing dose resulted in a greater than dose-proportional increase in $C_{\max}$ and $AUC_{0-24 \text{ hr}}$ values. Control contamination did not occur. Data is summarized in the following table (table taken from the sponsor submission).

Table 14. Toxicokinetic data in a rat fertility study

Study Day	Dose Group	Dose Level (mg/kg/day)	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-t} (ng·h/mL)	AUC ₀₋₂₄ (ng·h/mL)	C _{last} (ng/mL)	T _{last} (h)
0	2	10	M	202	2.00	1240	1300	11.0	12.0
	3	30	M	7200	6.00	53900	53900	10.6	24.0
	4	100	M	21900	6.00	277000	277000	4820	24.0
14	2	10	F	318	0.500	1890	2080	30.6	12.0
	3	30	F	9250	2.00	53500	53500	11.2	24.0
	4	100	F	32400	6.00	447000	447000	3440	24.0
28	2	10	M	475	2.00	3610	3610	7.47	24.0
			F	387	0.500	2180	2300	18.6	12.0
	3	30	M	9820	6.00	64000	64000	12.6	24.0
			F	4210	6.00	37300	37300	56.9	24.0
	4	100	M	28300	6.00	333000	333000	121	24.0
			F	28600	6.00	438000	438000	2080	24.0

Dose Formulation Analysis (homogeneity: 1st preparation; concentration: 1st, 3rd, 5th, and last preparation)

Acceptance criteria were met for homogeneity and concentration. Test article was not detected in control formulations.

8.2 Embryofetal Development

Title: Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetics Study with GS-9857 in Sprague-Dawley Rats (Study no. TX-338-2004)

Summary - Mortality, clinical signs/physical examinations, body weight, food consumption, gross pathology, Caesarian-section parameters, and toxicokinetics were evaluated in pregnant Sprague Dawley rats (assigned 8/group) administered oral doses of vehicle/control article [0.5% carboxymethylcellulose sodium (high viscosity), 0.5% Tween 20, 0.9% benzyl alcohol, 98.1% 50 mM phosphate buffer (pH 7.5)], 10, 30, or 100 mg/kg/day GS-9857 from GD 6 to 15. Fetal alterations were also evaluated. Drug-related effects were limited to decreases in maternal body weight parameters and food consumption in the mid- and high-dose groups. Overall, effects were considered non-adverse.

Based on a lack of adverse effects at any dose, the NOAEL for both maternal and developmental toxicity was 100 mg/kg/day (AUC_{0-t} = 365 µg·hr/mL; C_{max} = 30.5 µg/mL).

Title: Oral Gavage Dose Range-Finding Developmental Toxicity and Toxicokinetics Study with GS-9857 in Rabbits (Study no. TX-338-2005)

Summary - Mortality, clinical signs/physical examinations, body weight, food consumption, gross pathology, Caesarian-section parameters, and toxicokinetics were evaluated in pregnant New Zealand White rabbits (assigned 5/group) administered oral doses of vehicle/control article [0.5% carboxymethylcellulose sodium (high viscosity), 0.5% Tween 20, 0.9% benzyl alcohol, 98.1% 50 mM phosphate buffer (pH 7.5)], 100, 200, or 300 mg/kg/day GS-9857 from GD 7 to

19. Fetal alterations were also evaluated. Drug-related effects included decreases in maternal body weight parameters and decreased food consumption at all doses. With the exception of body weight changes in high-dose animals, effects occurred only during the treatment-free period. Decreased body weights were observed in fetuses from a single high-dose dam. Maternal effects in the high-dose group were considered adverse.

Based on the absence of adverse effects in the mid-dose group, the NOAEL for maternal toxicity was 200 mg/kg/day ($AUC_{0-t} = 0.67 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 0.12 \mu\text{g/mL}$). Based on the absence of adverse effects at any dose level, the NOAEL for developmental toxicity was 300 mg/kg/day ($AUC_{0-t} = 4.09 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 1.06 \mu\text{g/mL}$).

Title: Oral Gavage Embryo-Fetal Developmental Toxicity Study with GS-9857 in Rats (Study no. TX-338-2008)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	4/1/15
GLP Compliance:	yes, with exception of test article characterization and stability as well as collection of GD 0 bodyweights and clinical observations (provided by supplier)
QA Statement:	yes
Drug, Lot #, and % Purity:	GS-9857, lot PP-1027-5001, purity 99.5%

Key Findings

- No adverse effects were observed.
- Non-adverse effects included decreased body weight gain and food consumption.
- NOAEL for maternal toxicity and embryofetal development = 100 mg/kg/day

Summary - Mortality, clinical signs/physical examinations, body weight, food consumption, maternal gross pathology, reproductive performance, and Cesarean-section parameters were evaluated in pregnant Sprague Dawley rats (assigned 25/group) administered oral doses of vehicle/control article [0.5% carboxymethylcellulose sodium (high viscosity), 0.5% Tween 20, 0.9% benzyl alcohol, and 98.1% 50 mM phosphate buffer (pH 7.5 ± 0.2)], 10, 30, or 100 mg/kg/day of GS-9857 from GD 6 to 17. Fetal alterations and ossification sites were also evaluated. Drug-related decreases in body weight gain and food consumption were observed in the high-dose group.

Based on the absence of adverse findings at any dose, the NOAEL for maternal toxicity and embryofetal development was 100 mg/kg/day ($AUC_{0-t} = 365 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 30.5 \mu\text{g/mL}$).

Methods

Doses:

Group ^a	No. of Animals Mated Female	Dose Level (mg/kg/day)	Dose Concentration ^b (mg/mL)	Dosing Schedule Days of Gestation
Main Study Animals				
1 (Control)	25	0	0	6-17
2 (Low)	25	10	2	6-17
3 (Mid)	25	30	6	6-17
4 (High)	25	100	20	6-17

^a Group 1 received vehicle control article only.

^b Animals were dosed at a dose volume of 5 mL/kg.

Frequency of Dosing: once daily
 Route of Administration: oral
 Formulation/Vehicle: 0.5% (w/w) carboxymethylcellulose sodium (high viscosity), 0.5% (w/w) Tween 20, 0.9% benzyl alcohol (w/w) and 98.1% (w/w) 50 mM phosphate buffer (pH 7.5 ± 0.2)
 Species/Strain: rat/Crl:CD(SD) Sprague Dawley (b) (4),
 (b) (4)
 Study Design: Females were dosed daily from GD 6 to 17. All surviving animals were sacrificed on GD 21.

Dose Selection – Dose selection was based on results of the dose range-finding embryofetal development study (TX-338-2004).

Protocol Deviations: minor with no impact on study integrity

Observations and Results

Mortality

There were no drug-related effects.

Clinical Signs/Physical Examinations

There were no drug-related effects.

Body Weight

Drug-related decreases in body weight gain were observed in the high-dose group (-7% vs concurrent controls between GD 6 to 18). The effect did not persist after cessation of dosing. Overall, the decrease in body weight gain was considered non-adverse.

Food Consumption

Drug-related decreases in food consumption were noted in the high-dose group (≥ -8% vs concurrent controls over 2 day intervals during the dosing period). The effect did not persist after cessation of dosing. The minor effect was consistent with decreased body weight gain and considered non-adverse.

Gross Pathology (GD 21)

There were no drug-related effects.

Reproductive Performance/Pregnancy Rates (n = 25/group)

There were no drug-related effects.

Cesarean-Section (GD 21; n ≥ 24/group)

There were no drug-related effects on the following parameters:

- corpora lutea
- implantation sites
- pre-implantation loss
- placental abnormalities
- live/dead fetuses
- early/late resorptions
- post-implantation loss
- fetal weight
- fetal sex

Offspring (GD 21; n ≥ 24 litters/group)

There were no drug-related effects on the following parameters:

- gross external alterations (n ≥ 287 fetuses/group)
- soft tissues alterations (n ≥ 143 fetuses/group)
- skeletal alterations and ossification sites (n ≥ 144 fetuses/group)

Dose Formulation Analysis (1st and last preparation)

Acceptance criteria were met for homogeneity and concentration. Test article was not detected in control formulations.

Title: Oral Gavage Embryo-Fetal Developmental Toxicity and Toxicokinetic Study with GS-9857 in Rabbits (Study no. TX-338-2009)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	3/31/15
GLP Compliance:	yes, with exception of test article characterization and stability as well as collection of GD 0 bodyweights and clinical observations (provided by supplier)
QA Statement:	yes
Drug, Lot #, and % Purity:	GS-9857, lot PP-1027-5001, purity 99.5%

Key Findings

- No adverse effects were observed.
- Non-adverse effects included discolored urine (orange/red).
- NOAEL for maternal toxicity and embryofetal development = 600 mg/kg/day

Summary - Mortality, clinical signs/physical examinations, body weight, food consumption, maternal gross pathology, reproductive performance/pregnancy rates, Cesarean-section parameters, and toxicokinetics were evaluated in pregnant New Zealand White rabbits (assigned 22/group) administered oral doses of vehicle/control article [0.5% carboxymethylcellulose sodium (high viscosity), 0.5% Tween 20, 0.9% benzyl alcohol, and 98.1% 50 mM phosphate buffer (pH 7.5 ± 0.2)], 100, 300, or 600 mg/kg/day of GS-9857 from GD 7 to 19. Fetal alterations and ossification sites were also evaluated. Drug-related effects were limited to discolored urine (orange/red).

Based on the absence of adverse effects at any dose, the NOAEL for maternal toxicity and embryofetal development was 600 mg/kg/day ($AUC_{0-24 \text{ hr}} = 9.06 \mu\text{g}\cdot\text{hr/mL}$; $C_{\text{max}} = 1.26 \mu\text{g/mL}$).

Methods

Doses:

Group ^a	No. of Animals Mated Female	Dose Level (mg/kg/day)	Dose Concentration ^b (mg/mL)	Dosing Schedule Days of Gestation
Main Study Animals ^c				
1 (Control)	22	0	0	7-19
2 (Low)	22	100	20	7-19
3 (Mid)	22	300	60	7-19
4 (High)	22	600	120	7-19

a Group 1 received vehicle control article only.

b Animals were dosed at a dose volume of 5 mL/kg.

c Three of the Main Study Animals in each group were also used for collection of toxicokinetic (TK) blood samples.

Frequency of Dosing: once daily
 Route of Administration: oral
 Formulation/Vehicle: 0.5% (w/w) carboxymethylcellulose sodium (high viscosity), 0.5% (w/w) Tween 20, 0.9% benzyl alcohol (w/w) and 98.1% (w/w) 50 mM phosphate buffer (pH 7.5 ± 0.2)
 Species/Strain: rabbit/New Zealand White (b) (4)
 Study Design: Females were dosed daily from GD 7 to 19. All surviving animals were sacrificed on GD 29.

Dose Selection – Dose selection was based on results of a 7-day tolerability study (TX-338-2003; not reviewed) and dose range-finding embryofetal development study (TX-338-2005).

Protocol Deviations: minor with no impact on study integrity

Observations and Results

Mortality

There were no drug-related effects. Deaths in one low-dose rabbit (found dead on GD 26) and one mid-dose rabbit (found dead on GD 15) were attributed to an esophageal perforation and gavage error, respectively. One mid-dose animal aborted on GD 24 with 9 fetuses in the cage pan. This dam had fecal changes consistent with decreased food consumption and also experienced body weight loss (-6% vs. concurrent controls from GD 9 to 20). The abortion was a single occurrence in the mid-dose group and no abortions were observed in the dose-range finding study. Also, the effects on food consumption and body weight do not appear to be drug-related. The weight of evidence suggests the effect is associated with poor maternal health unrelated to GS-9857.

Clinical Signs/Physical Examinations

Drug-related effects included discolored urine (orange/red) in high-dose rabbits. The effect was non-adverse. Multiple incidences of few feces were noted in all groups, including controls.

Body Weights

There were no drug-related effects.

Food Consumption

There were no drug-related effects.

Gross Pathology (GD 29)

There were no drug-related effects.

Reproductive Performance/Pregnancy Rates

There were no drug-related effects.

Cesarean Section (GD 29; $n \geq 17$ /group)

There were no drug-related effects on the following parameters:

- corpora lutea
- implantation sites
- pre-implantation loss
- placental abnormalities
- live/dead fetuses
- early/late resorptions
- post-implantation loss
- fetal weight
- fetal sex

Offspring (GD 29; $n \geq 18$ litters/group and ≥ 149 fetuses/group)

There were no drug-related effects on the following parameters:

- gross external alterations
- soft tissues alterations
- skeletal alterations
- ossification sites

Toxicokinetic Analysis (GD 7 and 19)

GS-9857 systemic exposures in the mid- and high-dose groups substantially decreased upon repeat dosing. Increasing dose resulted in a greater than dose-proportional increase in C_{max} and $AUC_{0-24 \text{ hr}}$ values between the low- and mid-dose groups but approximately dose-proportional increase between the mid- and high-dose groups. Control contamination did not occur. Data is summarized in the following tables (tables taken from the sponsor submission).

Table 15. Toxicokinetic data in a rabbit embryofetal development study (GD 7)

Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
2	100	Mean	72.9	1.00	274	311	6.13	12.0
		SD	59.6	0	127	128	1.88	0
		N	3	3	3	3	3	3
3	300	Mean	6150	4.00	28900	29000	21.8	20.0
		SD	3640	0	14100	14100	20.8	6.93
		N	3	3	3	3	3	3
4	600	Mean	11700	4.67	56000	56000	38.5	24.0
		SD	7300	3.06	23200	23200	25.9	0
		N	3	3	3	3	3	3

Table 16. Toxicokinetic data in a rabbit embryofetal development study (GD 19)

Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
2	100	Mean	70.9	2.33	342	363	9.72	14.7
		SD	20.2	1.53	123	119	2.63	8.33
		N	3	3	3	3	3	3
3	300	Mean	512	2.67	2340	2450	18.3	12.0
		SD	407	1.15	1370	1360	12.4	0
		N	3	3	3	3	3	3
4	600	Mean	1260	2.33	9060	9060	73.4	24.0
		SD	266	1.53	2850	2850	84.5	0
		N	3	3	3	3	3	3

Dose Formulation Analysis (1st and last preparation)

With 3 exceptions, acceptance criteria were met for homogeneity and concentration. The GD 19 low-dose formulation primary and back-up top samples as well as the high-dose formulation primary top sample were slightly lower than expected. The out of specification investigation determined that inadequate stirring during sample collection was the cause. Because

formulations were stirred for 30 minutes prior to dosing, the out of specification results did not reflect actual experimental conditions. Therefore, the results had no impact on study integrity.

8.3 Prenatal and Postnatal Development

Title: An Oral (Gavage) Study of the Effects of GS-9857 on Pre- and Post-Natal Development, Including Maternal Function in Sprague Dawley Rats (Study no. TX-338-2011)

Study Report Location:	EDR
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	3/6/15
GLP Compliance:	yes, with exception of test article characterization and stability
QA Statement:	yes
Drug, Lot#, and Purity:	GS-9857, lot#PP-1027-5001, purity 99.5%

Key Findings

- No adverse effects were observed.
- Non-adverse decreases in F₁ pup birth weights continued to affect body weights through weaning.
- NOAEL for F₀ maternal toxicity, F₁ neonatal/developmental toxicity, F₁ parental systemic toxicity, F₁ reproductive toxicity, and F₂ neonatal/early postnatal toxicity = 100 mg/kg/day

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, gestation length and parturition, necropsy findings, and toxicokinetics were evaluated in female Sprague Dawley rats (assigned 25/group main study animals or 3 – 9/group toxicokinetic animals) administered oral doses of vehicle/control article [0.5% carboxymethylcellulose sodium (high viscosity), 0.5% Tween 20, 0.9% benzyl alcohol in 98.1% 50 mM phosphate buffer (pH 7.5 ± 0.2)], 10, 30, or 100 mg/kg/day GS-9857. Pregnant females were dosed from GD 6 through LD 20 or post-mating Day 24 (if no delivery). Litter parameters and survival, clinical signs/physical examinations, body weight, gross pathology, and toxicokinetics were evaluated in pre-weaning F₁ pups. F₁ post-weaning parameters included developmental landmarks, sensory function, neurobehavioral testing, reproductive performance, mortality, clinical signs/physical examinations, body weight, gestation length and parturition, and necropsy findings. Litter parameters and survival, clinical signs/physical examinations, body weight, and gross pathology were also evaluated in F₂ offspring. There were no drug-related effects observed in F₀ females, F₁ post-weaning or F₂ offspring. Effects in the F₁ generation pre-weaning were limited to decreases in pup birth weights that effected body weights through weaning. The effect was considered non-adverse. Toxicokinetic data indicated that significant exposure to F₁ pups occurred through lactation.

Based on the absence of adverse effects at any dose level, the NOAEL for F₀ maternal toxicity, F₁ neonatal/developmental toxicity, F₁ parental systemic toxicity, F₁ reproductive toxicity, and F₂ neonatal/early postnatal toxicity was 100 mg/kg/day (AUC_{0-24 hr} = 613 µg·hr/mL and C_{max} = 424

µg/mL in F₀ females on LD10; AUC_{0-24 hr} = 357 µg·hr/mL and C_{max} = 18.5 µg/mL in F₁ pups on PND10).

Methods

Doses:

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females	
				Main	TK
1	Vehicle Control	0	5	25	3
2	GS-9857	10	5	25	9
3	GS-9857	30	5	25	9
4	GS-9857	100	5	25	9

Main = Pre- and postnatal development phase.

TK = Toxicokinetic phase.

Frequency of Dosing: daily
 Route of Administration: oral
 Formulation/Vehicle: 0.5% (w/w) carboxymethylcellulose sodium (high viscosity), 0.5% (w/w) Tween 20, 0.9% (w/w) benzyl alcohol, in 98.1% (w/w) 50 mM phosphate buffer (pH 7.5 ± 0.2)
 Species/Strain: rat/Crl:CD(SD) (b) (4)
 Study Design: Main Study Animals – Pregnant females (F₀) were dosed daily from GD 6 through LD 20 or post-mating Day 24 (if no delivery). F₀ females who delivered were sacrificed on LD 21. A subset of offspring (F₁) were allowed to cohabitate and females were necropsied on LD 4 (or post-mating Day 25 if no delivery). F₁ males were necropsied following the last female necropsy. F₁ offspring (F₂) were sacrificed on PND 4.

Toxicokinetic Animals – Pregnant females (F₀) were dosed daily from GD 6 through LD 10. F₀ females were sacrificed on LD 11/PND 11 and F₁ pups were sacrificed following blood collection.

Dose Selection – Dose selection was based on results of the 13-week general toxicology study (TX-338-2001) and dose range-finding embryofetal development study (TX-338-2004).

Protocol Deviations: minor with no impact on study integrity

Observations and Results

F₀ Females

Mortality

There were no drug-related effects.

Clinical Signs/Physical Examinations

There were no drug-related effects.

Body Weight

There were no drug-related effects during gestation or lactation.

Food Consumption

There were no drug-related effects during gestation or lactation.

Gestation Length and Parturition (n ≥ 20/group)

There were no drug-related effects.

Necropsy (LD 21; n ≥ 20/group)

No drug-related effects on the following parameters:

- gross pathology
- former implantation sites
- unaccounted for sites

Toxicokinetics (GD 6 and LD 10)

Systemic exposures were similar on GD 6 and LD 10, indicating no accumulation occurred. Increasing dose resulted in a greater than dose-proportional increase in $C_{\max}$ and $AUC_{0-24 \text{ hr}}$ values. Control contamination was not detected. Data is summarized below (table taken from the sponsor submission).

Table 17. Toxicokinetic data in a rat pre-/post-natal study (maternal data)

Interval	Dose Group	Dose Level (mg/kg/day)	Sex	$C_{\max}$ (ng/mL)	$T_{\max}$ (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	T_{last} (h)
GD 6	2	10	F	269	4.00	1960	2080	12.0
	3	30	F	8720	4.00	69000	69000	24.0
	4	100	F	44900	8.00	575000	575000	24.0
LD 10	2	10	F	361	0.500	2400	2400	24.0
	3	30	F	6900	2.00	43000	43000	24.0
	4	100	F	42400	8.00	613000	613000	24.0

F₁ – Pre-Weaning (birth to PND 21)**Litter Parameters and Survival** (n ≥ 20 litters/group)

There were no drug-related effects on the following parameters:

- litter size
- live litter size
- sex

- gross necropsy (partial evaluation in pups found dead from PND 0 to 4)
- post-natal survival (multiple intervals from birth to PND 21)

Clinical Signs/Physical Examinations

There were no drug-related effects.

Body Weight

Drug-related decreases in birth weights were observed in high-dose males (-7.8% vs. concurrent controls) and females (-5.6% vs. concurrent controls). Decreased body weights were observed in high-dose males ($\geq -12\%$ vs. concurrent controls) and females ($\geq 9.4\%$ vs concurrent controls) at different intervals. Decreased body weight gain also occurred in high-dose males (-10% vs. concurrent controls) and females (-7.9% vs. concurrent controls) from PND 1 to 21. Body weights in the high-dose group fell within historical control values and were, therefore, considered non-adverse.

Gross Pathology (all unscheduled deaths after PND 4; scheduled necropsy)

There were no drug-related effects.

Toxicokinetics (PND 10)

GS-9857 toxicokinetics were similar in both males and females. Increasing dose resulted in a less than dose-proportional increase in $C_{\max}$ and $AUC_{0-24 \text{ hr}}$ values. The maternal:pup exposure ratio was similar across all dose groups. Detectable concentrations were present in 1 male and 1 female control pups. The values were slightly above the limit of detection and were substantially less than concentrations detected in the low-dose group. Therefore, the finding had no impact on study integrity. Data is summarized below (table taken from the sponsor submission).

Table 18. Toxicokinetic data in a rat pre/postnatal study (PND 10 data in F₁)

Dose Group	Dose Level (mg/kg/day)	Sex	$C_{\max}$ (ng/mL)	$T_{\max}$ (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	T_{last} (h)	M:P
2	10	M	635	24.0	5640	5640	24.0	0.425
		F	504	24.0	4910	4910	24.0	0.488
		MF	569	24.0	5270	5270	24.0	0.455
3	30	M	2790	8.00	36600	36600	24.0	1.18
		F	2770	1.00	32500	32500	24.0	1.33
		MF	2700	8.00	34500	34500	24.0	1.25
4	100	M	17500	2.00	352000	352000	24.0	1.74
		F	19000	2.00	360000	360000	24.0	1.70
		MF	18500	2.00	357000	357000	24.0	1.72

Note: MF data are based on the analysis of the combined concentration data for both males and females.

F₁ – Post-Weaning (after PND 21)**Developmental Landmarks, Sensory Function, and Neurobehavioral Testing** (n ≥ 18 litters/group)

There were no drug-related effects on the following parameters:

- balanopreputial separation (starting on PND 35; n = 2 rats/litter)
- vaginal patency (starting on PND 25; n = 2 rats/litter)
- auditory startle response (PND 20 and 60; n = 1 rat/sex/litter)
- motor activity (PND 21 and 61; n = 1 rat/sex/litter)
- swimming ability (PND 22 and 62; n = 1 rat/sex/litter)
- learning (PND 22 and 62; n = 1 rat/sex/litter)
- memory (PND 22 and 62; n = 1 rat/sex/litter)

Reproductive Performance (n ≥ 17/group)

There were no drug-related effects on the following parameters:

- male mating index
- male fertility index
- male copulation index
- female mating index
- female fertility index
- female conception index
- estrous cycle length
- pre-coital interval

Mortality

There were no drug-related effects. A single mid-dose male was sacrificed in poor condition on PND 31. This animal had misaligned incisors and low weight but no other effects. The death was considered unrelated to treatment.

Clinical Signs/Physical Examinations

There were no drug-related effects.

Body Weight

Drug-related effects on body weight parameters were noted in the high-dose group. The decrease occurred only at early timepoints and was considered a continuation of effects observed during the pre-weaning period (birth to PND 21). There were no drug-related effects during pre-mating (females and males from PND 21 to 84), the subsequent evaluation period in males (PND 21 to 126), or in maternal rats during gestation or lactation. Overall, the transient effect was considered non-adverse.

Gestation Length and Parturition ($n \geq 17/\text{group}$)

There were no drug-related effects.

Necropsy (females: LD 4 or post-mating Day 25 if no delivery; males: following the last female necropsy)

There were no drug-related effects on the following parameters in females:

- gross pathology
- corpora lutea
- implantation sites
- unaccounted for sites

There were no drug-related effects on gross pathology in males.

F₂ - Litter Data**Litter Parameters and Survival** ($n \geq 17$ litters/group)

There were no drug-related effects on the following parameters:

- litter size
- live litter size
- sex
- gross malformations
- postnatal survival (multiple intervals from birth to PND 4)

Clinical Signs/Physical Examinations

There were no drug-related effects.

Body Weight (PND 1 - 4)

There were no drug-related effects.

Gross Pathology (unscheduled deaths)

There were no drug-related effects.

Dose Formulation Analysis (homogeneity: 1st preparation; concentration: 1st and last preparation)

Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

9 Special Toxicology Studies

Title: Neutral Red Uptake Phototoxicity Assay of GS-9857 in BALB/c 3T3 Mouse Fibroblasts (Study no. TX-338-2012)

Summary – Phototoxicity was evaluated in BALB/c 3T3 fibroblasts exposed to ≤ 3.16 $\mu\text{g/mL}$ GS-9857 with and without ultraviolet radiation. The positive control (≤ 178 $\mu\text{g/mL}$ promethazine) induced the appropriate response. Based on a photoirritancy factor > 6.846 and mean photo effect = 0.443, GS-9857 was shown to possess phototoxic and cytotoxic potential.

Title: A Multiple Dose Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of GS-9857 on Eyes and Skin in Pigmented Rats (Study no. TX-338-2013)

Summary – Mortality, clinical observations/physical examinations, body weight, dermal effects, ophthalmic examinations, histopathology, and plasma concentrations were evaluated in skin and eyes of Long-Evans pigmented rats administered oral doses of vehicle/control article (13.34% ethanol, 8.88% propylene glycol, 33.34% Labrasol®, and 44.44% Kolliphor HS-15), 10, 30, or 100 mg/kg/day of GS-9857 for 3 days followed by exposure to UVA, UVB, and visible light. Drug-related effects included dehydration at the high-dose and decreased body weight parameters at all doses. There were no effects suggestive of cutaneous or ocular phototoxicity. Positive (15 mg/kg/day 8-methoxypsoralen) and negative controls (vehicle/control) induced the appropriate responses. Plasma concentration data is summarized in the following table (table taken from the sponsor submission).

Table 19. Plasma GS-9857 concentrations in a rat phototoxicity study

Treatment Description	Day	Average Concentration (ng/ml) Pre-dose on Day 3	Average Concentration (ng/ml) 6 hours post dose on Day 3
0 mg/kg	3	BLQ < (5.00)	BLQ < (5.00)
10 mg/kg	3	BLQ < (5.00)	347
30 mg/kg	3	50	7067
100 mg/kg	3	155	36500

BLQ: Below the limit of quantitation

Title: GS-9857: The Bovine Corneal Opacity and Permeability (BCOP) Assay (Study no. TX-338-2032)

Summary – Ocular irritation potential was evaluated in isolated bovine corneas exposed to 20% (w/v) GS-9857. Positive (20% w/v imidazole) and negative controls (0.9% w/v sodium chloride)

induced the appropriate responses. Based on an *in vitro* irritancy score = 6.4, GS-9857 was classified as a non-irritant.

Title: GS-9857: *In Vitro* EPIDERM™ Skin Corrosion Test (Study no. TX-338-2033)

Summary – Skin corrosion potential was evaluated in a human 3D epidermal model (EPIDERM™) exposed to GS-9857. Positive (8.0 N potassium hydroxide) and negative controls (sterile distilled water) induced the appropriate responses. Based on the lack of effect on % viability, GS-9857 was classified as non-corrosive.

Title: GS-9857: Local Lymph Node Assay in the Mouse – Individual Method (Study no. TX-338-2034)

Summary – Skin sensitization potential was evaluated in female CBA/Ca mice administered dermal doses of vehicle/control article (4:1 acetone:olive oil), 10%, 25%, or 50% GS-9857 for 3 days followed by injection of ³H-methyl thymidine on Day 6. Based on the lack of proliferative response in lymph nodes, GS-9857 was classified as a non-sensitizer.

Title: GS-9857: Determination of Skin Irritation Potential using the EPISKIN™ Reconstructed Human Epidermis Model (Study no. TX-338-2037)

Summary – Skin irritation potential was evaluated in reconstructed human epidermis model (EPIDERM™) exposed to GS-9857. Positive (5% w/v sodium dodecyl sulfate) and negative controls (Dulbecco's Phosphate Buffered Saline with Ca⁺⁺ and Mg⁺⁺) induced the appropriate responses. Based on the lack of effect on relative mean tissue viability, GS-9857 was classified as a non-irritant.

10 Integrated Summary and Safety Evaluation

This marketing application was submitted in support of a SOF/VEL/VOX FDC indicated for the treatment of adult patients with chronic HCV. SOF (NS5B polymerase inhibitor) and VEL (NS5A inhibitor) have been previously approved as part of combination therapies, while VOX (NS3/4A protease inhibitor) is a new molecular entity. The proposed clinical dosing regimen of SOF/VEL/VOX is 400/100/100 mg for 12 weeks.

The following sections summarize nonclinical results for VOX.

PK/ADME: The oral bioavailability of VOX was 83% in rat and 27% in dog. *In vitro* protein binding was >99% in all species, including human. Following an oral dose of ¹⁴C-VOX in rat, radioactivity was widely distributed with the highest concentrations found in liver, GI tissues, and kidney. The drug did not readily cross the blood:brain barrier, blood:eye barrier, blood:testis

barrier, or bind to melanin. Although VOX metabolites were not detected in plasma from rats or dogs, multiple minor metabolites were detected in urine and/or feces. A similar metabolite profile was observed in human. There were no significant circulating clinical metabolites (e.g., >10% of total drug-related exposure) requiring nonclinical safety assessment. Elimination primarily occurred through the biliary route in rats, dogs, and humans. VOX was transferred to rat pups during lactation.

VOX was also an *in vitro* inhibitor of OATP1B1 and OATP1B3. This inhibition represents a likely mechanism for the significant but non-adverse increases in bilirubin parameters noted in repeat-dose toxicology studies and in clinical trials.

Safety Pharmacology: VOX was not associated with clinically relevant adverse effects on safety pharmacology parameters. These studies included neurological and respiratory assessments in rats. Cardiovascular evaluations included both an *in vitro* study (i.e., hERG inhibition) and *in vivo* study in telemetered dogs.

Repeat-Dose Toxicology: General toxicology evaluations included exploratory and GLP repeat-dose studies of ≤ 26 weeks in rats and ≤ 39 weeks in dogs. Overall, VOX was not associated with clinically relevant adverse effects. However, non-adverse findings and/or findings of limited clinical relevance were noted in GI tract (rats and dogs), hepatobiliary system (rats and dogs), hematology (rats), and kidney (rats and dogs).

- GI Tract: In rat GLP studies, microscopic lesions of minimal to slight severity were observed in nonglandular stomach (edema, mucosa; infiltrate, mixed cell; hyperplasia/hyperkeratosis; fibrosis, submucosa; neovascularization) while lesions of minimal severity occurred in glandular stomach (infiltrate, mixed cell). In addition, minimal to slight hemorrhage was noted in an exploratory 5-day study. Effects in the nonglandular stomach were reversible, occurred regardless of study duration, and occurred primarily in high-dose groups (100 mg/kg/day for studies ≤ 13 weeks and 70 mg/kg/day for the 26-week study). Overall, the effects in nonglandular stomach were considered to be of limited clinical relevance as this organ is unique to rodents (Greaves, 2012), effects occurred only at very high exposures, and esophageal changes were not observed. The safety margin for GI effects in the pivotal 26-week rat study was 244x.

Drug-related emesis was consistently observed in high-dose group dogs (20 mg/kg/day for the 2-week study or 15 mg/kg/day for studies ≥ 13 weeks). Emesis was considered adverse in the 2-week study as it was accompanied by body weight loss and decreased food consumption. These effects occurred at exposures 160x of the clinical exposure. Emesis unaccompanied by body weight effects was considered non-adverse in the 13-week and 39-week studies. Microscopic lesions of minimal to slight severity were observed in the cecum (infiltrate, neutrophils; hemorrhage) and rectum (infiltrate, neutrophils with erosion) of high-dose animals in the 13-week study (15 mg/kg/day). Similar effects were not observed at the same dose in the 39-week study. The safety margin for GI effects in the pivotal 39-week dog study was 98x.

Clinical effects with the SOF/VEL/VOX combination included increased rates of Grade 1 nausea and diarrhea vs. SOF/VEL.

- Hepatobiliary System: VOX treatment resulted in increased liver weight and bilirubin parameters in rats. These reversible effects were primarily observed in high-dose animals (100 mg/kg/day studies $\leq$ 13-weeks and 70 mg/kg/day for the 26-week study). Increases in bilirubin parameters were consistent with OATP inhibition. Persistent bile duct hyperplasia of minimal severity was noted in high-dose females in the 13-week study (100 mg/kg/day) and mid- and high-dose for the 26-week study (30 and 70 mg/kg/day). The effect was considered non-adverse as lesions did not progress in severity with extended dosing duration and there were no additional hepatic lesions. This pathology is consistent with non-adverse typical bile duct hyperplasia described by Hailey *et al.* (2014). Safety margins for hepatobiliary effects in the pivotal 26-week rat study were 244x.

Hepatobiliary effects in dogs included partially reversible microscopic lesions of minimal to slight severity in the gall bladder (epithelium, hyperplasia; vacuoles, epithelium; infiltrate, mononuclear cell). These lesions were observed at doses $\geq$ 10 mg/kg/day. The effect did not progress in severity with extended dosing duration, was not accompanied by additional lesions and was, therefore, considered non-adverse. Reversible increases in bilirubin parameters, consistent with OATP inhibition, were limited to high-dose animals in the 2-week study (20 mg/kg/day). The safety margin for hepatobiliary effects in the pivotal 39-week dog study was 98x.

Consistent with expected outcome of OATP inhibition by VOX, slight increases in total bilirubin were noted in clinical trials.

- Hematology: Changes consistent with mild blood loss and compensatory response as well as inflammation were noted in high-dose rats (100 mg/kg/day for studies $\leq$ 13 weeks and 70 mg/kg/day for the 26-week study). Additional high-dose effects in studies $\leq$ 13 weeks included microscopic lesions of minimal to slight severity in spleen (hematopoiesis, extramedullary, increased) and bone marrow (cellularity, increased). Hematologic changes were likely secondary to pathological effects in the nonglandular stomach. The safety margin for hematological effects in the pivotal 26-week rat study was 244x.
- Kidney: Changes in serum electrolytes along with decreased urine specific gravity and increased urine volume occurred in all rat studies. Increased kidney weight parameters were also observed in the 2- and 13-week studies. Effects were observed in high-dose groups (100 mg/kg/day for studies $\leq$ 13 weeks and 70 mg/kg/day for the 26-week study) and were reversible. Safety margins for kidney effects in the pivotal 26-week rat study were 244x.
In dog, microscopic lesions of slight severity (vacuolation, tubule epithelial cell, increased) were observed at doses $\geq$ 10 mg/kg/day in the 13-week study. Similar effects were not observed at the same dose in the 39-week study. Overall, the safety margin for kidney effects in the pivotal 39-week study was 98x.

Genetic Toxicology and Carcinogenicity: VOX was not considered genotoxic based on negative results in the *in vitro* bacterial reverse mutation assay, *in vitro* mammalian chromosome aberration assay in human peripheral blood lymphocytes, and *in vivo* rat micronucleus assay. Because VOX was not genotoxic and clinical administration is for only 12 weeks, carcinogenicity studies were not required.

Developmental and Reproductive Toxicology: There were no clinically relevant adverse effects on male or female fertility in rats, embryofetal development in rats or rabbits, or pre/post-natal development in rats. A slight decrease in high-dose F₁ pup birth weights was observed in the pre/post-natal development study (100 mg/kg/day). The decreased birth weight continued to impact body weight parameters through the post weaning phase. However, body weights fell within historical control values and were, therefore, considered non-adverse. The safety margin for the F₁ neonatal/developmental toxicity was 238x.

Other Studies: VOX was not associated with ocular irritation, skin corrosion, skin sensitization, or skin irritation. The weight of evidence suggests that VOX is not phototoxic.

Excipients, Metabolites, and Impurities: Excipient exposures fell below maximum potencies listed for approved products in the FDA Inactive Ingredient Database and were, therefore, considered acceptable. All impurities were qualified per appropriate ICH guidelines. There were no significant circulating clinical metabolites (e.g., >10% of total drug-related exposure) requiring nonclinical safety assessment.

Combination Studies: Because there were no specific safety concerns with the individual SOF/VEL/VOX components, nonclinical studies with the combination were not warranted.

Safety Margins: Safety margins for individual components of the SOF/VEL/VOX combination are summarized in the following tables. Safety margins for SOF and VEL are based on reviews for NDA#204-671 and NDA#208-341, respectively.

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Table 20. SOF safety margins

Study	NOAEL	Nonclinical AUC ^a	Safety Margin ^b
General Toxicology			
6-month rat	500 mg/kg/day	66.0 µg·hr/mL ^c	5x
9-month dog	100 mg/kg/day	90 µg·hr/mL ^d	7x
Carcinogenicity			
mouse	200 mg/kg/day (M)	48.0 µg·hr/mL ^c	4x
	600 mg/kg/day (F)	214 µg·hr/mL ^c	17x
rat	750 mg/kg/day	111 µg·hr/mL ^f	9x
Developmental and Reproductive Toxicology			
<u>Segment 1</u>			
fertility and early embryonic development in rat	500 mg/kg/day	57.1 µg·hr/mL ^g	4x
<u>Segment 2</u>			
embryofetal development in rat	500 mg/kg/day	72.1 µg·hr/mL ^h	6x
embryofetal development in rabbit	300 mg/kg/day	200 µg·hr/mL ⁱ	16x
<u>Segment 3</u>			
pre-/post-natal development in rat	500 mg/kg/day	83.3 µg·hr/mL ^j	7x

^a based on GS-331007^b compared to clinical AUC_{tau} = 12.8 µg·hr/mL^c Day 178 data^d Day 273 data^e Day 1 data^f Day 180 data^g Day 24 data^h GD 18 dataⁱ GD 19 data^j LD 10 data

Table 21. VEL safety margins

Study	NOAEL	Nonclinical AUC	Safety Margin ^a
General Toxicology			
6-month rat	200 mg/kg/day	12.2 µg·hr/mL ^b	3x
9-month dog	100 mg/kg/day	27.8 µg·hr/mL ^c	7x
Developmental and Reproductive Toxicology			
<u>Segment 1</u>			
fertility and early embryonic development in rat	200 mg/kg/day	17.1 µg·hr/mL ^e	4x
<u>Segment 2</u>			
embryofetal development in mouse	1000 mg/kg/day	93.0 µg·hr/mL ^f	23x
embryofetal development in rat	200 mg/kg/day	17.1 µg·hr/mL ^g	4x
embryofetal development in rabbit	300 mg/kg/day	2.06 µg·hr/mL ^h	0.5x
<u>Segment 3</u>			
pre-/post-natal development in rat	200 mg/kg/day	13.9 µg·hr/mL ⁱ	3x

^a compared to clinical AUC_{tau} = 4.04 µg·hr/mL^b Week 26 data^c Week 39 data^d Week 4 data from range-finding study (data is for 1500 mg/kg/day; expect similar exposure to 1000 mg/kg/day)^e Day 14 data^f GD 15 data^g GD 17 data^h GD 20 dataⁱ LD 10 data

Table 22. VOX safety margins

Study	NOAEL	Nonclinical AUC	Safety Margin ^a
General Toxicology			
6-month rat	70 mg/kg/day ^b	631 µg·hr/mL ^c	244x
9-month dog	15 mg/kg/day	253 µg·hr/mL ^d	98x
Developmental and Reproductive Toxicology			
<u>Segment 1</u>			
fertility and early embryonic development in rat	100 mg/kg/day	385 µg·hr/mL ^e	149x
<u>Segment 2</u>			
embryofetal development in rat	100 mg/kg/day	365 µg·hr/mL ^f	141x
embryofetal development in rabbit	600 mg/kg/day	9.06 µg·hr/mL ^g	4x
<u>Segment 3</u>			
pre-/post-natal development in rat	100 mg/kg/day	613 µg·hr/mL ^h	238x

^a compared to clinical AUC_{tau} = 2.58 µg·hr/mL^b based on the absence of clinically relevant adverse effects^c Week 26 data^d Week 39 data^e Day 28 data^f GD 17 data^g GD 19 data^h LD 10 data

References

Greaves, P. (2012) Chapter 8: Digestive System, in *Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation*, 4th edition, pp 325-431, Academic Press, New York, New York.

Hailey, J.R., Nold, J.B., Brown, R.H., Cullen, J.M., Holder, J.C., Jordan, H.L., Ennulat, D., Miller, R.T., (2014) Biliary proliferative lesions in the Sprague Dawley rat: adverse/non-adverse. *Toxicol. Pathol.* 42:844-854.

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11 Appendix

Qualification of Sofosbuvir and Velpatasvir Drug Substances

Proposed specifications for SOF and VEL organic impurities, residual solvents, and elemental impurities are identical to or less than those accepted under NDA#204-671 and NDA#208-341, respectively.

Qualification of Voxilaprevir Drug Substance

Specified Impurities

Organic Impurities

The qualification of specified organic impurities in the VOX drug substance is based on results from general toxicology studies, experimental Ames assay data, and/or assessments of mutagenic potential using (quantitative) structure-activity relationship [(Q)SAR].

General Toxicology – Specified impurities were present in the drug lots evaluated in 2-week and 13-week rat studies (TX-338-2026 and TX-338-2001). Using NOAELs established in these studies, qualified impurity levels are adequate to support the proposed specifications. Summary information is provided below.

Table A1. VOX drug substance organic impurity specifications

(b) (4)

Genotoxicity – The VOX (b) (4) are covered by negative experimental results for the parent drug (i.e., Ames assay, *in vitro* chromosomal

aberration assay, *in vivo* micronucleus assay). (b) (4) was negative in *Salmonella* strains TA98, TA100, TA1535, TA1537, as well as *E. coli* strain WP2uvrA with and without metabolic activation at doses ≤ 5000 $\mu\text{g}/\text{plate}$ (Nicolette *et al.*, 2016).

Recommended testing for impurities with exposures exceeding the ICH Q3A qualification threshold (i.e., lower of (b) (4)) include a genetic toxicology evaluation consisting of Ames and *in vitro* mammalian cell assays. In contrast, ICH M7 indicates that genetic toxicology qualification based only on (Q)SAR predictions of mutagenic potential is acceptable for impurities with exposures (b) (4). Because proposed specifications for any individual impurity do not exceed (b) (4) (Q)SAR predictions of mutagenic potential are considered sufficient for qualification.

Per the sponsor, “*voxilaprevir potential and/or actual impurities*” were evaluated by (Q)SAR (Study no. PDM-2593). As the (Q)SAR models only account for 2D structure, evaluation of (b) (4) was excluded in cases where predictions were available for parent compounds. The sponsor’s (Q)SAR evaluation included Derek Nexus (v5.0.2 – Database Derek KB_2015_2.0) and Leadscape Model Applier [v2.1.2 models Genetic Microbial *in vitro* - *Salmonella* (v.3) and *E. coli* - *Sal* 102 A-T Mut (v.1)] and, in some cases, CASE Ultra (v1.6.0.3 - models GT1_A7B 1.6.0.0.3979.450 and PHARM_SALM 1.5.1.8.11337.500).

Table A2. (Q)SAR summary for specified VOX drug substance organic impurities

(b) (4)

Based on the sponsor’s (Q)SAR assessment, all specified impurities listed above are deemed to lack mutagenic potential.

Volatile Organic Impurities

The proposed specification for (b) (4) is (b) (4). Experimental data for this compound includes only genetic toxicology data. The European Chemical Agency (ECHA) registered substance database reports that (b) (4) is non-mutagenic in 2 experimental Ames assays. The first report was negative in *Salmonella* strains TA98, TA100, TA102, TA1535, and TA1537 while the second report was negative in TA98, TA100, TA1535, TA1537, and *E. coli* WP2uvrA. Both assays were conducted with and without metabolic activation over an appropriate dose range (e.g., reaching limit dose of 5000 $\mu\text{g}/\text{plate}$ or dose limited by cytotoxicity).

Although there are no published repeat-dose studies, there is data to support an overall safety assessment for (b) (4).

1. Toxtree (online version) predicts (b) (4) to have a low level of toxicity (b) (4) using (b) (4) classification rules. The threshold of toxicological concern (b) (4) is (b) (4). This provides a 9x safety factor compared to the proposed maximum clinical exposure (b) (4).
2. (b) (4) a closely related structural analog, is listed in ICH Q3C(R6) with a PDE = (b) (4). This provides a 250x safety factor compared to the proposed maximum clinical exposure.

(b) (4)

3. The proposed specification for (b) (4) is only slightly above the Q3A qualification threshold for drugs with a maximum dose of (b) (4) (b) (4).

Based on the totality of information available, the proposed specification (b) (4) does not appear to pose a substantial clinical risk.

Residual Solvents

All residual solvents in the VOX drug substance are listed in the ICH Q3C(R5) guideline. While the proposed specification for (b) (4) exceeds the Option 1 limit, potential clinical exposures are well below the Option 2 limit and there is minimal toxicological concern. Proposed specifications for the remaining listed solvents are all within Option 1 limits. Summary information is provided below.

Table A3. VOX drug substance residual solvent specifications

(b) (4)

Elemental Impurities

None

Unspecified Impurities

With few exceptions, all unspecified impurities were predicted to be non-mutagenic based on the (Q)SAR methodology previously described. The following were predicted to be negative by Derek Nexus but were out of domain or indeterminate in Leadscape Model Applier.

(b) (4) Although this impurity was out of domain in Leadscape Model Applier models, a valid negative prediction was obtained for (b) (4)

(b) (4)

(b) (4) the impurity was deemed non-mutagenic.

(b) (4)

(b) (4): These impurities were out of domain or indeterminate in Leadscape Model Applier. However, all structures with the exception of (b) (4) possessed an alert identified by CASE Ultra. Since the alert was also present in the Ames negative API, these impurities were deemed to be non-mutagenic per ICH M7 Class 4. (b) (4) did not possess a structural alert but is a substructure of the Ames negative API and is, therefore, considered non-mutagenic.

Several mutagenic compounds were identified as known or potential impurities. These include (b) (4)

(b) (4)

(b) (4). Through the manufacturing process, these potential impurities are controlled to levels below the appropriate TTC. Therefore, these impurities are not included in the drug substance specifications.

Qualification of the Sofosbuvir/Velpatasvir/Voxilaprevir Drug Product

Degradants

Proposed specifications for SOF and VEL degradants are identical to or less than those accepted under NDA#204-671 and NDA#208-341, respectively. Proposed specifications (at release and shelf-life) for unspecified VOX-related degradation products (b) (4) as well as unidentified, unspecified degradation products (b) (4) fall below the ICH Q3B(R2) qualification threshold for drugs with maximum daily dos 0 mg.

References

ECHA: Key Genetic Toxicity *In Vitro*.001

<https://echa.europa.eu/registration-dossier/-/registered-dossier/13609/7/7/2/?documentUUID=21c1db86-23ef-4528-8a71-0b5a8a0bdd49>

ECHA: Key Genetic Toxicity *In Vitro*.004

<https://echa.europa.eu/registration-dossier/-/registered-dossier/13609/7/7/2/?documentUUID=1448d153-e311-4c1f-857c-435729a6789d>

Hemingway, R., Fowkes, A., Williams, R.V. (2017) Carbamates and ICH M7 classification: making use of expert knowledge. *Reg. Toxicol. Pharmacol.* available online 4/4/17.

Kroes, R., Renwick, A.G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., Van Schothorst, F., Vos, J.G., Wurtzen, G. (2004) Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* 42:65-83.

(b) (4)

Toxtree online version accessed 3/27/17

<https://apps.ideaconsult.net/data/ui/toxtree>

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Structures of impurities qualified through experimental testing

(b) (4)

Title: 2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study with GS-9857 in Male Sprague Dawley Rats (Study no. TX-338-2026)

Summary - Mortality, clinical signs/physical examinations, body weights, food consumption, hematology, coagulation, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in male Sprague Dawley rats administered oral doses of vehicle/control article (13.34% ethanol, 8.88% propylene glycol, 33.34% Labrasol, and 44.44% Kolliphor HS-15), 15, or 30 mg/kg/day of GS-9857 spiked with impurities for 2 weeks. An additional group was administered 30 mg/kg/day of neat GS-9857. Treatment-related effects included non-adverse hematology changes (↑ reticulocytes, RCDW, WBCs, and/or lymphocytes). There were no substantial differences between spiked and neat GS-9857.

Based on the absence of adverse effects at any dose, the NOAEL for spiked GS-9857 was 30 mg/kg/day.

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/s/

MARK W POWLEY

05/05/2017

HANAN N GHANTOUS

05/05/2017

I concur with the conclusion of Dr. Mark Powley that the nonclinical data is sufficient to support marketing application approval of the Fixed-Dose combination of Sofosbuvir/Velpatasvir/Voxilaprevir.