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APPLICATION NUMBER:

208261Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208,261
Supporting document/s:	000, 048, 052, 059, 060, 062 and 063
Applicant's letter date:	May 28, 2015 (for 000)
CDER stamp date:	May 28, 2015 (for 000)
Product:	ZEPATIER™ is a fixed-dose combination (FDC) tablet of elbasvir (EBR), an HCV NS5A inhibitor, and grazoprevir (GZR), an HCV NS3/4 protease inhibitor
Indication:	ZEPATIER™ is indicated for the treatment of chronic hepatitis C (CHC) genotype 1 and 4 infection
Applicant:	Merck
Review Division:	Division of Antiviral Products
Reviewer:	Christopher Ellis, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, M.D.
Project Manager:	Nina Mani, Ph.D., MPH

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1 Executive Summary

1.1 Introduction

ZEPATIER™ is a once daily fixed-dose combination (FDC) tablet of elbasvir (EBR, MK-8742), an HCV NS5A inhibitor, and grazoprevir (GZR, MK-5172), an HCV NS3/4 protease inhibitor. Elbasvir and grazoprevir have EC_{90} values that vary from the low pM to low nM range in HCV replicon assays with HCV genotypes 1, 4 and 6. The nonclinical safety profile of EBR and GZR has been evaluated in: safety pharmacology studies in rats and dogs; repeat-dose toxicology studies in mice, rats and dogs for up to 3, 6 and 9 months duration, respectively; phototoxicity studies in rats; fertility and pre- and post-natal developmental studies in rats; embryo-fetal developmental studies in rats and rabbits; and genetic toxicology studies (Ames, *in vitro* chromosomal aberration and *in vivo* rat micronucleus assays). In addition, numerous *in vitro* and *in vivo* nonclinical pharmacokinetic studies evaluating the absorption, distribution, metabolism and excretion of EBR and GZR have been conducted.

1.2 Brief Discussion of Nonclinical Findings

Refer to original Pharmacology/Toxicology review for discussion of nonclinical findings. The non-clinical safety sections of the product label were reviewed (refer to Section 1.3.3 for suggested language).

1.3 Recommendations

1.3.1 Approvability

Yes, the applicant provided sufficient nonclinical safety information on elbasvir and grazoprevir in support of ZEPATIER™ approval for marketing in the U.S.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

No adequate human data are available to establish whether or not ZEPATIER poses a risk to pregnancy outcomes. In animal reproduction studies, no evidence of adverse developmental outcomes was observed with the components of ZEPATIER (elbasvir or grazoprevir) at exposures greater than those in humans at the recommended human dose (RHD) *[see Data in (8.1)]*. During organogenesis in the rat and rabbit, systemic exposures (AUC) were approximately 10 and 18 times (for elbasvir) and 117 and 41 times (for grazoprevir), respectively, the exposure in humans at the RHD. In rat pre/postnatal developmental studies, maternal systemic exposures (AUC) to elbasvir and grazoprevir were approximately 10 and 78 times, respectively, the exposure in humans at the RHD.

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The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

If ZEPATIER is administered with ribavirin, the combination regimen is contraindicated in pregnant women and in men whose female partners are pregnant. Refer to the ribavirin prescribing information for more information on use in pregnancy.

Data

Animal Data

Elbasvir: Elbasvir was administered orally at up to 1000 mg/kg/day to pregnant rats and rabbits on gestation days 6 to 20 and 7 to 20, respectively, and also to rats on gestation day 6 to lactation/post-partum day 20. No effects on embryo-fetal (rats and rabbits) or pre/postnatal (rats) development were observed at up to the highest dose tested. Systemic exposures (AUC) to elbasvir were approximately 10 (rats) and 18 (rabbits) times the exposure in humans at the RHD. In both species, elbasvir has been shown to cross the placenta, with fetal plasma concentrations of up to 0.8% (rabbits) and 2.2% (rats) that of maternal concentrations observed on gestation day 20.

Grazoprevir: Grazoprevir was administered to pregnant rats (oral doses up to 400 mg/kg/day) and rabbits (intravenous doses up to 100 mg/kg/day) on gestation days 6 to 20 and 7 to 20, respectively, and also to rats (oral doses up to 400 mg/kg/day) on gestation day 6 to lactation/post-partum day 20. No effects on embryo-fetal (rats and rabbits) or pre/postnatal (rats) development were observed at up to the highest dose tested. Systemic exposures (AUC) to grazoprevir were ≥78 (rats) and 41 (rabbits) times the exposure in humans at the RHD. In both species, grazoprevir has been shown to cross the placenta, with fetal plasma concentrations of up to 7% (rabbits) and 89% (rats) that of maternal concentrations observed on gestation day 20.

8.2 Lactation

Risk Summary

It is not known whether ZEPATIER is present in human breast milk, affects human milk production, or has effects on the breastfed infant. When administered to lactating rats, the components of ZEPATIER (elbasvir and grazoprevir) were present in milk, without effects on growth and development observed in nursing pups *[see Data in (8.2)]*.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ZEPATIER and any potential adverse effects on the breastfed child from ZEPATIER or from the underlying maternal condition.

If ZEPATIER is administered with ribavirin, the information for ribavirin with regard to nursing mothers also applies to this combination regimen. Refer to the ribavirin prescribing information for information on use during lactation.

Data

Elbasvir: No effects of elbasvir on growth and postnatal development were observed in nursing pups at up to the highest dose tested *[see Data in (8.1)]*. Maternal systemic exposure (AUC) to elbasvir was approximately 10 times the exposure in humans at the RHD. Elbasvir was excreted into the milk of lactating rats following oral administration (1000 mg/kg/day) from gestation day 6 to lactation day 14, with milk

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concentrations approximately 4 times that of maternal plasma concentrations observed 2 hours post-dose on lactation day 14.

Grazoprevir: No effects of grazoprevir on growth and postnatal development were observed in nursing pups at up to the highest dose tested *[see Data in (8.1)]*. Maternal systemic exposure (AUC) to grazoprevir was approximately 78 times the exposure in humans at the RHD. Grazoprevir was excreted into the milk of lactating rats following oral administration (up to 400 mg/kg/day) from gestation day 6 to lactation day 14, with milk concentrations of 54 and 87% that of maternal plasma concentrations observed 2 and 8 hours post-dose, respectively, on lactation day 14.

8.3 Females and Males of Reproductive Potential

If ZEPATIER is administered with ribavirin, the information for ribavirin with regard to pregnancy testing, contraception, and infertility also applies to this combination regimen. Refer to ribavirin prescribing information for additional information.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

Elbasvir and grazoprevir were not genotoxic in a battery of *in vitro* or *in vivo* assays, including microbial mutagenesis, chromosomal aberration in Chinese Hamster Ovary cells, and in *in vivo* rat micronucleus assays.

Carcinogenicity studies with elbasvir or grazoprevir have not been conducted.

If ZEPATIER is administered in a regimen containing ribavirin, the information for ribavirin on carcinogenesis and mutagenesis also applies to this combination regimen. Refer to the ribavirin prescribing information for information on carcinogenesis and mutagenesis.

Impairment of Fertility

No effects on mating, female or male fertility, or early embryonic development were observed in rats at up to the highest dose tested. Systemic exposures (AUC) to elbasvir and grazoprevir were approximately 8 and 114 times, respectively, the exposure in humans at the recommended human dose.

If ZEPATIER is administered with ribavirin, the information for ribavirin on impairment of fertility also applies to this combination regimen. Refer to the ribavirin prescribing information for information on impairment of fertility.

2 Drug Information

2.1 Drug

2.1.1 Elbasvir

CAS Registry Number	1370468-36-2
Generic Name	Elbasvir (EBR)

Reference ID: 3878691

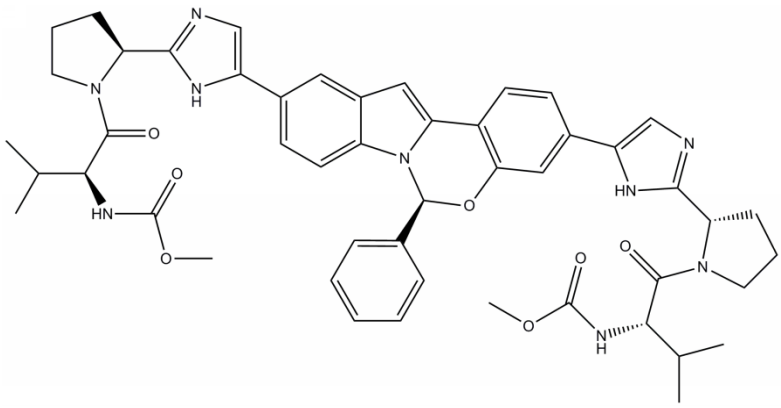
Code Name MK-8742 (also L-002469825)

Chemical Name Dimethyl N,N'-([(6S)-6-phenylindolo[1,2-c][1,3]benzoxazine-3,10-diyl]bis{1 H-imidazole-5,2-diyl-(2S)-pyrrolidine-2,1-diyl[(2S)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate (IUPAC)

Molecular Formula/Molecular Weight

$C_{49}H_{55}N_9O_7$/882.02 g/mol

Structure or Biochemical Description



Pharmacologic Class HCV NS5A inhibitor

2.1.2 Grazoprevir

CAS Registry Number 1350514-68-9

Generic Name Grazoprevir (GZR)

Code Name MK-5172 (L-002214070)

Chemical Name (1a*R* ,5*S* ,8*S* ,10*R* ,22a*R*)-*N* -[(1*R* ,2*S*)-1-[(Cyclopropylsulfonamido)carbonyl]-2-ethenylcyclopropyl]-14-methoxy-5-(2-methylpropan-2-yl)-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H* -7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-Carboxamide (IUPAC)

Molecular Formula/Molecular Weight

$C_{38}H_{50}N_6O_9S$/766.90 g/mol

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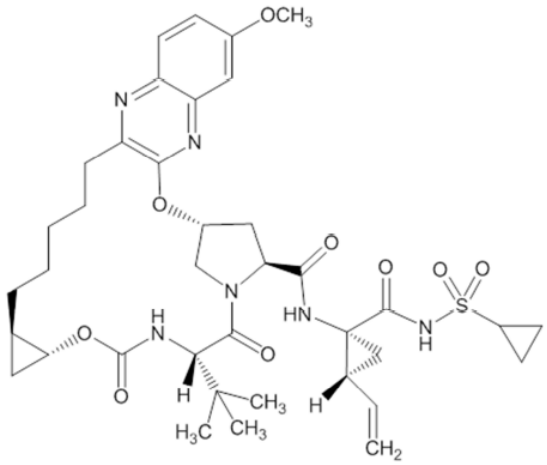
Structure or Biochemical Description



Pharmacologic Class HCV NS3/4 protease inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND-110,261 (for GZR and for EBR & GZR FDC) and IND-114,298 (for EBR)

2.3 Drug Formulation

EBR/GZR fixed dose combination (FDC) tablets are film-coated tablets (21.22 x 10.34 mm) containing 50 mg of EBR and 100 mg of GZR (refer to Applicant Table below).

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Table 1: Composition of EBR/GZR FDC tablets

Components	Reference	Function	mg/tablet
(b) (4)			
(b) (4)			(b) (4)
Grazoprevir	In-house	Active	100.0[T]
Sodium Lauryl Sulfate	USP-NF		(b) (4)
Copovidone	USP-NF		
	(b) (4)		(b) (4)
Mannitol	USP-NF		
Croscarmellose Sodium	USP-NF		
Sodium Chloride	USP-NF		
Colloidal Silicon Dioxide	USP-NF		
Magnesium Stearate	USP-NF		
	(b) (4)		(b) (4)
Elbasvir	In-house		
Hypromellose (b) (4)	USP-NF		(b) (4)
Vitamin E Polyethylene Glycol Succinate	USP-NF		
			(b) (4)
			(b) (4)
Cellulose, Microcrystalline	USP-NF		(b) (4)
Lactose Monohydrate	USP-NF		
Croscarmellose Sodium	USP-NF		
Sodium Chloride	USP-NF		
Colloidal Silicon Dioxide	USP-NF		
Magnesium Stearate	USP-NF		
	(b) (4)		(b) (4)
Film Coat (b) (4)			
(b) (4) (b) (4)			(b) (4)
Carnauba Wax	USP-NF	Wax	(b) (4)
			(b) (4)

2.4 Comments on Novel Excipients

All excipients are compendial (b) (4) all excipients of the coating agent are compendial (refer to footnote above). A Drug Master File (DMF (b) (4) for the coating agent has been submitted to the Agency for review.

2.5 Comments on Impurities/Degradants of Concern

Refer to original Pharmacology/Toxicology review for NDA-208,261.

2.6 Proposed Clinical Population and Dosing Regimen

EBR/GZR FDC is to be indicated for the treatment (single tablet once a day) of chronic hepatitis C (CHC) genotype 1 and 4 infection for 12 to 16 weeks in adult patients.

2.7 Regulatory Background

IND-110,261 (initially for GZR) and IND-114,298 (for EBR) were opened on December 22, 2010 and April 21, 2012, respectively.

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/s/

CHRISTOPHER E ELLIS
01/28/2016

HANAN N GHANTOUS
01/28/2016

Oct 29, 2015

Comments from A. Jacobs on NDA 208-261 Zeapatier (fixed dose combo of elbasvir and grozoprevir)

1. I concur that there are no pharm-tox issues regarding approval.

2. I have made a few suggestions to the reviewer and he will address them as appropriate.

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/s/

ABIGAIL C JACOBS
10/29/2015

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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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1 Executive Summary

1.1 Introduction

ZEPATIER™ is a once daily fixed-dose combination (FDC) tablet of elbasvir (EBR, MK-8742), an HCV NS5A inhibitor, and grazoprevir (GZR, MK-5172), an HCV NS3/4 protease inhibitor. Elbasvir and grazoprevir have EC_{90} values that vary from the low pM to low nM range in HCV replicon assays with HCV genotypes 1, 4 and 6. The nonclinical safety profile of EBR and GZR has been evaluated in: safety pharmacology studies in rats and dogs; repeat-dose toxicology studies in mice, rats and dogs for up to 3, 6 and 9 months duration, respectively; phototoxicity studies in rats; fertility and pre- and post-natal developmental studies in rats; embryo-fetal developmental studies in rats and rabbits; and genetic toxicology studies (Ames, *in vitro* chromosomal aberration and *in vivo* rat micronucleus assays). In addition, numerous *in vitro* and *in vivo* nonclinical pharmacokinetic studies evaluating the absorption, distribution, metabolism and excretion of EBR and GZR have been conducted.

1.2 Brief Discussion of Nonclinical Findings

EBR and GZR are bound highly to plasma protein, with >99.9% and 98.4% bound, respectively. Unchanged parent drug was the only EBR- and GZR-related component detected in plasma (rats, dogs and human subjects) and was also the predominant EBR-related component in bile and feces, with EBR-related metabolites also present (rats, dogs and human subjects). Unchanged parent drug was also the predominant GZR-related material in feces, with unchanged parent drug and GZR-related metabolites present at roughly equal amounts in bile (rats and dogs). Thus, the major routes of elimination for EBR and GZR were similar, involving biliary excretion/ intestinal secretion (for EBR) and oxidative metabolism/biliary excretion (for GZR) of primarily unchanged parent drug (rats and dogs).

<u>Elbasvir</u>:

No target organs of toxicity were identified, following oral EBR administration of up to 1000 mg/kg/day, in toxicology studies in mice, rats and dogs for up to 1, 6 and 9 months, respectively, at area under the curve (AUC) exposures ≥55 (mice), 9 (rats) and 8 (dogs) times higher than clinical exposure at the recommended dose. Non-adverse findings included increased urine volume (rats), infrequent emesis (dogs) and vacuolation of gut associated lymphoid tissue (GALT), lymph nodes, gall bladder and/or spleen (dogs), with these latter findings consistent with lysosomal phospholipid accumulation in macrophages (*i.e*. phospholipidosis). This lymphoid tissue phospholipidosis was reversible and was not associated with overt toxicity (*e.g*. lymphoid depletion, change in spleen weights) and so appears to be an adaptive rather than a toxic response.

Following EBR administration for one month in the rat fertility study, a slight decrease (~15%) in sperm count, without effects on testicular weight, sperm motility and morphology or male fertility parameters, was observed at an estimated AUC exposure 9 times higher than clinical exposure at the recommended dose, with no effects on sperm count seen at exposure 5 times higher than clinical exposure. No testicular toxicity was

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observed in rats administered EBR for up to 6 months at AUC exposure 11 times higher than clinical exposure at the recommended dose. This minor effect on sperm count is not considered to be biologically significant or of clinical concern.

Grazoprevir:

The main target organs of toxicity identified following oral GZR administration were the liver and gallbladder (mice and dogs), with related hepatobiliary findings also observed in clinical trials. The other identified target organs of toxicity were of questionable clinical relevance, given the high human exposure multiples where toxicity was observed, and included the testes (dogs), gastrointestinal (GI) tract (mice and rats), red blood cells (RBCs) (mice and dogs) and kidney (mice). Toxicity occurred at AUC exposures ≥74 (hepatobiliary), 163 (testicular), 282 (GI) and 747 (renal & hematologic) times higher than clinical exposure at the recommended dose, with no effects in these organ systems seen at exposures ≥3 (hepatobiliary), 37 (testicular), 163 (hematologic) and 179 (renal & GI) times higher than clinical exposure.

Hepatobiliary findings included increased serum taurine conjugated and total unconjugated bile acids, total (direct) bilirubin, ALT, AST, ALP (dogs), hepatocellular size and liver weights. Additional findings in mice included focal hepatic necrosis, hepatocyte cytoplasmic rarefaction or prominent lobular pattern, as well as distention, inflammation, epithelial hyperplasia, erosions and/or focal necrosis of the gallbladder. In dogs, yellow feces, microlithiasis in lumen of gallbladder and larger bile ducts in liver, distended gallbladder with abundant bile content, gallbladder wall thickened and yellowish discoloration of gingival mucosae, skin and/or eyes (bulbar conjunctiva) were also observed. In mice, ALT, AST and bilirubin elevations were first evident after ~2 weeks of GZR administration and resolved ~8 weeks into the treatment-free “recovery” period, with no histopathological effects noted following the recovery period. Although GZR is able to form chemically reactive metabolites, capable of covalently modifying hepatic proteins, bioactivation does not appear to be a major contributor to hepatobiliary toxicity (may be initiated due to OATP1B1, OATP1B3 and/or BSEP inhibition).

Testicular findings in dogs included seminiferous tubule degeneration, reduced testicular weights and decreased amount of sperm in epididymis. No testicular effects were seen at AUC exposures 231 and 37 times higher than clinical exposure at the recommended dose in the 1 and 9 month studies, respectively. In addition, testicular toxicity was not observed in mice and rats following oral GZR administration for up to 3 and 6 months, respectively, at AUC exposures 747 (mice) and 282 (rats) times higher than clinical exposure at the recommended dose.

Renal toxicity in mice consisted of degeneration of renal tubular epithelium, while hematological findings in mice and dogs included decreased RBCs, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Increased reticulocytes, hemosiderin content of spleen and sinusoidal cells of liver, extramedullary hematopoiesis in spleen and erythroid hyperplasia of bone marrow were also observed in dogs.

GI toxicity in mice included epithelial vacuolation, single cell necrosis, villous atrophy and regenerative epithelial hyperplasia primarily in the duodenum, suggesting a local effect of GZR-related material (possibly associated with inflow of bile). In male rats, GI findings consisted of focal hemorrhage in the glandular mucosa of stomach.

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1.3 Recommendations

1.3.1 Approvability

Yes, the applicant provided sufficient nonclinical safety information on elbasvir and grazoprevir in support of ZEPATIER™ approval for marketing in the U.S.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The draft product label provided by the applicant is under review.

2 Drug Information

2.1 Drug

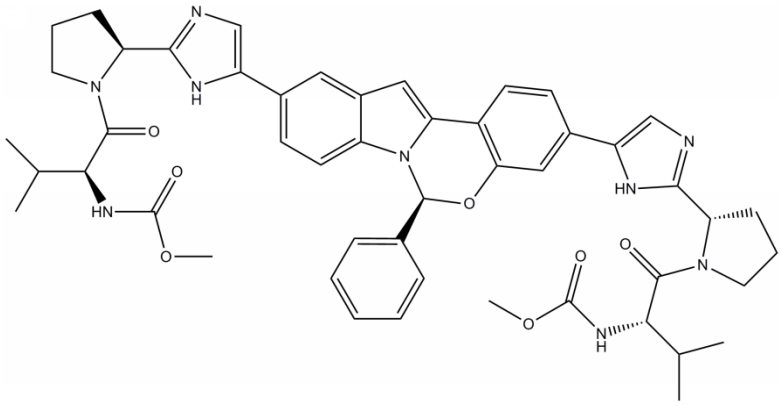
2.1.1 Elbasvir

CAS Registry Number	1370468-36-2
Generic Name	Elbasvir (EBR)
Code Name	MK-8742 (also L-002469825)
Chemical Name	Dimethyl N,N'-([(6S)-6-phenylindolo[1,2-c][1,3]benzoxazine-3,10-diyl]bis{1 H-imidazole-5,2-diyl-(2S)-pyrrolidine-2,1-diyl[(2S)-3-methyl-1-oxobutane-1,2-diyl]})dicarbamate (IUPAC)

Molecular Formula/Molecular Weight

$C_{49}H_{55}N_9O_7$/882.02 g/mol

Structure or Biochemical Description



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Pharmacologic Class HCV NS5A inhibitor

2.1.2 Grazoprevir

CAS Registry Number 1350514-68-9

Generic Name Grazoprevir (GZR)

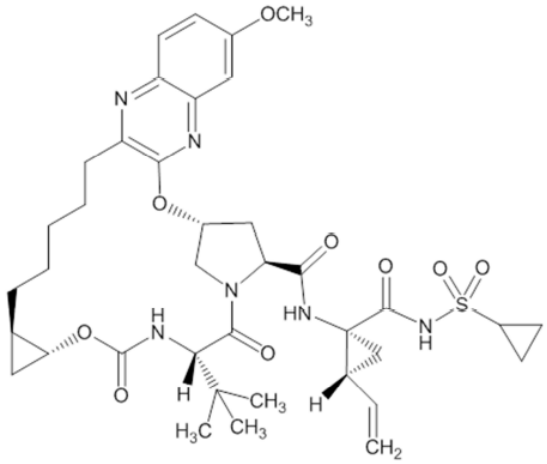
Code Name MK-5172 (L-002214070)

Chemical Name (1a*R* ,5*S* ,8*S* ,10*R* ,22a*R*)-*N* -[(1*R* ,2*S*)-1-[(Cyclopropylsulfonamido)carbonyl]-2-ethenylcyclopropyl]-14-methoxy-5-(2-methylpropan-2-yl)-3,6-dioxo-1,1a,3,4,5,6,9,10,18,19,20,21,22,22a-tetradecahydro-8*H* -7,10-methanocyclopropa[18,19][1,10,3,6]dioxadiazacyclononadecino[11,12-*b*]quinoxaline-8-Carboxamide (IUPAC)

Molecular Formula/Molecular Weight

$C_{38}H_{50}N_6O_9S$/766.90 g/mol

Structure or Biochemical Description



Pharmacologic Class HCV NS3/4 protease inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND-110,261 (for GZR and for EBR & GZR FDC) and IND-114,298 (for EBR)

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2.3 Drug Formulation

EBR/GZR fixed dose combination (FDC) tablets are film-coated tablets (21.22 x 10.34 mm) containing 50 mg of EBR and 100 mg of GZR (refer to Applicant Table below).

Table 1: Composition of EBR/GZR FDC tablets

Components	Reference	Function	mg/tablet
(b) (4)			
(b) (4)			(b) (4)
Grazoprevir	In-house	Active	100.0[†]
Sodium Lauryl Sulfate	USP-NF		(b) (4)
Copovidone	USP-NF		
			(b) (4)
Mannitol	USP-NF		(b) (4)
Croscarmellose Sodium	USP-NF		
Sodium Chloride	USP-NF		
Colloidal Silicon Dioxide	USP-NF		
Magnesium Stearate	USP-NF		
			(b) (4)
(b) (4)			
			(b) (4)
Elbasvir	In-house		
Hypromellose (b) (4)	USP-NF		
Vitamin E Polyethylene Glycol Succinate	USP-NF		
		(b) (4)	
			(b) (4)
Cellulose, Microcrystalline	USP-NF		(b) (4)
Lactose Monohydrate	USP-NF		
Croscarmellose Sodium	USP-NF		
Sodium Chloride	USP-NF		
Colloidal Silicon Dioxide	USP-NF		
Magnesium Stearate	USP-NF		
			(b) (4)
Film Coat (b) (4)			
			(b) (4)
Carnauba Wax	USP-NF	Wax	(b) (4)
		(b) (4)	
			(b) (4)

2.4 Comments on Novel Excipients

All excipients are compendial [redacted] (b) (4) all excipients of the coating agent are compendial (refer to footnote above). A Drug Master File (DMF [redacted] (b) (4) for the coating agent has been submitted to the Agency for review.

2.5 Comments on Impurities/Degradants of Concern

Qualification assessment of residual solvents, elemental, EBR- and GZR-related impurities (specified and unspecified process intermediates, degradants, starting materials etc.) in the EBR and GZR drug substance and/or drug product was conducted (refer to Dr. Mark Powley's pharmacology/toxicology review for specific details). The qualification of impurities in the EBR and GZR drug substance is based on results from repeat-dose toxicology studies, Ames assays and/or assessment of potential mutagenicity using (quantitative) structure-activity relationship [(Q)SAR] models. Overall, the proposed EBR and GZR specifications are considered acceptable from a pharmacology/toxicology perspective.

2.6 Proposed Clinical Population and Dosing Regimen

EBR/GZR FDC is to be indicated for the treatment (single tablet once a day) of chronic hepatitis C (CHC) genotype 1 and 4 infection for 12 to 16 weeks in adult patients.

2.7 Regulatory Background

IND-110,261 (initially for GZR) and IND-114,298 (for EBR) were opened on December 22, 2010 and April 21, 2012, respectively.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study #
Safety Pharmacology	
MK-8742: Effects of test articles on hERG (I_{Kr}), hKCNQ1/hKCNE1 (I_{Ks}), and hNa_v 1.5 (I_{Na}) Currents Stably Expressed in Mammalian Cells.	TT #10-3196
MK-8742: Electrophysiological Evaluation on hERG Channel Current Stably Expressed in CHO cells	TT #11-4703
MK-8742: A Pharmacological Assessment of the Effect of MK-8742 (L-002469825) on the Cardiovascular System of Male Beagle Dogs Using Telemetry	TT #10-5474
MK-8742: Cardiovascular Evaluation of Hemodynamic Effects in a Rat Telemetry Study (non-GLP)	TT #10-5352
MK-8742: Oral Respiratory Telemetry Study in Beagle Dogs	TT #11-5606

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MK-5172: Effect of L-002214070-001Z003 on hERG (I_{Kr}), hKCNQ1/hKCNE1 (I_{Ks}), and hNa_v 1.5 (I_{Na}) Currents Stably Expressed in Mammalian Cells. Exploratory Study conducted with PatchXpress 7000A.	TT #08-3064
MK-5172: Electrophysiological Evaluation on hERG Channel Current Stably Expressed in CHO Cells	TT #08-4721
MK-5172: Exploratory Cardiovascular Study in Anesthetized Dogs	TT #08-5918
MK-5172: Oral Cardiovascular and Respiratory Telemetry Study in Dogs	TT #08-5651
MK-5172: One month oral toxicity study in rats with a functional observation battery	TT #08-1152
MK-5172: One month oral toxicity study in rats with a functional observation battery with 2-week interim necropsy	TT #09-1120
MK-5172: In Vivo Liver Response Assay Report	TT #08-9637
Analytical Method Validation	
MK-8742: Validation Summary Report of the Bioanalytical Methods used to Support the MK-8742 Preclinical Program	PK025
MK-5172: Validation Summary Report of the Bioanalytical Methods used to Support the MK-5172 Preclinical Program	PK028
ADME/Pharmacokinetics	
MK-8742: Pharmacokinetic Studies of MK-8742 in Preclinical Species After Intravenous and Oral Administration	PK001
MK-8742: In Vitro Studies of MK-8742	PK002
MK-8742: In Vivo Metabolism and Excretion of MK-8742	PK003
MK-8742: MK-8742 in Dutch Belted Rabbits	PK005
MK-8742: Quantitative Whole-Body Autoradiography in Wistar Hannover and Long-Evans Rats Following a Single Oral Administration of [^{14}C]MK-8742 and Human Dosimetry Prediction	PK008
MK-8742: In Vivo Metabolism and Excretion of MK-8742 in Dogs Following IV Administration	PK011
MK-8742: Pharmacokinetics and Excretion Balance Study in Bile Duct Cannulated (BDC) Beagle Dogs Following Intravenous (IV) Administration of [^{14}C]L-002469825	PK016
MK-8742: Excretion of Radioactivity in Bile Duct Cannulated Rats Administered a Single IV Bolus Dose of [^{14}C]L-002469825	PK017
MK-8742: In Vivo Metabolism and Excretion of MK-8742 in Rats Following IV Administration	PK018

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MK-8742: Oral Placental Transfer Study in Rabbits	TT #14-7030
MK-8742: Oral Placental and Lactational Transfer Study in Rats	TT #14-7050
MK-5172: Pharmacokinetic Studies of MK-5172 in Wistar Hannover Rats and Beagle Dogs After Intravenous and Oral Administration	PK001
MK-5172: In Vivo Metabolism and Excretion of MK-5172	PK002
MK-5172: In Vitro Studies of MK-5172	PK003
MK-5172: MK-5172 in Dutch Belted Rabbits	PK004
MK-5172: Liver and Plasma Pharmacokinetic Studies of MK-5172 in Wister Hannover Rats and Beagle Dogs After Oral Administration	PK005
MK-5172: Covalent Protein Binding Study of MK-5172	PK012
MK-5172: Quantitative Whole-Body Autoradiography of Rats Following Oral Administration of ^{14}C-MK-5172	PK015
MK-5172: In Vivo Metabolism and Excretion of MK-5172 in Dogs	PK018
MK-5172: Intravenous Placental Transfer Study in Rabbits	TT #12-7420
MK-5172: Oral Placental and Lactational Transfer Study in Rats	TT #14-7040
General Toxicology/Toxicokinetics	
MK-8742: Exploratory 7-Day Tolerability Study in Male Rats	TT #10-2901
MK-8742: Fifteen-Day Oral Toxicokinetic Study in Rats	TT #11-6017
MK-8742: Fourteen-Day Oral Toxicity Study in Dogs	TT #11-6013
MK-8742: Fourteen-Day Oral Toxicity Study in Rats with Micronucleus and Functional Observational Battery Assays	TT #11-6014
MK-8742: One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice	TT #12-6047
MK-8742: Three-Month Oral Toxicity Study in Rats	TT #11-6024
MK-8742: Three-Month Oral Toxicity Study in Dogs	TT #11-6025
MK-8742: Six-Month Oral Toxicity Study in Rats	TT #12-6033
MK-5172: Exploratory Single-Dose Oral Toxicokinetic Study in Male CD1 Mice	TT #11-6043

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MK-5172: Exploratory Single and Multiple Dose Oral Toxicokinetic Study in Rats	TT #09-1102
MK-5172: Exploratory 7-Day Oral Tolerability Study in Female Rats	TT #08-2525
MK-5172: Exploratory 9-Day Oral Rising-Dose Tolerability Study in Dogs	TT #08-1119
MK-5172: One month oral toxicity study in rats with a functional observation battery	TT #08-1152
MK-5172: One month oral toxicity study in rats with a functional observation battery with 2-week interim necropsy	TT #09-1120
MK-5172: Six month oral toxicity study in rats	TT #10-6014 (& 12-9745)
MK-5172: One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice (Hybrid)	TT #11-6034
MK-5172: Three-Month Oral Range-Finding and Toxicokinetic Study in CD1 Mice	TT #11-6038
MK-5172: One month oral toxicity study in dogs	TT #08-1151
MK-5172: Nine-month oral toxicity study in dogs with a 3-month interim necropsy	TT #10-6015 (& 12-9746)
Genetic Toxicology	
MK-8742: Microbial Mutagenesis Assay	TT #10-8239 & 11-8060
MK-8742: Exploratory Assays for Micronucleus Induction In Vitro, in Chinese Hamster Ovary Cells	TT #10-8688
MK-8742: Assay for Chromosomal Aberrations In Vitro in Chinese Hamster Ovary Cells	TT #11-8536
MK-8742: Assay for Micronucleus Induction in Rat Bone Marrow from an Exploratory 4-Day Oral Biomarker and Gene Expression Study	TT #10-8730
MK-5172: Microbial Mutagenesis Assay	TT #09-8000 & 09-8011
MK-5172: Assay for Chromosomal Aberrations In Vitro in Chinese Hamster Ovary Cells	TT #08-8686 & 09-8600

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MK-5172: Assay for micronucleus induction in rat bone marrow from a 1-month oral toxicity study with a 2-week interim necropsy	TT #09-8734
MK-5172: Assay for Micronucleus Induction in Rat Bone Marrow From a 1-Month Oral Toxicity Study	TT #08-8813
Reproductive and Developmental Toxicology	
MK-8742: Oral Fertility Study in Female and Male Rats	TT #13-7290
MK-8742: Exploratory Oral Range-Finding Study in Pregnant Rats	TT #12-7175
MK-8742: Exploratory Oral Range-Finding Study in Pregnant Rabbits	TT #12-7185
MK-8742: Oral Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rats	TT #12-7170
MK-8742: Oral Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rabbits	TT #12-7180
MK-8742: Oral Pre- and Postnatal Developmental Toxicity Study in Rats	TT #14-7020
MK-5172: Oral Fertility Study in Female and Male Rats	TT#12-7260
MK-5172: Exploratory Oral Range-Finding Study in Pregnant Rats	TT#10-7055
MK-5172: Oral Developmental Toxicity Study in Rats With Prenatal Evaluation	TT#10-7050
MK-5172: Exploratory Oral Single Dose Toxicokinetic Study in Nonpregnant Rabbits	TT#10-7047
MK-5172: Exploratory Oral Single Dose Toxicokinetic Study in Nonpregnant Rabbits	TT#10-7048
MK-5172: Exploratory Oral Two-Day Toxicokinetic Study in Nonpregnant Rabbits	TT#10-7049
MK-5172: Exploratory Oral 9-Day Toxicokinetic Study in Nonpregnant Rabbits	TT#10-7046
MK-5172: Exploratory Oral Single Dose Toxicokinetic Study in Nonpregnant Rabbits	TT#10-7044

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MK-5172: Preliminary Oral Developmental Toxicity Study in Rabbits	TT#10-7040
MK-5172: Exploratory One-Week Oral Toxicokinetic Study in Nonpregnant Rabbits	TT#10-7042
MK-5172: Exploratory Five-Day Oral Toxicokinetic Study in Nonpregnant Rabbits	TT#11-7127
MK-5172: Exploratory 5-Day Intravenous Toxicokinetic and Tolerability Study in Nonpregnant Rabbits	TT#11-7126
MK-5172: Preliminary Intravenous Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rabbits	TT#12-7001
MK-5172: Intravenous Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rabbits	TT#12-7300
MK-5172: Oral Pre- and Postnatal Developmental Toxicity and Toxicokinetic Study in Rats	TT#14-7010
Toxicology Studies (other)	
MK-8742: Bovine Corneal Opacity and Permeability Test (BCOP)	TT#13-7807
MK-8742: MatTek EpiDerm™ MTT Viability Assay	TT#13-7806
MK-8742: Local Lymph Node Assay in Mice (LLNA)	TT#13-7805
MK-8742: In Vitro Hemolysis Assay of MK-8742 Formulation	TT#14-7800
MK-8742: Three Day Oral (Gavage) Phototoxicity and Bioanalysis Evaluation of MK-8742 in Male Pigmented Rats	TT#12-9042
MK-8742: One-Month Oral Toxicity Study in Dogs with a Three-Month Treatment-Free Period	TT#14-1031
MK-5172: Bovine Corneal Opacity and Permeability Test (BCOP)	TT#11-7866
MK-5172: Acute Dermal Irritation/Corrosion in Rabbits	TT#11-7865
MK-5172: Local Lymph Node Assay in Mice (LLNA)	TT#11-7864

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MK-5172: In Vitro Hemolysis Assay of MK-5172 Formulation	TT#11-7867
MK-5172: Exploratory 12-Week Oral Biomarker Study in rasH2 Wild-Type Mice (Hybrid) with a 12-Week Recovery Period	TT#12-6034 (& 12-9693)
MK-5172: Exploratory One-Month Oral Toxicity Study in C57BL/6 and BALB/c Mice	TT#12-6039 (& 12-9709)
MK-5172: Exploratory 8-Day Oral Tolerability Study in Rhesus Monkeys	TT#09-1059
MK-5172: Three Day Oral (Gavage) Phototoxicity and Bioanalysis Evaluation of MK-5172 in Male Pigmented Rats	TT#12-9002
MK-8742/MK-5172: One-Month Oral Combination Toxicity Study in Dogs	TT#12-6051

3.2 Studies Not Reviewed

All nonclinical safety and PK/ADME studies were reviewed. Note: *In vitro* PK studies using human matrices (cells, microsomes, blood, plasma etc.) were reviewed by the clinical pharmacology reviewer.

3.3 Previous Reviews Referenced

Some MK-5172 nonclinical safety studies, including repeat-dose toxicology and genetic toxicology studies have been reviewed by Dr. Ita Yuen to support the NDA, and are summarized (as appropriate) in sections of this review, with complete reviews of pivotal studies included within the review text.

4 Pharmacology

4.1 Primary Pharmacology

ZEPATIER™ is a once daily fixed-dose combination (FDC) tablet of elbasvir (EBR, MK-8742), an HCV NS5A inhibitor, and grazoprevir (GZR, MK-5172), an HCV NS3/4 protease inhibitor. Elbasvir and grazoprevir have EC_{90} values that vary from the low pM to low nM range in HCV replicon assays with HCV genotypes 1, 4 and 6. Please refer to the clinical virology review for a detailed description and review of the primary pharmacology data.

4.2 Secondary Pharmacology

Elbasvir (EBR, MK-8742) exhibited >100,000-fold selectivity with respect to off target binding and enzyme inhibition of 116 potential unintended targets (b) (4) EBR displayed some binding activity to the human ET_A and 5-HT_{2A} receptors (IC_{50}= 7.8 & 3.9 μM) and the guinea pig adenosine transporter (IC_{50}= 4.2 μM),

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while enzyme inhibition of rat PKC, bovine PKA and human ERK1 at IC_{50}~ 0.88, 1.7 & 2.0 μM, respectively, were also observed.

Grazoprevir (GZR, MK-5172) exhibited >25,000-fold selectivity with respect to HCV protease (GT1, 4 and 6) versus human serine protease inhibition, as evaluated with elastase and trypsin (IC_{50}>100 μM) and chymotrypsin (IC_{50}~1.5 μM). In addition, GZR exhibited similarly high selectivity with respect to enzyme and receptor binding of unintended targets (b) (4) with binding activity (IC_{50}<10 μM) only observed to matrix metalloproteinase-1 & 12 (IC_{50}~1.5 & 6.9 μM), lipoxygenase 5-LO (IC_{50}~2.8 μM), prostanoid FP (IC_{50}~6.5 μM) and hERG (IC_{50}~3.3 μM). Clinical significance of this unintended binding/activity of EBR and/or GZR appears unlikely.

4.3 Safety Pharmacology

4.3.1 Elbasvir

Neurological effects: No significant neurological effects were observed in male rats (6/group), evaluated in a functional observational battery (FOB), given oral doses of 0, 100, 300 or 2000 mg/kg/day MK-8742 (Study #TT11-6014). No test-article related changes in numerous standard observations (home-cage, hand-held & open-field), stimulus activity responses and other measurements (including grip strength, body temperature & hot plate latency) were noted ~4 hours post-dose on day 1 in this GLP study.

Cardiovascular effects:

In vitro studies:

- A minor effect of MK-8742 on membrane K^+ current was observed (5.2% reduction @ 10 μM) in stably hERG transfected CHO cells in a GLP study (Study #TT11-4703). Limited test-article solubility prevented examination of higher concentrations. This effect was not statistically significant and is not considered to be of clinical concern.
- Prior to this study, a non-GLP study was conducted using a higher throughput planar voltage-clamp technology [PatchXpress 7000A (Molecular Devices); FASTPatch® assay (ChanTest™)] to evaluate potential effects of MK-8742 on three cardiac ion channel currents [I_{kr} (hERG), I_{ks} and $hNa_v1.5$] in stably transfected CHO or HEK293 cells (Study #TT10-3196). A minor effect of MK-8742 on the $hNa_v1.5$ (I_{na}) channel was observed (17% inhibition 30 μM), while no significant effects on the other channels were observed at up to 30 μM of MK-8742.

In vivo studies:

- The NOEL is ≥50 mg/kg in telemetrized male dogs given single oral doses of 0, 0.5, 2 or 50 mg/kg because no test-article related effects on hemodynamic (HR and BP) or electrocardiographic parameters were noted by telemetry for up to 24 hours post-dose in this GLP study (Study #TT10-5474). This study used a Latin square cross-over design with a minimum duration of 3 days between doses.

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- The NOEL is ≥40 mg/kg in telemetrized female rats given single oral doses of 0, 10 or 40 mg/kg because no test-article related effects on blood pressure, heart rate and ECG intervals were observed for up to 20 hours post-dose in a non-GLP study (Study #TT10-5006).

Pulmonary effects: The NOEL is ≥50 mg/kg in conscious telemetrized dogs given single oral doses of 0, 2, 25 or 50 mg/kg because no test-article related effects on respiratory parameters [respiratory rate (bpm) or depth of respiration (mmHg)] or on body temperature were noted by telemetry for up to 24 hours post-dose in this GLP study (Study #TT10-5606).

4.3.2 Grazoprevir

Neurological effects: No significant neurological effects were observed in male rats (6/group), evaluated in a functional observational battery (FOB), given single oral doses of 0, 25, 50 or 1000 mg/kg MK-5172 (Study #TT08-1152) or oral doses of 0, 50, 200 (q.d.) or 200 (b.i.d.) mg/kg (Study #TT09-1120). Significantly greater MK-5172 exposures were achieved in the latter study and so exposures from this study were used for exposure multiple comparisons (refer to Section 11). No test-article related changes in numerous standard observations (home-cage, hand-held & open-field), stimulus activity responses and other measurements (including grip strength, body temperature & hot plate latency) were noted ~2 hours following the second of two doses on day 1 in this latter GLP study.

Cardiovascular effects:

In vitro studies:

- An effect of MK-5172 on membrane K^+ current was observed (IC_{50}=25 μM) in stably hERG transfected CHO cells in a GLP study (Study #TT08-4721) (refer to Applicant Table below). In addition to this inhibition, an effect on gating properties of the hERG current is suggested "by the apparent change in the kinetics of deactivation of the tail current at -50 mV, and by the increase in steady-state current at +20 mV, especially at 96 μM" (detailed examination of this effect was beyond scope of this study).
- Prior to this study, a non-GLP study was conducted using a higher throughput planar voltage-clamp technology [PatchXpress 7000A (Molecular Devices)] to evaluate potential effects of MK-5172 on three cardiac ion channel currents [I_{kr} (hERG), I_{ks} and $hNa_v1.5$] in stably transfected CHO or HEK293 cells (Study #TT08-3064). An effect of MK-5172 on the hERG channel was observed (IC_{50}=8.9 μM), while no effects on the other channels were observed at up to 30 μM of MK-5172.

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Table 2: Inhibition of the hERG channel current by MK-5172 in a GLP study

Concentration (μM)	% Inhibition (Mean)	SD	SEM	N
2.5	5.7	6.7	3.2	3
7.9†	26.6	8.1	3.5	4
27†	51.7	10.4	4.5	4
96†	51.4	6.4	3.0	3

SD = Standard deviation.
SEM = Standard error of the mean.
N = Sample size.
† Effect at this concentration is significantly different from control ($p \leq 0.05$).

In vivo studies:

- The NOEL is 5 mg/kg in conscious telemetrized dogs (n=2/sex) given escalating single oral ([redacted] (b) (4) doses of 5, 20 or 600 mg/kg MK-5172 or vehicle (100% PEG 400) (with a 3-day washout period between dose levels) because increased heart rate was observed at 20 (+42%) and 600 (+30%) mg/kg of MK-5172. Increased heart rate began ~3 hours post-dose and remained elevated until ~20 hours post-dose. Although coincident decreases in QT interval and/or PR interval (up to -9%) were observed, no effect on QTc interval (using Miyazaki correction method) was noted. No additional significant effects on cardiovascular parameters [arterial blood pressure, electrocardiographic parameters (or core body temperature)] were noted by telemetry for up to 48 hours post-dose in this GLP study (Study #TT08-5651).
- No significant effects were observed in an exploratory cardiovascular study in anesthetized, vagotomized and mechanically ventilated female dogs (n=3) given consecutive intravenous doses of 1, 2 and 2 mg/kg at 30 min intervals (for a total of 5 mg/kg) because no test-article related effects on blood pressure, heart rate and ECG intervals were observed (Study #TT08-5918). Plasma concentrations of 74.4±24.8 μM were achieved following the last dose.

Pulmonary effects: The NOEL is ≥600 mg/kg in conscious telemetrized dogs (n=2/sex) given escalating single oral ([redacted] (b) (4) doses of 5, 20 or 600 mg/kg MK-5172 or vehicle (100% PEG 400) (with a 3-day washout period between dose levels) because no test-article related effects on respiratory parameters [respiratory rate (bpm) or depth of respiration (mmHg)] were noted by telemetry for up to 48 hours post-dose in this GLP study (Study #TT08-5651).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Elbasvir

Absorption, distribution, metabolism and excretion of elbasvir (EBR, MK-8742) have been studied in numerous *in vitro* and *in vivo* studies. A summary of the significant findings from studies in rats, rabbits, dogs and monkeys is presented below.

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Absorption:

The oral bioavailability of EBR was estimated to be ~3-9 and 35% in rats and dogs, respectively, following administration by gavage in vehicle [rat: spray dried material (30% drug load) in 20 mM citrate buffer (pH=4) in 0.4% HPMC; dog: 10% Tween 80 & 90% PEG 400] (refer to Table below). Oral T_{max} values for EBR in rats, monkeys and dogs ranged from ~3.3 to 5 hours. The low bioavailability observed in rats and dogs was likely due to low absorption, with first-pass hepatic extraction also an important contributor in dogs. In addition, solubility limited absorption was likely responsible for the less than dose proportional increases in exposure observed following oral administration at higher dose levels in these species. Although saturation of absorption limited the exposure obtained in oral toxicology studies, absorption of EBR from the GI tract was sufficient to achieve adequate circulating EBR exposure levels in the toxicology studies submitted in these species.

Table 3: Pharmacokinetic parameters of EBR in male rats, dogs and cynomolgus monkeys

Species	Dose (mg/kg)	Route	$AUC_{0-\infty}$ (μM.hr)	CL_p (ml/min/kg)	Vd_{ss} (L/kg)	$T_{1/2}$ (hr)	C_{max} (μM)	T_{max} (hr)	F* (%)
Rat	5	IV	4.3	24	5.0	4.2	-	-	-
Rat	30	PO	2.3	-	-	-	0.4	3.3	9
Rat	300	PO	7.8	-	-	-	0.6	5.0	3
Dog	1	IV	2.4	8.4	3.0	7.7	-	-	-
Dog	2	PO	1.7	-	-	-	0.29	3.3	35
cyno	1	IV	3.6	5.2	2.7	16	-	-	-

*calculated using mean IV AUC and individual PO AUC values

Distribution:

In rats, dogs and monkeys, the steady state volume of distribution (Vd_{ss}) was 5.0, 3.0 and 2.7 L/kg, respectively, values greater than total body water volume (refer to Table above). In addition, EBR was bound highly to plasma protein in all species (>99.9% bound), including mice, rats, rabbits, dogs, monkeys and humans, as determined by equilibrium dialysis at EBR concentrations from 1-10 μM. These data with EBR appear consistent with that expected for a hydrophobic compound.

Tissue distribution of EBR and related metabolites was evaluated in male Wistar Hannover (non-pigmented) and Long Evans (pigmented) rats at 2, 6, 10, 36, 72 and 168 hours and 6, 10, 72 and 672 hours post-dose, respectively, following a single 30 mg/kg oral dose of ^{14}C-EBR using quantitative whole body autoradiography. Although ^{14}C-EBR-derived radioactivity distributed well to most tissues (*i.e.* tissue concentrations greater than or equal to blood concentrations) with the highest maximum concentrations of radioactivity observed in the GI tract, adrenal gland, liver and renal cortex (refer to Table below for non-GI tract tissue to plasma ratios), distribution to the brain, bone and eye lens was limited. In Wistar Hannover rats, concentration of EBR and related metabolites in liver were ~12 to 20 times that in plasma from 2 to 10 hours post-dose, while at 168 hours post-dose, EBR and related metabolites were almost completely

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absent from tissues and could only be detected in the spleen, liver, kidney (cortex) and Harderian gland. Differences between pigmented and non-pigmented rats were observed, most notably in the eye uveal tract. Concentration of EBR and related metabolites in the eye uveal tract in Long-Evans rats peaked at 10 hours post-dose (at ~33 times that in plasma) and was still detectable at 72 (3 days) and 672 hours (28 days) post-dose (both at a concentration ~50% that observed at 10 hours), while concentration in Wistar Hannover rats peaked at 2 hours post-dose and was undetectable by 10 hours post-dose. Interestingly, the distribution of EBR to pigmented versus non-pigmented skin in Long Evans rats was roughly equivalent, so direct binding of EBR to melanin appears unlikely.

Table 4: Selected tissue to plasma ratios (≥2-fold) of EBR and related metabolites from male Wistar Hannover rats administered a single 30 mg/kg dose of ^{14}C-EBR

Tissue	2 hr post-dose	6 hrs post-dose	10 hrs post-dose
Bile	162	280	231
Liver	13	20	15
Adrenal gland	13	18	14
Renal Cortex	8	17	18
Pituitary gland	5	14	27
Salivary gland	5	14	16
Renal Medulla	5	7	11
Spleen	5	10	11
Pancreas	4	9	7
Thyroid gland	5	10	6
Adipose (brown)	6	10	7
Preputial gland	3	11	16
Bone marrow	3	7	6
Heart (myocardium)	4	5	3
Lung	4	4	3
Diaphragm	3	5	NA
Urine	5	5	NA
Urinary Bladder	NA	NA	12
Harderian gland	NA	10	12
Thymus	NA	8	11
Lacrimal gland (exorbital)	NA	7	8
Lacrimal gland (intraorbital)	NA	6	9
Prostate gland	NA	5	7
Lymph Node(s)	NA	6	7
Adipose (white)	NA	5	7
Mammary gland (region)	NA	5	2
Choroid Plexus	NA	2	7
Skin (nonpigmented)	NA	3	4
Skeletal Muscle	NA	4	2
Eye (uveal tract)	NA	3	NA

NA=not applicable since <2-fold

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Placental transfer of EBR was investigated in pregnant rats and rabbits by measuring EBR concentration in maternal and fetal plasma at 2 and 24 hours post-dose on gestation day (GD) 20 following oral administration of 1000 mg/kg/day from GD 6 through 20. Fetal to maternal plasma concentration ratios were low (range from 0.006-0.022 in rat and 0.006-0.008 in rabbit) (refer to Applicant Table below). The results suggest that EBR can cross the placenta in both species, but fetal plasma levels are only 0.6-2% that of the maternal plasma concentrations under the conditions of these studies.

Table 5: Maternal and fetal EBR concentrations on gestation day (GD) 20 in pregnant rats and rabbits following oral EBR administration from GD 6 through 20

Species	Dose (mg/kg/day)	Time (hr)	Plasma Elbasvir Concentration (μM) (Mean ± SE)		Ratio (Fetal/Maternal)
			Maternal	Fetal	
Rat	1000	2	1.05 ± 0.161	0.00594 ± 0.000852	0.00608 ± 0.00108
		24	0.0522 ± 0.0522	0.00250 ± 0.000950	0.0220 ± ID
Rabbit	1000	2	1.60 ± 0.399	0.00917 ± 0.00319	0.00564 ± 0.000804
		24	1.22 ± 0.299	0.00889 ± 0.00207	0.00751 ± 0.000975

ID: insufficient data.
Oral Placental and Lactational Transfer Study in Rats. TT #14-7050 and Oral Placental Transfer Study in Rabbits. TT #14-7030, n=4.

Metabolism:

EBR was stable metabolically, as assessed in hepatic microsomes from rats, rabbits, dogs and humans and hepatocytes from mice, rats, dogs and humans, with mono-oxidative and double-hydroxylated metabolites (catalyzed primarily by CYP3A4) identified in-vitro and in-vivo (refer to Applicant Figure below). Unchanged parent drug was the only drug-related component detected in plasma following administration of ^{14}C-EBR to bile duct cannulated (BDC) male Wistar Hannover rats, BDC male Beagle dogs and healthy human subjects, with unchanged EBR also comprising the majority of drug-related material in Dutch-Belted rabbits (minor amounts of circulating oxidative metabolites also observed). Unchanged parent drug was also the major component in bile (in rats and dogs) and feces (in rats, rabbits, dogs and humans), while oxidative metabolites comprised significant percentages of total drug-related material eliminated in bile (~15% in rats) and feces (~4% in dogs and ~19% in humans) (refer to Applicant Table below).

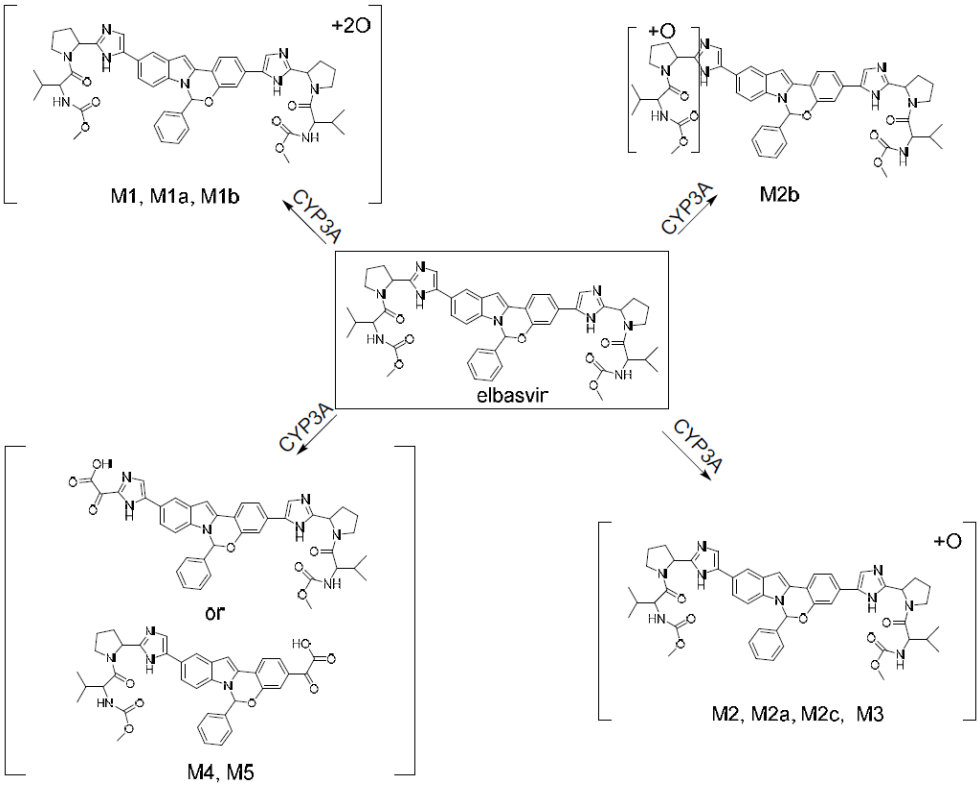

Figure 1: Proposed metabolic pathways of elbasvir in mice, rats, rabbits, dogs and humans

Table 6: Elbasvir and related metabolites in excreta in rats, rabbits, dogs and humans

Species	Rat		Rabbit		Dog		Human	
Biological fluid	B	F	B	F	B	F	B	F
Elbasvir	+	+	NS	+	+	+	NS	+
M2	+	-	NS	-	+	+	NS	+
M3	+	-	NS	-	+	+	NS	+

Doses: rats 5 mg/kg IV and 30 mg/kg P.O.; rabbits 100 mg/kg P.O.; dogs 1 mg/kg IV and 2 mg/kg P.O.; humans ~50 mg P.O. Also, no metabolites were detected in the plasma of rats, dogs, or humans with low metabolite levels detected in rabbit plasma.
B, bile; F, feces. M2 and M3 were also detected in rat urine following an IV dose.
+: Detected.
-: Not Detected.
NS: Sample not collected.

Excretion:

The systemic clearance (CL) of EBR was low to moderate in species evaluated with terminal half-lives of ~4 to 16 hours in rats, dogs and monkeys (refer to Table 3). The majority of radioactivity was recovered in feces and bile following administration of ^{14}C-EBR by oral and IV routes, respectively (refer to Applicant Table below). The low percentage of EBR and related metabolites in bile following oral (as compared to IV)

administration in rats and dogs is consistent with the low oral bioavailability observed with EBR. Although ~16% of EBR was eliminated as unchanged parent drug in rat urine following IV administration, only minor amounts of the total administered EBR dose was recovered in urine from dogs (and humans). Thus, biliary excretion and intestinal secretion of unchanged parent drug is the major route of elimination for EBR in rats and dogs.

Table 7: Excretion of elbasvir and related metabolites following IV and/or oral administration of ^{14}C-EBR in rats, rabbits, dogs and humans

Dose (mg/kg)	Route	Percent of Administered Dose			
		Rat (0-72 hr)			
		Urine	Bile	Feces	Total[a]
5	IV[b]	16.9 ± 1.8	41.0 ± 4.1	30.0 ± 4.5	89.0 ± 1.6
30	P.O.[b]	0.8 ± 0.4	7.8 ± 1.1	61.0 ± 18.7	69.8 ± 18.4
		Rabbit (0-72hr)			
100	P.O.[c]	1.2 ± 1.1	NC	69.8 ± 16.7	71.1 ± 17.1
		Dog (0-72hr)			
1	IV[d]	3.3	37.8	27.9	69.4
2	P.O.[c]	0.5 ± 0.1	8.7 ± 3.4	60.8 ± 29.4	70.5 ± 25.9
		Human (0-240 hr)			
50-51[e]	P.O.[f]	0.2 ± 0.1	NC	94.1 ± 5.7	94.3 ± 5.7

NC = not collected.
[a] Includes cage wash.
[b] Values represent mean ± SD (n=4).
[c] Values represent mean ± SD (n=3).
[d] Values represent mean ± SD (n=2).
[e] Dose in mg.
[f] Values represent mean ± SD (n=6).

Excretion of EBR and related metabolites into milk was also evaluated in lactating rats following oral administration of 1000 mg/kg/day of EBR from gestation day (GD) 6 to lactation day (LD) 14. Significant concentrations of EBR and related metabolites were present in rat milk as determined two hours post-dose on LD 14, with a milk to maternal plasma ratio of ~4.2 (refer to Applicant Table below).

Table 8: Maternal plasma and milk EBR concentrations on lactation day (LD) 14 in rats following oral EBR administration from GD 6 through LD 14

MK-8742 (mg/kg/day):	1000
Maternal Plasma Concentration	
2 hours	1.40 ± 0.150
Milk Concentration	
2 hours	5.75 ± 0.629
Milk/Maternal Plasma Ratio[a]	
2 hours	4.15 ± 0.264

[a] Values are the Mean ± SE of the individual milk concentration divided by the corresponding maternal plasma concentration.

PK Drug Interactions:

As determined in in-vitro studies, EBR is a substrate of CYP3A and P-gp and can inhibit P-gp, OATP1B1, OATP1B3 and BCRP. Refer to the clinical pharmacology review for a detailed description and review of the PK drug interaction-related data for EBR.

5.1.2 Grazoprevir

Absorption, distribution, metabolism and excretion of grazoprevir (GZR, MK-5172) have been studied in numerous *in vitro* and *in vivo* studies. A summary of the significant findings from studies in rats, rabbits and dogs is presented below.

Absorption:

The oral bioavailability of GZR was estimated to be 13 and 12% in rats and dogs, respectively, following administration by gavage in vehicle (100% PEG 400) (refer to Table below). Oral T_{max} values for GZR in rats and dogs ranged from ~2 to 8 hours, with the data suggesting a delayed absorption at higher dose levels in rats. The low bioavailability of GZR observed in rats and dogs was likely due to low absorption and first-pass hepatic extraction. Despite the low bioavailability, oral absorption of GZR was estimated to be at least 40% at these low dose levels, based on recovery of total drug-related material in urine and bile in BDC rats and dogs. In addition, oral absorption at intermediate oral dose levels was likely higher, given the greater than dose proportional increases in exposures observed.

Table 9: Pharmacokinetic parameters of GZR in male rats and dogs

Species	Dose (mg/kg)	Route	$AUC_{0-\infty}$ (μM.hr)	CL_p (ml/min/kg)	Vd_{ss} (L/kg)	$T_{1/2}$ (hr)	C_{max} (μM)	T_{max} (hr)	F (%)
Rat	2	IV	2.3	27.8	3.1	1.4	-	-	-
Rat	5	PO	0.7	-	-	-	0.3	4.0	13*
Rat	25	PO	61.0	-	-	-	12.3	4.0	-
Rat	100	PO	258	-	-	-	40.2	8.0	-
Dog	0.5	IV	2.3	5.2	0.7	3.0	-	-	-
Dog	1	PO	0.4^	-	-	-	0.1	4.7	12#
Dog	5	PO	41.3	-	-	-	7.1	2.0	-
Dog	10	PO	66.9	-	-	-	10.3	2.3	-
Dog	20	PO	237	-	-	-	25.6	2.7	-
Dog	30	PO	486	-	-	-	42.5	2.7	-

*calculated using mean IV AUC and mean PO AUC values; #calculated in individual animals using a cross-over design; ^represents AUC_{0-6hr} due to insufficient data points for extrapolation

Distribution:

In rats and dogs, the steady state volume of distribution (Vd_{ss}) was 3.1 and 0.7 L/kg, respectively (refer to Table above). In addition, GZR was bound highly to plasma protein in all species (>98.4% bound), including rats, rabbits, dogs and humans, as

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determined by equilibrium dialysis at GZR concentrations from 0.1-10 μM, with binding independent of GZR concentration.

Tissue distribution of GZR and related metabolites was evaluated in male Wistar Hannover (non-pigmented) and Long Evans (pigmented) rats at 4, 8, 24, 72, 168 and 336 hours and 8, 24, 168 and 672 hours post-dose, respectively, following a single 50 mg/kg oral dose of ^{14}C-GZR using quantitative whole body autoradiography. Limited distribution of ^{14}C-GZR-derived radioactivity was observed for most tissues, with the highest maximum concentrations of radioactivity observed in the GI tract, liver, intervertebral ligament and renal cortex (refer to Table below for non-GI tract tissue to plasma ratios) and radioactivity below the level of detection in CNS tissues. In Wistar Hannover rats, concentration of GZR and related metabolites in liver were 21 times that in plasma by 4 hours post-dose. By 72 hours post-dose, GZR and related metabolites were only detectable in liver and were below the level of detection by 168 hours post-dose. No significant differences between pigmented and non-pigmented rats were noted, with minimal distribution of GZR to pigmented and non-pigmented skin observed.

Table 10: Selected tissue to plasma ratios (≥1-fold) of GZR and related metabolites from male Wistar Hannover rats administered a single 50 mg/kg dose of ^{14}C-GZR

Tissue	4 hr post-dose	8 hrs post-dose	24 hrs post-dose
Liver	21	13	152
Bile	NA	16	449
Intervertebral ligament	1.2	2.5	NA
Renal Cortex	1.4	1.6	NA
Arterial wall	NA	1.4	NA
Adrenal gland	NA	1.2	NA
Adipose (brown)	NA	1.2	NA
Lacrimal gland (exorbital)	NA	1.2	NA
Lacrimal gland (intraorbital)	NA	1.1	NA
Renal Medulla	NA	1.1	NA
Harderian gland	NA	1.0	NA

NA=not applicable since <1-fold

In order to better understand the relationship between liver and plasma pharmacokinetics, additional single dose studies were conducted in rats and dogs utilizing multiple GZR dose levels (refer to Applicant Tables below). Robust distribution of GZR and related metabolites to the liver was observed at the lowest dose levels examined in rats and dogs. In both species, the liver to plasma ratios decreased as the dose levels increased, suggesting that GZR uptake is a saturable process. At the highest dose levels examined, the terminal liver half-lives were 8.4 and 9.4 hours in rats and dogs, respectively.

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Table 11: Summary of plasma and liver PK parameters following a single oral dose of GZR in rats

	5 mg/kg P.O.			25 mg/kg P.O.			100 mg/kg P.O.		
	Plasma	Liver	Liver/ Plasma	Plasma	Liver	Liver/ Plasma	Plasma	Liver	Liver/ Plasma
$AUC_{0-\infty}$ (μM·hr)	1.0	242	242	127	1697	13	352	3142	9
Terminal $T_{½}$ (hr)	0.9	3.0	-----	7.4	7.3	-----	8.7	8.4	-----

Table 12: Summary of plasma and liver PK parameters following a single oral dose of GZR in dogs

	1 mg/kg P.O.			5 mg/kg P.O.		
	Plasma	Liver	Liver/ Plasma	Plasma	Liver	Liver/ Plasma
$AUC_{0-\infty}$ (μM·hr)	3.5 ± 0.6	127 ± 18	36	44 ± 7	356 ± 63	8
Terminal $T_{½}$ (hr)	6.9 ± 0.4	7.0 ± 0.6	-----	8.8 ± 2.7	9.4 ± 2.6	-----

Placental transfer of GZR was investigated in pregnant rats and rabbits by measuring GZR concentration in maternal and fetal plasma at 2, 8 and 24 hours and 0.5, 2 and 24 hours post-dose, respectively, on GD 20 following oral administration of 400 mg/kg/day (200 mg/kg b.i.d.) in rats and intravenous administration of 100 mg/kg/day in rabbits from GD 6 through 20. Fetal to maternal plasma concentration ratios ranged from 0.0138 to 0.894 in rat and 0.0128 to 0.0706 in rabbit (refer to Applicant Table below). The results demonstrate that GZR can cross the placenta in both species. Additionally, although fetal exposure was less than maternal exposure in both species at all time-points, mean fetal plasma concentrations of up to ~7 (rabbits) and 89% (rats) that of maternal concentrations were achieved under the conditions of these studies.

Table 13: Maternal and fetal GZR concentrations on gestation day (GD) 20 in pregnant rats and rabbits following GZR administration from GD 6 through 20

Species	Dose (mg/kg)	Time (hr)	Plasma Grazoprevir Concentration (μM)		Ratio (Fetal/Maternal)
			Maternal	Fetal	
Rat	200, b.i.d., P.O.	2	28.3 ± 8.14	0.388 ± 0.110	0.0138 ± 0.000587
		8	39.1 ± 5.96	1.04 ± 0.113	0.0285 ± 0.00478
		24	0.0707± 0.0275	0.0473 ± 0.0143	0.894 ± 0.278
Rabbit	100, qd, IV	0.5	30.6 ± 2.58	0.391 ± 0.0752	0.0128 ± 0.00244
		2	8.43 ± 0.674	0.339 ± 0.0350	0.0408 ± 0.00505
		24	0.458 ± 0.0226	0.0310 ± 0.0110	0.0706 ± 0.0283

a Values are the mean ± SE.
Oral Placental and Lactational Transfer Study in Rats. TT #14-7040, n=4 or 5 per time point.
Intravenous Placental Transfer Study in Rabbits. TT #12-7420, n=12.

Note: Ratios calculated by dividing individual fetal plasma concentrations by the corresponding maternal plasma concentration

Metabolism:

GZR was stable metabolically, as assessed in hepatic microsomes and hepatocytes from rats, rabbits, dogs and humans and hepatic microsomes from mice, with metabolites resulting from hydroxylation (M1a, M4a, M4b, M7a & M9), oxidative O-dealkylation (M3), oxidative loss of vinylcyclopropylsulfonamide (M5) and oxidation and glutathione addition (M8) (catalyzed primarily by CYP3A4) identified in-vitro and in-vivo (refer to Applicant Figure below). In addition, several metabolites (M11a, M11b & M10) identified in human feces were not observed in-vitro, the likely result of gut bacterial metabolism. Although the M11a and M11b metabolites were only observed in humans (refer to Applicant Table below), they are likely eliminated from the GI tract without being absorbed. In addition, a human specific oxidative metabolite (M14), which is structurally similar to M5 (M14 is hydroxylated M5), was identified in human feces. This metabolite comprises ~4% of total GZR-related material eliminated in human feces. The presence of this minor human specific metabolite is not thought to pose a significant clinical safety risk.

Unchanged parent drug was the only drug-related component detected in plasma following administration of ^{3}H-GZR or ^{14}C-GZR to male Wistar Hannover rats, BDC male Beagle dogs and healthy human male subjects, with unchanged GZR also comprising the majority of drug-related material in Dutch-Belted rabbits (minor amounts of oxidative metabolites also present). In rats and dogs, unchanged parent drug and oxidative metabolites were present in roughly equivalent amounts in bile, while the predominant GZR-related material in feces was unchanged parent drug, with the M10 metabolite also detected in dog feces. In humans, unchanged parent drug and the M10 metabolite comprises 80% of total GZR-related material in feces, with various oxidative metabolites accounting for the remaining ~20%.

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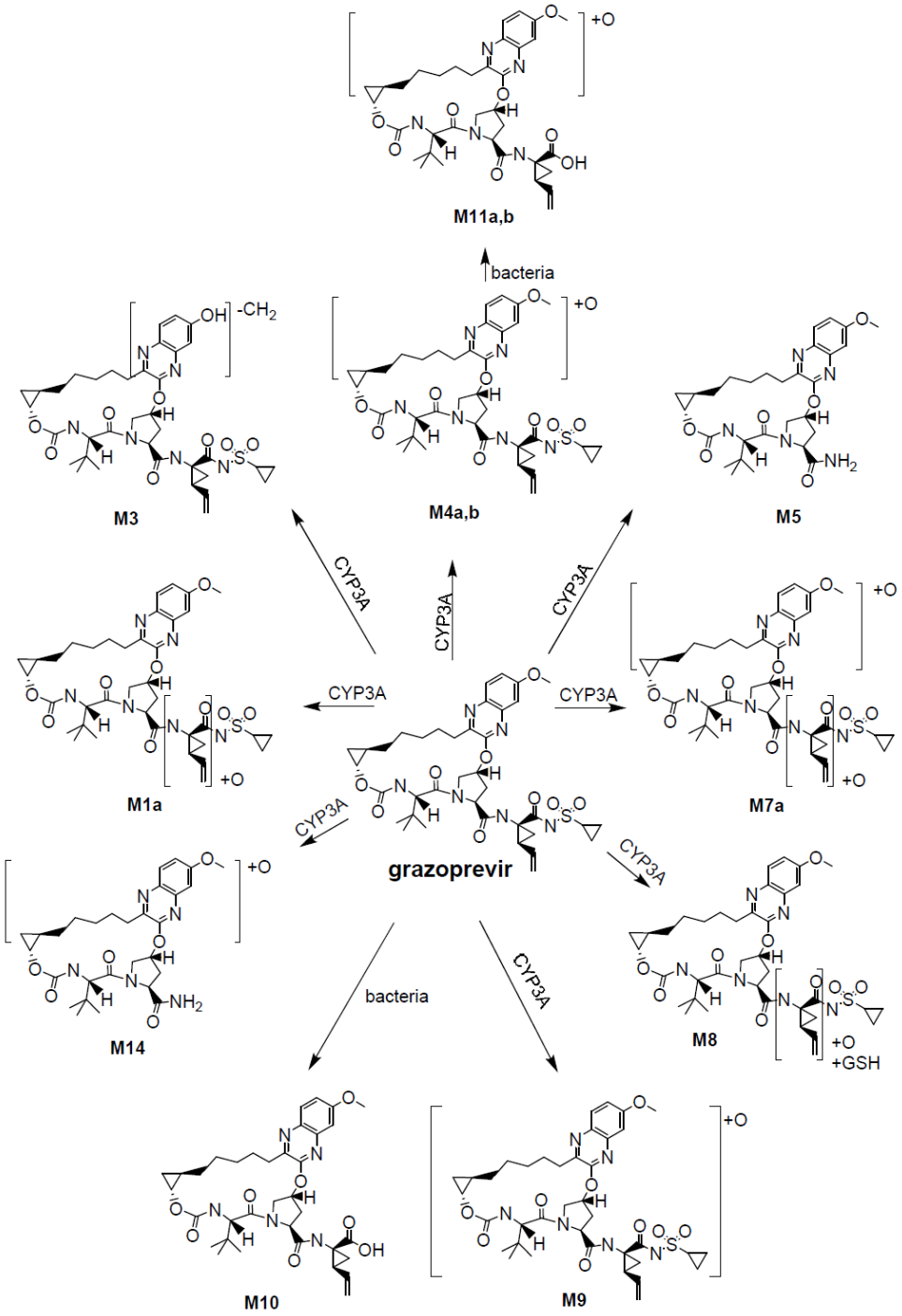

Figure 2: Proposed metabolic pathways of grazoprevir in mice, rats, rabbits, dogs and humans

Table 14: Grazoprevir and related metabolites in excreta in rats, rabbits, dogs and humans

Species	Rat		Rabbit		Dog		Human	
Biological Fluid	B	F	B	F	B	F	B	F
Grazoprevir	+	+	NS	+	+	+	NS	+
M1a	+	-	NS	+	-	-	NS	-
M3	+	+	NS	+	+	-	NS	-
M4a	+	+	NS	+	+	-	NS	+
M4b	+	+	NS	+	+	-	NS	+
M5	+	+	NS	+	-	-	NS	-
M7a	+	-	NS	+	+	-	NS	+
M8	+	-	NS	-	-	-	NS	-
M9	+	-	NS	+	-	-	NS	-
M10	-	-	NS	-	-	+	NS	+
M11a/b	-	-	NS	-	-	-	NS	+
M14	-	-	NS	-	-	-	NS	+

Doses: rats 5 mg/kg P.O.; rabbits 200 mg/kg P.O.; dogs 0.5 mg/kg IV and 1 mg/kg P.O.; humans 186-188 mg
B, bile; F, feces. Recovery of radioactive dose in urine was <4% in all species tested [Sec. 2.6.5.A.17] and therefore urine was not profiled in any species. Also, no metabolites were detected in the plasma of rats, dogs, or humans. Only minor oxidative metabolites were detected in the plasma of rabbits.
+: Detected.
-: Not Detected.
NS: Sample not collected.

The potential for GZR to form chemically reactive metabolites, capable of covalently modifying proteins, was evaluated using 10 μM ^{3}H-GZR or ^{14}C-GZR in rat and human liver microsomes and human hepatocytes incubated with or without reduced glutathione (GSH) or ritonavir. It was determined that GZR is capable of undergoing NADPH-dependent metabolic bioactivation to form chemically reactive intermediate(s) (refer to Applicant Table below). In incubations with GSH or ritonavir, the amount of covalent protein binding is reduced. This result along with the NADPH-dependence observed, indicates that CYP450 is important for metabolic bioactivation. However, the lack of detectable metabolites that are clearly indicative of metabolic bioactivation in human feces supports the hypothesis that bioactivation is a minor pathway in humans.

Table 15: Covalent protein binding of grazoprevir and related metabolites in rat and human liver microsomes and human hepatocytes

Conditions	NAPDH	5 mM GSH	Ritonavir	10 μM [^{3}H]grazoprevir, pmol equiv./mg protein	10 μM [^{14}C]grazoprevir, pmol equiv./mg protein
RLM	-	-	-	4.3 ± 0.2	-----
RLM	+	-	-	96.8 ± 1.4	-----
RLM	+	+	-	41 ± 3	-----
HLM	-	-	-	2.1 ± 0.3	48 ± 4
HLM	+	-	-	354 ± 46	451 ± 48
HLM	+	+	-	202 ± 11	-----
HLM	+	-	+	-----	53 ± 13
HH, 0 hr	-	-	-	-----	0 ± 0
HH, 4 hr	-	-	-	-----	53 ± 2

RLM = rat liver microsomes; HLM = human liver microsomes; HH = human hepatocytes; GSH = reduced glutathione; - represents not included; + represents included.
Data represent mean ± SD (n=3).

Excretion:

The systemic clearance (CL) of GZR was considered to be moderate, with terminal half-lives of 1.4 to 3 hours in rats and dogs. Although plasma CL was ~5 and 28 ml/min/kg in dogs and rats, respectively, blood clearance was estimated to be ~11 and 46 ml/min/kg after correcting for the blood to plasma partition ratios. The majority of radioactivity was recovered in feces and bile following administration of ^{14}C-GZR by oral and IV routes, respectively (refer to Applicant Table below). The low percentage of GZR and related metabolites in bile following oral (as compared to IV) administration in dogs is consistent with the low oral bioavailability observed. Only minor amounts of the total administered GZR dose was recovered in urine from all species evaluated including humans. Thus, both oxidative metabolism and biliary excretion of unchanged parent drug are major routes of elimination for GZR in rats and dogs, with intestinal secretion also contributing in dogs.

Table 16: Excretion of grazoprevir and related metabolites following IV and/or oral administration of ^{14}C-GZR in rats, rabbits, dogs and humans

Dose (mg/kg)	Route	Percent of Administered Dose			
		Rat (0-72 hr)[a]			
		Urine	Bile	Feces	Total
5	P.O.	0.4 ± 0.3	28.1 ± 2.2	72.8 ± 4.0	101.3 ± 5.3
		Rabbit (0-72hr)[a]			
200	P.O.	1.4 ± 2.3	NC	77.3 ± 14.3	78.7 ± 12.1
		Dog (0-72hr)[a]			
0.5	IV	0.5 ± 0.3[b]	74.3 ± 20.8	22.0 ± 24.5	97 ± 5
1	P.O.	3.4 ± 2.8	27.8 ± 9.0	26.3 ± 10.7	57.5 ± 9.5
		Human (0-576 hr)[c]			
186 – 188[d]	P.O.	0.29 ± 0.07	NC	110 ± 16	110 ± 16

NC = not collected.
[a] values represent mean ± SD (n=3).
[b] includes the cage rinse counts.
[c] values represent mean ± SD (n=6).
[d] dose in mg.

Excretion of GZR and related metabolites into milk was also evaluated in lactating rats following oral administration of GZR from gestation day (GD) 6 to lactation day (LD) 14. Significant concentrations of GZR and related metabolites were present in rat milk as determined 2 and 8 hours post-dose on LD 14, with milk concentrations ranging from 54 to 87% that measured in maternal plasma (refer to Applicant Table below).

Reference ID: 3839341

Table 17: Maternal plasma and milk GZR concentrations on lactation day (LD) 14 in rats following oral GZR administration from GD 6 to LD 14

	MK-5172		
	25 mg/kg/day	100 mg/kg/day	200 mg/kg b.i.d.
Maternal Plasma Concentration (µM)			
2 hours	3.81 ± 1.38	38.6 ± 5.65	22.3 ± 10.2
8 hours	NC	7.63 ± 3.24	56.6 ± 4.82
Milk Concentration (µM)			
2 hours	1.70 ± 0.591	31.7 ± 4.64	17.8 ± 8.80
8 hours	NC	4.66 ± 2.16	40.0 ± 3.45
Milk/Maternal Plasma Ratio[a]			
2 hours	0.538 ± 0.0941	0.836 ± 0.0548	0.866 ± 0.127
8 hours	NC	0.546 ± 0.0524	0.675 ± 0.0288

[a] Values are the Mean ± SE of the individual milk concentration divided by the corresponding maternal plasma concentration.
NC = Not calculated; no blood collection.
MK-5172 Dosing: From Gestation Day 6 to Lactation Day 14.
b.i.d. Dosing: The second dose was administered approximately 6 hours after the end of the first dosing.

PK Drug Interactions:

As determined in in-vitro studies, GZR is a substrate of CYP3A, P-gp, OATP1B1 and OATP1B3 and can inhibit OATP1B1, OATP1B3, BSEP and potentially MRP2, 3 and 4. Refer to the clinical pharmacology review for a detailed description and review of the PK drug interaction-related data for GZR.

5.2 Toxicokinetics

Review of the majority of this data is included in Section 6.

Exploratory toxicokinetic studies with MK-5172 were conducted in male CD1 mice (non-GLP) (11/group) administered a single oral (gavage) dose of 150 or 350 mg/kg of a spray-dried formulation in 0.5%(w/v) methylcellulose/ 0.02% (w/v) sodium lauryl sulfate with 5 mM HCl in deionized water (Study # TT11-6043) and in female SD rats administered single or multiple (14 days) oral (gavage) doses of 50, 300 or 1000 mg/kg of the potassium salt form in PEG 400 (Study # TT09-1102). No test-article related effects on mortality were observed.

Table 18: Mean plasma MK-5172 TK parameters in male CD1 mice following single dose administration

Dose (mg/kg)	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
150	953 ± 198	94.4 ± 10.9	4.0 ± NC
350	1420 ± 220	95.1 ± 7.65	4.0 ± NC

NC = Not Calculated
Exploratory Single-Dose Oral Toxicokinetic Study in Male CD1 Mice. TT #11-6043.
Table from applicant

Table 19: Mean plasma MK-5172 TK parameters in female rats following 14-days of administration

Dose (mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
50[a]	70.0 ± 32.8	11.4 ± 5.37	3.3 ± 0.67
300[a,b]	140 ± 56.6	24.4 ± 6.24	1.0 ± 0.0

[a] Stand alone formulations and samples protected from light.
[b] Animals received 1000 mg/kg/day until Study Day 10 and 300 mg/kg/day onwards.

Table from applicant

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose studies were conducted with MK-8742 or MK-5172.

6.2 Repeat-Dose Toxicity

6.2.1 Elbasvir

Exploratory repeat-dose toxicology studies with MK-8742 in rats (non-GLP) and dogs (GLP) are summarized briefly below. The toxicity of MK-8742 was evaluated in male rats (4 or 5/group) administered oral (gavage) doses of 10, 100 or 750 mg/kg or vehicle control (10% polysorbate 80 in deionized water) (Study # TT10-2901) for 7 days. Toxicity endpoints were evaluated including clinical signs, body weights, clinical pathology, micronucleus evaluation and limited gross and histopathology (liver, heart, kidneys, brain, skeletal muscle, small intestine, large intestine, bone marrow, spleen, stomach and lung). No test-article related effects were observed. The NOAEL was 750 mg/kg/day (C_{max}= 1.45 μM, $AUC_{0\text{-}24\ hr}$=16 μM·hr @ 7d). The toxicity and toxicokinetics of MK-8742 were evaluated in dogs (2/sex) administered an oral (gavage) dose of 25 mg/kg (Study # TT11-6013) for 14 days. Standard toxicity endpoints were evaluated including clinical signs, body weights, food consumption, electrocardiographic evaluations, clinical pathology and gross and histopathology. No test-article related effects were observed. Mean liver concentrations of MK-8742 were estimated to be ~4.3 μM. The NOAEL was 25 mg/kg/day (C_{max}= 1.14 μM, $AUC_{0\text{-}24\ hr}$=15.9 μM·hr @ 14d).

Reference ID: 3839341

Study title: MK-8742: Fourteen-Day Oral Toxicity Study in Rats with Micronucleus and Functional Observational Battery Assays

Study no.:	TT#11-6014
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Merck Research Laboratories Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	April 27, 2011
GLP compliance:	Yes (French Ministry of Health)
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G010 and 97.7%

Key Study Findings: The NOAEL is 2000 mg/kg/day ($AUC_{0\text{-}24\ hr}$=15.5 (♀) & 27 (♂) μM·hr @ 2 weeks) for MK-8742, the highest dose tested, since no adverse toxicological findings were observed, including no test-article related effects in a functional observational battery and negative results for micronuclei induction. Feces discoloration (due to the presence of unabsorbed MK-8742) was observed in all high-dose animals. In addition, reduced body weight gain, which correlated with reduced food consumption, was observed in mid-dose males and in high-dose males and females.

Table 20: Mean plasma MK-8742 TK parameters in rats following 2-weeks of administration

Dose (mg/kg/day)[a]	Sex	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
300	Female	7.84 ± 0.894	0.862 ± 0.0628	4.0 ± NC
	Male	14.3 ± 0.903	1.28 ± 0.159	6.0 ± NC
	All	11.1 ± 1.21	1.02 ± 0.0942	4.0 ± NC
1000 (b.i.d.)[b]	Female	15.5 ± 0.992	1.07 ± 0.0906	8.0 ± NC
	Male	27.0 ± 1.66	1.96 ± 0.121	8.0 ± NC
	All	21.3 ± 2.15	1.52 ± 0.209	8.0 ± NC

[a] MK-8742 concentrations in plasma from all control group animals were below the lower limit of quantitation (0.023 μM) at all timepoints.
NC = Not Calculated
[b] 6 hours apart (+/- 15 minutes)

Table from applicant

Reference ID: 3839341

Table 21: Mean liver MK-8742 concentrations (μM[a]) in rats following 2-weeks of administration

Dose (mg/kg/day)[b]	Female	Male
100	1.74 ± 1.02	1.92 ± 0.537
300	2.74 ± 1.14	2.91 ± 0.701
1000 (b.i.d.)[c]	6.35 ± 1.36	11.0 ± 2.33

a Assuming a liver density of 1.
b MK-8742 liver concentrations from all control animals were below the lower limit of quantitation (0.208-0.284 μM).
c 6 hours apart (+/- 15 minutes)

Table from applicant

Study title: MK-8742: Three-Month Oral Toxicity Study in Rats

Study no.: TT#11-6024
Study report location: 4.2.3.2
Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret
Merck Research Laboratories
Route de Marsat, Riom,
63963 Clermont-Ferrand Cedex 9, France
Date of study initiation: September 23, 2011
GLP compliance: Yes (French Ministry of Health)
QA statement: Yes
Drug, lot #, and % purity: MK-8742, L-002469825-000G015 and 98.9%

Key Study Findings: The NOAEL is 1000 mg/kg ($AUC_{0\text{-}24\ hr}$=17.3 μM·hr @ 13 weeks) for MK-8742, the highest dose tested, since no adverse toxicological findings were observed. Increased incidence of transient post-dosing salivation (in males and females) and reduced body weight gain (in males) was observed at all dose levels.

Table 22: Mean plasma MK-8742 TK parameters in rats following 13-weeks of administration

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
50	Female	2.75 ± 0.231	0.416 ± 0.0411	2.0 ± NC
	Male	5.96 ± 0.530	0.584 ± 0.0353	2.0 ± NC
	All	4.36 ± 0.608	0.500 ± 0.0404	2.0 ± NC
300	Female	8.64 ± 0.834	0.965 ± 0.116	4.0 ± NC
	Male	11.5 ± 1.15	0.916 ± 0.0567	4.0 ± NC
	All	10.4 ± 0.975	0.940 ± 0.0587	4.0 ± NC
1000	Female	15.1 ± 1.02	1.10 ± 0.0772	4.0 ± NC
	Male	19.5 ± 1.21	1.37 ± 0.0949	8.0 ± NC
	All	17.3 ± 1.23	1.14 ± 0.114	8.0 ± NC

NC = Not Calculated

MK-8742 concentrations in plasma from all control group animals at 4 hours postdose were below the lower limit of quantitation (LLQ = 0.023 μM).

TT #11-6024: Three-Month Oral Toxicity Study in Rats.

Table from applicant

Table 23: Mean liver MK-8742 concentrations (μM[a]) in rats following 13-weeks of administration

Dose (mg/kg/day)[b]	Female	Male
50	1.15 ± 0.542	1.31 ± 0.253
300	4.00 ± 1.06	5.69 ± 1.28
1000	6.05 ± 1.77	7.71 ± 1.99

[a] Assuming a liver density of 1

[b] MK-8742 liver concentrations from all control animals were below the lower limit of quantitation (0.226 μM).

Table from applicant

Study title: MK-8742: Six-Month Oral Toxicity Study in Rats

Study no.:	TT#12-6033
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Merck Research Laboratories Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	May 22, 2012
GLP compliance:	Yes (French Ministry of Health)
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G017 (weeks 1-9) and L-002469825-000G018 (weeks 9-27), ≥97.5%

Key Study Findings: The NOAEL is 1000 mg/kg ($AUC_{0\text{-}24\ hr}$=21.9 μM·hr @ 13 weeks) for MK-8742, the highest dose tested, since no adverse toxicological findings were observed. Increased incidence of transient post-dosing salivation, slight increases in mean urine volume (in males and females) and reduced body weight gain associated with decreased food consumption (in males) was observed at all dose levels.

Methods

Doses:	0, 30, 300 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	15 main study, 9 TK
Age:	5 weeks
Weight:	110.0-150.8 (♀) and 136.2-190.0 (♂) g
Satellite groups:	TK
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None due to MK-8742. One control and high-dose male were found dead on Day 79 following blood collection and Day 171 following dosing, respectively. These unscheduled deaths were considered accidental due to anesthesia and gavage error, respectively.

Clinical Signs (1x/day):

Reference ID: 3839341

- Post-dosing salivation (absent in controls) observed at all dose levels w/ increased frequency at ≥300 mg/kg/day. Appears to be a non-adverse MK-8742-related effect.

Body Weights: Note: Measured pre-test, once/week (week 1-14) and once every 4 weeks (week 15-26).

- Decreases in mean body weight gain observed in males at ≥300 mg/kg/day compared to controls (refer to Applicant Table below). Slight decrease in mean body weight gain also observed in males at 30 mg/kg/day (≤-7%).

Table 24: Mean body weight gain (% of control) in rats administered MK-8742 for 6 months

MK-8742 (mg/kg/day)					
Females			Males		
30	300	1000	30	300	1000
-	-	-	-	-11	-17
- = No test article-related change.					

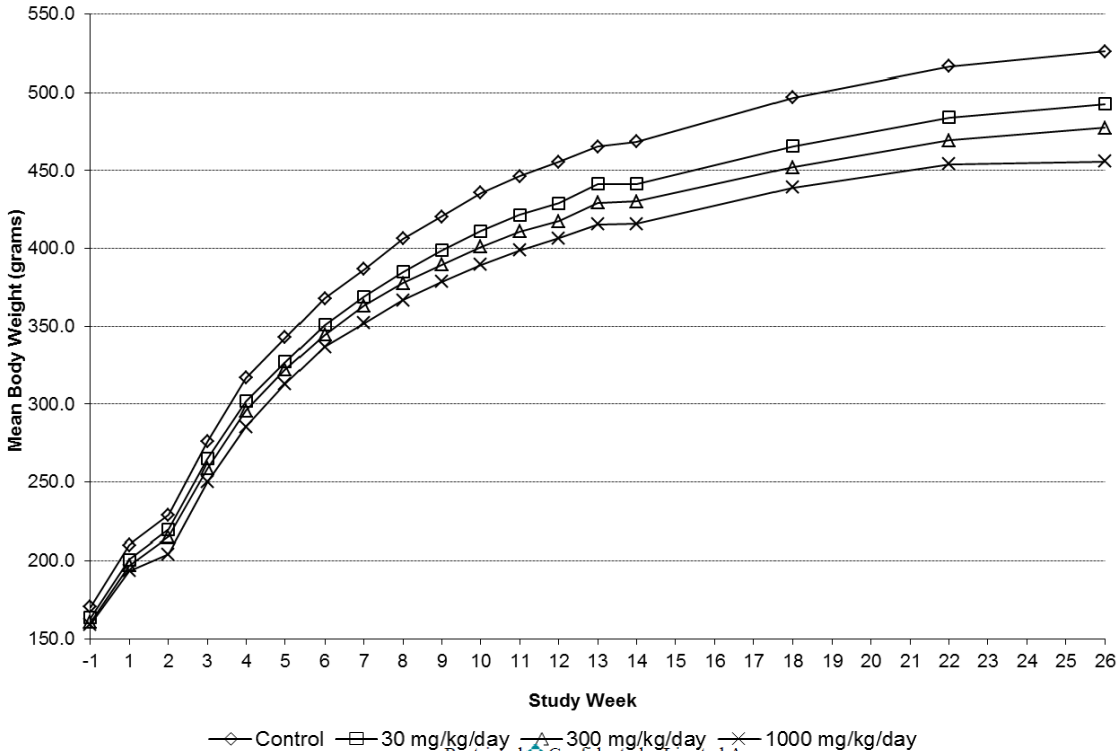

Figure 3: Summary of body weight changes in male rats administered MK-8742 over a six month period (from applicant)

Feed Consumption: Note: Measured once/week over a 3-6 day period (week 1-14) and once every 4 weeks (week 15-26).

- Decreases in food consumption observed in males at ≥300 mg/kg/day (compared to controls). Slight decrease in food consumption also observed in males at 30 mg/kg/day (≤-6%).

Ophthalmoscopy: No test-article related changes noted.

Hematology: Note: Measured on week 12 and 25.

- WBCs ↑~32% at 1000 mg/kg/day (♀) at weeks 12 and 25
- Neutrophils ↑76 & 48% at 1000 mg/kg/day (♀) at weeks 12 & 25, respectively
- Eosinophils ↑80% at 1000 mg/kg/day (♀) at week 25
- Monocytes ↑44% at 1000 mg/kg/day (♀) at week 12 only
- PT ↑10% at 1000 mg/kg/day at week 27 (♂)
- With the exception of the PT value, findings within the historical control range provided. Findings could be minor MK-8742-related effects.

Clinical Chemistry: Note: Measured on week 12 and 25. No test-article related changes noted.

Urinalysis: Note: Measured on week 12 and 25.

- Slight increases in mean urine volume (up to ~71%) observed in both sexes at all dose levels at 12 and 25 weeks. Finding appears to be a minor MK-8742-related effect.

Table 25: Mean urine volume (ml) in rats administered MK-8742 for 6-months

Sex	Week	Control	30 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
females	12	8 ± 2*	11 ± 4	9 ± 6	11 ± 6
	25	8 ± 3	10 ± 6	11 ± 6	12 ± 6
males	12	15 ± 7	16 ± 10	17 ± 8	22 ± 14
	25	14 ± 5	20 ± 6	18 ± 5	24 ± 8

*±S.D.

Gross Pathology: No test-article related changes noted.

Organ Weights:

- Liver weight ↓19% at 1000 mg/kg/day (♂). Liver weight within the provided historical control range.

Histopathology: Note: Only examined control and 1000 mg/kg/day dose groups.

Adequate Battery: yes

Peer Review: yes

Reference ID: 3839341

Histological Findings: No test-article related changes noted.

Special Evaluation: None

Toxicokinetics: Note: Blood sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose in Week 13 only (using 3 rats/sex/group for each time-point-so each animal sampled twice over 24 hrs). Liver collected for MK-8742 concentration determination at week 27 (collection time not clearly specified, perhaps 24 hrs post-dose?).

Table 26: Mean plasma MK-8742 TK parameters in rats following 13-weeks of administration in the 6-month study

Dose (mg/kg/day)	Sex	$AUC_{0-24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
30	Female	2.98 ± 0.476	0.392 ± 0.0569	1.0 ± NC
	Male	4.30 ± 0.365	0.498 ± 0.0415	2.0 ± NC
	All	3.64 ± 0.321	0.425 ± 0.0308	1.0 ± NC
300	Female	14.6 ± 4.22	1.12 ± 0.0338	2.0 ± NC
	Male	15.6 ± 2.64	1.20 ± 0.202	4.0 ± NC
	All	15.1 ± 2.24	1.12 ± 0.142	4.0 ± NC
1000	Female	19.8 ± 1.05	1.33 ± 0.0891	8.0 ± NC
	Male	24.1 ± 1.14	1.76 ± 0.110	8.0 ± NC
	All	21.9 ± 1.16	1.54 ± 0.114	8.0 ± NC

NC = Not Calculated

MK-8742 concentrations in plasma from all control animals at 4 hours postdose were below the lower limit of quantitation (LLQ = 0.023 μM).

TT #12-6033: Six-Month Oral Toxicity Study in Rats.

Table from applicant

Table 27: Mean liver MK-8742 concentrations (μM[a]) in rats following 6-months of administration

Dose (mg/kg/day)[b]	Female	Male
30	0.430 ± 0.138	0.942 ± 0.126
300	2.03 ± 0.477	6.83 ± 1.58
1000	6.34 ± 1.61	12.6 ± 1.99

[a] Assuming a liver density of 1.0 g/mL.

[b] MK-8742 liver concentrations from all control animals were below the lower limit of quantitation (LLQ = 0.229 μM).

TT #12-6033: Six-Month Oral Toxicity Study in Rats.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study Day 1 and in Week 9, and for concentration only in Week 13 and 26. Uniformity samples were within an acceptable range (≤10%) and all assay

Reference ID: 3839341

results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Study title: MK-8742: One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice

Study no.:	TT#12-6047
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Merck Research Laboratories Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	September 20, 2012
GLP compliance:	Yes (French Ministry of Health)
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G018 and 97.6%

Key Study Findings: The NOAEL is 1000 mg/kg ($AUC_{0-24\ hr}$= 120(♀) & 182(♂) μM·hr @ 4 weeks) for MK-8742, the highest dose tested, since no adverse toxicological findings were observed. Thus, a maximal tolerated dose (MTD) level was not achieved in this study.

Methods

Doses:	0, 10, 50, 300 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Mouse/Jic:CB6F1-nonTgrasH2@jcl
Number/Sex/Group:	10 (main), 11 (TK)
Age:	8 to 9 weeks
Weight:	19.4-23.6 (♀) and 23.3-29.2 (♂) g
Satellite groups:	TK only
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None

Clinical Signs (1x/day): No test-article related changes noted.

Body Weights: Note: Measured pre-test and once/week. No test-article related changes noted.

Reference ID: 3839341

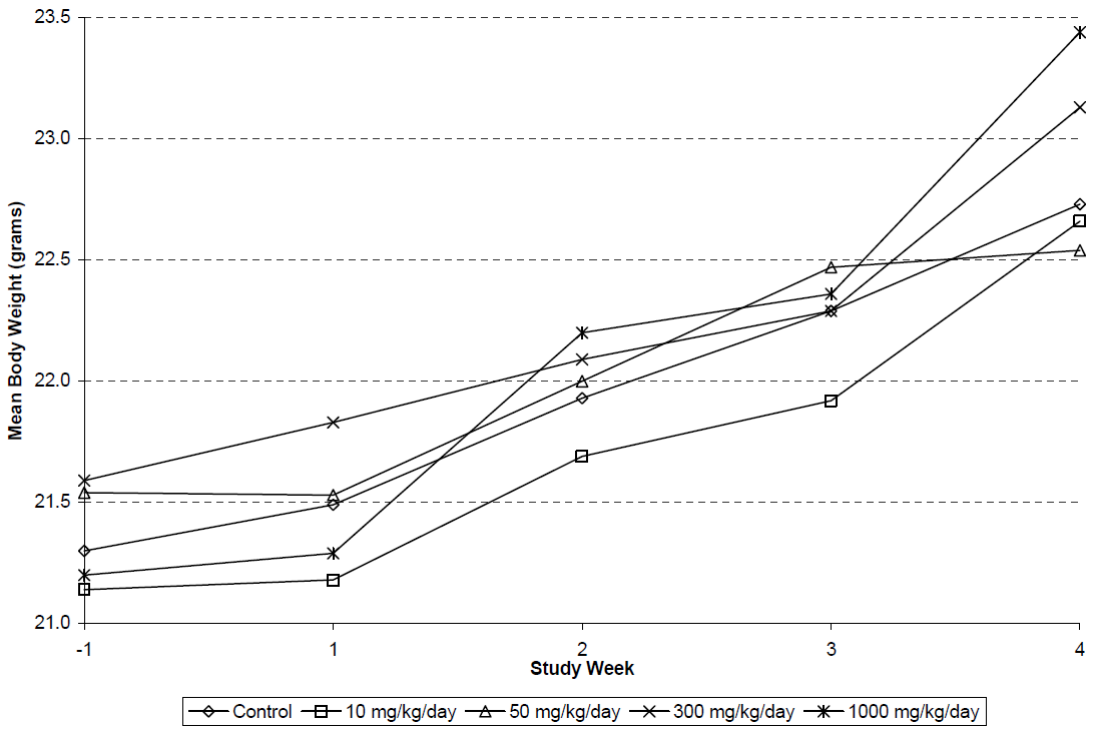

Figure 4: Summary of body weight changes in female mice administered MK-8742 over a one month period (from applicant)

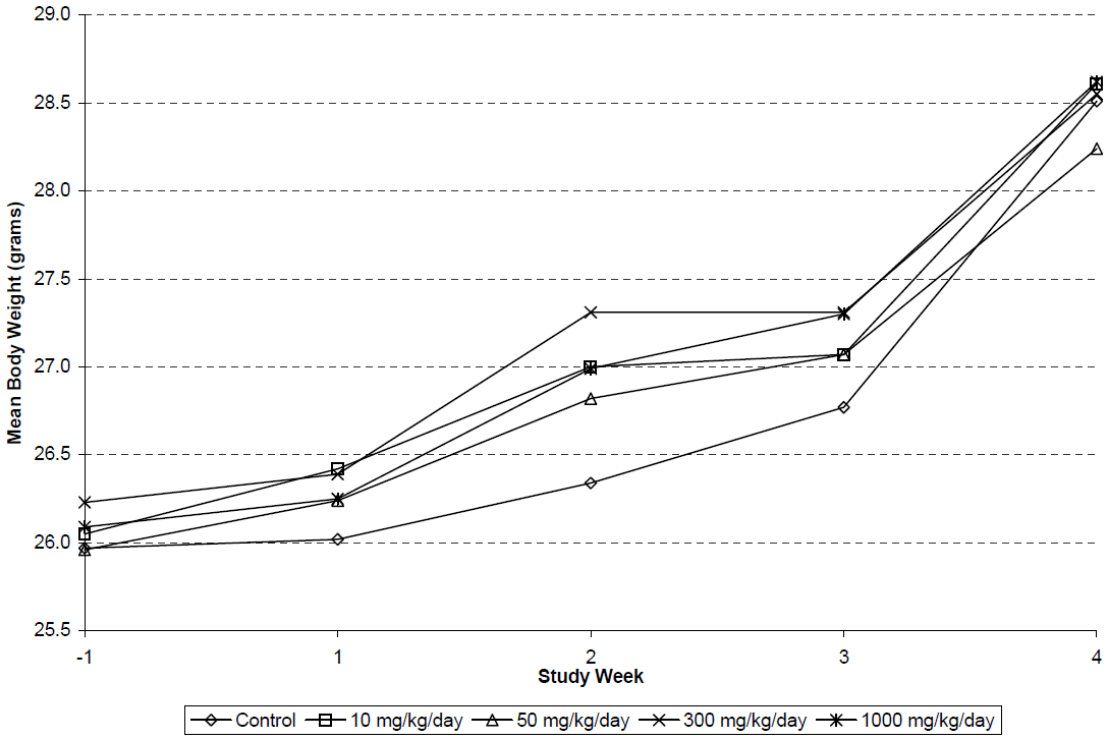

Figure 5: Summary of body weight changes in male mice administered MK-8742 over a one month period (from applicant)

Feed Consumption: Not evaluated

Ophthalmoscopy: Not evaluated

Hematology: No clear test-article related changes noted.

Clinical Chemistry:

- Cholesterol ↑12-14% at 300 & 1000 mg/kg/day (♂). Mean values in control, 300 & 1000 mg/kg groups were 91, 102 and 104 mg/dl (versus a historical control range of 65-98 mg/dl). Finding is a potential MK-8742-related effect but not considered adverse.

Urinalysis: Not evaluated

Gross Pathology: No clear test-article related changes noted.

- Decreased testis size in 1/10 at 1000 mg/kg/day associated with histopathology findings (male testis weight was 0.0435 g versus mean weight of 0.257 and 0.262 g in controls and the other nine high-dose males, respectively)

Organ Weights: No test-article related changes noted.

Histopathology: Note: Only examined control and 1000 mg/kg/day dose groups.

Adequate Battery: yes

Peer Review: yes

Histological Findings: No clear test-article related changes noted.

- Marked hypoplasia of testis in 1/10 at 1000 mg/kg/day (refer to gross pathology findings above)
- Very slight to slight focal inflammation of testis and epididymides w/ seminiferous tubule dilatation in 1/10 at 1000 mg/kg/day (not same male as described above)

Special Evaluation: None

Toxicokinetics: Note: Blood sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose in Week 4 (using 3 mice/sex/group for each timepoint-so each animal sampled twice over 24 hrs). Liver collected for MK-8742 concentration determination following last blood sampling timepoint (at 4, 8 or 24 hrs post-dose).

Reference ID: 3839341

Table 28: Mean (+/- SE) plasma MK-8742 TK parameters in mice following 4-weeks of administration

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
10	Female	2.33 ± 0.367	0.442 ± 0.0832	1.0 ± NC
	Male	3.47 ± 0.181	0.588 ± 0.0520	1.0 ± NC
	All	2.90 ± 0.301	0.515 ± 0.0547	1.0 ± NC
50	Female	13.6 ± 0.725	3.23 ± 0.186	1.0 ± NC
	Male	21.1 ± 0.828	3.59 ± 0.192	2.0 ± NC
	All	17.4 ± 1.25	3.34 ± 0.145	2.0 ± NC
300	Female	68.3 ± 14.6	6.76 ± 0.507	2.0 ± NC
	Male	76.0 ± 8.05	7.36 ± 0.197	4.0 ± NC
	All	72.1 ± 7.64	6.60 ± 0.320	2.0 ± NC
1000	Female	120 ± 13.9	9.62 ± 0.604	2.0 ± NC
	Male	182 ± 16.3	11.0 ± 1.21	4.0 ± NC
	All	151 ± 15.1	9.17 ± 1.12	4.0 ± NC

NC = Not Calculated
TT #12-6047: One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice.

Table from applicant

Table 29: Mean liver MK-8742 concentrations (μM[a]) in mice following 4-weeks of administration

Dose (mg/kg/day)	Time (hr) 4	8	24
10	2.38 ± 0.336	1.01 ± 0.292	0.00 ± 0.00
50	9.08 ± 1.26	4.21 ± 0.823	0.00 ± 0.00
300	40.0 ± 5.15	27.4 ± 4.22	0.928 ± 0.133
1000	59.1 ± 9.10	46.5 ± 5.19	20.9 ± 8.00

[a] Assuming a liver density of 1.0 g/mL.
TT #12-6047: One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration in Study Day 1 and for concentration only in Week 4. Uniformity samples were within an acceptable range (≤10%) and all assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Study title: MK-8742: Three-Month Oral Toxicity Study in Dogs

Study no.:	TT#11-6025
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Merck Research Laboratories Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	September 30, 2011
GLP compliance:	Yes (French Ministry of Health)
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G015 and 98.9%

Key Study Findings: The NOAEL is 1000 mg/kg ($AUC_{0\text{-}24\ hr}$=19.3 μM·hr @ 13 weeks) for MK-8742, the highest dose tested, since no adverse toxicological findings were observed. Feces discoloration (due to unabsorbed test-article) and infrequent liquid emesis were observed in animals administered 1000 mg/kg. In addition, vacuolation of gut associated lymphoid tissue (GALT) in small and/or large intestine, of mandibular and/or mesenteric lymph nodes and/or of spleen was observed in all animals administered 1000 mg/kg and in one male administered 25 mg/kg. Findings were characterized by the presence of an increased number of large vacuolated cells, morphologically consistent with macrophages in follicular areas of the lymphoid tissues, containing various amount of intracytoplasmic cell debris. These vacuolar changes correlated with the presence of myelin figures in macrophages observed by transmission electron microscopy (TEM) and are consistent with lysosomal phospholipid accumulation (phospholipidosis). Although reversibility was not evaluated, lymphoid tissue phospholipidosis was not associated with overt toxicity (with other histopathology findings in lymphoid tissue, change in spleen weights or with lymphoid depletion) and so may be an adaptive rather than a toxic response.

Reference ID: 3839341

Table 30: Mean plasma MK-8742 TK parameters in dogs following 13-weeks of administration

Dose (mg/kg/day)	Sex	$AUC_{0-24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
2	Female	ID	ID	ID
	Male	0.133 ± 0.0313	0.0341 ± 0.00390	1.3 ± 0.33
	All	ID	ID	ID
25	Female	5.48 ± 0.198	0.712 ± 0.233	2.3 ± 0.88
	Male	5.07 ± 1.15	0.345 ± 0.0824	1.7 ± 0.33
	All	5.28 ± 0.528	0.529 ± 0.138	2.0 ± 0.45
1000	Female	18.9 ± 2.59	1.36 ± 0.258	4.0 ± 0.0
	Male	19.7 ± 4.14	0.959 ± 0.210	11 ± 6.7
	All	19.3 ± 2.19	1.16 ± 0.174	7.3 ± 3.3

MK-8742 concentrations in plasma from all control group animals were below the lower limit of quantitation (LLQ = 0.023 µM).
ID = Insufficient Data

Table from applicant

Table 31: Mean liver MK-8742 concentrations (µM[a]) in dogs following 13-weeks of administration

Dose (mg/kg/day)	Female	Male
2	0 ± 0	0 ± 0
25	4.57 ± 1.47	16.7 ± 12.7
1000	14.5 ± 1.90	24.7 ± 3.07

[a] Assuming a liver density of 1
MK 8742 liver concentrations from all control animals were below the lower limit of quantitation (0.226 µM).

Table from applicant

Study title: MK-8742: Nine-Month Oral Toxicity Study in Dogs

Study no.:	TT#12-6030
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Merck Research Laboratories Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	April 3, 2012
GLP compliance:	Yes (French Ministry of Health)
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G015 (weeks 1-4), L-002469825-000G017 (weeks 5-15), L-002469825-000G018 (weeks 15-39) and ≥97.5%

Key Study Findings: The NOAEL is 1000 mg/kg ($AUC_{0\text{-}24\ hr}$=16.6 μM·hr @ 26 weeks) for MK-8742, the highest dose tested, since no adverse toxicological findings were observed. Feces discoloration (due to unabsorbed test-article) and reduced body weight gain (associated with infrequent liquid emesis and reduced food consumption) were observed in animals administered 1000 mg/kg. In addition, vacuolation of gut associated lymphoid tissue (GALT) in stomach, small and/or large intestine, lymph nodes and/or gall bladder, consistent with lysosomal phospholipid accumulation (phospholipidosis), was observed in all animals administered 1000 mg/kg and in two dogs administered 25 mg/kg. Although reversibility was not evaluated, lymphoid tissue phospholipidosis was not associated with overt toxicity (with other histopathology findings in lymphoid tissue including lymphoid depletion) and so may be an adaptive rather than a toxic response.

Methods

Doses:	0, 5, 25 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	26 to 30 weeks
Weight:	6.1-7.7 (♀) and 6.9-9.2 (♂) kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Reference ID: 3839341

Mortality (1x/day): None

Clinical Signs (1x/day):

- Feces discoloration (pale brown or white), typically starting on Day 2, observed frequently in all HD dogs. Appears to be due to unabsorbed test-article.
- Infrequent liquid emesis in all dogs administered 1000 mg/kg/day.

Body Weights (1x/week):

- BW gain reduced in HD ♀ (0.9±0.5 versus 1.7±0.3 kg in controls) due primarily to 1 of 4 HD animals (gained only 0.2 kg over 39 weeks w/ slight BW loss over first 34 weeks).
- BW gain reduced in 2 of 4 HD ♂ (gained 0.7 & 1.0 versus 1.9±0.5 kg in controls over 39 weeks). One of these HD males lost 17% of BW over first 3 weeks and (following food supplementation) attained pre-test BW by week 10.
- BW changes appear to be test-article related but are not considered adverse.

Feed Consumption (1x/day): Note: Performed qualitatively.

- Occasional unsatisfactory food consumption observed in all females and in 3 of 4 males at 1000 mg/kg/day. Food supplementation provided to one male due to inappetence and weight loss.

Ophthalmoscopy: Note: Performed pre-test and at weeks 25 & 38. No test-article related changes noted.

ECG: Note: Performed pre-test and 2-3 hours post-dose at weeks 26 & 37. Morphology, intervals and heart rates examined for each animal. No test-article related changes noted.

Hematology: Note: Performed pre-test and at weeks 2, 12, 25 & 38. No test-article related changes noted.

Clinical Chemistry: Note: Performed pre-test and at weeks 2, 12, 25 & 38. No test-article related changes noted.

Urinalysis: Note: Performed at weeks 12, 25 & 38. No test-article related changes noted.

Gross Pathology: No test-article related changes noted.

Organ Weights: No test-article related changes noted.

Histopathology: Note: Performed in control and HD only, except for stomach, lymphoid tissues of small (Peyer's patches) and large intestine, gall bladder and lymph nodes (examined in MD) and Peyer's patches and lymph nodes (examined in LD).

Reference ID: 3839341

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:

- Very slight to slight vacuolation of GALT [stomach, small (Peyer's patches) and/or large intestine], lymph nodes (mandibular & mesenteric) and/or gall bladder from all HD animals and 2 of 8 MD (see Table below). Note: Findings considered moderate (Peyer's patches) in one HD male.
- Vacuolar changes are consistent with lysosomal phospholipid accumulation (phospholipidosis). Note: TEM not conducted on tissues in this study (to confirm phospholipidosis) but similar findings were observed in 3-month study and correlated with the presence of myelin figures in macrophages observed by TEM.
- Findings not associated with any other histopathology finding in lymphoid tissues (lymphoid depletion etc.).

Table 32: Summary of the incidence of histopathology findings in lymphoid tissue following 39-weeks of MK-8742 administration in dogs

Dose, mg/kg/day	5	25	1000
Number of Animals	8	8	8
Histomorphology (Incidence)			
Lymphoid tissue, vacuolation			
Stomach	NE	-	3
Peyer's patches	-	1	7
Large intestine	NE	-	5
Gallbladder	NE	-	1
Lymph node	-	1	8
- = No noteworthy change. NE = Group not examined.			

TT #12-6030: Nine-Month Oral Toxicity Study in Dogs.

Table from applicant

Special Evaluation: None

Toxicokinetics: Note: Sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose after the first dose and on weeks 4, 13 and 26. Liver collected for MK-8742 concentration determination at week 39 (collection time not clearly specified, perhaps 24 hrs post-dose?).

Reference ID: 3839341

Table 33: Mean plasma MK-8742 TK parameters in dogs following 26-weeks of administration in the 9-month study

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
5	Female	0.879 ± 0.105	0.0796 ± 0.00764	1.0 ± 0.0
	Male	0.905 ± 0.300	0.0844 ± 0.0222	2.1 ± 0.72
	All	0.892 ± 0.147	0.0820 ± 0.0109	1.6 ± 0.39
25	Female	4.45 ± 0.809	0.638 ± 0.109	1.5 ± 0.29
	Male	3.78 ± 0.499	0.311 ± 0.0480	2.0 ± 0.0
	All	4.11 ± 0.458	0.474 ± 0.0830	1.8 ± 0.16
1000	Female	17.6 ± 2.04	1.50 ± 0.279	8.5 ± 5.2
	Male	15.6 ± 6.24	1.11 ± 0.252	7.8 ± 5.5
	All	16.6 ± 3.06	1.31 ± 0.189	8.1 ± 3.5

MK-8742 concentrations in plasma from all control group animals were below the lower limit of quantitation (LLQ = 0.023 μM) of the bioanalytical method.
TT #12-6030: Nine-Month Oral Toxicity Study in Dogs.

Table from applicant

Table 34: Mean liver MK-8742 concentrations (μM[a]) in dogs following 39-weeks of administration

Dose (mg/kg/day)	Female	Male	All
5	1.76 ± 0.975	0.507 ± 0.148	1.13 ± 0.514
25	1.78 ± 0.584	2.09 ± 0.808	1.93 ± 0.465
1000	9.62 ± 3.01	18.4 ± 7.01	14.0 ± 3.90

[a] Assuming a liver density of 1.0 g/mL.

MK-8742 liver concentrations from all control animals were below the lower limit of quantitation (0.229 μM) of the bioanalytical method.
TT #12-6030: Nine-Month Oral Toxicity Study in Dogs.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study Day 1 and in Weeks 5 and 16, and for concentration only in Weeks 4, 13, 26 and 38. Uniformity samples were within an acceptable range (≤10%) and all assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

6.2.2 Grazoprevir

Exploratory repeat-dose toxicology studies in rats (non-GLP) and dogs (non-GLP) were conducted with MK-5172. Briefly, female Wistar Hannover rats (5/group) administered oral (gavage) doses of 20, 40 or 200 mg/kg/day or vehicle control (100% PEG 400) (Study # TT08-2525) for 7 days were evaluated for clinical signs, body

weights, serum biochemistry and limited gross and histopathology. Test-article related effects were limited to a very slight increase in triglycerides at 40 and 200 mg/kg/day. The NOAEL was considered to be 200 mg/kg/day (C_{max}= 118 μM, $AUC_{0\text{-}24\ hr}$=635 μM·hr @ 7d). Two male beagle dogs were administered escalating oral ([redacted] (b) (4) doses of 3, 30 and 300 mg/kg/day of MK-5172 in 100% PEG 400 for 3 days each (Study # TT08-1119) for a total of 9 days. Toxicity assessment included clinical signs, body weights, food consumption and limited clinical pathology, gross and histopathology evaluations. Test-article related effects consisted of emesis, slight bilirubin increase (3-fold in one male) and albumin and calcium decreases at 300 mg/kg/day. Of note, testes were not examined. The NOAEL was estimated to be 300 mg/kg/day (C_{max}= 111 μM, $AUC_{0\text{-}24\ hr}$=1,689 μM·hr @ 9d).

Study title: MK-5172: One month oral toxicity study in rats with a functional observation battery

Study no.:	TT#08-1152
Study report location:	4.2.3.2
Conducting laboratory and location:	Merck Research Laboratories, West Point, PA, USA & Laboratoires Merck Sharp & Dohme-Chibret Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	December 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z014 and 98.4%

Key Study Findings: The NOAEL is 1000 mg/kg/day ($AUC_{0\text{-}24\ hr}$=24 μM·hr @ 1 month) for MK-5172, the highest dose tested, since no adverse toxicological findings were observed, including no test-article related effects in a functional observational battery in males. Saturation of absorption was reached at 50 mg/kg/day, since AUC values at this dose level were similar to those observed at 1000 mg/kg/day.

Reference ID: 3839341

Table 35: Mean plasma MK-5172 TK parameters in rats following 1-month of administration

Dose (mg/kg/day)[a]	Sex	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
25	Female	7.44 ± 2.62	2.93 ± 1.68	2.0 ± NC
	Male	10.1 ± 2.84	5.24 ± 1.87	2.0 ± NC
	All	8.76 ± 1.92	4.08 ± 1.24	2.0 ± NC
50	Female	29.6 ± 8.84	10.6 ± 3.79	1.0 ± NC
	Male	16.5 ± 6.40	8.13 ± 4.08	2.0 ± NC
	All	23.0 ± 5.66	8.97 ± 2.90	2.0 ± NC
1000	Female	26.4 ± 5.68	8.11 ± 2.49	2.0 ± NC
	Male	22.2 ± 6.86	7.03 ± 3.66	2.0 ± NC
	All	24.3 ± 4.09	7.57 ± 2.06	2.0 ± NC

NC = Not Calculated

[a] No drug levels above the lower limit of quantitation in control plasma samples at 2 hours postdose.

Table from applicant

Study title: MK-5172: One month oral toxicity study in rats with a functional observation battery with 2-week interim necropsy

Study no.:	TT#09-1120
Study report location:	4.2.3.2
Conducting laboratory and location:	Merck Research Laboratories, West Point, PA, USA & Laboratoires Merck Sharp & Dohme-Chibret Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	May 30, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z014 and 98.4%

Key Study Findings: The NOAEL is 400 mg/kg/day (200 mg/kg b.i.d.) ($AUC_{0\text{-}24\ hr}$= 156(♀) & 267(♂) μM·hr @ 4 weeks) for MK-5172, the highest dose tested, since no adverse toxicological findings were observed, including no test-article related effects in a functional observational battery in males. Saturation of absorption, which was observed in the previous study at 50 mg/kg/day, was not observed in this study despite identical formulations (MK-5172 in 100% PEG 400).

Table 36: Mean plasma MK-5172 TK parameters in rats following 2 or 4-weeks of administration

	Male			Female		
Dose	**50 mg/kg/day**	**200 mg/kg/day**	**200 mg/kg/b.i.d.**	**50 mg/kg/day**	**200 mg/kg/day**	**200 mg/kg/b.i.d.**
T_{max} (hr) Week 2	2	-	1	2	-	2
Week 4	2	2	2	2	1	8
C_{max} (μM)						
Week 2	18.6 ± 9.16	-	23.2 ± 5.83	23.6 ± 1.62	-	28.5 ± 14.6
Week 4	15.4 ± 8.53	34.0 ± 12.9	22.9 ± 12.5	7.72 ± 4.17	21.4 ± 3.39	17.7 ± 4.36
AUC_{0-24h} (μM-hr)						
Week 2	73.1 ± 24.0	-	188 ± 51.8	74.4 ± 10.9	-	300 ± 87.2
Week 4	38.4 ± 17.0	142 ± 58.0	267 ± 118	25.8 ± 9.75	92.4 ± 13.3	156 ± 57.2

Note: The following study review was taken directly from the original IND-110,261 review by Dr. Ita Yuen.

Study title: MK-5172: Six month oral toxicity study in rats

Study no.: TT#10-6014
Study report location: 4.2.3.2
Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret
Route de Marsat, Riom,
63963 Clermont-Ferrand Cedex 9, France
Date of study initiation: April 13, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-5172, L-002214070-001Z015 (weeks 1-10 & 23-27) & L-002214070-001Z016 (weeks 10-23) and ≥99%

Key Study Findings: Fifteen Wistar Hannover rats/sex/dose received 0, 50, 200 mg/kg/day or 200 mg/kg/b.i.d (400 mg/kg/day) MK-5172 for six months. In general, systemic exposures increased dose proportionally. Unlike the results from the one month study, twice daily dosing did not increase the systemic exposures. There was no accumulation following 26 weeks of repeated daily dosing and no gender difference in the toxicokinetic parameters analyzed. Transient salivation following dose administration in all dose groups (dose related increases in the incidence and duration) was observed. Focal hemorrhage in the glandular mucosa of stomach, likely corresponding to red foci or discoloration in the stomach, was seen in 8 out of 15 males in the 400 mg/kg/day (200 mg/kg/b.i.d.) dose group. This toxicity was not dose limiting. The NOAEL was 200 mg/kg/day, equivalent to an AUC value of ~321 μM-hr. The results of this study also indicated that maximum tolerated dose was not achieved.

Reference ID: 3839341

Methods

Doses:	0, 50, & 200 mg/kg/day and 200 mg/kg/b.i.d.
Frequency of dosing:	Once (200 mg/kg/day group) or twice daily (control and 200 mg/kg/b.i.d. groups) where doses were administered 6-7 hours apart
Route of administration:	Oral gavage
Dose volume:	2 ml/kg
Formulation/Vehicle:	100% polyethylene glycol 400
Species/Strain:	Wistar Hanover rats
Number/Sex/Group:	15
Age:	5 weeks old
Weight:	F: 98.1–157.5 g; M: 131.2–197.3 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that affected the interpretation of the study results

Observations and Results

Mortality

Animals were observed for mortality once daily. No treatment related death was observed. However, there were 4 deaths, 3 associated with anesthetic accidents. These 3 deaths included one control female that died in week 12, one male in the 200 mg/kg/day and 1 female in the 200 mg/kg/b.i.d. in week 24. In addition, one control male was found dead in week 25 without apparent cause of death.

Clinical Signs

Animals were observed for physical signs once daily, except for weekends and holidays. Post-dose salivation was seen in all dose groups but incidence and duration were dose-related. Unformed feces were seen in all dose groups with highest incidence in control and 200 mg/kg/b.i.d. male groups that received twice a day dose administrations, suggesting that they were the results of high amount of PEG400 administered and males were more sensitive than females.

Body Weights

Body weights were recorded weekly from weeks 1 to 14, then every 4 weeks thereafter until sacrifice. No treatment effect was observed.

Feed Consumption

Food consumption was recorded weekly over a 2- to 3-day period from weeks 1 to 14, then every 4 weeks thereafter. No treatment effect was observed.

Ophthalmoscopy

Ophthalmic examinations were done on all control and high dose animals in study weeks 11 and 26. No treatment effect was observed.

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Hematology

Blood samples were collected in study weeks 12 and 24. No treatment effect was observed.

Clinical Chemistry

Blood samples were collected in study weeks 12 and 24. There were several statistically significant changes (above those in the historical control values) in bilirubin, sodium, chloride, and potassium levels in the 200 mg/kg/b.i.d. groups. However, most of the changes were seen in one sex only, in week 12 only, and small in magnitude, their toxicological significance was unclear.

Urinalysis

Overnight urine was collected in study weeks 12 and 24. No treatment effect was observed.

Gross Pathology

Necropsy was scheduled at termination in week 26. Dose-related increases in the incidence of red foci was seen in the stomach of males. In contrast, only one low dose and one high dose females had this macroscopic finding. Corresponding microscopic finding was the very slight focal hemorrhage in the glandular mucosa of the stomach.

Table 37: Gross pathology findings in stomach in rats following 6-months of MK-5172 administration

	Male				Female			
Dose (mg/kg/day)	**0**	**50**	**200**	**400***	**0**	**50**	**200**	**400***
Stomach								
Discoloration	0	0	0	4	0	0	0	0
Focus	1	1	2	5	0	1	0	1
* The 400 mg/kg/day dose was given as two daily doses that were separated by at least 6 hours apart.								

Organ Weights

Organ weights of adrenals, liver, brain, heart, ovaries, kidneys, thyroids, pituitary, prostate, spleen, and testes were determined at necropsy. No treatment effect was observed.

Histopathology

Adequate Battery: Yes, histopathological evaluation was performed on the organs/tissues of the control and 200 mg/kg/b.i.d. (400 mg/kg/day) groups only. They included brain (including cerebral cortex, subcortical white matter, basal ganglia, thalamus, hippocampus, midbrain, cerebellum, and medulla oblongata), spinal cord (cervical), peripheral nerve (sciatic), eye, optic nerve, pituitary, adrenals, thyroids, parathyroids, Harderian gland, salivary gland (submandibular/sublingual), heart, aorta, skeletal muscle (quadriceps), small intestine (duodenum, jejunum, and ileum), Peyer's patches, esophagus, large intestine (colon and cecum), stomach (glandular and nonglandular portions), kidneys, urinary bladder, skin (from inguinal mammary region), mammary gland, liver, lungs, trachea, spleen, thymus, prostate, seminal vesicles, lymph nodes (mesenteric and cervical), pancreas, testes, epididymides, uterus, cervix,

Reference ID: 3839341

ovaries, vagina, bone (femur, tibia, and femorotibial joint), bone marrow (in bone section) from the control and high dose groups only.

Peer Review: Yes

Histological Findings: Eight out 15 males in the 200 mg/kg/b.i.d had focal hemorrhage in the glandular mucosa of the stomach. This finding was characterized by small collections of red blood cells and occasional siderocytes in the lamina propria near the surface of the mucosa. It also corresponded to the macroscopic observation and the increased incidence and frequency of unformed feces in the same dose group.

Toxicokinetics

Blood samples were collected at 1, 2, 4, 6, 8, 10, and 24 hours post-dose in study weeks 4, 9, and 13. Liver samples were collected at necropsy. No gender difference was observed in all of the toxicokinetic parameters analyzed. Absorption was rapid to moderate rapid with C_{max} achieved between 1 to 8 hours post dosing. Unlike the results from the previous one-month study, systemic exposures in the 200 mg/kg once and twice daily dose groups were similar and were dose proportional between the 50 and 200 mg/kg/day. In addition, the C_{max} and AUC values in this study were consistently higher than those in the previous one-month study (up to 4-fold higher especially for the 50 and 200 mg/kg/day doses). No accumulation was observed after 13 weeks of repeated daily dose administration.

Table 38: Mean plasma MK-5172 TK parameters and liver concentrations in rats following 4, 9 or 13-weeks and 6-months of administration, respectively

		Male			Female		
Dose		**50 mg/kg/day**	**200 mg/kg/day**	**200 mg/kg/b.i.d.**	**50 mg/kg/day**	**200 mg/kg/day**	**200 mg/kg/b.i.d.**
T_{max} (hr)	Week 4	2	2	8	2	2	8
	Week 9	2	1	8	2	2	2
	Week 13	2	2	8	2	2	2
C_{max} (μgM)							
	Week 4	18.1 ± 5.77	36.3 ± 15.6	39.6 ± 3.85	21.0 ± 3.09	62.9 ± 14.6	60.1 ± 11.5
	Week 9	18.1 ± 3.69	30.1 ± 1.86	37.2 ± 13.0	29.4 ± 1.05	60.0 ± 8.19	40.6 ± 8.29
	Week 13	29.5 ± 6.75	52.9 ± 3.12	43.0 ± 10.7	20.4 ± 0.525	40.0 ± 7.03	29.0 ± 15.8
$AUC_{0\text{-}24h}$ (μM-hr)							
	Week 4	63.2 ± 18.0	260 ± 81.5	232 ± 45.3	108 ± 24.4	476 ± 134	390 ± 85.0
	Week 9	116 ± 27.1	179± 35.9	341 ± 60.3	105 ± 25.1	563 ± 182	392 ± 82.2
	Week 13	119 ± 27.3	419 ± 68.4	525 ± 168	88.0 ± 15.7	321 ± 77.2	365 ± 81.7
Liver Conc. (μM)*		38.5 ± 6.39	41.7 ± 13.3	24.8 ± 5.60	21.0 ± 3.20	42.0 ± 6.62	23.9 ± 4.14

* Liver samples from the vehicle control group were also analyzed. Liver sample from one female rat had a concentration of 0.271 μM, above the LLQ level of 0.110 μM. MK-5172 was not detected in any other plasma or liver samples from the control group. Thus, the finding was probably an anomaly and did not affect the overall interpretation of the results.

Dosing Solution Analysis

The result of the analysis was acceptable.

Reference ID: 3839341

Study title: MK-5172: One-Month Oral Range-Finding and Toxicokinetic Study in rasH2 Wild-Type Mice (Hybrid)

Study no.:	TT#11-6034
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	November 21, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-011D003 (spray-dried), L-002214070-010B005 (free acid) and ~50% & ≥99.8%, respectively

Key Study Findings: The NOEL/NOAEL is 20/100 mg/kg/day ($AUC_{0\text{-}24\ hr}$= 5/164 μM·hr @ 1 month) for MK-5172 since adverse toxicological findings were observed at higher dose levels. The most predominant of these findings were body weight loss, renal tubule degeneration, villus atrophy of the small intestine and hepatobiliary (increase liver/hepatocyte weight/size, ALT, AST, bilirubin with gall bladder distention and cytoplasmic rarefaction in periportal region of the liver).

Methods

Doses:	0, 20, 100, 200 and 500 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Spray-dried formulation containing 50% (w/w) free acid and 50% (w/w) hydroxypropylmethylcellulose phthalate (HPMCP) in 0.5% methylcellulose/0.02% sodium lauryl sulfate w/ 5 mM HCl in deionized water (pH=7.0) (vehicle control: 50 mg/ml HPMCP in 0.5% methylcellulose/0.02% sodium lauryl sulfate w/ 5 mM HCl in deionized water)
Species/Strain:	Mouse/Jic:CB6F1-nonTgrasH2@Jc1
Number/Sex/Group:	10 (main), 11 (TK)
Age:	6 to 7 weeks
Weight:	18.3-21.4 (♀) and 21.4-29.0 (♂) g
Satellite groups:	TK
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality (1x/day): None

Reference ID: 3839341

Clinical Signs (1x/day): No test-article related changes noted.

Body Weights (1x/week): Test-article related changes noted (refer to Applicant Tables below).

- Transient BW loss between weeks one & two at 500 mg/kg/day (♀)
- BW loss (-4%) at 500 mg/kg/day (♂)
- ↓ mean body weight gain at ≥20 mg/kg/day (compared to controls) (♂)
  - 20 (-11%), 100 (-26%) and 200 (-44%) mg/kg/day
  - Mean BW gain was 3.4±1.1, 3.1±0.9, 2.5±0.8 and 1.9±1.4 g in control, 20, 100 and 200 mg/kg/day groups

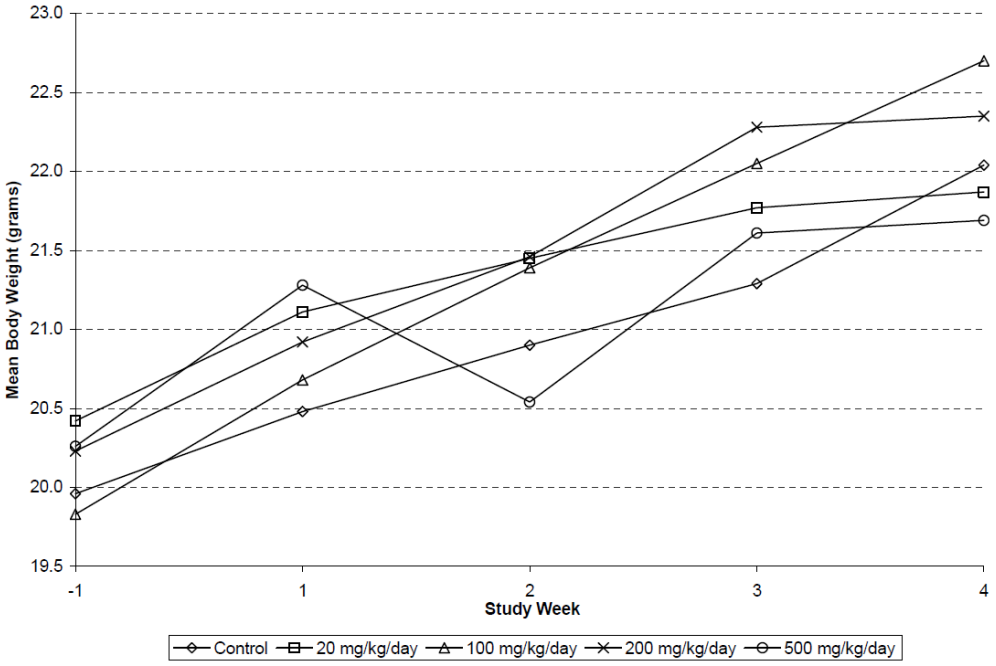

Figure 6: Summary of body weight changes in female mice administered MK-5172 over a one month period (from applicant)

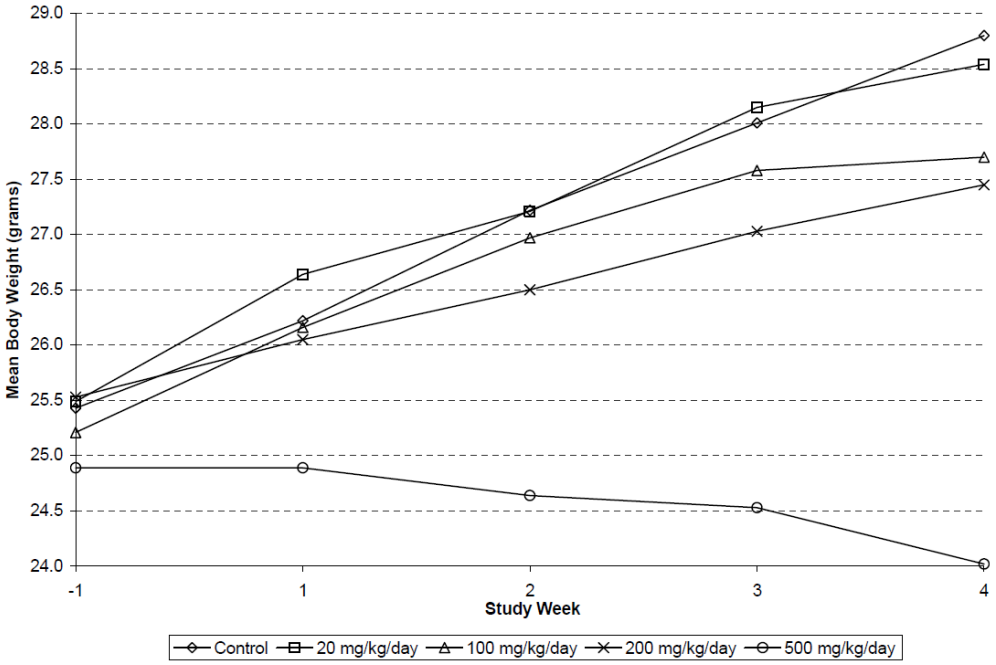

Figure 7: Summary of body weight changes in male mice administered MK-5172 over a one month period (from applicant)

Feed Consumption: Not evaluated (group housed)

Hematology: Test-article related changes noted (refer to Applicant Table below).

Table 39: Hematological findings in mice administered MK-5172 for 4-weeks

Parameter	MK-5172 (mg/kg/day)							
	Females				Males			
	20	100	200	500	20	100	200	500
RBC	-	-	-	I	-	-	-	I
MCV	-	-	-	-2	-	-	-	-3
MCH	-	-	-	-6	-	-	-	-6
MCHC	-	-	-	-4	-	-	-	-3
Platelets	-	-	-	+17	-	-	+19	+19
WBC	-	-29	-41	-37	-	-	-	+63
Neutrophils	-	-	-	-	-	-	-	+53
Lymphocytes	-	-32	-41	-43	-	-	-	+61
Eosinophils	-	-	-	-	-	-	-	+350

- = No test article-related change.
I = Increased based on incidence and/or severity.
MCH = Mean corpuscular hemoglobin.
MCHC = Mean corpuscular hemoglobin concentration.
MCV = Mean corpuscular volume.
RBC = Red blood cell count.
WBC = White blood cell count.

Note: Values= % difference in mean values from control

Clinical Chemistry: Test-article related changes noted (refer to Applicant Table below).

Table 40: Clinical chemistry findings in mice administered MK-5172 for 4-weeks

Parameter	MK-5172 (mg/kg/day)							
	Females				Males			
	20	100	200	500	20	100	200	500
Glucose	-	-	-	+20	-	-	+10	+16
BUN	-	-	-	-	-	-	-	I
Total Protein	-	-	-6	-9	-	-	-	-10
Albumin	-	-	-6	-9	-	-	-	-9
Globulin	-	-	-7	-7	-	-	-	-7
AST	-	-	-	+135	-	-	-	+39
ALT	-	-	+83	+430	-	-	-	+204
ALP	-	-	I	I	-	-	-	+37
Phosphorus	-	-	+26	+27	-	-	-	+44
Cholesterol	-	I	I	+39	-	I	I	I
Total bilirubin	-	-	+200	+1900[a]	-	I	+700[a]	+4600[a]

- = No test article-related change.
[a] Direct bilirubin level was determined in animals with a total bilirubin level above or equal to 1.0 mg/dL. In these individuals, direct bilirubin was the main component of total bilirubin.
ALP = Alkaline phosphatase activities.
ALT = Alanine aminotransferase activity.
AST = Aspartate aminotransferase activity.
BUN = Blood urea nitrogen.
I = Increased based on incidence and/or severity.

Note: Values= % difference in mean values from control

Urinalysis: Not evaluated

Gross Pathology:

- ↑ liver size (3 of 10) & prominent lobular pattern (2 of 10) at 500 mg/kg/day (♂)
- ↑ liver size (3 of 10) at 500 mg/kg/day (♀)
- ↓ thymus size (4 of 10) at 500 mg/kg/day (♂)
- ↑ gallbladder size (2 of 10 ♂ & 1 of 10 ♀) at 500 mg/kg/day

Organ Weights: Test-article related changes noted (refer to Applicant Table below).

- ↓ thymus (-45%), heart (-21%) and kidney (-12%) weight (% of brain weight) at 500 mg/kg/day (♂)

Table 41: Summary of select organ weight changes and histopathology findings in mice administered MK-5172 for 4-weeks

	Females				Males			
Dose, mg/kg/day	20	100	200	500	20	100	200	500
Number of Animals	10	10	10	10	10	10	10	10
Organ Weights[a, b]								
Liver	-	+21*	+26*	+77*	-	-	+12*	+34*
Histomorphology (incidence)								
Kidney,	NE	NE	-		NE	NE	-	
Tubule, Epithelial Degeneration				3				8
Liver,	NE	NE	-		NE	-		
Hepatocyte, increased size				9			7	9
Periportal region, Cytoplasmic rarefaction				3				
Small Intestine	NE	NE	-		NE	NE	-	
Villus atrophy				7				10

[a] Values are the mean percent change from concurrent controls of organ weight relative to brain weight.
[b] Statistics performed by trend assessment by sexes separate; * = $p \leq 0.05$.
- = No test article-related change.
NE = Not examined.

Histopathology: Note: Performed in control and HD only, except for adrenals, small intestine, kidneys, liver, thymus, ovaries, testes and epididymis (examined in 200 mg/kg/day group) and liver and thymus (examined in 100 mg/kg/day group).

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: Test-article related changes noted (refer to Applicant Table above).

- very slight (♀) to moderate (♂) degeneration of renal tubular epithelium "characterized mainly by tubular basophilia and dilatation of tubuli with scattered epithelial single cell necrosis, with and without presence of cellular debris in dilated tubules" observed at 500 mg/kg/day. Findings were restricted to a few foci in subcapsular areas or in medullary rays for very slight cases, while the moderate cases had involvement of large parts of the outer medulla.
- very slight to slight increase in hepatocellular size at 200 and/or 500 mg/kg/day accompanied by a slightly increased cytoplasmic rarefaction in the periportal region (♀) associated with large amounts of granular cytoplasmic structures (compatible with glycogen) in periportal and centrilobular hepatocytes (confirmed by PAS staining and TEM). Increased hepatocellular size correlated with increased mean liver weights and/or increased organ size and/or prominent lobular pattern (♂) observed by gross pathology.
- very slight (♀) to slight (♂) atrophy of the villi in the small intestine "largely restricted to the duodenum and generally characterized by blunting and fusion of villi associated with epithelial single cell necrosis and/or vacuolation and regenerative hyperplasia of the crypt epithelium" at 500 mg/kg/day. Jejunum changes much less prominent (than in the duodenum) suggesting a local effect of test article-related material (possibly assoc. w/ inflow of bile into the duodenum).

- distention of gallbladder present in 8 of 10 (♂) at 500 mg/kg/day (versus 3 of 10 controls)
- Potential "stress" (*i.e.* ↓ food consumption (presumed)/BW) associated findings at 500 mg/kg/day include:
  - very slight to slight lymphoid depletion in thymus (8 of 20) (assoc. w/ decreased thymus weight in ♂)
  - very slight adrenal cortical hypertrophy (6 of 10 ♂) and X zone involution present (5 of 10 ♀)
  - slight decrease in number of corpora lutea in the ovaries (6 of 10)
  - very slight increase in number of residual bodies in testes (4 of 10)
  - Although ovarian and testicular changes have been observed in some strains of mice upon food/calorie restriction [refer to Koizumi A. *et al*, 49:93-104 (1989) and Chapin, R.E. *et al*, 20:15-22 (1993)], the possibility that these changes are direct test-article related effects cannot be ruled out (particularly in females given the lack of clinical signs and the fact that body weight loss was transient).

Special Evaluation: None

Toxicokinetics: Note: Sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose at week 4 (3 mice/sex/timepoint). Liver collected at 4, 8 and 24 hours post-dose for MK-5172 concentration determination at week 4.

Table 42: Mean plasma MK-5172 TK parameters in mice following 4-weeks of administration

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
20	Female	5.34 ± 0.472	2.16 ± 0.572	0.50 ± NC
	Male	4.66 ± 0.510	1.97 ± 0.278	1.0 ± NC
	All	5.00 ± 0.334	1.91 ± 0.265	1.0 ± NC
100	Female	190 ± 89.0	36.2 ± 10.1	1.0 ± NC
	Male	138 ± 22.5	45.4 ± 6.54	1.0 ± NC
	All	164 ± 41.9	40.8 ± 5.74	1.0 ± NC
200	Female	335 ± 82.2	61.5 ± 9.04	1.0 ± NC
	Male	332 ± 61.8	83.0 ± 11.0	2.0 ± NC
	All	333 ± 49.5	63.7 ± 10.1	2.0 ± NC
500	Female	2140 ± 375	234 ± 72.9	0.50 ± NC
	Male	3470 ± 1140	290 ± 43.3	2.0 ± NC
	All	2810 ± 574	242 ± 31.1	2.0 ± NC

NC = Not Calculated

Table from applicant

Table 43: Mean liver-gallbladder MK-5172 concentrations (μM[a]) in mice following 4-weeks of administration

Dose (mg/kg/day)	Time (hr)		
	4	8	24
20	123 ± 6.30	75.1 ± 9.20	3.31 ± 0.904
100	274 ± 29.0	294 ± 52.7	51.3 ± 20.4
200	340 ± 30.2	361 ± 24.6	169 ± 25.8
500	680 ± 57.2	444 ± 85.5	490 ± 47.0

[a]Assuming liver-gallbladder density of 1

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study Day 1 and for concentration only in Week 4. Uniformity samples were within an acceptable range (≤10%) and assay results were within an acceptable concentration range (±15%) except for the 2 mg/ml formulation on Day 1, which was only ~49% of expected (reevaluated on Day 3 and determined to be ~103% of expected). Formulation most likely inadvertently prepared from the 10 mg/ml formulation (instead of the 20 mg/ml formulation) and so low-dose animals were administered 10 mg/kg on Day 1 (instead of 20 mg/kg). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Study title: MK-5172: Three-Month Oral Range-Finding and Toxicokinetic Study in CD1 Mice

Study no.:	TT#11-6038
Study report location:	4.2.3.2
Conducting laboratory and location:	Laboratoires Merck Sharp & Dohme-Chibret Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	December 15, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-011D005 (spray-dried), L-002214070-010B009 (free acid) and ~50% & ~100%, respectively

Key Study Findings: The NOEL/NOAEL is 20/100 mg/kg/day ($AUC_{0\text{-}24\ hr}$= 8/451 μM·hr @ 13 weeks) for MK-5172 since adverse toxicological findings were observed at higher dose levels. The most predominant of these findings were mortality (two males at 500 mg/kg/day), renal tubule degeneration, hematological (decrease in RBCs, Hg & Hct) and hepatobiliary (increase liver/hepatocyte weight/size, ALT, ALP, bilirubin with gall bladder inflammation and cytoplasmic rarefaction in periportal region of the liver).

Reference ID: 3839341

Methods

Doses:	0, 20, 100, 200 and 500 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Spray-dried formulation containing 50% (w/w) free acid and 50% (w/w) hydroxypropylmethylcellulose phthalate (HPMCP) in 0.5% methylcellulose/0.02% sodium lauryl sulfate w/ 5 mM HCl in deionized water (pH=7.0) (vehicle control: 50 mg/ml HPMCP in 0.5% methylcellulose/0.02% sodium lauryl sulfate w/ 5 mM HCl in deionized water)
Species/Strain:	Mouse/CRL:CD1(ICR)
Number/Sex/Group:	10 (main), 11 (TK)
Age:	~6 weeks
Weight:	22.9-31.3 (♀) and 30.6-38.8 (♂) g
Satellite groups:	TK
Unique study design:	None
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality (1x/day): There were eight unscheduled deaths (1, 0, 2, 1 and 4 deaths in 0, 20, 100, 200 and 500 mg/kg/day groups, respectively). Six deaths were due clearly to intubation accidents (confirmed by pathology assessments). One male in the main study group administered 500 mg/kg/day was found dead on Day 15, with clinical signs just prior to death consisting of distended abdomen. The cause of death in this male was unclear (no significant gross or histopathology findings noted). In addition, one male in the TK group administered 500 mg/kg/day was found dead on Day 10, with the cause of death undetermined.

Clinical Signs (1x/day): No clear test-article related changes noted.

- Distended abdomen observed infrequently in 2/10 (♂) at 100 mg/kg/day and 1/10 (♀) & 3/10 (♂) at 500 mg/kg/day.

Body Weights (1x/week): Test-article related changes noted (refer to Applicant Figures below).

- ↓ mean BW gain @ 100 (-20% ♂), 200 (-17% ♂) and 500 mg/kg/day (-14% ♀, -42% ♂)

Reference ID: 3839341

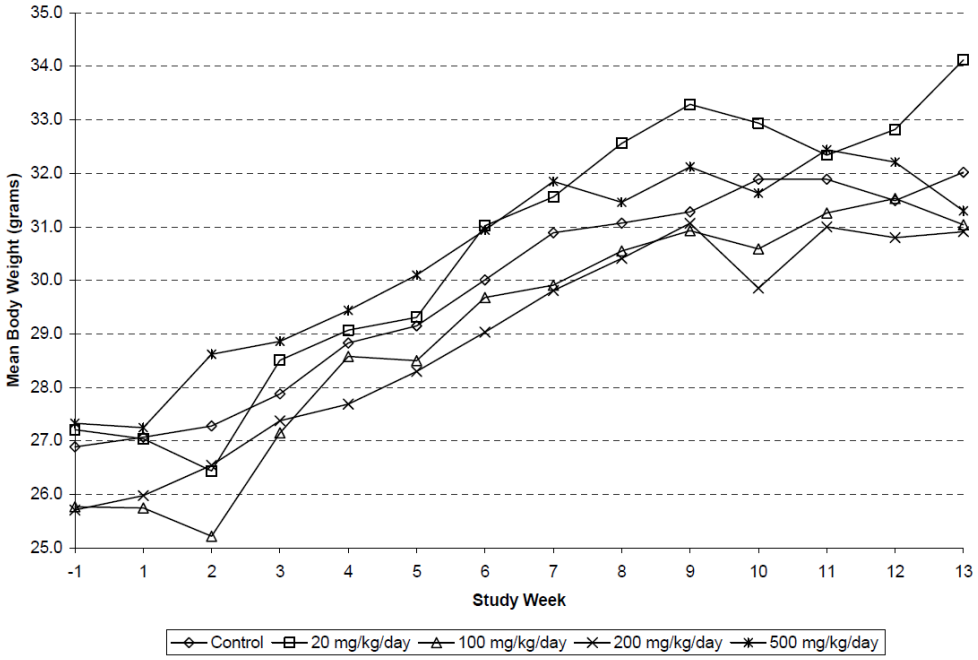

Figure 8: Summary of body weight changes in female mice administered MK-5172 for 13-weeks (from applicant)

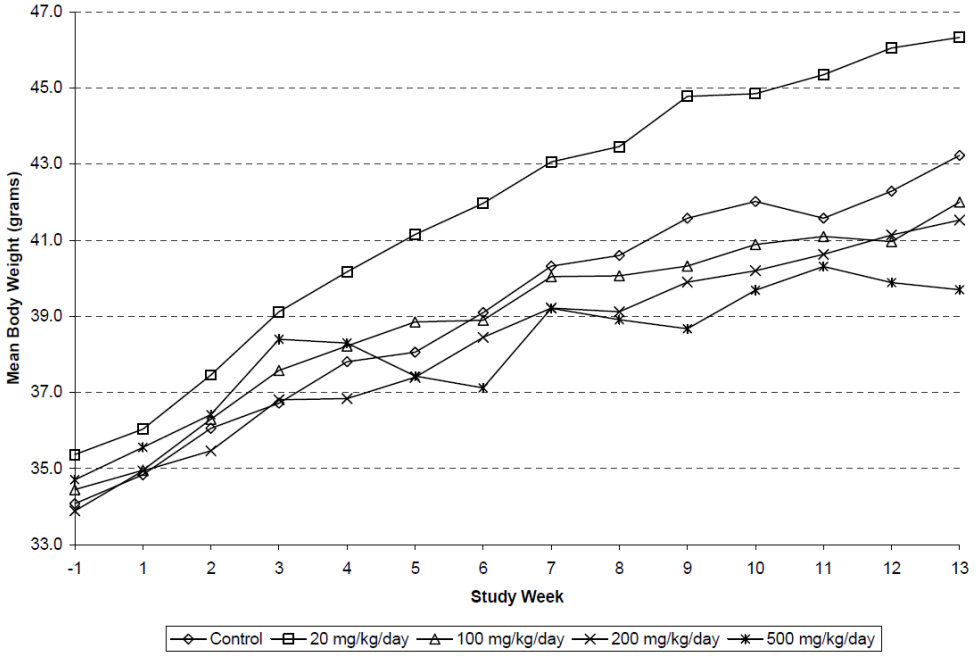

Figure 9: Summary of body weight changes in male mice administered MK-5172 for 13-weeks (from applicant)

Feed Consumption: Not evaluated (group housed)

Hematology: Test-article related changes noted (refer to Applicant Table below).

Table 44: Hematological findings in mice administered MK-5172 for 13-weeks

Parameter	MK-5172 (mg/kg/day)							
	Females				Males			
	20	100	200	500	20	100	200	500
RBC	-	-	-	-4	-	-	-	-8*
Hemoglobin	-	-	-	-14*	-	-	-	-20*
Hematocrit	-	-	-	-8*	-	-	-	-15*
MCV	-	-	-	-5*	-	-	-	-8*
MCH	-	-	-	-11*	-	-	-	-13*
MCHC	-	-	-	-7*	-	-	-	-6*
WBC	-	-	-	+52	-	-	-	-
Neutrophils	-	-	-	+96	-	-	-	-
Lymphocytes	-	-	-	+45	-	-	-	-

- = No test article-related change.
* = p-value ≤ 0.05 in Dunnett's multiple comparisons with the concurrent control group.
MCH = Mean corpuscular hemoglobin.
MCHC = Mean corpuscular hemoglobin concentration.
MCV = Mean corpuscular volume.
RBC = Red blood cell count.
WBC = White blood cell count.

Note: Values= % difference in mean values from control

Clinical Chemistry: Test-article related changes noted (refer to Applicant Table below).

Table 45: Clinical chemistry findings in mice administered MK-5172 for 13-weeks

Parameter	SW	MK-5172 (mg/kg/day)							
		Females				Males			
		20	100	200	500	20	100	200	500
Glucose	8	NE	NE	NE	NE	NE	NE	NE	NE
	12	NE	NE	NE	NE	NE	NE	NE	NE
	14	-	-	-	+21	-	-	+11	+18
ALT	8	-	-	-	-	-	-	-	+62*
	12	-	-	+81*	+91*	-	-	-	-
	14	-	-	-	-	-	-	-	+83*
ALP	8	-	-	-	-	-	-	+38*	+55*
	12	-	-	-	+46*	-	-	+54*	+70*
	14	-	-	+45*	+45	-	-	+54*	+65*
Cholesterol	8	-	-	-	-	-	-	-	-
	12	-	-	-	+46*	-	-	-	-
	14	-	-	-	+40*	-	-	-	-
Total Bilirubin	8	-	-	+100*	+1900*[a]	-	+100	+200*	+3100*[a]
	12	-	-	+200*	+2100*[a]	-	I[a]	+100*	+2100*[a]
	14	-	-	-	+400*[a]	-	-	+100*	+1000*[a]

[a] Direct bilirubin level was determined in animals with a total bilirubin level above or equal to 1.0 mg/dL. In these individuals, direct bilirubin was the main component of total bilirubin.
- = No test article-related change.
* = p-value ≤0.05 in Dunnett's multiple comparisons with the concurrent control group.
ALP = Alkaline phosphatase activities.
ALT = Alanine aminotransferase activity.
NE = Not Examined.
SW = Study Week.
I = Increased total bilirubin in a single male (#11-0052).

Note: Values= % difference in mean values from control

Urinalysis: Not evaluated

Gross Pathology:

- ↑ liver size (2 of 10) & prominent lobular pattern (3 of 10) at 500 mg/kg/day (♂)
- prominent lobular pattern (1 of 10) at 200 mg/kg/day (♂)
- ↑ liver size at 200 (1 of 10 ♀) and 500 mg/kg/day (4 of 10 ♀)
- ↓ thymus size at 20 (2 of 10 ♂), 200 (1 of 10 ♂) and 500 mg/kg/day (4 of 10 ♂)
- ↓ thymus size at 500 mg/kg/day (1 of 10 ♀)
- ↑ gallbladder size at 500 mg/kg/day (2 of 10 ♀) (also in 1 of 10 controls)

Organ Weights: Test-article related changes noted (refer to Applicant Table below).

Table 46: Summary of select organ weight changes and histopathology findings in mice administered MK-5172 for 13-weeks

	Females				Males			
Dose, mg/kg/day	20	100	200	500	20	100	200	500
Number of Animals	10	10	10	10	10	10	10	10
Organ Weights[a, b]								
Liver	-	-	+19*	+73*	-	-	+23*	+56*
Histomorphology (incidence)								
Kidney,	NE	NE			NE	NE		
Tubule, Epithelial Degeneration			-	3			-	6
Liver,	NE				NE			
Hepatocyte, increased size		-	2	8		-	10	9
Periportal region, Cytoplasmic rarefaction		-	1	7		-	-	-
Gallbladder	NE	NE			NE	NE		
Inflammation			-	1			-	1

[a] Values are the mean percent change from concurrent controls of organ weight relative to brain weight.
[b] Statistics performed by trend assessment by sexes separate; * = p ≤0.05.
- = No test article-related change.
NE = Not examined.

Histopathology: Note: Performed in control and HD only, except for adrenals, kidneys, liver and ovaries (examined in 200 mg/kg/day group) and liver (examined in 100 mg/kg/day group).

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: Test-article related changes noted (refer to Applicant Table above).

- very slight to marked degeneration of renal tubular epithelium "characterized mainly by tubular basophilia and dilatation with scattered epithelial single cell necrosis, with and without presence of cellular debris in dilated tubules" observed at 500 mg/kg/day. Findings were restricted to a few foci in subcapsular areas or in medullary rays for very slight cases, while the marked cases had involvement of large parts of the outer medulla.
- very slight to moderate increase in hepatocellular size at 200 and 500 mg/kg/day, accompanied by very slight to slight cytoplasmic rarefaction in the periportal

Reference ID: 3839341

region (♀) associated with large amounts of glycogen in periportal and centrilobular hepatocytes (confirmed by PAS staining and TEM). Increased hepatocellular size correlated with increased mean liver weights and/or increased organ size and/or prominent lobular pattern (♂) observed by gross pathology.

- very slight to slight focal inflammation of the gallbladder at 500 mg/kg/day, characterized by wall thickening due to neutrophilic cellular infiltration and/or fibroplasia and "overlying epithelial hyperplasia with superficial epithelial necrosis in contact with amorphous eosinophilic debris present in the lumen".
- potential "stress" associated findings at 500 mg/kg/day include:
  - very slight adrenal cortical hypertrophy (7 of 10 ♂) & (6 of 10 ♀)
  - very slight decrease in number of corpora lutea in the ovaries (6 of 10) (also in 1 & 2 of 10 in controls and at 200 mg/kg/day, respectively)
  - Although ovarian changes have been observed in some strains of mice upon food/calorie restriction [refer to Koizumi A. *et al*, 49:93-104 (1989)], the possibility that these changes are direct test-article related effects cannot be ruled out (given the lack of clinical signs and the fact that the decrease in body weight gain was mild).

Special Evaluation: None

Toxicokinetics: Note: Sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose at week 13 (3 mice/sex/timepoint). Liver collected at 4, 8 and 24 hours post-dose for MK-5172 concentration determination at week 13.

Table 47: Mean plasma MK-5172 TK parameters in mice following 13-weeks of administration

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
20	Female	9.02 ± 2.70	1.56 ± 0.647	0.50 ± NC
	Male	6.91 ± 1.67	1.47 ± 0.743	0.50 ± NC
	All	7.96 ± 1.54	1.52 ± 0.441	0.50 ± NC
100	Female	448 ± 116	51.6 ± 8.42	0.50 ± NC
	Male	455 ± 146	38.5 ± 12.3	1.0 ± NC
	All	451 ± 83.5	39.4 ± 8.13	1.0 ± NC
200	Female	957 ± 141	67.4 ± 13.8	8.0 ± NC
	Male	683 ± 165	50.9 ± 7.29	1.0 ± NC
	All	820 ± 109	56.3 ± 10.8	8.0 ± NC
500	Female	1550 ± 161	150 ± 41.8	2.0 ± NC
	Male	1390 ± 286	108 ± 26.7	2.0 ± NC
	All	1470 ± 173	129 ± 24.1	2.0 ± NC

NC = Not Calculated

Table from applicant

Table 48: Mean liver-gallbladder MK-5172 concentrations (μM[a]) in mice following 13-weeks of administration

Dose (mg/kg/day)	Time (hr)		
	4	8	24
20[b]	168 ± 14.7	93.6 ± 17.3	2.38 ± 0.828
100[b]	316 ± 34.3	389 ± 33.1	46.9 ± 22.7
200	453 ± 19.1	442 ± 30.8	67.2 ± 15.6
500	477 ± 32.6	482 ± 43.3	187 ± 29.5

[a]Assuming liver or liver-gallbladder density of 1

[b]At the 4-hour timepoint, only liver tissue was collected.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study Day 1 and for concentration only in Week 13. Uniformity samples were within an acceptable range (≤10%) and assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Study title: MK-5172: One month oral toxicity study in dogs

Study no.:	TT#08-1151
Study report location:	4.2.3.2
Conducting laboratory and location:	Merck Research Laboratories, West Point, PA, USA & Laboratoires Merck Sharp & Dohme-Chibret Route de Marsat, Riom, 63963 Clermont-Ferrand Cedex 9, France
Date of study initiation:	December 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z014 and 98.4%

Key Study Findings: The NOAEL is considered to be 20 & 600 mg/kg/day in males and females, respectively, ($AUC_{0\text{-}24\ hr}$= 429(♂) & 3,300(♀) μM·hr @ week 5) for MK-5172, since adverse testicular toxicity (consisting of minimal to mild tubular epithelial degeneration) was observed in all males at 600 mg/kg/day (the highest dose tested). Emesis, decreases in erythroid parameters and increases in total bilirubin were observed in both sexes at 600 mg/kg/day. A slight increase in bilirubin was also noted in one male at 20 mg/kg/day.

Reference ID: 3839341

Table 49: Mean plasma MK-5172 TK parameters in dogs following single or multiple dose administration for one month

	Male			Female		
Dose (mg/kg/day)	**5**	**20**	**600**	**5**	**20**	**600**
T_{max} (hr)						
Day 1	2.0 ± 0.0	3.3 ± 0.67	4.0 ± 0.0	2.0 ± 0.0	4.0 ± 0.0	4.2 ± 2.2
Week 5	2.0 ± 0.0	2.0 ± 0.0	4.0 ± 0.0	2.7 ± 0.67	3.3 ± 0.67	5.3 ± 1.3
C_{max} (μM) Day 1	11.1 ± 1.10	43.8 ± 5.51	110 ± 17.9	11.9 ± 1.22	41.8 ± 6.20	99.6 ± 41.5
Week 5	7.49 ± 0.220	39.3 ± 5.12	168 ± 16.5	10.1 ± 0.627	45.2 ± 10.8	178 ± 30.5
$AUC_{0\text{-}24h}$ (μM-hr)						
Day 1	60.5 ± 5.45	566 ± 87.7	1560 ± 329	76.3 ± 9.14	544 ± 77.5	1550 ± 698
Week 5	47.6 ± 5.22	429 ± 54.5	2560 ± 125	83.1 ± 2.40	564 ± 178	3300 ± 624

Study title: <u>MK-5172: Nine-month oral toxicity study in dogs with a 3-month interim necropsy</u>

Study no.: TT#10-6015
Study report location: 4.2.3.2
Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret
Route de Marsat, Riom,
63963 Clermont-Ferrand Cedex 9, France
Date of study initiation: April 23, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-5172, L-002214070-001Z015 (weeks 1-9 and 22-39) & L-002214070-001Z016 (weeks 9-22) and ≥99.0%

Key Study Findings: The <u>NOAEL</u> is <u>5 mg/kg/day</u> ($AUC_{0\text{-}24\ hr}$= 69.3 μM·hr @ week 12) for MK-5172, since adverse toxicological findings were observed including emesis, unformed/liquid/yellow feces, jaundice, body weight loss and mortality at 300 mg/kg/day. Additional adverse findings observed at 15 and/or 300/100 mg/kg/day included testicular (seminiferous tubule degeneration with decreased testes weight and sperm content in epididymis), hematological (decreased RBCs, Hg, Hct & mean corpuscular Hg & volume associated with increased hemosiderin content of spleen and liver, as well as increased reticulocytes, erythroid hyperplasia of bone marrow and increased extramedullary hematopoiesis in spleen) and hepatobiliary (increase liver weight, sporadic ALT, AST & ALP elevations, increased serum and urine bilirubin, distended gallbladder with abundant bile content, gallbladder wall thickening and microlithiasis of gall bladder and hepatic bile ducts).

Reference ID: 3839341

Methods

Doses:	0, 5, 15, or 300/100 mg/kg/day (dose lowered to 100 mg/kg on day 79)
Frequency of dosing:	Once daily
Route of administration:	Oral (b) (4)
Dose volume:	2 mL/kg (in 2 to 4 (b) (4)
Formulation/Vehicle:	100% polyethylene glycol 400
Species/Strain:	Beagle dogs
Number/Sex/Group:	4
Age:	39 to 54 weeks
Weight:	F: 7.1–11.5 kg; M: 8.5–11.9 kg
Satellite groups:	3 (3-month interim sacrifice at 5 mg/kg/day dose)
Unique study design:	No
Deviation from study protocol:	None that affected the interpretation of the study results

Observations and Results

Mortality (1x/day): One male and one female in the 300 mg/kg/day group were sacrificed in weeks 7 and 11, respectively, because of marked body weight loss (-2.3 kg/-21% and -1.8 kg/-22%, respectively) and persistent poor food consumption (even after canned food supplementation). These animals had decreased activity, coolness to touch, thinness and/or red ocular discoloration (also observed transiently in one other HD male). Thus, the 300 mg/kg/day dose was determined to exceed the MTD and was lowered to 100 mg/kg/day on study day 79 (week 12).

Findings in common with these early sacrificed animals included distended gallbladder with abundant bile content, very slight pigmentation (hemosiderin) of liver and/or bone marrow, and lymphoid depletion of spleen, thymus, Peyer's patches and/or lymph nodes considered secondary effects due to the poor condition of these animals prior to sacrifice. Findings observed only in the male included yellow discoloration of skin and eyes (bulbar conjunctiva), yellowish discoloration of mucosae, very slight focal inflammation of duodenum and pale flaccid testes associated with marked degeneration of seminiferous tubules (characterized by loss of spermatids and spermatozoa and marked decrease in sperm in epididymis).

Clinical Signs (1x/day):

- ↑ incidence and frequency of emesis (♂&♀) and unformed/liquid feces (♀) at 300 mg/kg/day.
- Occasional yellow discoloration of feces in all animals at 300 mg/kg/day.
- Above findings also present after dose reduction (to 100 mg/kg/day at 12 weeks)

Body Weights: Note: Measured pre-test, once/week in weeks 1-2 & 19-38 and twice/week in weeks 3-18.

Reference ID: 3839341

- BW loss in all females (-12 to -22%) and males (-6 to -21%) administered 300 mg/kg/day. Food supplementation occurred in all animals.
- BW loss (-12% @ week 24) in one male administered 100 mg/kg/day, but gained weight starting at week 24 (food supplementation from week 24-37).

Feed Consumption (1x/day):

- Unsatisfactory consumption occurred in all animals with BW loss.

Ophthalmoscopy: Note: Evaluated pre-test and in weeks 11, 26 and 38. No test-article related changes noted.

ECG: Note: Evaluated pre-test and 3 to 4 hours post-dose in weeks 11, 12, 25 and 37. No test-article related changes noted.

Hematology: Note: Evaluated pre-test and in weeks 3/4, 11, 16 (HD group only), 26 and 38. Test-article related changes noted (refer to Applicant Table below).

Table 50: Hematological findings in dogs administered MK-5172 for up to 39 weeks

Parameter	Study Week	MK-5172 (mg/kg/day)					
		Females			Males		
		5	15	300/100[a]	5	15	300/100[a]
Erythrocyte count	3/4[b]	-	-	D[c]	-	-	-15[c]
	11	-	-	D	-	-	-6
	16[d]	NE	NE	-	NE	NE	-
	26	-	-	-	-	-	-9
	38	-	-	-	-	-	+10
Nucleated erythrocytes	3/4[b]	-	-	-	-	-	-
	11	-	-	-	-	-	-
	16[d]	NE	NE	-	NE	NE	P[e]
	26	-	-	-	-	-	P[e]
	38	-	-	P[e]	-	-	P[e]

Absolute reticulocyte count	3/4[b]	-	-	-	-	-	-17[c]
	11	-	-	D	-	-	D
	16[d]	NE	NE	-	NE	NE	-
	26	-	-	-	-	-	+808[e]
	38	-	-	I	-	-	+312[e]
Hemoglobin concentration	3/4[b]	-	-	-16[c]	-	-	-18[c]
	11	-	-	-20	-	-	-24
	16[d]	NE	NE	-13[c]	NE	NE	-20[c]
	26	-	-	-18	-	-	-38
	38	-	-	-25	-	-	-24
Hematocrit	3/4[b]	-	-	D[c]	-	-	-19[c]
	11	-	-	-17	-	-	-19
	16[d]	NE	NE	-9[c]	NE	NE	-14[c]
	26	-	-	-13	-	-	-28
	38	-	-	-20	-	-	-18
Mean corpuscular volume	3/4[b]	-	-	-2[c]	-	-	-5[c]
	11	-	-	-12	-	-	-15
	16[d]	NE	NE	-10[c]	NE	NE	-15[c]
	26	-	-	-13	-	-	-18
	38	-	-	-17	-	-	-26
Mean corpuscular hemoglobin	3/4[b]	-	-	-4[c]	-	-	-3[c]
	11	-	-	-14	-	-	-20
	16[d]	NE	NE	-13[c]	NE	NE	-21[c]
	26	-	-	-17	-	-	-31
	38	-	-	-21	-	-	-31
Mean corpuscular hemoglobin concentration	3/4[b]	-	-	-	-	-	-
	11	-	-	-3	-	-	-5
	16[d]	NE	NE	-3[c]	NE	NE	-7[c]
	26	-	-	-5	-	-	-15
	38	-	-	-5	-	-	-7
Fibrinogen	3/4[b]	-	-	I[c]	-	-	+49[c]
	11	-	-	I	-	-	I
	16[d]	NE	NE	I[c]	NE	NE	-
	26	-	-	-	-	-	-
	38	-	-	-	-	-	-
Platelet count	3/4[b]	-	-	-	-	-	+37[c]
	11	-	-	+52	-	-	+66
	16[d]	NE	NE	+58[c]	NE	NE	+77[c]
	26	-	-	+82	-	-	+158
	38	-	-	+48	-	-	+96

a Due to unscheduled deaths, the high dose was lowered from 300 mg/kg/day to 100 mg/kg/day in Study Week 12.
b Due to early antemortem findings, examination of the high-dose group was brought forward from Study Week 4 to Study Week 3.
c Change relative to mean or individual pretest value (no concurrent control value available in Study Weeks 3 and 16).
d Examination performed only on the high-dose group, approximately 1 month after the lowering of the dose level.
e Nucleated erythrocytes were observed notably in male #10-0004 in Study Week 26 and male #10-0012 in Study Week 38 and, to a lesser extent, in male #10-0012 in Study Week 16 and female #09-0193 in Study Week 38.
- = No test article-related change.
D/I = Individual decrease/increase in females #09-0193, #10-0003 (early sacrificed in Study Week 11) or #10-0013, and male #10-0012.
NE = Group not examined.
P = Present.

Table from applicant

Clinical Chemistry: Note: Evaluated pre-test and in weeks 3/4, 11, 16 (HD group only), 26 and 38. Test-article related changes noted (refer to Applicant Table below).

- ↑ AST (3.7-fold) and ALT (4.6-fold) at week 3 in early sacrificed female
- ↑ ALT (~11-fold) in 1 of 3 females administered 300/100 mg/kg/day at week 26 only

Table 51: Clinical chemistry findings in dogs administered MK-5172 for up to 39 weeks

Parameter	Study Week	MK-5172 (mg/kg/day)					
		Females			Males		
		5	15	300/100[a]	5	15	300/100[a]
Total bilirubin	3/4[b]	-	I	+400[c]	-	-	+800[c]
	11	-	+200	+600	-	-	+1000
	16[d]	NE	NE	+200[c]	NE	NE	+300[c]
	26	-	-	I	-	I	+400
	38	-	+50	+200	-	I	+600
Alkaline phosphatase	3/4[b]	-	-	-	-	-	I[c]
	11	-	-	-	-	-	+222
	16[d]	NE	NE	I[c]	NE	NE	+109[c]
	26	-	-	-	-	-	+256
	38	-	-	-	-	-	+214
Cholesterol	3/4[b]	-	-	D[c]	-	-	-49[c]
	11	-	-	D	-	-	-
	16[d]	NE	NE	-	NE	NE	D[c]
	26	-	-	-	-	-	-
	38	-	-	-	-	-	-

[a] Due to unscheduled deaths, the high dose was lowered from 300 mg/kg/day to 100 mg/kg/day in Study Week 12.
[b] Due to early antemortem findings, examination of the high-dose group was brought forward from Study Week 4 to Study Week 3.
[c] Change relative to mean or individual pretest value (no concurrent control value available in Study Weeks 3 and 16).
[d] Examination performed only on the high-dose group, approximately 1 month after the lowering of the dose level.
- = No test article-related change.
D/I = Individual decreases/increases.
NE = Group not examined.

Table from applicant

Urinalysis: Note: Evaluated in weeks 11, 26 and 38/39.

- ↑ urine bilirubin at 300 mg/kg/day at week 11 (1 of 3 ♀ & 3 of 3 ♂) and at 300/100 mg/kg/day at week 38/39 (1 of 3 ♂)

Gross Pathology

- distended gallbladder with abundant bile content in all animals administered 300/100 mg/kg/day
- gallbladder wall thickened (1 of 3 ♀ & 2 of 3 ♂) at 300/100 mg/kg/day

Reference ID: 3839341

- yellowish discoloration of gingival mucosae, white adipose tissue (2 of 3 ♂) and aorta (1 of 3 ♂) administered 300/100 mg/kg/day

Organ Weights: Test-article related changes noted (refer to Applicant Table below).

- ↑ heart weight (~18% ♀ & 22% ♂) at 300/100 mg/kg/day

Table 52: Summary of select organ weight changes and histopathology findings in dogs administered MK-5172 for 39-weeks

	Females			Males		
Dose, mg/kg/day	5	15	300/100	5	15	300/100
Number of Animals	4	4	3	4	4	3
Organ Weights[a]						
Liver[b]	-	-	+42.5*	-	-	+40.3*
Testis[b]	NA	NA	NA	-	-14.5	-33.9*
Histomorphology (Incidence)						
Liver,						
Pigmentation	-	-	1	-	-	3
Bile duct, microlithiasis	-	1	1	-	-	-
Gallbladder						
Microlithiasis	-	1	3	-	-	1
Spleen	NE			NE		
Extramedullary hematopoiesis, increased		-	1		-	1
Pigmentation, increased		-	-		-	2
Bone marrow	NE			NE		
Hyperplasia, erythroid		-	-		-	1
Testis	NA	NA	NA			
Seminiferous tubule degeneration				-	1	3
Epididymis	NA	NA	NA	NE		
Sperm, decreased amount					-	3

[a] Values are the mean percent change from concurrent controls of organ weight relative to brain weight.
[b] Statistics performed sexes combined by Dunnett's Pairwise Comparison Test * = p ≤0.05.
- = No noteworthy change.
NE = Group not examined.
NA= Not Applicable

Table from applicant

Histopathology

Adequate Battery: yes

Peer Review: yes

Histological Findings: Test-article related changes noted (refer to Applicant Table above).

- very slight to slight pigmentation (hemosiderin) of spleen and sinusoidal cells in liver
- very slight microlithiasis in the liver noted in larger bile ducts consisting of finely granular basophilic material in lumen
- very slight microlithiasis in the lumen of gallbladder

- slight erythroid hyperplasia in bone marrow
- very slight (15 mg/kg/day) and moderate to marked (300/100 mg/kg/day) seminiferous tubule degeneration of testes correlating with reduced testicular weights at both dose levels and with decreased amount of sperm in epididymis at the high dose. Testicular change at the high dose was wide-spread and characterized by "loss of spermatids and spermatozoa with intraluminal degenerated and frequently multinucleated spermatogenic epithelial cells".

Special Evaluation: none

Toxicokinetics

Table 53: Mean plasma MK-5172 TK parameters in dogs following 12-weeks of administration in the 9-month study

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
5	Female	69.8 ± 9.45	11.2 ± 1.36	1.6 ± 0.20
	Male	68.9 ± 8.17	10.6 ± 0.917	1.7 ± 0.18
	All	69.3 ± 6.00	10.9 ± 0.793	1.6 ± 0.13
15	Female	431 ± 80.0	42.1 ± 6.81	2.5 ± 0.50
	Male	303 ± 103	26.7 ± 9.34	2.6 ± 0.85
	All	367 ± 65.0	34.4 ± 6.09	2.6 ± 0.46
300	Female	3340 ± 1610	200 ± 92.8	5.3 ± 1.3
	Male	1900 ± 142	126 ± 6.11	5.3 ± 1.3
	All	2620 ± 792	163 ± 44.8	5.3 ± 0.84

MK-5172 concentrations below lower limit of quantitation in control plasma samples.

Table from applicant

Table 54: Mean plasma MK-5172 TK parameters in dogs following 26-weeks of administration in the 9-month study

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
100[a]	Female	2420 ± 771	150 ± 38.9	5.3 ± 1.3
	Male	2170 ± 893	133 ± 42.8	4.0 ± 0.0
	All	2300 ± 531	142 ± 26.2	4.7 ± 0.67

[a] The original dose (300 mg/kg/day) was lowered to 100 mg/kg/day from Study Day 79 (Study Week 12).

Table from applicant

Table 55: Mean liver MK-5172 concentrations (μM[a]) in dogs following 39-weeks of administration

Dose (mg/kg/day)	Time (hr) Necropsy
5	6.14 ± 1.27
15	39.3 ± 5.23
100[b]	136 ± 26.1

[a] Assuming liver density of 1
[b] The original dose (300 mg/kg/day) was lowered to 100 mg/kg/day from Study Day 79 (Study Week 12).
All MK-5172 concentrations in liver from control group animals were below the lower limit of quantitation (LLQ = 0.104 μM).

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study weeks 1, 9 and 12 (HD group only) and for concentration only in week 3/4, 12, 16 (HD group only), 26 and 39. Uniformity samples were within an acceptable range (≤10%) and assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

7 Genetic Toxicology

Note: The following GZR (MK-5172) study reviews were taken directly from the original IND-110,261 review by Dr. Ita Yuen.

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

Study title: Microbial Mutagenesis Assay

Study no.:	TT #10-8239 & 11-8060
Study report location:	4.2.3.3.1
Conducting laboratory and location:	For 11-8060: Merck Research Laboratories Merck & Co., Inc. West Point, Pennsylvania 19486 U.S.A. For 10-8239: (b) (4) [redacted]
Date of study initiation:	October 25, 2010 (10-8239) & June 16, 2011 (11-8060)
GLP compliance:	Yes (11-8060 only)
QA statement:	Yes (11-8060 only)
Drug, lot #, and % purity:	MK-8742, L-002469825-000G010 and 97.7% (for 11-8060); L-002469825-000G006 and 97.8% (for 10-8239)

Key Study Findings: MK-8742 was evaluated for mutagenic potential in a microbial mutagenesis test system using mutant strains of *Salmonella typhimurium* (TA1535, TA97a, TA98, and TA100) and *Escherichia coli* (WP2 uvrA pKM101) treated for 48 hours. The final concentrations tested were 100, 300, 1000, 3000 and 5000 μg/plate with and without S-9 metabolic activation. Precipitate was seen on plates at test concentrations of 1000 µg/plate and higher, but did not interfere with scoring of the plates. No inhibition of bacterial lawn was noted at any concentration tested; however, inhibition of revertant growth was noted at the highest concentrations tested in some test strains. MK-8742 did not produce any 2-fold or greater increases in revertants relative to solvent control (DMSO) in these studies, while the positive control (2-aminoanthracene) and diagnostic mutagens (sodium azide, 4-nitroquinoline-N-oxide, 2-nitrofluorene & ICR-191) showed appropriate S-9- and strain-dependent increases in revertants.

Study title: Microbial Mutagenesis Assay

Study no.:	TT#09-8000 & TT#09-8011
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Merck Research Laboratories West Point, PA
Date of study initiation:	January 6 & 27, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z014 and

Reference ID: 3839341

98.4%

Key Study Findings: MK-5172 was considered negative in the bacterial reverse mutation assay at the concentrations up to 5000 μg/plate.

Methods

Strains:	*Salmonella*: TA97a, TA98, TA100, & TA1535 *E. coli*: WP2 uvrA
Concentrations in definitive study:	30 (in one assay only), 100, 300, 1000, 3000, & 5000 μg/plate
Basis of concentration selection:	Precipitation seen at concentrations ≥3000 μg/plate without interfering with scoring
Negative control:	DMSO
Positive control:	In the presence of S-9 activation: 2-aminoanthracene: 1 & 2 μg/plate with S-9 activation for TA98; 2 & 5 μg/plate for WP2 uvrA In the absence of S9 activation: 1 μg/plate 2-nitrofluorene for TA98 0.75 μg/plate sodium azide for TA100 & TA1535 1 μg/plate 4-nitroquinoline-N-oxide for WP2*uvr*A 1.5 μg/plate ICR-191 for TA97a
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 hours

Study Validity

All doses were evaluated in triplicate plates. All positive and negative control induced revertant frequencies that were within the preset values.

Results

MK-5172 was considered negative under the assay conditions.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Assay for Chromosomal Aberrations In Vitro in Chinese Hamster Ovary Cells

Study no.:	TT #11-8536
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Merck Research Laboratories Merck & Co., Inc. West Point, Pennsylvania 19486 U.S.A.
Date of study initiation:	October 26, 2011
GLP compliance:	Yes

Reference ID: 3839341

QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G010 and 97.7%

Key Study Findings: MK-8742 was evaluated for its potential to cause chromosomal aberrations in Chinese hamster ovary (CHO) cells. Precipitate was found at the highest MK-8742 concentration tested (40 µM) and so dose levels for the chromosomal aberration assay were limited by test article solubility. The treatment levels of MK-8742 scored for aberrations were 10, 20, and 30 µM with and without S-9 (3- and 20-hour treatments). Cell growth at 20 hours was 98, 83, and 95% of concurrent solvent controls, respectively. The pos tive controls (cyclophosphamide and mitomycin C) induced significant increases in aberrations over the solvent controls (DMSO). There was no significant increase in structural chromosome aberrations in MK-8742 treated cells and so the assay was negative.

Study title: Assay for Chromosomal Aberrations In Vitro in Chinese Hamster Ovary Cells

Study no.:	TT#08-8686 and TT#09-8600
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Merck Research Laboratories, West Point, PA
Date of study initiation:	January 5 & 9, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z014 and 98.4%

Key Study Findings: MK-5172 was considered negative for potential to induce chromosomal aberration in the mammalian cells.

Methods

Cell line:	CHO cells
Concentrations in definitive study:	5 (in the presence of S-9 activation only), 10, 20, 25, 30, 40, 50, 60, & 80 (in the absence of S-9 activation only) µM
Basis of concentration selection:	No viable cells observed at concentrations ≥125 µM with S-9 activation and ≥62.5 µM without S-9 activation
Negative control:	DMSO
Positive control:	5 & 10 µM cyclophosphamide with S-9 activation 0.5 & 1.5 µM mitomycin C without S-9 activation
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 and 20 hours (without S-9 activation only)

Reference ID: 3839341

Study Validity

MK-5172 was cytotoxic at doses ≥50 and 25 μM, respectively, in the presence and absence of S-9 activation for 3-hour treatment and ≥20 μM in the absence of S-9 activation for 20-hour treatment. The positive controls induced significant increases in aberrations over the concurrent negative controls.

Results

MK-5172 was considered negative under the assay conditions.

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

Note: Refer to study TT#11-6014 in Section 6.2 for MK-8742 data summary.

Study title: Assay for micronucleus induction in rat bone marrow from a 1-month oral toxicity study with a 2-week interim necropsy

Study no:	TT#09-8734
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Merck Research Laboratories, West Point, PA
Date of study initiation:	June 16, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, not provided

Key Study Findings: MK-5172 was negative for micronucleus induction in rat bone marrow at oral doses up to 200 mg/kg/b.i.d.

Methods

Doses in definitive study:	0, 50 mg/kg/day, or 200 mg/kg/b.i.d. (10/sex/dose) for 2 weeks 0, 50, 200 mg/kg/day, or 200 mg/kg/b.i.d. (10/sex/dose) for one month
Frequency of dosing:	Once or twice daily
Route of administration:	Oral gavage
Dose volume:	2 ml/kg
Formulation/Vehicle:	100% PEG 400
Species/Strain:	Wistar Hanover rats
Number/Sex/Group:	10 or 20
Satellite groups:	None
Basis of dose selection:	Doses were those used in the one-month general toxicity study in rats whose bone marrow cells were used for this study
Negative control:	100 % PEG 400
Positive control:	0.6 and 1.6 mg/kg mitomycin C

Reference ID: 3839341

Study Validity

The mean incidences of MN-PCE for the vehicle control were within the range for historical vehicle controls. The high dose positive control, 1.6 mg mitomyocin, induced marked increases in micronuclated PCE.

Results

MK-5172, at the tested doses up to 200 mg/kg/b.i.d., was negative for micronucleus induction in bone marrow in rats.

7.4 Other Genetic Toxicity Studies

Multiple exploratory microbial mutagenesis assays were conducted with EBR and GZR drug substance-related impurities. Refer to Dr. Mark Powley's pharmacology/ toxicology review for details.

8 Carcinogenicity

Given the intended treatment duration (<6 months) and lack of a specific cause for concern, studies to evaluate the carcinogenic potential of EBR or GZR are not being conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Oral Fertility Study in Female and Male Rats

Study no.:	TT#13-7290
Study report location:	4.2.3.5.1
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	July, 7, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G018 and 99.2%

Key Study Findings: Potential MK-8742 related effects on fertility were evaluated in male and female rats (20/sex/group) administered oral doses of 50, 300, 1000 mg/kg/day or vehicle (10% polysorbate 80 in deionized water) control, beginning 15 days before cohabitation. The NOEL for female and male fertility parameters was ≥1000 and 300 mg/kg/day, respectively. Although a slight decrease (~15%) in sperm count was observed at 1000 mg/kg/day, no effects on testicular weight, sperm motility and morphology or male fertility parameters were noted. MK-8742-related effects consisted of transient decreases in food consumption in females and males at 300 and 1000 mg/kg/day, with effects on body weight gain only observed in males.

Reference ID: 3839341

Methods

Doses:	0, 50, 300 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	20
Age:	~9 (♀) and 11 (♂) weeks
Weight:	180 to 223 (♀) and 281 to 343 (♂) g
Satellite groups:	none
Study design:	Males and females treated once daily beginning 15 days before cohabitation, during cohabitation and continuing through GD7 (♀) or the day prior to euthanasia (6 weeks total in ♂). Females euthanized on GD15 to 17.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: None

Clinical Signs (2x/day): According to the summary information provided, no test-article related changes noted. Clinical evaluation data from individual or groups of animals was not provided.

Body Weight (2x/week ♂): Note: Females measured pre-mating day (PMD) 1, 4, 8, 11 & 15, during cohabitation on PMD 22, 25 & 29 and GD 0, 2, 4, 6, 8, 12 and 15.

- mean BW gain ↓29 and 33% from week 1-3 at 300 and 1000 mg/kg/day (♂)

Feed Consumption: Note: Females: measured over 3-day intervals ending on PMD 4 & 11 and GD 5 & 12. Males: measured over 3-day intervals during weeks 1, 2 and 6.

- mean consumption ↓11 and 16% at 300 and 1000 mg/kg/day at interval ending on PMD4 (♀)
- mean consumption ↓16 and 18% in week 1 and ↓9 and 10% in week 2 at 300 and 1000 mg/kg/day, respectively (♂)

Toxicokinetics: not evaluated

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study week 1 and for concentration only in week 6. Uniformity samples were within an acceptable range (≤10%) and assay results were within an acceptable concentration range (±15%) except for the 10 mg/ml formulation on week 6,

Reference ID: 3839341

which was only ~79% of expected. Thus, the 50 mg/kg/day dose group may have been under-dosed slightly (~40 mg/kg/day). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Necropsy

Gross pathology: No test-article related changes noted.

Organ weights: No test-article related changes on testicular weight noted.

Histopathology: not conducted

Spermatological parameters (viability, motility & morphology):

- 14 & 16%↓ in sperm count/gram cauda epididymis & sperm count/cauda epididymis, respectively, at 1000 mg/kg/day vs control (refer to Applicant Table below)
- Although mean values in the 1000 mg/kg/day group are outside of the historical control data range provided, the concurrent control values are similar to the lowest control values seen previously (refer to Applicant Table below). Findings are statistically significant (p=0.0030 in two tailed t-test) and appear to be a minor test-article related effect given the potential dose/exposure relationship observed.

Table 56: Summary of cauda epididymal sperm count and vas deferens sperm motility in rats administered MK-8742

	Control	50 mg/kg/day	300 mg/kg/day	1000 mg/kg/day M/ Untreated F
SPERM COUNT / CAUDA EPIDIDYMIS (× 10^6) ± S.D.	244.2 ± 38.2 (20)	241.3 ± 51.0 (20)	243.6 ± 52.2 (20)	206.0 ± 38.1 (20)
SPERM COUNT / GRAM CAUDA EPIDIDYMIS (× 10^6) ± S.D.	814.0 ± 114.8 (19)	777.3 ± 159.0 (20)	789.1 ± 127.2 (20)	701.2 ± 143.9 (20)
% SPERM MOTILITY ± S.D.	86.8 ± 4.2 (19)	85.7 ± 7.2 (20)	85.1 ± 9.6 (20)	87.1 ± 6.3 (20)

VALUES ARE MEANS ± S.D.
(N) = GROUP SIZE. SEE INDIVIDUAL TABLE FOR EXCLUSIONS.
NE = NOT EVALUATED

Table 57: Historical control data (2007-2012) for sperm assessment in Wistar Hannover rats

Sperm Parameter	Studies	Mean	SD	50.0 Percentile	Lowest value	Highest value
Age at Necropsy (weeks)	11	20	3.4	18	17	24
Sperm Count / Cauda Epididymis (× 10^6)	11	263	10.1	267	243	279
Sperm Count / Gram Cauda Epididymis (× 10^6)	11	861	46.7	844	814	959
% Sperm Motility	11	89	5.1	90	74	91

Mating performance and fertility parameters (mating/fecundity/fertility index): No test-article related changes noted.

Estrus cycle determination and mating (time to insemination): Note: Estrus cycle not evaluated. No test-article related changes in insemination time noted.

C-section (@ 15-17d):

Pregnancy status: No test-article related changes noted (refer to Table below). Note: Treated high-dose males and females were mated to untreated animals, while the lower dose groups were both administered MK-8742.

Table 58: Pregnancy status of rats administered MK-8742 or vehicle control

Control	50 mg/kg	300 mg/kg	1000 mg/kg(♀)	1000 mg/kg(♂)
18 of 20	19 of 20	17 of 20	19 of 20	18 of 20

Corpora lutea: No test-article related changes noted.
Implantation sites: No test-article related changes noted.
Pre-implantation loss: No test-article related changes noted.
Post-implantation loss: No test-article related changes noted.
Early intrauterine deaths: No test-article related changes noted.
Late intrauterine deaths: No test-article related changes noted.
Dead embryos: No test-article related changes noted.
Viable embryos: No test-article related changes noted.

Study title: Oral Fertility Study in Female and Male Rats

Study no.:	TT#12-7260
Study report location:	4.2.3.5.1
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	June 7, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z016 and 99.0%

Key Study Findings: Potential MK-5172-related effects on fertility were evaluated in male and female rats (20/sex/group) administered oral doses of 50, 200, 400 mg/kg/day or vehicle (100% PEG 400) control, beginning 15 days before cohabitation. The NOEL for female and male fertility parameters was ≥400 mg/kg/day, since no significant MK-5172-related effects were observed.

Methods

Doses:	0, 50, 200 q.d. and 200 b.i.d. mg/kg
Frequency of dosing:	Once or twice daily
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	100% PEG 400 in deionized water
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	20
Age:	~10 (♀) and 12 (♂) weeks
Weight:	173 to 241 (♀) and 299 to 375 (♂) g
Satellite groups:	none
Study design:	Males and females treated beginning 15 days before cohabitation, during cohabitation and continuing through GD7 (♀) or the day prior to euthanasia (6 weeks total in ♂). Females euthanized on GD15 to 17.
Deviation from study protocol:	None

Observations and Results

Mortality: None

Clinical Signs (2x/day):

- According to the summary information provided, vehicle-related increases in unformed feces were observed with b.i.d. dosing, with incidence increased with dosing duration. Clinical evaluation data from individual or groups of animals was not provided.

Body Weight (2x/week ♂): Note: Females measured pre-mating day (PMD) 1, 4, 8, 11 & 15, during cohabitation on PMD 22, 25 & 29 and GD 0, 2, 4, 6, 8, 12 and 15. No test-article related changes noted.

Feed Consumption: Note: Females: measured over 3-day intervals ending on PMD 4 & 11 and GD 4 & 11. Males: measured over 3-day intervals during weeks 1, 2 and 6. No test-article related changes noted.

Toxicokinetics: not evaluated

Dosing Solution Analysis: All dosing formulations were analyzed for concentration on Study week 1 and 6. Assay results were within an acceptable concentration range (±10%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Necropsy

Reference ID: 3839341

Gross pathology: No test-article related changes noted.

Organ weights: No test-article related changes on testicular weight noted.

Histopathology: not conducted

Spermatological parameters (viability, motility & morphology): No test-article related changes noted.

Mating performance and fertility parameters (mating/fecundity/fertility index): No test-article related changes noted.

Estrus cycle determination and mating (time to insemination): Note: Estrus cycle not evaluated. No test-article related changes in insemination time noted.

C-section (@ 15-17d):

Pregnancy status: No test-article related changes noted (refer to Table below).
Note: Treated high-dose males and females were mated to untreated animals, while the lower dose groups were both administered MK-5172.

Table 59: Pregnancy status of rats administered MK-5172 or vehicle control

Control	50 mg/kg	200 mg/kg	200 mg/kg b.i.d. (♀)	200 mg/kg b.i.d. (♂)
17 of 20	18 of 20	17 of 20	17 of 20	19 of 20

Corpora lutea: No test-article related changes noted.
Implantation sites: No test-article related changes noted.
Pre-implantation loss: No test-article related changes noted.
Post-implantation loss: No test-article related changes noted.
Early intrauterine deaths: No test-article related changes noted.
Late intrauterine deaths: No test-article related changes noted.
Dead embryos: No test-article related changes noted.
Viable embryos: No test-article related changes noted.

9.2 Embryonic Fetal Development

9.2.1 Elbasvir

Exploratory embryo-fetal developmental studies with MK-8742 were conducted in rats (Study# TT#12-7175) and rabbits (Study# TT#12-7185) as summarized briefly below. Time-mated WI(HAN) rats and Dutch belted rabbits (6 presumed pregnant females/group) received 30, 100, 300, or 1000 mg/kg/day MK-8742 or vehicle (10% polysorbate 80 in deionized water) from GD 6 (rats) or 7 (rabbits) through 20. Assessment of maternal and developmental toxicity was based on mortality, physical signs, body weights and food consumption and embryonic/fetal viability, fetal weights, sex ratios (rats only) and external fetal morphology at GD 21 (rats) or 28 (rabbits),

Reference ID: 3839341

respectively. No evidence of maternal or developmental toxicity was noted in rats or rabbits at up to the limit dose of 1000 mg/kg/day.

Study title: Oral Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rats

Study no.:	TT #12-7170
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	June 13, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G018 and 99.2%

Key Study Findings: Potential MK-8742 related effects on embryo-fetal development (EFD) were evaluated in pregnant rats (28/group) administered oral doses of 50, 300, 1000 mg/kg/day or vehicle (10% polysorbate 80 in deionized water) control from GD 6 to 20. The NOEL for maternal and developmental toxicity was 300 mg/kg/day and ≥1000 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$= 21.8 μM·hr @ GD 15), respectively. MK-8742-related maternal effects consisted of slight decreases in body weight gain at 1000 mg/kg/day.

Methods

Doses:	0, 50, 300 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	28 (24 control)
Age:	~10 weeks
Weight:	172 to 266 g
Satellite groups:	TK
Study design:	Females treated once daily from GD 6 to 20 and euthanized at GD 21. Potential for skeletal malformations/variations assessed by both Alizarin red staining and micro-CT scanning.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: none

Reference ID: 3839341

Clinical Signs (1x/day): No test-article related changes noted.

Body Weight: Note: Measured on GD 6, 8, 10, 12, 16, 18, 20 and 21.

- ↓13% in mean maternal BW gain from GD 6 to 21 at 1000 mg/kg/day
- ↓19% in mean maternal BW gain from GD 6 to 21 at 1000 mg/kg/day (after adjusting for total fetal weight)

Feed Consumption: Note: Measured over 2-day intervals ending on GD 8, 14 and 20. No test-article related changes noted.

Toxicokinetics: Note: Sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose on GD 15 (3 rats/timepoint).

Table 60: Mean maternal plasma MK-8742 TK parameters in rats at gestation day 15 following 10 days of administration

Dose (mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
50	4.85 ± 0.957	0.932 ± 0.412	2.0 ± NC
300	11.4 ± 2.47	1.12 ± 0.229	2.0 ± NC
1000	21.8 ± 1.79	1.45 ± 0.144	8.0 ± NC

NC = Not Calculated

MK-8742 concentrations in maternal plasma from all control animals at 4 hours postdose were below the lower limit of quantitation (LLQ = 0.023 µM) of the bioanalytical method.

TT #12-7170: Oral Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rats.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study week 1 and for concentration only on GD 15. Assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Necropsy: Note: Only thoracic and abdominal viscera evaluated. No test-article related changes noted.

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

Reference ID: 3839341

Table 61: Cesarean section summary of rats administered MK-8742

Treatment Group	Control	50 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Mated Females	20	20	20	20
Pregnant	20	19	19	20
Examined Live Litter	20	19	19	20
Resorbed or Dead Litter	0	0	0	0
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Not Pregnant	0	1	1	0
Live	0	1	1	0
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Corpora Lutea	233	223	239	247
Corpora Lutea/Pregnant Female ± SD	11.7 ± 1.4	11.7 ± 1.4	12.6 ± 1.5	12.4 ± 1.6
% Peri-Implantation Loss, Litter Mean ± SD	11.5 ±20.1	12.1 ±19.9	13.2 ±16.7	19.7 ±23.6
Implants	209	193	208	197
Implants/Pregnant Female ± SD	10.5 ± 2.8	10.2 ± 2.1	10.9 ± 2.6	9.9 ± 3.0
Resorptions	11	6	10	11
% Resorptions/Implants, Litter Mean ± SD	5.1 ±11.0	3.1 ± 6.0	4.7 ± 7.2	5.4 ±11.4
Dead Fetuses	0	0	0	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0	0.0	0.0
% Postimplantation Loss, Litter Mean ± SD	5.1 ±11.0	3.1 ± 6.0	4.7 ± 7.2	5.4 ±11.4
Live Fetuses	198	187	198	186
Females	92	105	109	93
Males	106	82	89	93
Sex Ratio, Litter Mean ± SD	0.48 ± 0.19	0.56 ± 0.15	0.56 ± 0.13	0.52 ± 0.15
Live Fetuses/Pregnant Female ± SD	9.9 ± 3.0	9.8 ± 2.2	10.4 ± 2.6	9.3 ± 3.1
Live Fetal Weight (g), Litter Mean ± SD				
Females	4.84 ± 0.45	4.93 ± 0.47	4.92 ± 0.27	4.88 ± 0.43
Males	5.12 ± 0.47	5.15 ± 0.51	5.31 ± 0.30	5.13 ± 0.45

% Peri-Implantation Loss = ([No. Corpora Lutea - No. Implants] / No. Corpora Lutea) X 100.
% Postimplantation Loss = ([No. Resorptions + No. Dead Fetuses] / No. Implants) X 100.
Sex Ratio = Total No. Live Female Fetuses / (Total No. Live Female Fetuses + Total No. Live Male Fetuses).

Table from applicant

Placental morphology: No test-article related changes noted.

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

Study title: Oral Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rabbits

Study no.:	TT #12-7180
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	June 8, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G018 and 99.2%

Key Study Findings: Potential MK-8742 related effects on embryo-fetal development (EFD) were evaluated in pregnant rabbits (23/group) administered oral doses of 30, 100, 1000 mg/kg/day or vehicle (10% polysorbate 80 in deionized water) control from

Reference ID: 3839341

GD 7 to 20. The NOEL for maternal and developmental toxicity was ≥1000 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$= 39.4 μM·hr @ GD 15).

Methods

Doses:	0, 30, 100 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Dutch Belted rabbits
Number/Sex/Group:	23
Age:	26-29 weeks
Weight:	1642 to 2283 g
Satellite groups:	TK
Study design:	Females treated once daily from GD 7 to 20 and euthanized at GD 28.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: none

Clinical Signs (1x/day): No test-article related changes noted.

Body Weight: Note: Measured on GD 7, 9, 11, 13, 15, 17, 19, 21, 24 and 28. No clear (statistically significant) test-article related changes noted.

- ↓11-12% in mean maternal BW gain from GD 7 to 21 at 100 and 1000 mg/kg/day (191±55 in control vs 168±29 & 170±37g at 100 & 1000 mg/kg/day, respectively)
- ↓16-17% in mean maternal BW gain from GD 6 to 21 at 30, 100 and 1000 mg/kg/day (after adjusting for total fetal weight) (99.7±53.1 in control vs 82.7±52.6, 84.2±46.9 & 83.0±42.4 at 30, 100 & 1000 mg/kg/day, respectively)

Feed Consumption: Note: Measured over 24-hour intervals ending on GD 5, 8, 13, 17, 21, 25 and 28. No test-article related changes noted.

Toxicokinetics: Note: Sampling times were 0.5, 1, 2, 4, 8 & 24 hrs post-dose on GD 15 (4 females/timepoint).

Reference ID: 3839341

Table 62: Mean maternal plasma MK-8742 TK parameters in rabbits at gestation day 15 following 10 days of administration

Dose (mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
30	1.27 ± 0.0649	0.333 ± 0.0842	1.8 ± 0.75
100	4.16 ± 1.57	0.517 ± 0.140	1.5 ± 0.29
1000	39.4 ± 9.20	1.93 ± 0.503	8.8 ± 5.3

MK-8742 concentration in plasma from 1/4 control animals at 8 hours post-dose was above the lower limit of quantitation (LLQ = 0.023 μM) and was less than 11% of the mean C_{max} value of the low-dose group.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for uniformity and concentration on Study week 1 and for concentration only on GD 15. Assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Necropsy: Note: Only thoracic and abdominal viscera evaluated. No test-article related changes noted.

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

Table 63: Cesarean section summary of rabbits administered MK-8742

Treatment Group	Control	30 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
Mated Females	19	19	19	19
Pregnant	19	19	19	19
Examined Live Litter	19	19	19	19
Resorbed or Dead Litter	0	0	0	0
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Not Pregnant	0	0	0	0
Live	0	0	0	0
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Corpora Lutea	146	152	150	147
Corpora Lutea/Pregnant Female ± SD	7.7 ± 1.3	8.0 ± 1.4	7.9 ± 1.1	7.7 ± 1.2
% Peri-Implantation Loss, Litter Mean ± SD	7.3 ±10.0	3.9 ± 7.4	13.1 ±10.0	8.4 ±10.5
Implants	135	146	131	135
Implants/Pregnant Female ± SD	7.1 ± 1.4	7.7 ± 1.4	6.9 ± 1.4	7.1 ± 1.4
Resorptions	2	2	3	8
% Resorptions/Implants, Litter Mean ± SD	1.3 ± 3.9	1.4 ± 4.2	2.2 ± 5.1	5.9 ±10.3
Dead Fetuses	0	0	0	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0	0.0	0.0
% Postimplantation Loss, Litter Mean ± SD	1.3 ± 3.9	1.4 ± 4.2	2.2 ± 5.1	5.9 ±10.3
Live Fetuses	133	144	128	127
Females	65	74	66	69
Males	68	70	62	58
Sex Ratio, Litter Mean ± SD	0.48 ± 0.20	0.51 ± 0.24	0.51 ± 0.22	0.55 ± 0.17
Live Fetuses/Pregnant Female ± SD	7.0 ± 1.3	7.6 ± 1.5	6.7 ± 1.4	6.7 ± 1.6
Live Fetal Weight (g), Litter Mean ± SD				
Females	31.4 ± 3.0	31.0 ± 3.2	32.6 ± 3.4	31.4 ± 2.3
Males	32.3 ± 3.8	32.3 ± 2.9	33.3 ± 2.7	32.2 ± 2.9

% Peri-Implantation Loss = ([No. Corpora Lutea - No. Implants] / No. Corpora Lutea) X 100.
% Postimplantation Loss = ([No. Resorptions + No. Dead Fetuses] / No. Implants) X 100.
Sex Ratio = Total No. Live Female Fetuses / (Total No. Live Female Fetuses + Total No. Live Male Fetuses).

Table from applicant

Placental morphology: No test-article related changes noted.

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

- Cervical vertebra variation observed in 4 and 3 fetuses (0 in controls) at 100 and 1000 mg/kg/day, resulting in mean incidence (% litter) of 2.7 and 2.3%, respectively. Although falling slightly outside of the historical control range (0-1.389%) in this laboratory, it is unclear if this is a MK-8742-related effect given the low incidence and lack of a clear dose/exposure relationship.
- Increase in incomplete ossification of cervical vertebra observed in 5 fetuses (2 in controls) at 100 and 1000 mg/kg/day, resulting in mean incidence (% litter) of 5.0 and 3.5%, respectively. Although the incidence at 100 mg/kg/day falls slightly outside of the historical control range (0-3.571%), it appears doubtful that this is a MK-8742-related effect given the lack of a clear dose/exposure relationship.
- Increase in incomplete ossification of metacarpal bones observed in 7 fetuses (2 in controls) at 1000 mg/kg/day, resulting in a mean incidence (% litter) of 4.7%, which falls within the historical control range (0-5.485%) for this laboratory.

9.2.2 Grazoprevir

Exploratory dose range-finding toxicokinetic and/or embryo-fetal developmental studies with MK-5172 were conducted in pregnant rats (Study# TT#10-7055) and

pregnant and non-pregnant rabbits (Study# TT#10-7040, 10-7042, 10-7044, 10-7046, 10-7047, 10-7048, 10-7049, 11-7126, 11-7127 & 12-7001) as summarized briefly below. Time-mated WI(HAN) rats (6 presumed pregnant females/group) received 50, 100, 200 mg/kg/day or 200 mg/kg b.i.d. MK-5172 or vehicle (100% PEG 400) from GD 6 through 20. Assessment of maternal and developmental toxicity was based on mortality, physical signs, body weights and food consumption and embryonic/fetal viability, fetal weights, sex ratios and external fetal morphology at GD 21, respectively. No evidence of maternal or developmental toxicity was noted in rats at up to 400 mg/kg/day (200 mg/kg b.i.d.), a dose level determined previously to achieve maximum MK-5172 exposure.

In rabbits, multiple exploratory studies were conducted in non-pregnant rabbits in an attempt to identify an oral formulation and dosing regimen that could be used for the pivotal study. Typically, a 100% PEG 400 containing formulation (dosed twice daily at 1 ml/kg/dose) provided the highest exposures. However, excessive vehicle-induced toxicity (including body weight loss and mortality) was observed with this formulation/dosing regimen when administered twice daily to pregnant rabbits in the preliminary oral embryo-fetal development study. Although no evidence of maternal or developmental toxicity was noted in rabbits at 200 mg/kg/day (200 mg/kg q.d. dose), systemic exposure was low ($AUC_{0\text{-}24hr}$=1.08 μM.hr). Based on these studies, the Applicant concluded that none of the oral formulations achieved well-tolerated reproducible systemic exposure levels that provide adequate multiples of human exposure and so the IV formulation was developed and used for the preliminary and pivotal rabbit embryo-fetal developmental studies.

In the preliminary rabbit IV embryo-fetal developmental study, time-mated Dutch Belted rabbits (12 presumed pregnant females/group) received 50, 100 or 200 mg/kg/day of a nanosuspension of MK-5172 or vehicle (0.15% polyvinylpyrrolidone K16-18/0.036% sodium deoxycholate/5% mannitol in deionized water) by IV injection (2 ml/kg, 1.5 ml/min) from GD 7 through 20. Assessment of maternal and developmental toxicity was based on mortality, physical signs, body weights and food consumption and embryonic/fetal viability, fetal weights, sex ratios and external, visceral and skeletal fetal morphology at GD 28, respectively. MK-5172-related physical signs (convulsion-like activity, lateral recumbency, decreased activity and/or splayed legs) and death were observed at 200 mg/kg/day in two females at GD 7 and 8 and so this dose level exceeded the MTD and remaining animals in this group were euthanized. No evidence of maternal or developmental toxicity was noted in rabbits at 100 mg/kg/day ($AUC_{0\text{-}24hr}$=83.3 μM.hr).

Reference ID: 3839341

Study title: Oral Developmental Toxicity Study in Rats With Prenatal Evaluation

Study no.:	TT #10-7050
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	August 6, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-001Z016 and 99.0%

Key Study Findings: Potential MK-5172 related effects on embryo-fetal development (EFD) were evaluated in pregnant rats (28/group) administered oral doses of 50, 200, 400 mg/kg/day or vehicle (100% PEG 400) control from GD 6 to 20. The NOEL for maternal and developmental toxicity was ≥400 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=217 μM·hr @ GD 15).

Methods

Doses:	0 (b.i.d.), 50, 200 and 400 (200 mg/kg b.i.d.) mg/kg/day
Frequency of dosing:	Once or twice (~6 hrs apart) daily
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	100% PEG 400
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	28 (24 control)
Age:	~10 weeks
Weight:	175 to 215 g
Satellite groups:	TK
Study design:	Females treated once or twice daily from GD 6 to 20 and euthanized at GD 21. Potential for skeletal malformations/variations assessed by both Alizarin red staining and micro-CT scanning.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: none

Clinical Signs (1x/day): No test-article related changes noted.

Reference ID: 3839341

Body Weight: Note: Measured on GD 0, 6, 8, 10, 12, 14, 16, 18, 20 and 21. No test-article related changes noted.

Feed Consumption: Note: Measured over 2-day intervals ending on GD 8, 14 and 20. No test-article related changes noted.

Toxicokinetics: Note: Sampling times were 1, 2, 4, 6, 10 & 24 hrs post-dose on GD 15 (3 rats/timepoint).

Table 64: Mean maternal plasma MK-5172 TK parameters in rats at gestation day 15 following 10 days of oral administration in EFD study

Dose	$AUC_{0\text{-}24\ hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
50 (mg/kg/day)	160 ± 53.5	23.4 ± 4.93	2.0 ± NC
200 (mg/kg/day)	133 ± 67.5	17.2 ± 9.41	2.0 ± NC
200 (mg/kg/b.i.d.)	217 ± 23.5	48.1 ± 3.28	8.0 ± NC

NC = Not Calculated
The second daily dose was administered at least 6 hours after the first dosing.
MK-5172 concentrations in maternal plasma from all control group animals at 2 hours postdose were below the lower limit of quantitation (LLQ = 0.0137 µM)

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for concentration on Study week 1 and 2. Assay results were within an acceptable concentration range (±10%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Necropsy: Note: Only thoracic and abdominal viscera evaluated. No test-article related changes noted.

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

Reference ID: 3839341

Table 65: Cesarean section summary of rats administered MK-5172

Treatment Group	Control b.i.d.	50 mg/kg/day	200 mg/kg/day	200 mg/kg b.i.d.
Mated Females	20	20	20	20
Pregnant	18	18	17	19
Examined Live Litter	18	18	17	18
Resorbed or Dead Litter	0	0	0	1
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Not Pregnant	2	2	3	1
Live	2	2	3	1
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Corpora Lutea	211	201	194	225
Corpora Lutea/Pregnant Female ± SD	11.7 ± 0.9	11.2 ± 1.4	11.4 ± 1.4	11.8 ± 2.3
% Peri-Implantation Loss, Litter Mean ± SD	17.6 ±20.6	9.1 ±15.3	12.8 ±21.4	16.7 ±20.8
Implants	173	183	169	191
Implants/Pregnant Female ± SD	9.6 ± 2.4	10.2 ± 2.2	9.9 ± 2.7	10.1 ± 3.4
Resorptions	7	8	4	9
% Resorptions/Implants, Litter Mean ± SD	5.9 ±10.4	4.4 ± 6.0	2.1 ± 3.9	8.8 ±22.8
Dead Fetuses	0	0	0	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0	0.0	0.0
% Postimplantation Loss, Litter Mean ± SD	5.9 ±10.4	4.4 ± 6.0	2.1 ± 3.9	8.8 ±22.8
Live Fetuses	166	175	165	182
Females	77	90	82	94
Males	89	85	83	88
Sex Ratio, Litter Mean ± SD	0.44 ± 0.20	0.51 ± 0.18	0.50 ± 0.21	0.51 ± 0.14
Live Fetuses/Pregnant Female ± SD	9.2 ± 2.7	9.7 ± 2.3	9.7 ± 2.5	9.6 ± 3.3
Live Fetuses/Live Litter ± SD	9.2 ± 2.7	9.7 ± 2.3	9.7 ± 2.5	10.1 ± 2.4

% Peri-Implantation Loss = ([No. Corpora Lutea - No. Implants] / No. Corpora Lutea) X 100.
% Postimplantation Loss = ([No. Resorptions + No. Dead Fetuses] / No. Implants) X 100.
Sex Ratio = Total No. Live Female Fetuses / (Total No. Live Female Fetuses + Total No. Live Male Fetuses).

Table from applicant

Placental morphology: No test-article related changes noted.

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

Study title: Intravenous Embryo-Fetal Developmental Toxicity and Toxicokinetic Study in Rabbits

Study no.:	TT #12-7300
Study report location:	4.2.3.5.2
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	June 28, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-5172, L-002214070-010B014 and ~100%

Key Study Findings: Potential MK-5172 related effects on embryo-fetal development (EFD) were evaluated in pregnant rabbits (23/group) administered intravenous doses of 25, 50, 100 mg/kg or vehicle (0.15% polyvinylpyrrolidone K16-18/0.036% sodium deoxycholate/5% mannitol in deionized water) control from GD 7 to 20.The NOEL for maternal and developmental toxicity is considered to be ≥100 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=76.1 μM·hr @ GD 15).

Reference ID: 3839341

Methods

Doses:	0, 25, 50 and 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 ml/kg
Route of administration:	IV (1.5 ml/min)
Formulation/Vehicle:	nanosuspension in 0.15% polyvinylpyrrolidone K16-18/0.036% sodium deoxycholate/5% mannitol in deionized water
Species/Strain:	Dutch Belted rabbits
Number/Sex/Group:	23
Age:	~26 weeks
Weight:	1656 to 2188 g
Satellite groups:	TK
Study design:	Females treated once daily from GD 7 to 20 and euthanized at GD 28. Potential for skeletal malformations/variations assessed by both Alizarin red staining and micro-CT scanning.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

Mortality: none

- One (of 23) female at 100 mg/kg/day euthanized (GD 16) due to excessive BW loss (9.8% between GD 7 & 15) and a second female euthanized (GD 16) after aborted tissue found in cage pan. Findings not considered clearly test-article related due to the low incidence (in 1 of 35 including animals in the preliminary study).

Clinical Signs (1x/day): No test-article related changes noted.

Body Weight: Note: Measured on GD 7, 9, 11, 13, 15, 17, 19, 21, 24 and 28. No test-article related changes noted.

Feed Consumption: Note: Measured over 24-hour intervals ending on GD 5, 8, 13, 17, 21, 25 and 28. No test-article related changes noted.

Toxicokinetics: Note: Sampling times were 0.08, 0.5, 2, 4, 8 & 24 hrs post-dose on GD 15.

Reference ID: 3839341

Table 66: Mean maternal plasma MK-5172 TK parameters in rabbits at gestation day 15 following 10 days of intravenous administration

Dose (mg/kg/day)	$AUC_{0\text{-}24\,hr}$ (µM•hr)	C_{max} (µM)	T_{max} (hr)
25	6.14 ± 0.586	7.36 ± 1.75	0.080 ± 0.0
50	24.4 ± 4.81	33.8 ± 8.71	0.080 ± 0.0
100	76.1 ± 7.44	67.2 ± 3.92	0.080 ± 0.0

IV administration from GD 7 to GD 15 with an injection rate of 1.5 mL/min

MK-5172 concentrations in plasma from all control group animals were below the lower limit of quantitation (LLQ = 0.013 µM) of the bioanalytical method.

Table from applicant

Dosing Solution Analysis: All dosing formulations were analyzed for concentration on GD 7 and 15. Assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.

Necropsy: Note: Only thoracic and abdominal viscera evaluated. No test-article related changes noted.

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

Table 67: Cesarean section summary of rabbits administered MK-5172

Treatment Group	Control	25 mg/kg/day	50 mg/kg/day	100 mg/kg/day
Mated Females	19	19	19	19
Pregnant	17	19	19	17
Examined Live Litter	17	19	18	15
Resorbed or Dead Litter	0	0	0	0
Found Dead	0	0	0	0
Sacrificed	0	0	1	1
Aborted	0	0	0	1
Not Pregnant	2	0	0	2
Live	2	0	0	2
Found Dead	0	0	0	0
Sacrificed	0	0	0	0
Corpora Lutea	120	138	139	104
Corpora Lutea/Pregnant Female ± SD	7.1 ± 1.3	7.3 ± 1.1	7.7 ± 1.4	6.9 ± 1.2
% Peri-Implantation Loss, Litter Mean ± SD	11.0 ±13.6	13.3 ±17.7	10.5 ± 8.8	6.0 ± 7.8
Implants	108	121	124	98
Implants/Pregnant Female ± SD	6.4 ± 1.8	6.4 ± 1.8	6.9 ± 1.3	6.5 ± 1.3
Resorptions	7	0	6	2
% Resorptions/Implants, Litter Mean ± SD	8.2 ±15.0	0.0	4.4 ± 7.5	1.9 ± 5.2
Dead Fetuses	0	0	0	0
% Dead Fetuses/Implants, Litter Mean ± SD	0.0	0.0	0.0	0.0
% Postimplantation Loss, Litter Mean ± SD	8.2 ±15.0	0.0	4.4 ± 7.5	1.9 ± 5.2
Live Fetuses	101	121	118	96
Females	59	64	60	48
Males	42	57	58	48
Sex Ratio, Litter Mean ± SD	0.57 ± 0.18	0.56 ± 0.25	0.51 ± 0.17	0.52 ± 0.21
Live Fetuses/Pregnant Female ± SD	5.9 ± 2.2	6.4 ± 1.8	6.6 ± 1.2	6.4 ± 1.3
Live Fetal Weight (g), Litter Mean ± SD				
Females	31.2 ± 2.8	31.9 ± 4.5	31.6 ± 2.7	30.8 ± 2.4
Males	32.5 ± 3.2	31.9 ± 2.8	33.8 ± 2.9	31.1 ± 4.4

% Peri-Implantation Loss = ([No. Corpora Lutea - No. Implants] / No. Corpora Lutea) X 100.
% Postimplantation Loss = ([No. Resorptions + No. Dead Fetuses] / No. Implants) X 100.
Sex Ratio = Total No. Live Female Fetuses / (Total No. Live Female Fetuses + Total No. Live Male Fetuses).

Reference ID: 3839341

Table from applicant

Placental morphology: No test-article related changes noted.

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

- Cervical rib variation observed in 3 fetuses from one litter (0 in controls) at 100 mg/kg/day, resulting in mean incidence (% litter) of 2.9%, respectively. Although falling slightly outside of the historical control range (0-1%) in this laboratory, it is unclear if this is a MK-5172-related effect given the low incidence.
- Increase in incomplete ossification observed in 4, 7 and 4 litters (in the torso and limb; vs 2 controls) and in 4, 3 and 2 fetuses/litters (in the head; vs 0 controls) at 25, 50 and 100 mg/kg/day, respectively, resulting in mean incidences (% litter) of 3.3, 2.5 and 2.3% in the latter. Although the historical control ranges were not provided, it appears doubtful that this is a MK-5172-related effect given the low incidence and a lack of a clear dose/exposure relationship.

9.3 Prenatal and Postnatal Development

Study title: Oral Pre- and Postnatal Developmental Toxicity Study in Rats

Study no.:	TT #14-7020
Study report location:	4.2.3.5.3
Conducting laboratory and location:	Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation:	February 21, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MK-8742, L-002469825-000G018 and 99.2%

Key Study Findings: Potential MK-8742 related effects on pre- and post-natal development (PPND) were evaluated in pregnant rats (20/group) administered oral doses of 50, 300, 1000 mg/kg or vehicle (10% polysorbate 80 in deionized water) control from GD6 to lactation day 20 (LD20).The NOEL for maternal and PPND toxicity is considered to be 300 and ≥1000 mg/kg/day, respectively. MK-8742-related maternal effects consisted primarily of transient decreases in body weight gain and food consumption at 1000 mg/kg/day.

Reference ID: 3839341

Methods

Doses:	0, 50, 300 and 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	10% polysorbate 80 in deionized water
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	20
Age:	~10 weeks
Weight:	165.2 to 246.7 g
Satellite groups:	none
Study design:	F0 females treated once daily from GD 6 to LD 20 and euthanized at LD 21. F1 animals were mated starting PN (post-natal) week 12 and bred females sacrificed between GD 15 and 17.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

F_0 Dams

Survival:	No test-article related changes noted.
Clinical signs (2x/day):	No test-article related changes noted.
Body weight:	Note: Measured on GD 6, 8, 10, 12, 14, 16, 18, 20, 21, 22 & 24 and on LD 0, 3, 7, 10, 14, 17 and 21. Transient decreases in mean BW gain compared to control observed between GD 6 and 8 (-47%) only at 1000 mg/kg/day. Greater BW loss compared to control (-16.6 vs -5.7%) observed between LD 14 and 21 at 1000 mg/kg/day.
Feed consumption:	Note: Measured over 2-day intervals ending on GD 8, 14 and 20. Mean food consumption decreased on GD 8 (-13%) versus controls at 1000 mg/kg/day.
Uterine content:	No test-article related changes noted.
Necropsy observation:	No test-article related changes noted following gross examination of maternal thoracic and abdominal viscera.
Toxicokinetics:	Not conducted
Dosing Solution Analysis	All dosing formulations were analyzed for uniformity and concentration in Study week 2 (first week of dosing) and for concentration only in Study week 7 (last week of dosing). Assay results were within an acceptable concentration range (±15%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.
Other:	None

Reference ID: 3839341

Table 68: Summary of uterine content in F0 dams administered MK-8742

	0 (vehicle)	50 mg/kg	300 mg/kg	1000 mg/kg
# of dams that delivered:	19	19	20	19
Metrial glands/female	11.8	10.7	11.0	11.9
Post-implantation survival (%):	92.3	92.7	95.0	96.1
Live litter size (PND 0)/litter:	10.9	9.9	10.5	11.4
Gestation length (days):	21.8	22.0	21.7	21.7
% female:	48	48	47	53

F_1 Generation

Survival: No test-article related changes noted.
Clinical signs (2x/wk): No test-article related changes noted.
Body weight (1x/wk): No test-article related changes noted.
Feed consumption: No test-article related changes noted.
Physical development: No test-article related changes noted regarding time to achieve vaginal opening or preputial separation.
Neurological assessment: No test-article related changes noted regarding passive avoidance (PND 35-36 & 42-43), auditory startle habituation (PND 62-64) or open-field motor activity (PND 69-71).
Reproduction: No test-article related changes noted regarding reproductive performance or fertility (*i.e*. mating, fecundity and fertility indexes).
Other: None

Table 69: Summary of survival, body weight and selected developmental parameters in the F1 generation

	0 (vehicle)	50 mg/kg	300 mg/kg	1000 mg/kg
# of live pups delivered:	197	189	209	217
# of dead pups delivered:	0	0	0	0
Live litter size (PND 0)/litter:	10.9	9.9	10.5	11.4
Live litter size (PND 3)/litter:	10.8	9.9	10.3	11.4
Live litter size (PND 7)/litter:	7.7	7.6	7.2	8.0
Live litter size (PND 21)/litter:	7.7	7.6	7.2	8.0
Mean pup weight on PND 0 (♂):	6.0 g	6.2 g	5.8 g	5.7 g
Mean pup weight on PND 0 (♀):	5.6 g	5.8 g	5.6 g	5.5 g
Mean pup weight on PND 21 (♂):	51.3 g	53.8 g	51.7 g	49.9 g
Mean pup weight on PND 21 (♀):	48.7 g	51.9 g	50.2 g	48.9 g
Balanopreputial separation (PND):	43.6	44.1	43.6	44.9
Vaginal patency (PND):	34.4	34.2	33.6	34.1

Reference ID: 3839341

Table 70: Summary of uterine content in F1 rats

	0 (vehicle)	50 mg/kg	300 mg/kg	1000 mg/kg (♀)*	1000 mg/kg (♂)*
# of gravid females:	16	19	18	18	18
Corpora lutea/gravid ♀:	12.4	13.2	12.6	12.8	12.8
Implantation sites/ gravid ♀:	11.4	10.8	11.0	11.0	10.6
% resorptions/implant:	3.3	5.2	3.9	6.2	2.8
% post-implantation loss:	3.3	6.4	4.3	6.2	2.8
Live litter size (PND 0)/litter:	11.1	10.3	10.5	10.6	10.3

*F1 males & females mated to control animals

F_2 Generation:

Survival: No test-article related changes noted.
Body weight: Not evaluated
External evaluation: Not evaluated
Male/Female ratio: Not evaluated
Other: None

Study title: Oral Pre- and Postnatal Developmental Toxicity and Toxicokinetic Study in Rats

Study no.: TT #14-7010
Study report location: 4.2.3.5.3
Conducting laboratory and location: Merck Research Laboratories West Point, Pennsylvania 19486
Date of study initiation: February 7, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-5172, L002214070-001Z016 and 96.2%

Key Study Findings: Potential MK-5172-related effects on pre- and post-natal development (PPND) were evaluated in pregnant rats (28/group) administered oral doses of 25, 100, 400 mg/kg/day or vehicle (100% PEG 400) control from GD6 to lactation day 20 (LD20).The NOEL for maternal and PPND toxicity is considered to be ≥400 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=146 μM·hr @ GD 15).

Reference ID: 3839341

Methods

Doses:	0 (b.i.d.), 25, 100 and 400 (200 mg/kg b.i.d.) mg/kg/day
Frequency of dosing:	Once or twice (~6 hrs apart) daily
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	100% PEG 400
Species/Strain:	Wistar Hannover rats
Number/Sex/Group:	28 (24 controls)
Age:	~10 weeks
Weight:	189.1 to 251.9 g
Satellite groups:	TK
Study design:	F0 females treated once daily from GD 6 to LD 20 and euthanized at LD 21. F1 animals were mated starting PN (post-natal) week 12 and bred females sacrificed between GD 15 and 17.
Deviation from study protocol:	None that affected the integrity or conclusions of the study.

Observations and Results

F_0 Dams

Survival:	No test-article related changes noted.
Clinical signs (2x/day):	No test-article related changes noted.
Body weight:	Note: Measured on GD 6, 8, 10, 12, 14, 16, 18, 20, 21, 22 & 24 and on LD 0, 3, 7, 10, 14, 17 and 21. No test-article related changes noted.
Feed consumption:	Note: Measured over 2-day intervals ending on GD 8, 14 and 20. No test-article related changes noted.
Uterine content:	No test-article related changes noted.
Necropsy observation:	No test-article related changes noted following gross examination of maternal thoracic and abdominal viscera.
Toxicokinetics:	Note: Samples collected 1, 2, 4, 6, 8, 10 and 24 hours post-dose on GD 15 (4 females/group).
Dosing Solution Analysis	All dosing formulations were analyzed for concentration in Study week 2 (first week of dosing), 3 and 6 (last week of dosing). Assay results were within an acceptable concentration range (±10%). In addition, the vehicle and the compound in this vehicle were stable under the conditions of this study.
Other:	None

Reference ID: 3839341

Table 71: Mean maternal plasma MK-5172 TK parameters in rats at gestation day 15 following 10 days of oral administration in PPND study

Dose	Sex	$AUC_{0-24\,hr}$ (μM*hr)	Cmax (μM)	Tmax (hr)
25 (mg/kg/day)	Female	20.1 ± 7.02	4.73 ± 1.79	2.0 ± NC
100 (mg/kg/day)		155 ± 52.7	26.8 ± 9.26	2.0 ± NC
200 (mg/kg b.i.d.)		146 ± 37.7	16.5 ± 7.71	2.0 ± NC
NC = Not Calculated.				

MK-5172 dosing: from Gestation Day 6 to Gestation Day 15.
b.i.d. dosing: The second dose was administered at least 6 hours after the end of the first dosing.
MK-5172 concentrations in plasma from all control group animals at 2 hours postdose were below the lower limit of quantitation (LLQ = 0.0132 μM) of the bioanalytical method.

Table from applicant

Table 72: Summary of uterine content in F0 dams administered MK-5172

	0 (vehicle)	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d
# of dams that delivered:	22	28	28	27
Metrial glands/female:	11.7	11.4	11.1	9.8
Post-implantation survival (%):	94.6	96.6	95.4	96.2
Live litter size (PND 0)/litter:	11.1	11.0	10.6	9.6
Gestation length (days):	21.8	21.7	21.8	21.8
% female:	52	52	56	48

F_1 Generation

Survival: No test-article related changes noted.
Clinical signs (2x/wk): No test-article related changes noted.
Body weight (1x/wk): No test-article related changes noted.
Feed consumption: No test-article related changes noted.
Physical development: No test-article related changes noted regarding time to achieve vaginal opening or preputial separation.
Neurological assessment: No test-article related changes noted regarding passive avoidance (PND 35-36 & 42-43), auditory startle habituation (PND 62-64) or open-field motor activity (PND 69-71).
Reproduction: No test-article related changes noted regarding reproductive performance or fertility (*i.e.* mating, fecundity and fertility indexes).
Other: None

Table 73: Summary of survival, body weight and selected developmental parameters in the F1 generation

	0 (vehicle)	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d
# of live pups delivered:	199	219	211	183

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# of dead pups delivered:	1	1	0	1
Live litter size (PND 0)/litter:	11.1	11.0	10.6	9.6
Live litter size (PND 3)/litter:	10.5	10.9	10.5	8.7
Live litter size (PND 7)/litter:	7.5	8.0	7.6	6.3
Live litter size (PND 21)/litter:	7.5	8.0	7.6	6.3
Mean pup weight on PND 0 (♂):	5.7 g	5.9 g	6.1 g	6.1 g
Mean pup weight on PND 0 (♀):	5.4 g	5.6 g	5.7 g	5.8 g
Mean pup weight on PND 21 (♂):	49.3 g	50.6 g	51.2 g	46.9 g
Mean pup weight on PND 21 (♀):	47.8 g	49.0 g	49.2 g	45.9 g
Balanopreputial separation (PND):	43.0	43.2	42.2	42.5
Vaginal patency (PND):	34.0	33.6	33.1	33.5

Table 74: Summary of uterine content in F1 rats

	0 (vehicle)	25 mg/kg/d	100 mg/kg/d	400 mg/kg/d (♀)*	400 mg/kg/d (♂)*
# of gravid females:	13	19	19	14	13
Corpora lutea/gravid ♀:	13.1	13.2	13.7	12.7	13.5
Implantation sites/ gravid ♀:	12.0	11.1	13.2	10.9	13.0
% resorptions/implant:	7.1	2.8	6.0	11.5	2.9
% post-implantation loss:	7.1	2.8	6.0	11.5	2.9
Live litter size (PND 0)/litter:	11.2	10.7	12.4	9.8	12.6

*F1 males & females mated to control animals

F_2 Generation:

Survival: No test-article related changes noted.
Body weight: Not evaluated
External evaluation: Not evaluated
Male/Female ratio: Not evaluated
Other: None

10 Special Toxicology Studies

10.1 Exploratory hepatobiliary toxicity studies

Exploratory oral toxicity studies were conducted in rasH2 wild-type (Study # TT12-6034), C57BL/6 and BALB/c (Study # TT12-6039) mice primarily to further evaluate the hepatobiliary toxicity observed with MK-5172. Toxicity of MK-5172 was evaluated in C57BL/6 and BALB/c mice (10/sex/group) administered oral (gavage) doses of 200 or 500 mg/kg/day or vehicle control [HPMCP in 0.5%(w/v) methylcellulose, 0.02% (w/v) SLS with 5 mM HCl in deionized water] for up to one month. Toxicity endpoints were evaluated including clinical signs, body weights, serum biochemistry and gross and histopathology. Mortality was observed at 500 mg/kg/day in 4 and 3 C57BL/6 and BALB/c mice, respectively (cause of death undetermined). Although the

Reference ID: 3839341

severity and incidence differed to some extent, the findings were fairly consistent between sexes and strains of mice and typically included decreased body weight gain/body weight loss, serum biochemistry changes [moderate to severe increases in total (direct) bilirubin, up to slight increases in ALT and/or ALP, slight to moderate increases in glucose and cholesterol, up to slight decreases in total protein and/or triglycerides], increased liver weight, focal hepatic necrosis, periportal cytoplasmic rarefaction (females), gall bladder inflammation, renal tubular epithelial degeneration and epithelial vacuolation/single cell necrosis or villous atrophy in small intestine. The NOAEL was <200 mg/kg/day given the adverse hepatobiliary findings observed.

Toxicity of MK-5172 was also evaluated in rasH2 wild-type mice (50/sex/group) administered oral (gavage) doses of 200 or 500 mg/kg/day or vehicle control [HPMCP in 0.5%(w/v) methylcellulose, 0.02% (w/v) SLS with 5 mM HCl in deionized water] for 12 weeks (also included a 12-week treatment-free “recovery” period). Toxicity endpoints were evaluated including clinical signs, body weights, limited serum biochemistry (albumin, AST, ALT, ALP, GLDH and total/direct bilirubin) and limited gross and histopathology (liver, gallbladder, small and large intestines). Mortality was observed at 500 mg/kg/day in 6 animals (cause of death undetermined). Other findings included decreased body weight gain/body weight loss, serum biochemistry changes [very slight to moderate increases in AST, ALT, ALP and/or GLDH, slight to marked increase in total (direct) bilirubin, very slight decreases in albumin] increased liver weight, periportal cytoplasmic rarefaction (females), erosions or focal necrosis of gallbladder wall (and adjacent liver tissue), epithelial hyperplasia of gallbladder, neutrophilic cellular infiltration of gallbladder, epithelial vacuolation (at 200 and 500 mg/kg/day) and/or villous atrophy (500 mg/kg/day only) in small intestine. Of note, serum biochemistry changes were first evident after ~2-weeks of administration and had resolved ~8-weeks into the treatment-free period. In addition, no MK-5172 related histopathological effects were noted following the treatment-free period. The NOAEL was <200 mg/kg/day given the adverse hepatobiliary findings observed.

Exploratory analysis of bile acids (all species), transcriptional endpoints for bile acid transport and metabolism (mice), miRNA species (mice) and serum cytokines (rasH2 wild-type mice only) (Study # TT12-9693, 12-9709, 12-9745 and 12-9746) were conducted in liver and/or serum (also ileum from rasH2 wild-type mice) samples collected from previously conducted studies in mice (Study # TT12-6034 and 12-6039), rats (Study # TT10-6014) and dogs (Study # TT10-6015). MK-5172-related effects included significant increases in serum taurine conjugated bile acids (including taurocholic acid) as well as total unconjugated bile acids in dogs and in all three strains of mice (but not in rats). In addition, consistent changes in mRNA expression for multiple transporters were observed in all mouse strains. Additional details for these non-GLP studies are beyond the scope of this review and so are not included.

10.2 Phototoxicity studies

Study title: Three Day Oral (Gavage) Phototoxicity and Bioanalysis Evaluation of MK-8742 in Male Pigmented Rats

Reference ID: 3839341

Study no.: TT# 12-9042
Study report location: 4.2.3.7
Conducting laboratory and location: (b) (4)
Date of study initiation: Not provided (protocol signed October 23, 2012)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-8742, L-002469825-000G018 and 99.2%

Key Study Findings: A 3-day multiple dose oral (gavage) phototoxicity study was conducted in pigmented male Long Evans (LE) rats (5/group) administered MK-8742 once a day at 100 and 1000 mg/kg/day or a vehicle control (10% polysorbate 80 in deionized water) (refer to Applicant Table below). Toxicity endpoints evaluated including clinical signs, body weight, dermal scoring, ophthalmology (pre-dose and on Day 6) and ocular histopathology (cornea, lens, bulbar conjunctiva, vitreous & aqueous chambers, optic nerve, retina, uvea, sclera, iris, ciliary body and choroid evaluated in both eyes on Day 6). Following three days of MK-8742 administration, rat eyes were exposed to a Xenon lamp (emits light at wavelengths throughout the UVB, UVA, visible and into the near IR spectrum) for ~30 min, either 4 or 6 hours after the third dose. Reduction in body weight gain and body weight loss was observed during dosing phase at 100 and 1000 mg/kg/day, respectively, while these groups gained weight during Days 4 to 6. No skin or eye reactions indicative of phototoxicity occurred in pigmented male rats administered oral doses of MK-8742 up to 1000 mg/kg for 3 days followed by a single exposure to solar-simulated UVR. Thus, the NOEL for dermal and ocular phototoxicity was ≥1000 mg/kg/day (estimated C_{max}=1.63 μM @ 8 hrs) for MK-8742.

Table 75: Experimental design summary of MK-8742 phototoxicity study in male Long Evans rats

Group No.	No. of Animals	Test Material	Dose Level (mg/kg/day)	Conc. (mg/mL)	Dose Volume (mL/kg)	UVR Exposure	
						Instrumental UVR Dose (MED)[a]	Interval Between Formulation Administration and UVR Exposure (Hours ± Minutes)[b]
1	5 + 3[c]	Control Article	0	0	5	0.5	4 ± 10
2	5 + 3[c]	MK-8742	100	20	5	0.5	4 ± 10
3	5 + 3[c]	MK-8742	1000	200	5	0.5	6 ± 10

Conc. = concentration
[a] Minimal Erythema Dose is a UVR dose considered adequate to elicit a barely perceptible response in the skin.
[b] The timing of the start of the UVR exposure was based on the median dosing time for each group.
[c] Rats assigned to the bioanalysis phase. These rats were not anesthetized or exposed to UVR.
[d] 10% Polysorbate 80 in deionized water [Polysorb 80/DI Water (10:90w/w)]

Reference ID: 3839341

Table 76: Individual plasma MK-8742 concentrations (μM) in male Long Evans rats following 3 days of administration of MK-8742 at 100 (group 2) or 1000 (group 3) mg/kg/day or vehicle (group 1)

Group	Animal	2 hours	4 hours	6 hours	8 hours
1	2816	Below limit of quantitation			
	2817	Below limit of quantitation			
	2818	Below limit of quantitation			
2	2819	0.975	1.06	0.811	0.640
	2820	1.02	1.06	0.809	0.708
	2821	0.801	0.901	0.719	0.571
3	2822	1.26	1.15	1.16	1.17
	2823	1.23	1.43	1.57	1.58
	2824	1.79	1.96	2.01	2.14

Study title: <u>Three Day Oral (Gavage) Phototoxicity and Bioanalysis Evaluation of MK-5172 in Male Pigmented Rats</u>

Study no.: TT# 12-9002
Study report location: 4.2.3.7
Conducting laboratory and location: (b) (4)
Date of study initiation: Not provided (protocol signed January 18, 2012)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-5172, L-002214070-001Z016 and 99.0%

Key Study Findings: A 3-day multiple dose oral (gavage) phototoxicity study was conducted in pigmented male Long Evans (LE) rats (5/group) administered MK-5172 once or twice a day at 50 and 200 mg/kg/dose or a vehicle control (100% PEG 400) (<u>refer to Applicant Table below</u>). Toxicity endpoints evaluated including clinical signs, body weight, dermal scoring, ophthalmology (pre-dose and on Day 6) and ocular histopathology (cornea, lens, bulbar conjunctiva, vitreous & aqueous chambers, optic nerve, retina, uvea, sclera, iris, ciliary body and choroid evaluated in both eyes on Day 6). Following three days of MK-5172 administration, rat eyes were exposed to a Xenon lamp (emits light at wavelengths throughout the UVB, UVA, visible and into the near IR spectrum) for ~30 min, 2 hours after first dose on day 3. No MK-5172 related effects on clinical signs or body weight were observed. In addition, no skin or eye reactions indicative of phototoxicity occurred in pigmented male rats administered oral doses of MK-5172 up to 200 mg/kg/dose (5 total doses) for 3 days followed by a single exposure to solar-simulated UVR. Thus, the NOEL for dermal and ocular phototoxicity was ≥400 mg/kg/day for MK-5172.

Reference ID: 3839341

Table 77: Experimental design summary of MK-5172 phototoxicity study in male Long Evans rats

Group No.	No. of Animals	Test Material	Dosage Level (mg/kg/dose)	Conc. (mg/mL)	Dosage Volume (mL/kg)	UVR Exposure	
						Instrumental UVR Dosage (MED)[a]	Interval Between Formulation Administration and UVR Exposure (Minutes)[b]
1	5 + 3[c]	Control Article (PEG 400)	0[d]	0	2	0.5	120 ± 10
2	5 + 3[c]	MK-5172	50	25	2	0.5	120 ± 10
3	5 + 3[c]	MK-5172	200[d]	100	2	0.5	120 ± 10

a. Minimal Erythema Dose is a UVR dose considered adequate to elicit a barely perceptible response in the skin.
b. The timing of the start of the UVR exposure was based on the median dosing time for each group.
c. Rats assigned to the bioanalysis phase. These rats were not anesthetized or exposed to UVR.
d. Test article or control article was administered twice daily on Days 1 and 2 (6 to 7 hours between daily doses); test article or control article was administered once on Day 3 prior to UVR exposure.

10.3 Reversibility studies

Study title: <u>MK-8742: One-Month Oral Toxicity Study in Dogs with a Three-Month Treatment-Free Period</u>

Study no.: TT#14-1031
Study report location: 4.2.3.7
Conducting laboratory and location: Merck Research Laboratories
West Point, Pennsylvania 19486
Date of study initiation: May 16, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-8742, L-002469825-000G018 and 99.2%

Key Study Findings: This study was conducted to evaluate potential reversibility of MK-8742 induced phospholipidosis in female dogs administered 1000 mg/kg/day [or vehicle (10% polysorbate 80 in deionized water)] for one month. Feces discoloration (due to unabsorbed test-article), infrequent liquid emesis and skin turgor and/or thin appearance associated with body weight loss were observed. In addition, vacuolation of gut associated lymphoid tissue (GALT) in small and/or large intestine, of mandibular and/or mesenteric lymph nodes and/or of spleen was observed in MK-8742 treated animals. Similar to previous studies, these findings were characterized by the presence of increased numbers of large vacuolated macrophages predominantly in follicular areas of the lymphoid tissues, with transmission electron microscopy (TEM) findings consistent with lysosomal phospholipid accumulation (phospholipidosis). Findings consistent with phospholipidosis were not observed in animals following a 3-month treatment-free period.

Reference ID: 3839341

Table 78: Mean plasma MK-8742 TK parameters in female dogs following a single MK-8742 dose of 1000 mg/kg

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (µM*hr)	C_{max} (µM)	T_{max} (hr)
1000	Female	27.1 ± 4.68	2.12 ± 0.294	12 ± 2.4
Plasma MK-8742 concentrations in all control samples were below the lower limit of quantitation (LLQ = 0.0227 µM).				

Table from applicant

10.4 Combination toxicology studies

Study title: MK-8742/MK-5172: One-Month Oral Combination Toxicity Study in Dogs

Study no.: TT#12-6051
Study report location: 4.2.3.7
Conducting laboratory and location: Laboratoires Merck Sharp & Dohme-Chibret
Merck Research Laboratories
Route de Marsat, Riom,
63963 Clermont-Ferrand Cedex 9, France
Date of study initiation: September 13, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-8742, L-002469825-000G018 and 97.6%; MK-5172, L-002214070-001Z016 and 96.2%

Key Study Findings: This study was conducted to evaluate the potential toxicity and toxicokinetic profiles of MK-8742 and MK-5172 when co-administered at 25 and 5 mg/kg/day, respectively, for one month. Dogs were administered (once a day) each drug individually, in combination (MK-5172 [redacted] (b) (4) followed by MK-8742 gavage) or a combined vehicle control [[redacted] (b) (4) containing 100% PEG 400 followed by gavage (10% polysorbate 80 in deionized water)] (refer to Applicant Table below). Toxicity assessment included all standard endpoints, including electrocardiographic and ophthalmology evaluation. Vacuolation in lymph nodes and gut associated lymphoid tissue (GALT) in small and/or large intestine was observed in females treated with MK-8742 with and without MK-5172. In addition, a trend for increased circulating exposures and liver concentrations were observed for MK-5172 when given in combination with MK-8742, which may not be significant in females given inter-animal variability (refer to Applicant Tables below). No other toxicologically significant findings were noted in any of the groups.

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Table 79: Experimental design summary of one month MK-8742/MK-5172 combination toxicity study in dogs

Group	Females	Males
Control: 100% PEG 400[a] - 10% Polysorbate 80[b] 2 mL/kg/day - 5 mL/kg/day	3	3
MK-5172[a] - 10% Polysorbate 80[b]: 5 mg/kg/day - 5 mL/kg/day	3	3
100% PEG 400[a] - MK-8742[b]: 2 mL/kg/day - 25 mg/kg/day	3	3
MK-5172[a]/MK-8742[b]: 5 mg/kg/day - 25 mg/kg/day	3	3

[a] Oral administration by capsule followed by
[b] Oral administration by gavage.
PEG 400 = Polyethylene Glycol 400.
MK-5172 was given as a formulation in 100% PEG 400 (2 mL/kg/day).
MK-8742 was given as a formulation in 10% polysorbate 80 (5 mL/kg/day).

Table 80: Mean plasma MK-5172 TK parameters in dogs following administration of MK-5172 alone or in combination with MK-8742 for one month

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
5[a]	Female	35.4 ± 12.2	7.54 ± 3.18	2.3 ± 0.88
	Male	25.2 ± 2.43	4.80 ± 0.287	2.0 ± 0.0
	All	30.3 ± 6.01	6.17 ± 1.55	2.2 ± 0.40
5/25[b]	Female	71.1 ± 32.7	7.14 ± 3.43	2.7 ± 0.67
	Male	49.3 ± 2.24	6.10 ± 2.07	4.7 ± 1.8
	All	60.2 ± 15.4	6.62 ± 1.81	3.7 ± 0.95

[a] MK-5172: 5 mg/kg/day
[b] MK-5172/ MK-8742: 5/ 25 mg/kg/day
MK-5172 concentrations in plasma from all control group animals were below the lower limit of quantitation (LLQ = 0.013 μM) of the bioanalytical method.

Table 81: Mean plasma MK-8742 TK parameters in dogs following administration of MK-8742 alone or in combination with MK-5172 for one month

Dose (mg/kg/day)	Sex	$AUC_{0-24\ hr}$ (μM•hr)	C_{max} (μM)	T_{max} (hr)
5/25[a]	Female	9.87 ± 3.27	0.890 ± 0.289	3.3 ± 0.67
	Male	5.66 ± 0.566	0.471 ± 0.0349	3.0 ± 1.0
	All	7.76 ± 1.76	0.680 ± 0.160	3.2 ± 0.54
25[b]	Female	14.7 ± 3.67	1.36 ± 0.133	3.3 ± 0.67
	Male	4.48 ± 1.15	0.522 ± 0.0553	2.7 ± 0.67
	All	9.59 ± 2.86	0.943 ± 0.199	3.0 ± 0.45

[a] MK-5172/ MK-8742: 5/ 25 mg/kg/day

[b] MK-8742: 25 mg/kg/day

MK-8742 concentration in plasma from one control animal was above the lower limit of quantitation (LLQ = 0.023 μM) at 2 hours postdose. This value was less than 4% of the mean C_{max} of the 5/25 mg/kg/day (MK-5172/MK-8742) dose group.

Table 82: Mean (±SE) liver MK-5172 concentrations (μM[c]) in dogs following 4-weeks of administration of MK-5172 alone or in combination with MK-8742

Dose (mg/kg/day)	Time (hr) Necropsy
Vehicle	0.00 ± 0.00
5[a]	6.20 ± 1.51
5/25[b]	11.7 ± 2.50

[a] MK-5172: 5 mg/kg/day

[b] MK-5172/ MK-8742: 5/ 25 mg/kg/day

[c] Assuming a liver density of 1.0 g/mL

MK-5172 concentrations in liver from all control group animals were below the lower limit of quantitation (LLQ = 0.104 μM) of the bioanalytical method.

Table 83: Mean (±SE) liver MK-8742 concentrations (μM[c]) in dogs following 4-weeks of administration of MK-8742 alone or in combination with MK-5172

Dose (mg/kg/day)	Time (hr) Necropsy
Vehicle	0.00 ± 0.00
5/25[a]	6.32 ± 2.00
25[b]	6.05 ± 1.32

[a] MK-5172/ MK-8742: 5/ 25 mg/kg/day

[b] MK-8742: 25 mg/kg/day

[c] Assuming a liver density of 1.0 g/mL

MK-8742 concentrations in liver from all control group animals were below the lower limit of quantitation (LLQ = 0.227 μM) of the bioanalytical method.

11 Integrated Summary and Safety Evaluation

ZEPATIER™ is a once daily fixed-dose combination (FDC) tablet of elbasvir (EBR, MK-8742), an HCV NS5A inhibitor, and grazoprevir (GZR, MK-5172), an HCV NS3/4 protease inhibitor. Elbasvir and grazoprevir have EC_{90} values that vary from the low pM to low nM range in HCV replicon assays with HCV genotypes 1, 4 and 6.

Pharmacokinetics

Elbasvir:

The oral bioavailability of EBR was estimated to be ~3-9 and 35% in rats and dogs, respectively, while oral T_{max} values in rats, monkeys and dogs ranged from ~3.3 to 5 hours. The low bioavailability observed was likely due to low absorption, with first-pass hepatic extraction also an important contributor in dogs. In addition, solubility limited absorption was likely responsible for the less than dose proportional increases in exposure observed following oral administration at higher dose levels in these species. Although saturation of absorption limited the exposure obtained in oral toxicology studies, absorption of EBR from the GI tract was sufficient to achieve adequate circulating EBR exposure levels in the toxicology studies submitted in these species.

In rats, dogs and monkeys, the steady state volume of distribution (Vd_{ss}) was 5.0, 3.0 and 2.7 L/kg, respectively, values greater than total body water volume. In addition, EBR was bound highly to plasma protein in all species examined (>99.9% bound). Tissue distribution of EBR and related metabolites was evaluated in male Wistar Hannover (WH) and Long Evans (LE) rats following a single 30 mg/kg oral dose of ^{14}C-EBR using quantitative whole body autoradiography. The highest maximum concentrations of radioactivity were observed in the GI tract, adrenal gland, liver and renal cortex, while distribution to the brain, bone and eye lens was limited. In WH rats, concentration of EBR and related metabolites in liver were 20 times that in plasma at 6 hours post-dose, and could only be detected in spleen, liver, kidney (cortex) and Harderian gland by 168 hours post-dose.

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Unchanged parent drug was the only drug-related component detected in plasma following administration of ^{14}C-EBR to WH rats, dogs and healthy human subjects, with unchanged EBR also comprising the majority of drug-related material in rabbits. Unchanged parent drug was also the major component in bile and feces, while mono-oxidative and/or double-hydroxylated metabolites (catalyzed primarily by CYP3A4) comprised significant percentages of total drug-related material eliminated in bile and/or feces in these species.

The systemic clearance of EBR was low to moderate in species evaluated with terminal half-lives of ~4 to 16 hours in rats, dogs and monkeys. The majority of radioactivity was recovered in feces and bile following administration of ^{14}C-EBR by oral and IV routes, respectively. The low percentage of EBR and related metabolites in bile following oral (as compared to IV) administration in rats and dogs is consistent with the low oral bioavailability observed with EBR. Although ~16% of EBR was eliminated as unchanged parent drug in rat urine following IV administration, only minor amounts of the total administered EBR dose was recovered in urine from dogs (and humans). Thus, biliary excretion and intestinal secretion of unchanged parent drug is the major route of elimination for EBR in rats and dogs.

Grazoprevir:

The oral bioavailability of GZR was estimated to be 13 and 12% in rats and dogs, respectively, while oral T_{max} values for GZR in rats and dogs ranged from ~2 to 8 hours, with the data suggesting a delayed absorption at higher dose levels in rats. The low bioavailability of GZR observed in rats and dogs was likely due to low absorption and first-pass hepatic extraction. Despite the low bioavailability, oral absorption of GZR was estimated to be at least 40% at the dose levels examined, based on recovery of total drug-related material in urine and bile in bile duct cannulated (BDC) rats and dogs.

In rats and dogs, the steady state volume of distribution (Vd_{ss}) was 3.1 and 0.7 L/kg, respectively. In addition, GZR was bound highly to plasma protein in all species examined (>98.4% bound), with binding independent of GZR concentration. Tissue distribution of GZR and related metabolites was evaluated in male WH and LE rats following a single 50 mg/kg oral dose of ^{14}C-GZR using quantitative whole body autoradiography. Limited distribution of ^{14}C-GZR-derived radioactivity was observed for most tissues, with the highest maximum concentrations of radioactivity observed in the GI tract, liver, intervertebral ligament and renal cortex and radioactivity below the level of detection in CNS tissues. In WH rats, concentration of GZR and related metabolites in liver were 21 times that in plasma by 4 hours post-dose and were below the level of detection in all tissues except liver by 72 hours post-dose and all tissues by 168 hours post-dose. Additional single dose studies were conducted in rats and dogs to better understand the relationship between liver and plasma pharmacokinetics. The liver to plasma ratios decreased as the dose levels increased, suggesting that GZR uptake is a saturable process in rats and dogs. At the highest dose levels examined, the terminal liver half-lives were ~9 hours.

Unchanged parent drug was the only drug-related component detected in plasma following administration of ^{3}H-GZR or ^{14}C-GZR to male WH rats, dogs and healthy human male subjects, with unchanged GZR also comprising the majority of drug-related material in rabbits. GZR related metabolites (catalyzed primarily by CYP3A4), resulting

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from hydroxylation, oxidative O-dealkylation, oxidative loss of vinylcyclopropylsulfonamide and oxidation and glutathione addition, were identified, with two metabolites identified only in human feces. These two human specific metabolites were the result of gut bacterial metabolism and are likely eliminated from the GI tract without being absorbed. In addition, a human specific oxidative metabolite (M14), comprising ~4% of total GZR-related material eliminated, was identified in human feces. The presence of this minor human specific metabolite is not thought to pose a significant clinical safety risk. In rats and dogs, unchanged parent drug and oxidative metabolites were present in roughly equivalent amounts in bile, while the predominant GZR-related material in feces was unchanged parent drug, with the M10 metabolite also detected in dog feces. In humans, unchanged parent drug and the M10 metabolite comprises 80% of total GZR-related material in feces, with various oxidative metabolites accounting for the remaining ~20%.

The systemic clearance (CL) of GZR was considered to be moderate, with terminal half-lives of 1.4 to 3 hours in rats and dogs. Although plasma CL was ~5 and 28 ml/min/kg in dogs and rats, respectively, blood clearance was estimated to be ~11 and 46 ml/min/kg after correcting for the blood to plasma partition ratios. The majority of radioactivity was recovered in feces and bile following administration of ^{14}C-GZR by oral and IV routes, respectively. The low percentage of GZR and related metabolites in bile following oral (as compared to IV) administration in dogs is consistent with the low oral bioavailability observed. Only minor amounts of the total administered GZR dose was recovered in urine from all species evaluated including humans. Thus, both oxidative metabolism and biliary excretion of unchanged parent drug are major routes of elimination for GZR in rats and dogs, with intestinal secretion also contributing in dogs.

Safety Pharmacology

Elbasvir:

No significant neurological effects were observed in male rats following administration of single oral EBR doses of up to 2,000 mg/kg/day (estimated C_{max}~1.96 μM for MK-8742), providing an ~14-fold rat to human exposure multiple at the recommended EBR dose. This C_{max} value (Day 14) was obtained from male rats in the 2-week toxicology study (Study #TT11-6014). In addition, no significant EBR-related cardiovascular [hemodynamic (HR and BP) or electrocardiographic parameters] or respiratory [respiratory rate (bpm) or depth of respiration (mmHg)] effects were noted by telemetry for up to 24 hours post-dose in male dogs given single oral doses of EBR up to 50 mg/kg (estimated C_{max}>0.566 μM for MK-8742), providing an >4-fold dog to human exposure multiple at the recommended EBR dose. This C_{max} value (Day 1) was obtained from male dogs administered a 25 mg/kg dose in the 3-month toxicology study (Study #TT11-6025). A minor effect of EBR on membrane K^+ current was observed (5.2% reduction @ 10 μM) at the maximal feasible concentration (based on solubility limitations) in stably hERG transfected CHO cells. This effect was not statistically significant and is not considered to be of clinical concern. No clear EBR-related cardiovascular effects have been described in clinical trials.

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Grazoprevir:

No significant neurological effects were observed in male rats following administration of oral GZR doses of up to 400 mg/kg/day (200 mg/kg b.i.d.) (estimated C_{max}~23.2 μM for MK-5172), providing an ~105-fold rat to human exposure multiple at the recommended GZR dose. This C_{max} value (Week 2) was obtained from male rats in the 1-month toxicology study (Study #TT09-1120). In addition, mean heart rate was increased up to 42%, beginning ~3 hours post-dose and remained elevated until ~20 hours post-dose, in conscious telemetrized dogs administered oral doses of GZR at 20 and 600 mg/kg (estimated C_{max}~42.8 μM for MK-5172 at 20 mg/kg), providing an ~195-fold dog to human exposure multiple at the recommended GZR dose. No significant effects on heart rate were noted at a 5 mg/kg dose (estimated C_{max}~11.5 μM for MK-5172), providing an ~52-fold dog to human exposure multiple at the recommended GZR dose. Additionally, no significant GZR-related effects on other cardiovascular (arterial blood pressure or electrocardiographic) or respiratory [respiratory rate (bpm) or depth of respiration (mmHg)] parameters were noted by telemetry for up to 48 hours post-dose in dogs given single oral ((b) (4) doses of GZR up to 600 mg/kg (estimated C_{max}~104.8 μM for MK-5172), providing an ~476-fold dog to human exposure multiple at the recommended GZR dose. These C_{max} values (Day 1) were obtained from dogs in the 1-month toxicology study (Study #TT08-1151). An effect of GZR on membrane K^+ current was observed (IC_{50}=25 μM) in stably hERG transfected CHO cells. In addition to this inhibition, an effect on gating properties of the hERG current was suggested "by the apparent change in the kinetics of deactivation of the tail current at -50 mV, and by the increase in steady-state current at +20 mV, especially at 96 μM". These effects are not considered to be of significant clinical concern, given that GZR is highly bound to plasma protein so that this IC_{50} value is estimated to be >7,500-fold higher than the maximal plasma concentration (free fraction) in humans at the recommended GZR dose. No clear GZR-related cardiovascular effects have been described in clinical trials.

Repeat-Dose Toxicology Studies

Elbasvir:

Repeat-dose toxicology studies were conducted in mice, rats and dogs administered EBR by oral gavage at up to 1000 mg/kg/day, which is typically considered an appropriate limit dose [refer to ICH M3(R2)]. The NOAEL was 1000 mg/kg/day in rasH2 WT mice ($AUC_{0\text{-}24\ hr}$=120(♀) & 182(♂) μM·hr @ 4 weeks) and rats ($AUC_{0\text{-}24\ hr}$=19.8(♀) & 24.1(♂) μM·hr @ 13 weeks) administered oral EBR doses for 1 and 6 months, respectively. The mouse/rat to human EBR AUC exposure multiples at the NOAEL are estimated to be ~55 and 9 (♀) and 83 and 11 (♂), for mice and rats respectively, at the recommended EBR dose. In rats, non-adverse findings included increased incidence of transient post-dosing salivation, slight increases in mean urine volume (in males and females) and reduced body weight gain associated with decreased food consumption (in males) at all dose levels examined.

The NOAEL was considered to be 1000 mg/kg/day in dogs administered oral EBR doses for 3 ($AUC_{0\text{-}24\ hr}$=19.3 μM·hr @ 13 weeks) and 9 ($AUC_{0\text{-}24\ hr}$=16.6 μM·hr @ 26 weeks) months. The dog to human EBR AUC exposure multiples at the NOAEL are estimated to be ~9 and 8, for the 3 and 9 month studies respectively, at the recommended EBR dose. Findings observed in animals administered 1000 mg/kg

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consisted of feces discoloration (due to unabsorbed test-article), infrequent liquid emesis and, in animals administered EBR for 9 months, reduced body weight gain associated with reduced food consumption. In addition, vacuolation of gut associated lymphoid tissue (GALT) in stomach, small and/or large intestine, of mandibular and/or mesenteric lymph nodes, gall bladder and/or spleen was observed in all animals administered 1000 mg/kg/day and in one and two dogs administered 25 mg/kg/day for 3 and 9 months, respectively. Findings were characterized by the presence of an increased number of large vacuolated cells, morphologically consistent with macrophages in follicular areas of the lymphoid tissues, containing various amount of intracytoplasmic cell debris. These vacuolar changes correlated with the presence of myelin figures in macrophages observed by transmission electron microscopy (TEM) and are consistent with lysosomal phospholipid accumulation (*i.e*. phospholipidosis). A one month study in female dogs administered 1000 mg/kg/day was conducted to evaluate potential reversibility of EBR induced phospholipidosis. In addition to feces discoloration, infrequent liquid emesis and GALT vacuolation (similar to that observed in longer duration studies), skin turgor and/or thin appearance associated with body weight loss were observed unexpectedly. However, findings consistent with phospholipidosis were not observed in animals following a 3-month treatment-free period. Thus, lymphoid tissue phospholipidosis was considered to be reversible and was not associated with overt toxicity (with other histopathology findings in lymphoid tissue, change in spleen weights or with lymphoid depletion) and so appears to be an adaptive rather than a toxic response.

Grazoprevir:

Repeat-dose toxicology studies were conducted in rasH2 wild-type (WT) and CD1 mice, rats and dogs administered oral doses of GZR for up to 1, 3, 6 and 9 months, respectively. In addition, exploratory oral toxicity studies with GZR were conducted in rasH2 WT (for 3-months), C57BL/6 and BALB/c mice (for 1-month), with the most significant toxicity findings for all species described below.

Hepatobiliary toxicity:

The NOEL for hepatobiliary toxicity was 20 mg/kg/day ($AUC_{0\text{-}24\ hr}$= 5 μM·hr @ 1 month) in rasH2 WT mice administered oral GZR doses for 1 month, since hepatobiliary findings were observed at 100 ($AUC_{0\text{-}24\ hr}$=190(♀) & 138(♂) μM·hr @ 1 month), 200 ($AUC_{0\text{-}24\ hr}$=333 μM·hr @ 1 month) and 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$=2140(♀) & 3470(♂) μM·hr @ 1 month). The mouse to human GZR AUC exposure multiple at the NOEL is ~3 at the recommended GZR dose, while the exposure multiples at 100, 200 and 500 mg/kg/day are ~74, 179 and 1,865 in males and ~102, 179 and 1,150 in females, respectively. Hepatobiliary findings consisted of increased bilirubin, ALT and/or AST, with severity (and incidence) of findings increasing with dose level (500 mg/kg/day: up to 4,600, 430 and 135% increases in bilirubin, ALT and AST, respectively; 200 mg/kg/day: up to 700 and 83% increases in bilirubin and ALT, respectively; 100 mg/kg/day(♂): individual animals had 100% increases in bilirubin). These findings were associated with increased hepatocellular size at 200 and 500 mg/kg/day accompanied by increased cytoplasmic rarefaction in the periportal region (♀) associated with large

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amounts of granular cytoplasmic structures (compatible with glycogen) in periportal and centrilobular hepatocytes. Increased hepatocellular size correlated with increased mean liver weights (up to 21, 26 and 77% increases at 100, 200 and 500 mg/kg/day) and/or organ size and by prominent lobular pattern (♂). In addition, distention of gallbladder was present in 8 of 10 (♂) at 500 mg/kg/day.

The NOEL for hepatobiliary toxicity was 20 ($AUC_{0\text{-}24\ hr}$=8 μM·hr @ 13 weeks) and 100 mg/kg/day ($AUC_{0\text{-}24\ hr}$=451 μM·hr @ 13 weeks) in male and female CD1 mice respectively, administered oral GZR doses for 3 months, since hepatobiliary findings were observed at 100 ($AUC_{0\text{-}24\ hr}$=451 μM·hr @ 13 weeks) in males and 200 ($AUC_{0\text{-}24\ hr}$=957(♀) & 683(♂) μM·hr @ 13 weeks) and 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$=1,550(♀) & 1,390(♂) μM·hr @ 13 weeks) in males and females. The mouse to human GZR AUC exposure multiples at the NOEL are ~4 and 242 in males and females respectively, at the recommended GZR dose, while the exposure multiples at 100 (♂), 200 and 500 mg/kg/day are ~242, 367 and 747 in males and ~515 and 833 in females, respectively. Hepatobiliary findings consisted of increased bilirubin, ALT and/or ALP, with severity (and incidence) of findings increasing with dose level (500 mg/kg/day: up to 3,100, 91 and 70% increases in bilirubin, ALT and ALP, respectively; 200 mg/kg/day: up to 200, 81 and 54% increases in bilirubin, ALT and ALP, respectively; 100 mg/kg/day(♂):up to 100% increase in bilirubin). Similar to that observed in rasH2 WT mice, findings at 200 and 500 mg/kg/day were associated with increased hepatocellular size, cytoplasmic rarefaction in the periportal region (♀), increased mean liver weights (up to 23 and 73% increases at 200 and 500 mg/kg/day) and/or organ size and by a prominent lobular pattern (♂). In addition, focal inflammation of the gallbladder was observed at 500 mg/kg/day only, characterized by wall thickening due to neutrophilic cellular infiltration and/or fibroplasia and "overlying epithelial hyperplasia with superficial epithelial necrosis in contact with amorphous eosinophilic debris present in the lumen".

The NOEL for hepatobiliary toxicity was 5 mg/kg/day ($AUC_{0\text{-}24\ hr}$=69 μM·hr @ week 12) in dogs administered oral GZR doses for 9 months, since hepatobiliary findings were observed at 15 ($AUC_{0\text{-}24\ hr}$=431(♀) & 303(♂) μM·hr @ week 12) and 300/100 mg/kg/day ($AUC_{0\text{-}24\ hr}$=2420(♀) & 2170(♂) μM·hr @ week 26). The dog to human GZR AUC exposure multiple at the NOEL is ~37 at the recommended GZR dose, while the exposure multiples at 15 and 300/100 mg/kg/day are ~232 and 1,301 in females and ~163 and 1,167 in males, respectively. Hepatobiliary findings consisted of sporadic ALT, AST & ALP elevations and increased serum and urine bilirubin, with severity (and incidence) of findings increasing with dose level (300/100 mg/kg/day: up to 1,000, 1,100, 370 and 256% increases in bilirubin, ALT, AST and ALP, respectively; 15 mg/kg/day: up to 200% increases in bilirubin). These findings were associated with unformed/liquid/yellow feces and microlithiasis in the lumen of the gallbladder and in larger bile ducts in the liver (consisting of finely granular basophilic material in lumen) at 15 and 300/100 mg/kg/day. In addition, increased liver weight (up to 43%), distended gallbladder with abundant bile content, gallbladder wall thickened and yellowish discoloration of gingival mucosae, white adipose tissue and/or aorta were observed at 300/100 mg/kg/day, with jaundice-like findings [yellow discoloration of skin and eyes (bulbar conjunctiva)] seen in one early sacrificed animal. In dogs administered oral GZR doses for 1 month, hepatobiliary findings consisting of increases in bilirubin (up to 5-fold) and sporadic ALT/ALP elevations (up to 7-fold) were observed at 20 ($AUC_{0\text{-}24}$

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$_{hr}$=429(♂) μM·hr @ week 5) in males and 600 mg/kg/day ($AUC_{0-24\ hr}$=3,300(♀) & 2,560(♂) μM·hr @ week 5) in males and females. Thus, the NOEL for hepatobiliary findings was 5 ($AUC_{0-24\ hr}$=47.6 μM·hr @ week 12) and 20 mg/kg/day ($AUC_{0-24\ hr}$=564 μM·hr @ week 12) in males and females, respectively. The dog to human GZR AUC exposure multiples at the NOEL in the one month study are ~26 and 303 in males and females respectively, at the recommended GZR dose, while the exposure multiples at 20(♂) and 600 mg/kg/day are ~231 and 1,376 in males and ~1,774 in females, respectively. Additionally, hepatobiliary findings consisting of increases in total bilirubin (up to 400% in 6 of 30 animals) were observed at 400 mg/kg/day (200 mg/kg b.i.d.) ($AUC_{0-24\ hr}$~445 μM·hr @ week 12) in rats administered oral GZR doses for 6 months. The rat to human GZR AUC exposure multiple at the NOEL for hepatobiliary findings is ~200 at the recommended GZR dose, while the exposure multiple at 400 mg/kg/day is ~240.

In addition to studies described above, exploratory oral toxicity studies were conducted in rasH2 WT (12-week), C57BL/6 (1-month) and BALB/c mice (1-month), in part, to further evaluate the hepatobiliary toxicity observed with GZR. Although the severity and incidence differed to some extent, the findings were fairly consistent between sexes and strains of mice and typically included moderate to severe increases in total (direct) bilirubin, increases in ALT and/or ALP, increased liver weight, periportal cytoplasmic rarefaction (♀) and gall bladder inflammation. Notable exceptions to the generally similar findings described in all strains of mice included BALB/c mice that also had focal hepatic necrosis at 200 and 500 mg/kg/day, and rasH2 WT mice that had erosions or focal necrosis of the gallbladder wall (and adjacent liver tissue) and epithelial hyperplasia of gallbladder. In the 12-week study in rasH2 WT mice, the serum transaminase and bilirubin elevations were first evident after ~2-weeks of administration and had resolved ~8-weeks into the treatment-free “recovery” period. In addition, no GZR-related histopathological effects were noted following the treatment-free period.

Exploratory analysis of bile acids (all species), transcriptional endpoints for bile acid transport and metabolism (mice), miRNA species (mice) and serum cytokines (rasH2 WT mice only) were also conducted in liver and/or serum samples collected from previously conducted repeat-dose toxicology studies in rasH2 WT (12-week), C57BL/6 and BALB/c mice (1-month), rats (6-month) and dogs (9-month). GZR-related effects included significant increases in serum taurine conjugated bile acids (including taurocholic acid) as well as total unconjugated bile acids in dogs and in all three strains of mice (but not in rats), and changes in mRNA expression for multiple transporters in all mouse strains.

To further evaluate mechanisms for hepatotoxicity, the potential for GZR to form chemically reactive metabolites, capable of covalently modifying proteins, was evaluated using 10 μM ^{3}H-GZR or ^{14}C-GZR in rat and human liver microsomes and human hepatocytes incubated with or without reduced glutathione (GSH) or ritonavir. It was determined that GZR is capable of undergoing NADPH-dependent metabolic bioactivation to form chemically reactive intermediate(s). In incubations with GSH or ritonavir, the amount of covalent protein binding is reduced. This result along with the NADPH-dependence observed, indicates that CYP450 is important for metabolic bioactivation. However, the lack of detectable metabolites that are clearly indicative of metabolic bioactivation in human feces supports the hypothesis that bioactivation is a

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minor pathway in humans and so likely is not a major contributor to the hepatobiliary toxicity observed in mice, dogs and humans. Thus, hepatobiliary findings may be initiated primarily by transporter inhibition, since GZR can inhibit OATP1B1, OATP1B3 and BSEP in-vitro. Refer to the clinical pharmacology review for details. In addition, refer to the clinical reviews of Dr. Sarita Boyd and Dr. Prabha Viswanathan, since hepatobiliary toxicity has been observed in clinical trials with GZR.

Testicular toxicity:

The NOEL for testicular toxicity was 20 ($AUC_{0\text{-}24\ hr}$=429 μM·hr @ week 5) and 5 mg/kg/day ($AUC_{0\text{-}24\ hr}$=69.3 μM·hr @ week 12) in dogs administered oral GZR doses for 1 and 9 months, respectively. Testicular findings were observed at 600 mg/kg/day ($AUC_{0\text{-}24\ hr}$=2,560 μM·hr @ week 5) in the 1 month study and at 15 ($AUC_{0\text{-}24\ hr}$=303 μM·hr @ week 12) and 300/100 mg/kg/day ($AUC_{0\text{-}24\ hr}$=2,170 μM·hr @ week 26) in the 9-month study. The dog to human GZR AUC exposure multiples at the NOEL are ~231 and 37 at 1 and 9 months respectively, at the recommended GZR dose, while the exposure multiples at 15, 300/100 and 600 mg/kg/day are ~163, 1,167 and 1,376 respectively. Testicular findings consisted of seminiferous tubule degeneration of testes, with severity and incidence of findings increasing with dose level and duration (600 mg/kg/day: minimal to mild in 3 of 3; 300/100 mg/kg/day: moderate to marked in 3 of 3; 15 mg/kg/day: very slight in 1 of 3), reduced testicular weights (14.5 and 33.9% @ 15 and 300/100 mg/kg/day, respectively) and decreased amount of sperm in epididymis at 300/100 mg/kg/day (in all animals). The testicular toxicity at 300/100 mg/kg/day was wide-spread and characterized by "loss of spermatids and spermatozoa with intraluminal degenerated and frequently multinucleated spermatogenic epithelial cells". Although the potential for recovery was not evaluated, one could expect that the testicular toxicity at 300/100 mg/kg/day would be irreversible, while toxicity at the other dose levels may be expected to recover after cessation of treatment. Testicular toxicity was not observed in CD1 mice or rats at dose levels up to 500 ($AUC_{0\text{-}24\ hr}$=1,390 μM·hr @ 13 weeks) and 400 mg/kg/day ($AUC_{0\text{-}24\ hr}$=525 μM·hr @ 13 weeks) for up to 3 and 6 months, respectively. The mouse/rat to human GZR AUC exposure multiples at the NOEL for testicular toxicity are ~747 and 282, for mice and rats respectively, at the recommended GZR dose. The potential for GZR-related testicular toxicity has not been evaluated in clinical trials.

Renal toxicity:

The NOEL for renal toxicity was 200 mg/kg/day ($AUC_{0\text{-}24\ hr}$=333 μM·hr @ 1 month) in rasH2 WT mice administered oral GZR doses for 1 month, since adverse kidney findings were observed at 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$=2,140(♀) & 3,470(♂) μM·hr @ 1 month). The mouse to human GZR AUC exposure multiple at the NOEL is ~179 at the recommended GZR dose, while the exposure multiples in females and males at 500 mg/kg/day are ~1,150 and 1,865, respectively. Kidney findings consisted of very slight (♀) to moderate (♂) degeneration of renal tubular epithelium "characterized mainly by tubular basophilia and dilatation of tubuli with scattered epithelial single cell necrosis, with and without presence of cellular debris in dilated tubules". Findings were restricted

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to a few foci in subcapsular areas or in medullary rays for very slight cases, while the moderate cases had involvement of large parts of the outer medulla.

The NOEL for renal toxicity was also 200 mg/kg/day ($AUC_{0\text{-}24\ hr}$=957(♀) & 683(♂) μM·hr @ 13 weeks) in CD1 mice administered oral GZR doses for 3 months, since adverse kidney findings were observed at 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$=1,550(♀) & 1,390(♂) μM·hr @ 13 weeks). The mouse to human GZR AUC exposure multiples at the NOEL are ~515 and 367 for females and males respectively, at the recommended GZR dose, while the exposure multiples in females and males at 500 mg/kg/day are ~833 and 747, respectively. Kidney findings were similar to those observed in rasH2 WT mice, with very slight to marked degeneration of renal tubular epithelium observed. Of note, similar findings were also observed in exploratory oral toxicity studies in C57BL/6 and BALB/c mice administered GZR at 500 mg/kg/day. No clear GZR-related renal safety signals have been described in clinical trials.

Hematological toxicity:

The NOEL for hematological toxicity was 200 mg/kg/day ($AUC_{0\text{-}24\ hr}$=957(♀) & 683(♂) μM·hr @ 13 weeks) in CD1 mice administered oral GZR doses for 3 months, since hematological findings were observed at 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$=1,550(♀) & 1,390(♂) μM·hr @ 13 weeks). The mouse to human GZR AUC exposure multiples at the NOEL are ~515 and 367 for females and males respectively, at the recommended GZR dose, while the exposure multiples in females and males at 500 mg/kg/day are ~833 and 747, respectively. Hematological findings consisted of decreased RBCs (4-8%), hemoglobin (Hg) (14-20%), hematocrit (Hct) (8-15%), and mean corpuscular (MC) volume (MCV) (5-8%), hemoglobin (MCH) (11-13%) and hemoglobin concentration (MCHC) (6-7%) in males and females.

The NOEL for hematological toxicity was 15 mg/kg/day ($AUC_{0\text{-}24\ hr}$= 431(♀) & 303 (♂) μM·hr @ week 12) in dogs administered oral GZR doses for 9 months, since hematological findings were observed at 300/100 mg/kg/day ($AUC_{0\text{-}24\ hr}$= 2,420(♀) & 2,170(♂) μM·hr @ week 26). The dog to human GZR AUC exposure multiples at the NOEL are ~232 and 163 for females and males respectively, at the recommended GZR dose, while the exposure multiples in females and males at 300/100 mg/kg/day are ~1,301 and 1,167, respectively. Hematological findings consisted of decreased RBCs (up to 15%), Hg (13-38%), Hct (9-28%), MCV (5-26%), MCH (3-31%) and MCHC (3-15%) in males and females. These clinical pathology findings were associated with increased hemosiderin content of spleen and sinusoidal cells of liver, increased reticulocytes (up to 808%), erythroid hyperplasia of bone marrow and increased extramedullary hematopoiesis in spleen. Although RBC counts actually recovered during treatment (↑10% by week 38 in ♂), the severity of other clinical pathology parameters progressed over the 9 month treatment duration. No clear GZR-related hematological safety signals have been described in clinical trials.

Gastrointestinal (GI) toxicity:

The NOEL for GI toxicity was 200 mg/kg/day ($AUC_{0\text{-}24\ hr}$=333 μM·hr @ 1 month) in rasH2 WT mice administered oral GZR doses for 1 month, since GI toxicity findings

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were observed at 500 mg/kg/day ($AUC_{0\text{-}24\ hr}$=2140(♀) & 3470(♂) μM·hr @ 1 month). The mouse to human GZR AUC exposure multiple at the NOEL is ~179 at the recommended GZR dose, while the exposure multiples in females and males at 500 mg/kg/day are ~1,150 and 1,865, respectively. GI findings consisted of very slight (♀) to slight (♂) villous atrophy in the small intestine described as "largely restricted to the duodenum and generally characterized by blunting and fusion of villi associated with epithelial single cell necrosis and/or vacuolation and regenerative hyperplasia of the crypt epithelium". Jejunum changes were much less prominent than in the duodenum, suggesting a potential local effect of GZR-related material (possibly associated with inflow of bile). Of note, similar findings were also observed in exploratory oral toxicity studies in C57BL/6 and BALB/c mice administered GZR at 500 mg/kg/day.

The NOEL for GI toxicity was 200 mg/kg/day ($AUC_{0\text{-}24\ hr}$=419 μM·hr @ 13 weeks) in male rats administered oral GZR doses for 6 months, since GI toxicity findings were observed at 400 mg/kg/day (200 mg/kg b.i.d.) ($AUC_{0\text{-}24\ hr}$=525 μM·hr @ 13 weeks). GI toxicity was not observed in female rats at 400 mg/kg/day (200 mg/kg b.i.d.) ($AUC_{0\text{-}24\ hr}$=365 μM·hr @ 13 weeks). The rat to human GZR AUC exposure multiples at the NOEL are ~196 and 225, for females and males respectively, at the recommended GZR dose, while the exposure multiple in males at 400 mg/kg/day is ~282. GI findings consisted of focal hemorrhage in the glandular mucosa of stomach (in 8 of 15 males), correlating with red foci or discoloration observed by gross pathology. Only mild GI symptoms (nausea, diarrhea) have been described in clinical trials with GZR.

Reproductive and Developmental Toxicology

Elbasvir:

The NOEL for female and male fertility parameters was ≥1000 mg/kg/day (estimated $AUC_{0\text{-}24hr}$~15.1 μM.hr for MK-8742) and 300 mg/kg/day (estimated $AUC_{0\text{-}24hr}$~11.5 μM.hr for MK-8742), respectively, in rats administered oral EBR doses of up to 1000 mg/kg/day. EBR-related effects consisted of transient decreases in food consumption in females and males at 300 and 1000 mg/kg/day, with effects on body weight gain only observed in males. Although a slight decrease (~15%) in sperm count was observed at 1000 mg/kg/day (estimated $AUC_{0\text{-}24hr}$~19.5 μM.hr for MK-8742), no effects on testicular weight, sperm motility and morphology or male fertility parameters were noted. In addition, no testicular toxicity was observed in rats administered EBR for up to 6 months at 1000 mg/kg/day ($AUC_{0\text{-}24hr}$=24.1 μM.hr for MK-8742). The rat to human EBR AUC exposure multiples at the NOEL are estimated to be ~7 and 5, for females and males respectively, at the recommended EBR dose, while the exposure multiples in males at 1000 mg/kg are estimated to be ~9 and 11 in the fertility and 6-month toxicology studies, respectively. This minor decrease in sperm count is not considered to be of biological significance. Since TK were not conducted in the fertility study, exposures at the 300 and 1000 mg/kg/day dose levels were estimated based on data obtained at these dose levels following 13-weeks of EBR administration (Study #TT11-6024).

The NOEL for maternal and embryo-fetal developmental (EFD) toxicity was 300 mg/kg/day and ≥1000 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$= 21.8 μM·hr @ GD 15), respectively, in pregnant rats administered oral EBR doses of up to 1000 mg/kg/day. EBR-related maternal effects consisted of slight decreases in body weight gain at 1000

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mg/kg/day. The NOEL for maternal and EFD toxicity was ≥1000 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=39.4 μM·hr @ GD 15), in pregnant rabbits administered oral EBR doses of up to 1000 mg/kg/day. The rat/rabbit to human EBR AUC exposure multiples at the NOEL are estimated to be ~10 and 18, for rats and rabbits respectively, at the recommended EBR dose. Of note, EBR and related metabolites can cross the placenta in pregnant rats and rabbits following oral administration (from GD 6 to 20), with fetal plasma levels of only 0.6-2% that of maternal plasma concentrations achieved under the conditions studied.

The NOEL for maternal and pre- and post-natal developmental (PPND) toxicity is considered to be 300 and ≥1000 mg/kg/day, respectively. EBR-related maternal effects consisted primarily of transient decreases in body weight gain and food consumption at 1000 mg/kg/day, the highest dose level tested. The rat to human EBR AUC exposure multiple at the NOEL in the PPND study is estimated to be ~10 at the recommended EBR dose. Of note, no developmental effects occurred despite the fact that EBR and related metabolites are excreted into milk, as evaluated in lactating rats following oral administration [from GD 6 to lactation day (LD) 14], with significant concentrations present in milk two hours post-dose on LD 14 (milk to maternal plasma ratio ~4.2).

Grazoprevir:

The NOEL for female and male fertility parameters was ≥400 mg/kg/day (estimated $AUC_{0\text{-}24hr}$~156(♀) and 267(♂) μM.hr for MK-5172), in rats administered oral GZR doses of up to 400 mg/kg/day (200 mg/kg b.i.d.). The rat to human GZR AUC exposure multiples at the NOEL are estimated to be ~84 and 144, for females and males respectively, at the recommended GZR dose. Since TK were not conducted in the fertility study, exposure at the 400 mg/kg/day dose level was estimated based on data obtained at this dose following 4-weeks of GZR administration (Study #TT09-1120).

The NOEL for maternal and EFD toxicity was ≥400 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=217 μM·hr @ GD 15), in rats administered oral GZR doses of up to 400 mg/kg/day (200 mg/kg b.i.d.), and ≥100 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=76.1 μM·hr @ GD 15), in rabbits administered intravenous (IV) GZR doses of up to 100 mg/kg/day. The rat/rabbit to human GZR AUC exposure multiples at the NOEL are estimated to be ~117 and 41, for rats and rabbits respectively, at the recommended GZR dose. Multiple exploratory studies were conducted in rabbits to identify an oral formulation and dosing regimen that could be used for the pivotal EFD study. However, it was concluded that none of the oral formulations achieved well-tolerated reproducible systemic exposure levels that would provide adequate multiples of human exposure, and so an IV formulation was developed. The high-dose level for the pivotal EFD study was selected based on results from the preliminary IV study where GZR-related physical signs (convulsion-like activity, lateral recumbency, decreased activity and/or splayed legs) and death were observed at 200 mg/kg/day (dose level exceeded the MTD). Of note, GZR and related metabolites can cross the placenta in pregnant rats and rabbits following oral (rats) or IV (rabbits) administration (from GD 6 through 20), with fetal plasma concentrations of up to ~7 (rabbits) and 89% (rats) that of maternal concentrations achieved under the conditions studied.

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The NOEL for maternal and PPND toxicity is considered to be ≥400 mg/kg/day (maternal $AUC_{0\text{-}24\ hr}$=146 μM·hr @ GD 15) in rats administered oral GZR doses of up to 400 mg/kg/day (200 mg/kg b.i.d.). The rat to human GZR AUC exposure multiple at the NOEL in the PPND study is estimated to be ~78 at the recommended GZR dose. Of note, no developmental effects occurred despite the fact that GZR and related metabolites were excreted into milk, as evaluated in lactating rats following oral administration (from GD 6 to LD 14), with significant concentrations present in milk two and eight hours post-dose on LD 14 (milk concentrations from 54 to 87% that in maternal plasma).

Phototoxicity

Elbasvir:

Tissue distribution of EBR and related metabolites was evaluated in male Wistar Hannover (WH) (non-pigmented) and Long Evans (LE) (pigmented) rats following a single 30 mg/kg oral dose of ^{14}C-EBR using quantitative whole body autoradiography. Differences between pigmented and non-pigmented rats were observed, most notably in the eye uveal tract. Concentration of EBR and related metabolites in the eye uveal tract in LE rats peaked at 10 hours post-dose (at ~33 times that in plasma) and was still detectable at 672 hours (28 days) post-dose (at a concentration ~50% that observed at 10 hours), while concentration in WH rats peaked at 2 hours post-dose and was undetectable by 10 hours post-dose. Interestingly, the distribution of EBR to pigmented versus non-pigmented skin in LE rats was roughly equivalent, so direct binding of EBR to melanin appears unlikely. Given this accumulation of EBR in the uveal tract of the eye in pigmented rats and since EBR absorbs ultraviolet (UV) light, a 3-day multiple dose phototoxicity study was conducted in male LE rats administered EBR. No skin or eye reactions indicative of phototoxicity occurred in pigmented male rats administered oral doses of EBR up to 1000 mg/kg for 3 days followed by a single exposure to solar-simulated UVR. Thus, the NOEL for dermal and ocular phototoxicity was ≥1000 mg/kg/day (estimated C_{max}=1.63 μM @ 8 hrs). The rat to human EBR C_{max} and AUC exposure multiples at the NOEL are estimated to be ~12 and 9, respectively, at the recommended EBR dose. AUC exposure ($AUC_{0\text{-}24\ hr}$=19.5 μM·hr @ 13-weeks) was estimated based on data obtained at this dose in males (Study #TT11-6024).

Grazoprevir:

Tissue distribution of GZR and related metabolites was evaluated in male WH and LE rats following a single 50 mg/kg oral dose of ^{14}C-GZR using quantitative whole body autoradiography. No significant differences between pigmented and non-pigmented rats were noted, with minimal distribution of GZR to the uveal tract of the eye and to pigmented and non-pigmented skin observed. A 3-day multiple dose oral phototoxicity study was conducted in male LE rats administered GZR once or twice a day. No skin or eye reactions indicative of phototoxicity occurred in pigmented male rats administered oral doses of GZR up to 200 mg/kg/dose (5 total doses) for 3 days followed by a single exposure to solar-simulated UVR. Thus, the NOEL for dermal and ocular phototoxicity was ≥400 mg/kg/day. The rat to human GZR AUC exposure multiple at the NOEL is estimated to be ~101 at the recommended GZR dose. GZR

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exposure at 400 mg/kg/day ($AUC_{0\text{-}24\ hr}$=188 μM·hr @ 2-weeks) was estimated based on data obtained at this dose in males (Study #TT09-1120).

Combination toxicology study

A study was conducted to evaluate the potential toxicity and toxicokinetic profiles of EBR and GZR when co-administered at 25 and 5 mg/kg/day, respectively, for one month. Dogs were administered (once a day) each drug individually, in combination or a combined vehicle control. Toxicity assessment included all standard endpoints, including electrocardiographic and ophthalmology evaluation. Vacuolation in lymph nodes and gut associated lymphoid tissue (GALT) in small and/or large intestine was observed in females treated with EBR with and without GZR. In addition, a trend for increased (~2-fold) circulating AUC exposures and liver concentrations were observed for GZR when given in combination with EBR, which may not be significant in females given inter-animal variability. No other toxicologically significant findings were noted in any of the groups.

Genetic Toxicology and Carcinogenicity

Elbasvir and grazoprevir were not mutagenic or clastogenic as tested in the Ames assay, the *in vitro* chromosomal aberration assay in CHO cells and an *in vivo* rat micronucleus assay. In addition, mouse and rat carcinogenicity studies with EBR and GZR are not being conducted, given the intended treatment duration (<6 months) and lack of a specific cause for concern.

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Table 84: Summary of Systemic Exposure Multiples for Elbasvir Toxicology Studies

Species	Study Type/ Duration/Toxicity	Dose (mg/kg/day)	Exposure Multiple Based on MK-8742 AUC*
Mouse (rasH2 WT)	1-month RD	1000 (NOAEL)	55 (♀) 83 (♂)
Rat	RD Ocular Phototoxicity	1000 (♂ NOEL)	9
	6-month RD	1000 (NOAEL)	9 (♀), 11 (♂)
	↓ sperm count Fertility & EED	1000 (♂) 300 (♂ NOEL) 1000 (♀ NOEL)	9 5 7
Rat (pregnant)	EFD	1000 (NOEL)	10
	PPND	1000 (NOEL)	10
Rabbit (pregnant)	EFD	1000 (NOEL)	18
Dog	9-month RD	1000 (NOAEL)	8

*$AUC_{0\text{-}24hr}$ in HCV-infected human subjects given FDC (EBR & GZR): 2,180 nM.hr at 50 mg QD; RD=repeat-dose toxicology study; EFD=embryo-fetal development study; PPND=pre- and post-natal development study; EED=early embryonic development; GD=gestational day; NOEL=no effect level; NOAEL=no adverse effect level

Table 85: Summary of Systemic Exposure Multiples for Grazoprevir Toxicology Studies

Species	Study Type/ Duration/Toxicity	Dose (mg/kg/day)	Exposure Multiple Based on MK-5172 AUC*
Mouse (rasH2 WT)	GI & Renal Hepatobiliary 1-month RD	500 200 100 (NOAEL) 20 (NOEL)	1150(♀),1865(♂) 179 102 (♀), 74 (♂) 3
Mouse (CD1)	Renal & Hematological Hepatobiliary 3-month RD	500 200 100 (NOAEL) 20 (NOEL)	833(♀),747(♂) 515(♀),367(♂) 242 4
Rat	RD Ocular Phototoxicity	400 (♂ NOEL)	101
	GI toxicity 6-month RD	400 (♂) 400 (♀ NOAEL) 200 (♂ NOAEL)	282 196 225
	Fertility & EED	(♂ NOEL) (♀ NOEL)	144 84
Rat (pregnant)	EFD	400 (NOEL)	117
	PPND	400 (NOEL)	78
Rabbit (pregnant)	EFD	100 (NOEL)	41
Dog	Hematological Hepatobiliary & Testicular 9-month RD	300/100 15 5 (NOEL)	1301(♀),1167(♂) 232(♀),163(♂) 37

*$AUC_{0\text{-}24hr}$ in HCV-infected human subjects given FDC (EBR & GZR): 1,860 nM.hr at 100 mg QD; RD=repeat-dose toxicology study; EFD=embryo-fetal development study; PPND=pre- and post-natal development study; EED=early embryonic development; GD=gestational day; NOEL=no effect level; NOAEL=no adverse effect level

12 Appendix/Attachments

12.1 Histopathology inventory

Study	12-6047	12-6033	12-6030	11-6034	11-6038	10-6015
Species	mice	rats	dogs	mice	mice	dogs
Adrenals	X	*, X	*, X	X	X	*, X

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Aorta	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X
Bone (femur)	X	X	X	X	X	X
Brain	*, X^	*, X^	*, X†	*, X#	*, X#	*, X‡
Cecum	X	X	X	X	X	X
Cervix	X	X	X	X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						
Gall bladder	X		X	X	X	X
Gross lesions	X	X	X	X	X	X
Harderian gland	X	X		X	X	
Heart	*, X	*, X	*, X	*, X	*, X	*, X
Ileum	X	X	X	X	X	X
Injection site						
Jejunum	X	X	X	X	X	X
Kidneys	*, X	*, X	*, X	*, X	*, X	*, X
Lachrymal gland						
Larynx	X					
Liver	*, X	*, X	*, X	*, X	*, X	*, X
Lungs	X	X	X	X	X	X
Lymph nodes, cervical						
Lymph nodes mandibular	X	X	X	X	X	
Lymph nodes, mesenteric	X	X	X	X	X	X
Lymph nodes, popliteal						
Lymph nodes, retropharyngeal						X
Lymph nodes, unilateral						
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves	X	X	X	X	X	X
Ovaries	X	*, X	*, X	X	X	*, X
Pancreas	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X
Peripheral nerve	X	X	X	X	X	X

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Peyer's patches	X	X	X	X	X	X
Pharynx						
Pituitary	X	*, X	*, X	X	X	*, X
Prostate	X	*, X	*, X	X	X	*, X
Rectum						
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X	X		X	X	
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	*, X	*, X	*, X	*, X	*, X	*, X
Sternum						
Stomach	X	X	X	X	X	X
Testes	*, X	*, X	*, X	*, X	*, X	*, X
Thymus	*, X	X	X	*, X	*, X	X
Thyroid	X	*, X	*, X	X	X	*, X
Tongue						
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus	X	X	X	X	X	X
Vagina	X	X	X	X	X	X
Zymbal gland						

X, histopathology performed; *, organ weight obtained;
^, included anterior commissure, septal nuclei, basal nuclei, cerebral cortex, corpus callosum, internal/external (b) (4) optic tract, amygdala, hippocampus, thalamus, hypothalamus, cerebral peduncles, midbrain, pons, pyramids, cerebellum, reticular formation, trigeminal nuclei, medulla oblongata, and choroid plexus; †, included basal nuclei, cerebral cortex, corpus callosum, internal/external (b) (4) hypothalamus, amygdala, thalamus, hippocampus, optic tract, midbrain, pons, pyramids, cerebellum, medulla oblongata, and choroid plexus; #, included cerebral cortex, subcortical white matter, basal ganglia, cerebellum, and pons; ‡, included cerebral cortex, subcortical white matter, basal ganglia, thalamus, hippocampus, midbrain, cerebellum, and medulla oblongata Note: some organs weighed together

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/s/

CHRISTOPHER E ELLIS
10/28/2015

HANAN N GHANTOUS
10/28/2015
I concur with Dr. Christopher Ellis on the recommendation of approval of ZEPATIER™ (elbasvir and grazoprevir) for marketing in the U.S.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 208-261

Supporting document/s:

Supporting Document	Sponsor Submission Date	CDER Received Date
1	5/28/15	5/28/15

Product: Grazoprevir/Elbasvir Fixed-Dose Combination

Indication: treatment of HCV infection

Applicant: Merck Sharp & Dohme Corp.

Review Division: Division of Antiviral Products

Reviewer: Mark W. Powley, Ph.D.

Supervisor/Team Leader: Hanan Ghantous, Ph.D., DABT

Division Director: Debra B. Birnkrant, M.D.

Project Manager: Nina Mani, Ph.D., MPH

Disclaimer

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1 Executive Summary

1.1 Introduction

Merck Sharp & Dohme Corp. has submitted an NDA to support the fixed dose combination of grazoprevir (GZR or MK-5172; HCV NS3/4A protease inhibitor) and elbasvir (EBR or MK-8742; HCV NS5A inhibitor) for the treatment of chronic HCV infection in patients with or without cirrhosis. The proposed dosing regimen includes 100 mg/day GZR + 50 mg/day EBR for (b) (4) to 16 weeks.

This review focuses on qualification of organic impurities, residual solvents, and elemental impurities. Regulatory decisions utilize recommendations from ICH M7 "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk", ICH Q3A(R2) "Impurities in New Drug Substances", ICH Q3B(R2) "Impurities in New Drug Products", ICH Q3C(R5) "Impurities: Guideline for Residual Solvents", and ICH Q3D "Guideline for Elemental Impurities".

Overall, proposed specifications (or lack of specifications) are considered acceptable from a pharmacology/toxicology perspective. This conclusion is based on the results of general toxicology studies, experimental Ames assays, and/or (quantitative) structure-activity relationship [(Q)SAR] predictions of mutagenicity.

Note that the Sponsor's (Q)SAR approach utilizes predictions from Derek for Windows or Derek Nexus with additional analysis by MultiCASE or Leadscope software on a case by case basis. Regardless of whether impurities were assessed by a single or multiple (Q)SAR programs, the approach is consistent with ICH M7 recommendations during the guideline implementation period.

2 Qualification of Grazoprevir Drug Substance

2.1 Specified Impurities

2.1.1 Organic Impurities

The qualification of specified impurities in the GZR drug substance is based on results from general toxicology studies, experimental Ames assay data, and/or assessment of mutagenic potential using (Q)SAR.

General Toxicology: The (b) (4) impurities were present in drug lots used in a 1-month study in rats (TT#09-1120; reviewed by Dr. Chris Ellis). Using the NOAEL established in the 1-month study, qualified levels of these impurities are deemed adequate to support the proposed specifications. The proposed specification for (b) (4) is acceptable as it does not exceed the ICH Q3A(R2) qualification threshold. Summary information is provided below.

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Table 1. Grazoprevir drug substance organic impurity specifications

Organic Impurities	Toxicology Study Content	NOAEL	Qualified Levels[a]	Proposed Specification
[redacted]				(b) (4)

Genotoxicity: Recommendations for impurities with exposures exceeding the (b) (4) qualification threshold (b) (4) include an Ames assay and an *in vitro* mammalian cell assay. In contrast, (b) (4) indicates that (Q)SAR is sufficient to evaluate the mutagenic potential for impurities with exposures (b) (4) mg/day. Because the highest proposed specification yields a clinical exposure of (b) (4) mg/day (b) (4) (Q)SAR is considered adequate to evaluate the mutagenic potential of the specified impurities. Based on the Sponsor's (Q)SAR assessment, all specified impurities are deemed to lack mutagenic potential.

2.1.2 Residual Solvents

All specified residual solvents in the GZR drug substance are listed in (b) (4) The proposed specification for (b) (4) is within the (b) (4). While proposed specifications for (b) (4) and (b) (4) exceed the respective (b) (4) t, potential clinical exposure are well below the (b) (4) Therefore, there is minimal toxicological concern. Summary information is provided below.

Table 2. Grazoprevir drug substance residual solvent specifications

Residual Solvents	(b) (4) Concentration Limit (Option 1)	(b) (4) PDE (Option 2)	Proposed Specification Concentration	Proposed Specification Clinical Exposure[a]
[redacted]				(b) (4)

2.1.3 Elemental Impurities

The proposed specification for (b) (4) in the GZR drug substance is less than the (b) (4) recommended PDE of (b) (4) µg/day for oral drugs.

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2.2 Unspecified Impurities

In addition to the specified impurities described above, per the Sponsor, *"All identified impurities in each starting material, intermediate, and GZR were also evaluated for structural alerts"*. Impurities predicted positive by (Q)SAR were evaluated in experimental Ames testing or qualified based on comparison with Ames negative structural analogs.

(b) (4)

Several mutagenic impurities are reported by the Sponsor. (b) (4)

(b) (4)

A summary of GZR unspecified impurity structures and Ames results as well as reviews of Ames study reports are included in the Appendix.

3 Qualification of Elbasvir Drug Substance

3.1 Specified Impurities

3.1.1 Organic Impurities

The qualification of specified impurities in the EBR drug substance is based on results from general toxicology studies, experimental Ames assay data, and/or assessment of mutagenic potential using (Q)SAR.

General Toxicology: The (b) (4) impurities were present in drug lots used in a 6-month study in rats (TT#12-6033; reviewed by Chris Ellis). Using the NOAEL established in the 6-month study, qualified levels of these impurities are deemed adequate to support the proposed specifications. The proposed specifications for the remaining specified impurities are acceptable as they do not exceed the ICH Q3A(R2) qualification threshold. Summary information is provided below.

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Table 3. Elbasvir drug substance organic impurity specifications

Organic Impurities	Toxicology Study Content	NOAEL	Qualified Levels[a]	Proposed Specification
[redacted]				(b) (4)

3.1.2 Residual Solvents

Both [redacted] (b) (4) are listed in ICH Q3C(R5). While proposed specifications for each solvent exceed the respective Option 1 limit, potential clinical exposure are well below the Option 2 limits. Therefore, there is minimal toxicological concern. Summary information is provided below.

Table 4. Elbasvir drug substance residual solvent specifications

	ICH Q3C(R5) Limit		Proposed Specification	
Residual Solvents	Concentration Limit (Option 1)	PDE (Option 2)	Concentration	Clinical Exposure[a]
[redacted]				(b) (4)

3.1.3 Elemental Impurities

The proposed specification for [redacted] (b) (4) in the EBR drug substance is less than the [redacted] (b) (4) recommended PDE of [redacted] (b) (4) µg/day for oral drugs.

3.2 Unspecified Impurities

In addition to the specified impurities described above, per the Sponsor, *"All identified impurities in each starting material, intermediate, and drug substance were also evaluated for structural alerts"*. With the exception of [redacted] (b) (4) impurities predicted positive by (Q)SAR were evaluated in experimental Ames testing.

[redacted] (b) (4) is non-mutagenic based on results of experimental Ames testing. [redacted] (b) (4) are mutagenic according to follow-up experimental Ames testing. [redacted] (b) (4) is considered mutagenic based solely on the (Q)SAR evaluation. Published reports

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indicate that (b) (4) are Ames positive. These mutagenic impurities are (b) (4)

Two mutagenic rodent carcinogens, (b) (4) as well as two Ames negative rodent carcinogens, (b) (4)

A summary of EBR unspecified impurity structures and Ames results as well as reviews of Ames study reports are included in the Appendix.

4 Qualification of the Grazoprevir/Elbasvir Drug Product

4.1 Degradants

There are no specified impurities for the grazoprevir/elbasvir drug product. Summary information is provided below.

Table 5. Drug product impurity proposed specifications

Degradant	Qualified Levels	Proposed Specification	
		Release	Shelf Life
Grazoprevir			
individual, unspecified			
total			
Elbasvir			
individual, unspecified			
total			

(b) (4)

(b) (4)

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Appendix

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames) – Summary Data

Table 6. Grazoprevir drug substance unspecified impurities Ames testing results

Impurity	Ames Result	Source

(b) (4)

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/s/

MARK W POWLEY
10/26/2015

HANAN N GHANTOUS
10/26/2015