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APPLICATION NUMBER:

201917Orig1s000

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: 201917
Supporting document/s: 000, 0003, 0005, 0033, 0040, 0044
Applicant's letter date: June 24, 2010
CDER stamp date: June 24, 2010
Product: Telaprevir
Indication: HCV in adult patients with compensated liver disease
Applicant: Vertex Pharmaceuticals Inc.
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1 Executive Summary

1.1 Introduction

Telaprevir (VX-950) is an HCV NS3•4A protease inhibitor developed as a direct acting antiviral agent against the hepatitis C virus. Vertex has submitted an NDA in support of a combination therapy of telaprevir, ribavirin, and pegylated interferon alpha to treat adult patients infected with chronic hepatitis virus who have compensated liver disease. The combination therapy is indicated for both treatment-naïve and treatment-experienced patients. The proposed clinical dose of telaprevir is 750 mg q8hr (2250 mg day).

Telaprevir exists as the S-diastereomer and is converted nonenzymatically, both in vitro and in vivo, to its less active R-diastereomer, VRT-127394. The nonclinical development of telaprevir included numerous formulations. The primary focus of this review is on evaluations using ^{(b) (4)}

1.2 Brief Discussion of Nonclinical Findings

The oral bioavailability of telaprevir was 33 to 52% in rat, 43 to 67% in fasted dog, 70 to 95% in fed dog, and <22% in rabbit. Following an oral dose of ¹⁴C-telaprevir in rat, radioactivity was widely distributed with the highest concentrations found in GI tissues and liver. Radioactivity was also detected in testes at low concentrations. Telaprevir was metabolized by CYP3A4 in vitro. While repeat-dose studies in rat showed induction of CYP3A activity by telaprevir, CYP3A4 activity was not induced in human hepatocytes.

Studies showed qualitatively similar in vivo metabolite profiles in rat, dog, and human. Three metabolites (i.e., VRT-127934, pyrazinoic acid, and VRT-922061) each achieved exposures > 10% of total drug-related exposure in human. All were considered qualified based on their formation in nonclinical studies following oral administration of telaprevir, empirical testing or computational evaluation for genotoxic potential, clinical experience, the clinical indication (i.e., treatment of hepatitis C virus), and because telaprevir will be administered in combination with other toxic drugs (i.e., ribavirin and pegylated interferon). Note that pyrazinoic acid is structurally similar to several molecules associated with rash and pruritus. These effects were reported in clinical trials with telaprevir+ribavirin+pegylated interferon. In addition, a minor clinical metabolite, VRT-841125, was considered to be a skin sensitizer.

No adverse drug-related effects on neurological activity or respiratory parameters were detected in rats. Minor effects occurred in the in vitro cardiotoxicity evaluations but lacked in vivo correlates in dogs or humans.

The following were targets of telaprevir toxicity in pivotal repeat-dose studies in rat and dog:

Hematopoietic System: Hematology effects observed in both rat and dog included decreases in red blood cell associated parameters (e.g., red blood cells, hemoglobin, hematocrit, etc.). A compensatory increase in circulating reticulocytes was also detected. Changes in bone marrow cytology (e.g., decreased total granulocytic cells and M:E

ratio; increased total erythrocytic cells), increased spleen weight, and/or histopathological lesions in spleen (increased red pulp hematopoiesis) occurred and were consistent with the compensatory response to decreases in red blood cell associated parameters. With the exception of increased spleen weight in rat, the hematopoietic effects appeared to be reversible in both rat and dog. Anemia was a common adverse event in clinical trials with telaprevir.

Liver: Liver effects were noted in both species. Effects in the rat included clinical pathology indicators of hepatotoxicity (primarily increased aminotransferases), increased organ weight, and histopathological lesions (hepatocellular hypertrophy; single cell hepatocellular necrosis). These changes were consistent with the induced CYP3A activity and decreased systemic exposure observed in repeat-dose studies. Increased aminotransferases and microscopic lesions persisted through the recovery period. Hepatotoxicity occurred at exposures <0.63-fold those in human at the recommended clinical dose.

Effects in the dog included reversible changes in clinical pathology (no change in aminotransferases) and histopathology (mixed perivascularitis; sinusoidal hypercellularity; increased eosinophilic pigmentation in Kupffer cells). Telaprevir exposures at the no observed adverse effect level (NOAEL) for liver effects were 0.17- to 0.25-fold the exposures in human at the recommended clinical dose.

Indicators of hepatotoxicity (e.g., increased aminotransferases) were not observed in clinical trials.

Male Reproductive System: Drug-related effects on the male reproductive system were observed in the rat for both repeat-dose toxicology and fertility studies. Testicular toxicity was noted as macroscopic (small; soft) and microscopic lesions (degeneration, germinal epithelium; degeneration/necrosis, individual germ cells; degeneration, tubules, multifocal; increased/enlarged residual bodies; retained Step 19 spermatids, stage IX and X tubule; sloughed/necrotic intraluminal germ cells; vacuolation, Sertoli cells) with a correlating decrease in organ weight. Decreased organ weight accompanied by microscopic changes were also detected in the epididymis (exfoliated germ cell; hypospermia; aspermia; exfoliated spermatogenic cells/residual bodies). A decrease in the % of motile sperm as well as an increase in nonmotile sperm count also occurred. These male reproductive effects likely contributed to the changes noted during Caesarian-section evaluation in the fertility study. Notable effects included increased % preimplantation loss, % of dams with nonviable embryos, and % nonviable conceptuses/litter. In general, effects on male reproductive system appear reversible. The NOAEL for reproductive organ toxicity occurred at exposures 0.17-fold the human exposures at the recommended clinical dose. The sperm evaluation was conducted at a single dose level with an exposure 0.30-fold the clinical exposure.

Chronic active vasculitis in the epididymis was observed in a single dog after 9 months of treatment. This lesion occurred in multiple organs in the 9 month dog study and is described in detail below.

The Sponsor notes that the testicular toxicity in rat appeared to be species specific and suggested questionable human relevance for this effect. Measurements of inhibin-b, LH, and FSH from clinical trials were provided to support this claim. Mean changes in these proposed hormonal biomarkers were comparable between telaprevir-treated subjects and those administered placebo. The proposed species-specific nature of the testicular toxicity is biologically plausible but has not been fully demonstrated.

Vasculitis and Secondary Effects: Chronic-active vasculitis was observed only in the dog. This microscopic lesion was observed in stomach, epididymis, heart, and ovary. Vasculitis was not observed in recovery animals. Additional microscopic lesions including several in bone and bone marrow (myelofibrosis; hypocellularity; necrosis; dysplasia, cartilaginous) were considered secondary to the chronic-active vasculitis. Primary and secondary lesions associated with this effect were generally reversible. The drug-induced vasculitis is consistent with canine polyarteritis (Hayes et al., 1989) which is typically considered to have questionable human relevance (Clemo et al., 2003). Vasculitis was not reported in clinical studies with telaprevir, further supporting the species-specific nature of this lesion. An NOAEL for vasculitis was established in the female at exposures 0.17-fold the human exposure at the recommended clinical dose. Vasculitis occurred at all dose levels in males, the lowest dose yielding exposure 0.25-fold of the clinical dose in human.

Telaprevir was not considered genotoxic based on negative results in the in vitro bacterial mutation assay, in vitro mammalian chromosome aberration assay, and in vivo rat micronucleus assay. Because telaprevir was not genotoxic and will be administered to humans for only 12 weeks, carcinogenicity studies were not required.

There were no adverse drug-related effects on embryofetal development in mouse or rat. Embryofetal development was not studied in rabbit due to an inability to achieve substantial systemic exposure. A fertility study in rat found male reproductive system toxicity and effects on Caesarian-section parameters (previously described). While the fertility effects were due, in part, to the male, contributions of the female cannot be ruled out due to study design limitations. Drug-related findings in a perinatal/postnatal evaluation were limited to a decrease in pup weight/litter prior to weaning in offspring from telaprevir-treated dams.

There were multiple impurities present in the final drug substance or drug product. The proposed specifications were deemed acceptable based on a 13-week toxicology study in rat, evaluation of genotoxic potential (i.e., empirical testing or computational analysis), as well as the clinical indication and dosing regimen previously described.

1.3.1 Approvability

The Sponsor submitted sufficient nonclinical safety data to support approval for marketing.

1.3.2 Additional Non Clinical Recommendations

None

(b) (4)



2 Drug Information

2.1 Drug

CAS Registry Number
402957-28-2

Generic Name
Telaprevir

Code Name
VX-950

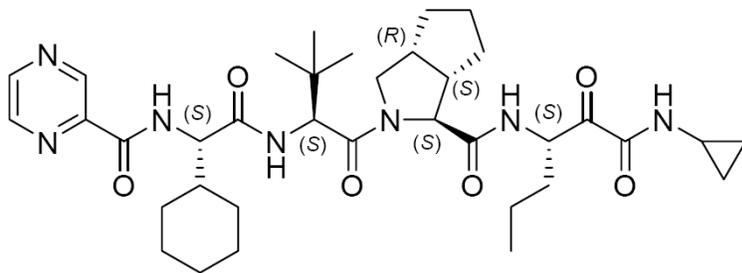
Chemical Name

(1*S*, 3*aR*, 6*aS*)-2-[(2*S*)-2-[[[(2*S*)-cyclohexyl[(pyrazinylcarbonyl)amino]acetyl]amino]-3,3-dimethylbutanoyl]-*N*-[(1*S*)-1-(cyclopropylamino)oxoacetyl]butyl]octahydrocyclopenta[*c*]pyrrole-1-carboxamide

Molecular Formula/Molecular Weight

C₃₆H₅₃N₇O₆ /679.85

Structure or Biochemical Description



Pharmacologic Class

HCV NS3•4A protease inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND#71,832

2.3 Drug Formulation

Table 1. Drug formulation components (taken from Sponsor submission)

Component	Quality Reference	Component Function	Amount per Tablet (mg)	Content (%w/w)
	(b) (4)			
Telaprevir drug substance	Section 3.2.S.4.1	Active pharmaceutical ingredient	375	37.3
Hypromellose acetate succinate (HPMCAS)	USP/NF ^c			(b) (4)
Sodium lauryl sulfate (SLS)	USP/NF			
(b) (4)	USP/NF			
	USP/NF			
	USP			
Dibasic calcium phosphate, anhydrous	USP			
Microcrystalline cellulose	USP/NF			
Croscarmellose sodium	USP/NF			
Colloidal silicon dioxide	USP/NF			
Sodium stearyl fumarate	USP/NF			
(b) (4)	DMF No. (b) (4)			
	USP			
Total	--	--	1005.7	100.0
				(b) (4)

Table 2. Film coating components (taken from Sponsor submission)

(b) (4)				
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2.4 Comments on Novel Excipients

Although some excipients are present at levels exceeding those listed for marketed oral products, all inactive drug product components are listed in the FDA’s Inactive Ingredient Database. Given the indication and proposed dosing regimen, the levels of individual components are considered acceptable.

2.5 Comments on Impurities/Degradants of Concern

Regulatory recommendations in regards to impurities are provided by “ICH Q3A(R2) – Impurities in New Drug Substance”, “ICH Q3B(R2) – Impurities in New Drug Products”, and “Draft FDA Guidance for Industry – Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches”.

General Toxicology: A 13-week study in rats with VX-950 containing spiked impurities (i.e., (b) (4)) was the basis of general toxicity qualification. Toxicities observed with 1000 mg/kg/day telaprevir spiked with impurities were consistent with 1000 mg/kg/day telaprevir alone. While it is preferable to calculate qualified levels using an NOAEL, adverse effects were observed at all doses in this study. In the absence of an NOAEL and in consideration of the indication as well as the dosing regimen, it is acceptable to base calculations on the highest dose level tested. Doing so provides qualified levels of impurities that exceed the Sponsor's proposed specifications.

Note that VRT-127394 is qualified based on its presence as a major telaprevir metabolite.

Genetic Toxicology: The genotoxic potential of (b) (4) was evaluated in an Ames test. Because this impurity is also a major metabolite of telaprevir, it was present at substantial concentrations in the in vivo micronucleus study and likely formed in the in vitro chromosomal aberration test. On the basis of negative results in all these studies, (b) (4) is considered to be non-genotoxic.

(b) (4) was demonstrated to be non-genotoxic in an Ames test and in vitro chromosomal aberration test.

(b) (4) was also evaluated for genotoxicity by the Sponsor in two Ames tests and an in vitro mammalian cell chromosomal aberration test. While negative in the Ames tests, (b) (4) was positive for chromosomal aberrations. There are also two publications describing Ames tests with (b) (4) however, due to inadequate study designs these are not acceptable for regulatory purposes. As such it is being regulated as a potentially genotoxic impurity. The acceptance criterion is being set as close as is reasonably practical to the threshold of toxicological concern described in "Draft FDA Guidance for Industry – Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches". Given the clinical indication and proposed dosing regimen, the Sponsor's proposed specification for (b) (4) is acceptable.

(b) (4) were evaluated for genotoxic potential using computational methodology. The Sponsor's analysis with Derek for Windows did not identify genotoxic structural alerts in any of these impurities. The Draft FDA Guidance recommends no further action for impurities below the ICH Q3A(R2) and Q3B(R2) qualification threshold that lack structural alerts. Although two impurities have specifications greater than the ICH qualification threshold, the computational analysis is acceptable for qualification because of the indication and proposed dosing regimen.

2.6 Comments on Metabolites of Concern

Regulatory recommendations in regards to metabolites are provided by "ICH M3 (R2) – Guidance on Nonclinical Safety Studies for the Conduct of Clinical Trials and Marketing Authorization for Pharmaceuticals" and "FDA Guidance for Industry – Safety Testing of Drug Metabolites".

Table 3 summarizes metabolite exposures as % of total drug-related material following single and repeat dosing of VX-950 in the clinic (Study no. VX05-950-104EU; table taken from Sponsor report). Note that $AUC_{0-4\text{ hr}}$ is not an optimal basis of comparison since T_{max} was up to 4 hr. This suggests AUC calculated for such a short duration is unlikely to represent true exposure as it will not adequately cover the elimination phase. There were two metabolites that reached the ICH M3(R2) threshold of >10% of drug-related exposure following a single dose and three metabolites that reached the threshold at presumed steady-state. Other metabolites were detected but did not approach this threshold. The three metabolites reaching the stated threshold at steady-state are VRT-127394, pyrazinoic acid, and VRT-0922061. Nonclinical data supporting the safety of these metabolites is presented below.

Table 3. Clinical metabolite profile

Metabolite	% of TDM			
	Day 1		Day 85	
	$AUC_{0-4\text{hr}}$	C_{max}	$AUC_{0-4\text{hr}}$	C_{max}
PZA	2	4	22	23
VRT-126036	2	3	1	1
VRT-127394	20	22	22	20
VRT-753137	6	6	4	4
VRT-753138	1	1	1	1
VRT-753130	1	1	1	1
VRT-842291	0	0	0	1
VRT-0922061	3	4	11	10
VRT-922076	0	0	0	0
Telaprevir	65	60	37	38

N=11; TDM: Total drug related material quantified

VRT-127394: In addition to being a major clinical and nonclinical metabolite, this molecule is an epimer of telaprevir. At the highest dose tested in a 9-month dog study, systemic exposure exceeded that of human. Achieving equivalent exposure at the nonclinical NOAEL compared to human at the recommended clinical dose is preferred; however, in consideration of the indication (i.e., hepatitis C virus) as well as the proposed dosing regimen (i.e., VX-950 will be co-administered with the toxic drugs ribavirin and pegylated interferon), it is acceptable to base general toxicology qualification on the highest dose level tested in dog. Systemic exposure at the NOAEL for embryofetal development in mice was nearly equivalent to that observed clinically. In contrast, human systemic exposure was disproportionately greater than rat for the highest dose tested in both the 6-month and embryofetal development studies. The genotoxic potential of VRT-127394 was evaluated in an Ames test. Because this impurity is also a major metabolite of VX-950, it was present at substantial concentrations in the in vivo micronucleus study and likely present in the in vitro chromosomal aberration test. On the basis of negative results in all these studies, VRT-127394 is considered to be non-genotoxic. Data is summarized in Table 4.

Table 4. Animal/human exposure margins for VRT-127394

Species	AUC _{0-t} (ng·hr/mL)		Animal/Human Exposure Margin ^a	
	male	female	male	female
rat ^b	2343	4889	0.15x	0.32x
pregnant rat ^c	-	20,200	-	0.43x
pregnant mouse ^d	-	38,970	-	0.85x
dog ^e	95,718	81,750	2.10x	1.8x

^a AUC_{0-8 hr} = 15,310 ng·hr/mL; AUC_{0-24 hr} = 45,930 ng·hr/mL (calculated as AUC_{0-8 hr} x 3); Study no. VX-950-TiDP24-C208; Day 57 data for clinical dose (2250 mg/day)

^b AUC_{0-8 hr}; Study no. VX-950-TX-020; Day 182 data for highest dose tested (300 mg/kg/day BID)

^c AUC_{0-24 hr}; Study no. VX-950-TX-018; presumed Gestation Day 17 (1st dose administered on Day 7) data for highest dose tested (500 mg/kg/day BID)

^d AUC_{0-24 hr}; Study no. VX-950-TX-022; presumed Gestation Day 15 (1st dose administered on Day 6) for NOAEL (1000 mg/kg/day BID)

^e AUC_{0-24 hr}; Study no. VX-950-TX-021; Day 266 data for highest dose tested (100 mg/kg/day BID)

Pyrazinoic Acid: *Pyrazinoic acid is also a major metabolite of pyrazinamide, a marketed treatment for tuberculosis (information below regarding pyrazinamide is from Rifater® label). Pyrazinamide was negative for bacterial mutagenicity and was not carcinogenic in rodents (although evaluation in female mouse was inadequate). However, chromosomal aberrations were induced in vitro following treatment with pyrazinamide and in vivo and in lymphocytes of patients treated with combinations of rifampin, isoniazid, pyrazinamide, and streptomycin, rifampin, isoniazid, and pyrazinamide. Nonclinical reproductive toxicity studies have not been conducted with pyrazinamide. Among the clinical adverse effects noted were hepatotoxicity as well as rash and pruritus. Rash and pruritus are common adverse events of telaprevir+ribavirin+pegylated-interferon. Structurally similar compounds include niacin and a number of drugs containing a pyrazinoic acid like moiety that could be released during metabolic conversion. These drugs include amiloride, bortezomib, eszopiclone, and glipizide. All the structurally similar molecules are also associated with rash and pruritus. While the Sponsor indicates that peak clinical concentrations of pyrazinoic acid are greater for pyrazinamide compared to telaprevir, the possible contribution of this metabolite to telaprevir associated rash and pruritus cannot be ruled out.*

The animal/human exposure margin for pyrazinoic acid was 0.49 in the 6-month rat study and 0.64 in the 13-week dog study (data summarized in Table 5). These exposure margins were based on the highest dose levels tested in the nonclinical studies. Following a single dose of telaprevir, mice had slightly greater exposure than humans administered the recommended clinical dose. Although the data was generated following a single dose in non-pregnant animals, the results show that mice have the capacity to form pyrazinoic acid and suggest that the metabolite would be formed in the embryofetal development study. Taking into account the data for pyrazinamide and telaprevir as well as the indication and proposed dosing regimen, the metabolite is considered qualified.

Table 5. Animal/human exposure margins for pyrazinoic acid

	AUC _{0-4 hr} (nmol·hr/mL)	Animal/Human Exposure Margin ^a
rat ^b	5.58	0.49x
mouse ^c	12.4	1.1x
dog ^d	7.27	0.64x

^a AUC_{0-4 hr} = 11.4 nmol·hr/mL; Study no. VX-05-950-104EU; Day 85 data for clinical dose (750 mg q8hr)

^b Study no. VX-950-TX-020; Day 91 for highest dose tested (300 mg/kg/day BID)

^c Study no. VX-950-TX-017; Day 43 data for highest dose tested (100 mg/kg/day BID)

^d Study no. VX-950-TX-010; Day 1 data for highest dose tested (2000 mg/kg BID)

VRT-922061: *Compared to telaprevir, this metabolite had little pharmacologic activity. A computation evaluation of genotoxic potential did not identify any structural alerts. Because exposure to this pharmacologically inactive metabolite was marginal in humans (i.e., only 11% compared to the >10% drug-related exposure threshold), and taking into account the clinical indication and proposed dosing regimen for telaprevir, further evaluation to qualify the metabolite was not deemed necessary.*

2.7 Proposed Clinical Population and Dosing Regimen

Telaprevir is indicated for patients with HCV genotype 1. The dosing regimen is 750mg q8hr (taken with food) ribavirin, and pegylated interferon alfa for 12 weeks. Ribavirin and pegylated interferon alfa are administered for an additional 12 or 36 weeks.

2.8 Regulatory Background

IND#71,832 was originally submitted to DAVP in 2005. NDA#201,917 was provided as a rolling submission in 2010 with priority review.

3 Studies Submitted

3.1 Studies Reviewed

Safety Pharmacology

Cardiovascular

Study no. VX-950-TX-005	Effects on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers
Study no. VX-950-TX-006	Effects on hERG Tail Current Recorded from Stably Transfected HEK193 Cells
Study no. VX-950-TX-015	The Cardiovascular Effects of Oral Administration of VX-950 in Conscious, Telemetered Beagle Dogs – A Repeat Study

Neurological

Study no. VX-950-TX-012	The Effects of VX-950 in the Irwin Test in Sprague-Dawley Rats
Study no. VX-950-TX-013	Effect of VX-950 on Locomotor Activity in the Rat

Respiratory

Study no. VX-950-TX-011

The Effects of VX-950 on Respiration Rate and Tidal Volume in Sprague Dawley Rats

PK/ADME

Study no. B207

Effect of Food on the Pharmacokinetics of VX-950 Following Oral Administration of VX-950 (b) (4)

Study no. VX-950-DMPK-DM-016

(b) (4) to Male Beagle Dogs

Absorption, Metabolism, Distribution, and Excretion of ¹⁴C-VX-950 Following Oral or Intravenous Administration to Intact and Bile Duct-Cannulated Rats

Study no. C105

Pharmacokinetic Characteristics of VX-950 in New Zealand White Rabbits Following Single Oral or Intravenous Dose of VX-950 (b) (4)

Study no. VX-950-DMPK-PK-023

Lacteal and Placental Transfer of VX-950 Following Administration of a Single Oral Dose to Lactating and Pregnant Rats

Study no. VX-950-DMPK-PK-024

Placental Transfer of VX-950 Following Administration of a Single Oral Dose to Pregnant Mice

Study no. 6536-464

Pharmacokinetics, Excretion/Mass Balance, Bile Excretion/Mass Balance and Volatile Collections in Male Beagle Dogs After a Single Administration of [¹⁴C]VX-950

Study no. 6536-307

Evaluation of CYP450 Induction Using Primary Cultures of Human Hepatocytes

Study no. 03-VERT.Po9R1~
Report 5CYP Reaction Phenotyping of VX-950 Using Supersomes
In Vitro Binding of ¹⁴C-VX-950 to Mouse, Rat, Dog, and Human Plasma Proteins, and Protein Binding Displacement Interactions Between VX-950 and Ritonavir or Warfarin

Study no. 6536-430

General ToxicologySingle Dose

Study no. VX-950-TX-007

Acute Oral Toxicity and Toxicokinetic Study of VX-950 in Mice

Study no. VX-950-TX-008

Acute Oral Toxicity and Toxicokinetic Study of VX-950 in Sprague-Dawley Rats

Repeat Dose

Study no. VX-950-TX-001

Twenty-Eight Day Toxicity and Toxicokinetic Study on VX-950 in Sprague-Dawley Rats with a Twenty-Eight Day Recovery Period

Study no. VX-950-TX-014

Twenty-Eight Oral Day Toxicity and Toxicokinetic Study of VX-950 in Beagle Dogs with a Fourteen-Day Recovery Period – A Repeat Study

Study no. VX-950-TX-016	A Thirteen-Week Oral Toxicity and Toxicokinetic Study of VX-950 in Sprague-Dawley Rats with a Twenty-Eight Day Recovery Period
Study no. FXU00003	A Low Dose Toxicity and Toxicokinetic Study in Male Sprague-Dawley Rats
Study no. VX-950-TX-017	A Thirteen-Week Oral Toxicity and Toxicokinetic Study of VX-950 in Beagle Dogs with a Twenty-Eight Day Recovery Period
Study no. VX-950-TX-020	VX-950: A Six-Month Oral Toxicity and Toxicokinetic Study in Sprague-Dawley Rats with a Three-Month Recovery Period
Study no. VX950-TX-021	VX-950: A Nine-Month Oral Toxicity and Toxicokinetic Study in Beagle Dogs with a Three-Month Recovery Period

Genetic Toxicology

Study no. VX-950-TX-003	Bacterial Reverse Mutation Assay
Study no. VX-950-TX-004	<i>In Vitro</i> Mammalian Chromosome Aberration Test
Study no. VX-950-TX-010	<i>In Vivo</i> Mammalian Erythrocyte Micronucleus Test

Developmental and Reproductive Toxicology

Study no. VX-950-TX-018	Oral (Gavage) Developmental Toxicity Study in Rats
Study no. VX-950-TX-019	Oral (Gavage) Fertility and General Reproduction Study in Rats
Study no. VX-950-TX-022	Oral (Gavage) Dose-Range Developmental Toxicity Study in Mice
Study no. VX-950-TX-023	Oral (Gavage) Developmental Toxicity Study in Mice
Study no. VX-950-TX-025	Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral/Functional Evaluation

Special Toxicology

Metabolites

Study no. E046	Activity of VRT-0842291 (M12) and VRT-0922061, Two Metabolites of Telaprevir (VX-950), in HCV NS3 Protease Enzyme and Replicon Assays
Study no. VRT-127394-TX-001	Bacterial Reverse Mutation Assay

Impurities

Study no. VX-950-TX-036	Impurity and Degradation Product Qualification Toxicity Study Conducted with Spiked Material Administered by Oral Gavage to Sprague-Dawley Rats for Thirteen Weeks with a 28-Day Recovery Period
Study no. VRT- ^{(b) (4)} -TX-001	Bacterial Reverse Mutation Test
Study no. VRT- ^{(b) (4)} -TX-002	<i>In Vitro</i> Mammalian Chromosome Aberration Test in Human Lymphocytes
Study no. ^{(b) (4)} -TX-001	<i>Salmonella</i> Plate Incorporation Mutagenicity Assay

Study no. (b) (4)-TX-002
 Study no. (b) (4)-TX-003

Bacterial Reverse Mutation Assay
In Vitro Mammalian Chromosome Aberration Test

Mechanistic

Study no. 1022388

(b) (4) Pharmacology Data Report on
 Compounds (b) (4) VX-950 Bulk Drug Substance to
 (b) (4), VRT-127394 for Vertex Pharmaceuticals
 Incorporated

Study no. AL-4355-G

Binding Study of M-424 (VX-950) to Dog Androgen
 Receptor

Study no. 9R474

An Evaluation of Effects of MP-424 (VX-950) on Human
 Erythrocytes *In Vitro*

Antigenicity

Study no. VX-950-TX-028

Assessment of Skin Sensitization Potential Using the Local
 Lymph Node Assay in the Mouse

Study no. VRT-126032-TX-014

Assessment of Skin Sensitization Potential Using the Local
 Lymph Node Assay in the Mouse

Study no. VRT-841125-TX-001

Assessment of Skin Sensitization Potential Using the Local
 Lymph Node Assay in the Mouse

Study no. VRT-841125-TX-002

A Sensitization Study of VRT-841125 and VRT-126032
 Administered by the Dermal Route to Guinea Pigs

3.2 Studies Not Reviewed

Studies that were repeated as well as those conducted using substances or formulations deemed irrelevant to the final telaprevir drug product were not reviewed.

3.3 Previous Reviews Referenced

Several genetic toxicology studies were reviewed under IND#71,832.

4 Pharmacology

4.1 Primary Pharmacology

See virology review.

4.2 Secondary Pharmacology

None

4.3 Safety Pharmacology

Cardiovascular

Title: Effects on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibers (Study no. VX-950-TX-005)

Key Study Findings

Action potential duration at 60% and 90% repolarization, maximum rate of depolarization, upstroke amplitude, and resting membrane potential were evaluated in isolated dog Purkinje fibers perfused with 1, 10, and 50 μ M VX-950. A slight increase in action potential duration at 90% repolarization occurred at the high-dose but was considered of questionable biological relevance.

Title: Effects on hERG Tail Current Recorded from Stably Transfected HEK193 Cells (Study no. VX-950-TX-006)

Key Study Findings

hERG potassium channel tail current was evaluated in HEK293 cells stably transfected with hERG cDNA perfused with 8, 30, and 80 μ M VX-950. Inhibition of hERG tail current was observed ($IC_{25} = 54.95 \mu$ M; $IC_{50} > 80 \mu$ M).

Title: The Cardiovascular Effects of Oral Administration of VX-950 in Conscious, Telemetered Beagle Dogs – A Repeat Study (Study no. VX-950-TX-015)

Key Study Findings

Clinical signs, arterial blood pressure, heart rate, ECGs, and toxicokinetics were evaluated in telemetered Beagle dogs orally administered 25, 75, and 250 mg/kg VX-950. Adverse effects were limited to VX-950-related emesis observed within 2 hr post-dose in the mid- and high-dose groups.

Neurological

Title: The Effects of VX-950 in the Irwin Test in Sprague-Dawley Rats (Study no. VX-950-TX-012)

Key Study Findings

Irwin test parameters (gross behavioral and physiological state) were evaluated in male Sprague-Dawley rats orally administered 100, 300, and 1000 mg/kg VX-950. Although minor and transient, drug-related effects on cage dispersion, vocalization, and decreased startle response were primarily observed in the high dose group. These effects were not considered to be adverse.

Title: Effect of VX-950 on Locomotor Activity in the Rat (Study no. VX-950-TX-013)**Key Study Findings**

Locomotor activity (total, fine, ambulatory, and rearing) was evaluated in male Sprague-Dawley rats orally administered 100, 300, and 1000 mg/kg VX-950. There were no drug-related effects observed.

Respiratory**Title: The Effects of VX-950 on Respiration Rate and Tidal Volume in Sprague Dawley Rats (Study no. VX-950-TX-011)****Key Study Findings**

Respiration rate and tidal volume were evaluated in male Sprague-Dawley rats orally administered 100, 300, and 1000 mg/kg VX-950. There were no drug-related effects observed.

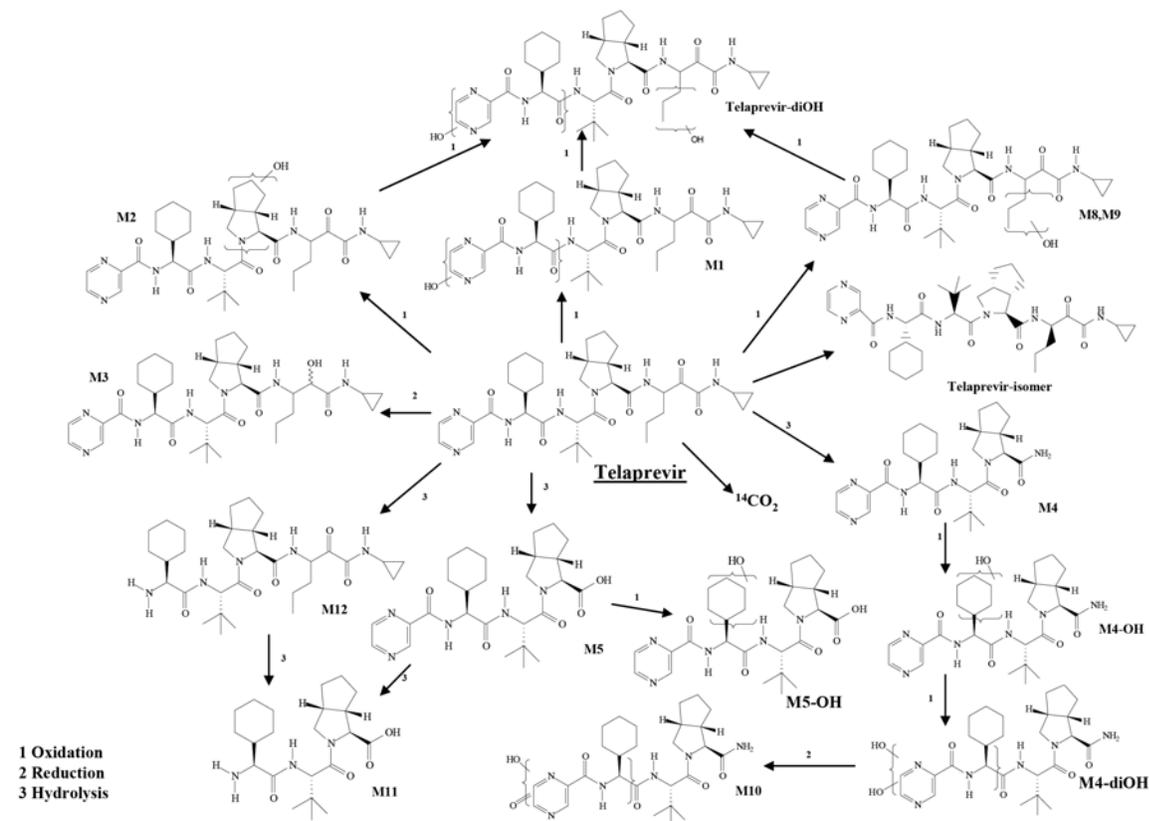
5 Pharmacokinetics/ADME/Toxicokinetics**5.1 PK/ADME**

Figure 1. VX-950 metabolism in rat, dog, and human

Title: Effect of Food on the Pharmacokinetics of VX-950 Following Oral Administration of VX-950 Solid Dispersion Suspended in 1% HPMCAS/10% Vit E TPGS/0.01% Simethicone in Water to Male Beagle Dogs (Study no. B207)

Key Study Findings

Pharmacokinetics were evaluated in fasted and fed male Beagle dogs administered a single oral dose of 25 and 250 VX-950. (Study no B207). Oral bioavailability at 250 mg/kg was 43 to 67% for fasted animals and 70 to 95% for fed animals. The $t_{1/2}$ in plasma for was 1.0 to 2.2 hr at 25 mg/kg and 2.5 to 4.3 hr at 250 mg/kg. Note that i.v. data from Study B176 was used to calculate bioavailability.

Title: Absorption, Metabolism, Distribution, and Excretion of ^{14}C -VX-950 Following Oral or Intravenous Administration to Intact and Bile Duct-Cannulated Rats (Study no. VX-950-DMPK-DM-016)

Key Study Findings

Pharmacokinetics, absorption, metabolism, distribution, and excretion were evaluated in intact and bile-duct cannulated Sprague-Dawley rats administered a single dose of 6 mg/kg (i.v.) or 30 mg/kg (oral) of radiolabeled VX-950. Distribution was also evaluated in Long Evans rats administered a single oral dose of 30 mg/kg radiolabeled VX-950. Oral bioavailability was 33.4% in males and 52% in females. Following oral dosing the $t_{1/2}$ in plasma for males was 3.1, 4.9, and 27 hr for VX-950, VRT-127394, and total radioactivity, respectively. For females, $t_{1/2}$ in plasma was 2.7 hr for VX-950, 3.35 hr for VRT-127394, and 17 hr for total radioactivity. In Sprague-Dawley rats, radioactivity was widely distributed throughout the body after oral administration with the highest concentrations found in GI tissues and liver. Although at lower concentrations, radioactivity was also detected in testes. Peak concentrations were generally achieved by 24 hr with tissue $t_{1/2}$ ranging from 25.5 hr (prostate) to 70.1 hr (liver). Radioactivity exposures in skin and eyes was similar for both rat strains suggesting minimal binding to melanin. As described above, VX-950 was extensively metabolized. Metabolites formed are included in Figure 1 (taken from Sponsor submission). Excretion occurred primarily in feces (46 to 49%) followed by bile (23 to 32%), expired air as CO_2 (18 to 22%), and urine (4 to 6%).

Title: Pharmacokinetic Characteristics of VX-950 in New Zealand White Rabbits Following Single Oral or Intravenous Dose of VX-950 as Solid Dispersion (Study no. C105)

Key Study Findings

Pharmacokinetics were evaluated in male New Zealand rabbits administered a single dose of 6 mg/kg (i.v.) or 6, 25, 75, and 250 mg/kg (oral) VX-950. Oral bioavailability was $\leq 21.7\%$. The $t_{1/2}$ in plasma was 0.32 to 2.1 hr for oral dosing and 0.98 hr following i.v. administration.

Title: Lacteal and Placental Transfer of VX-950 Following Administration of a Single Oral Dose to Lactating and Pregnant Rats (Study no. VX-950-DMPK-PK-023)

Key Study Findings

Maternal and fetal toxicokinetics were evaluated in Sprague-Dawley rats orally administered 250 mg/kg VX-950. Pregnant females were dosed on approximately gestation Day 16 while lactating females were dosed approximately 7 to 9 days postpartum. Systemic exposure in fetus was 5 % of that in human. Concentrations in milk were 2-fold that observed in maternal plasma.

Table 6. Placental and lactational transfer in rat

Transfer	VX-950	
	AUC _{0-24 hr} (ng·hr/g)	C _{max} (ng/g)
placental ^a		
maternal plasma	131,000	10,300
fetal plasma	6190	522
lactational ^b		
maternal plasma	112,0000	10,500
milk	222,000	16,700
pup plasma	68.1	5.16

^a single dose administered on presumed Gestation Day 16

^b single dose administered ~10 days postpartum

Title: Placental Transfer of VX-950 Following Administration of a Single Oral Dose to Pregnant Mice (Study no. VX-950-DMPK-PK-024)

Key Study Findings

Maternal and fetal toxicokinetics were evaluated in pregnant CD-1 mice orally administered 500 mg/kg VX-950 16 days post-conception. Systemic exposure in fetus was 7% of that in human for VX-950 and VRT-127394.

Table 7. Placental transfer in mouse

Transfer	VX-950	
	AUC _{0-24 hr} (ng·hr/g)	C _{max} (ng/g)
placental ^a		
maternal	120,000	11,700
fetal	8190	614

^a single dose administered on presumed Gestation Day 16

Title: Pharmacokinetics, Excretion/Mass Balance, Bile Excretion/Mass Balance and Volatile Collections in Male Beagle Dogs After a Single Administration of [¹⁴C]VX-950 (Study no. 6536-464)

Key Study Findings

Pharmacokinetics, absorption, distribution, metabolism, and excretion were evaluated in intact and bile-duct cannulated male Beagle dogs administered a single dose of 1 mg/kg (i.v.) or 30 mg/kg (oral) of radiolabeled VX-950. Following oral dosing the $t_{1/2}$ in plasma for males was 7.3, 4.5, and 76 hr for VX-950, VRT-127394, and total radioactivity, respectively. Metabolites formed in dog are included in Figure 1 (figure taken from Sponsor submission). Excretion following oral dosing in intact animals occurred primarily in feces (80%) with little contribution from urine. Bile-cannulated data was not considered reliable.

Title: Evaluation of CYP450 Induction Using Primary Cultures of Human Hepatocytes (Study no. 6536-307)

Key Study Findings

CYP induction capacity of VX-950 and VRT-127394 were evaluated in human hepatocytes. Neither substrate induced the activity of CYP1A1, CYP2C, or CYP3A.

Title: CYP Reaction Phenotyping of VX-950 Using Supersomes (Study no. 03- VERT.Po9R1~Report 5)

Key Study Findings

CYP reaction phenotyping was evaluated in human supersomes expressing major human CYP450 enzymes. Substantial VX-950 metabolizing capacity was detected only in CYP3A4.

Title: *In Vitro* Binding of ¹⁴C-VX-950 to Mouse, Rat, Dog, and Human Plasma Proteins, and Protein Binding Displacement Interactions Between VX-950 and Ritonavir or Warfarin (Study no. 6536-430)

Key Study Findings

Plasma protein binding was evaluated in multiple species. Binding was similar in plasma protein from mouse (53 to 71%), rat (82 to 85%), dog (62 to 67%), and human (59 to 76%). VX-950 bound to both human serum albumin and α_1 -acid glycoprotein. VX-950 binding in human plasma proteins was displaced by both ritonavir and warfarin.

5.2 Toxicokinetics

Analyte concentrations in matrix from various collection sites were determined using LC-MS/MS. Details from pivotal repeat-dose toxicology as well as developmental and reproductive toxicology studies are summarized below. Discussion of nonclinical data regarding major clinical metabolites is provided in section 2.6 (Comments on Metabolites of Concern).

Title: A Six-Month Oral Toxicity and Toxicokinetic Study in Sprague-Dawley Rats with a Three-Month Recovery Period (Study no. VX-950-TX-020)

Key Study Findings

Toxicokinetics were evaluated in Sprague-Dawley rats following oral administration of 30, 100, and 300 mg/kg/day of VX-950 for 6 months (BID doses separated by at least 8 hr). Compared to males, systemic exposure in females was up to ~2.5-fold higher. AUC_{0-8 hr} values at 100 and 300 mg/kg/day were greater on Day 1 compared to later sampling periods. A decrease in systemic exposure following repeat-dosing is consistent with enzyme induction. This is supported by both increased CYP activity noted in the 13-week study as well as liver histopathology commonly found in the repeat dose studies. Increasing dose generally resulted in an approximately dose-proportional or slightly less than dose-proportional increase in systemic exposure for plasma. In contrast, systemic exposure in liver was generally less than dose-proportional in liver tissue. Compared to orbital sinus plasma, systemic exposure values were greater for hepatic vein plasma and liver tissue suggesting preferential hepatic distribution. Toxicokinetic data for non-pregnant rat is summarized in Tables 8 and 9.

Table 8. VX-950 plasma toxicokinetics in non-pregnant rat

Dose (mg/kg/day)	Day	VX-950			
		AUC _{0-8 hr} (ng·hr/mL)		C _{max} (ng/mL)	
		male	female	male	female
30	1	1066	2218	465	864
	91	608	1190	245	541
	182	844	1910	503	703
100	1	6175	10,559	1757	3050
	91	2751	4727	1165	1500
	182	3271	5286	1770	2497
300	1	22,876	35,297	5650	6560
	91	8274	12,318	3227	4647
	182	10,006	16,368	4550	7223

Table 9. VX-950 plasma vs. tissue toxicokinetics in non-pregnant rat^a

Dose (mg/kg/day)	site ^b	VX-950			
		AUC _{0-8 hr} (ng·hr/mL)		C _{max} (ng/mL)	
		male	female	male	female
30	os	1117	1688	457	1020
	hpv	2160	3261	1210	2040
	liv	9907	15,826	4433	7867
100	os	4306	7641	1470	2380
	hpv	9116	12,234	4800	4270
	liv	31,032	47,349	13,500	15,633
300	os	7551	16,126	1570	3660
	hpv	13,399	31,078	3300	6030
	liv	40,005	60,307	11,400	14,800

^a Day 183 data^b os = orbital sinus plasma; hpv = hepatic portal vein plasma; liv = liver tissue**Title: Oral (Gavage) Developmental Toxicity Study in Rats (Study no. VX-950-TX-018)****Key Study Findings**

Toxicokinetics were evaluated in Sprague-Dawley rats following oral administration of 50, 150, 250, and 500 mg/kg/day of VX-950 (BID doses separated by at least 8 hr). Pregnant females were dosed from presumed Gestation Day 7 to 17. The kinetic profile in pregnant rats was similar to that generated in non-pregnant rats. Repeat dosing resulted in a lower AUC_{0-24 hr}. Increasing dose led to a dose-proportional or slightly less than dose-proportional increase in systemic exposure. Data is summarized in below.

Table 10. VX-950 plasma toxicokinetics in pregnant rat

Dose (mg/kg/day)	Day ^a	VX-950	
		AUC _{0-24 hr} (ng·hr/mL)	C _{max} (ng/mL)
50	7	15,400	1990
	17	9870	1310
150	7	41,300	5070
	17	34,300	2340
250	7	69,200	5400
	17	38,100	3850
500	7	98,300	10,000
	17	51,900	5520

^a presumed gestation day (1st dose administered on Day 7)^b AUC_{0-12 hr}

Title: Acute Oral Toxicity and Toxicokinetic Study of VX-950 in Mice (Study no. VX-950-TX-007)

Key Study Findings

Toxicokinetics were evaluated in non-pregnant CD-1 mouse following a single oral dose 100, 500, and 1000 mg/kg (VX-950-TX-007). There were no substantial differences in systemic exposure between male and females. Overall, increasing dose resulted in a less than dose-proportional increase in systemic exposure. An increase in VX-950 dose led to a greater than dose-proportional increase in $AUC_{0-8\text{ hr}}$ between 100 and 500 mg/kg but approximately dose-proportional increase between 500 and 1000 mg/kg. The increase in C_{\max} values were approximately dose-proportional across the dose range. Data is summarized below.

Table 11. VX-950 plasma toxicokinetics in non-pregnant mouse

Dose (mg/kg/day)	Day	VX-950			
		$AUC_{0-8\text{ hr}}$ (ng·hr/mL)		C_{\max} (ng/mL)	
		male	female	male	female
100	1	11,400	12,900	5160	6030
500	1	38,800	35,900	11,600	13,500
1000	1	50,700	71,700	17,500	20,300

Title: Oral (Gavage) Dose-Range Developmental Toxicity Study in Mice (Study no. VX-950-TX-022)

Key Study Findings

Toxicokinetics were evaluated in pregnant CD-1 mice following oral administration of 100, 300, 600, and 1000 mg/kg/day (BID doses separated by at least 8 hr). Repeat dosing resulted in a decrease in $AUC_{0-24\text{ hr}}$ at doses ≥ 300 mg/kg/day. Pregnant females were dosed from presumed Gestation Day 6 to 15. This suggests possible enzyme induction as was observed for rat. In general, increasing dose resulted in a less than dose-proportional increase in VX-950 systemic exposures. Data is summarized below.

Table 12. VX-950 plasma toxicokinetics in pregnant mouse

Dose (mg/kg/day)	Day ^a	VX-950	
		AUC _{0-24 hr} (ng·hr/mL)	C _{max} (ng/mL)
100	6	26,050	4610
	15	24,494	3193
300	6	107,021	8767
	15	52,904	4507
600	6	159,082	14,800
	15	85,353	8517
1000	6	251,665	13,867
	15	157,712	10,363

^a presumed gestation day (1st dose administered on Day 6)

Title: *In Vivo* Mammalian Erythrocyte Micronucleus Test (Study no. VRT-950-TX-010)

Key Study Findings

Toxicokinetics were evaluated in ICR mice administered 2 doses of 500, 1000, and 2000 mg/kg of VX-950 (BID doses separated by 3 hr). Data is summarized below.

Table 13. VX-950 plasma toxicokinetics in micronucleus assay

Dose (mg/kg/day)	Day	VX-950			
		AUC _{0-15 hr} (ng·hr/mL)		C _{max} (ng/mL)	
		male	female	male	female
500	1	49,400	49,500	11,500	13,800
1000	1	88,100	66,500	19,000	14,300
2000	1	133,000	140,000	23,600	18,500

Title: A Nine-Month Oral Toxicity and Toxicokinetic Study in Beagle Dogs with a Three-Month Recovery Period (Study no. VX-950-TX-021)

Key Study Findings

Toxicokinetics were evaluated in Beagle dogs following oral administration of 25, 50, and 100 mg/kg/day of VX-950 for 9 months (BID doses separated by at least 8 hr). Although males generally had slightly higher values, there were no substantial sex differences in systemic exposure for either plasma or liver tissue. AUC_{0-24 hr} values were slightly greater in later sampling periods compared to Day 1 suggesting limited potential for accumulation. Enzyme inhibition, as observed in the 13-week study, may be partially responsible for the increased

systemic exposure with repeat dosing. Increasing dose generally resulted in a greater than dose-proportional increase in $AUC_{0-24\text{ hr}}$ and C_{\max} values in plasma and liver. Systemic exposure values were similar between jugular plasma, hepatic portal vein plasma, and liver tissue. Data is summarized in Tables 13 and 14.

Table 14. VX-950 plasma toxicokinetics in dog

Dose (mg/kg/day)	Day	VX-950			
		$AUC_{0-24\text{ hr}}$ (ng·hr/mL)		C_{\max} (ng/mL)	
		male	female	male	female
25	1	14,843	15,508	2140	2119
	91	31,209	23,719	4119	3582
	182	36,381	29,330	4353	4334
	266	21,149	14,463	3048	2102
50	1	71,899	52,624	6517	5207
	91	102,178	60,283	8720	6377
	182	100,975	70,558	9147	7052
	266	76,929	61,615	6658	5532
100	1	128,508	121,073	11,113	9179
	91	184,217	204,075	13,391	15,213
	182	207,163	243,322	16,178	18,089
	266	201,011	184,112	15,177	13,487

Table 15. VX-950 plasma vs. tissue toxicokinetics in dog

Dose (mg/kg/day)	site ^a	VX-950							
		Day 183				Day 274			
		$AUC_{1-8\text{ hr}}$ (ng·hr/mL)		C_{\max} (ng/mL)		$AUC_{1-8\text{ hr}}$ (ng·hr/mL)		C_{\max} (ng/mL)	
		male	female	male	female	male	female	male	female
25	jv	15,311	11,185	5480	7700	8515	6230	4460	2590
	hpv	17,823	10,738	5120	8680	13,204	11,167	6500	7690
	liv	9626	6220	2700	3633	8606	6525	3533	2910
50	jv	48,995	27,200	10,300	17,700	22,490	14,683	7220	4760
	hpv	50,565	30,622	12,100	18,800	18,900	15,258	4610	5960
	liv	36,780	15,587	4400	8900	30,910	18,907	7233	6633
100	jv	76,150	80,370	35,800	18,800	62,510	101,325	15,800	21,800
	hpv	81,090	87,460	35,900	26,700	73,785	106,360	25,600	21,600
	liv	57,600	57,700	13,267	11,533	71,817	58,800	18,700	15,100

^ajv = jugular vein; hpv = hepatic portal vein; liv = liver

6 General Toxicology

6.1 Single-Dose Toxicity

Title: Acute Oral Toxicity and Toxicokinetic Study of VX-950 In Mice (Study no. VX-950-TX-007)

Key Study Findings

Mortality, clinical signs, body weights, gross pathology, and toxicokinetics was conducted in CD-1 mice following a single oral administration of 100, 500, and 1000 mg/kg/day of VX-950. Control groups received deionized water or VX-950 placebo. There were no drug-related effects observed.

Based on a lack of adverse effects in any dose group, the NOAEL was ≥ 1000 mg/kg/day VX-950.

Title: Acute Oral Toxicity and Toxicokinetic Study of VX-950 In Sprague-Dawley Rats (Study no. VX-950-TX-008)

Key Study Findings

Mortality, clinical signs, body weights, gross pathology, and toxicokinetics were evaluated in Sprague-Dawley rats following a single oral administration of 100, 500, and 1000 mg/kg/day of VX-950. Control groups received deionized water or VX-950 placebo. There were no drug-related effects observed.

Based on a lack of adverse effects in any dose group, the NOAEL was ≥ 1000 mg/kg.

6.2 Repeat-Dose Toxicity

Title: Twenty-Eight Day Toxicity and Toxicokinetic Study of VX-950 in Sprague-Dawley Rats with a Twenty-Eight Day Recovery Period (Study no. VX-950-TX-001)

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical exams, ophthalmoscopy, clinical pathology, bone marrow cytology, gross pathology, organ weights, histopathology, CYP450 induction/inhibition, and toxicokinetics were evaluated in Sprague-Dawley rats following oral administration of 100, 300, and 1000 mg/kg/day of VX-950 for 28 days followed by a 28 day recovery period. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). There were no drug-related effects observed.

Based on a lack of adverse effects in any dose group, the NOAEL was ≥ 1000 mg/kg/day.

Title: Twenty-Eight Day Oral Toxicity and Toxicokinetic Study of VX-950 in Beagle Dogs with a Fourteen-Day Recovery Period – A Repeat Study (Study no. VX-950-TX-014)

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical exams, ophthalmoscopy, ECGs, clinical pathology, bone marrow cytology, gross pathology, organ weights, histopathology, CYP450 induction/inhibition, and toxicokinetics were evaluated in Beagle dogs following oral administration of 50, 150, and 500/300 mg/kg/day of VX-950 for 28 days followed by a 14 day recovery period. VX-950, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). As a result of toxicity noted in animals administered 500 mg/kg/day, these animals were not dosed from Days 8 to 14 and the high dose was subsequently lowered to 300 mg/kg/day on Day 15. Drug-related mortality was observed in high-dose males. VX-950-related clinical/physical signs (e.g., decreased activity, thin, and inappetence) occurred. A decrease in body weight parameters correlated with decreased food consumption. Changes in hematology and coagulation parameters were numerous and included a decrease in red blood cell parameters, white blood cell types, platelets, and APTT with an increase in reticulocytes. Effects on bone marrow cytology were consistent with hematology changes. Clinical chemistry effects included decreased albumin and A:G as well as increased globulin, ALP, cholesterol, triglycerides, bilirubin, AST, and ALT. Macroscopic changes were noted in the heart (red or dark focus), lung (mottled; dark focus), and thymus (small). There was a drug-related increase in liver weight parameters. Chronic-active vasculitis and associated secondary effects were noted in numerous tissues (bone, male and female reproductive tissues, esophagus, eye, gall bladder, heart, GI tract, kidney, lung, sciatic nerve, skin, thymus, thyroid gland, trachea, and urinary bladder). Effects considered secondary to the overall poor health of the animal were observed in the liver (bile retention in canaliculi; increased eosinophilic pigmentation in Kupffer cells), spleen (increased hematopoiesis in red pulp; increased brown pigment in red pulp), and thymus (atrophy). Additional microscopic lesions were noted in the bone (cartilaginous dysplasia), liver (mixed perivascularitis; sinusoidal hypercellularity), and lung (chronic-active inflammation). Clinical chemistry and organ weight effects appeared to correlate with the microscopic findings in liver. Based on effects on probe substrate metabolism, there were drug-treated reductions in the activities of CYP1A1/2, CYP2B11, CYP2E1, and CYP3A12 in the liver. Effects attributed to VX-950 were primarily observed at the high dose while the mid-dose level was impacted to a lesser degree. A few minor changes were noted in the low-dose. The incidence of some drug-related effects improved during recovery; however, not all changes were reversible.

Based on the presence of only non-adverse effects on food consumption, hematology, and liver weight in the low-dose group, the NOAEL was 50 mg/kg/day.

Title: A Thirteen-Week Oral Toxicity and Toxicokinetic Study of VX-950 in Sprague-Dawley Rats with a Twenty-Eight Day Recovery Period (Study no. VX-950-TX-016)

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical exams, ophthalmoscopy, clinical pathology, bone marrow cytology, gross pathology, organ weights, histopathology, CYP450 induction/inhibition, and toxicokinetics were evaluated in Sprague-Dawley rats following oral administration of 100, 300, and 1000 mg/kg/day of VX-950 for 13-weeks followed by a 28-day recovery period. VX-950, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Decreased red blood cell associated parameters as well as increased reticulocytes and white blood cell types were noted. APTT was also increased. Clinical chemistry changes such as decreased albumin with increased ALT, AST, and GGT were suggestive of hepatotoxicity. Increased liver weights and microscopic lesions (hepatocellular hypertrophy; single cell hepatocellular necrosis) were consistent with clinical chemistry indicators of liver effects. VX-950-related macroscopic lesions were limited to testes (small; soft) which correlated with decreased organ weight and microscopic lesions (germinal epithelium degeneration). Decreased epididymis weights were also accompanied by microscopic changes (increased exfoliated germ cell; hypospermia; aspermia). Additional increased organ weight parameters were observed in spleen. Based on effects on probe substrate metabolism, there were drug-treated increases in CYP3A1/2 and CYP2E1 activity as well as decreased CYP2B1/2 activity. Overall, drug-related effects generally limited to the mid- and high-dose groups. However, hepatotoxicity was observed in low-dose males. Several clinical pathology effects as well as liver and reproductive tissue lesions persisted.

Based on the presence of non-adverse hematology changes in low-dose females, the NOAEL for this sex was 100 mg/kg/day. An NOAEL was not established for male rats due to hepatic toxicity in all dose groups.

Title: A Low Dose Toxicity and Toxicokinetic Study in Male Sprague-Dawley Rats (Study no. FXU00003)

Key Study Findings

Mortality, clinical signs, body weights, food consumption, clinical chemistry, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in male Sprague-Dawley rats following oral administration of 1, 3, and 10 mg/kg/day of VX-950 for 13-weeks. VX-950 and VX-950 placebo were given BID (doses separated by at least 8 hr). A single high-dose male had macroscopic testicular lesion (small; soft) accompanied by microscopic lesions (seminiferous tubule degeneration; diffuse germinal epithelium). Pathological evaluation of the testis was not performed on other animals. However, this lesion routinely is found in control rats and testicular toxicity was not observed at doses up to 100 mg/kg/day in repeat-dose rat studies. Although it is not possible to rule out an association with VX-950, it appears likely that this is a spurious result.

Based on the lack of adverse effects in the high-dose group, the NOAEL in male rats was \geq 10 mg/kg/day.

Title: A Thirteen-Week Oral Toxicity and Toxicokinetic Study of VX-950 in Beagle Dogs with a Twenty-Eight Day Recovery Period (Study no. VX-950-TX-017)

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical examinations, ophthalmoscopy, ECGs, clinical pathology, bone marrow cytology, gross pathology, organ weights, histopathology, CYP450 inhibition/induction and toxicokinetics were evaluated in Beagle dogs following oral administration of 25, 50, and 100 mg/kg/day of VX-950 for 13-weeks. VX-950, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related decreased body weight parameters were noted in high-dose males. Hematology effects included decreases in red blood cell associated parameters and an increase in reticulocytes. Bone marrow cytology effects were consistent with hematology changes. Changes in clinical chemistry were limited to decreased albumin and A:G ratio with increased globulin, as well as increased cholesterol and ALP. Increased liver weight parameters were noted. Drug-related decrease in CYP3A12, CYP2E1, and CYP2B11 activities consistent with decreased levels of P450 protein. There were no histopathological correlates for any drug-related effect and most changes appeared reversible. Overall, drug-related effects were generally observed in high dose animals; however, effects occasionally impacted the low- and mid-dose groups as well.

Based on the presence of non-adverse effects on body weights, hematology, bone marrow cytology, clinical chemistry, and liver weights at the high-dose, the NOAEL was 100 mg/kg/day.

Title: A Six-Month Oral Toxicity and Toxicokinetic Study in Sprague-Dawley Rats with a Three-Month Recovery Period (Study no. VX-950-TX-020)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 16, 2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	VX-950, lot# 17QB01.HQ00002, purity 99.4%

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical exams, ophthalmoscopy, clinical pathology (hematology, coagulation, clinical chemistry, urinalysis, bone marrow cytology), gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Sprague-Dawley rats following oral administration of 30, 100, and 300 mg/kg/day of VX-950 for 6 months followed by a 3-month recovery period. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related effects included decreases in red blood cell associated parameters with increases in reticulocytes, white blood cell types, and coagulation parameters. The clinical chemistry evaluation identified decreased triglyceride as well increases in aminotransferases, GGT, ALP, and cholesterol. Changes in protein parameters (e.g., decreased albumin and A:G ratio with increased globulin) and elevated creatinine and phosphorus were also noted. A macroscopic lesion in testis (small; soft) was consistent with decreased organ weight parameters and microscopic change (degeneration,

germinal epithelium). Increased spleen weight parameters correlated with histopathological effects (increased red pulp hematopoiesis) and increased reticulocytes in peripheral blood. Liver effects, consistent with the clinical chemistry data, were also noted as both increased organ weights with microscopic findings (hepatocellular hypertrophy; single cell hepatocellular necrosis). The epididymis was identified as a target organ based on decreased organ weight parameters and microscopic lesions (increased exfoliated germ cell; hypospermia; aspermia). In general, effects were reversible and primarily observed in the mid- and high-dose groups. However, indicators of hepatotoxicity (clinical pathology and histopathology) were observed at all doses and persisted through the recovery period.

Based on hepatotoxicity in the low dose group, an NOAEL was not defined in this study.

Methods

Doses:

Group Number	Daily Dose Level of VX-950 (mg/kg/day)	Dose Level Per Dose of VX-950 ^a (mg/kg/dose)	Dose Concentration of VX-950 (mg/gram)	Number of Main Study Animals ^b		Number of Toxicokinetic Animals		Number of Biomarker Animals		Dosing Days
				Males	Females	Males	Females	Males	Females	
1	0	0	0	10	10	0	0	0	0	1-182 ^c
2	0	0	0	15	15	6	6	5	5	
3	30	15	1.5	10	10	6	6	0	0	
4	100	50	5	10	10	6	6	0	0	
5	300	150	15	15	15	6	6	5	5	

^aOral doses were administered based on the weight of the formulated test article. A dose weight of 10 grams/kg/dose ($\pm 5\%$) was administered to each animal.

^bFive animals/sex/group (if surviving) in Groups 2 and 5 were held for a three-month recovery period following the treatment period.

^cThe Toxicokinetic animals were administered one additional dose on Study Day 183.

Frequency of dosing:	BID (separated by at least 8 hr)
Route of administration:	oral
Dose weight:	10 g/kg/dose
Formulation/Vehicle:	1% (w/v) HPMC-AS in deionized water with 10% (w/v) vitamin E-TPGS containing 0.01% (w/v) simethicone
Species/Strain:	rat/Crl:CD® (SD) out-bred albino rats ((b) (4))
Age at study start:	~8 weeks old
Weight at study start:	males = 208 to 281 g females = 182 to 228 g
Unique study design:	none
Deviations from study protocol:	minor with no impact on study integrity

Observations and Results

Mortality

No drug-related effects. One high-dose group female was euthanized on Day 121, likely as the result of a gavage accident. A single mid-dose female was found dead on Day 6. This death was attributed to hydrothorax.

Clinical Signs

No drug-related effects.

Body Weights

No drug-related effects. Mid-dose and high-dose animals had decreased body weights vs. placebo control; however, there was no difference compared to water control. Therefore, this finding is considered of uncertain relationship to treatment.

Food Consumption

No drug-related effects.

Physical Examinations

No drug-related effects.

Ophthalmoscopy (pre-test, Weeks 13 and 26)

No drug-related effects.

Hematology (main study - Weeks 13 and 27; recovery - Day 274)

Drug-related effects included decreases in hemoglobin, hematocrit, MCV, and MCH with increases in reticulocytes, white blood cells, lymphocytes, and monocytes. With the exception of effects on white blood cell types in males, changes were generally observed in both sexes in Weeks 13 and 27. Effects were primarily noted in high dose animals; however, decreased hemoglobin and hematocrit with increased reticulocytes were noted at the low- and/or mid-dose. Hematological changes appeared to be reversible. VX-950-related effects are summarized in the table below (table taken from the Sponsor report).

Table 16. Hematology changes in 6-month rat study

Parameter	Direction and Magnitude of Change (%) Relative to Placebo	Dosage Level (mg/kg/day)	Sex	Study Day
Reticulocyte Absolute	↑ 18, 17, 26	30, 100, 300	Male	86
Reticulocyte Absolute	↑ 35	300	Male	183
Reticulocyte Absolute	↑ 24, 39, 36	30, 100, 300	Female	86
Reticulocyte Absolute	↑ 21, 29, 23	30 ^{NS} , 100, 300	Female	183
Hemoglobin, Hematocrit	↓ 7	300	Both	183
Hemoglobin, Hematocrit	↓ 3-4, 5-6	30, 300	Female	86
Mean Corpuscular Volume	↓ 2-6	300	Both	86, 183
Mean Corpuscular Hemoglobin	↓ 6	300	Male	183
Mean Corpuscular Hemoglobin	↓ 3, 5	300	Female	86, 183
Leukocyte count	↑ 32	300	Male	183
Lymphocyte count	↑ 36	300	Male	183
Monocyte count	↑ 48, 40	300	Male	86, 183
Large Unstained Cells	↑ 46	300	Male	86

NS = Not statistically significant

Coagulation (main study - Weeks 13 and 27; recovery - Day 274)

Drug-related increases in APTT were observed in mid- and high-dose males as well as high-dose females. PT effects were mixed with mid- and high-dose males exhibiting an increase but high-

dose females had a decrease. Changes were noted during both sampling periods but were not present after the recovery period. VX-950-related effects are summarized in the table below (table taken from the Sponsor report).

Table 17. Coagulation changes in 6-month rat study

Parameter	Direction and Magnitude of Change (%) Relative to Placebo	Dosage Level (mg/kg/day)	Sex	Study Day
APTT	↑ 35, 24	300	Male	86, 183
APTT	↑ 17	100	Male	86
APTT	↑ 18	300	Female	183
PT	↑ 20, 9	300	Male	86, 183
PT	↑ 12	100	Male	86
PT	↓ 6, 7	300	Female	86, 183

Clinical Chemistry (main study - Weeks 13 and 27; recovery - Day 274)

Drug-related increases in ALT, AST, and GGT were observed in males at all dose levels. In females, elevated AST and GGT were limited to the mid- and high-dose groups. Decreased albumin and triglycerides occurred in mid- and high-dose males. Decreased albumin and A:G ratio with increased globulin were observed in high-dose females. Additional drug-related changes include increased ALP, cholesterol, creatinine, and phosphorus in high-dose males. Most changes were observed during both sampling periods during the dosing phase but were reversible during recovery. However, ALT values were still slightly greater than placebo at the end of recovery. VX-950-related effects are summarized in the table below (table taken from the Sponsor report).

Table 18. Clinical chemistry changes in 6-month rat study

Parameter	Direction and magnitude of change *	Dosage Level (mg/kg/day)	Sex	Study Day
ALT	↑ 1.9X, 2.8X, 3.4X	30, 100, 300	Male	86
ALT	↑ 4.1X, 5.6X, 6.1X	30, 100, 300	Male	183
AST	↑ 1.5X, 2.2X, 3X	30, 100, 300	Male	86
AST	↑ 3.3X, 4.4X, 5.2X	30, 100, 300	Male	183
AST	↑ 1.4X, 1.5X	100, 300	Female	86
AST	↑ 1.56X, 1.62X	100, 300	Female	183
AST	↑ 1.1X [@]	Placebo	Female	86
GGT	↑	300	Male, Female	86, 183
GGT	↑	30, 100	Male	183
GGT	↑	100	Female	86, 183 ^{NS}
ALP	↑ 22%, 50%	300	Male	86 ^{NS} , 183
Albumin	↓ 7%, 4%	100, 300	Male	86
Albumin	↓ 6%	300	Female	183 ^{NS}
Globulin	↑ 9%, 4%	300	Female	86, 183 ^{NS}
A/G Ratio	↓ 10%, 10%	300	Female	86, 183
Cholesterol	↑ 17%, 22%	300	Male	86, 183
Triglyceride	↓ 29%, 45%	300	Male	86 ^{NS} , 183
Triglyceride	↓ 33%, 39%	100	Male	86 ^{NS} , 183
Creatinine	↑ 17%	300	Male	183
Inorganic Phosphorus	↑ 9%	300	Male	183

* Relative to Group 2 placebo controls

@ Relative to Group 1 water control

^{NS} = Not statistically significant

Urinalysis (main study - Weeks 13 and 27; recovery - Day 274)

No drug-related effects.

Bone Marrow Cytology (scheduled necropsies)

No drug-related effects.

Gross Pathology (scheduled necropsies)

Drug-related effects on testis (small; soft) were noted in high-dose males and were consistent with organ weight changes and microscopic lesions. This gross effect was not observed following the recovery period.

Organ Weights (scheduled necropsies)

Drug-related reductions in epididymis and testis weights were observed at the high-dose. An increase in liver weight was noted in high-dose males and females. Increased spleen weight parameters were observed in females at all doses but were limited to the high-dose group in males. All affected organs had microscopic correlates at the terminal necropsy. Testis and spleen weights appeared to be affected at the end of recovery. Organ weight effects are summarized below.

Table 19. Organ weight changes in 6-month rat study^a

	Males			Females		
	30 mg/kg	100 mg/kg	300 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
epididymis						
absolute	-	-	-16.7%*	-	-	-
% body weight	-	-	-9.40%	-	-	-
% brain weight	-	-	-14.5%*	-	-	-
spleen						
absolute	-	-	+12.8%	+10.8%	+3.93%	+9.05%
% body weight	-	-	+22.6%*	+9.32%	+3.25%	+14.9%
% brain weight	-	-	+16.3%	+9.68%	+6.60%	+10.7%
testis						
absolute	-	-	-17.5%	-	-	-
% body weight	-	-	-11.0%	-	-	-
% brain weight	-	-	-15.2%	-	-	-
liver						
absolute	-	-	-	-	-	+14.0%
% body weight	-	-	+10.3%*	-	-	+22.0%*
% brain weight	-	-	-	-	-	+15.8%

^a % change vs. placebo controls

* statistically significant difference compared to placebo controls

Histopathology (scheduled necropsies)

Adequate Battery – yes (some recommended tissues not examined)

Peer Review - no

Histological Findings - *VX-950-treatment resulted in several microscopic lesions. Drug-related microscopic lesions are discussed by tissue and summarized in the tables below (taken from Sponsor report).*

Liver: Hepatocellular hypertrophy was observed at all doses. Single cell hepatocellular necrosis occurred at all doses in males but was limited to mid- and high-dose females. These lesions correlated with clinical chemistry and/or organ weight changes. Both lesions were still present at the end of the recovery period.

Spleen: Increased red pulp hematopoiesis occurred in high-dose males and females at all doses. Increased hematopoiesis was consistent with the increases in reticulocytes and organ weight data. Lesions appeared reversible.

Testis: Degeneration of the germinal epithelium was detected at the high-dose. This lesion correlated with decreased organ weight as well as the macroscopic finding of small, soft testis and was reversible.

Epididymis: Increased exfoliated germ cell, hypospermia, and aspermia occurred in the high-dose. Decreased organ weight accompanied this lesion, which was determined to be reversible.

Table 20. Histopathological lesions in 6-month rat study (terminal necropsy)

Finding	Group (Number of Animals)	Males					Females				
		1 (10)	2 (10)	3 (10)	4 (10)	5 (10)	1 (10)	2 (10)	3 (10)	4 (10)	5 (10)
Liver		N=10									
Hypertrophy, hepatocellular											
minimal		0	0	3	3	2	0	0	3	7	3
mild		0	0	7	7	8	0	0	2	3	7
Necrosis, single cell, hepatocellular											
minimal		0	0	3	8	2	0	0	0	5	5
mild		0	0	0	1	8	0	0	0	0	2
Spleen		N=10									
Hematopoiesis, increased, red pulp											
minimal		2	4	7	7	5	0	1	5	6	7
mild		0	0	0	0	2	0	0	0	0	0
Testis		N=10	N=10	N=10	N=10	N=10	NA	NA	NA	NA	NA
Degeneration, germinal epithelium											
bilateral, minimal		0	0	0	0	2					
bilateral, mild		0	0	0	0	1					
bilateral, moderate		0	0	0	0	1					
bilateral, marked		0	0	0	0	1					
Epididymis		N=10	N=10	N=10	N=10	N=10	NA	NA	NA	NA	NA
Exfoliated germ cell, increased											
bilateral, minimal		0	0	0	0	4					
Hypospermia, bilateral		0	0	0	0	1					
Aspermia, bilateral		0	0	0	0	1					

N = Number of animals examined

NA = Not applicable

Table 21. Histopathological lesions in 6-month rat study (recovery necropsy)

Finding	Group (Number of Animals)	Males		Females	
		2 (5)	5 (5)	2 (5)	5 (4)*
Liver		N=5	N=5	N=5	N=4*
Hypertrophy, hepatocellular					
minimal		0	5	0	2
mild		0	0	0	2
Necrosis, single cell, hepatocellular					
minimal		0	5	0	0

N = Number of animals examined

*One Group 5 female (animal #934) that was designated for the three-month recovery period was euthanized in a moribund condition on Study Day 121.

Toxicokinetics

A detailed description of the toxicokinetic data is included in section 5.2 (Toxicokinetics). Both VX-950 and its epimer metabolite (VRT-127394) were present at detectable concentrations in control samples; however, levels were relatively low and few animals were affected. Therefore, the control contamination profile was not substantial and did not impact the integrity of the study.

Dosing Solution Analysis

Formulation stability of at least 24 hr was established in a separate study. Acceptance criteria was met for both concentration (i.e., $\pm 15\%$ of expected) and homogeneity (i.e., $\leq 10\%$ RSD between layers).

Title: A Nine-Month Oral Toxicity and Toxicokinetic Study in Beagle Dogs with a Three-Month Recovery Period (Study no. VX-950-TX-021)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 16, 2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	VX-950, lot# 17QB01.HQ00002, purity 99.4%

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical exams, ophthalmoscopy, ECGs, clinical pathology, bone marrow cytology, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Beagle dogs following oral administration of 25, 50, and 100 mg/kg/day of VX-950 for 9 months followed by a 3-month recovery period. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related observations included clinical signs (e.g., decreased activity, rigid body, hunched posture, involuntary spasms, tilted head, and cold to touch) and decreased body weight parameters at the high-dose. Decreased red blood cell associated parameters with increased reticulocytes were also noted. Several clinical chemistry parameters were affected including decreased albumin and A:G ratio with increased cholesterol, ALP, and globulin. Bone marrow cytology changes were consistent with hematological effects. A macroscopic cardiac lesion (epicardium, white focus) was associated with microscopic finding of vasculitis. Organ weight changes were limited to liver weight. In addition to the heart, chronic-active vasculitis was observed in stomach, epididymis, and ovary. This lesion is similar to canine polyarteritis. Microscopic findings potentially secondary to the vasculitis were noted in bone and bone marrow (myelofibrosis; hypocellularity; necrosis; cartilaginous dysplasia). The liver (mixed perivascularitis; sinusoidal hypercellularity; Kupffer cell, increased eosinophilic pigmentation) and spleen (red pulp, increased hematopoiesis) were also identified as a histopathological target. Microscopic findings in the spleen correlated with the mild anemia and increased reticulocytes observed in peripheral blood. Effects were primarily noted in the high-dose group;

however, some changes in clinical pathology, organ weight, vasculitis, and hepatotoxicity were noted in the mid-dose group. The low-dose group was rarely affected; however, vasculitis was observed in the heart. While most effects appeared to be reversible, changes in clinical chemistry, liver:body weight ratio, and myelofibrosis in bone marrow were still present at the end of the recovery period.

Based on the presence of only minor changes in clinical chemistry and bone marrow cytology in the low-dose group, the NOAEL was 25 mg/kg/day in females. Due to vasculitis in the heart in the low-dose group, an NOAEL was not established for male.

Methods

Doses:

Group Number	Test Article	Daily Dose Level of VX-950 ^a (mg/kg/day)	Dose Level Per Dose of VX-950 (mg/kg/dose)	Number of Main Study Animals ^b		Necropsy Day
				Males	Females	
1	Water Control	0	0	9	9	183, 274
2	Placebo	0	0	12	12	183, 274, 365
3	VX-950 Low-dose	25	12.5	9	9	183, 274
4	VX-950 Mid-dose	50	25	9	9	183, 274
5	VX-950 High-dose	100	50	12	12	183, 274, 365

^aAdministered as two equally divided daily doses given orally by gavage at least eight hours apart.

^bFour animals/sex/group in all groups were dosed for six months and necropsied on Study Day 183.

Up to five animals/sex/group in all groups were dosed for up to nine months and necropsied on Study Day 274. The remaining animals in Groups 2 and 5 were designated as Recovery animals and were held without treatment for a three-month recovery period and necropsied on Study Day 365.

Frequency of dosing:	BID (separated by at least 8 hr)
Route of administration:	oral
Dose amount:	5 g/kg/dose
Formulation/Vehicle:	1% (w/v) HPMC-AS in deionized water with 10% (w/v) vitamin E-TPGS containing 0.01% (w/v) simethicone
Species/Strain:	dog/Beagle (from (b) (4))
Age at study start:	6 to 7 months old
Weight at study start:	males = 8.2 to 11.1 kg females = 5.5 to 9.8 kg
Unique study design:	none
Deviation from study protocol:	minor with no impact on study integrity

Observations and Results

Mortality

No drug-related effects. A total of 11 animals (1 placebo control male; 3 placebo control females; 2 low-dose females; 1 mid-dose male; 1 mid-dose female; 2 high-dose males; 1 high-dose female) were found dead or sacrificed early during the study. These deaths were considered the result of gavage accidents.

Clinical Signs

Increased incidence of discolored stool was observed in high-dose animals. In addition, a number of drug-related signs were recorded for 2 high-dose females. These included

inappetence, thin, decreased activity, rigid body, hunched posture, involuntary spasms (neck, head, and shoulder), tilted head, and/or cold to touch which were consistent with canine polyarteritis.

Body Weights

Drug-related effects were limited to high-dose males. At multiple timepoints, body weights were $\leq -16.9\%$ lower in high-dose males compared to placebo controls. There were also sporadic decreases in body weight gain for this group. Decreases in body weight were reversible.

Food Consumption

No drug-related effects.

Physical Examinations (pre-test, Weeks 13, 26, and 39)

Pale-mucous membrane was noted in a single high-dose female during Week 13. This observation correlated with effects on clinical signs and histopathological evaluation.

Ophthalmoscopy (pre-test, Weeks 13, 26, and 38/39)

No drug-related effects.

ECGs (pre-test, Weeks 13, 26, and 38)

No drug-related effects. A statistically significant +7.11% increase in mean QT_c (16 msec) was observed in high-dose males during Week 13. This difference was not observed during subsequent sampling periods and is considered of uncertain relationship to treatment.

Hematology (pre-test, Weeks 13, 26, 38, and 52)

Drug-related effects included decreased red blood cells, hemoglobin, hematocrit, MCH, MCHC, and basophils with increased platelets, reticulocytes, and lymphocytes. Effects on red blood cell associated parameters and reticulocytes were observed in high dose males and females throughout the treatment-period. All other changes occurred only during 1 or 2 sampling periods. Transient decreases in red blood cells, hemoglobin, hematocrit, and MCHC were also noted in mid-dose males. All drug-related effects appeared to be reversible. Hematological effects are summarized in the following table.

Table 22. Hematology changes in 9-month dog study^a

	Males			Females		
	25 mg/kg	50 mg/kg	100 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
red blood cells						
Week 13	-	-5.35%*	-5.23%*	-	-	-8.08%*
Week 26	-	-	-6.16%	-	-	-10.5%*
Week 38	-	-	-10.2%*	-	-	-10.0%
hemoglobin						
Week 13	-	-5.32%*	-7.36%*	-	-	-9.58%*
Week 26	-	-	-9.92%*	-	-	-13.0%
Week 38	-	-	-13.0%*	-	-	-11.0%
hematocrit						
Week 13	-	-5.21%*	-6.56%*	-	-	-7.98%
Week 26	-	-	-8.27%*	-	-	-11.8%
Week 38	-	-	-11.9%*	-	-	-8.53%
MCH						
Week 26	-	-	-3.79%*	-	-	-
MCHC						
Week 13	-	-	-	-	-	-2.01%*
Week 26	-	-1.43%*	-1.66%*	-	-	-
Week 38	-	-	-	-	-	-2.80%*
platelets						
Week 26	-	-	+26.3%*	-	-	-
reticulocytes						
Week 13	-	-	+56.0%*	-	-	+55.1%
Week 26	-	-	+37.3%	-	-	+38.0%
Week 38	-	-	+55.4%	-	-	+51.8%
lymphocytes						
Week 26	-	-	+28.9%*	-	-	-
basophils						
Week 38	-	-	-	-	-	-43.7%*

^a % change vs. placebo controls

* statistically significant difference compared to placebo controls

Coagulation (pre-test, Weeks 13, 26, 38, and 52)*No drug-related effects.***Clinical Chemistry** (pre-test, Weeks 13, 26, 38, and 52)

Drug-related decreases in albumin and A:G ratio with increases in cholesterol, ALP, and globulin were observed. Effects were detected throughout the treatment-period, primarily at the high-dose. Protein parameter changes occurred in mid-dose females while increased cholesterol and ALP affected both sexes at the mid-dose as well as low-dose females. These effects in the low- and mid-dose groups were observed during only 1 or 2 sampling periods. In general, drug-related changes improved during the recovery period but did not fully reverse. Clinical chemistry effects are summarized in the following table.

Table 23. Clinical chemistry changes in 9-month dog study^a

	Males			Females		
	25 mg/kg	50 mg/kg	100 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
albumin						
Week 13	-	-	-6.73%*	-	-	-14.1%*
Week 26	-	-	-10.0%*	-	-	-13.4%*
Week 38	-	-	-13.3%*	-	-10.1%	-16.0%*
Week 52	-	-	-9.71%	-	-	-
cholesterol						
Week 13	-	-	+28.4%*	-	-	-
Week 26	-	+21.4%*	+30.7%*	+43.7%*	+43.9%*	+31.4%*
Week 38	-	+28.6%*	+42.3%*	-	-	-
Week 52	-	-	+43.2%*	-	-	+35.7%
ALP						
Week 13	-	-	+65.1%*	-	-	+131%
Week 26	-	-	+149%*	-	+51.8%*	+125%*
Week 38	-	-	+143%	+55.4%	+56.7%	+84.9%
A:G ratio						
Week 13	-	-	-12.7%*	-	-	-24.8%*
Week 26	-	-	-16.0%*	-	-	-25.0%*
Week 38	-	-	-12.5%	-	-17.8%	-22.4%
Week 52	-	-	-20.9%	-	-	-
globulin						
Week 13	-	-	+7.44%	-	-	+12.6%
Week 26	-	-	+8.38%	-	-	+17.4%*
Week 38	-	-	+3.25%	-	+10.9%	+11.9%
Week 52	-	-	+10.5%	-	-	-

^a % change vs. placebo controls

* statistically significant difference compared to placebo controls

Urinalysis (pre-test, Weeks 13, 26, 38, and 52)*No drug-related effects.***Bone Marrow Cytology** (scheduled necropsies)

Drug-related changes in total erythroid cells, total granulocytic cells, and M:E ratio were noted in Week 40 (end of treatment necropsy). The increases in erythroid cells is consistent with effects noted in peripheral blood (decreases in red blood cell associated parameters and increased reticulocytes). There were no drug-related effects at the end of the recovery period.

Table 24. Bone marrow cytology changes in 9-month dog study

	Males			Females		
	25 mg/kg	50 mg/kg	100 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
total granulocytic cells						
Week 40	-	-	-28.4%	-22.6%	-26.9%	-28.2%
total erythrocytic cells						
Week 40	-	-	+17.8%	+27.0%	+30.6%	+34.4%
M:E ratio						
Week 40	-	-	-37.5%	-36.4%	-45.4%	-45.4%

Gross Pathology (scheduled necropsies)

At the Week 27 necropsy, one high-dose female had a drug-related cardiac lesion (epicardium, white focus). This animal also had a corresponding microscopic lesion.

Organ Weights (scheduled necropsies)

Drug-related increases in liver:body weight ratio were observed at all necropsies. At the 6-month necropsy males were affected at all dose groups ($\leq +28.2\%$) while mid- and high-dose females were also affected ($\leq +34.2\%$). At the 9-month necropsy liver:body weight was increased in both sexes at the high-dose ($\leq +30.7\%$). With the exception of liver weight remaining increased in high-dose females (+28.3%), there were no substantial effects observed after the recovery period. Additional organ weight parameter changes in epididymides, heart, lung, and pituitary gland were noted in high dose males. Because these organs were not affected during the treatment-period necropsies, the changes are considered of uncertain relationship to treatment.

Histopathology (scheduled necropsies)

Adequate Battery – yes (some recommended tissues not examined)

Peer Review - no

Histological Findings - VX-950-treatment resulted in several microscopic lesions. Drug-related microscopic effects are discussed by lesion or tissue and summarized in the tables below (taken from Sponsor report).

Vasculitis, Chronic-Active: Vasculitis, similar to canine polyarteritis, was observed in numerous tissues during the 6- and 9-month necropsies. Effects in stomach and epididymis were limited to the high-dose. Vasculitis in heart was noted at the low- and high-dose while the ovary was a target in the mid-dose. The microscopic cardiac effect was consistent with the gross lesion (epicardium, white focus). Vasculitis was not observed in recovery animals.

Bone and Bone Marrow: At both the 6-month and 9-month necropsies there were lesions observed in femur, sternum, and/or sternum bone marrow. Myelofibrosis, hypocellularity, necrosis, and cartilaginous dysplasia were observed in high-dose animals. Lesions were reversible with the exception of myelofibrosis in sternum bone marrow for females. The Sponsor suggests that these lesions were potentially secondary to vasculitis.

Liver: Mixed perivascularitis, sinusoidal hypercellularity, and increased eosinophilic pigmentation in Kupffer cells were noted in females at the high-dose at the 6-month necropsy. At the 9-month necropsy mixed perivascularitis was detected in a high-dose female while the Kupffer cell effect was observed in mid- and high-dose males as well as high-dose females. All hepatic lesions were reversible.

Spleen: Increased red pulp hematopoiesis was observed at the high-dose in females only. This lesion occurred during both treatment-period necropsies and was consistent with both mild anemia and increased reticulocytes in the peripheral blood. This was a reversible effect.

Table 25. Histopathological lesions in 9-month dog study (6-month necropsy)

Group Findings (Number of Animals)	Males					Females				
	1 (4)	2 (4)	3 (4)	4 (4)	5 (4)	1 (4)	2 (4)	3 (4)	4 (4)	5 (4)
Bone Marrow, Sternum	N=4									
Myelofibrosis	0	0	0	0	1	0	0	0	0	1
Hypocellularity	0	0	0	0	1	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0	0	1
Bone, Femur	N=4									
Myelofibrosis	0	0	0	0	0	0	0	0	0	1
Hypocellularity	0	0	0	0	0	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0	0	1
Bone, Sternum	N=4									
Dysplasia, cartilaginous	0	0	0	0	1	0	0	0	0	0
Heart	N=4									
Vasculitis, chronic-active*	0	0	0	0	1	0	0	0	0	1
Liver	N=4									
Perivasculitis, mixed**	0	0	0	0	0	0	0	0	0	1
Hypercellularity, sinusoidal	0	0	0	0	0	0	0	0	0	1
Pigmentation, eosinophilic, increased, Kupffer cell	0	0	0	0	0	0	0	0	0	1
Spleen	N=4									
Hematopoiesis, increased, red pulp	0	0	0	0	0	0	0	0	0	1
Stomach	N=4									
Vasculitis, chronic-active*	0	0	0	0	0	0	0	0	0	1

N = Number examined

* Vasculitis, chronic-active = Inflammation, chronic-active, vascular

** Perivasculitis, mixed = Inflammation, mixed, perivascular

Table 26. Histopathological lesions in 9-month dog study (9-month necropsy)

Group Findings (Number of Animals)	Males					Females				
	1 (5)	2 (5)	3 (5)	4 (5)	5 (4 ^a)	1 (5)	2 (4 ^a)	3 (5)	4 (5)	5 (4 ^a)
Bone Marrow, Sternum	N=5	N=5	N=5	N=5	N=4	N=5	N=4	N=5	N=5	N=4
Hypocellularity	0	0	0	0	0	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0	0	1
Bone, Femur	N=5	N=5	N=5	N=5	N=4	N=5	N=4	N=5	N=5	N=4
Myelofibrosis	0	0	0	0	0	0	0	0	0	1
Hypocellularity	0	0	0	0	0	0	0	0	0	1
Necrosis	0	0	0	0	0	0	0	0	0	1
Heart	N=5	N=5	N=5	N=5	N=4	N=5	N=4	N=5	N=5	N=4
Vasculitis, chronic-active*	0	0	1	0	0	0	0	0	0	1
Ovary	NA	NA	NA	NA	NA	N=5	N=4	N=5	N=5	N=4
Vasculitis, chronic-active*						0	0	0	1	0
Epididymis	N=5	N=5	N=5	N=5	N=4	NA	NA	NA	NA	NA
Vasculitis, chronic-active*	0	0	0	0	1					
Liver	N=5	N=5	N=5	N=5	N=4	N=5	N=4	N=5	N=5	N=4
Perivasculitis, mixed**	0	0	0	0	0	0	0	0	0	1
Pigmentation, eosinophilic, increased, Kupffer cell	0	0	0	1	3	0	0	0	0	3
Spleen	N=5	N=5	N=5	N=5	N=4	N=5	N=4	N=5	N=5	N=4
Hematopoiesis, increased, red pulp	0	0	0	0	0	0	0	0	0	2

N = Number examined

NA = Not applicable

^aThere were only four Study Day 274 Group 5 males, Group 2 females, and Group 5 females because a Group 5 male (#791) was found dead on Study Day 207, a Group 2 female (#740) was found dead on Study Day 157, and a Group 5 female (#792) was euthanized in a moribund condition on Study Day 57.

*Vasculitis, chronic-active = Inflammation, chronic-active, vascular

** Perivasculitis, mixed = Inflammation, mixed, perivascular

Table 27. Histopathological lesions in 9-month dog study (recovery necropsy)

Group Findings (Number of Animals)	Males		Females	
	1 (3)	5 (3)	1 (3)	5 (3)
Bone Marrow, Sternum	N=3	N=3	N=3	N=3
Myelofibrosis	0	0	0	1

N = Number examined

Toxicokinetics

A detailed description of the toxicokinetic data is included in section 5.2 (Toxicokinetics). Both VX-950 and VRT-127394 were present at detectable concentrations in control samples; however, levels were relatively low and few animals were affected. Therefore, the control contamination profile was not substantial and did not impact the integrity of the study.

Dosing Solution Analysis (Days 1, 91, 182, 197, 266, and 274)

Formulation stability of at least 24 hr was established in a separate GLP study. With one exception, acceptance criteria was met for both concentration (i.e., $\pm 15\%$ of expected) and homogeneity (i.e., $\leq 10\%$ RSD between layers) A single high-dose formulation sample for concentration analysis was out of specification (-51.3% of expected) likely due to a dilution error. This was an isolated incidence and did not impact the study.

7 Genetic Toxicology

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

Title: Bacterial Reverse Mutation Assay (Study no. VX-950-TX-003)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 5, 2004
GLP compliance:	Yes
QA statement:	included
Drug, lot #, and % purity:	VX-950, lot 03-VZK-001X, purity 94.8%

Key Study Findings

Mutagenicity was evaluated in Salmonella strains TA98, TA100, TA1535, and TA1537 as well as E. coli strain WP2 uvrA with $\leq 5000 \mu\text{g}/\text{plate}$ VX-950 \pm S9. There were no drug-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2 *uvrA*
Concentrations in definitive study: 75, 200, 600, 1800, and 5000 µg/plate
Basis of concentration selection: standard top dose of 5000 µg/plate w/ and w/out S9
Negative control: DMSO
Positive control:

Strain	S9	Control	Dose
TA98	-	2-nitrofluorene	1.0 µg/plate
	+	2-aminoanthracene	1.0 µg/plate
TA100	-	sodium azide	1.0 µg/plate
	+	2-aminoanthracene	1.0 µg/plate
TA1535	-	sodium azide	1.0 µg/plate
	+	2-aminoanthracene	1.0 µg/plate
TA1537	-	9-aminoacridine	75 µg/plate
	+	2-aminoanthracene	1.0 µg/plate
WP2 <i>uvrA</i>	-	methyl methansulfonate	1000 µg/plate
	+	2-aminoanthracene	10 µg/plate

Formulation/Vehicle: DMSO
Incubation & sampling time: 4-72 hr w/ or w/out metabolic activating system in plate incorporation method. Metabolic activation system contained 10% S9 fraction from Arochlor 1254-induced male rat liver.

Study Validity: Criteria for a valid study and a positive response are as follows:

- All *Salmonella* strains must demonstrate presence of the deep rough mutation (*rfa*) and deletion of the *uvrB* gene. Cultures of TA98 and TA100 must have the pKM101 plasmid R-factor. All WP2 *uvrA* cultures must have deleted *uvrA* gene.
- Spontaneous Revertants: Mean revertants/plate for vehicle controls must fall within the following: TA98 = 10-50; TA100: 80-240; TA1535 = 5-45; TA1537: 3-21; WP2 *uvrA* = 10-60. Spontaneous revertants must demonstrate histidine and tryptophan dependence.
- Tester Strain Titers: Must be $\geq 0.3 \times 10^9$ cells/mL.
- Positive Control Values: Positive controls must induce ≥ 3 -fold increase in revertants/plate vs. concurrent vehicle control.
- Minimum # of Dose Levels: A minimum of 3 non-toxic dose levels is required. Toxicity is defined as 1) a > 50 % reduction in mean revertants/plate vs. mean vehicle control accompanied by abrupt dose dependent-drop in revertant count or 2) a moderate reduction in background lawn.
- Positive Response: A dose-related increase in mean revertants/plate for at least 1 test strain over 2 concentrations. The increase in revertant frequency must be ≥ 2 -fold increase vs. vehicle control for TA98, TA100, and WP2*uvrA* or ≥ 3 -fold increase for TA1535 and TA1537.

Results

No drug-related effects. Precipitate was observed at 5000 µg/plate. Criteria for a valid study were met.

7.2 *In Vitro* Assays in Mammalian Cells

Title: *In Vitro* Mammalian Chromosome Aberration Test (Study no. VX-950-TX-004)

Study report location: EDR
Conducting laboratory and location: (b)(4)
Date of study initiation: April 5, 2004
GLP compliance: Yes
QA statement: included
Drug, lot #, and % purity: VX-950, lot 03-VZK-001X, purity 94.8%

Key Study Findings

Clastogenicity was evaluated in Chinese hamster ovary cells at VX-950 concentrations ≤ 1500 $\mu\text{g}/\text{mL}$ following 4 hr \pm S9 and ≤ 125 $\mu\text{g}/\text{mL}$ following 20 hr $-$ S9. There were no drug-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Methods

Cell line: Chinese hamster ovary cells

Concentrations in definitive study:

S9	time	Doses
+	4 hr	125, 250, 500, 1000, 1500, 1750, and 2000 $\mu\text{g}/\text{mL}$
-	4 hr	125, 250, 500, 1000, 1500, 1750, and 2000 $\mu\text{g}/\text{mL}$
	20 hr	15.6, 31.3, 62.5, 125, 250, and 500 $\mu\text{g}/\text{mL}$

Basis of concentration selection: Top dose selected based on $\geq 50\%$ toxicity (cell growth inhibition compared to control) with adequate number of metaphase cells.

Negative control: DMSO

Positive control:

S9	Control	Dose
+	cyclophosphamide	10 and 20 $\mu\text{g}/\text{mL}$
-	mitomycin C	0.1 and 0.2 $\mu\text{g}/\text{mL}$

Formulation/Vehicle: DMSO

Incubation & sampling time: 4 hr with or without and 20 hr without metabolic activation. All incubations were at 37 ± 1 °C. Colcemid® (0.1 $\mu\text{g}/\text{mL}$) was present for the last 2 hr. Metabolic activation system contained S9 fraction from Arochlor 1254-induced male rat liver.

Study Validity. Criteria for a valid study and a positive response are as follows:

- Vehicle Controls: Must fall within historical control range for vehicle controls.
- Positive Control: Must produce a significant increase ($p \leq 0.05$) in the % of cells with chromosomal aberrations vs. vehicle control.
- Positive Response: Requires the percentages of cells with aberrations be increased in a dose-responsive manner, with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$). Values that are statistically significant but do not exceed the range of historical solvent controls may be judged as not biologically significant.
- Negative Response: A non-statistically significant increase in aberrations.

Results

Chromosomal Aberration Assay: *No drug-related effects. The concentrations selected for analysis were 250, 500, and 1500 $\mu\text{g}/\text{mL}$ following 4 hr incubation with or without S9 and 31.3, 62.5, and 125 $\mu\text{g}/\text{mL}$ 20 hr incubation without S9. The top doses evaluated were based on cytotoxicity (4 hr w/ S9: 69% cell growth inhibition; 4 hr w/out S9: 60% cell growth inhibition; 20 hr w/out S9: 66% cell growth inhibition). Criteria for a valid study were met.*

Dosing Solution Analysis: *The observed results for the 2 highest concentrations were within 10% of expected; however, the lower concentration (1.56 mg/mL) was 127.2% of target. Detectable levels of VX-950 were not found in control formulations. Because the top dose levels met the acceptance criteria, the out of specification results did not affect the integrity of the study.*

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

Title: *In Vivo* Mammalian Erythrocyte Micronucleus Test (Study no. VRT-950-TX-010)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 9, 2004
GLP compliance:	Yes
QA statement:	included
Drug, lot #, and % purity:	VX-950 (b) (4) (b) (4), lot DV040065, purity 97.7%

Key Study Findings

Clastogenicity was evaluated in ICR mice administered oral doses of 500, 1000, or 2000 mg/kg of VX-950. VX-950 was administered BID (doses separated by 3 hr). There were no drug-related effects. Criteria for a valid study were met.

Methods**Doses in definitive study:**

Treatment (2 x 20 mL/kg)	Number of Mice/Sex Dosed	Number of Mice/Sex Used for Bone Marrow Collection After Dose Administration	
		24 hr	48 hr
Untreated Control	12	6	6
Vehicle Control: Placebo	12 + 3*	6	6
Test Article: VX-950			
Low dose (500 mg/kg)	6 + 21*	6	0
Mid dose (1000 mg/kg)	6 + 21*	6	0
High dose (2000 mg/kg)	12 + 21*	6	6
Positive Control: CP (50 mg/kg)**	6	6	0

*Animals assigned for blood collection; ** CP administered only once at a volume of 20 mL/kg

Frequency of dosing:	once daily
Route of administration:	oral
Dose volume:	20 mL/kg
Formulation/Vehicle:	1% (w/v) HPMC and 0.67% (w/w) simethicone (0.3% emulsion)
Species/Strain:	Mouse/ICR ([REDACTED]) (b) (4)
Basis of dose selection:	Standard maximum high dose of 2000 mg/kg.
Negative control:	[REDACTED] (b) (4)
Positive control:	50 mg/kg cyclophosphamide

Study Validity. Criteria for a valid study and a positive response are as follows:

- Vehicle Controls: Micronucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%).
- Positive Control: Micronucleated polychromatic erythrocytes must be significantly increased relative to the vehicle control group (p<0.05, Kastenbaum-Bowman Tables).
- Positive Response: Requires a dose-responsive increase in micronucleated polychromatic erythrocytes be observed and one or more doses statistically elevated relative to the vehicle control (p ≤ 0.05, Kastenbaum-Bowman Tables) at any sampling time. However, values that were statistically significant but did not exceed the range of historical negative or vehicle controls were judged as not biologically significant.
- Negative Response: Requires absence of a statistically significant increase in micronucleated polychromatic erythrocytes above the concurrent vehicle control values and lack of dose response at any sampling time.

Results**Micronucleus Assay**

No drug-related effects. Criteria for a valid study were met.

Toxicokinetics

A detailed description of the toxicokinetic data is included in section 5.2 (Toxicokinetics).

Dosing Solution Analysis: *The observed results for both dose formulation concentration and homogeneity were within $\pm 15\%$ of expected.*

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Because telaprevir was not genotoxic and will be administered to humans for 12 weeks, carcinogenicity studies were not required.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Title: Oral (Gavage) Fertility and General Reproduction Toxicity Study in Rats (Study no. VX-950-TX-019)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 13, 2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	VX-950 powder for suspension (solid dispersion), lot DV050032, purity 95.8% as-is

Key Study Findings

Mortality, clinical signs, body weights, food consumption, estrus cycle/mating behavior, gross pathology, organ weights, sperm parameters, and Caesarian-section parameters were evaluated in Sprague-Dawley rats orally administered VX-950. Males received doses of 30, 100, and 300 mg/kg/day for 70 days prior to 1st cohabitation through completion of the 2nd cohabitation. Females received 150, 250, and 500 mg/kg/day for 15 days prior to cohabitation through Gestation Day 7. Treated males were mated with treated females (1st cohabitation period) and untreated females (2nd cohabitation period). A recovery group of males was mated with untreated females 3-months after the cessation of dosing (3rd cohabitation). A satellite group of males was administered 500 mg/kg/day in a manner similar to main study animals but were not mated with females. Test article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Main study males were sacrificed at the completion of the 2nd and 3rd cohabitation while satellite animals were sacrificed at various timepoints. Females were sacrificed on Gestation Day 13. Drug-related testicular effects were observed as gross lesions (small; purple; flaccid), decreased organ weights, decreased % motile sperm, increased nonmotile sperm count, and numerous degenerative microscopic lesions (degeneration/necrosis, individual germ cells; degeneration, tubules, multifocal; increased/enlarged residual bodies; retained Step 19 spermatids, stage IX and X tubule; sloughed/necrotic intraluminal germ cells;

vacuolation, Sertoli cells). Lesions in the epididymides were detected both macroscopically (small) and microscopically (exfoliated spermatogenic cells/residual bodies). Decreased seminal vesicle without fluid weights were also observed. Effects in males were primarily observed in the satellite group, although organ weight changes were observed in mid- and high-dose main study groups. Drug-related effects seen during Caesarian-section included increased % preimplantation loss, % of dams with nonviable embryos, and % nonviable conceptuses/litter. Caesarian-section endpoints were affected in high-dose or untreated females mated with high-dose males, suggesting an association with male reproductive effects. With the exception of some organ weight changes, there were no drug-related effects associated with the 3rd cohabitation period, indicating reversibility of most changes.

Since sperm evaluations were only conducted in males assigned to satellite or recovery groups the possibility of adverse effects occurring at lower doses exists. However, the incidence of sperm staging changes decreased or reversed during recovery and effects noted in Caesarian-section evaluation were only associated with high dose males and were reversible. Therefore, the paternal reproductive toxicity NOAEL was 100 mg/kg/day. Due to limitations in study design (i.e., no treated female mated with untreated male groups) a maternal reproductive toxicity NOAEL was not established.

Methods

Doses: Males

Dose Group	BID Dose (mg/kg/dose)	Total Daily Dose (mg/kg/day)	Concentration (mg/g)	Weight per Dose (g/kg) ^a	Number of Male Rats
I	0 (Control Article)	0 (Control Article)	0	10	25
II	0 (Placebo)	0 (Placebo)	0	10	25
III	15	30	1.5	10	25
IV	50	100	5	10	25
V	150	300	15	10	25
VI	0 (Control Article)	0 (Control Article)	0	10	10 ^b
VII	150	300	15	10	30 ^c

^a Doses were administered based on the weight of the formulated test article.

^b Male rats assigned to Group VI were treated as an age matched control group for the biomarker analysis.

^c Male rats assigned to Group VII were treated as a satellite group to establish potential biomarkers.

Females

Dose Group	BID Dose (mg/kg/dose)	Total Daily Dose (mg/kg/day)	Concentration (mg/g)	Weight per Dose (g/kg) ^a	Number of Female Rats
VIII	0 (Control Article)	0 (Control Article)	0	5	25
IX	0 (Placebo)	0 (Placebo)	0	5	25
X	75	150	15	5	25
XI	125	250	25	5	25
XII	250	500	50	5	25

^a doses were administered based on the weight of the formulated test article

Frequency of dosing: BID (at least 8 hr apart)

Route of administration: oral

Formulation/Vehicle: 1% (w/v) HPMC-AS in deionized water with 10% (w/v) vitamin E-TPGS containing 0.01% (v/v) simethicone

Species/Strain: rat/Crl:CD(SD) (b) (4)

Study design: Dosing – Main study males were dosed from 70 days prior to cohabitation through the completion of 2 cohabitation periods. These males were sacrificed following the 2nd cohabitation or after a 3-month recovery period and subsequent 3rd cohabitation. A satellite group of males was dosed similar to the main study groups and sacrificed at timepoints throughout the study. Female rats were dosed 15 days prior to the 1st cohabitation until presumed Gestation Day 7 (females in the 2nd and 3rd cohabitation periods were not dosed) and sacrificed on presumed Gestation Day 13.

 Reproductive/Developmental endpoints evaluated

Males	Females
gross pathology ^{a,b}	estrus cycle/mating behavior
reproductive organ weights	gross pathology
sperm evaluation	Caesarian section
number ^a	corpora lutea
motility ^a	implantation sites
sperm staging ^b	viable/nonviable embryos
^a recovery and satellite only	
^b satellite only	

Deviation from study protocol: minor with no impact on study integrity

Observations and Results

Mortality

No drug-related effects. In main study animals, a single male from each of the placebo control, low-dose, and mid-dose groups was found dead. In addition, one male from each of the control article, mid-dose, and high-dose groups was sacrificed due to poor condition. While observations in several animals were consistent with gavage error, the cause of death was not determined in all cases. In animals where a cause of death was not identified, mortality was also not considered drug-related due to the lack of dose-response. A single control article male in the satellite group was sacrificed due to poor condition.

Clinical Signs

Although not considered adverse, drug-related excess salivation as well as red and/or orange perioral substance was observed. These changes were noted in main study males and females from the mid- and high-dose groups as well as high-dose male satellite males.

Body Weight

No drug-related effects.

Food Consumption

There were minor, sporadic increases in absolute and/or relative food consumption in main study mid- and high-dose males as well as females at all doses.

Estrus Cycle/Mating Behavior

No drug-related effects in males and females.

NecropsyMale

Gross Pathology – Drug-related lesions in the testes (small; purple; flaccid) and epididymis (small) were noted in high-dose satellite animals sacrificed ~1 month after cessation of treatment.

Organ Weights – Decreased testes weight parameters were observed in the mid- (-4.36 to -7.58% vs. concurrent placebo control) and high-dose (-9.93 to -14.9% vs. concurrent placebo control). Decreases in testes and seminal vesicle without fluid weight parameters were also observed in the high-dose satellite group sacrificed ~ 1 and 3 months after cessation of dosing. Because concurrent controls were not available, this determination is based on a comparison with satellite group values obtained at other timepoints (see table below).

Table 28. Organ weight changes in rat fertility study

	Day 30	Day 70	Day 105	Day 149 (~1 month recovery)	Day 218 (~3 month recovery)
<i>testes, left</i>					
<i>absolute (g)</i>	1.7971	1.7757	1.8116	1.5749	1.6813
<i>body weight (%)</i>	0.400	0.346	0.303	0.265	0.264
<i>testes, right</i>					
<i>absolute (g)</i>	1.7515	1.7430	1.8192	1.5667	1.6875
<i>body weight (%)</i>	0.388	0.336	0.305	0.270	0.266
<i>seminal vesicles (without fluid)</i>					
<i>absolute (g)</i>	1.0087	1.1005	1.2417	0.8623	0.8209
<i>body weight (%)</i>	0.222	0.214	0.210	0.130	0.130

Sperm Evaluation – Drug-treated effects were observed in high-dose satellite group animals sacrificed ~1 month after cessation of treatment. Because concurrent controls were not available, this conclusion is based on a comparison with satellite group values obtained at other timepoints. Effects included a decrease in % of motile sperm (69.2% vs. 87.0 to 95.0% at other timepoints) as well as an increase in nonmotile sperm count (136.0 vs. 17.5 to 56.6 at other timepoints). This effect was not observed in main study males following 3 months of recovery.

Drug-related microscopic effects in testes and epididymides were noted in the high-dose satellite group at the various necropsies. Numerous testicular effects were degenerative in nature (degeneration/necrosis, individual germ cells; degeneration, tubules, multifocal; increased/enlarged residual bodies; retained Step 19 spermatids, stage IX and X tubule; sloughed/necrotic intraluminal germ cells; vacuolation, Sertoli cells). Lesions in the epididymides (exfoliated spermatogenic cells/residual bodies) were also observed. Histopathological lesions correlated with effects noted in gross pathology, organ weight parameters, and sperm motility and count. It is noteworthy that sperm staging was only

conducted in satellite males, therefore, it is not possible to determine effects at doses < 300 mg/kg/day.

Degenerative changes in testes and epididymides were reversible or occurred at a lower incidence during the recovery period compared to the dosing phase. Additional lesions in the testes (atrophy, seminiferous tubules, diffuse) and epididymides (hypospermia) were observed during the recovery period but considered of uncertain relationship to treatment. Microscopic effects are summarized below (tables constructed from Sponsor report).

Table 29. Histopathological lesions in rat fertility study (high-dose satellite group)

Dose Group:	VIIA	VIIB	VIIC	VIID	VIIIE
Sex:	M	M	M	M	M
Number of Animals/Group:	5	5	10	5	5
TESTES:					
NO. EXAMINED	5	5	10	5	5
NO. NORMAL	0	0	2	2	3
-degeneration/necrosis, individual germ cells					
minimal	3	3	3	0	0
mild	1	1	0	0	0
Total Incidence, All Grades	4	4	3	0	0
-atrophy, seminiferous tubules, diffuse marked	0	0	1	1	1
Total Incidence, All Grades	0	0	1	1	1
-degeneration, tubules, multifocal					
minimal	0	0	3	1	1
mild	0	2	1	0	0
moderate	0	0	0	1	0
Total Incidence, All Grades	0	2	4	2	1
-hyperplasia, interstitial-cell, multifocal					
minimal	0	0	0	0	1
Total Incidence, All Grades	0	0	0	0	1
-increased/enlarged residual bodies					
minimal	3	3	4	0	0
mild	2	1	0	0	0
Total Incidence, All Grades	5	4	4	0	0
-multinucleated giant cells					
minimal	0	0	0	1	0
Total Incidence, All Grades	0	0	0	1	0
-retained Step 19 spermatids, Stage IX and X tubules	4	5	4	0	1
-sloughed/necrotic intraluminal germ cells					
minimal	1	1	3	0	1
Total Incidence, All Grades	1	1	3	0	1
-vacuolation, Sertoli cells					
minimal	0	1	1	0	0
mild	1	0	1	0	0
Total Incidence, All Grades	1	1	2	0	0
-vasculitis, multifocal					
minimal	0	0	0	0	1
Total Incidence, All Grades	0	0	0	0	1

Group VIIA: Necropsy Date: 03/15/06

Group VIID: Necropsy Date: 07/12/06

Group VIIB: Necropsy Date: 04/24/06

Group VIIIE: Necropsy Date: 09/19/06

Group VIIC: Necropsy Date: 05/29/06

Dose Group:	VIA	VIIB	VIIC	VIID	VIE
Sex:	M	M	M	M	M
Number of Animals/Group:	5	5	10	5	5
EPIDIDYMIDES:					
NO. EXAMINED	5	5	10	5	5
NO. NORMAL	0	0	0	3	1
-exfoliated spermatogenic cells/residual bodies					
minimal	2	1	5	0	2
mild	2	2	2	0	0
moderate	1	2	2	2	1
marked	0	0	1	0	0
Total Incidence, All Grades	5	5	10	2	3
-hyperplasia, epididymal tubules, focal					
mild	0	0	0	1	1
Total Incidence, All Grades	0	0	0	1	1
-hypospermia					
mild	0	0	0	1	0
moderate	1	0	0	0	0
marked	0	0	1	1	1
Total Incidence, All Grades	1	0	1	2	1
-infiltration, mononuclear-cell, focal					
minimal	1	2	1	0	1
mild	0	0	0	0	1
Total Incidence, All Grades	1	2	1	0	2
-multinucleated giant cells					
minimal	0	0	1	1	0
Total Incidence, All Grades	0	0	1	1	0
-vasculitis, multifocal					
mild	0	0	0	0	1
Total Incidence, All Grades	0	0	0	0	1
<hr/> Group VIIA: Necropsy Date: 03/15/06 Group VIIB: Necropsy Date: 04/24/06 Group VIIC: Necropsy Date: 05/29/06 Group VIID: Necropsy Date: 07/12/06 Group VIE: Necropsy Date: 09/19/06					

Female

Gross Pathology – No drug-related effects.

Cesarean Section Data – Following the 1st cohabitation, high-dose females mated with high-dose males had an increase in % pre-implantation loss (6.3% vs. 2.2% in placebo control). Additional effects in this group included statistically significant decreases in the number of implantation sites (14.0 vs. 16.2 in placebo control), litter size (14.0 vs. 16.2 in placebo control), and number of viable embryos (11.7 vs. 14.5 in placebo control). Values for number of implantation sites, litter size, and number of viable embryos were within historical control

range and are considered of uncertain relationship to treatments. Untreated females mated with high-dose males during the 2nd cohabitation period had an increase in the % of dams with nonviable embryos (95.6% vs. 75.0% in placebo control) and percentage of nonviable conceptuses/litter (16.1% vs. 9.3% in placebo control). Effects were not observed in females mated with males during the 3rd cohabitation period (recovery).

Dosing Solution Analysis

Formulation stability for 6 days was established in a separate study. Acceptance criteria was met for both concentration (i.e., $\pm 15\%$ of expected) and homogeneity ($\leq 10\%$ RSD between layers).

9.2 Embryonic Fetal Development

Title: Oral (Gavage) Developmental Toxicity Study in Rats (Study no. VX-950-TX-018)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 16, 2005
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	VX-950 powder for suspension (solid dispersion), lot# DV050032, purity 95.3% as-is

Key Study Findings

Mortality, clinical signs, body weights, food consumption, gross pathology, Caesarian-section parameters, and toxicokinetics were evaluated in Sprague-Dawley rats following oral administration of 50, 150, 250, and 500 mg/kg/day of VX-950. Fetal weight, sex, and alterations were also evaluated. Pregnant females were dosed from presumed Gestation Day 7 to 17 and sacrificed on Day 21. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related adverse effects were not observed.

Based on a lack of adverse findings in any dose group, the NOAEL for maternal toxicity and embryofetal development was ≥ 500 mg/kg/day.

Methods

Doses:

Dose Group	BID Dose (mg/kg/dose)	Total Daily Dose (mg/kg/day)	Concentration (mg/g)	Weight per Dose (g/kg) ^a	Number of Rats
I	0 (Control Article)	0 (Control Article)	0	5	25
II	0 (Placebo)	0 (Placebo)	0	5	25 + 9 ^b
III	25	50	5	5	25 + 9 ^b
IV	75	150	15	5	25 + 9 ^b
V	125	250	25	5	25 + 9 ^b
VI	250	500	50	5	25 + 9 ^b

a. Doses were administered based on the weight of the formulated test article.

b. Nine rats assigned to toxicokinetic sample collection.

Frequency of dosing: BID (at least 8 hr apart)
Route of administration: oral
Formulation/Vehicle: 1% (w/v) HPMC-AS in deionized water with 10% (w/v) vitamin E-TPGS containing 0.01% (v/v) simethicone
Species/Strain: rat/Crl:CD(SD) (b) (4)

Study design: Dosing - Pregnant females were dosed twice daily from presumed Gestation Days 7 through 17. The animals were sacrificed on presumed Gestation Day 21.

Reproductive/Developmental endpoints evaluated

Females	Offspring
gross pathology	fetal weight
Caesarian section	sex
corpora lutea	alterations
implantation sites	gross external
live/dead fetuses	soft tissue
early/late resorptions	skeletal

Deviation from study protocol: minor with no impact on study integrity

Observations and Results

Mortality

No drug-related effects. One high-dose female designated to the toxicokinetic evaluation was sacrificed early. This early death was likely due to an eye injury resulting from the bleeding technique (i.e., collection from orbital sinus).

Clinical Signs

Drug-related effects included sparse hair coat, excess salivation, as well as red and/or clear perioral substance. Sparse hair coat was limited to the high-dose while the other signs were observed in all dose groups.

Body Weight

No drug-related effects.

Food Consumption

No drug-related effects.

Necropsy

Maternal Gross Pathology – *No drug-related effects.*

Cesarean Section Evaluation – *No drug-related effects.*

Offspring – *No drug-related effects.*

Toxicokinetics

A detailed description of the toxicokinetic data is included in section 5.2 (Toxicokinetics). Both VX-950 and VRT-127394 were present at detectable concentrations in control samples; however, levels were relatively low and few animals were affected. Therefore, the control contamination profile was not substantial and did not impact the integrity of the study.

Dosing Solution Analysis

Formulation stability of at least 24 hr was established in a separate study. Acceptance criteria was met for both concentration (i.e., $\leq \pm 15\%$ of expected) and homogeneity (i.e., $\leq 10\%$ RSD between layers).

Title: Oral (Gavage) Dose-Range Developmental Toxicity Study in Mice (Study no. VX-950-TX-022)

Key Study Findings

Mortality, clinical signs, body weights, mating, gross pathology, Caesarian-section parameters, and toxicokinetics were evaluated in CD-1 mice following oral administration of 100, 300, 600, and 1000 mg/kg/day VX-950. Gross external fetal features were also evaluated. Pregnant females were dosed from presumed Gestation Day 6 to 15 and sacrificed on Day 18. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related effects were limited to decreased body weight gain in high-dose. A detailed description of the toxicokinetic data is included in the PK/ADME section of this review.

Based on the lack of adverse findings, the NOAEL for maternal health was 600 mg/kg/day. Based on the lack of adverse effects in any dose group, the NOAEL for embryofetal development was ≥ 1000 mg/kg/day.

Title: Oral (Gavage) Developmental Toxicity Study in Mice (Study no. VX-950-TX-023)

Study report location: EDR
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: July 28, 2006
GLP compliance: yes
QA statement: yes
Drug, lot #, and % purity: VX-950 [REDACTED] (b) (4), lot
17QB01.HQ00008, purity 98.4% as-is

Key Study Findings

Mortality, clinical signs, body weights, gross pathology, and Cesarean-section parameters were evaluated in CD-1 mice following oral administration of 100, 300, and 1000 mg/kg/day of VX-950. Fetal weight, sex, and alterations were also evaluated. Pregnant females were dosed from presumed Gestation Day 6 through 15 and euthanized on Gestation Day 18. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related adverse effects were not observed.

Based on a lack of adverse findings in any dose group, the NOAEL for maternal health and embryofetal development was ≥ 1000 mg/kg/day.

Methods

Doses:

Dose Group	BID Dose ^a (mg/kg/dose)	Total Daily Dose (mg/kg/day)	Concentration (mg/g)	Weight per Dose (g/kg) ^b
I	0 (Control Article)	0 (Control Article)	0 (Control Article)	10
II	0 (Placebo)	0 (Placebo)	0 (Placebo)	10
III	50	100	5	10
IV	150	300	15	10
V	500	1000	50	10

a. Twice daily at least 8 hours apart.

b. Doses were administered based on the weight of the formulated test article.

Frequency of dosing: BID (at least 8 hr apart)
 Route of administration: oral
 Formulation/Vehicle: 1% (w/v) HPMC-AS in deionized water with 10% (w/v) vitamin E-TPGS containing 0.01% (v/v) simethicone
 Species/Strain: mouse/Crl:CD1(ICR) (b) (4)
 Study design: Dosing - Females were dosed twice daily from presumed Gestation Days 6 through 15. The animals were sacrificed on presumed Gestation Day 18.

Reproductive/Developmental endpoints evaluated

Females	Offspring
gross pathology	fetal weight
Caesarian section	sex
corpora lutea	alterations
implantation sites	gross external
live/dead fetuses	soft tissue
early/late resorptions	skeletal

Deviation from study protocol: minor with no impact on study integrity

Observations and Results

Mortality

No drug-related effects. One water control and 3 low-dose animals were found dead during the study. Observations in 2 of the low-dose animals were consistent with gavage accident. A cause of death was not identified for the 3rd low-dose female; however, the death was not considered drug-related due to the lack of dose-response.

Clinical Signs

No drug-related effects.

Body Weight

No drug-related effects.

Necropsy

Maternal Gross Pathology – *No drug-related effects.*

Cesarean Section Data – *No drug-related effects.*

Offspring – *There was a statistically significant increase in the number of cervical ribs for fetuses in the low- and high-dose groups. Effects on ossification sites included a statistically significant increase for thoracic vertebrae and a decrease for lumbar vertebrae at the mid- and high-dose. In general, values were slightly outside of the range of historical controls. These effects are associated with supernumerary ribs, a common finding in CD-1 mice. An additional observation was a statistically significant increase in 2-segment ribs in high-dose animals. These minimal changes are of uncertain relationship to treatment. Due to the lack of dose-response and the nature of the effects, especially findings associated with supernumerary ribs, are not considered adverse.*

Dosing Solution Analysis

Formulation stability of at least 24 hr was established in a separate study. Acceptance criteria was met for both concentration (i.e., $\leq \pm 15\%$ of expected) and homogeneity (i.e., $\leq 10\%$ RSD between layers). A single 15 mg/g replicate was out of specification (-27% of expected); however, the matching sample as well as average of the 2 replicates were within $\pm 15\%$ of expected.

9.3 Prenatal and Postnatal Development

Title: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral/Functional Evaluation (Study no. VX-950-TX-025)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 22, 2006
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	VX-950 (b) (4), lot 17QB01.HQ00008, purity 98.4% as-is

Key Study Findings

Mortality, clinical signs, body weights, delivery observations, and gross pathology were evaluated in female Sprague-Dawley rats mice following oral administration of 50, 150, and 500 mg/kg/day VX-950. Pregnant females were dosed from presumed Gestation Day 7 through Lactation Day 20 or Gestation Day 24 (if no delivery). F₁ pups (pre-weaning) were observed for clinical signs, body weights, reflex/physical development, and gross pathology while mortality, clinical signs, body weight, food consumption, sexual maturation, behavioral assessments, estrus cycle/mating behavior, gross pathology, organ weights, and Caesarian section data were evaluated postweaning. Fetal weight, sex, and gross external alterations were evaluated in F₂. Test-article, deionized water, and VX-950 placebo were given BID (doses separated by at least 8 hr). Drug-related adverse effects were limited to decrease in pup weight/litter for F₁ offspring prior to weaning. Effects were primarily observed in maternal high-dose animals.

Based on the lack of adverse body weight effects in the mid-dose group, the NOAEL for perinatal/postnatal development was 150 mg/kg/day.

Methods

Doses: F₀ Generation

Dose Group	BID Dose ^a (mg/kg/dose)	Total Daily Dose (mg/kg/day)	Concentration (mg/g)	Weight per Dose (g/kg) ^b	Number of Rats
I	0 (Control Article)	0 (Control Article)	0	5	25
II	0 (Placebo)	0 (Placebo)	0	5	25
III	25	50	5	5	25
IV	75	150	15	5	25
V	250	500	50	5	25

a. Twice daily at least 8 hr apart

b. Doses were administered based on the weight of the formulated test article.

F₁ Generation

Dose Group	Maternal Dose (mg/kg/day)	Number of Rats Per Sex
I	0 (Control Article)	25
II	0 (Placebo)	25
III	50	25
IV	150	25
V	500	25

Frequency of dosing: BID (at least 8 hr apart)

Route of administration: oral

Formulation/Vehicle: 1% (w/v) HPMC-AS in deionized water with 10% (w/v) vitamin E-TPGS containing 0.01% (v/v) simethicone

Species/Strain: rat/Crl:CD(SD) (b) (4)Study design: Dosing – Pregnant females (F₀) were dosed twice daily from presumed Gestation Day 7 through Lactation Day 20 or Gestation Day 24 (if no delivery). F₀ rats were sacrificed on Lactation Day 21 or on Gestation Day 25 (if no delivery). Offspring (F₁) were allowed to cohabitate, and males were subsequently sacrificed. F₁ females were sacrificed on Gestation Day 21.

Reproductive/Developmental endpoints evaluated

F₀ Females	F₁ Pre-weaning	F₂
delivery observations	reflex/physical development	fetal weight
adverse signs	surface righting reflex	sex
duration of gestation	pinna unfolding	alterations
litter size	eye opening	gross external
viability	acoustic startle	
cannibalized pups	air righting reflex	
gross pathology	pupil constriction	
	F₁ Post-weaning	
	sexual maturation	
	vaginal patency	
	testes descent	
	balano-preputial separation	
	behavioral	
	passive avoidance	
	open field observations	
	(alertness, posture, and gait)	
	motor activity	
	watermaze	
	estrus cycle/mating behavior	
	gross pathology	
	organ weights	
	testes	
	epididymides	
	Caesarian section	
	corpora lutea	
	implantation sites	
	live/dead fetuses	
	early/late resorptions	

Deviation from study protocol: minor with no impact on study integrity

Observations and Results

F₀ Females

Mortality

No drug-related effects. One placebo control female was sacrificed because of difficult labor. In addition, 1 high-dose was found dead during the lactation period. While no cause of death was identified, there were no signs of toxicity in this animal.

Clinical Signs

Excess salivation and sparse hair coat on the back was observed in high-dose animals during the gestation and/or lactation periods.

Body Weight

A transient but statistically-significant reduction in body weight gain (-52.1% vs. placebo control) was noted for high-dose females during Days 7 to 10 of the gestation period. Body weight gain was similar for all groups from gestation Days 0 to 20. At the end of the lactation period, an increase in body weight was observed in the high-dose (+4.04% vs. placebo control). This was primarily due to an increase in body weight gain from Days 14 to 21 contrasting with a decrease in all other dose groups. Over the course of the lactation period, the high-dose animals had a statistically significant increase in body weight gain (+59.8% vs. placebo control).

Food Consumption

A transient decrease in food consumption (-16.7% vs. placebo control) occurred at the high-dose from gestation Days 7 to 10. This effect correlated with a concurrent decrease in body weight gain.

Delivery Observations

No drug-related effects.

Necropsy

Gross Pathology - *No drug-related effects.*

F₁ Preweaning**Clinical Signs**

No drug-related effects.

Body Weight

Drug-related decrease in pup weight/litter were noted in the mid-dose group on Days 14 and 21 (\leq - 6.87% vs. placebo control) and high-dose group on Days 4, 7, 14, and 21 (\leq - 18.6% vs. placebo control).

Reflex and Physical Development

No drug-related effects.

Necropsy

Gross Pathology - *No drug-related effects.*

F₁ Postweaning**Mortality**

No drug-related effects. A single maternal low-dose group male was found dead of an unidentifiable cause. In addition, 1 maternal mid-dose group female and 1 maternal high-dose group male were sacrificed early due to injury (i.e., broken palate).

Clinical Signs

No drug-related effects.

Body Weight

Drug-related decreases in body weight gain of $\leq -11.8\%$ (vs. placebo control group) were observed for maternal mid- and high-dose group males at various times through the postweaning period. Compared to placebo controls, the difference in body weight gain from postweaning Day 1 to termination was -5.82% for maternal mid-dose group and -8.02% for maternal high-dose group males. However, body weight gain was similar in all drug-treated groups as well as the water control group over this time period.

Body weight gain decreases of $\leq -14.6\%$ vs. placebo controls were observed in maternal high-dose group females at various times of the postweaning period. The difference between postweaning Days 1 and precohabitation was -6.03% for the maternal high-dose group.

Although effects on postweaning body weights appears to be drug-related, the effects are likely the result of decreased preweaning body weights.

Food Consumption

Compared to the placebo control group, increases in food consumption relative to body weight of $\leq +9.13\%$ were observed in maternal high-dose group males and females at various times through the postweaning period. The difference in relative food consumption from postweaning Day 1 to termination was $+5.01\%$ for males. Between postweaning Days 1 and 71, high-dose maternal group females increased $+5.24\%$.

Sexual Maturation

No drug-related effects.

Behavioral Assessment

No drug-related effects.

Estrus Cycle/Mating Behavior

No drug-related effects.

NecropsyMale

Gross Pathology - No drug-related effects.

Testes and Epididymides Weight – No drug-related effects.

Female

Gross Pathology – No drug-related effects.

C-section – No drug-related effects.

Offspring (F₂) – No drug-related effects.

Dosing Solution Analysis

Formulation stability of at least 24 hr was established in separate studies. Acceptance criteria was met for both concentration (i.e., $\leq \pm 15\%$ of expected) and homogeneity (i.e., $\leq 10\%$ RSD between layers).

10 Special Toxicology Studies**10.1 Metabolites**

Title: Bacterial Reverse Mutation Assay (Study no. VRT-127394-TX-001)

Key Study Findings

Mutagenicity was evaluated in *Salmonella* strains TA98, TA100, TA1535, TA1537 as well as *E. coli* strain WP2 *uvrA* with $\leq 5000 \mu\text{g}/\text{plate}$ VRT-12739 ± 9 . There were no test article-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Title: Activity of VRT-0842291 (M12) and VRT-0922061, Two Metabolites of Telaprevir (VX-950), in HCV NS3 Protease Enzyme and Replicon Assays (Study no. E046)

Key Study Findings

When compared to VX-950, both VRT-0842291 and VRT-0922061 had IC_{50} values less than 10% of that for VX-950.

10.2 Impurities

Title: Impurity and Degradation Product Qualification Toxicity Study Conducted with Spiked Material Administered by Oral Gavage to Sprague-Dawley Rats for Thirteen Weeks with a 28-Day Recovery Period (Study no. VX-950-TX-036)

Key Study Findings

Mortality, clinical signs, body weights, food consumption, physical examinations, clinical pathology (hematology, coagulation, clinical chemistry, urinalysis, bone marrow cytology), gross pathology, organ weights, and histopathology were evaluated in Sprague-Dawley rats following oral administration of 100, 300, and 1000 mg/kg/day VX-950 spiked with process impurities and degradation products (Table 1 taken from Sponsor report). For comparison, a group was also given 1000 mg/kg/day of VX-950 alone. Spiked VX-950, VX-950, and VX-950 placebo were given BID (doses separated by at least 8 hr). Decreased red blood cell associated parameters as well as increased reticulocytes and white blood cell types were noted. APTT was also increased. Bone marrow cytology changes were consistent with hematology. Clinical chemistry changes were extensive involving decreases in A:G ratio and triglycerides with increases in aminotransferases, GGT, cholesterol, and globulin. Effects suggestive of hepatotoxicity were consistent with increased liver weight parameters and histopathology (hepatocellular hypertrophy; single cell hepatocellular necrosis). Decreased epididymis weight correlated with microscopic lesions (increased exfoliated germ cell; hypospermia; aspermia). Decreased organ weight in testes correlated with both macroscopic (soft; small) and macroscopic effects (degeneration, germinal epithelium). Increased spleen weight was observed but had no histopathological correlate. Effects were observed primarily in the VX-950 alone group as well as the mid- and high-dose VX-950 + impurity group. However, changes in hematology, bone marrow cytology, and clinical chemistry indicators of hepatotoxicity were noted in the low-dose VX-950 + impurity group. Histopathological evaluation was limited to the high dose VX-950 spiked with impurities or VX-950 alone. Several clinical pathology effects as well as liver and reproductive tissue lesions persisted through the recovery period.

Administration of VX-950 spiked with impurities was consistent with effects noted in the concurrent 1000 mg/kg/day VX-950 alone group as well as results from a previously conducted 13-week study.

Table 30. Impurity levels in 13-week rat study



(b) (4)

Title: Bacterial Reverse Mutation Test (Study no. VRT- (b) (4) -TX-001)**Key Study Findings**

Mutagenicity was evaluated in Salmonella strains TA98, TA100, TA1535, TA1537 as well as E. coli strain WP2 uvrA with 5000 µg/plate VRT- (b) (4) ±S9. There were no test article-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Title: In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes (Study no. VRT- (b) (4) -TX-002)**Key Study Findings**

An evaluation of clastogenicity was conducted in human lymphocytes at VRT- (b) (4) concentrations ≤ 1250 µg/mL following 3 hr -S9, ≤ 625 µg/mL following 20 hr -S9, and ≤ 1500 µg/plate following 3 hr +S9. There were no test article-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Title: Salmonella Plate Incorporation Mutagenicity Assay (Study no. (b) (4) -TX-001)**Key Study Findings** (reviewed under IND#71,832)

Mutagenicity were evaluated in Salmonella strains TA98 and TA100, with ≤ 5000 µg/plate (b) (4) ±S9. There were no test article-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Title: Bacterial Reverse Mutation Assay (Study no. (b) (4) -TX-002)**Key Study Findings** (reviewed under IND#71,832)

Mutagenicity was evaluated in Salmonella strains TA98, TA100, TA1535, TA1537 as well as E. coli strain WP2 uvrA with ≤ 5000 µg/plate (b) (4) ±S9. There were no test article-related effects in the presence or absence of metabolic activation. Criteria for a valid study were met.

Title: In Vitro Mammalian Chromosome Aberration Test (Study no. (b) (4) -TX-003)**Key Study Findings** (reviewed under IND#71,832)

Clastogenicity was evaluated in Chinese hamster ovary cells at (b) (4) concentrations ≤ 400 µg/mL following 4 hr incubation -S9, ≤ 100 µg/mL following 4 hr incubation +S9 and 20 hr incubation -S9. A test article-related increase in chromosomal aberrations was detected at 250 µg/plate following 4 hr incubation with metabolic activation. Criteria for a valid study were met.

10.3 Mechanistic

Title: (b) (4) Pharmacology Data Report on Compounds (b) (4), VX-950 Bulk Drug Substance to (b) (4), VRT-127394 for Vertex Pharmaceuticals Incorporated (Study no. 1022388), Binding Study of M-424 (VX-950) to Dog Androgen Receptor (Study no. AL-4355-G), and An Evaluation of Effects of MP-424 (VX-950) on Human Erythrocytes In Vitro (Study no. 9R474)

Key Study Findings (from Sponsor submission)

Species / Strain	Method of Administration	Duration of Dosing	Dose (mg/kg) ^a	Gender and No. per Group	Noteworthy Findings	Report Number
Mechanistic Studies						
Testosterone binding	In vitro	-	0.1-10 µM	-	No significant responses noted.	1022388
Dog androgen binding	In vitro	-	0.1-10 µM	-	No appreciable binding or inhibition observed.	AL-4355-G
Effects on human erythrocytes	In vitro	-	80 µM	-	No appreciable effects observed for cell health parameters evaluated	9R474

Note for Study no. 1022388: While a significant response was not observed in the testosterone binding studies, a low degree of inhibition was detected for the rat testosterone receptor with both VX-950 and VRT-127394.

10.4 Antigenicity

Title: Assessment of Skin Sensitization Potential Using the Local Lymph Node Assay in the Mouse (Study no. VX-950-TX-028), Assessment of Skin Sensitization Potential Using the Local Lymph Node Assay in the Mouse (VRT-126032-TX-014), Assessment of Skin Sensitization Potential Using the Local Lymph Node Assay in the Mouse (Study no. VRT-841125-TX-001), and A Sensitization Study of VRT-841125 and VRT-126032 Administered by the Dermal Route to Guinea Pigs (Study no. VRT-841125-TX-002)

Key Study Findings (from Sponsor submission)

Species / Strain	Method of Administration	Duration of Dosing	Dose (mg/kg) ^a	Gender and No. per Group	Noteworthy Findings	Report Number
Antigenicity						
CBA/ Ca Mice	Dermal	3 days	25 µl (10%, 25%, and 50% w/v)	4F	VX-950 is not regarded as a potential skin sensitizer	VX-950-TX-028
CBA/ Ca Mice	Dermal	3 days	25 µl (10%, 25%, and 50% w/v)	4F	VRT-126032 showed the potential to induce skin sensitization (delayed contact hypersensitivity)	VRT-126032-TX-014
CBA/ Ca Mice	Dermal	3 days	25 µl (5%, 10%, and 25% w/v, 25% v/v HCA ^b)	4F	VRT-841125 is not regarded as a potential skin sensitizer	VRT-841125-TX-001
Hartley-derived albino guinea pig	Subcutaneous and Dermal	1 week intradermal induction; 1 week topical induction; challenge 2 weeks later; rechallenge 1 week later	Intradermal induction: 5% VRT-841125 or VRT-126036 and FCA ^c Topical induction: 100% VRT-841125 or VRT-126032 Challenge: 100% VRT-841125 or VRT-126032 Rechallenge: 100% and 5% VRT-841125 or 10% and 1% VRT-126032 Second rechallenge: 0.5% and 0.1% VRT-126032	10M/10F	VRT-841125 meets the criteria to be considered a contact sensitizer. Due to noted irritation, VRT-126032 results were equivocal in determining potential for contact sensitization.	VRT-841125-TX-002

M = Males; F = Females; - = Not applicable

^a Unless otherwise specified; ^b Hexyl connamic aldehyde (positive control); ^c Freund's Complete Adjuvant

Note: VRT-841125 is a VX-950 metabolite and VRT-126032 is a structurally similar molecule with known reactivity.

11 Integrated Summary and Safety Evaluation

Telaprevir (VX-950) is an HCV NS3•4A protease inhibitor developed as a direct acting antiviral agent against the hepatitis C virus. Vertex has submitted an NDA for approval of combination therapy with telaprevir, ribavirin, and pegylated interferon alpha for treating adult patients infected with chronic hepatitis virus who have compensated liver disease. The combination therapy is proposed for both treatment-naïve and treatment-experienced patients. The clinical dose of telaprevir is 750 mg q8hr (i.e., 2250 mg day).

Telaprevir exists as the S-diastereomer and is converted nonenzymatically, both in vitro and in vivo, to its less active R-diastereomer, VRT-127394. VRT-127394 is treated as (b) (4) a clinical

metabolite (b) (4). The nonclinical development of telaprevir included numerous formulations. (b) (4)

The oral bioavailability of telaprevir was 33 to 52% in rat, 43 to 67% in fasted dog, 70 to 95% in fed dog, and <22% in rabbit. The elimination $t_{1/2}$ was up to 3.1 hr in rat and 4.3 in dog. Following an oral dose of ^{14}C -telaprevir in rat, radioactivity was widely distributed throughout the body with the highest concentrations found in GI tissues and liver. Although at lower concentrations, radioactivity was also detected in testes. Studies in rodents confirmed placental and lactational transfer. In vitro plasma protein binding was similar between mouse, rat, dog, and human. Reaction phenotyping studies demonstrated that CYP3A4 was the only CYP450 enzyme capable of metabolizing telaprevir in vitro. Telaprevir and VRT-127394 did not induce activity of CYP1A1, CYP2C, or CYP3A4 in human hepatocytes. While repeat-dose studies in rat showed induction of CYP3A activity by telaprevir. Studies showed qualitatively similar in vivo metabolite profiles in rat, dog, and human. Three metabolites (i.e., VRT-127934, pyrazinoic acid, and VRT-922061) accounted for > 10% of total drug-related exposure in human. Excretion occurred primarily in feces from all species.

In chronic studies, female rats had greater systemic exposure than males while values were similar for both sexes in dog. A decrease in systemic exposure was observed upon repeat dosing in rat, an effect consistent with enzyme induction. This is supported by both increased CYP activity noted in the 13-week study as well as liver histopathology found in the repeat-dose studies. Slight potential for accumulation was observed after repeat-dosing in dogs. Enzyme inhibition, as observed in the 13-week study, may be partially responsible for the increased systemic exposure with repeat dosing. In the rat, increasing dose generally resulted in an approximately dose-proportional or slightly less than dose-proportional increase in telaprevir for plasma. In contrast, increasing dose generally resulted in a greater than dose-proportional increase for dog. Telaprevir was preferentially distributed to the liver in the rat only.

There were no adverse drug-related effects on neurological activity and respiratory parameters in rats, or cardiovascular parameters in telemetered dogs. In vitro evaluations of cardiotoxicity indicated a minor action potential duration increase in isolated dog Purkinje fibers and inhibition of hERG potassium channel tail currents in HEK293 cells. These in vitro changes lacked in vivo correlates in both telemetry and repeat-dose toxicity studies conducted in dogs as well as clinical studies.

Hematopoietic System: Hematology effects observed in both rat and dog included decreases in red blood cell associated parameters (e.g., red blood cells, hemoglobin, hematocrit, etc.). A compensatory increase in circulating reticulocytes was also detected. Changes in bone marrow cytology (e.g., decreased total granulocytic cells and M:E ratio; increased total erythrocytic cells), increased spleen weight, and/or histopathological lesions in spleen (increased red pulp hematopoiesis) occurred and were consistent with the compensatory response to decreases in red blood cell associated parameters. With the exception of increased spleen weight in rat, the hematopoietic

effects appeared to be reversible in both rat and dog. Anemia was a common adverse event in clinical trials with telaprevir.

Liver: Liver effects were noted in both species. Effects in the rat included clinical pathology indicators of hepatotoxicity (primarily increased aminotransferases), increased organ weight, and histopathological lesions (hepatocellular hypertrophy; single cell hepatocellular necrosis). These changes were consistent with the induced CYP3A activity and decreased systemic exposure observed in repeat-dose studies. Increased aminotransferases and microscopic lesions persisted through the recovery period. Hepatotoxicity occurred at exposures <0.63-fold those in human at the recommended clinical dose.

Effects in the dog included reversible changes in clinical pathology (no change in aminotransferases) and histopathology (mixed perivascularitis; sinusoidal hypercellularity; increased eosinophilic pigmentation in Kupffer cells). Telaprevir exposures at the no observed adverse effect level (NOAEL) for liver effects were 0.17- to 0.25-fold the exposures in human at the recommended clinical dose.

Indicators of hepatotoxicity (e.g., increased aminotransferases) were not observed in clinical trials.

Male Reproductive System: Drug-related effects on the male reproductive system were observed in the rat for both repeat-dose toxicology and fertility studies. Testicular toxicity was noted as macroscopic (small; soft) and microscopic lesions (degeneration, germinal epithelium; degeneration/necrosis, individual germ cells; degeneration, tubules, multifocal; increased/enlarged residual bodies; retained Step 19 spermatids, stage IX and X tubule; sloughed/necrotic intraluminal germ cells; vacuolation, Sertoli cells) with a correlating decrease in organ weight. Decreased organ weight accompanied by microscopic changes were also detected in the epididymis (exfoliated germ cell; hypospermia; aspermia; exfoliated spermatogenic cells/residual bodies). A decrease in the % of motile sperm as well as an increase in nonmotile sperm count also occurred. These male reproductive effects likely contributed to the changes noted during Caesarian-section evaluation in the fertility study. Notable effects included increased % preimplantation loss, % of dams with nonviable embryos, and % nonviable conceptuses/litter. In general, effects on male reproductive system appear reversible. The NOAEL for reproductive organ toxicity occurred at exposures 0.17-fold the human exposures at the recommended clinical dose. The sperm evaluation was conducted at a single dose level with an exposure 0.30-fold the clinical exposure.

Chronic active vasculitis in the epididymis was observed in a single dog after 9 months of treatment. This lesion occurred in multiple organs in the 9 month dog study and is described in detail below.

The Sponsor notes that the testicular toxicity in rat appeared to be species specific and suggested questionable human relevance for this effect. Measurements of inhibin-b, LH, and FSH from clinical trials were provided to support this claim. Mean changes in these

proposed hormonal biomarkers were comparable between telaprevir-treated subjects and those administered placebo. The proposed species-specific nature of the testicular toxicity is biologically plausible but has not been fully demonstrated.

Vasculitis and Secondary Effects: Chronic-active vasculitis was observed only in the dog. This microscopic lesion was observed in stomach, epididymis, heart, and ovary. Vasculitis was not observed in recovery animals. Additional microscopic lesions including several in bone and bone marrow (myelofibrosis; hypocellularity; necrosis; dysplasia, cartilaginous) were considered secondary to the chronic-active vasculitis. Primary and secondary lesions associated with this effect were generally reversible. The drug-induced vasculitis is consistent with canine polyarteritis (Hayes et al., 1989) which is typically considered to have questionable human relevance (Clemo et al., 2003). Vasculitis was not reported in clinical studies with telaprevir, further supporting the species-specific nature of this lesion. An NOAEL for vasculitis was established in the female at exposures 0.17-fold the human exposure at the recommended clinical dose. Vasculitis occurred at all dose levels in males, the lowest dose yielding exposure 0.25-fold of the clinical dose in human.

Telaprevir was not considered genotoxic based on negative results in the in vitro bacterial mutation assay, in vitro mammalian chromosome aberration assay, and in vivo rat micronucleus assay. Because telaprevir was not genotoxic and will be administered to humans for only 12 weeks, carcinogenicity studies were not required.

There were no adverse drug-related effects on embryofetal development in mouse or rat. Embryofetal development was not studied in rabbit due to an inability to achieve substantial systemic exposure. A fertility study in rat found male reproductive system toxicity and effects on Caesarian-section parameters (previously described). While the fertility effects were due, in part, to the male, contributions of the female cannot be ruled out due to study design limitations. Drug-related findings in a perinatal/postnatal evaluation were limited to a decrease in pup weight/litter prior to weaning in offspring from telaprevir-treated dams.

There were multiple impurities present in the final drug substance or drug product. The proposed specifications were deemed acceptable based on a 13-week toxicology study in rat, evaluation of genotoxic potential (e.g., empirical testing, computational analysis), the clinical indication (i.e., treatment of hepatitis C virus), and because telaprevir will be administered in combination with other toxic drugs (i.e., ribavirin and pegylated interferon).

Two clinical metabolites met the ICH M3(R2) threshold of >10% of drug-related exposure following a single dose while three clinical metabolites exceeded this threshold at steady-state. All were considered qualified based on their formation in nonclinical studies following oral administration of telaprevir, empirical testing or computational evaluation for genotoxic potential, clinical experience, as well as the clinical indication and dosing regimen previously described. Note that pyrazinoic acid is structurally similar to several molecules associated with rash and pruritus. These effects were reported in clinical trials with telaprevir+ribavirin+pegylated interferon. In addition, a minor clinical metabolite, VRT-841125, was considered to be a skin sensitizer.

A summary of exposure margins, based on available AUC data, is presented below.

Table 31. Telaprevir exposure margins

Species	Dose (mg/kg/day)		AUC _{0-24 hr} (ng·hr/mL)		Animal/Human Exposure Margin ^a	
	Male	Female	Male	Female	Male	Female
Rat						
6-month ^b	30 (lowest dose tested)	30 (lowest dose tested)	27,700	53,900	0.32	0.63
fertility ^c overall	100 (NOAEL)	N/E	14,700	N/E	0.17	N/E
sperm effects	300 (only dose tested)	N/A	25,600	N/A	0.30	N/A
embryofetal ^d	N/A	500 (NOAEL)	N/A	51,900	N/A	0.60
peri/postnatal ^e	N/A	150 (NOAEL)	N/A	34,300	N/A	0.40
Dog						
9-month ^f	25 (lowest dose tested)	25 (NOAEL)	21,149	14,463	0.25	0.17
Mouse						
embryofetal ^g	N/A	1000 (NOAEL)	N/A	157,712	N/A	1.84

^a Study no. VX-950-TiDP24-C208; AUC_{0-8 hr} = 28,600 ng·hr/mL; AUC_{0-24 hr} = 85,800 ng·hr/mL (calculated as AUC_{0-8 hr} x 3)

^b Study no. VX-950-TX-020 (Day 182 data)

^c Study no. VX-950-TX-016 (Day 91 data)

^d Study no. VX-950-TX-018 (Day 17 data; 1st dose administered on Day 7)

^e Study no. VX-950-TX-018 (Day 17 data; 1st dose administered on Day 7)

^f Study no. VX-950-TX-021 (Day 266 data)

^g Study no. VX-950-TX-022 (Day 15 data; 1st dose administered on Day 6)

N/A - not applicable

N/E – not established due to limitations of study design

References

Clemo, F.A.S., Evering, W.E., Snyder, P.W., Albassman, M.A. (2003) Differentiating spontaneous from drug-induced vascular injury in the dog. Toxicol. Pathol. 31(suppl):25-31.

Damiani, E., Greci, L., Hrelia, P. (2000) Cyto- and genotoxic effects of novel aromatic nitroxide radicals in vitro. Free Radic. Biol. Med. 28:330-336.

Gallez, B., DeMeester, C., Debuyst, R., Dejehet F., Dumont P. (1992) Mutagenicity of nitroxyl compounds: structure-activity relationships. Toxicol. Letters 63:35-45.

Hayes, T.J., Roberts G.K.S., Halliwell W.H. (1989) An idiopathic febrile necrotizing arteritis syndrome in the dog: Beagle pain syndrome. Toxicol. Pathol. 17(1 pt 2):129-137.

12 Appendix/Attachments

Organ Weights (paired organs weighed together)

Tissue	6-Month Rat Study (VX- 950-TX-020)	9-Month Rat Study (VX- 950-TX-021)
adrenal gland	✓	✓
brain	✓	✓
epididymis	✓	✓
heart	✓	✓
kidney	✓	✓
liver	✓	✓
lung*	✓	✓
ovary	✓	✓
pituitary gland*	✓	✓
prostate gland*	✓	✓
salivary gland*	✓	✓
seminal vesicle	✓	
spleen	✓	✓
testis	✓	✓
thymus	✓	✓
thyroid/parathyroid	✓	✓
uterus	✓	✓

* in addition to recommended tissue list

Histopathology

Tissue	6-Month Rat Study (VX- 950-TX-020)	9-Month Rat Study (VX- 950-TX-021)
adrenal gland	✓	✓
aorta	✓	✓
artery (mesentery)*	✓	
bone (femur)	✓	✓
bone (sternum)		✓
bone marrow (sternum)	✓	✓
brain (brain stem, cerebellum, and cerebrum)	✓	✓
cecum	✓	✓
cervix	✓	✓
colon	✓	✓
duodenum	✓	✓
epididymis	✓	✓
esophagus	✓	✓
eye	✓	✓
fallopian tube		
gall bladder		✓
gross lesions	✓	✓
gut associated lymphoid tissue (Peyer's patch)	✓	✓
Harderian gland	✓	
heart	✓	✓
ileum	✓	✓
jejunum	✓	✓
kidney	✓	✓

lacrimal gland		✓
liver	✓	✓
lung	✓	✓
lymph node (mandibular)	✓	✓
lymph node (mesenteric)	✓	✓
mammary gland	✓	✓
muscle (skeletal)	✓	✓
nasal cavity/turbinates		
nerve (optic)	✓	✓
nerve (sciatic)	✓	✓
ovary	✓	✓
pancreas	✓	✓
pharynx		
pituitary gland	✓	✓
prostate gland	✓	✓
rectum	✓	✓
salivary gland	✓	✓
seminal vesicle	✓	
skin/subcutis	✓	✓
spinal cord (cervical, lumbar, and thoracic)	✓	✓
spleen	✓	✓
stomach	✓	✓
testis	✓	✓
thymus	✓	✓
thyroid/parathyroid	✓	✓
tongue	✓	✓
trachea	✓	✓
urinary bladder	✓	✓
uterus	✓	✓
vagina	✓	✓
Zymbal's gland		

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/s/

MARK W POWLEY
04/22/2011

HANAN N GHANTOUS
04/22/2011

Comments on N201-917 telaprevir
From: Abigail Jacobs. Assoc Dir Pharm/tox
Date: 4/15/11

1. I agree that there are no outstanding pharm/tox issues, that the pregnancy category for telapavir is appropriate, and that label warnings for use of the drug are acceptable.
2. I have discussed some other comments with the reviewer and he will incorporate the changes into his review, as appropriate.

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/s/

ABIGAIL ABBY C C JACOBS
04/18/2011

**Division of Antiviral Products
Center for Drug Evaluation and Research**

Date: April 15, 2011
Reviewer: Hanan Ghantous, PhD, DABT
Supervisory Interdisciplinary Scientist
NDA #/SS#/date: 201-917/000/6/24/2010
Sponsor: Vertex Pharmaceuticals Inc.
Drug Product: Telaprevir (VX-950)
Indication: Treatment of Chronic hepatitis C
Recommended Action: Nonclinical data support approval

Telaprevir (VX-950) is an HCV NS3•4A protease inhibitor developed as a direct acting antiviral agent against the hepatitis C virus. Telaprevir will be administered with ribavirin, and pegylated interferon alpha to treat adult patients infected with chronic hepatitis virus who have compensated liver disease.

The safety of telaprevir was investigated in a number of toxicology studies including repeat-dose nonclinical toxicity studies (rats and dogs), genetic toxicity, and reproductive and developmental toxicity studies.

The primary target organs of toxicity in nonclinical studies were the hematopoietic system, the liver and the male reproductive system.

Hematology effects observed in both rat and dog included decreases in red blood cell parameters and compensatory increase in circulating reticulocytes. Changes in bone marrow cytology, increased spleen weight, and/or histopathological lesions in spleen (increased red pulp hematopoiesis) occurred and were consistent with the compensatory response to decreases in red blood cell associated parameters. With the exception of increased spleen weight in rat, the hematopoietic effects appeared to be reversible in both rat and dog. Anemia was a common adverse event in clinical trials with telaprevir.

Liver effects were noted in both species. Effects in the rat included increased aminotransferases, increased organ weight, and histopathological lesions. These changes

were consistent with the induced CYP3A activity and decreased systemic exposure observed in repeat-dose studies. Increased aminotransferases and microscopic lesions were not reversible. Hepatotoxicity occurred at exposures <0.63-fold those in human at the recommended clinical dose. Effects in the dog included reversible changes in clinical pathology and histopathology. Telaprevir exposures at the no observed adverse effect level (NOAEL) for liver effects were 0.17 to 0.25-fold the exposures in human at the recommended clinical dose. Indicators of hepatotoxicity (e.g., increased aminotransferases) were not observed in clinical trials.

Drug-related effects on the male reproductive system were observed in the rat for both repeat-dose toxicology and fertility studies. Testicular toxicity was noted as macroscopic and microscopic lesions with decreased organ weight. These male reproductive effects likely contributed to the increased % preimplantation loss, % of dams with nonviable embryos, and % nonviable conceptuses/litter in the reproductive studies. In general, effects on male reproductive system appear reversible and appeared to be species specific. The NOAEL for reproductive organ toxicity occurred at exposures 0.17-fold the human exposures at the recommended clinical dose. Mean changes in proposed hormonal biomarkers from clinical trials (inhibin-b, LH, and FSH) were comparable between telaprevir-treated subjects and those administered placebo.

Telaprevir was not considered genotoxic and since it will be administered to humans for only 12 weeks, carcinogenicity studies were not required.

There were no adverse drug-related effects on embryofetal development in mouse or rat. Embryofetal development was not studied in rabbit due to an inability to achieve substantial systemic exposure. Drug-related findings in a perinatal/postnatal evaluation were limited to a decrease in pup weight/litter prior to weaning in offspring from telaprevir-treated dams.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Mark Powley that the nonclinical data support an approval action for telapavir.

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/s/

HANAN N GHANTOUS
04/25/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 201917 Applicant: Vertex

Stamp Date: 11/22/10

Drug Name: Telaprevir NDA/BLA Type: Priority

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	✓		- carcinogenicity studies not needed per meeting minutes from April 17, 2008
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			- not applicable - components of core tablet are listed in IIG, amounts need to be compared to other marketed oral drugs
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	✓		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	✓		- included in toxicology written summary
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	✓		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

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

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

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

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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/s/

MARK W POWLEY
12/22/2010

HANAN N GHANTOUS
12/22/2010