2.6 NONCLINICAL SUMMARY

SECTION 2.6.6—TOXICOLOGY WRITTEN SUMMARY

EMTRICITABINE/RILPIVIRINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (FTC/RPV/TAF [F/R/TAF] FDC)

Gilead Sciences

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CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

λ_{max}	absorption maxima
ACTH	adrenocorticotropic hormone
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
APPT	activated partial thromboplastin time
ARV	antiretroviral
AST	aspartate amino transferase
B-ALP	bone alkaline phosphatase
BCOP	bovine corneal opacity permeability
BID	twice daily
BMD	bone mineral density
BUN	blood urea nitrogen
cAMP	adenosine monophosphate
CC_{50}	concentration that results in 50% cytotoxicity
不純物FD*	不純物FD*
CHL	Chinese hamster lung
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese Hamster ovary
CK	creatinine kinase
COBI	cobicistat
CO_2	carbon dioxide
CYP	cytochrome P450
CSF	cerebrospinal fluid
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
Dpd	deoxypyridinoline
dpm	disintegrations per minute
DXA	dual-emission X-ray absorptiometry
EC3	estimated concentration of 3is the concentration of test substance which would result in a SI of 3 in the LLNA
EC_{50}	estimated concentration of drug for a half maximal response
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EMA	European Medicines Agency
EU	European Union
EVG	elvitegravir (JTK-303, GS-9137)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil (coformulated); E/C/F/TDF, Stribild

LIST OF ABBREVIATIONS (CONTINUED)

F_0	parental generation
F_1	first generation
f.b.e.	free base equivalents
FDC	fixed dose combination
FDA	United States Food and Drug Administration
FTC	Emtricitabine, Emtriva® (Gilead), FTC, TP-006, GS-9019, 524W91
FTC/TDF	emtricitabine/tenofovir DF (Truvada®, Gilead Sciences), TVD
不純物FA*	不純物FA*
GALT	gut-associated lymphoid tissue
GD	gestation day
GGT	gamma glutamyltransferase
GI	gastrointestinal
GLP	Good Laboratory Practice
Hb	hemoglobin
HCT	hematocrit
HIV	human immunodeficiency virus
HPMC	hydroxypropylmethylcellulose
^{3}HTdR	^{3}H-methyl Thymidine
ICH	International Conference on Harmonization
IgM	immunoglobulin M
LC/MS	liquid chromatography/mass spectrometry
LD	lactation day
LNC	lymph node cell
LLNA	local lymph node assay
LV	left ventricular
MAA	marketing authorization application
MATE1	multidrug and toxin extrusion protein 1
MATE2-K	multidrug and toxin extrusion protein 2-K
MC	methylcellulose
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MRP4	multi-drug resistance-associated protein-4
MTD	maximum tolerated dose
mtDNA	mitochondrial DNA
NAG	N-acetyl-β-glucosaminidase
NCE	normochromatic erythrocyte
NA	not applicable
NK	natural killer
NNRTI	nonnucleoside reverse transcriptase inhibitor

LIST OF ABBREVIATIONS (CONTINUED)

NOAEL	no observed adverse effect level
NOEL	no observed effect level
NRTI	nucleoside reverse transcriptase inhibitor
OECD	Organization for Economic Cooperation and Development
PBMC	peripheral blood mononuclear cell
PCE	polychromatic erythrocyte
PEG 400	polyethylene glycol 400
P-gp	P-glycoprotein
PII	Primary Irritation Index
PK	pharmacokinetic
PM	premating
PND	postnatal day
PP	postpairing
PR	interval between the peak of the P wave and the peak of the R wave on ECG
PRTD	proximal renal tubular dysfunction
PT	prothrombin time
PTH	parathyroid hormone
QT	interval between the start of the Q wave and the end of the T wave on ECG
RBC	red blood cell
RDW	red cell distribution width
RPV	rilpivirine
S9	post-mitochondrial supernatant fraction from homogenized liver
sALP	bone specific alkaline phosphatase
SC	subcutaneous
SI	Stimulation Index
SIV	simian immunodeficiency virus
STB	Stribild®
STR	single tablet regimen combination
T_3	triiodothyronine
T_4	thyroxine
TAF	tenofovir alafenamide, GS-7340
TAF fumarate	tenofovir alafenamide fumarate, GS-7340-03
TDF	tenofovir disoproxil fumarate (TDF), 不純物XH* fumarate, GS-4331-05, PMPA prodrug (Viread®, Gilead)
TFV	tenofovir, GS-1278, PMPA
TFV-DP	tenofovir diphosphate
TID	3 times a day
tk	thymidine kinase
TK	toxicokinetic

LIST OF ABBREVIATIONS (CONTINUED)

TMC278	rilpivirine
TSH	thyroid-stimulating hormone
TTC	threshold of toxicological concern
TVD	Truvada®
USP	United States Pharmacopeia
v/v	volume concentration
WBC	white blood cell
w/v	weight-to-volume ratio

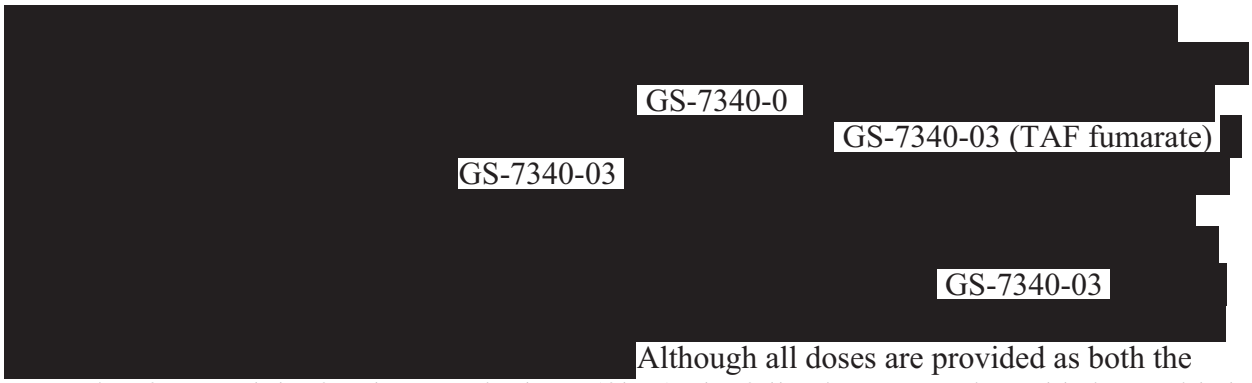
Abbreviations are in accordance with those accepted by *Toxicology and Applied Pharmacology*.

PHARMACOKINETIC ABBREVIATIONS

λ_z	Terminal elimination rate constant, estimated by linear regression of the terminal elimination phase of the serum, plasma, or peripheral blood mononuclear cells (PBMCs) concentration of drug versus time curve
AUC_{inf}	The area under the serum, plasma, or PBMC concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$
AUC_{x-xx}	Partial area under the serum, plasma, or PBMC concentration versus time curve from time "x" to time "xx"
AUC_{ss}	The area under the serum, plasma, or PBMC concentration versus time curve at steady state
AUC_{tau}	The area under the serum, plasma, or PBMC concentration versus time curve over the dosing interval
CL	The systemic clearance of the drug after intravenous administration
CL/F	The apparent oral clearance after administration of the drug: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug
CL_{ss}/F	The apparent oral clearance at steady state: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug
C_{max}	The maximum observed concentration of drug in serum, plasma, or PMBC
$T_{½}$	An estimate of the terminal elimination half-life of the drug in serum, plasma, or PBMCs, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
T_{max}	The time (observed time point) of C_{max}

NOTE TO REVIEWER

This application is being submitted for a fixed dose combination (FDC) that contains nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®), the nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (RPV, R, Edurant®) and and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide: the F/R/TAF FDC (200/25/25 mg) tablet. All nonclinical studies to support the F/R/TAF FDC application are included with no cross referencing to data previously submitted. This comprises all nonclinical tests utilizing FTC, RPV or TAF, including relevant combination studies, eg, FTC/tenofovir disoproxil fumarate (TDF); and other studies necessary to support the proposed product labeling for the treatment of human immunodeficiency virus type 1 (HIV-1) infection. Links to other modules and study reports are in blue text.



Although all doses are provided as both the respective form and the free base equivalents (f.b.e.), the following conversion table is provided as a reference.

GS-7340 Equivalents		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

The following conversions are also provided to aid the reviewer:

- FTC 1 µM = 0.247 µg/mL
- RPV 1 µM = 0.366 µg/mL
- TAF (GS-7340) 1 µM = 0.477 µg/mL
- TFV 1 µM = 0.287 µg/mL

1. BRIEF SUMMARY

This dossier is being submitted in support of a fixed dose combination (FDC) of emtricitabine (FTC, F, Emtriva®), rilpivirine (RPV, R, Edurant®) and tenofovir alafenamide (TAF, GS-7340): the FTC/RPV/TAF (200/25/25 mg) FDC.

Emtricitabine (Emtriva) is a nucleoside reverse transcriptase inhibitor (NRTI). Rilpivirine (Edurant®) is a nonnucleoside reverse transcriptase inhibitor (NNRTI). Tenofovir disoproxil fumarate (TDF, Viread®) is the first generation prodrug of tenofovir (TFV). Tenofovir alafenamide is a prodrug of TFV that, compared to TDF, has a longer plasma half-life and achieves higher levels of TFV into target cells at lower exposure of circulating TFV. TAF has a unique metabolism that provides enhanced delivery of TFV to HIV-target cells including lymphocytes and macrophages, resulting in higher intracellular levels of the active phosphorylated metabolite tenofovir-diphosphate (TFV-DP), and, at the 10-mg dose, yields approximately 90% lower circulating levels of TFV relative to TDF {7415}, {13119}, {22029}. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are known risks with TDF administration {21762}, {22031}, {30895}.

The FTC/RPV/TAF FDC contains the same dose of FTC (200 mg) that is currently approved within Emtriva®, FTC/TDF (Truvada®), FTC/efavirenz/TDF (Atripla®), FTC/RPV/TDF (Complera®/Eviplera®) and elvitegravir/cobicistat/FTC/TDF (Stribild®) and the same dose of RPV (25mg) currently approved within Edurant® and Complera®/Eviplera®. Comprehensive programs of nonclinical toxicology studies with FTC, RPV, and TAF have been conducted (Table 1). All of the definitive toxicology and toxicokinetic studies reported in this summary were conducted in accordance with the Good Laboratory Practice (GLP) procedures promulgated by international health authorities. Pilot, exploratory, and mechanistic studies were not all conducted under strict GLP procedures, but were conducted using appropriate protocols and documentation to assure data integrity. The nonclinical data discussed within this document support the proposed use of the FTC/RPV/TAF FDC for the treatment of HIV-1 infection.

FTC

Emtricitabine is an NRTI that is marketed as a once-daily capsule (Emtriva 200 mg) and as an oral solution (Emtrival Oral Solution 10 mg/mL) for the treatment of HIV-1 infection. Emtricitabine is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine that is structurally similar to lamivudine (Epivir®). Intracellularly, FTC is phosphorylated by cellular enzymes to form the active metabolite, emtricitabine triphosphate.

The general systemic (single and repeat dose) toxicity, genotoxicity, carcinogenicity, reproductive toxicity, and immunotoxicity of FTC have been characterized in a variety of in vitro and in vivo studies.

The nonclinical toxicity studies demonstrated that FTC was well tolerated after oral dosing for up to a year at doses producing systemic exposure levels in animals much greater than those anticipated in patients treated with the recommended clinical dose.

Treatment-related effects were confined to high-dose groups only and included changes in red blood cell (RBC) parameters, interpreted as a mild, reversible anemia (mice 1 month, 6 months; rat 3 months; monkey 1 year); changes in various organ weights without any associated adverse histopathological effects in rodents (mouse 1 month, 6 months; rat 3 months), increased urine output (mice 6 months), and soft feces (monkeys 1 month, 3 months). No observed effect levels (NOELs) could be established for all treatment-related effects, and in several cases the minor effects observed were reversible after a recovery period.

Emtricitabine was not genotoxic or clastogenic. Long-term carcinogenicity studies in mice and rats showed no evidence of carcinogenic potential at exposures greater than 25 times those observed in humans at the therapeutic dose. Emtricitabine did not adversely affect reproduction or embryo-fetal development. It was relatively noncytotoxic to human cells in vitro, including bone marrow progenitor cells, and did not produce mitochondrial toxicity.

The impurities and degradation products in the FTC drug substance have been identified and qualified in toxicology studies.

RPV

Rilpivirine (TMC278) is an NNRTI active against wild type and NNRTI-resistant HIV-1 that is marketed as a once-daily tablet (Edurant 25 mg) for the treatment of HIV-1 infection and is a component of Complera®/Eviplera® (FTC/RPV/TDF). The following convention is applied throughout this module: reference is made to “TMC278” when the hydrochloride (HCl) salt was administered and to “TMC278 base” when the base was administered. The dose or concentration is always given as base equivalent. The analyte in bioanalytical determinations is referred to as “TMC278.”

Rilpivirine base was used in the early phases of development and TMC278 in the later phases, after selection of the final chemical form. The objectives of the toxicology program were to evaluate the toxicological profile of TMC278, its metabolites, and impurities in nonclinical test systems. Furthermore, attempts were made to elucidate the mechanism of action of the effects observed. All in vivo studies were done by oral administration, with the exception of the sensitization and dermal irritation studies. For oral dosing, TMC278 base was dissolved in polyethylene glycol 400 (PEG400) usually with 100 mg/mL citric acid (CA) to improve exposure, with the exception of the rabbit studies in which TMC278 base was suspended in 0.5% (m/v; mass per volume) aqueous hydroxypropylmethylcellulose (HPMC). TMC278 was suspended in 0.5% (m/v) aqueous HPMC in the mouse, rat, and dog studies. In the studies with cynomolgus monkeys, the vehicle was 1% (m/v) aqueous HPMC with 0.5% v/v Tween 20.

No formal single-dose studies were done as single dose evaluations were part of the initial dose range finding studies or, in the case of mice, part of the bone marrow micronucleus test. The single-dose toxicity of TMC278 in mice, rats, and dogs appeared to be low. The maximal feasible dose did not induce significant toxicity or effects.

Repeat-dose toxicity studies were done in mice as preparation for a 3-month dose range finding carcinogenicity study; in rats for up to 6 months; in nonpregnant rabbits for 5 days as preparation for the dose range finding early embryonic development studies; in dogs up to 12 months; and in

immature female cynomolgus monkeys up to 8 weeks as part of the assessment of the effects of TMC278 on juvenile animals. Juvenile rats were used in a 2-week oral dosing study starting on lactation Day 12 (LD 12). The dogs in studies up to 1-month duration are also considered immature. Since the animals were 6.5 to 8 months old at the start of these studies they were not yet sexually mature at the end of the dosing period. Reversibility (after a 1-month recovery period) of the effects of TMC278 on the high dose group was investigated in the 6-month rat study and the 1-month dog study.

The targets of toxicity of TMC278 identified in the repeat-dose studies were: RBCs (mouse, rat, and dog); coagulation (rat); liver (rat and dog); kidneys (mouse and dog); thyroid gland with secondary effects on the pituitary gland (rat); adrenal glands (mouse, rat, dog, and cynomolgus monkey); testes (dog); and ovaries (dog, in immature females with secondary effects on other tissues of the genital tract and on mammary glands). The majority of the induced effects appeared to be completely reversible after a 1-month postdosing period. The effects on thyroid gland and coagulation in rats and on liver and serum alkaline phosphatase (ALP) in dogs showed signs of recovery, but this was not complete at the end of the 1-month postdosing period. A number of targets were affected at the low dose tested in dogs and cynomolgus monkeys preventing establishment of no observed adverse effect levels (NOAELs) in these 2 species.

TMC278 did not show a potential for genotoxicity in the in vitro bacterial reverse mutation (Ames) tests, mouse lymphoma assays or in the in vivo mouse bone marrow micronucleus test. No potential for carcinogenicity by a direct interaction with deoxyribonucleic acid (DNA) was concluded in the 2-year studies in mice and rats. The hepatocellular adenomas and carcinomas seen in mice are considered to be induced by liver enzyme induction, an epigenetic mechanism. The hepatocellular adenomas and thyroid follicular cell adenomas and carcinomas in rats are also considered to derive from an epigenetic mechanism as result of liver enzyme induction and a likely associated increased clearance of thyroid hormones leading to a continuous stimulation of the thyroid gland by thyroid stimulating hormone (TSH). No neoplastic lesions associated with the other targets of TMC278 were detected.

TMC278 did not show a teratogenic potential and did not affect fertility, fecundity, early embryonic development, maternal behavior at parturition and during lactation, or peri- and postnatal development of offspring from dams (rats) treated with TMC278.

TMC278 had similar targets or induced the same type of adverse effects in juvenile or sexually immature rats, dogs, and cynomolgus monkeys as in adult animals. The TMC278-related effects on the female genital tract and mammary glands in dogs at the end of the 1-month studies differed from the effects in longer duration studies in which animals were sexually mature animals at the end of the dosing period. The activation of ovaries in dogs treated with TMC278 in the 1-month studies led to secondary activation noticed in the other parts of the genital tract and mammary glands. These secondary effects occur normally in sexually mature animals during estrous cycle. Therefore, they were not noted as a difference between TMC278-treated and control animals in longer duration studies. However, in the 1-month study, the control animals were still dormant (i.e., no activation of ovaries and therefore no secondary effects). In the evaluation relative to control animals, the secondary effects were noted. Activation of ovaries was not induced during an 8-week study in immature cynomolgus monkeys at the age of approximately 18 months at the start of the study.

TMC278 tested negative for the potential to cause phototoxicity, skin irritation, and delayed-type hypersensitivity and to induce an immunotoxic effect on the challenge of rats with sheep RBCs. TMC278 was classified as a moderate eye irritant in an in vitro test.

The drug substance contains 3 impurities that were qualified according to Guideline Q3A of the International Conference on Harmonization (ICH), entitled: "Impurities in New Drug Substances." Two of these, 不純物A* and 不純物B*, have been evaluated upon spiking to the drug substance at a level of 4%. Qualification comprised a bacterial reverse mutation Ames test, a mouse lymphoma assay, and a 1-month oral rat study. The presence of the impurities at 4% did not modify the effects of TMC278 in any of the tests. The third impurity, 不純物C*, is the [redacted] isomer of TMC278. This isomer was present in all drug substance batches involved in pivotal nonclinical studies at the level of minimally 0.61%. In view of the close structural relationship with TMC278 and the overage between the lowest nonclinical dose (5 mg/kg/day) and the recommended clinical dose of 25 mg once daily, separate qualification of 不純物C* is not considered relevant.

Three further (potential) impurities, 不純物G*, 不純物E* , and 不純物F* , which contain moieties with a mutagenic alert, are present in the drug substance at levels that do not warrant qualification according to International Conference on Harmonization (ICH) Q3A(R2) (Impurities in Drug Substances). The mutagenic potentials of the HCl salts of 不純物G*, 不純物FA* , and of 不純物E* and 不純物F* were tested in an Ames test. Only 不純物H* showed a mutagenic potential. 不純物G* is considered a genotoxic impurity. The maximum allowable level of 不純物G* in a daily dose of 25 mg TMC278 was calculated to be [redacted] ppm, on the basis of the Threshold of Toxicological Concern (TTC) approach with daily treatment for 1-10 years per ICH M7 (Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 2014). The level of 不純物G* in drug substance and drug product has been controlled at less than [redacted] ppm.

TAF

Tenofovir alafenamide is a prodrug of TFV, an NtRTI. Tenofovir alafenamide is more stable in plasma than TDF, allowing for it to circulate for a longer period of time and interact with target cells. Tenofovir alafenamide provides enhanced delivery of TFV to the liver and HIV-target cells and tissues, resulting in higher intracellular levels of the active phosphorylated metabolite TFV-diphosphate (TFV-DP). In lymphocytes and macrophages, TAF is predominantly hydrolyzed intracellularly to TFV by cathepsin A (CatA) {13119}. Once released intracellularly, TFV is metabolized to the active metabolite, TFV-DP {1574}, a competitive inhibitor of HIV-1 reverse transcriptase that terminates the elongation of the viral DNA chain {21}, {1131}. The increased stability of TAF in plasma combined with the enhaced delivery of TFV to the target cells and tissues resulting in subsequent formation of 4-fold (3- to 7-fold at 90% confidence interval) higher intracellular levels of the active phosphorylated metabolite TFV-DP in peripheral blood mononuclear cells (PBMCs) and 90% lower circulating levels of TFV relative to TDF {17137}. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are known risks with TDF administration {21762}, {22031}, {30895}.

Blood was collected for plasma concentration analysis of TAF and/or TFV in repeat-dose and developmental and reproductive toxicity studies. Based on the PK profile of TAF in CD-1 mice, Sprague-Dawley rats, and TgRasH2 wild-type mice, TAF has been shown to be rapidly converted to TFV. In vitro plasma studies confirmed that TAF is rapidly hydrolyzed in rat ($t_{1/2}$ = 31 minutes) and mouse ($t_{1/2}$ < 3 minutes). Unlike TDF, TAF is relatively stable in human plasma ($t_{½}$ ~75 minutes), but rapidly converts to TFV inside cells. Shorter duration repeat-dose rodent toxicity studies had TFV but not TAF blood level determinations; repeat-dose nonrodent toxicity studies evaluated both TAF and TFV toxicokinetics. Both TAF and TFV toxicokinetics were evaluated in the rat and rabbit developmental and reproductive toxicity studies. Protein binding of GS-7340-02 was determined; the free fraction in human and beagle dog plasma was 46.8% and 48.0%, respectively (m2.6.4, Section 4.1.3).

The toxicology program for TAF, the only unapproved component of the FTC/RPV/TAF FDC, is provided in Table 1. TAF was evaluated in mouse, rat, dog, and monkey single- and/or repeat-dose toxicity studies for up to 39 weeks in duration. In addition, the mouse was used for an in vivo genetic toxicity test and rat and rabbit were used for developmental and reproductive toxicity studies. All in vivo studies utilized oral administration, the clinical route of administration, with the exception of the sensitization and dermal irritation studies. The rat and dog were demonstrated to have similar in vivo metabolic profiles to humans (m2.6.4, Section 5.1.3). The vehicle used for toxicity studies was 1) 25 mM citric acid, 2) 0.5% polysorbate 20, 0.5% carboxymethylcellulose, 0.9% benzyl alcohol, or 3) 0.1% (volume concentration [v/v]) Tween 20 and 0.1% (v/v) hydroxypropylmethylcellulose (HPMC).

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day), the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decreased serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in rats (≥ 25 mg/kg/day) and dogs (≥ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss, but these changes were accompanied by a slight but significant decrease in serum 1,25 dihydroxy vitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

TAF administered by oral gavage for up to 13 weeks to mice at ≥10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs, or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

TAF had no discernible electrocardiograph effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (approximately 13% to 24%), which was associated with significant decreases in triiodothyronine (T3) {29101}, {29104}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study.

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. These infiltrates were seen primarily in perivascular regions in the ciliary body, and less frequently in the choroid layer subjacent to the border of the peripheral retina with the ciliary body at the level of the ora ciliaris retinae (equivalent to the ora serrata in humans). Based on their bilateral nature and typical locations in perivascular regions of the ciliary body and junction of the choroid layer with the margin of the peripheral retina, these infiltrates were considered to be consistent with an increased incidence and/or size of minor, random perivascular infiltrates of mononuclear cells commonly associated with maintenance of the blood-eye barrier. This suggests that the microscopic findings in dogs are of limited clinical relevance to humans. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), nonhuman primates (4 weeks), or in the 4-week dog toxicology study. The minimal infiltration of histiocytes in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. Based on these data from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mitochondrial DNA levels up to 1μM (approximately 2-fold higher than C_{max}), the highest concentration tested, in HepG2 cells in a 10-day assay (PC-120-2006). The active metabolite of TAF, TFV-DP is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, adenosine triphosphate (> 10,000 fold) {4923}. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli*), and a L5178Y gene mutation assay in mouse lymphoma cells. The in vivo evaluation consisted of a mouse bone marrow micronucleus study (at oral doses of 500, 1000, and 2000 mg/kg).

Per separate agreements with the Food and Drug Administration (FDA) and Committee for Medicinal products for Human Use (CHMP), [redacted]

[redacted].
Toxicokinetic data obtained following dosing of TAF to the transgenic mouse and rat clearly demonstrate an inability to measure plasma concentrations of the prodrug, TAF. Although significant plasma levels of the active drug, TFV, were obtained in the rat following chronic dosing with TAF, the TFV exposure was less than that already tested in the TDF carcinogenicity study. Lifetime daily administration of TDF at dosage levels of up to 300 mg/kg/day given for 104 weeks did not reveal any evidence of carcinogenicity in Swiss Crl:CDR-1(ICR)BR mice. At 600 mg/kg/day, a low incidence of duodenal tumors, possibly treatment-related, were observed. Lifetime daily administration of TDF at dosage levels up to 300 mg/kg/day did not reveal any evidence of carcinogenicity in Sprague-Dawley rats.

Reproductive tissues were examined in repeat-dose toxicology studies in the rat, dog, and monkey. There were no treatment-related histologic alterations or changes in organ weights in the rat and the dog following chronic daily dosing, or in the monkey following 28 days of daily oral administration.

The TAF oral rat fertility study data indicated dose-related decreases in body weight gain in males and females, but no drug-related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions, and live fetuses at doses up to 160 mg free base equivalents/kg/day. The NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of TAF up to 250 mg/kg/day or in pregnant rabbits administered TAF up to 100 mg/kg/day. The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, $AUC_{0-\tau}$ values for TAF and TFV on Day 17 were 0.2 and 17.4 µg·h/mL, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, $AUC_{0-\tau}$ values for TAF and TFV on Day 20 were 11.0 and 27.3 µg·h/mL, respectively.

Per separate agreements with the FDA and CHMP, [redacted] As with the carcinogenicity study rationale, there is an inability to measure plasma concentrations of TAF in rats, and TFV exposure after TAF administration was less than that already tested in the TDF peri/postnatal study. The peri/postnatal studies with TDF clearly characterized the potential postnatal toxicity of TFV.

The peri/postnatal study of TDF showed increased rat pup mortality at maternally toxic doses ≥ 450 mg/kg/day. No TDF-related deaths occurred. The maternal NOEL of TDF was 50 mg/kg/day. The 150, 450, and 600 mg/kg/day dosages caused adverse clinical observations, reductions in body weight gain during gestation and increases in body weight gain during lactation, and the 450 and 600 mg/kg/day dosages reduced gestation and lactation feed consumption values. The developmental NOEL was 150 mg/kg/day. The 450 and 600 mg/kg/day dosages resulted in increased peri/postparturn pup mortality, reduced pup survival, and reduced pup body weights. The NOEL for general toxicity of TDF in the first generation (F_1) generation was 50 mg/kg/day (mortality occurred or body weights and/or feed consumption were reduced in

the 150-, 450-, and 600-mg/kg/day dosage groups). The F_1 male and female NOEL for behavioral, reproductive, and developmental toxicity of TDF was 150 mg/kg/day (effects on sexual maturation were observed at dosages of 450 and/or 600 mg/kg/day).

Evaluation of local tolerance to the GI tract was conducted during the repeat-dose oral toxicity studies. No clinical or histopathological changes occurred in the GI tract in the TAF repeat-dose toxicity studies in mice, rats, or monkeys. After 39 weeks of dosing dogs at 18/12 mg/kg/day, observations included atrophy of gut-associated lymphoid tissue (GALT), accompanied by an infiltrate of macrophages; mucosal atrophy of the fundic gland; mucosal hyperplasia of the pyloric gland; and mucosal degeneration and/or regeneration in the cecum and colon. GI toxicity was not dose-limiting.

Local toxicity studies concluded that TAF fumarate was predicted to be a noncorrosive/nonsevere eye irritant, and nonirriating/noncorrosive to rabbit skin under semi-occluded conditions. TAF fumarate did not show the potential to cause skin sensitization with repeated application to the dorsal surface of mouse ears.

The impurities and degradation products in the TAF drug substance have been identified and qualified in toxicology studies.

FTC/RPV/TAF

Based on the well-characterized toxicity profiles of FTC, RPV and TAF, the low potential for toxicologic interaction noted in the combination toxicology studies with FTC+TDF; and the clinical safety data available for TVD (Truvada), and other FTC/TDF-containing regimens including FTC/RPV/TDF, the data demonstrate an acceptable benefit/risk profile for the proposed use of the FTC/RPV/TAF FDC product for the treatment of HIV-1 infection.

In accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008), nonclinical safety studies with the FTC/RPV /TAF combination were not conducted. There are no anticipated clinically relevant PK or toxicological interactions expected in the FTC/RPV/TAF combination.

The impurities and degradation products in the FTC, RPV and TAF, and in FTC/RPV/TAF tablets have been identified and qualified per International Conference on Harmonization (ICH) guidelines in toxicology studies.

Table 1. FTC/RPV/TAF Toxicology Program

Study Type & Duration	Route of Administration	Species	Compound Administered
Single-Dose Toxicity			
FTC	Oral, Intravenous	Mouse, rat	FTC
RPV	Oral	Mouse, rat, dog	RPV base
TAF	Oral	Rat, dog	TAF
Repeat-Dose Toxicity			
FTC			
2 Weeks	Oral	Mouse	FTC
4 Weeks	Oral	Mouse, monkey	FTC
13 Weeks	Oral	Rat	FTC
26 Weeks	Oral	Mouse	FTC
1 Year	Oral	Monkey	FTC
RPV			
5 Days	Oral	Rat, rabbit, dog	RPV base
7 Days	Oral	Dog	RPV base
2 Weeks	Oral	Mouse, rat	RPV base (rat), RPV (mouse, rat)
4-Weeks	Oral	Mouse, rat, dog	RPV base (rat, dog), RPV (mouse, rat, dog)
8 Weeks	Oral	Monkey	RPV
13-Weeks	Oral	Mouse	RPV
26-Weeks	Oral	Dog, rat	RPV base
1 Year	Oral	Dog	RPV base
TAF			
6-15 days	Oral	Mouse, rat	TAF
4 weeks	Oral	Rat, dog, monkey	TAF
13 weeks	Oral	Mouse	TAF
26 weeks	Oral	Rat	TAF
39 weeks	Oral	Dog	TAF
FTC/TDF			
14 Days	Oral	Rat	FTC/TDF
28 Days	Oral	Dog	FTC/TDF

Study Type & Duration	Route of Administration	Species	Compound Administered
Genotoxicity			
FTC			
In vitro nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	FTC
In vitro mammalian cell system (chromosome aberration)	In vitro	Mouse lymphoma cells	
In vivo: Micronucleus Assay	Oral	Mouse	FTC
RPV			
In Vitro: Nonmammalian Cell System	—	Bacteria	RPV base, RPV
In Vitro: Mammalian Cell System	—	Mouse	RPV base, RPV
In Vivo: Micronucleus Assay	Oral	Mouse	RPV base
TAF			
In vitro: nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	TAF
In vitro: mammalian cell system (forward mutation)	In Vitro	Mouse lymphoma cells	TAF
In vivo: Micronucleus Assay	Oral	Mouse	TAF
FTC/TDF			
In vitro: nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	FTC/TDF
In vitro: mammalian cell system (forward mutation)	In Vitro	Mouse lymphoma cells	FTC/TDF
Carcinogenicity			
FTC			
2 Years	Oral	Mouse, rat	FTC
RPV			
2 Years	Oral	Mouse, Rat	RPV
TDF			
2 Years	Oral	Mouse, rat	TDF
Reproductive Toxicity			
FTC			
Fertility and early embryonic development	Oral	Mouse, rat	FTC
Embryo-fetal development	Oral	Mouse, rabbit	FTC
Prenatal and postnatal development	Oral	Mouse	FTC

Study Type & Duration	Route of Administration	Species	Compound Administered
RPV			
Fertility & Early Embryonic Development	Oral	Rat	RPV
Embryo-Fetal Development	Oral	Rat, rabbit	RPV base
Prenatal & Postnatal Development	Oral	Rat	RPV
TAF			
Fertility and early embryonic development	Oral	Rat	TAF
Embryo-fetal development	Oral	Rat, rabbit	TAF
TDF			
Prenatal and postnatal development	Oral	Rat	TDF
Local Tolerance			
Phototoxicity - In Vitro: Mammalian Cell System	—	Mouse	RPV base
Eye, Skin Irritation Tests	Topical	Bovine, rabbit	RPV
TAF			
Eye irritation	Topical	Bovine ex vivo	TAF
Skin irritation	Topical	Rabbit	TAF
Other Studies			
FTC			
Immunotoxicity	Oral	Rat	FTC
Impurities – qualification studies	Oral	Mouse	FTC
Genotoxicity qualification assays	In Vitro	Bacteria, Chinese hamster ovary cells	FTC
RPV			
Antigenicity	Topical	Mouse	RPV
Mechanistic Studies – In Vitro	—	–	RPV
Mechanistic Studies – In Vivo	Oral	Dog	RPV base
Impurities - Genotoxicity Mutation Assays	In vitro	–	RPV/不純物A* / 不純物B* / 不純物E* / 不純物F*
Oral Qualification Studies	Oral	Rat	RPV/不純物A*, 不純物B*
TAF			
Antigenicity - local lymph node assay	Topical	Mouse	TAF
Bone evaluation	Oral	Rat, dog	TAF
Renal effects	Oral	Rat	TAF
Impurity evaluation	Oral	Rat	TAF

2. SINGLE DOSE TOXICITY

2.1. FTC

2.1.1. Single Oral Dose Toxicity Study in the Mouse

Five male and 5 female CD-1 mice were orally administered 4000-mg/kg FTC (m2.6.7, Section 5.1, TTEP/93/0020). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings. Emtricitabine was well tolerated and the NOEL was > 4000 mg/kg.

2.1.2. Single Intravenous Dose Toxicity Study in the Mouse

Five male and 5 female CD-1 mice were intravenously administered 200 mg/kg FTC (m2.6.7, Section 5.1, TTEP/93/0023). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings. Emtricitabine was well tolerated and the NOEL was > 200 mg/kg.

2.1.3. Single Oral Dose Toxicity Study in the Rat

Five male and 5 female CD rats were orally administered 4000 mg/kg FTC (m2.6.7, Section 5.1, TTEP/93/0021). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings. Emtricitabine was well tolerated and the NOEL was 4000 mg/kg.

2.1.4. Single Intravenous Dose Toxicity Study in the Rat

Five male and 5 female CD rats were intravenously administered 200 mg/kg FTC dissolved in 0.9% saline (m2.6.7, Section 5.1, TTEP/93/0024). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings and FTC was well tolerated. It was concluded that the approximate lethal dose in rats dosed intravenously was > 200 mg/kg.

2.2. RPV

2.2.1. Single Oral Dose Toxicity Study in the Mouse

In a bone marrow micronucleus study (m2.6.7, Section 9.2, TMC278-Exp5538 and Addendum [FK4259]), a single oral dose of TMC278 base dissolved in PEG400 with citric acid was administered by gavage to 10 male and 10 female CD-1 mice per group. The doses were 0 (vehicle), 100, 400, and 1600 mg/kg given in a volume of 20 mL/kg. The dose of 1600 mg/kg was the maximally feasible dose on the basis of the maximally feasible volume for a single dose study, 20 mL/kg, and a supersaturated and highly viscous solution of 80 mg/mL. No premature deaths occurred.

2.2.2. Single Oral Dose Toxicity Study in the Rat

The highest feasible concentration of an oversaturated solution of TMC278 base in PEG400 was 80 mg/mL, in terms of viscosity for administration by gavage to rats. This concentration was administered by oral gavage at a volume of 10 mL/kg to 6 male and 6 female Sprague Dawley rats, rendering a dose of 800 mg/kg (m2.6.7, Section 5.2, TMC278-Exp5559). Mortality and clinical observations were evaluated for 1 day following the administration. Four males and 4 females were sampled to assess exposure.

TMC278 did not cause any mortality. All males and 2 out of 6 females showed salivation during the day of dosing. At necropsy, no TMC278-related gross lesions were recorded. The mean C_{max} was 8.0 and 18.0 µg/mL and the AUC_{inf} was 86 and 233 µg.h/mL in males and females, respectively.

2.2.3. Single Oral Dose Toxicity Study in the Dog

For dogs, the highest feasible concentration of an oversaturated solution of TMC278 base in PEG400 with or without CA was 100 mg/mL in terms of viscosity for the administration by gavage. The dogs were pretreated with the vehicles applied in this study. Polyethylene glycol 400 was administered by oral gavage at 0.8 mL/kg to 2 groups of 1 male and 1 female beagle dog and PEG400 + CA to another group (m2.6.7, Section 5.2, TMC278-Exp5461). The next day, the PEG400-pretreated animals were dosed with TMC278 base dissolved in PEG400 at a single dose of 40 or 80 mg/kg. The PEG400 + CA pretreated animals received TMC278 base dissolved in PEG400 + CA at a single dose of 80 mg/kg. The animals were observed for 4 days after dosing. No clinical pathology or necropsy was performed on these animals.

TMC278 did not cause any mortality. Only in the group dosed with 80 mg/kg dissolved in PEG400 did a higher incidence of vomiting occur for 2 days after dosing. Slightly soft feces were recorded for the male at Day 3 after dosing and for the female at Days 2 and 3 after dosing. The animals dosed with 40 mg/kg were transferred to the repeat-dose arm of the study 3 weeks after the end of the single dose observation period. Exposure data are presented in Table 2.

Table 2. Exposure to TMC278 (C_{max} and AUC) in an Oral Single-Dose Study with TMC278 Base in Dogs

Dose (mg/kg/day)	Vehicle	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M (n = 1)	F (n = 1)	M (n = 1)	F (n = 1)
40	PEG400	1.1	0.95	21	19
80	PEG400	0.88	0.81	17	16
80	PEG400 + CA	2.1	2.2	42	49

PEG = polyethylene glycol, CA = citric acid, M = males, F = females

2.3. TAF

2.3.1. Single Oral Dose Toxicity in the Rat

Male and female Sprague-Dawley rats (5/sex/group) were administered single oral dose (15 mL/kg) of GS-7340-02 at doses of 0 (vehicle), 100, 300, or 1000 mg/kg (80, 240, 800 mg/kg free base equivalents [f.b.e.]/kg) followed by a 14-day observation period (m2.6.7, Section 5.3, R990185). The NOAEL for single oral dose GS-7340-02 in the rat was determined to be > 1000 mg/kg.

2.3.2. Single Oral Dose Toxicity in the Dog

Male and female beagle dogs (1/sex/group) were administered a single oral dose (15 mL/kg) of GS-7340-02 at doses of 0 (vehicle), 30, 90, or 270 mg/kg (24, 72, 216 mg f.b.e./kg) followed by a 14-day observation period (m2.6.7, Section 5.3, D990181). Once prior to the commencement of treatment and on Days 2 and 14, hematology, clinical biochemistry, and urinalysis were performed on all animals. Mild clinical signs (salivation, vomiting, reduced activity, tremors, incoordination) observed at 270 mg/kg resolved 2 days following dosing. There was a transient increase in blood urea nitrogen in animals administered 270 mg/kg (present on study Day 2, not study Day 14). There were no other significant changes in clinical chemistry, hematology, or urinary parameters 14 days after dosing. Thymus weights were reduced in all treated groups compared with controls, and thymic atrophy was present in males administered 90 and 270 mg/kg. Renal tubular changes characterized by basophilia and/or karyomegaly were present in the male administered 270 mg/kg and the females administered 270 mg/kg and 90 mg/kg. The NOAEL for single oral dose of TAF in the dog was determined to be 30 mg/kg.

2.4. FTC/RPV/TAF

No single-dose studies have been performed with the combination of FTC, RPV, and TAF. Single-dose toxicity studies indicated that FTC, RPV, and TAF had low acute toxicity. With no overlapping toxicities, coadministration is unlikely to change the acute toxicity profile.

3. REPEAT-DOSE TOXICITY

3.1. FTC

3.1.1. 2-Week Oral (Gavage) Toxicity Study in Mice

Four groups of 15 male and 15 female CD-1 mice were administered FTC, suspended in 0.5% methyl cellulose, at doses of 0, 120, 600, and 3000 mg/kg/day (m2.6.7, Section 7.1.1, TOX001). The dose was administered by gavage and as a divided dose with 6 hours between the morning and afternoon dose. Ten mice/sex/group were necropsied at the end of the exposure period; 5/sex/group were necropsied at the end of a 2-week postdose recovery period. There were no important differences in levels of FTC measured when males were compared to females and Day 2 values were compared to Day 14 values. Mean concentrations for FTC were proportional to dose. At the high dose, mean concentrations on Day 2 were 318 and 358 μg/mL for males and females, respectively; and on Day 14 were 389 and 405 μg/mL, respectively. There were no signs of toxicity.

3.1.2. 4-Week Oral Toxicity Study in Mice

Groups of 14 male and female mice were orally administered (once daily) FTC suspended in 0.5% methylcellulose at 0, 120, 600, and 3000 mg/kg/day for 30 days, with 4 males and females from each group held for an additional 13-day recovery period (m2.6.7, Section 7.1.2, TOX599). Also exposed to the same dosing regimen were groups of 10 males and females, which were used for clinical pathology evaluations prestudy and on Days 31 to 32. Groups of 38 male and female satellite toxicokinetic animals were dosed at 120, 600, and 3000 mg/kg/day and drug level determinations made from samples collected on Days 3 and 32. There were no clinical signs of toxicity that were thought to be treatment related. Two high dose male mice died of undetermined causes, and a relationship to treatment cannot be ruled out. No treatment-related changes in feed consumption or body weight were seen. At the end of the study, hemoglobin (Hb) and hematocrit (HCT) were reduced in high-dose females, and total erythrocyte count (RBC) was decreased in both sexes, whereas MCV, MCH, and red cell distribution width (RDW) were increased in both sexes. All the hematology changes were reversed in females following the recovery period, but high-dose males experienced a further decline in HCT and RBC, with some improvement in MCV and RDW. There were no changes in clinical chemistry values, urinalysis parameters, or ocular toxicity endpoints. Pituitary, heart, spleen, and ovary weights were increased in high-dose females and the spleen weight was increased in high-dose males, whereas the testes and thymus weights were decreased. There were no accompanying histopathologic correlates noted. A NOEL for hematological changes was established at 600 mg/kg/day. Day 32 toxicokinetic AUC_{inf} values for high dose males and females were 7830 and 6130 μM·h (1680 and 1320 μg·h/mL), respectively. Day 3 values were similar.

3.1.3. 6-Month Oral (Gavage) Toxicity Study in Mice with 3-Month Interim Sacrifice

Groups of 30 male and 30 female CD-1 mice were administered FTC at 0, 167, 500 and 1500 mg/kg/day, given as a single oral dose each day for 26 weeks (m2.6.7, Section 7.1.3, TOX022). After 13 weeks of treatment, 10 males and 10 females from each group were necropsied. After 26 weeks of treatment, 15 males and 15 females from each group were necropsied. The remaining 5 males and 5 females from each group went untreated for 1 month and were then necropsied. Satellite groups of 40 males and 40 females per dose group were used for toxicokinetic blood collections after 13 and 26 weeks. Clinical observations were made daily and ophthalmoscopic exams at predose and Weeks 13 and 26. Unfasted blood samples were taken at each necropsy for clinical pathology evaluations. At necropsy, organs were examined grossly, weighed, and processed for histopathologic evaluations.

Compared to controls, there were no significant clinical observations, body weight changes, ophthalmoscopic findings, blood chemistry alterations, organ weight changes, or gross and histopathologic findings. Mean corpuscular volume and MCH were elevated in the high-dose group after 26 weeks of exposure, more so in females than males. These changes were still evident after the 1-month recovery period.

At 3 and 6 months of exposure, plasma samples were collected from 3 satellite animals/sex/group at predose, 0.5, 1, 2, 5, and 8 hours after the daily dose. With the exception of females in the low dose group, mice at all doses had measurable concentrations of FTC in the predose blood samples. Peak plasma concentrations (C_{max}) of FTC were reached by 30 to 60 minutes after dosing. Following 3 months exposure, C_{max} values at 167 mg/kg/day were 44.0 and 37.2 µg/mL; at 500 mg/kg/day were 128.0 and 88.8 µg/mL; and at 1500 mg/kg/day were 225.0 and 187.1 µg/mL for males and females, respectively. Following 6 months exposure, C_{max} values at 167 mg/kg/day were 41.7 and 48.6 µg/mL; at 500 mg/kg/day were 87.3 and 111.7 µg/mL; and at 1500 mg/kg/day were 310.9 and 306.0 µg/mL for males and females, respectively.

The systemic exposure to FTC over the steady state dosing interval ($AUC_{0–24h}$) increased proportionally as the dose increased. Following 3 months exposure, $AUC_{0–24h}$ increased from 63.8 and 67.9 µg·h/mL at 167 mg/kg/day to 513.4 and 569.5 µg·h/mL at 1500 mg/kg/day for males and females, respectively. Following 6 months exposure, $AUC_{0–24h}$ increased from 82.3 and 93.1 µg·h/mL at 167 mg/kg/day to 732.1 and 899.4 µg·h/mL at 1500 mg/kg/day for males and females, respectively. A NOEL of 500 mg/kg/day was established (equivalent to an $AUC_{0–24h}$ of 266.4 µg·h/mL; sexes combined).

3.1.4. 6-Month Oral Toxicity Study (with a 3 Month Interim Sacrifice) in Mice

Groups of 25 male and 25 female CD-1 mice were administered once daily doses of FTC at 0, 120, 600, or 3000 mg/kg for up to 6 months (m2.6.7, Section 7.1.4, TOX628).
Five mice/sex/dose were subjected to a 3-week recovery period following 6-months of dosing. An additional 28/sex/group were used to assess plasma concentrations of drug, and 10/sex/dose (20 in the control group) were used for clinical pathology evaluations. The following parameters

were evaluated for evidence of toxicity: body weights, feed consumption, clinical signs, hematology, clinical chemistry, urinalysis, ophthalmoscopic examinations, organ weights, gross and histopathology, and electron microscopy.

After 6-months of exposure at the high dose, there were reversible decreases in RBCs, and reversible increases in MCV and urine quantity for both sexes. In high dose females, there was a reversible increase in absolute and relative thyroid weight, which was not associated with histopathological changes. Electron microscopy of liver, skeletal and cardiac muscle did not reveal any ultrastuctural changes. The AUC_{0-24h} for sexes combined on Day 175 was 142.5, 538.5, and 1787.5 μg·h/mL for the low-, mid-, and high-dose groups, respectively. The no-effect dose was 600 mg/kg. Females had statistically significantly lower ($p < 0.05$) plasma AUC values than did males at all dose levels. At the NOEL (600 mg/kg/day), AUC_{0-24h} values were 2369 μM·h (509 μg·h/mL) for males and 1985 μM·h (427 μg·h/mL) for females.

3.1.5. 3-Month Oral Gavage Study for Bioassay Dose Selection in Rats

To evaluate the repeat dose toxicity in rats of FTC and help select doses for a 2-year carcinogenicity bioassay, Charles River CD rats (10/sex/dose) were administered 0, 120, 600, or 3000 mg/kg/day for 3 months, euthanized, and necropsied (m2.6.7, Section 7.1.5, TOX097). An additional 18 rats/sex/dose were administered the same doses to evaluate systemic exposure after the second and ninetieth doses. During the dosing period, rats were evaluated for clinical signs of toxicity, and body weight and food consumption were measured. Urine samples were collected for urinalysis at the end of the dosing period. At necropsy, blood was collected for clinical pathology studies; organs were weighed; rats were examined for gross pathologic lesions; and tissues were collected for light microscopic examination.

Rats tolerated daily FTC doses up to 3000 mg/kg/day for 3 months without clinical signs of toxicity or effects on body weight gain, food consumption, or clinical chemistry parameters. Mild anemia was noted at 3000 mg/kg/day. No gross pathologic or histopathologic lesions occurred. Pituitary and thyroid glands weighed less at 3000 mg/kg/day, but there were no histopathologic changes to correlate with the reduced weights.

Emtricitabine was rapidly absorbed, with females absorbing the compound more rapidly than males (T_{max} = 1–2 hours). Day 90 C_{max} was 20% to 50% greater in females than males. Day 90 mean C_{max} was greater than Day 2 C_{max} for both sexes (5% to 30% in males, 20% to 60% in females). Day 90 mean AUC_{0-24h} was 20% to 40% greater than Day 2 AUC_{0-24h} in females, but about the same as Day 2 AUC_{0-24h} in males. Mean $t_{1/2}$ was 2.4 to 3.8 hours on Day 2 and 3.6 to 6.5 hours on Day 90. Dose-normalized C_{max} tended to decrease with increasing dose, but dose-normalized AUC_{0-24h} remained relatively constant, suggesting a linear relationship between systemic exposure and daily dose of FTC in rats from 120 to 3000 mg/kg/day.

3.1.6. 1-Month Oral Toxicity Study in Cynomolgus Monkeys

In a 1-month oral toxicity study, cynomolgus monkeys (5/sex/dose) were administered FTC at 0, 80, 400, or 2000 mg/kg/day in 0.5% methylcellulose, as equal divided doses 6 hours apart via nasogastric intubation for 28 days, after which 3/sex/dose were necropsied (m2.6.7,

Section 7.1.6, TOX600). Remaining monkeys (2/sex/dose) were held without dosing for 3 weeks, then necropsied to evaluate recovery from treatment-related effects. The following parameters were monitored for signs of toxicity: clinical signs, appetite, body weights, ophthalmology, electrocardiography, neurophysiology, hematology, clinical chemistry, urinalysis, plasma drug concentrations, cerebrospinal fluid (CSF) drug concentrations, organ weights, gross pathology and histopathology, and neurohistopathology. Samples of skeletal and cardiac muscle from control and high dose animals were examined by transmission electron microscopy.

There were no changes in any of the observed parameters suggestive of treatment-related effects. The only possibly treatment-related clinical sign was an increased incidence of soft feces in females at 2000 mg/kg/day. Systemic exposure to FTC was similar between sexes and between Days 3 and 27. The C_{max} and the AUC values increased in proportion to the dose. No significant drug accumulation occurred over the dosing period. Drug levels in CSF measured 1 hour after dosing in the third week of the study (together with a companion blood sample) demonstrated that CSF drug levels rose proportionately with plasma drug levels and were 4.3%, 3.7%, and 3.7% of plasma drug levels in the 80-, 400-, and 2000-mg/kg/day groups, respectively. A NOEL of 400 mg/kg/day was established; corresponding systemic exposure levels ($AUC_{0–6h}$) at 3 days were 687 to 688 μM·h (148 μg·h/mL) for males and females, respectively, and at Day 27 were 793 to 664 μM·h (170 to 143 μg·h/mL) for males and females, respectively.

3.1.7. 3-Month Oral Toxicity Study in Cynomolgus Monkeys

In a 3-month GLP oral toxicity study, cynomolgus monkeys (5/sex/dose) were administered FTC at 0, 40, 200, or 1000 mg/kg/day in 0.5% methylcellulose, as equal divided doses 6 hours apart for 3 months, then 3/sex/dose were killed (m2.6.7, Section 7.1.7**,** TOX627). The tissues were fixed by perfusion and the monkeys were necropsied. Remaining monkeys (2/sex/dose) were allowed to recover for 3 weeks, and evaluated for reversibility of treatment-related effects. The following parameters were observed for signs of toxicity: clinical signs, body weight, ophthalmology, electrocardiography, neurophysiology and neurohistopathology, hematology, clinical chemistry, urinalysis, and plasma drug concentrations. At terminal necropsy, organs were weighed and gross and histopathology were performed. Liver, cardiac, and skeletal muscle was examined by transmission electron microscopy.

There were no treatment-related changes in any of the observed parameters at any dose. A NOEL of 1000 mg/kg/day was established. C_{max} and AUC increased with dose and there was no evidence of accumulation. Systemic exposure to FTC was similar between sexes and between Days 3 and 87. In the 1000-mg/kg group, $AUC_{0–6h}$ values on Day 3 for males and females were 1339 and 942 μM·h (288 and 203 μg·h/mL), respectively, and on Day 87 were 1630 and 1109 μM·h (350 and 238 μg·h/mL), respectively.

3.1.8. 52-Week Oral Toxicity Study in Cynomolgus Monkeys with a 4-Week Recovery Period

In a 1-year oral toxicity study with a 3-month interim necropsy, cynomolgus monkeys were administered FTC at 0, 50, 200, or 500 mg/kg/day in 0.5% methylcellulose (n = 8, 4, 4, and 8 males and females, respectively) as equal divided doses at least 5 hours apart, by nasogastric intubation, daily for 52 weeks (m2.6.7, Section 7.1.8, TOX032). After dosing for 3 months, 2 monkeys/sex at 0 and 500 mg/kg/day were necropsied. At the end of dosing, 4 monkeys/sex/dose were necropsied. Remaining monkeys (2/sex at 0 and 500 mg/kg/day) were allowed to recover for a month and evaluated for reversibility of treatment-related effects. Data collected were body weights, food consumption, clinical signs, ophthalmic examinations, ECG recordings (with measurement of QT interval), neurophysiology parameters, hematology, clinical chemistry, plasma FTC concentrations (Day 1 and Weeks 13, 26, and 52), gross pathology, organ weights, and histopathology findings.

There were no signs of toxicity after dosing for 3 months at any dose. At the end of 52 weeks of dosing, the only treatment-related effects were a slight (nonsignificant) decrease in RBC count and increased MCH at 500 mg/kg/day in females only, which were absent at the end of the recovery period. No other signs of toxicity occurred at any dose. Based on these findings, the NOEL was 200 mg/kg/day.

Emtricitabine was rapidly and well absorbed with peak plasma concentrations occurring between 0.5 to 2 hours postdosing. Plasma half-life was 2 to 4 hours for all dose levels. Half-life may have been underestimated because of the short (6 hour) sampling period. A longer half-life was expected, based on the measurable predose drug concentrations and the slightly higher plasma FTC concentrations after repeated dosing. No major differences were noted in plasma FTC exposure between male and female monkeys. Plasma C_{max}, $AUC_{0–6h}$, and estimated steady-state $AUC_{0–24h}$ (Weeks 13, 26, and 52) increased linearly over the dose range of 50 to 500 mg/kg/day. Systemic exposure to FTC was similar between sexes and at Weeks 13, 26, and 52. Week 52 $AUC_{0–24h}$ values at the NOEL of 200 mg/kg/day were 97.2 and 97.8 μg·h/mL in males and females, respectively. Corresponding C_{max} values were 17.4 and 21.3 μg/mL, respectively.

3.2. RPV

Oral studies in mice with TMC278 were conducted in preparation of the carcinogenicity study. In a 2-week study with CD-1 mice, toxicity and exposure was determined, following administration by gavage (in aqueous HPMC) and via diet. In a 1-month study, the feasibility of the transgenic CB6F1-non TgrasH2 strain was tested. The final carcinogenicity dose range finding study was a 3-month study in CD-1 mice with administration by gavage.

3.2.1. 2-Week Oral Toxicity Study in Mice

In a 2-week study with CD-1 mice (m2.6.7, Section 6.1, TMC278-NC118), TMC278 was administered by gavage or via diet in doses up to 2000 and 5000 mg/kg/day, respectively.

No compound-related mortality was noted up to 400 mg/kg/day by either route. The high doses, 2000 (gavage) and 5000 mg/kg/day (diet), were not tolerated.

Almost exclusively at the highest tolerated dose of 400 mg/kg/day, either by gavage or diet, body weight gain was decreased up to 29%, whereas food consumption (diet administration only) was increased maximally 49%.

Hematology showed RBC, Hb, and HCT (all decreased 8%) and increased reticulocyte count (23%) and reduced eosinophil counts in females when administered by diet (also at 40 mg/kg/day).

Increased serum concentrations were observed for total bilirubin (up to 3-fold at 400 mg/kg/day, 77% at 40 mg/kg/day), ALP (up to 3-fold), ALT (up to 3-fold), AST (72%), albumin (up to 22%), total protein (13%), cholesterol (up to 48%), calcium (up to 8%), and urea (up to 40%) and decreased concentration of triglycerides (up to 73%).

At necropsy, increased liver weight was noted accompanied by pale, dark, and/or swollen aspect associated with hepatocellular hypertrophy, marginal to slight focal necrosis, and marginal single cell necrosis, marginally increased mononuclear phagocytic system (MPS)-aggregates with necrotic hepatocytes, and prominent mitoses (females on diet, only).

Increased kidney weight was accompanied by pale and swollen aspect (females by gavage, only). Histopathology showed tubular dilatation and subchronic nephropathy and exudative nephritis.

Systemic exposure expressed as C_{max} and AUC values determined at the end of the dosing period are given in Table 3 below.

Table 3. Exposure to TMC278 (C_{max} and AUC) in a 2-Week Oral Study with TMC278 in Mice

Dose (mg/kg/day)	Route	C_{max} (µg/mL)		$AUC_{0–24h}$ (µg.h/mL)	
		M (n = 3-9)	F (n = 3-9)	M (n = 3-9)	F (n = 3-9)
40	diet	8.9	8.3	150	148
400	diet	57	63	1066	1320
5000	diet				
400	gavage	63	94	687	1233
2000	gavage				

M = males, F = females, Shaded = not sampled; dose not tolerated

3.2.2. 1-Month Oral Toxicity Study in Transgenic Mice

In a 1-month study, TMC278 was administered orally by gavage to transgenic CB6F1-nonTgrasH2 mice at 0 (vehicle), 20, 80, or 320 mg/kg/day (m2.6.7, Section 6.1, TMC278-NC121).

In the group dosed with 320 mg/kg/day, 2 mortalities occurred and signs of deteriorating clinical condition were noted. Almost exclusively at 320 mg/kg/day, body weight was decreased up to 13% and food consumption increased up to 22%.

Hematology showed reduced RBC, Hb, and HCT (all up to 6%); reduced reticulocyte count (56%, females only); and increased number of platelets (up to 24%).

Increased serum concentrations were observed for total bilirubin (3-fold, females), ALP (up to 3-fold), albumin (17%, males), total protein (up to 18%), cholesterol (up to 100%), calcium (27%, females), inorganic phosphate (42%, females), and urea (up to 72%).

At necropsy, increased weight of liver (up to 100% at 320 mg/kg/day, 29% at 80 mg/kg/day), spleen (up to 40%), and kidneys (14%, females) and decreased weight of thymus (73%, females) and ovaries (40%) was noted. Liver and spleen were enlarged and showed discoloration.

Upon histopathology, hepatocellular hypertrophy in all TMC278-treated groups was noted and at 320 mg/kg/day; this was associated with coagulative hepatocellular necrosis. Moreover, degenerative/necrotic nephropathy (also at 80 mg/kg/day); lymphoid depletion in thymus; myeloid cell hyperplasia in bone marrow; erythro/myelopoiesis in spleen; almost complete absence of ovulation; and uterus atrophy occurred.

Systemic exposure expressed as C_{max} and AUC values are given in Table 4 below.

Table 4. Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Study with TMC278 in Mice

Dose (mg/kg/day)	Day of Sampling	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 3)	F (n = 3)	M (n = 3)	F (n = 3)
20	1	15	14	52	45
	29	13	15	63	54
80	1	30	28	199	192
	29	30	37	250	240
320	1	45	42	643	667
	29	66	69	1090	942

a. $AUC_{inf.}$ on Day 1 otherwise AUC_{0-24h}, M: males, F: females

3.2.3. 3-Month Oral Toxicity and Carcinogenicity Dose Range Finding Study with TMC278.HCl in Mice

TMC278 was administered once daily by oral gavage for 3 months to CD-1 mice. Groups of 25 animals per sex (10 for the main study and 15 satellite animals for toxicokinetics) were given 0 (vehicle; 12 satellite animals per sex), 20, 80, or 320 mg/kg/day in 10 mL/kg (m2.6.7, Section 7.2.1, TMC278-NC119). All animals were observed regularly for clinical signs, morbidity, and mortality. Body weight of all animals was recorded, but assessed for main study animals only. For main study animals, food consumption and body weight gain were determined. Main animals were sampled for hematology and serum chemistry, and necropsied and inspected for gross lesions at the end of the study. A range of organs was weighed and a complete set of organs and tissues was preserved for microscopic examination. For possible electron microscopic

evaluation, liver samples from 5 male and 5 female mice of the control group and the group treated with 320 mg/kg/day were fixed in phosphate buffer containing 3% glutaraldehyde. Blood samples for toxicokinetic analysis were drawn from 3 satellite animals per gender per time point at 5 time points within 24 hours on Days 1, 31, and 87. After the last sampling these animals were killed.

There were no mortalities. No clinical signs were noted, except abdominal distention in 5/10 males and 7/10 females dosed with 320 mg/kg/day from Week 6 of treatment onwards.

Body weight at the end of the dosing period was increased up to 11% in the males and females dosed with 320 mg/kg/day. This increase was associated with a body weight gain over the whole dosing period of slightly more than 150%. The effects on body weight and body weight gain were in line with a 20% higher food consumption essentially throughout the dosing period in males and females dosed with 320 mg/kg/day.

Red blood cell count, Hb, and HCT were 10% lower in males and females dosed with 320 mg/kg/day. In females, this reduction in RBC parameters was associated with a 37% increase in reticulocyte count. In males treated with 320 mg/kg/day, leukocyte count was only 53% of control value. Lymphocyte, eosinophil, and to a lesser extent neutrophil counts contributed to this lower value.

Alkaline phosphatase and ALT activities in serum were more than 2-fold higher in males and females dosed with 320 mg/kg/day. Also in this group, females showed a 25% increase in urea. In females dosed with 80 and 320 mg/kg/day, cholesterol had increased 29% and 42%, respectively, whereas triglycerides had decreased almost 40% and more than 60%, respectively. Total protein and albumin had increased by 6% to 14% predominantly in females given 80 or 320 mg/kg/day. Calcium and inorganic phosphate had increased 7% to 18% in animals dosed with 320 mg/kg/day.

Liver weight was increased in a dose-related fashion in animals treated with 80 (20% and 37% in males and females, respectively) and 320 mg/kg/day (2.1- and 2.7-fold higher in males and females, respectively). Mean spleen weight in females treated with 320 mg/kg/day was 167% of control value. This increase was not statistically significant, but correlated to the swollen aspect noted in 3 out of 10 females in this group and the extramedullar hematopoiesis upon microscopic evaluations.

Histopathology confirmed that the liver was the major target for TMC278. The most prominent effect was hepatocellular hypertrophy with a dose-related increase in incidence and severity in the animals dosed with 80 and 320 mg/kg/day. In animals given the higher dose, this effect was associated with hepatocellular vacuolization, single cell necrosis, and pigmentation and proliferation of Kupffer cells; all to a slight to moderate degree. Electron microscopy revealed peroxisome proliferation in the animals dosed with 320 mg/kg/day.

In half the number of the female mice treated with 320 mg/kg/day, minimal to moderate nephropathy was noted. The nephropathy was characterized by slight to marked multifocal tubular basophilia; minimal to slight glomerulopathy (atrophic glomeruli with thickened Bowman’s capsule amidst basophilic tubules); minimal to moderate mononuclear cell infiltration; minimal to slight interstitial fibrosis; minimal tubular dilatation; and slight cortical mineralization.

At 320 mg/kg/day, a marginally increased incidence was noted of swollen cells and/or cells with dense cytoplasm in the adrenal zona fasciculata in males, whereas females showed a marginal decrease of a clear X-zone with increased brown degeneration in that zone.

In females treated with the high dose, a marginal decrease of the number of corpora lutea and generations of corpora lutea was noted in ovaries. Also, granulocyte infiltration in the endometrium was marginally decreased in this group. It cannot be excluded that these gonadal effects indicate a reduced cyclic activity.

Moreover, animals dosed with 320 mg/kg/day showed extramedullar hematopoiesis in liver (marginal) and spleen (slight to moderate), and slight to moderate increase of the myeloid/erythroid ratio in bone marrow. These effects are likely associated with the effects on RBC parameters.

Based on the findings in animals with 80 mg/kg/day in liver, the NOAEL was considered to be 20 mg/kg/day. Systemic exposure expressed as C_{max} and AUC values are given in Table 5 below.

Table 5. Exposure to TMC278 (Cmax and AUC) in a 3-Month Oral Study with TMC278 in Mice

Dose (mg/kg/day)	Day of Sampling	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 15)	F (n = 15)	M (n = 15)	F (n = 15)
20	1	14	13	71	59
	31	14	18	61[b]	74
	87	18	19	80	61
80	1	28	32	236	250
	31	38	37	263	313
	87	34	42	210	313
320	1	63	55	1010	707
	31	63	84	860	1170
	87	61	90	665	1360

a. AUC_{inf} on Day 1 and AUC_{0-24h} on Days 31 and 87, M: males, F: females, [b]: AUC_{0-8h}.

3.2.4. Non-Pivotal Pilot Oral Toxicity and Dose Range Finding Carcinogenicity, and Free Base – Salt Bridging Studies

For RPV, the pivotal studies with administration durations ranging from 1 to 6 months were preceded by pilot studies. Following the selection of the HCl-salt for further final development, a 1-month bridging study with TMC278 base and TMC278 was done. Two 2-week studies with TMC278 base and TMC278 administered by gavage or via diet were done for the selection of the test article and the route of administration in the oral carcinogenicity study.

3.2.4.1. 5-Day Oral Tolerance Study with TMC278

TMC278 base dissolved in PEG400 was administered once daily by oral gavage for 5 days at 0 (vehicle), 40, or 400 mg/kg/day in a dose volume of 8 mL/kg to 5 male Sprague Dawley rats/group (m2.6.7, Section 6.1, TMC278-TOX5463).

There was no mortality and treatment was well tolerated. There were no relevant clinical signs or effects on body weight or on hematology. There were no relevant histopathological changes in the examined tissues. Systemic exposure after repeated oral dosing of 400 mg/kg/day was shown by a C_{max} of 2 µg/mL and an AUC_{inf} of 28 µg.h/mL.

3.2.4.2. 2-Week Oral Toxicity Study with TMC278 Base in Rats

TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 2 weeks to Sprague Dawley rats at 0 (water), 0 (vehicle), 40, 120, or 400 mg/kg/day in 10 mL/kg (m2.6.7, Section 6.1, TMC278-TOX5535). All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with TMC278 base had an additional 4 males and 4 females for toxicokinetic analysis.

There were no mortalities in this study. Increased serum concentrations of total protein (3%, males) and albumin (7%, males) were noted in animals dosed with 400 mg/kg/day. Moreover, decreased concentrations of thyroxine (T_4; maximally 66%) were determined at all doses in males and/or females associated with increased thyroid stimulating hormone (TSH; up to 100%) in animals dosed with 120 and 400 mg/kg/day. At necropsy, increased thyroid gland weight (19%) in females given 400 mg/kg/day and hypertrophy of the thyroid follicular epithelium in male and/or female rats at all doses were noted.

Systemic exposure expressed as C_{max} and AUC values are given in Table 6 below.

Table 6. Exposure to TMC278 (C_{max} and AUC) in a 2-Week Oral Study with TMC278 Base in Rats

Dose (mg/kg/day)	Day of Sampling	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 4)	F (n = 4)	M (n = 4)	F (n = 4)
40	1	1.6	3.2	13	29
	14	2.2	5.7	16	42
120	1	2.8	4.7	30	55
	14	3.6	7.8	35	88
400	1	10	14	86	128
	14	8.4	15	84	152

a. $AUC_{inf;}$ on Day 1 otherwise $AUC_{0–24h}$, M: males, F: females

3.2.4.3. 2-Week Oral Study with TMC278 and TMC278.HCl by Gavage and via Diet in Rats

TMC278 was administered once daily to Sprague Dawley rats by oral gavage suspended in 0.5% (m/v) aqueous HPMC at a dose of 400 mg/kg/day or by dietary administration at 400 or 1200 mg/kg/day (m2.6.7, Section 6.1, TMC278-NC136). TMC278 base was administered by diet at 400 mg/kg/day. The control group received unmedicated diet. All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with TMC278 base had additionally 6 males and 6 females for toxicokinetic analysis.

No mortality was noted during this study. There were no toxicologically relevant differences between groups dosed with TMC278 base or TMC278 at 400 mg/kg/day by gavage or through the diet. With both forms, decreased WBC count (maximally 23%), and decreased Hb and HCT (maximally 6%) occurred. Increased ALT activity in serum (up to 44%) in males treated with 400 mg/kg/day and decreased concentration of cholesterol (29%) in males given TMC278 by gavage were determined.

Microscopy showed increased diffuse thyroid follicular hypertrophy; an increase in the number of swollen/vacuolated cells in the pars distalis of the pituitary gland in male rats; and hepatocellular hypertrophy in male and female rats at 1200 mg/kg/day.

Systemic exposure expressed as C_{max} and AUC values are given in Table 7 below.

Table 7. Exposure to TMC278 (C_{max} and AUC) in a 2-Week Oral Study in Rats with TMC278 Base and TMC278

Dose (mg/kg/day)	Form	Route	Days of Sampling	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
				M (n = 6)	F (n = 6)	M (n = 6)	F (n = 6)
400	Base	Diet	12	3.7	4.3	57	86
400	Salt	Diet	12	5.2	7.2	82	137
400	Salt	Gavage	12	7.3	12	51	103
1200	Salt	Diet	12	11	13	180	266

M: males, F: females

3.2.4.4. 2-Week Oral Pilot Carcinogenicity Dose Range Finding Study with TMC278.HCl in Rats

TMC278 suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage, for 2 weeks to Sprague Dawley rats at 0 (vehicle), 400, 1500, or 2000 mg/kg/day in 10 mL/kg (m2.6.7, Section 6.1, TMC278-NC177). All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with TMC278 had additionally 3 males and 3 females for toxicokinetic analysis.

There were no mortalities in this study. Increased activated partial thromboplastin time (APPT) and prothrombin time (PT, both up to 20%) were determined in males from 1500 mg/kg/day and above. Serum chemistry showed decreased serum concentrations of chloride (3%), glucose (up to 18%), urea nitrogen (33%), and creatinine (maximally 23%) in females given 1500 or 2000 mg/kg/day. In the latter group, males had decreased concentrations of cholesterol (30%). Increased urinary volume (up to 100%) was recorded in animals given 1500 and 2000 mg/kg/day.

At necropsy, increased weight of the thyroid gland in females of all groups treated with TMC278 was noted in association with hypertrophy of follicular cells. In males given 2000 mg/kg/day, hypertrophy of follicular cells was associated with multifocal vacuolated cells in the pars distalis of the pituitary gland.

Systemic exposure expressed as C_{max} and AUC values are given in Table 8 below.

Table 8. Exposure to TMC278 (C_{max} and AUC) in a 2-Week Oral Study in Rats with TMC278

Dose (mg/kg/day)	Day of Sampling	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 3)	F (n = 3)	M (n = 3)	F (n = 3)
400	1	5.4	12	50	78
	14	5.2	12	42	96
1500	1	18	12	153	152
	14	9.5	12	86	115
2000	1	10	17	103	206[b]
	14	7.7	14	77	147

a. $AUC_{inf.}$ on Day 1 otherwise AUC_{0-24h}, [b]: n=2, M: males, F: females.

3.2.4.5. 1-Month Bridging Study with TMC278 Base and TMC278 in Rats

This study served to bridge the toxicity and toxicokinetics after administration of TMC278 base with those after TMC278 (m2.6.7, Section 6.1, TMC278-NC117). Both compounds were administered once daily, by oral gavage, to Sprague Dawley rats for 4 weeks at 0 (vehicle), 10, or 400 mg/kg/day in 10 mL/kg. TMC278 base was dissolved in PEG400 + CA and TMC278 was suspended in 0.5% (m/v) aqueous HPMC. All groups comprised 10 male and 10 female rats for the toxicological evaluations and the groups treated with TMC278 base and TMC278 had additionally 6 males and 6 females for toxicokinetic analysis.

No compound related mortality was noted. No effects were noted in either group dosed with 10 mg/kg/day. There were no significant differences in the toxicity parameters of both forms dosed at 400 mg/kg/day. Hematology showed decreased HCT (3%) and increased RBC count (4%). Increased serum concentrations of albumin (up to 7%, males) and total protein (5%, males), and decreased levels of cholesterol (up to 22%, females), triglycerides (35%, females), urea (up to 18%), potassium (13%, females), and chloride (2%) were determined. Moreover,

serum concentration of T_4 was decreased up to 61%, whereas those of triiodothyronine (T_3) and TSH were increased up to 57% (males only) and up to 51%, respectively. Urinary volume was increased up to 98%.

At necropsy, increased weight of thyroid gland (up to 60%, females) and liver (up to 24%) were recorded. Hepatocellular hypertrophy in liver, diffuse follicular hypertrophy in thyroid gland, and an increase in swollen/vacuolated cells in the pars distalis of the pituitary gland was noted.

Systemic exposure expressed as C_{max} and AUC values are given in Table 9 below.

Table 9. Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Bridging Study with TMC278 Base and TMC278 in Rats

TMC278 base (mg/kg/day)	Sampling day	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 6)	F (n = 6)	M (n = 6)	F (n = 6)
10	1	0.87	1.5	6.3	10
	24	0.74	1.4	5.4	8.6
400	1	8.3	12	101	123
	24	7.7	13	90	149
TMC278 (mg/kg/day)					
10	1	0.90	1.6	5.5	8.6
	24	0.76	1.7	4.5	7.8
400	1	7.0	9.5	52	100
	24	4.8	9.0	33	86

a. $AUC_{inf.}$ on Day1 otherwise AUC_{0-24h}, M: males, F: Females

3.2.5. 1-Month Oral Toxicity Study Including Immunotoxicity Evaluation in Rats (Pivotal Study)

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 4 weeks to groups of 18 male and 18 female Sprague Dawley rats at 0 (water, negative control), 0 (vehicle), 10, 40, or 160 mg/kg/day in 10 mL/kg (m2.6.7, Section 7.2.2, TMC278-Exp5692); 8 male and 8 female satellite animals per group were used for the plaque forming cells (PFC) assay. TMC278-treated groups had 4 more males and females for toxicokinetic analysis. Regular observations were made for clinical signs, morbidity, and mortality. Body weight, food consumption, and body weight gain were determined throughout the dosing period. Ophthalmic examinations were performed prior to dosing and at the end of the dosing period (negative control, vehicle, and 160 mg/kg/day animals only). All main animals were sampled for hematological, serum chemistry, and urinalysis parameters at the end of the dosing period. The PFC satellites received on 4 consecutive days an intravenous injection with sheep RBCs. Thereafter, they were killed and a suspension of spleen cells was prepared and incubated with sheep RBCs and guinea pig complement. The resulting plaques are considered to be antibody-producing cells. At necropsy, gross lesions and organ weight were recorded, and tissues and organs were sampled for histopathology. Blood samples were drawn for toxicokinetics on Days 1 and 29.

There were no mortalities associated with TMC278, relevant clinical signs, or effects on body weight or food consumption; no treatment-related ophthalmic effects; and no effects on the direct PFC assay. A decrease in glucose (10%) was seen in females given 160 mg/kg/day. Higher thyroid gland weights (maximally 25%) and liver weights (maximally 23%) compared to the vehicle group were recorded in the groups dosed with 40 and 160 mg/kg/day. The weight of the pituitary gland was slightly increased in males (16%) and females (14%) dosed with 160 mg/kg/day. The increase in thyroid gland weight was associated with minimal follicular hypertrophy in the majority of the males and 4 out of 10 females given 160 mg/kg/day and in 2 out of 10 males given 40 mg/kg/day.

In view of the absence of findings of toxicological relevance in the group treated with 10 mg/kg/day, this dose was the NOAEL. Moreover, the absence of effects in the PFC assay indicated that TMC278 has no immunotoxic potential. Systemic exposure expressed as C_{max} and AUC values is given in Table 10 below.

Table 10. Exposure to TMC278 (C_{max} and AUC) in a 1-Month Toxicity Study of TMC278 Base in Rats

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 4)	F (n = 4)	M (n = 4)	F (n = 4)
10	1	0.94	2.0	7.5	14
	29	0.88	1.6	7.2	14
40	1	2.0	3.2	23	30
	29	2.6	5.8	27	42
160	1	6.5	9.6	52	77
	29	6.7	8.8	51	89

a. $AUC_{inf:}$ on Day 1 otherwise $AUC_{0–24h}$, M: males, F: females

3.2.6. 6-Month Oral Toxicity Study Including 1-Month Recovery in Rats

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 6 months to groups of 20 male and 20 female Sprague Dawley rats (m2.6.7, Section 7.2.3, TMC278-NC101) at 0 (vehicle), 40, 120, or 400 mg/kg/day in 10 mL/kg. There were 6 satellite rats of each sex per TMC278-treated group for toxicokinetic analysis, and 10 rats of each sex in the control and high dose group for assessment of reversibility of the findings at 1 month postdose. Regular observations were made for clinical signs, morbidity, and mortality. Body weight, food consumption, and body weight gain were determined throughout the dosing and recovery periods. Ophthalmic examinations were performed prior to dosing and at the end of the dosing and recovery. Samples for hematological, serum chemistry, and urinalysis parameters were taken after 3 months of treatment and at the end of dosing and recovery periods. In addition, serum concentrations of TSH, T_3, T_4, adrenocorticotropic hormone (ACTH), corticosterone, and progesterone were determined in samples taken prior to the autopsies. At necropsy, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology. Blood samples were drawn for toxicokinetics on Days 1, 31, and 87. At necropsy, blood and liver samples were taken for determination of TMC278.

Increasing difficulties with the daily administration by gavage, morbidity, and deaths started to occur after the first 2 months of dosing with comparable incidences in all groups including controls. For this reason, the dosing regimen was changed from once daily to BID dosing (5 mL/kg with an interval of 1.5 hours) from Day 84 onwards.

The cause of death (reaching 50% in some of the groups) was concluded to be associated with the relatively high volume and viscosity of the formulation, necessitating a long gavage time during which the animals physically opposed the manual restraint. This led to gavage errors, regurgitation, and restraint trauma. In the 1-month postdosing period, no further mortalities occurred.

Compound-related clinical signs included salivation in females at all dose levels and in males at 400 mg/kg/day, and wet urogenital region in both genders at 400 mg/kg/day. There were no relevant effects on ophthalmic examinations, body weight, or food consumption. In hematology, increases in APTT (up to 23%) and PT (up to 27%) were noted in TMC278-treated males of all groups, without a dose-related trend, at all sampling times, including the end of the recovery period. At the end of the dosing period, RBC count, Hb, and HCT of males dosed with 400 mg/kg/day were reduced by 5%. The eosinophil count in TMC278-treated females were 25% to 33% lower than control values without a clear dose-related trend. Red blood cell parameters and eosinophil counts showed complete recovery after termination of dosing.

Serum chemistry after 3 months of treatment and at the end of the dosing period showed increases in total protein (4%, in females at 400 mg/kg/day), and albumin (up to 10%, at 120 and 400 mg/kg/day). Decreases up to approximately 40% were recorded in triglycerides and total bilirubin levels in all TMC278-treated groups, without a clear relationship to dose. Alkaline phosphatase displayed a dose and treatment-duration related increase in males at 120 and 400 mg/kg/day. The increase was 30% and 53%, respectively, after 3 months of treatment and 32% and 70%, respectively, at the end of the dosing period. Inorganic phosphate was slightly higher (maximally 11%) after 3 months of dosing in females at all dose levels (dose-related) and in males at 120 and 400 mg/kg/day. Increases in urea (10%, in males at 120 and 400 mg/kg/day) and creatinine (12%, males of all TMC278-treated groups without a dose relationship) were noted at the end of treatment. All serum chemistry changes showed complete reversibility at the end of the recovery period.

Urinalysis showed no differences between the treated groups and the control group. The 2.5-fold higher concentration in urinary ketones at the end of the 1-month recovery period in males treated with 400 mg/kg/day is not likely to be treatment-related.

Thyroid stimulating hormone levels in serum showed a more or less dose-related increase from 45% and 77% in males and females, respectively, in the group dosed with 40 mg/kg/day, and up to 23% and 2.5-fold, respectively, in the group treated with 400 mg/kg/day. This effect was associated with a similarly statistically significant and dose-related decrease of serum T_4 ranging from 36% and 25%, respectively, in males and females dosed with 40 mg/kg/day, and up to 45% and 46% at the 400 mg/kg/day dose. In contrast, T_3 showed lesser and equivocal effects: 30% decrease in males dosed with 40 mg/kg/day and 34% increase in males dosed with 400 mg/kg/day. At the end of the postdosing period, no parameters, except T_4, showed differences with control values. Thyroxine levels in females treated with 400 mg/kg/day were 138% of control value, indicating that thyroid homeostasis had not yet completely recovered.

Corticosterone, progesterone, and ACTH levels were highly variable. The overall trend indicated a decrease of corticosterone levels and an increase in ACTH and progesterone concentrations in the groups dosed with 120 and 400 mg/kg/day. No trend of any effect was observed at the end of the postdosing period.

At necropsy, liver weight was increased without a clear dose relationship in animals treated with 120 and 400 mg/kg/day. Thyroid gland weight of animals in all groups was increased. The increase was dose-dependent ranging from 20% to 50% and was statistically significant in all groups. Reversibility of the effect in females was not complete at 1 month after dosing as the weight of the organ was still 40% higher than controls.

Livers of the animals treated with 400 mg/kg/day showed pronounced lobulation. Upon microscopy, a dose-related increase of hepatocellular hypertrophy was noted in the animals of the groups treated with 120 and 400 mg/kg/day. This hypertrophy was associated with a decrease in hepatocellular necrosis and hemorrhages, and in females with a decrease of vacuoles physiologically associated with fatty storage. The hepatocellular hypertrophy was considered recovered at the end of the 1-month postdosing period. The histopathological findings and recovery are in line with the serum chemistry effects and the effects on liver weight.

In the thyroid gland, a dose-related increase of diffuse follicular hypertrophy was observed in males and females of all groups. This hypertrophy was associated with an increase in the number of small follicles. In addition, unilateral focal follicular hyperplasia was noted in 1 animal dosed with 40 mg/kg/day and 1 animal dosed with 120 mg/kg/day, and in 3 animals dosed with 400 mg/kg/day. The follicular hypertrophy and small follicles were still noted in the animals allowed to recover from treatment with 400 mg/kg/day for 1 month, although to a lesser extent and incidence than in the animals killed at the end of the dosing period. The histopathological findings and incomplete recovery are in line with the thyroid-associated endocrinology data and the effects on the weight of the organ. In the pituitary gland of males from all groups, the number of swollen/vacuolated cells in the pars distalis was increased. These cells are known to produce TSH {15704}. This effect was not noted in animals killed at the end of the postdosing period, indicating complete recovery. This finding and its complete recovery are in line with the effects on TSH levels and the effects at the thyroid gland level.

In 20% to 25% of the males and females treated with 400 mg/kg/day, the macrophages that spontaneously form aggregates in the mesenteric lymph nodes had a swollen-vacuolated appearance. This effect was also noted in 30% to 33% of the animals at the end of the postdosing period.

In view of the effects on coagulation parameters and the thyroid and pituitary glands observed in the low dose group treated with 40 mg/kg/day, a NOAEL could not be established in this study. Systemic exposure expressed as C_{max} and AUC values are given in Table 11 below.

Table 11. Exposure to TMC278 (C_{max} and AUC) in a 6-Month Oral Toxicity Study with TMC278 Base in Rats

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M (n = 3)	F (n = 3)	M (n = 3)	F (n = 3)
40	1	2.9	6.5	19	32
	84	3.4	8.2	19	41
	175	1.7	6.6	12	50
120	1	6.4	8.5	53	83
	84	3.4	11	41	100
	175	3.0	8.8	35	116
400	1	9.1	17	92	160
	84	3.9	15	57	184
	175	6.2	16	73	244

a. $AUC_{inf.}$ on Day 1 otherwise $AUC_{0–24h}$, M: male, F: female

3.2.7. 5-Day Oral Dose Range Finding Toxicity Study with TMC278 Base in Rabbits

This nonpivotal study was part of the dose range finding studies for the embryo-fetal development study with TMC278 base in rabbits. For studies with pregnant rabbits, see Section 6.2.4.

TMC278 base suspended in 0.5% (m/v) aqueous HPMC was administered by oral gavage for 5 days to New Zealand white rabbits (m2.6.7, Section 6.1, TMC278-NC126). Groups of 5 nonpregnant females received 0 (vehicle), 100, 300, or 1000 mg/kg in 10 mL/kg. Daily observations were made for clinical signs, morbidity, and mortality. Body weight and food consumption were determined at the end of the dosing period. Blood samples for hematology, serum chemistry, and toxicokinetic analyses were taken at the end of the dosing period. At necropsy, gross lesions of external features and body cavities were recorded, but no organs were weighed and no organs or tissues were taken for histopathology.

No mortalities occurred. Food consumption was strongly reduced in a dose-related fashion. The animals dosed with 100 mg/kg/day consumed less than 75% of the food and those given 1000 mg/kg/day less than 10% of the food consumed by the control animals. Consequently, fecal output and body weight were significantly reduced. Mean reticulocyte counts were strongly and dose-dependently reduced and mean creatinine levels were increased in animals dosed with 300 and 1000 mg/kg/day. No relevant changes were noted at necropsy. Toxicokinetics showed mean C_{max} values of 57.3, 124, and 138 µg/mL and mean $AUC_{0–24h}$ values of 1120, 2695, and 2971 µg.h/mL in groups dosed with 100, 300, and 1000 mg/kg/day, respectively.

3.2.8. Non-Pivotal Pilot Oral Toxicity Study with TMC278 Base and TMC278 – TMC278 Bridging Studies

The pivotal studies with administration durations ranging from 1 to 12 months were preceded by pilot studies. Following the selection of the HCl salt for further final development, a 1-month bridging study with TMC278 base and TMC278 was done.

3.2.8.1. 5-Day Pilot Oral Toxicity Study in Dogs

TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 5 days to groups of beagle dogs each comprising 1 male and 1 female at 0 (vehicle) and 80 mg/kg/day in 0.8 mL/kg, after a wash-out period of 7 days following a similar single dose (m2.6.7, Section 5.2, TMC278-Exp5461).

No test article-related effects were noted on clinical signs, body weight, clinical pathology, and postmortem parameters. Exposure data on Day 5 showed a C_{max} value for the male of 13 µg/mL and for the female of 8.0 µg/mL. The respective $AUC_{0–24h}$ values were 262 and 154 µg.h/mL.

3.2.8.2. 7-Day Pilot Oral Toxicity Study in Dogs

TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 7 days to groups of 4 male beagle dogs at 0 (negative control, water), 0 (vehicle), 20, 40, or 80 mg/kg/day in 1 mL/kg (m2.6.7, Section 6.1, TMC278-TOX5534).

There were no mortalities. Serum levels of total bilirubin (111% and 108% at 40 and 80 mg/kg/day, respectively), and glucose and cholesterol (11% and 10%, respectively, both at 80 mg/kg/day) were increased. The AUCs of cortisol concentrations in serum were decreased 56% to 75% in all groups treated with TMC278. Multifocal inflammatory cell infiltration and multifocal vacuolated cells in the zona fasciculata of the adrenal glands were noted in all treated groups. Systemic exposure expressed as C_{max} and $AUC_{0–24h}$ values are given in Table 12 below.

Table 12. Exposure to TMC278 (C_{max} and AUC) in a 7-Day Oral Toxicity Study with TMC278 Base in Dogs

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
		M (n = 4)	
20	1	1.6	18
	7	3.7	39
40	1	1.7	27
	7	7.7	159
80	1	1.5	19
	7	7.8	147

M: males

3.2.8.3. 1-Month Bridging Study with TMC278 Base and TMC278 in Dogs

This study served to bridge the toxicity and toxicokinetics of TMC278 base dissolved in PEG400 + CA with those of TMC278 suspended in 0.5% (m/v) aqueous HPMC. Both forms were administered once daily by oral gavage to groups of 3 male and 3 female beagle dogs for 1 month at 0 (vehicle), 5, or 40 mg/kg/day in 1 mL/kg (m2.6.7, Section 6.1, TMC278-NC116).

No mortalities occurred in this study. There were no significant differences in the toxicity parameters. Effects were almost exclusively observed in the groups dosed with both forms at 40 mg/kg/day. Reduced RBC (7%, females) and reticulocyte (up to 61%, males) counts, and HCT (15%) were noted. Increased serum concentrations of bilirubin (up to 2-fold) and increased activities of ALT (up to 6-fold), AST (14%, females), ALP (up to 2-fold), and gamma-glutamyltransferase (GGT; up to 6-fold) were recorded. Moreover, serum concentration of triglycerides (up to 53%) and inorganic phosphate (up to 11%) were decreased. Endocrinology showed increased serum concentrations of ACTH (92%), progesterone (up to 11-fold, males), and 17α-hydroxyprogesterone (also in males given 5 mg/kg/day; up to 34-fold in males dosed with 40 mg/kg/day); and decreased levels of cortisol (up to 54%).

At necropsy, increased weights of liver (up to 19%) and adrenal glands (up to 32%) were recorded. Histopathology lesions comprised swollen cells with densely stained cytoplasm in zona reticularis/fasciculata and presence of foamy cells in the zona fasciculata of adrenal glands. Moreover, an increase in the number of cystic luteinized follicles or tertiary follicles in the ovaries was noted, with a tendency towards an increase in glandular development in the uterus. In testes, prominent Leydig cells were noted in 1 animal. Males and females showed centrilobular/periportal inflammation, brown pigmented macrophages, single cell death, and oval cell proliferation in liver.

Systemic exposure expressed as C_{max} and $AUC_{0–24h}$ values are given in Table 13 below.

Table 13. Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Bridging Study with TMC278 Base and TMC278 in Dogs

TMC278 base (mg/kg/day)	Sampling days	C_{max} (µg/mL)		$AUC_{0–24h}$ (µg.h/mL)	
		M (n = 3)	F (n = 3)	M (n = 3)	F (n = 3)
5	1	1.0	0.66	12	8.1
	28	1.4	1.1	23	14
40	1	1.0	3.5	17	59
	28	3.8	2.5	73	43
TMC278 (mg/kg/day)					
5	1	0.49	0.53	6.9	6.2
	28	0.89	0.70	13	7.5
40	1	1.3	1.5	20	22
	28	4.1	5.8	76	81

M: males, F: females

3.2.9. 1-Month Oral Toxicity Study Including 1-Month Recovery in Dogs

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to groups of 3 male and 3 female beagle dogs at 0 (water, negative control), 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg (m2.6.7, Section 7.2.4, TMC278-Exp5650). To evaluate reversibility of the effects of TMC278 in a 1-month postdosing period, 2 males and 2 females were added to the negative control group and the group treated with 40 mg/kg/day. Regular observations were made for clinical signs, morbidity, and mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations and electrocardiogram (ECG) recordings were performed and samples for hematology, serum chemistry, and urinalysis were taken prior to dosing and at the end of the 1-month dosing and postdosing periods. Serum concentrations of progesterone, cortisol, aldosterone, ACTH, and estradiol (females, only) were determined in samples taken on Days 1 and 16, and towards the end of the dosing and postdosing periods. For the determination of AUCs for ACTH, cortisol, and aldosterone, 4 samples were taken over a 24-hour period. Progesterone and estradiol were determined in predosing and 4-hour samples. Blood samples for toxicokinetics were taken on Day 1 and towards the end of the dosing and postdosing periods. Single blood samples for trough level and elimination determinations were taken regularly during the dosing and recovery periods. At necropsy, blood and adrenal gland samples were taken for determination of TMC278. Moreover, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology. Adrenal gland samples of the dogs of the negative and vehicle groups, and the group dosed with 40 mg/kg/day, were examined by electron microscopy.

No mortalities occurred in this study. Red vaginal discharge was noted in 2 out of 3 females dosed with 10 mg/kg/day and 1 out of 5 dosed with 40 mg/kg/day. Soft feces and vomiting occurred in all groups except the negative control group indicating this effect was vehicle related rather than test article related. There were no relevant effects on ophthalmoscopy, heart rate, ECGs, or hematology. Male dogs dosed with 40 mg/kg/day lost body weight during the first 3 weeks of dosing. Female dogs in this group did not gain body weight throughout the dosing period. These effects were associated with a reduced food intake.

Red blood cell count, Hb, and HCT of samples from males and females treated with 40 mg/kg/day taken together were marginally (less than 5%) lower than values from the vehicle group. White blood cell count in this group was 12% higher than that of the vehicle group. Albumin and total protein concentrations in samples of males and females taken together in the groups dosed with 10 and 40 mg/kg/day were marginally lower than those of the vehicle group, whereas triglyceride concentrations were 14% and 21% lower, respectively. The concentrations of cholesterol (32%) and total bilirubin (136%) of animals dosed with 40 mg/kg/day were higher than those of the vehicle group. In the same group, the activities of ALP and ALT were respectively 120% and almost 3-fold higher than those of the vehicle group.

Progesterone concentrations were increased in a more or less dose-related fashion in the trough level samples, more pronounced in the samples taken 4 hours after dosing on Day 16, and even more towards the end of the dosing period in males and females treated with TMC278. The increases were almost 30- and 10-fold in males and females, respectively, dosed with 40 mg/kg/day. The AUCs of ACTH were increased at the end of the dosing period, without a

clear dose relationship or statistical significance. The AUCs of cortisol showed a tendency for decrease in the animals dosed with 10 and 40 mg/kg/day. After a 1-month postdosing period, all parameters showed similar values in the control group and the group treated with 40 mg/kg/day.

Mean absolute weight of ovaries showed a more or less dose-related increase ranging from 33% in the group dosed with 5 mg/kg/day up to 160% and 138% in the groups given 10 and 40 mg/kg/day, respectively, compared to the vehicle group. At necropsy, a swollen aspect of the female genital tract comprising cervix, ovaries, uterus, and vagina and of the mammary glands was noted in the majority of the animals dosed with 10 and 40 mg/kg/day.

In the adrenal cortex, histopathology showed an increase in the number of swollen cells with dense cytoplasm and reduced Oil red O-staining indicative of a reduction of neutral fat content in the animals dosed with 10 and 40 mg/kg/day compared with the vehicle group. The ultrastructural evaluation of adrenal gland samples from males and females treated with 40 mg/kg/day showed a reduction in the number of cytoplasmic lipid vacuoles in endocrine cells. This explains and confirms the light microscopy observations of less Oil red O-staining and dense cytoplasm. In addition to these findings, an increase of macrophage-like cells containing many small- and large-sized lipid vacuoles and secondary lysosomes with lipid and lamellar material was noted in the zona fasciculata. The higher number of these cells containing material representing membrane detritus indicates that TMC278 may have a mild degenerative effect in this zone.

The female genital tract showed increased activation in the groups treated with 10 and 40 mg/kg/day. In the ovaries, corpora lutea were detected in 2 animals dosed with 10 mg/kg/day and in 1 animal that received 40 mg/kg/day. This finding was associated with prominent high epithelium in the oviduct; increased glandular development in the uterus; cornification with increased thickness of the epithelium of the cervix and vagina; and with active alveolar development in mammary glands. More prominent tertiary follicles were noted in all test article-treated animals that had not ovulated at the end of dosing period.

In liver, minimal to moderate centrilobular perivascular inflammatory reaction was observed in males. Minimal increase in the number of multifocally dispersed centrilobular hepatocytes with a clear appearance was noted in the groups treated with 10 and 40 mg/kg/day. Moreover, a slight to moderate increase of MPS-aggregates; slight to minimal centrilobular hepatocellular single cell necrosis and multifocal centrilobular perivascular fibrosis; and minimal multifocal bile duct proliferation occurred in males and females dosed with 10 and 40 mg/kg/day.

All adverse effects that were seen at 40 mg/kg/day were completely reversible within a 1-month recovery period, except for the changes in the liver and the increased level of ALP in the serum. However, the histological changes in the liver showed signs of reversibility.

Given the dose-related trend in endocrinology results and ovarian weight already evident in the group treated with the low dose, 5 mg/kg/day, a NOEL was not established. Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 14 below. The mean adrenal gland to plasma ratios of the concentrations of TMC278 ranged from 1.3 to 3.6, without a clear dose relationship. After 1 month of recovery, the concentration of TMC278 in adrenal gland, as well as in plasma, was below the limit of quantification.

Table 14. Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Toxicity Study Including 1-Month Recovery with TMC278 Base in Dogs

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M (n = 3)	F (n = 3)	M (n = 3)	F (n = 3)
5	1	0.94	1.3	13	15
	29	1.5	2.0	27	37
10	1	1.3	1.3	22	14
	29	5.6	2.5	103	47
40	1	2.8	2.4	51	40
	29	12	9.5	204	160

M: males, F: females

3.2.10. 6-Month Oral Toxicity Study Including 3-Month Interim Evaluation with TMC278

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 6 months to groups of 6 male and 6 female beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg (m2.6.7, Section 7.2.5, TMC278-NC115). Regular observations were made for clinical signs, morbidity, and mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations, ECG recordings, and samples for clinical pathology were taken prior to dosing, prior to 3-month interim evaluation, and at the end of the dosing period. Clinical pathology comprised a standard set of hematological, serum chemistry, and urinalysis parameters. In addition, serum concentrations of 17α-hydroxyprogesterone, progesterone, cortisol, and ACTH were determined. For the determination of AUCs for ACTH and cortisol, 4 samples were taken over a 24-hour period from the first 3 male and 3 female animals in each group. Blood samples for toxicokinetics were taken toward the 3-month interim evaluation, and towards the end of the dosing period. In addition, on the day of necropsy, just prior to the sacrifice, a single blood sample and samples of liver and adrenal gland were taken for the determination of TMC278. After 3 months of treatment, the last 2 males and 2 females of each group were killed for the interim evaluation. The remaining animals were killed at the end of the 6-month treatment period. At both necropsies, gross lesions and organ weight were recorded, and tissues and organs were sampled for histopathology.

No mortalities occurred in this study. The males and females dosed with 40 mg/kg/day lost body weight during the first 4 weeks of treatment. Thereafter, their weekly body weight gain was not different from that of the control animals. The effects on body weight are considered associated with the reduction of food consumption noted in the animals treated with 40 mg/kg/day during the first 4 weeks of treatment. There were no relevant effects on heart rate, ECG, ophthalmology, hematology, and urinalysis.

Serum chemistry towards 3 months of dosing showed 40% and 23% increases in cholesterol concentrations of males and females, respectively, dosed with 40 mg/kg/day. Total bilirubin was increased 46% in females treated with 10 mg/kg/day, and 31% and 77% in males and females, respectively, dosed with 40 mg/kg/day. Alkaline phosphatase activity was increased 85% in the

females dosed with 5 and 10 mg/kg/day, and almost 3.5-fold in females dosed with 40 mg/kg/day. The increase in ALP activity was 28% and 72% in males treated with 10 and 40 mg/kg/day, respectively. The same parameters were increased at the end of the 6-month treatment period to an almost similar extent as after 3 months of treatment, except ALP. The ALP enzyme activity in males and females dosed with 40 mg/kg/day had increased 130% in males and almost 5.5-fold in females.

Cortisol precursor 17α-hydroxyprogesterone showed a more or less dose-related increase in males only, from more than 4-fold in the group treated with 5 mg/kg/day, and up to almost 50-fold after 3 months of dosing and 20-fold after 6 months of dosing with 40 mg/kg/day. The assessment of the progesterone levels in females after 3 months of treatment was hampered by high and highly variable levels in the vehicle group, which were probably associated with the phase of the estrous cycle of these animals. At the end of the 6-month dosing period, control levels in both males and females were similar and a dose-related increase in the groups treated with TMC278 base was noted, ranging from 100% in the animals dosed with 10 mg/kg/day to 4- to 5-fold in the animals dosed with 40 mg/kg/day. The AUCs of cortisol showed a dose-related decrease, which was similar after 3 and 6 months of treatment. This decrease was more prominent in males, ranging from 30% (males only) in the group dosed with 5 mg/kg/day and up to 50% to 60% in the group dosed with 40 mg/kg/day. The AUCs of ACTH showed clear increases from 80% in males dosed with 5 mg/kg/day for 6 months up to almost 3-fold in the high dose males at the end of the dosing period. Females showed similar effects on cortisol and ACTH, but to a lower extent than the males after 3 months of treatment. However, at the end of the dosing period females did not show a clear effect on these hormones.

Histopathology showed that the genital tract, adrenal glands, liver, gall bladder, and thymus of male and females were affected by TMC278. In the zona fasciculata and zona reticularis of the adrenal cortex, an increase of swollen cells with densely stained cytoplasm and a lower number of droplets stainable with Oil Red O was noted in the groups treated with 10 and 40 mg/kg/day after 3 and 6 months of treatment. At the end of the dosing period, a single male and female treated with 5 mg/kg/day also showed these effects. Thymus of 1 male and 1 female treated for 3 months with 40 mg/kg/day showed slight to marginal signs of involution. A minimal number of macrophages laden with presumably lipogenic (Perl's negative) pigment were noted perivascularly in liver in 1 male dosed for 3 months with 40 mg/kg/day, in 2 males dosed for 6 months with 10 mg/kg/day, and in 2 males and 2 females dosed for 6 months with 40 mg/kg/day. Brown pigmentation of the gall bladder epithelium was noted to a minimal degree in 1 male dosed with 10 mg/kg/day, and in the majority of males and females dosed with 40 mg/kg/day at the end of the dosing period. In testes, Leydig cell hypertrophy occurred in 1 animal dosed with 10 mg/kg/day (minimal), in the 2 animals dosed with 40 mg/kg/day (minimal to slight) after 3 months of treatment, and in 2 animals (minimal) treated for 6 months with 40 mg/kg/day. Ovaries were increased in weight in all TMC278-treated groups after 3 months, and in the animals that received 10 and 40 mg/kg/day (100%) after 6 months of dosing. In the majority of the animals treated with 40 mg/kg/day for 6 months, ovaries, uterus, and vagina had a swollen aspect. Histopathology showed a slight increase in the number of atretic follicles of animals treated with 10 and 40 mg/kg/day for 3 and 6 months. In animals treated with 40 mg/kg/day, the number of regressive corpora lutea was slightly increased at the 3- and 6-month autopsies. The number of tertiary follicles was increased in animals of all groups treated with TMC278 for 3 and 6 months.

Given the changes seen in adrenals and ovaries at the lowest dose of 5 mg/kg/day, no NOAEL was established. Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 15 below. The mean adrenal gland to plasma ratios of the concentrations of TMC278 ranged from 4.1 to 8.1 over the groups, without a clear dose relationship. Liver to plasma ratios of the concentrations of TMC278 ranged from 7.7 to 13. Adrenal and liver to plasma ratios did not change between Day 93 and Day 184/185 of treatment.

Table 15. Exposure to TMC278 (C_{max} and AUC) in a 6-Month Oral Toxicity Study with TMC278 Base in Dogs

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M (n = 3)	F (n = 3)	M (n = 3)	F (n = 3)
5	1	1.0	0.70	11	9.2
	86	1.4	1.1	21	18
	177	1.5	1.4	21	17
10	1	1.3	1.2	22	20
	86	2.0	2.2	28	27
	177	2.0	1.9	26	32
40	1	1.5	0.82	23	10
	86	2.5	3.1	41	52
	177	4.0	2.9	68	43

M: males, F: females

3.2.11. 12-Month Oral Toxicity Study with TMC278

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 12 months to groups of 4 male and 4 female beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg (m2.6.7, Section 7.2.6, TMC278-NC107). Regular observations were made of clinical signs, morbidity, and mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations and ECG recordings were made and samples for hematological, serum chemistry, and urinalysis were taken prior to dosing; after 1 week (ECGs only); after 1, 3, 6, and 9 months of treatment; and at the end of the dosing period. Serum concentrations of 17α-hydroxyprogesterone, progesterone, estradiol, testosterone (in males only), and luteinizing hormone (LH, in males only) were determined in samples taken prior to the first administration (no samples for testosterone and LH); prior to dosing after 3 (no samples for testosterone and LH), 6, and 9 months of treatment; and at the end of the treatment period. For the determination of AUCs for ACTH and cortisol, 4 samples were taken over a 24-hour period before the first administration and after 3, 6, 9, and 12 months of treatment at 1, 2, 4, and 24 hours after a daily dose of TMC278. Blood samples for toxicokinetics were taken on Day 1, after 3 and 6 months of treatment, and prior to necropsy. At necropsy, gross lesions and organ weight were recorded, and tissues and organs were sampled for histopathology. The female animal dosed with 10 mg/kg/day that was killed for humane reasons following a gavage error on Day 21 was replaced.

No test article-related mortalities occurred. Five animals, including 1 control male (Day 360), 2 males (Days 155 and 333) and 1 female (Day 20, replaced) dosed with 10 mg/kg/day, and 1 female (Day 54) treated with 40 mg/kg/day, were killed for humane reasons in a moribund condition. Postmortem evaluations indicated that their condition was caused by respiratory tract abnormalities probably due to the presence of the applied formulation.

Body weight gain in males at the end of the study was reduced in a dose-related fashion in all groups (40% reduction in males dosed with 40 mg/kg/day). In females, a dose-dependent reduction of body weight gain occurred in groups treated with 10 and 40 mg/kg/day. At 40 mg/kg/day, the reduction was more than 50%. Food consumption in males was hardly affected by treatment. However, in females, food consumption varied considerably throughout the treatment period and was frequently reduced in the animals dosed with 10 and 40 mg/kg/day.

No test article-related abnormalities were detected at ophthalmic examinations or on ECGs. Hematology, serum chemistry, and urinalysis parameters were affected only in samples taken at the end of the dosing period from animals treated with 40 mg/kg/day. Red blood cell count, Hb, and HCT in males were 11% to 13% lower. Serum calcium concentrations in males and females were decreased 10% and 7%, respectively. Inorganic phosphate concentration in females was increased 17%. Creatinine level in males was 14% higher than the control value. Total bilirubin concentrations were 15% and 42% higher in males and females, respectively. Alkaline phosphatase activity in males was almost twice as high as in controls, whereas the enzyme activity in females was 55% higher. Urinary volume in males was 3.5-fold higher.

Hormone analyses showed a dose-related increase ranging from 3- to 20-fold in the serum concentrations of both progesterone and 17α-hydroxyprogesterone in males in all groups treated with TMC278 base throughout the dosing period. Evaluation of these hormones in females was hampered by interference of the estrous cycle, but the serum concentrations showed an increased trend similar to what was evident in males. The AUCs for cortisol in males showed a considerable variation, but for females a dose-related decrease ranging from minimally 14% to 80% was determined over all test article-treated groups, which was statistically significant in females dosed with 10 and 40 mg/kg/day throughout the dosing period. The AUCs for ACTH showed no effects, except for an incidental increase in the group treated with 40 mg/kg/day. At the end of the treatment period, LH and testosterone levels in males showed no treatment-related effects. Estradiol was essentially undetectable in the males of all groups throughout the study. In females, the concentrations of this hormone were highly variable due to the estrous cycle, but did not show any trend of an effect and were consistent with progesterone levels on an individual basis.

Terminal body weight showed a dose-related reduction in males, notably in all groups treated with TMC278. Mean adrenal gland weight was increased in animals treated with 40 mg/kg/day. The absolute increase was 23% in both males and females. The increase relative to body weight was 50% in males and 46% in females. This increased organ weight is probably associated with the (slight to moderate) increase in the size of the zona fasciculata in the adrenal cortex of 1 male and 3 females treated with 40 mg/kg/day and the bilateral focal hypertrophy of cells in this zone in 1 male of the same group. Moreover, histopathology showed dense cytoplasm of the cells in the zona fasciculata and/or reticularis of all females treated with TMC278, including the

premature death animals; a single male dosed with 10 mg/kg/day; and the majority of the males treated with 40 mg/kg/day. This effect was usually associated with a minimal reduction of cellular neutral fat deposits. In the high dose group, half of the males and females showed pigment deposits in the adrenal cortex.

The weight of ovaries showed a dose-related increase in females dosed with 10 and 40 mg/kg/day. The absolute increase was respectively 40% and 120% of control values. The increase relative to body weight was respectively 48% and 170% of control values. These weight effects are likely associated with the increase in the number of antral follicles and the prominent corpora lutea in the animals dosed with 10 and 40 mg/kg/day. In liver, yellow pigmentation in hepatocytes and canaliculi was noted in all males and females given 40 mg/kg/day and in 1 female given 10 mg/kg/day. Prominent brown pigment in the epithelium of the gall bladder was noted in males given 40 mg/kg/day. In testes, minimal hypertrophy of the Leydig cells was recorded in 2 males given 40 mg/kg/day. However, this effect had no impact on Sertoli cell functioning or spermatogenesis as an evaluation of the main stages of the spermatogenic cycles did not indicate any changes. Kidney changes were only seen in the group treated with 40 mg/kg/day and included acute interstitial nephritis in 2 males and minimal to slight corticomedullary mineralization in all terminally killed females.

As a consequence of the adrenal changes at the low dose group, no NOAEL was established. Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 16 below.

Table 16. Exposure to TMC278 (C_{max} and AUC) in a 12-Month Oral Toxicity Study of TMC278 Base in Dogs

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M (n = 4)	F (n = 4)	M (n = 4)	F (n = 4)
5	1	0.70	0.75	11	9.7
	90	1.2	1.4	18	21
	273	1.1	1.1	16	18
	363	1.1	1.5	17	19
10	1	0.90	1.2 [c]	15	15[c]
	90	2.0	2.3	29	29
	273	1.3[a]	2.3	19[a]	31
	363	1.3[b]	2.2	24[b]	36
40	1	2.4	2.5	37	41
	90	3.6	5.5[a]	60	88[a]
	273	3.2	3.5[a]	60	51[a]
	363	4.1	5.5[a]	65	61[a]

M = males, F = females, [a]: n = 3, [b]: n = 2; [c]: n=5 samples of dog humanely killed on Day 21 and of replacement dog are included

3.2.12. Rilpivirine: 8-Week Oral Toxicity Study in Juvenile Female Cynomolgus with TMC278.HCl

Nonhuman primates, like humans, have a significant adrenal androgenic pathway which is not present in rodents and dogs. For that reason, the effects of TMC278 on that pathway were evaluated in cynomolgus monkeys. Estrous cycle physiology in cynomolgus monkeys resembles that of human better than dogs. Rilpivirine has demonstrated inhibition of 21-hydroxylase or CYP21 in dogs, both in vitro and in vivo, and in rats in vivo (m2.6.7, Section 17.2, TMC278-FK4790, Section 7.2.3, TMC278-NC101, Section 7.2.4, TMC278-Exp5650, Section 7.2.5, TMC278-NC115, Section 7.2.6, TMC278-NC107,). The decreased output of cortisol or corticosterone because of this inhibition is compensated by an increased release of adrenocorticotropic hormone (ACTH) by the pituitary gland. As a consequence of this increased ACTH stimulation, higher levels of steroids, upstream of CYP21, in particular progesterone and 17-hydroxy (OH) progesterone, occur in the circulation. In species with an adrenal androgenic pathway, such an overall stimulation of the adrenal gland by ACTH may lead to increased levels of adrenal androgens in the circulation. Increased circulating androgen levels may have a masculinizing effect, particularly in prepubertal girls. For this reason, this study in immature cynomolgus monkeys served to assess the safety of RPV in the pediatric population. Eight animals per group were treated for 8 weeks with vehicle, a high RPV dose of 250-mg base equivalents (eq)/kg twice daily and a lower RPV dose of 100-mg base eq/kg twice daily. Cortisol, progesterone, 17-OH progesterone, dehydroepiandrosterone (DHEA), and androstenedione levels were determined in plasma at various time points as basal values and after stimulation with ACTH. Also, basal

ACTH values were determined. In addition, all standard general toxicology parameters were assessed in this study.

The second objective of this toxicity study in cynomolgus monkeys was to assess whether premature ovulation, noted in prepubertal dogs (TMC278-Exp5650), occurred in an animal model that was physiologically more similar to humans. Cynomolgus monkeys have an estrous cycle similar to that of women whereas that of dogs is quite different. The immature, ie, prepubertal, animals were treated for a period of 8 weeks covering thereby 2 estrous cycles, allowing a full assessment of any potential effect of RPV on premature ovulation. During that period estradiol and luteinizing hormone levels in plasma were determined and daily vaginal swabs were taken to monitor the occurrence of menses. In conclusion, to assess the above-described objectives, species widely used in toxicology studies, such as rats and dogs, would not have been suitable. Therefore, the study was conducted in nonhuman, immature primates.

The doses for the toxicity study in cynomolgus monkeys were selected on the basis of a toxicokinetic study described in m2.6.4, Section 3.3.2.5. For the repeat dose part of that study (TMC278-NC326), the animals were habituated to restraining chairs for ECG recordings. TMC278 was administered for 15 days to 3 groups of 3 young mature cynomolgus monkeys at 0 (vehicle), 200, or 500 mg/kg/day. Recordings were made on Days 2 and 15. No abnormalities were noted on RR, PR, or QT (also corrected according to Fridericia {15856} intervals, on the form of the QRS complex, or the T-wave. Moreover, no treatment-related effects were noted on the systolic, diastolic, or mean arterial blood pressure. Mean heart rate at all times was similar in all groups, ranging from 226 to 248 beats/minute.

TMC278 suspended in 1% m/v aqueous HPMC with 0.5% Tween 20 was administered for 8 weeks to 3 groups of 8 immature female cynomolgus monkeys at 0 (vehicle), 200, or 500 mg/kg/day. Animals were dosed 0, 100, or 250 mg/kg BID, with a 6-hour interval, at a volume of 5 mL/kg (m2.6.7, Section 7.2.7, TMC278-NC248). Regular observations of clinical signs, menses, morbidity, and mortality were made. Weekly, body weights were determined. Blood samples were taken for clinical pathology including hematology, clinical chemistry, and urinalysis prior to the start of dosing and towards the end of the dosing period. Blood samples for endocrinology (progesterone, estradiol, and LH) were taken prior to the start of the dosing period and weekly prior to the TMC278 administration (at approximately the same time as the samples were taken during the dosing period). Adrenocorticotropic hormone stimulation tests including determination of serum levels of ACTH (only prior to challenge), cortisol, 17α-hydroxyprogesterone, progesterone, androstenedione, and dehydroepiandrosterone (DHEA) prior to ACTH challenge and 15, 30, and 60 minutes after challenge were done prior to the start of the dosing period and 2, 4, and 6 weeks after the start of dosing. Samples for toxicokinetics were taken the day before necropsy at 10 time points over a 24-hour period. At necropsy at the end of the dosing period, gross lesions were recorded. A range of organs was weighed and a complete set of organs and tissues was examined microscopically.

No mortalities occurred in this study. No adverse or relevant effects were observed on body weight, clinical pathology, organ weights, or gross lesions. In (trough level) samples taken prior to the daily dosing, increased levels of 17α-hydroxyprogesterone (up to 100%) and progesterone (up to 57%) were noted throughout the dosing period in a more or less dose-related fashion. Reduced concentrations of androstenedione (maximally 31%) were evident throughout the dosing period in both dose groups to a similar extent. Decreased DHEA concentrations (maximally 20%) were noted only in the group dosed with 500 mg/kg/day after 4 and 6 weeks of dosing. No effects were noted on cortisol and ACTH in these samples taken at TMC278-trough level.

Upon ACTH challenge, serum concentrations of 17α-hydroxyprogesterone showed a very strong dose-related response throughout the dosing period, with C_{max} values of the groups treated with 200 and 500 mg/kg/day being 2.5- and 4-fold higher, respectively, than in the control group at 4 and 6 weeks of treatment. A similar pattern was visible for progesterone upon challenge. C_{max} values of the groups treated with 200 and 500 mg/kg/day were 2.5- and 3-fold higher, respectively, than in the vehicle group. Androstenedione and DHEA levels in the vehicle group showed only limited response to challenge. The C_{max} values of androstenedione and DHEA at 500 mg/kg/day were maximally 50% lower. Cortisol C_{max} values at 500 mg/kg/day were maximally 56% lower at the end of the dosing period.

Only incidental vaginal bleedings not indicative of menses were recorded. Moreover, no follicular or ovulatory effects were noted on serum levels of progesterone, estradiol, or LH. Microscopic evaluation of ovaries did not show any indication of activation. Minimal follicular cell hypertrophy in the thyroid gland was scored in 1 control animal, 3 animals dosed with 200 mg/kg/day, and 4 animals dosed with 500 mg/kg/day.

Since at the lowest dose tested several hormonal effects were evident, no NOAEL was established. Systemic exposure expressed as C_{max} and AUC values are given in Table 17 below.

Table 17. Exposure to TMC278 (C_{max} and AUC) in an 8-Week Oral Study with TMC278 in Juvenile Female Cynomolgus Monkeys

Dose (mg/kg/day)	Sampling Day	C_{max1} (µg/mL)	C_{max2} (µg/mL)	$AUC_{0–24h}$ (µg.h/mL)
200	Day 55	0.14	0.18	2.7
500	Day 55	0.31	0.31	4.6

C_{max1} = C_{max} after first daily dose, C_{max2} = C_{max} after second daily dose

3.3. TAF

3.3.1. 2-Week Oral Toxicity Study in Mice

Daily oral administration (10 mL/kg) of GS-7340-02 at 100, 500, or 1000 mg/kg/day (80, 400, 800 mg f.b.e./kg/day) to Crl:CD1(ICR) mice (10/sex/group) for up to 14 days resulted in early death for 10 and 20 mice administered 500 or 1000 mg/kg/day, respectively (m2.6.7, Section 6.3, TX-120-2006). Based on the number of confirmed gavage errors, the viscosity of the formulation, the number of abnormal lung findings noted at gross necropsy, and histopathologic changes consistent with nonfatal gavage injury and secondary inflammation, it is hypothesized that the early deaths for animals administered 500 or 1000 mg/kg/day were due in some undeterminable part to the character of the dose formulation concentrations of 50 and 100 mg/mL, respectively.

Minimal to moderate clinical pathology changes occurred in animals administered 500 or 1000 mg/kg/day and included mildly lower absolute reticulocyte counts, minimally to moderately higher total protein and albumin. Nonadverse microscopic changes in the rectal epithelium (minimal to slight vacuolation/apoptosis of the epithelium were observed in males administered ≥ 100 mg/kg/day and females administered 500 or 1000 mg/kg/day, which were not considered adverse as the epithelium remained intact and there was no resulting inflammation or necrosis). The NOAEL for TAF administered by oral gavage for 14 days to male and female mice was 100 mg/kg/day.

The TAF C_{max} at 100 mg/kg/day on Day 14 was 21.2 ng/mL for both sexes combined; AUC_{0-t} could not be calculated due to the lack of sufficient data points from which to estimate AUC (Table 18). TAF was rapidly converted to its metabolite TFV. The TFV C_{max} at this dose level on Day 14 was 1.24 µg/mL for both sexes combined, and AUC_{0-t} was 5.74 µg·h/mL for both sexes combined.

Table 18. Mean Repeat-Dose Toxicokinetics of TFV Following Oral Administration of GS-7340-02 in Male and Female Mice for 2 Weeks

GS-7340-02 (mg/kg/day)	Study Day	Toxicokinetic Parameters			
		TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
100	Day 1	0.931	5.34	0.012	NC
	Day 14	1.24	5.74	0.021	NC
500	Day 1	4.13	25.9	6.14	13.02
	Day 14	3.93	29.4	2.76	1.91
1000	Day 1	4.46	48.2	8.77	22.5
	Day 8	6.28	5.67	23.3	17.8

NC = insufficient data to calculate

3.3.2. 13-Week Oral Toxicity Study in Mice

Crl:CD1(ICR) mice (15/sex/group) were administered daily oral doses (10 mL/kg) of GS-7340-02 at 10, 30 or 100 mg/kg/day (8, 24, 80 mg f.b.e./kg/day) for up to 13 weeks (m2.6.7, Section 7.3.1, TX-120-2007). Administration of TAF resulted in nonadverse minor effects on body weight gain for males at 100 mg/kg/day and females at all dose levels. A transient decrease in food consumption occurred at the start of dosing for animals administered 30 or 100 mg/kg/day. There were no test article-related clinical signs or effects on clinical pathology, organ weight parameters, or macroscopic observations. Data indicate increased incidence and severity of test article-related microscopic findings present in the nasal turbinates (minimal to slight infiltrates of neutrophils in respiratory and olfactory mucosa, and minimal to moderate [high dose only] degeneration of olfactory epithelium) of males and females at ≥ 10 mg/kg/day; an additional adverse finding in the nasal turbinates (exudate in the lumen) was present in males and females administered ≥ 30 mg/kg/day. Minimal infiltrates and minimal olfactory degeneration were observed at a lower incidence in control animals. A test article-related microscopic finding also occurred in the rectum (minimal increased apoptosis) of males and females at 100 mg/kg/day.

Based on the increased incidence and severity of the microscopic findings for the nasal turbinates, the NOAEL for TAF administered for 13 weeks to mice could not be determined in this study. Due to limited concentration data for TAF, AUC values could not be calculated. At Week 13, the TFV AUC_{tau} (combined sexes) was 0.213 µg·h/mL at 10 mg/kg/day (Table 19).

Table 19. Mean Repeat-Dose Toxicokinetics of TFV Following Oral Administration of GS-7340-02 in Mice for 13 Weeks

GS-7340-02 (mg/kg/day)	Study Day/Week	TFV[a]	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
10	Day 1	0.060	0.171
	Week 13	0.069	0.213
30	Day 1	0.292	1.28
	Week 13	0.330	1.51
100	Day 1	1.01	6.53
	Week 13	0.863	7.40

Toxicokinetic parameters for TAF could not be calculated due to limited concentration data.

3.3.3. 4-Week Oral Toxicity Study in Rats

Daily oral administration of GS-7340-02 (15 mL/kg) at 1.5, 6.25, 25, 100, and 400 mg/kg/day (1.2, 5, 20, 80, 320 mg f.b.e/kg/day) to CD (Crl: CD®(SD)BR) rats (10/sex/group) for 28 days resulted in treatment-related effects including (at 400 mg/kg/day) decreased body weight gain, reduced food consumption, decreases in white blood cell (WBC) and RBC parameters, calciuria, decreased bone mineral density (BMD), decreased 1,25 dihydroxy vitamin D_3, renal karyomegaly, thymic atrophy, and atrophy of cancellous bone of the femur (m2.6.7, Section 7.3.2, R990182). Most effects were restricted to the 400-mg/kg/day group; however, selected changes were noted as low as 25 mg/kg/day with a nonsignificant decrease in 1,25 dihydroxy vitamin D_3 observed at 6.25 mg/kg/day. Based on changes in WBCs, the TAF NOAEL was considered to be 6.25 mg/kg/day (no change in WBC count was observed at doses up to 100 mg/kg/day in the subsequent 26-week rat toxicity study).

3.3.4. 26-Week Oral Toxicity Study in Rats

Daily oral administration of GS-7340-02 (10 mL/kg) at 5, 25, and 100 mg/kg/day (4, 20, 80 mg f.b.e./kg/day) to Crl: CD® (SD) BR rats (15/sex/group) for 26 weeks resulted in treatment-related effects that included minimal renal cortical tubular karyomegaly (100 mg/kg/day) and minimal to slight tibial cancellous bone atrophy (females, 100 mg/kg/day), changes in bone density measurements (100 mg/kg/day) and changes in biochemical markers of bone turnover (25 and 100 mg/kg/day) (m2.6.7, Section 7.3.3, TOX-120-001). These effects were not observed at 5 mg/kg/day. GS-7340-02 appeared to dose-dependently increase biochemical markers of bone turnover in males and dose-independently decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in males and females at the 25- and 100-mg/kg/day dose levels.

There were no treatment-related mortality or treatment-related clinical signs in any dose group during the 26-week treatment period. There were no significant effects on body weight, food consumption, ophthalmology, hematology, routine biochemistry, or urinalysis parameters. There were no treatment-related effects on organ weights or macroscopic findings. As the effects (increases in biochemical markers of bone turnover and changes in related hormones) seen at 25 mg/kg/day were minimal, it was concluded that the NOAEL was 25 mg/kg/day.

Toxicokinetic analysis of plasma samples demonstrated that TAF was rapidly absorbed after oral dosing and was rapidly converted to TFV (Table 20). No consistent differences in plasma pharmacokinetics were found between male and female rats. Tenofovir was eliminated from the plasma with half-lives ranging from 7 to 13 hours. Mean TFV C_{max} and AUC values for combined sex groups increased dose proportionally over the dose range of 5 mg/kg/day to 100 mg/kg/day at each study period.

Table 20. Mean Repeat-Dose Pharmacokinetics of TFV Following Oral Administration of GS-7340-02 in Male and Female Rats for 26 Weeks

GS-7340-02 (mg/kg/day)	Study Day/Week	TFV[a]	
		C_{max} (µg/mL)	AUC (µg·h/mL)
5	Day 1	0.309	0.604
	Week 13	0.344	0.727
	Week 26	0.267	0.670
25	Day 1	1.284	3.364
	Week 13	1.464	3.724
	Week 26	1.523	3.758
100	Day 1	4.944	12.415
	Week 13	5.514	15.044
	Week 26	4.911	15.534

a Toxicokinetic parameters for TAF could not be calculated due to limited concentration data

3.3.5. 1-Week Oral Toxicity Study in Rabbits

This nonGLP study evaluated the toxicity and determined the toxicokinetics of TAF (GS-7340-02), when administered daily via oral gavage to female New Zealand White rabbits, for 7 consecutive days (m2.6.7, Section 6.3, TX-120-2003). The study provided information on dose selection for a subsequent developmental toxicity study.

Animals (3 per group) were administered vehicle control (0.1% [v/v] Tween 20, 0.1% [w/v] HPMC K100LV prepared in ultrapure water) or GS-7340-02 at doses of 20, 50 or 75 mg/kg/day for 7 days using a dose volume of 10 mL/kg/day. Assessment of toxicity was based on mortality, clinical signs, body weight, body weight change, food consumption, and clinical pathology parameters. Toxicokinetic blood sampling was performed on Days 1 and 7.

All animals survived to their scheduled sacrifice. There were no test article-related effects on clinical condition or food consumption. There was a slight reduction in mean cumulative body weight gains at 50 and 75 mg/kg/day (74% and 57%, respectively compared to controls), but there was no decrease in body weight. There were no test article-related changes in clinical pathology parameters or macroscopic findings.

Plasma exposure to TAF and TFV generally increased with increase in dose level from 20 to 75 mg/kg/day. Values for mean C_{max} and AUC_{0-t} of TFV were generally higher on Day 7 than on Day 1. TAF was rapidly and extensively converted to TFV. The mean TAF AUC_{0-t} on day 7 was unable to be calculated for the 20 mg/kg/day dose (due to values below the lower limit of quantitation of 1.00 ng/mL) and was 0.252 and 1.174 µg·hr/mL for the 50- and 75-mg/kg/day groups, respectively. The mean TFV AUC_{0-t} on Day 7 was 2.256, 5.741 and 10.070 µg hr/mL for the 20-, 50-, and 75-mg/kg/day groups, respectively.

In conclusion, daily oral administration of GS-7340-02 at 20, 50 and 75 mg/kg/day to female rabbits, for 7 days, was generally well tolerated. There were no test article-related effects on food consumption, clinical pathology parameters or macroscopic findings. There was a slight decrease in cumulative body weight gain at 50 and 75 mg/kg/day, compared to controls, but there was no effect on body weight. The NOAEL was 75 mg/kg/day; the highest dose administered. Exposure to TAF and TFV generally increased with the increasing GS-7340 dose from 20 to 75 mg/kg/day. At 75 mg/kg/day, mean AUC_{0-t} values were 1.2 and 10.1 µg·hr/mL for TAF and TFV respectively.

3.3.6. 4-Week Oral Toxicity Study in Dogs

Daily oral administration of GS-7340-02 (10 mL/kg) at 0.1, 0.3, 1, 3, or 10 mg/kg/day (0.08, 0.24, 0.8, 2.4, 8 mg f.b.e./kg/day) (m2.6.7, Section 7.3.4, D990175) to male and female beagle dogs (4/sex/group) for 28 days resulted in a marginal rise in AST in high-dose females only (10 mg/kg/day) of questionable toxicologic significance, and renal tubular karyomegaly and/or basophilia in all males and females at 10 mg/kg/day and 1 male and 1 female at 3 mg/kg/day. There were no other adverse effects. Mean values for bone specific alkaline phosphatase, N telopeptide, parathyroid hormone, 1,25 dihydroxyvitamin D and 25 hydroxyvitamin D were generally similar among all groups on each occasion. There were no TAF effects on peripheral quantitative computed tomography-derived bone densitometry parameters (eg, bone mineral content and bone mineral density of the total slice and trabecular and cortical/subcortical compartments). The TAF NOAEL was 1 mg/kg/day.

At the lower doses, only C_{max} and T_{max} values for TAF were determined as most values were below the lower limit of quantitation of the assay. TAF was rapidly absorbed on Day 1, with median peak values within 0.25 to 0.5 hours of 18.5, 38.7, and 0.582 µg/mL at 1.0, 3.0, and 10 mg/kg/day, respectively. Peak TFV concentrations occurred within 1 hour. For the 10-mg/kg/day dose group, Day 28 TFV C_{max} and AUC_{tau} were 0.44 µg/mL and 5.26 µg h/mL, respectively (males and females combined). Comparisons between Day 1 and Day 28 for the 10-mg/kg/day dose group showed potential accumulation upon repeat dosing. Tenofovir in PBMCs was measurable (18.6 µg/mL) after 28 days of 10 mg/kg/day TAF (m2.6.4, Section 3.3.3, AD-D990175-PK).

Table 21. Median Repeat-Dose Pharmacokinetics of TFV and TAF Following Oral Administration of GS-7340-02 in Beagle Dogs for 28 Days

GS-7340-02 (mg/kg/day)	Study Day	TFV		TAF (GS-7340)		TFV PBMC (µg/mL)
		C_{max} (µg/mL)	AUC_{tau} (µg·h/mL)	C_{max} (µg/mL)	AUC_{tau} (µg·h/mL)	
10	Day 1	0.385	NC	0.582	NC	NC
	Day 28	0.444	5.26	1.28	0.985	18.6

NC = insufficient data to calculate
PBMC = peripheral blood mononuclear cell

3.3.7. 39-Week Oral Toxicity Study in Dogs

Male and female beagle dogs were administered daily oral doses (10 mL/kg) of GS-7340-02 at 2, 6, or 18/12 mg/kg/day (1.6, 4.8, 14.4/9.6 mg f.b.e./kg/day) for 13 weeks (2/sex/group) or 39 weeks (4/sex/group) with a 3-month post dose recovery phase (2/sex at 18/12 mg/kg/day) (m2.6.7, Section 7.3.5, TOX-120-002). Daily oral administration of GS-7340-02 for 39-weeks resulted in a dose-related decrease in body weight gain at 39 weeks for all male treated groups and for females receiving 18/12 mg/kg/day. The dose level in the high-dose group was reduced from 18 to 12 mg/kg/day on Days 45 and 51, for males and females, respectively, due to the occurrence of severe clinical signs and reduced body weight.

There were 2 unscheduled deaths of high-dose males. One of these was considered to be due to a gavage accident and not treatment related. A different high-dose male was euthanized on Day 45 due to its deteriorating clinical condition, considered to be treatment related. Prior to necropsy the animal had shown reduced body weight; reduced food consumption; increased AST, globulin levels, triglyceride, cholesterol, total bilirubin; and decreased monocyte and platelet counts. Macroscopically, there was bilateral enlargement of the submandibular lymph nodes, which histologically had slight inflammation and plasmacytosis. Histopathology findings included a mild, mononuclear infiltrate in the ocular posterior uvea; renal cortical tubular degeneration; atrophy of GALT, mesenteric lymph node, and thymus accompanied by an infiltrate of macrophages; mucosal atrophy of the fundic gland; mucosal hyperplasia of the pyloric gland; and mucosal degeneration and/or regeneration in the cecum and colon.

Hematology, serum chemistry and urinalysis parameters remained within normal historical ranges, with the exception of increased mean AST (~2.6x compared to control) and total bilirubin (~1.6x compared to control) in dogs administered 18/12 mg/kg/day. No ECG changes occurred at 2 mg/kg/day. At Week 39, a dose-related prolongation of PR interval was observed in the 6- (~ +13%) and 18/12-mg/kg/day (~ +24%) groups. GS-7340 appeared to reversibly reduce heart rate with an associated mild QT interval prolongation in the high-dose group only. These changes were associated with decreases in serum T3 {29101}, {29104}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals.

All bone markers showed age-related decreases. After 3 months of treatment, there were some small differences noted among mean values for bone formation (skeletal alkaline phosphatase [sALP]) and bone resorption markers (urinary free deoxypyridinoline and N telopeptide) for

groups administered GS-7340-02 compared to controls. After 9 months of GS-7340-02 administration, statistically significant increases in mean values for the bone resorption marker urinary N telopeptide were noted for males and females treated with GS-7340-02 at 18/12 mg/kg/day ($p \leq 0.05$), compared to controls. A similar though not statistically significant trend was noted in animals treated with GS-7340-02 at 6 mg/kg/day, suggesting a dose-related response. No significant changes in free deoxypyridinoline were observed in animals treated with GS-7340-02 with no consistent effects (increases) among treated groups. For the formation marker, serum sALP values in treated animals were comparable to vehicle controls except for one high dose male value (Animal 401), which was outside the control ranges. At the end of the recovery period, bone marker values returned to below the control range consistent with an age effect and recovery from treatment.

Treatment with GS-7340-02 at 18/12 mg/kg/day administered once daily to young beagle dogs for 39 weeks resulted in changes in bone densitometry parameters (by dual-energy x-ray absorptiometry [DXA] analysis) considered to reflect primarily effects on bone growth. These changes were considered secondary to the effects on body weight.

Compound-related histopathology alterations were noted in the kidneys, eyes, lungs, and spleen after both 13-week interim and 39-week treatment phases. The liver and possibly the adrenal glands were additional target organs identified after the longest treatment duration. After 13-weeks of treatment, findings of renal cortical tubular degeneration/regeneration and karyomegaly were limited to animals administered either 6 or 18/12 mg/kg/day; findings after 39 weeks of treatment were similar. These changes were minimal to slight (Grade 1 to 2) in affected mid-dose (6 mg/kg/day) males and females. In high-dose males (18/12 mg/kg/day) severity ranged from mild to moderate (Grade 2 to 3). After 39-weeks of treatment, similar lesions (karyomegaly and tubular degeneration) but of only minimal (Grade 1) severity were also present in 2 low-dose males administered 2 mg/kg/day. A minimal to slight (Grade 1 to 2) infiltration of mononuclear cells in the ocular posterior uvea was noted in some animals administered 18/12 mg/kg/day after both treatment periods. Alveolar histiocytosis was the sole compound-related finding present in the lungs after 13-weeks of treatment in animals administered 18/12 mg/kg/day. Additional pulmonary findings were noted following 39-weeks of treatment and included a macrophage accumulation with pigment, which could be detected predominantly in high-dose (18/12 mg/kg/day) group animals and in few animals in 6- or 2-mg/kg/day dose groups. An infiltration of macrophages laden with pigment was very frequently seen in the splenic white pulp of animals administered 18/12 mg/kg/day after both treatment periods. After 39 weeks of treatment centrilobular hepatocellular cytoplasmic acidophilic inclusions were identified in dogs administered 18/12 mg/kg/day, whereas pigment deposits in hepatic macrophages and/or sinusoidal cells (Kupffer cells) could be found in animals treated with 6 or 18/12 mg/kg/day. Also, similar pigment deposits in the sinusoidal cells (tissue macrophages) of the adrenal glands were seen in a few dogs administered 18/12 mg/kg/day. The cause of the intracellular pigment in tissue macrophages in the lung, liver, spleen, and adrenal is not known but could represent accumulation of the test article and/or test article metabolite(s) in these cells of the mononuclear phagocyte system. After a 13-week recovery period, treatment-related histological changes were still observed in the kidneys, lungs, and liver but were of reduced incidence and severity.

Based on these findings, the NOAEL of GS-7340-02 after 39 weeks of treatment in this study was determined to be 2 mg/kg/day. Treatment-related findings were completely or partially reversible following a 13-week recovery period.

Toxicokinetic analysis showed that TAF was rapidly absorbed and converted to TFV following oral dose administration, with peak plasma concentrations of TAF and TFV occurring 0.5 and 1 hour after dosing, respectively. The systemic exposure of TAF was dose dependent. Plasma C_{max} and AUC values for GS-7340 increased more than proportionally over the dose range of 2 to 18/12 mg/kg/day. The systemic exposure of TFV, as measured by plasma C_{max} and AUC, increased roughly in proportion to the administered dose. There was some accumulation of TFV following repeat dosing (approximately 3-fold) as expected based on its terminal phase half-life. There was no effect of sex on plasma pharmacokinetics of TAF and TFV after oral administration.

Tenofovir concentrations in PBMCs were measurable at 24 hours after dosing for all dose groups. The median terminal phase half-life of total TFV in PBMCs was estimated to be 31 hours (similar to the TFV plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total TFV increased more than dose proportionally during Week 39/40. The mean toxicokinetic parameters for TAF and TFV are summarized in Table 22.

Table 22. Mean Pharmacokinetics of TFV and TAF Following Repeat-Dose Oral Administration of GS-7340-02 in Beagle Dogs for 39 Weeks

GS-7340-02 (mg/kg/day)	Study Week	TFV		TAF (GS-7340)		TFV PBMC AUC_{tau} (ng·h/10^6 cells)
		C_{max} (µg/mL)	AUC_{tau} (µg h/mL)	C_{max} (µg/mL)	AUC_{tau} (µg h/mL)	
2	Day 1	0.08	0.40	0.08	0.03	NA
	Week 13	0.16	1.21	0.13	0.07	NA
	Week 39/40	0.18	1.18	0.14	0.08	258.2
6	Day 1	0.25	1.31	0.72	0.37	NA
	Week 13	0.56	4.10	1.10	0.66	NA
	Week 39/40	0.54	4.45	1.42	1.03	1263.5
18	Day 1	0.80	3.80	3.60	2.07	NA
12	Week 13	0.54	12.48	3.24	2.23	NA
	Week 39/40	1.32	13.73	2.62	1.95	3118.2

NA = not applicable (only Week 39/40 samples were analyzed), PBMC = peripheral blood mononuclear cell

3.3.8. 4-Week Oral Toxicity Study in Rhesus Monkey

Daily oral administration of GS-7340-02 at 3 or 30 mg/kg/day (2.4, 24 mg f.b.e./kg/day) or TFV at 15 mg/kg/day to male and female rhesus monkeys (3/sex/group, 10 mL/kg/day) for 28 days resulted in no adverse clinical signs and no clearly treatment-related effects on body weight,

serum chemistry, plasma chemistry, hematology (including lymphocyte subsets determined by flow cytometry), standard urinalysis parameters, organ weights, and bone-related or histologic parameters. There was 1 death in the 30-mg/kg/day TAF group, which was not treatment related. Kidney, liver, and skeletal muscle samples assayed for indicators of mitochondrial integrity showed no effects (m2.6.7, Section 7.3.6, P2000114). The NOAEL for TAF administered orally to rhesus monkeys for 28 consecutive days was ≥ 30 mg/kg/day.

The TAF C_{max} values were nonlinear with dose, with greater than expected increases in C_{max} with dose (Table 23) (m2.6.5, Section 4.3.3, P2000114-PK). The TAF AUC_{tau} could only be calculated for the 30-mg/kg/day group, with a mean value of 1.03 µg·h/mL and a terminal elimination half-life of 0.335 hours. No differences between sexes were observed.

Table 23. Mean Pharmacokinetics of TAF Following Repeat-Dose Oral Administration of GS-7340-02 in Male and Female Rhesus Monkeys, Day 28

GS-7340-02 (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{tau} (µg·h/mL)	CL_{ss}/F (mL/h/kg)
3	0.0188	0.917	NC	NC	NC
30	1.37	0.500	0.335	1.03	44600

NC = insufficient data to calculate

Day 28 TFV C_{max} and AUC exhibited slightly greater than proportional increases with increasing dose (Table 24). Comparison between Days 1 and 28 showed no statistical difference for C_{max} or AUC indicating no change in clearance over time. No differences between sexes were observed.

Table 24. Mean Pharmacokinetics of TFV Following Repeat-Dose Oral Administration of GS-7340-02 in Male and Female Rhesus Monkeys, Day 28

GS-7340-02 (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{tau} (µg·h/mL)	CLss/F (mL/h/kg)
3	0.0504	1.67	13.5	0.352	4250
30	0.963	0.700	16.1	5.87	2710

3.4. FTC/TDF

3.4.1. 14-Day Oral Gavage Toxicity Studies Comparing Non-Degraded and Degraded FTC/TDF in Rats

Two 14-day oral gavage GLP studies were conducted to investigate the potential toxicity of nondegraded and degraded FTC/TDF formulations following daily oral administration to rats for a minimum of 14 days (m2.6.7, Section 17.4, TX-164-2001 and m2.6.7, Section 7.4.1, TX-164-2005). There were no toxicologically significant differences between groups treated with nondegraded and degraded TDF/FTC, and no exacerbation of toxicity with the FTC/TDF combination compared to data with the individual agents.

3.4.2. 4-Week Oral Gavage Toxicity and Toxicokinetic Study with FTC/TDF in Male Dogs with a 4-Week Recovery Period

The purpose of this study was to evaluate the toxicity and to determine the toxicokinetics of the test articles FTC and TDF, alone or in combination, when administered daily via oral gavage to dogs for at least 4 weeks and to assess the reversibility, persistence, or delayed occurrence of any effects after a 4-week recovery (m2.6.7, Section 7.4.1, TX-164-2004).

Male purebred beagles were assigned to 5 groups (n = 4/group) and administered vehicle (50 mM citrate buffer in reverse osmosis water), 20 mg/kg/day FTC, 30 mg/kg/day TDF, 2/3 mg/kg/day FTC/TDF, or 20/30 mg/kg/day FTC/TDF in combination. Two additional animals administered 20/30 mg/kg/day FTC/TDF were designated for recovery sacrifice and underwent 4 weeks of recovery following 29 days of treatment.

Assessment of toxicity was based on mortality, clinical observations, ophthalmic examinations, body weights, food consumption, and clinical and anatomic pathology. Samples were also collected for toxicokinetic evaluation. In addition, immunophenotyping and natural killer (NK) cell analysis were conducted.

All animals survived to scheduled sacrifice. No clear test article-related effects on clinical signs and ophthalmic examinations were observed. Decreases in body weight gain and food consumption were noted in animals administered 30 mg/kg/day of TDF and 20/30 mg/kg/day of FTC/TDF respectively; however, these were not statistically significant.

Between Days 1 and 27, no accumulation of FTC occurred when FTC was administered alone at 20 mg/kg/day or in combination with 30 mg/kg/day TDF. The mean AUC values for Days 1 and 27 were 20.85 and 25.28 μg·h/mL, respectively, when administered alone and 24.46 and 32.33 μg·h/mL, respectively, when administered in combination. During the same period, no unexpected accumulation of TFV occurred when TDF was administered alone at 30 mg/kg/day or in combination with 20 mg/kg/day FTC. The mean AUC values for Days 1 and 27 were 7.60 and 18.41 μg·h/mL, respectively, when administered alone and 8.85 and 20.87 μg h/mL, respectively, when administered in combination.

TDF administration at 30 mg/kg/day, alone or in combination with FTC administration, was associated with minimally increased activated partial thromboplastin time (APPT) and creatinine. Both effects appeared to be reversible following recovery. Urinalysis test results appeared unaffected.

No remarkable changes were observed for immunophenotyping or NK cell assay values for test article-treated groups as compared with the vehicle control group.

No test article-related macroscopic findings or organ weight variations were present at either the terminal or recovery sacrifice.

Test article-related microscopic findings at the terminal sacrifice were seen in the kidneys of animals in 2 groups. Minimal tubular epithelial necrosis and an increased incidence and mean severity of tubular epithelial regeneration were seen in animals administered 30 mg/kg/day of

TDF alone or in combination with 20 mg/kg/day of FTC. Kidney lesions were morphologically similar in the 2 affected groups, and there were no overall differences in incidences and mean severities of tubular necrosis and regeneration noted between the 2 groups.

No test article-related microscopic changes were observed at the recovery sacrifice, indicating that the kidney findings noted microscopically at dosing termination were reversible.

In conclusion, administration of FTC alone at a dose of 20 mg/kg/day, or FTC/ TDF combination at 2/3 mg/kg/day, was well tolerated by dogs for 4 weeks. TDF alone at 30 mg/kg/day and FTC/TDF at 20/30 mg/kg/day resulted in microscopic kidney lesions, which in the case of FTC/TDF at 20/30 mg/kg/day were reversible after a 4-week recovery period. Based on the clinical and anatomic pathology findings, the NOAEL was 20 mg/kg/day for FTC alone and 2/3 mg/kg/day for the FTC/TDF combination. A NOAEL for TDF could not be determined at the doses used in the study.

3.5. FTC/RPV/TAF

Administration of TAF in combination with FTC and RPV is unlikely to exacerbate known toxicities of the individual agents. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH in monkeys at large multiples of clinical exposure (137-fold in mice; 20-fold in monkeys); therefore, these hematological findings are not considered relevant to clinical use. Extensive nonclinical investigations of the toxicity of TAF have shown that unlike FTC, the bone marrow is not a target for this agent, and that the target organs for TAF are distinctly different (bone and kidney). FTC and RPV have not shown any potential for bone toxicity; thus, exacerbation of any TAF effects on bone is not expected.

The repeat-dose toxicity studies with RPV have identified a series of targets of toxicity: RBC in mouse, rat, and dog; coagulation in rat; liver in rat and dog; kidneys in mouse and dog; thyroid gland with secondary effects on the pituitary gland in rat; adrenal glands in mouse, rat, dog, and cynomolgus monkey; testes in dog; ovaries in dog; and in immature females, secondary effects on other parts of the genital tract and on mammary glands compared to immature control animals. The majority of these effects appeared to be completely reversible after a recovery period. The effects on thyroid gland and coagulation in rats showed signs of recovery but this was not complete at the end of the 1-month postdosing period. For mice and rats, a NOAEL could be established. However, dogs and cynomolgus monkeys showed effects at the level of the adrenal glands at the low dose tested. Moreover, dogs also showed effects on ovaries in almost all pivotal studies at the low dose administered. These effects prevented establishment of NOAELs in these species.

Given that kidney effects have been observed with RPV in mice and dogs only at high dose levels and exposures, and that the routes of excretion differ for RPV (feces) and TFV (renal excretion), renal toxicity is not anticipated to be an issue with the FTC/RPV/TAF combination product. From in vitro data, pharmacokinetic studies in dogs and clinical experience, there are no anticipated pharmacokinetic interactions between FTC, RPV, and TAF. The ample nonclinical safety database on these drugs strongly indicates that little potential for drug interaction exists and further toxicological investigations are unlikely to yield new data relevant to humans.

Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT (TQT) study in healthy subjects (see m2.6.2, Section 4.2.2). At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the cardiovascular effect seen with RPV alone. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with bone and renal toxicity and included significant decreases in T3 {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.8, D2000006) or in the thorough QT study (m2.7.2, Section 2.3.2.2.1 [Study GS-US-120-0107]). No additional studies are considered necessary with the FTC/RPV/TAF combination.

The combination of FTV, RPV, and TAF is not anticipated to exacerbate known toxicities or lead to new toxicities. Emtricitabine has an established clinical safety profile with no significant toxicities observed. Potential toxicities related to RPV that were observed preclinically, but have not been observed in clinical studies include affects on the adrenal system and associated changes in 17-OH progesterone and cortisol levels, and liver and thyroid toxicity. The known clinical toxicities for TAF are renal and bone toxicity. Comprehensive nonclinical safety databases on these drugs, including combination toxicity studies with FTC and TDF, strongly indicate that further toxicological investigations are unlikely to yield new data relevant to humans. Further studies of longer duration with the combinations are not warranted because of the lack of significant overlapping toxicities, and the use of additional animals that would be required to obtain such information. Extensive clinical safety data are available for FTC and RPV. In addition, the data for the FTC/TDF FDC product (Truvada), and the FTC/RPV/TDF product (Complera/Eviplera), supports the use of these agents. The clinical data, along with the lack of overlapping toxicity in animals, support the safety of the new combination product for HIV-1 infection.

4. GENOTOXICITY

4.1. FTC

4.1.1. In Vitro

4.1.1.1. Mutagenicity Test with FTC in the *Salmonella – Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

FTC was dissolved in DMSO and tested for mutagenic activity in the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay (m2.6.7, Section 8.1, 18637-0-409R). The concentrations tested, along with vehicle and positive control test substances, were 5000, 3300, 1000, 333, and 100 μg/plate in the presence and in the absence of metabolic activation. The tester strains were *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2*uvr*A. Emtricitabine did not cause a positive increase in the number of revertants per plate in any of the tester strains in either the presence or absence of metabolic activation. The confirmatory assay, at the concentrations of FTC listed above, also gave negative results for mutagenic activity. It was concluded that FTC was negative in the reverse mutation (Ames) assay.

4.1.1.2. *Salmonella*/Mammalian Microsome Assays with FTC

Emtricitabine was tested for its ability to induce reverse mutations in 2 Ames *Salmonella*/mammalian-microsome assays, the *Salmonella* plate incorporation assay and the *Salmonella* preincubation assay (m2.6.7, Section 8.2, MUT203). The tester strains used were TA1535, TA1537, TA1538, TA98, and TA100, both in the presence and absence of Arochlor-induced rat liver S9 metabolic action. The range of concentrations of FTC were 100 to 5000 μg/plate.

There was no detectable mutagenic activity at any dose of FTC when tested by either Ames assay method.

4.1.1.3. Mutagenicity Test of FTC using Microorganisms

The ability of FTC to induce mutations was investigated by using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *Escherichia coli* strain WP2 *uvrA* with a preincubation method in the presence and absence of a metabolic activation system (S9 mix) (m2.6.7, Section 8.3, K01-3154). The mutagenicity of the test substance was judged negative because the number of revertant colonies in the test substance groups was less than twice that of each negative control in all test strains. It is concluded that FTC has no ability to induce mutations under the conditions of the assay.

4.1.1.4. In Vitro Mammalian Cell Gene Mutation Test

The free base of FTC was dissolved in sterile distilled water and tested for its potential to induce forward mutations at the TK locus in the mouse lymphoma L5178Y cell line (m2.6.7, Section 8.4, TOX012). Exposures to FTC were in the presence and in the absence of Aroclor-induced rat liver S9 metabolic activation. A preliminary experiment was used to select FTC concentrations ranging from 0.5 to 5000 µg/mL for testing. Cells at test concentrations of 1000, 2000, 3000, 4000, and 5000 µg/mL were cloned and evaluated with and without S9. For FTC, no cloned cultures exhibited mutant frequencies which were at least 55 mutants/10^6 clonable cells over that of sterile distilled water. Consistent with the low order of cytotoxicity established for FTC, no toxicity in the cloned cultures, i.e., total growth of ≤ 50% of the distilled water vehicle, was observed at any dose level. The colony sizing for the methyl methanesulfonate positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants. It was concluded that FTC was negative in the L5178Y/$TK^{+/-}$ mouse lymphoma mutagenesis assay at concentrations up to, and including, 5000 µg/mL.

4.1.2. In Vivo

4.1.2.1. Mammalian Erythrocyte Micronucleus Test

In this study, the free base of FTC was dissolved in sterile distilled water and administered by gavage to groups of 5 male ICR mice (m2.6.7, Section 9.1, TOX011). The doses administered were 0, 500, 1000, and 2000 mg/kg. Bone marrow was aspirated from the femur at 24 and 48 hours postdose. The slides of bone marrow were stained with May-Gruenwald-Giemsa stain. Light microscopy was used to count the number of micronuclei per 2000 PCEs. The data were analyzed using a 1-way analysis of variance and pairwise comparisons were made using Dunnett's t-test. The positive control, cyclophosphamide (60 mg/kg by gavage), produced a marked and significant increase in micronuclei. No significant increase in micronucleated PCEs was observed, at either time point or at any dose, in mice administered FTC. It was concluded that FTC was negative in the mouse in vivo micronucleus assay.

4.2. RPV

4.2.1. In Vitro

4.2.1.1. Ames Tests with Rat Liver S9

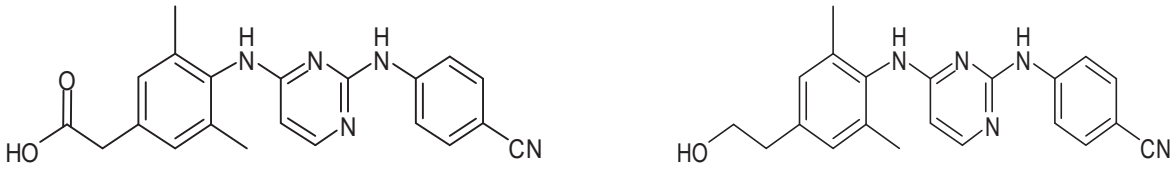
TMC278 base and TMC278 dissolved in DMSO (final concentration 0.1%) were tested in bacterial reverse gene mutation tests (Ames Test) (m2.6.7, Section 8.5, TMC278-Exp5540, Section 8.6, TMC278-Exp5693, and Section 8.7, TMC278-NC335). The Ames tests were done, in triplicate, with 5 histidine-requiring strains of *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, and TA102. On the basis of precipitation that limited scoring in range finding studies with plate incorporation and concentrations up to 5000 µg/plate, TMC278 base was tested in 2 main studies. Each of the studies comprised 2 independent repeat assays using the plate incorporation method up to 500 µg/plate. TMC278 was studied in 1 experiment with 2 independent repeat assays, one using the plate incorporation method and the other the preincubation method, up to 250 µg/plate.

In all of these studies, TMC278 base or TMC278 tested at the highest scorable concentration did not induce a biologically significant increase (more than 2 times the frequency of the vehicle) in the mutation frequency in any of the tested strains, irrespective of the presence of metabolic activation. (Note: In TMC278-NC279 (m2.6.7, Section 8.7), the preincubation method was used. Under these conditions, no precipitation occurred at the highest tested concentration in the absence of S9.) In all of these studies, the positive controls gave the anticipated results, indicating the validity of the test system.

4.2.1.2. Ames Test with Human Liver S9

The metabolic pathway of TMC278 generating metabolites M30 and M31 (Figure 1) encompasses potentially reactive metabolites (epoxide) due to Michael addition at the [redacted] moiety {15699} (see Figure 1 and m2.6.4, Section 5.1.2).

Figure 1. Metabolites M30 (Left) and M31 (Right)



Usually, metabolic pathways are evaluated in in vitro genotoxicity studies due to the presence of the S9 fraction of Aroclor-induced rat liver. However, rat liver S9 appeared not to yield either of these metabolites (m2.6.4, Section 5.2.2.1) and for that reason it was unclear whether the metabolic pathway with the potentially reactive metabolites had been adequately evaluated. In contrast, some batches of human liver S9 did yield M30 and M31. Moreover, M30 was detected in feces at 3% of the administered dose in the human metabolism study TMC278-TiDP6-C119. For these reasons, TMC278 base was tested in an Ames test in the presence of human liver S9 (m2.6.7, Section 8.7, TMC278-NC279). TMC278 base dissolved in DMSO was tested in 4 independent assays up to 500 µg/plate (the highest scorable concentration due to precipitations). The concentration of M30 + M31 (incomplete separation in liquid chromatography) indicated that the human S9 metabolic enhancer was active. The validity of the test system was evaluated by the response of positive controls used in the absence of a metabolic enhancer upon plate incorporation or preincubation, separate from each of the 4 assays. In parallel, TMC278 metabolites M30 and M31, the carboxylic and hydroxyl metabolites of the [redacted] moiety of TMC278, respectively, were determined by means of radiolabel LC-MS. In the first parallel assay, only the presence of M30 and M31 could be established due to the low radioactive dose. In the subsequent parallel incubations, a higher specific radioactivity of [^{14}C]TMC278 was applied allowing the quantitative determination of the sum of M30 and M31.

It is concluded that TMC278, under conditions that it is metabolized via a pathway encompassing potentially reactive intermediates such an epoxide, did not induce a biologically significant increase of the mutation frequency. The experimental conditions in the Ames test yielded M30 + M31 at 1.4% to 2.6% of the dose applied.

4.2.1.3. In Vitro Mammalian Cell System

TMC278 base and TMC278 dissolved in DMSO were tested in mouse lymphoma L5178Y cells for their potential to induce forward mutations at the TK locus (5-trifluorothymidine resistance; m2.6.7, Sections 8.8 and 8.9, TMC278-Exp5539 and TMC278-NC336, respectively). Prior to the main studies, a cytotoxicity range-finding experiment was performed for both compounds.

The maximum tested concentration of TMC278 base, on the basis of precipitation that limited scoring, was 500 µg/mL for a 3-hour exposure period without metabolic activation; 35 µg/mL for a 3-hour exposure period with metabolic activation; and 100 µg/mL for a 24-hour exposure period without metabolic activation (m2.6.7, Sections 8.8, TMC278-Exp5539).

TMC278 was tested in 1 study with 2 independent repeat assays up to 50 µg/mL for a 3-hour exposure period without metabolic activation; maximally 20 µg/mL for a 3-hour exposure period with metabolic activation; and up to 50 µg/mL for a 24-hour exposure period without metabolic activation (m2.6.7, Sections 8.9, TMC278-NC336).

TMC278 base and TMC278 did not induce an increase in the number of large or small colonies of L5178Y cells. Accordingly, it was concluded that these compounds did not induce an increased mutation frequency at the TK locus of L5178Y cells or large genetic damage like chromosomal aberrations.

4.2.2. In Vivo

4.2.2.1. Mammalian Erythrocyte Micronucleus Test

To evaluate the clastogenic and aneuploidic effect on early forms of erythrocytes in bone marrow, TMC278 base dissolved in PEG400 + CA was administered to mice in an in vivo micronucleus test (m2.6.7, Sections 9.2, TMC278-Exp5538). Single oral (gavage) doses of 0 (vehicle control), 100, 400, and 1600 mg/kg in 20 mL/kg were administered to 10 male and 10 female CD-1 mice per group. The dose of 1600 mg/kg was the maximally feasible dose on the basis of the maximally feasible volume for a single dose study, 20 mL/kg, and a supersaturated and highly viscous solution of 800 mg/mL. Bone marrow was sampled from 5 males and 5 females per group at 24 and 48 hours after dosing. Systemic exposure in terms of C_{1h} and $AUC_{0–6h}$ was determined. A positive control group of 5 male and 5 female CD-1 mice received a single oral dose of 40 mg cyclophosphamide per kg body weight, and was sampled for bone marrow 48 hours after dosing.

TMC278 base up to a single maximal feasible dose of 1600 mg/kg did not induce structural or numerical chromosome aberrations. At this dose, no bone marrow toxicity or signs of systemic toxicity were noted. Exposure data are presented in Table 25.

Table 25. Exposure to TMC278 (C_{1h} and AUC) in a Single Oral Dose Bone Marrow Micronucleus Study with TMC278 Base in Mice

Dose (mg/kg)	C_{1h} (µg/mL)		$AUC_{0–6h}$ (µg.h/mL)	
	M (n = 3 – 5)	F (n = 3 – 5)	M (n = 3 – 5)	F (n = 3 – 5)
100	39	40	158	130
400	59	68	262	258
1600	60	58	307	287

M: males, F: females

TMC278 did not show genotoxic potential when evaluated in a standard battery of in vitro and in vivo tests at the highest feasible concentration or dose.

4.3. TAF

4.3.1. In Vitro

4.3.1.1. Mutagenicity Test in the *Salmonella-Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay

The mutagenic potential of GS-7340-02 was evaluated in vitro in the Salmonella-Escherichia coli/mammalian microsome reverse mutation assay and GS-7340-02 up to 5000 µg/mL (4000 µg f.b.e./mL) did not cause a positive increase in the mean number of revertants per plate with any of the tester strains (m2.6.7, Section 8.10, V990212).

4.3.1.2. Mutagenicity Test in the L5178Y TK+/- Mouse Lymphoma forward Mutation Assay with a Confirmatory Assay

GS-7340-02 was evaluated for its ability to induce forward mutations at the tk locus in the mouse lymphoma L5178Y cell up to 5000 µg/mL (4000 µg f.b.e/mL) following exposure times of either 4 hours or 24 hours (m2.6.7, Section 8.11, V990213). All tests were performed either in the presence or in the absence of microsomal enzymes prepared from Aroclor™-induced rat liver. GS-7340-02 was evaluated as negative for inducing forward mutations at the tk locus in L5178Y mouse lymphoma cells under the nonactivating and activating conditions used.

4.3.2. In Vivo

4.3.2.1. Mouse Micronucleus Assay

Crl:CD-1®(CR) BR mice were administered GS-7340-02 to evaluate in vivo clastogenic activity and/or disruption of the mitotic apparatus in the bone marrow (m2.6.7, Section 9.3, M2000113). Five males administered 500 and 1000 mg/kg (400 and 800 mg f.b.e./kg) and 5 animals administered the positive control article were euthanized ~24 hours postdosing and the bone marrow harvested and evaluated for micronuclei. Five males administered 2000 mg/kg and 5 animals administered the vehicle control article were euthanized ~24 and 48 hours postdosing and the bone marrow harvested and evaluated for micronuclei. GS-7340-02 induced no signs of

clinical toxicity. Cytotoxicity to the bone marrow (statistically significant decrease in the polychromatic erythrocyte:normochromatic erythrocyte [PCE:NCE] ratio) was noted at the 2000 mg/kg dose group at the 24- and 48-hour harvest time points. No statistically significant increase in micronucleated PCEs occurred. TAF was negative for genotoxicity in this study. Statistically significant increases were apparent in the positive control animals.

4.4. FTC/TDF

4.4.1. In Vitro

4.4.1.1. Bacterial Reverse Mutation Assay with FTC/TDF

A combination of FTC and TDF was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9 (m2.6.7, Section 8.12, TX-164-2002). The assay was performed in 2 phases using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article. Dimethylsulfoxide was selected as the solvent of choice based on compatibility with the target cells. Dosing solutions of the 2 test article components were prepared in a fixed ratio of 2 parts of FTC to 3 parts of TDF. Each component was dissolved separately in DMSO and then combined. Subsequent dilutions of the combination were prepared by serial dilution in DMSO. The dose levels refer to the test article combination.

The dose levels tested in the initial toxicity-mutation assay were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg per plate. No positive mutagenic responses were observed. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assays, the dose levels tested in the confirmatory mutagenicity assay were 50, 150, 500, 1500, and 5000 µg per plate. In the confirmatory mutagenicity assay, no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Under the conditions of this study, the combination of FTC and TDF were concluded to be negative in the Bacterial Reverse Mutation Assay.

4.4.1.2. In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay) with FTC/TDF

The test article, FTC and TDF at a fixed ratio of 2:3 (FTC/TDF), was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9 (m2.6.7, Section 8.13, TX-164-2003). The preliminary toxicity assay was used to establish the concentration range for the mutagenesis assay. The mutagenesis assay was used to evaluate the mutagenic potential of the test article. Dimethylsulfoxide was selected as the solvent of choice based on solubility information and compatibility with the target cells.

In the preliminary toxicity assay, the maximum concentration of FTC/TDF was 3575 µg/mL. No visible precipitate was present at any concentration in treatment medium; however, substantial toxicity (i.e., suspension growth of ≤ 50% of the solvent control) was observed at ≥ 500 µg/mL with and without S9 activation (4-hour exposure) and at ≥ 150 µg/mL without activation (24-hour exposure). Based on the results of the preliminary toxicity assay, the concentrations

chosen for treatment in the mutagenesis assay ranged from 15 to 1000 µg/mL for the nonactivated cultures and 15 to 1500 µg/mL for the S9-activated cultures. No visible precipitate was present at any concentration in treatment medium. The concentrations chosen for cloning were 15, 50, 100, 150, and 200 µg/mL without activation, and 50, 100, 200, 300, 400, and 500 µg/mL with S9 activation. There was a concentration-related increase in mutant frequency in both the nonactivated and activated assays with a 4-hour exposure (mutant frequencies ≥ 100 mutants per 10^6 clonable cells over that of the solvent control). Toxicity in the cloned cultures (total growth of ≤ 50% of the solvent control) was observed at concentrations ≥ 100 µg/mL without activation and ≥ 300 µg/mL with activation.

The trifluorothymidine-resistant colonies for the cloned cultures and for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. There was an increase in the frequency of small colonies when the treated cultures were compared to the solvent control cultures. An increase in the frequency of small colonies is consistent with damage to multiple loci on chromosome 11 in addition to functional loss of the TK locus. Under the conditions of this study, the test article FTC/TDF was concluded to be positive in the L5178Y/$TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

4.5. FTC/RPV/TAF

FTC, RPV and TAF are considered nongenotoxic agents. The combination of the 3 components is therefore not expected to have an altered genotoxicity profile as compared with that of the individual agents.

5. CARCINOGENICITY

Per separate agreements with the FDA and CHMP, [redacted]. Toxicokinetic data obtained following dosing of TAF to the transgenic mouse and rat clearly demonstrate an inability to measure plasma concentrations of the prodrug, TAF. Summaries of the carcinogenicity studies with TDF are provided below.

5.1. FTC

5.1.1. 2-Year Oral Oncogenicity Study with FTC in CD-1 Mice

This study was conducted to evaluate the oncogenic potential of FTC and to characterize any chronic toxicities in Charles River CD-1 mice following 104 weeks of daily oral gavage administration (m2.6.7, Section 10.1, TOX109). The main study consisted of 3 groups of 60 male and 60 female mice that received the test article at a dose level of 80, 250, or 750 mg/kg/day, and an additional group of 60 male and 60 female mice that served as the control and were administered the vehicle (0.5% aqueous methylcellulose in deionized distilled water). Three additional treatment groups each containing 40 animals per sex per group were designated for toxicokinetic evaluation at dose levels of 80, 250, and 750 mg/kg/day. In addition, a sentinel group (nondosed) containing 25 animals per sex were used to evaluate fecal parasites and serology during the course of the study. The dose volume used in all groups was 10 mL/kg.

All animals were observed at least twice daily (BID) for morbidity, mortality, injury, overt clinical signs, and availability of food and water. Body weight and food consumption measurements were recorded for all animals during the course of the study. Detailed observations for clinical signs were recorded weekly for main study animals during the course of the study. Various hematologic evaluations were conducted at study termination for main study animals. Blood was collected for toxicokinetic evaluations at various time points during Weeks 2, 26, and 104. At study termination, complete necropsy examinations were performed and selected tissues were microscopically examined.

No effect of treatment was seen in any parameter evaluated, including survival, clinical and mass findings, body weight, food consumption, hematology, and macroscopic and microscopic pathology. No treatment-related microscopic changes suggestive of a carcinogenic effect were observed.

In summary, the daily administration of FTC for 2 years to Charles River CD-1 mice at dose levels at 80, 250, and 750 mg/kg/day did not produce any evidence of a carcinogenic effect.

Pharmacokinetic Evaluation

Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring at 0.5 to 1 hour postdose (see m2.6.4, Section 3.3.1.1, TOX-109). $AUC_{0–24h}$ and C_{max} increased proportionally with doses over the range of 80 to 750 mg/kg/day. In general, the

exposures ($AUC_{0–24h}$) in male mice were similar to those in female mice at all doses. $AUC_{0–24h}$ and C_{max} values were higher at Week 26 compared to Week 2. At Week 104, FTC concentrations were below the limit of quantitation in most control animals. The average exposure in mice in this oncogenicity study ranged from 2.6- to 26.3-fold greater (at 80 and 750 mg/kg/day, respectively) than in humans receiving 200 mg/day.

5.1.2. 2-Year Oral Oncogenicity Study with FTC in Rats

This study was conducted to evaluate the oncogenic potential of FTC and to characterize any chronic toxicities in CD (Crl: CD® [SD]IGS BR) rats following 104 weeks of daily oral gavage administration (m2.6.7, Section 10.2, TOX108). The main study consisted of 3 groups of 60 male and 60 female rats that received the test article at dose levels of 60, 200, or 600 mg/kg/day, and an additional group of 60 male and 60 female rats that served as the control and were administered the vehicle (0.5% aqueous methylcellulose in deionized distilled water). Three additional treatment groups each containing 20 animals per sex per group were designated for toxicokinetic evaluation at dose levels of 60, 200, or 600 mg/kg/day. The dose volume was 10 mL/kg.

All animals were observed at least BID for morbidity, mortality, injury, overt clinical signs, and availability of food and water. Body weight and food consumption measurements were recorded for all animals during the course of the study. Detailed observations for clinical signs were recorded weekly for main study animals during the course of the study. Various hematologic evaluations were conducted at study termination for main study animals. Blood was collected for toxicokinetic evaluations at various time points during Weeks 2, 26, and 104. At study termination, complete necropsy examinations were performed and selected tissues were microscopically examined.

No effect of treatment was seen in any parameter evaluated including survival, clinical and mass findings, body weight, food consumption, hematology, and macroscopic and microscopic pathology. No treatment-related microscopic changes suggestive of a carcinogenic effect were observed.

In summary, the daily administration of FTC for 2 years to CD rats at dose levels of 60, 200, and 600 mg/kg/day did not produce any evidence of a carcinogenic effect.

Pharmacokinetic Evaluation

Emtricitabine was rapidly absorbed following all doses, with peak plasma concentrations occurring at 0.5 hours postdose. $AUC_{0–24h}$ and C_{max} results are summarized in m2.6.4, Section 3.3.1.2 (TOX108). $AUC_{0–24h}$ and C_{max} increased with dose over the range of 60 to 600 mg/kg/day. In general, exposures ($AUC_{0–24h}$) in male rats were similar to those in female rats. $AUC_{0–24h}$ and C_{max} values were higher at Week 26 compared to Week 2. The average exposure, based on Week 2 and Week 26 $AUC_{0–24h}$ values, in rats in this oncogenicity study ranged from 3.9- to 31.3-fold greater (at 60 and 600 mg/kg/day, respectively) than in humans receiving 200 mg/day.

5.2. RPV

5.2.1. Rilpivirine: 2-Year Carcinogenicity Study in Mice

TMC278 formulated in 0.5% m/v aqueous HPMC was administered once daily by oral gavage for 24 months (m2.6.7, Sections 10.3, TMC278-NC120). One control and 3 treated groups were given 0 (vehicle), 20, 60 or 160 mg/kg/day at a dose volume of 10 mL/kg. Each group consisted of 60 male and 60 female CD-1 mice (main animals) along with 24 male and 24 female satellite animals for toxicokinetic analysis (18 animals/sex in control group). The intended treatment duration was 104 weeks. Stop-dosing and termination rules communicated and agreed by FDA were applied due to increased mortality rates (Table 26).

Regular observations were made of mortality, clinical signs, palpable masses, body weight, and food consumption. Sampling for hematology was done at the end of the treatment period. A full necropsy was performed on all animals, including those found dead or sacrificed moribund during the study, and all gross lesions were recorded. A selection of organs was weighed. Tissues and organs indicated by protocol were sampled and preserved for histological examination. Samples of any abnormal tissues were also retained and processed for examination. In those cases where a lesion was not clearly delineated, contiguous tissue was fixed with the grossly affected region and sectioned as appropriate. In addition to any masses, the lymph nodes draining the regions adjacent to those masses were also preserved. Blood samples were collected for toxicokinetic analysis on Day 1 and during Week 28.

The trend test for mortality with dose, when all treated groups were included, was statistically significant for male mice. Upon exclusion of the group treated with 160 mg/kg/day, the trend test was still significant. Pair-wise comparisons of the control group with the groups treated with 60 and 160 mg/kg/day were statistically significant. For females, the trend test for mortality with dose was not statistically significant. In male and female mice dosed with 160 mg/kg/day, hepatic tumors caused death more frequently than in males and females of the control group. Treatment-unrelated causes for mortalities included urogenital tract disease; glomerulonephritis and/or chronic nephropathy; malignant lymphoma; and ovarian cyst.

Table 26. Mortality in a Mouse Carcinogenicity Study with TMC278

	0		20		60		160	
Dose (mg/kg/day)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Initial number	60	60	60	60	60	60	60	60
Unscheduled deaths	25	40	35	37	39	30	42	43
Survival (%)	58	33	42	38	35	50	30	28
Week of treatment completed	104	102	104	102	104	102	100[a]	98
Week of termination	104	103	104	103	104	103	104	99

M: males, F: females, [a]: kept off dose until week 104.

There were no relevant changes in clinical signs. TMC278 did not affect the incidence or the time of appearance of palpable masses.

Treatment with TMC278 at doses up to 160 mg/kg/day was associated with a slightly higher body weight gain in all treated males and females at 60 and 160 mg/kg/day compared to control animals. Compared to controls, slightly higher food consumption in animals given 160 mg/kg/day and minimally higher food consumption in females at 60 mg/kg/day was evident.

No relevant changes in hematology were observed.

Gross lesions and organ weights indicated an increase in the incidence of liver masses in all groups treated with TMC278. In males and females treated with 160 mg/kg/day, the incidences of enlarged livers and pale areas in liver were also increased. Absolute and body weight-adjusted liver weight was increased (up to 3-fold) in males and females from all groups treated with TMC278, respectively.

In the liver, a statistically significant dose-related increase in total hepatocellular tumors (adenomas and carcinomas combined) was seen in males dosed with TMC278 from 20 mg/kg/day onwards. The dose-related increase in liver adenomas alone reached statistical significance in males treated at 60 and 160 mg/kg/day. An increase in the males with liver carcinomas that was not statistically significant was seen in all groups treated with TMC278 (see Table 27). The incidences of carcinomas and of adenomas for all groups treated with TMC278 and for the groups dosed with 60 and 160 mg/kg/day, respectively, were above the ranges expected from background data of the testing facility. The incidences increasing over background data are considered treatment related. The incidences of liver adenomas and of adenomas and carcinomas combined were statistically significantly increased and above the background data range in female mice treated with 60 and 160 mg/kg/day (Table 27).

Table 27. Neoplastic Liver Changes in a Mouse Carcinogenicity Study with TMC278

	0		20		60		160	
Dose (mg/kg/day)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals	60	60	60	60	60	60	60	60
Hepatocellular adenomas	8	1	14	0	21	8	19	16
Hepatocellular carcinomas	4	0	7	0	7	2	9	7

M: males, F: females

In addition, other treatment-related liver changes included eosinophilic, basophilic, and clear cell foci; centrilobular and generalized eosinophilic hypertrophy; and brown pigmented macrophages/Kupffer cells. A number of these changes were recorded at a higher incidence in males and females given 160 mg/kg/day and in females dosed with 60 mg/kg/day.

The degree and/or incidence of nephropathy/glomerulonephritis were slightly enhanced in females that received 160 mg/kg/day when compared with control females. These changes accounted for the granular appearance of kidneys noted at necropsy.

It was concluded that oral administration of TMC278 to CD-1 mice at doses of 20, 60, or 160 mg/kg/day produced a dose-related increase in total hepatocellular tumors (adenoma and carcinoma) in males dosed with 20 mg/kg/day and in males and females that received 60 and 160 mg/kg/day. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as C_{max} and AUC values is presented in Table 28 below.

Table 28. Exposure to TMC278 (C_{max} and AUC) in a Mouse Carcinogenicity Study with TMC278

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M	F	M	F
20	Day 1	9.9	13	59	61
	Week 28	9.8	9.9	76	51
60	Day 1	23	24	239	182
	Week 28	22	29	230	278
160	Day 1	41	38	440	345
	Week 28	36	58	505	766

a. $AUC_{inf.}$ on Day 1 otherwise AUC_{0-24h}, M: males, F: females

5.2.2. 2-Year Carcinogenicity Study in Rats

TMC278 formulated in 0.5% m/v aqueous HPMC was administered once daily by oral gavage for 24 months (m2.6.7, Sections 10.4, TMC278-NC123). One control and 4 treated groups were given 0 (vehicle), 40, 200, 500, or 1500 mg/kg/day at a dose volume of 10 mL/kg. Each group consisted of 65 male and 65 female Sprague Dawley rats (main animals) along with 9 male and 9 female satellite animals for toxicokinetic analysis (6 animals/sex in the control group). The intended treatment duration was 104 weeks. Dosing of the females from Group 2 was completed in Week 98. All female groups were terminated in Week 99 due to increased mortality rates according to stop-dosing and termination rules in agreement with the FDA.

Regular observations were made of mortality, clinical signs, body weight, and food consumption. The onset and progression of any palpable masses were evaluated after 6 months of treatment. Ophthalmic examinations were carried out in Weeks 26 and 52, and prior to termination. Samples for clinical laboratory investigations (hematology, coagulation, blood chemistry, and urinalysis) were taken after 12 and 24 months of dosing in the surviving males and females. A full necropsy was performed on all main animals, including those found dead or sacrificed moribund during the study, and all gross lesions were recorded. A selection of organs was weighed. Tissues and organs indicated by protocol were sampled and preserved for histological examination. Samples of any abnormal tissues were also retained and processed for examination. In those cases where a lesion was not clearly delineated, contiguous tissue was fixed with the grossly affected region and sectioned as appropriate. In addition to any masses, the lymph nodes draining the regions adjacent to those masses were also preserved. Blood samples for toxicokinetic analysis were taken on the first day of dosing and after 27 and 39 weeks of dosing.

There was no effect of treatment on mortality (see Table 29). No treatment-related pathologies contributing to death and no adverse clinical signs were observed.

Body weight, food consumption, and food conversion efficiency were not affected by treatment and the ophthalmic examination did not reveal any treatment-related lesions.

The hematological investigations did not indicate any changes in the peripheral blood that were related to the administration of TMC278, in particular there was no evidence of any treatment-related leukemias.

Table 29. Mortality in the Rat Carcinogenicity Study with TMC278

	0		40		200		500		1500	
Dose (mg/kg/day)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Initial number	65	65	65	65	65	65	65	65	65	65
Unscheduled deaths	40	45	40	46	38	35	37	33	44	34
Survival (%)	39	31	39	29	42	46	43	49	32	48
Week of treatment completed	104	99	104	98	104	99	104	99	104	99
Week of termination	104	99	104	99	104	99	104	99	104	99

M: males, F: females

Serum chemistry samples at Week 52 showed increased activities of ALP, ALT, AST, and GGT and increased concentration of creatinine and to a lesser extent of urea in males. Moreover, ALP activity in females and the albumin concentration and A/G ratio in males and females were higher than the respective control values. Serum concentrations of α2-globulin in males; α1-globulin in females; and cholesterol, triglycerides, and β-globulin in males and females were lower that the corresponding control values. The effects generally attained statistical significance at doses of 500 and 1500 mg/kg/day. The effects on creatinine, triglycerides, the globulins, and the A/G ratio showed a statistically significant difference from controls from a dose of 200 mg/kg/day, and above. The lowering of α2-globulin was already statistically significant at a dose of 40 mg/kg/day.

At termination, ALP activity and glucose concentration were elevated in males; potassium in females; and creatinine in males and females. Cholesterol and β-globulin concentrations were reduced in males, as were the triglycerides concentrations in males and females. The effects generally attained statistical significance at doses of 500 and 1500 mg/kg/day. The effect on cholesterol was statistically significant from a dose of 200 mg/kg/day, and above, and the decrease of β-globulin was statistically significantly different from control at a dose of 40 mg/kg/day.

Urinalysis showed lower potassium concentrations in females throughout the study and in males at termination. The effect in females attained statistical significance in the groups given 500 and 1500 mg/kg/day, whereas that in males was statistically significant from a dose of 200 mg/kg/day, and above. Chloride concentration in urine was strongly elevated in a dose-related fashion from 150% of the control value in females that received 200 mg/kg/day up to a more than a 4-fold increase in the males and females dosed with 1500 mg/kg/day. The effect in females was more prominent than in males. Urinary pH was significantly lower in the group given 1500 mg/kg/day (in females throughout the dosing period and in males at termination). Urinary protein was significantly higher in females dosed with 1500 mg/kg/day at Week 52.

At necropsy, white or pale contents of the GI tract were recorded in animals dosed with 200 mg/kg/day and higher doses. This observation is related to the color of the formulation and is associated with the recording of pale feces at clinical observations. A minimal increase in thyroid gland weight was recorded in high dose animals. Increased incidences of masses were noted in the liver of all groups of females dosed with TMC278 and in thyroid gland of males that received TMC278, in a non-dose-related fashion. No effects were noted on organ weights, except for a statistically significant increase of the absolute weight of the thyroid plus the parathyroid glands in males and females dosed with 1500 mg/kg/day.

In liver, an increase in hepatocellular adenomas was seen in males and females given 200, 500, or 1500 mg/kg/day, and also in females given 40 mg/kg/day. There was no apparent dose-related trend and the differences from control values did not reach statistical significance. When compared with the historical background data, the incidence of tumors in females was above the background level in all groups treated with TMC278; whereas in the males, the incidence of tumors was equal to the maximum historical background incidence recorded. These changes are considered to be related to the administration of TMC278. There were no nonneoplastic findings associated with the liver tumors.

In thyroid gland, when compared with the controls, a statistically significant increase of the number of follicular cell adenomas and of adenomas and carcinomas combined was seen in all groups of male and female rats treated with TMC278, with only a marginally apparent dose-related trend. The incidence of follicular cell adenomas was above background levels in the males and females at 200, 500, or 1500 mg/kg/day. The incidence of the carcinomas was above background levels in males only at 200 and 1500 mg/kg/day. These neoplastic changes presented in Table 30 are considered to be treatment related. Nonneoplastic changes related to these tumors included increased incidences of follicular cell hypertrophy and cystic follicular cell hyperplasia in females of all groups treated with TMC278. In males dosed with 200, 500, or 1500 mg/kg/day, the incidence of follicular cell hypertrophy was increased. None of the liver or thyroid gland tumors was considered contributory to death in any rat.

Table 30. Neoplastic Liver and Thyroid gland Changes in a Rat Carcinogenicity Study with TMC278

	0		40		200		500		1500	
Dose (mg/kg/day)	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Initial number	65	65	65	65	65	65	65	65	65	65
Liver										
Hepatocellular adenomas	0	1	1	5	3	5	3	6	3	4
Thyroid gland										
Follicular cell adenoma	1	0	3	0	4	3	5	3	5	4
Follicular cell carcinoma	0	0	0	1	2	1	1	0	2	1

M: males, F: females

Inflammatory exudate in the nasal passages, with associated changes in nasal mucosa (including hyperplasia and/or inflammatory cell infiltration of the respiratory epithelium and respiratory metaplasia of the olfactory epithelium), were seen in males and females given 1500 mg/kg/day. When compared with the controls, there was an increased incidence and/or severity of foamy alveolar macrophages (and associated alveolar epithelial hyperplasia) in the lungs of females given 200, 500, or 1500 mg/kg/day. In the liver, there was an increased incidence of sinusoidal dilatation in females given 1500 mg/kg/day.

It was concluded that the oral administration of TMC278 to Sprague Dawley rats at doses of 40, 200, 500, or 1500 mg/kg/day produced neoplastic changes in liver and thyroid gland. Increased incidences of liver adenomas were seen in males and females given 200 mg/kg/day or above and in females at 40 mg/kg/day without attaining statistical significance. Moreover, a statistically significant increase in follicular cell adenoma and/or carcinoma was seen in the thyroid gland among all groups of males and females treated with TMC278, generally without a dose-related trend. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as C_{max} and AUC values is presented in Table 31 below.

Table 31. Exposure to TMC278 (C_{max} and AUC Values) in a Rat Carcinogenicity Study with TMC278

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC (µg.h/mL)[a]	
		M	F	M	F
40	Day 1	1.6	2.2	19	17
	Week 27	0.85	2.2	4.5	15
	Week 39	0.82	2.1	6.3	14
200	Day 1	2.6	4.2	34	40 [b]
	Week 27	1.2	5.2	11	36
	Week 39	1.3	4.7	8.2	41
500	Day 1	4.5	6.5	45	63
	Week 27	2.0	5.7	14	70
	Week 39	1.8	8.5	14	46
1500	Day 1	6.1	7.0	58	81
	Week 27	2.7	10.	20	81
	Week 39	2.2	9.4	18	84

a. $AUC_{inf.}$ on Day 1 otherwise AUC_{0-24h}, M: males, F: females; [b]: AUC_{0-24h}

5.3. TDF

5.3.1. 104-Week Oral Carcinogenicity Study in Mice with TDF

In a carcinogenicity study of TDF, Swiss Crl:CD1(ICR) BR mice (60/sex/group) received daily doses of 0, 100, 300, or 600 mg/kg/day by oral gavage for 104 weeks (m2.6.7, Section 10.5, M990205).

Treatment-related clinical signs were restricted to salivation. Salivation following dose administration was noted for all animals receiving 300 or 600 mg/kg/day, with a higher incidence being seen in animals receiving 600 mg/kg/day.

A reduced group mean body weight was recorded for animals receiving 300 (4.2% and 0.6% for males and females, respectively) or 600 mg/kg/day (4.6% and 3.3% for males and females, respectively) when compared with the concurrent controls over the 104-week treatment period. The differences from the controls were dosage related and on occasion attained statistical significance. There were no effects on body weight in animals receiving 100 mg/kg/day.

There were no treatment-related effects on food consumption, hematology parameters, or ophthalmoscopic findings.

Treatment-related effects were observed in the bone marrow, duodenum, kidneys, liver, and testes. The results of the histopathology examination revealed a low incidence of treatment-related changes in the duodenum (adenoma/adenocarcinoma) at 600 mg/kg/day. Nonneoplastic lesions were observed in duodenum (epithelial hyperplasia) at 100, 300, and 600 mg/kg/day; in liver (eosinophilic and basophilic cell foci, hepatocellular hypertrophy, nuclear inclusions, cyto/karyomegaly, single cell necrosis, proliferation of sinusoidal cells) and testes (vascular mineralization) at 300 or 600 mg/kg/day; and in kidneys (karyomegaly) and bone marrow (hypercellularity: myeloid) at 600 mg/kg/day. An NOEL for nonneoplastic lesions (by histological criteria) was not established in this study.

Concentrations of TFV in plasma were determined during the study (m2.6.7, Section 10.5, M990205). Blood samples were taken from mice (3 mice/sex/time point) on Day 1 and Day 180 following daily oral dosing of 600 mg/kg/day TDF. Time points included predose; 5 (Day 1 only), 15, and 30 minutes; and 1, 2, 4, 8 (on Day 180, 2 males and 2 females were used for sampling), 12 and 24 hours postdose. Samples were processed for plasma and concentrations of TFV in plasma were determined using a validated liquid chromatography/mass spectrometry (LC/MS) method.

Following the first oral dose in male and female mice, concentrations of TFV reached mean peak values of 11.5 and 17.6 µg/mL, respectively. The corresponding mean calculated AUC_{inf} values in male and female mice were 50.4 and 48.7 µg·h/mL, respectively. The extent of exposure ($AUC_{0–24h}$) of TFV appeared to be similar between the male and female mice on Days 1 and 180. AUC_{inf} on Day 1 appeared to be comparable to $AUC_{0–24h}$ on Day 180 in both the male and female mice, suggesting TFV possesses linear toxicokinetic properties at a dose of 600 mg/kg/day.

In conclusion, lifetime daily administration of TDF at dosage levels of up to 300 mg/kg/day administered for 104 weeks did not reveal any evidence of carcinogenicity in Swiss Crl:CD^R-1(ICR)BR mice. At 600 mg/kg/day, a low incidence of duodenal tumors, possibly treatment-related, was observed.

5.3.2. 104-Week Oral Carcinogenicity Study in Rats with TDF

In a carcinogenicity study of TDF, Sprague-Dawley CD rats (60/sex/group) received daily doses of 0, 30, 100, or 300 mg/kg/day by oral gavage for 103/104 weeks (m2.6.7, Section 10.6, R990204). The high dose animals (300 mg/kg/day) were initially assigned as the low-dose group and received 10 mg/kg/day until Day 10 when the dose was increased to 300 mg/kg/day.

Survival ranged from 27% to 47% at termination, which was within the historical range for the laboratory. Treatment-related clinical signs included salivation in the 100 and 300 mg/kg/day animals. The number of rats with clinically observed masses was similar between control and treated groups. Lower mean body weights were recorded for males (100 and 300 mg/kg/day) and females (300 mg/kg/day) during the first 3 quarters of the study (with differences reaching statistical significance on occasion). Mean food consumption for all treated groups was comparable to controls. There were no changes in hematology (RBC, WBC) at Months 12, 18, or 24.

A dose-related increase in the incidence of renal tubular karyomegaly was observed in treated animals. This finding is consistent with previous observations in repeat-dose studies of TDF in rats.

Once daily oral administration of TDF to rats for 103/104 consecutive weeks did not result in any increase in the incidence of neoplastic lesions in various organ and tissue systems examined. The benign and malignant tumors observed followed no pattern, appeared to be spontaneous in nature, and were within the limits reported for aging CD Sprague-Dawley rats.

Concentrations of TFV in plasma were obtained during the study (m2.6.7, Section 10.6 R990204). Blood samples were collected from rats (3 rats/sex/time point) on Day 1 and Day 180 following daily oral dosing of 30, 100, and 300 mg/kg TDF. Time points included predose and 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose. Samples were processed for plasma and concentrations of TFV in plasma were determined using a validated LC/MS method.

Following the first oral dose in male rats, concentrations of TFV in plasma reached mean peak values of 0.54, 1.31, and 1.99 µg/mL at the 30, 100, and 300 mg/kg/day dose levels, respectively. The corresponding calculated mean AUC_{inf} values in male rats were 2.53, 5.82, and 19.36 µg·h/mL. For female rats, the C_{max} following the first dose was 0.77, 1.42, and 6.61 µg/mL at the 30, 100, and 300 mg/kg/day dose levels, respectively. The corresponding calculated mean AUC_{inf} values in female rats were 2.39, 5.27, and 14.18 µg·h/mL. On Day 180, mean plasma $AUC_{ss(0–24h)}$ at dose levels of 30, 100, and 300 mg/kg/day were 2.70, 6.60, and 16.69 µg·h/mL, respectively, for male rats; and 3.06, 7.46, and 15.72 µg·h/mL, respectively, for female rats. The extent of exposure ($AUC_{0–24h}$) of TFV appeared to be similar between the male and female rats on Days 1 and 180. C_{max} values were generally comparable between males and females. Exposure was linear within each dose level, but did not increase in a dose-proportional fashion when doses were increased from 30 to 300 mg/kg/day.

In conclusion, lifetime daily administration of TDF at dosage levels up to 300 mg/kg/day did not reveal any evidence of carcinogenicity in Sprague-Dawley rats.

5.4. FTC/RPV/TAF

No clinically relevant increases in tumor incidence were found in mice or rats administered FTC in long-term carcinogenicity studies. No drug related increases in tumor incidence were found in mice, except at very high doses or rats administered TDF. Exposure to TFV is higher after TDF than TAF administration, which supports the low carcinogenic potential of TAF. Therefore, FTC and TDF have demonstrated low carcinogenic potential in conventional 2-year bioassays at exposures that exceeded (TDF) or far exceeded (FTC) human exposures at the therapeutic dose (m2.4, Section 4.9.2). Since RPV is not genotoxic in a battery of in vitro and in vivo assays, the neoplastic lesions in liver observed in the mouse and rat carcinogenicity studies are considered a consequence of a nongenotoxic mechanism of action of RPV, rather than an expression of a direct carcinogenic potential of the compound. Combination dosing would not likely change these profiles as no exposure difference would be expected and no exacerbation of toxicity/genotoxicity is expected. Based on the overall lack of genotoxicity and the data from the 2-year carcinogenicity studies with the individual agents, the conduct of carcinogenicity studies with the FTC/RPV/TAF combination are not considered necessary nor would they add to the safety evaluation.

6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

6.1. FTC

6.1.1. Fertility and Early Embryonic Development

6.1.1.1. Oral Fertility and Early Embryonic Development Study of FTC in Mice

Four groups of 20 to 21 male and 20 female mice received vehicle or 250, 500, or 1000 mg/kg/day of FTC by oral gavage (m2.6.7, Section 12.1, TOX036). The dose was administered as 2 equal installments given approximately 6 hours apart. Male mice were dosed 28 days prior to breeding, 21 days during mating, and 21 days postmating for a total of 70 days. Females were dosed 14 days prior to breeding, 21 days during mating, and 7 days during gestation for a total of 42 days. There were no treatment-related deaths and all mice were observed to be normal during all observation periods. Body weight, body weight change, and food consumption were also unaffected by treatment. There was no indication of reproductive toxicity. Mating and fertility indices were unaffected by treatment, as was epididymal sperm number and motility. There were no gross pathological findings at necropsy. Embryonic development was also unaffected by treatment with FTC; there was no effect on the number of ovarian corpora lutea or uterine implantation sites per dam; the number or percent of resorptions and nonlive implants per litter; the number and percent of litters with nonlive implants; the number of live implants per litter; the number or percent of litters with resorptions; or the percent preimplantation or postimplantation loss per litter. In this study, the NOAEL of FTC for male and female mouse systemic, reproductive, and developmental toxicity was considered to exceed 1000 mg/kg/day.

6.1.1.2. Oral Fertility Study of FTC in Male Rats

The potential for adverse effects of orally administered FTC on the reproductive performance of male Sprague-Dawley rats was evaluated (m2.6.7, Section 12.2, TTEP/95/0028). Male rats were administered once daily doses at 0, 150, 750, or 3000 mg/kg/day for 73 days prior to mating and throughout the mating period. Body weight, feed consumption, organ weights, gross lesions, histopathology of reproductive organs, sperm parameters, and fertility were evaluated. Naive females were cohabited with treated males and monitored for evidence of mating. On Gestation Day 13 (GD 13), the females were inspected for pregnancy by cesarean section. The number of viable fetuses, number early and late resorptions, and number of deaths were then quantified. The only treatment-related effect was salivation immediately following dosing at 3000 mg/kg/day. There were no adverse effects on fertility, reproductive performance, sperm count or motility, and there were no pathological changes in the male reproductive organs. Based on these findings, the NOEL was 750 mg/kg/day.

6.1.2. Embryo-Fetal Development

6.1.2.1. Oral Dose Range-Finding Embryo/Fetal Development Study of FTC in Mice

A dose range-finding study was conducted to study the potential maternal and developmental toxicity of FTC in CD-1 mice for the purpose of selecting doses for the definitive study (m2.6.7, Section 11.1, TOX033). On GDs 6 to 15, FTC was orally administered at 0, 250, 500, and 1000 mg/kg/day as a divided dose with 6 hours between doses (0.5% methylcellulose vehicle). On GD 18, laparohysterectomies were performed on all females and standard endpoints were measured. One high-dose female delivered 2 pups of 13 total on GD 18, just prior to the scheduled necropsy. All other animals progressed normally to the GD 18 necropsy. There were no treatment-related internal findings at any dose level, including gravid uterine weight, numbers of fetuses, fetus weights, sex ratios, external developmental malformations and variations, early and late resorptions, total implantations, and corpora lutea numbers. Maternal body weight gain and feed consumption were unaffected at all dose levels. Based upon these results, doses of 250, 500, and 1000 mg/kg/day were selected for the definitive mouse teratology study.

6.1.2.2. Oral Embryo/Fetal Development Study of FTC in Mice

The potential for FTC to cause developmental and maternal toxicity was evaluated in this GLP-compliant study (m2.6.7, Section 13.1, TOX037). Groups of 25 pregnant mice were orally administered FTC suspended in 0.5% aqueous methylcellulose or vehicle alone, given BID as a divided dose with 6 hours between doses, from GDs 6 through 15. Doses tested were 0, 250, 500, and 1000 mg/kg/day (5 mL/kg/dose; 10 mL/kg/day). Body weights and feed consumption were recorded at the specified intervals. On GD 18, laparohysterectomies were performed on all females and data for maternal and fetal indices collected. Dams in satellite groups for each dose level were treated similarly, except that on GD 15 following a single dose of 250, 500, or 1000 mg/kg, they were exsanguinated at 0.5 hour postdose and plasma was harvested for toxicokinetic evaluations. All maternal animals survived to the necropsy on GD 18; there were no treatment-related maternal findings at that time. Feed and body weight data were all normal. Intrauterine growth and survival of fetuses were not affected by FTC at any dose level, and there were no external, visceral, or skeletal malformations or developmental variations observed at any dose. Toxicokinetic evaluations demonstrated dose proportional exposures to the drug with mean plasma values at 0.5 hour postdose of 52 ± 15, 88 ± 14, and 186 ± 14 µg/mL for the low-, mid-, and high-dose groups, respectively. Based on the results of this study, a dose level of 1000 mg/kg/day was considered to be the NOAEL for maternal and developmental toxicity of FTC when administered BID to mice.

6.1.2.3. Oral Dose Range-Finding Embryo/Fetal Development Study of FTC in Rabbits

A GLP-compliant dose range-finding study of TP-0006 (FTC) for a developmental toxicity study was conducted in New Zealand White rabbits (m2.6.7, Section 11.1, TOX034). Groups of 5 female artificially inseminated rabbits were dosed with FTC in 0.5% methylcellulose at 0, 250, 500, and 1000 mg/kg/day, administered in two equally divided doses with 6 hours between doses (GDs 7 to 19; GD 0 = day of insemination). Standard data parameters (body weights, feed consumption) were collected throughout the study. On GD 29, a laparohysterectomy was

performed and the uteri and their contents examined. All females survived to the scheduled necropsy with no evidence of toxic or pharmacologic effects. Mean maternal body weight gain and feed consumption were normal. Growth and survival of the conceptuses were also normal. There were no external malformations or variations observed in fetuses at any dose level.

6.1.2.4. Oral Embryo/Fetal Development Study of FTC in Rabbits

The potential for FTC to cause developmental and maternal toxicity were evaluated in rabbits (m2.6.7, Section 13.2, TOX038). Groups of 20 artificially inseminated rabbits were orally dosed with FTC in 0.5% aqueous methylcellulose from GDs 7 through 19. Dose levels were 0, 100, 300, and 1000 mg/kg/day. Dosing occurred BID, with approximately 6 hours between doses, at a dose volume of 5 mL/kg/dose (10 mL/kg/day). Clinical observations for signs of toxicity, body weights, and feed consumption were recorded. On GD 29, a laparohysterectomy was performed on all rabbits and the uteri and ovaries examined. Standard fetal evaluations were made to evaluate any effects on external, visceral, or skeletal development. Plasma concentrations of drug in maternal and fetal circulation was evaluated in a satellite group of rabbits (n = 5/group) on GDs 19 (maternal exposure) and 20 (fetal/maternal exposure). There were no test article-related deaths or abortions. The only test article-related clinical observation was decreased defecation in the high-dose group. Body weight gain and feed consumption were reduced in the 300 and 1000 mg/kg/day groups during GDs 11 to 15 (high dose only), 15 to 18, and/or 18 to 20. Intrauterine growth and survival were unaffected by FTC, and there were no treatment-related developmental malformations or variations in fetuses at any dose.

Toxicokinetic evaluation demonstrated that FTC was rapidly absorbed with C_{max} occurring generally within 1 hour postdose. AUC and C_{max} increased linearly with dose. Plasma elimination $t_{1/2}$ was 3 to 4 hours at all dose levels. Mean maternal exposures ($AUC_{0–24h}$) on GD 19 were 87, 315, 1258 µg.h/mL for the low-, mid-, and high-dose groups, respectively. Fetal/maternal exposure ratios were around 0.4 to 0.5 at 1 hour after dosing (at T_{max}) for all dose levels.

Based on the results of this study, a dose level of 100 mg/kg/day was considered to be the NOAEL for maternal toxicity and a dose level of 1000 mg/kg/day was considered the NOAEL for developmental toxicity of FTC when administered BID to rabbits.

6.1.3. Prenatal and Postnatal Development Including Maternal Function

6.1.3.1. Oral Pre- and Postnatal Development Study of FTC in CD-1® Mice

Groups of 25 pregnant female CD-1 mice were administered FTC by oral gavage at 0, 250, 500, and 1000 mg/kg/day (5 mL/kg/dose; 10 mL/kg/day) (m2.6.7, Section 14.1, TOX039). Doses were administered to F_0 dams BID, with approximately 6 hours between equally divided doses, from GD 6 through PND 20. The F_0 dams raised their pups through weaning at PND 21, at which time the F_1 weanlings were allowed to mature to at least 10 weeks of age. During maturation, attainment of sexual development (preputial separation and vaginal patency) and neurodevelopmental milestones (motor skills, learning, and memory) were monitored. Immediately prior to mating, estrous cyclicity of F_1 females was evaluated. F_1 animals were then cohabited within dose groups (sibling matings avoided) and the resulting F_2 litters were evaluated and the pups sacrificed.

There was no treatment-related toxicity observed in the F_0 dams during gestation or lactation, including mortality, body weight changes, or reduced feed consumed. The F_1 litters were normal at all doses tested with respect to number of pups, pup weights, and sex ratios. Maturation of F_1 pups through weaning proceeded normally. Attainment of neurodevelopmental and sexual development milestones were all normal. Body weights of F_1 animals during the postweaning maturation phase were normal across all groups. F_1 dams in the 1000-mg/kg/day group had slightly longer estrous cycles (0.64 days) than controls. Survival, body weights, and sex ratios of F_2 litters were normal across all groups. Necropsy of F_1 animals yielded no treatment-related findings. In summary, the NOAEL for reproductive toxicity in the mouse under the conditions of this study was found to be > 1000 mg/kg/day.

Exposure following oral administration of FTC to pregnant mouse dams and the exposure of murine fetuses to FTC was examined in a separate study (m2.6.4, Section 4.3.1.1, TOX103). On GD 15 following approximately 10 days administration of 500 mg/kg of FTC BID, pregnant mice and their viable fetuses had measurable concentrations of FTC at 1 hour following administration of the first 500-mg/kg dose. The mean (± SD) plasma concentration in the pregnant mice was 137.1 ± 28.0 µg/mL. The mean concentration of FTC in pooled fetal homogenate was 55.7 ± 10.4 µg/g. The mean fetal/maternal concentration ratio was 0.41 ± 0.04.

6.2. RPV

6.2.1. Female Rat Fertility Study and Early Embryonic Development Study

TMC278 suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage, to groups of 24 female Sprague Dawley rats at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (m2.6.7, Section 12.4, TMC278-NC125). Treatment started 2 weeks prior to mating, during mating, and until Day 7 of pregnancy. The females were paired 1:1 for mating with untreated males. Successful mating was established on the basis of sperm detection in vaginal smears. Regular observations were made for clinical signs, morbidity, and mortality. Female body weights were determined weekly until pregnancy, and on pregnancy Days 0 (day of sperm detection), 8, and 14. Food consumption was determined weekly during the premating period and pregnancy. Estrous cycle was determined on vaginal smears taken daily during the mating period and the precoital interval was calculated. The females were sacrificed on Day 14 of pregnancy for evaluation of pregnancy status and determination of copulation and fertility rates (e.g., number of corpora lutea, implantations, and live fetuses).

No mortalities were noted. Moreover, there were no relevant clinical signs and no relevant effects on body weight, food consumption, gross pathology, estrous cycle, mating or pregnancy rate, the number of corpora lutea, implantations or live fetuses, or early embryonic development indices. It was concluded that there was no effect on female fertility, fecundity, or early embryonic development up to 400 mg/kg.

Therefore, the NOAEL for female fertility, fecundity, and early embryonic development was at least 400 mg/kg/day.

6.2.2. Male Rat Fertility Study

TMC278 suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage, to groups of 25 male Sprague Dawley rats at 0 (vehicle), 100, 400, or 1600 mg/kg/day in a dose volume of 10 mL/kg (m2.6.7, Section 12.3, TMC278-NC124). Treatment started 10 weeks prior to mating, during mating, and for 3 to 4 weeks after the mating period. The males were paired 1:1 for mating with untreated females. Successful mating was established on the basis of sperm detection in vaginal lavage. Regular observations were made for clinical signs, morbidity, and mortality. Male body weight was recorded weekly throughout the dosing period and food consumption during the premating period. At necropsy of the males, 3 to 4 weeks after the end of the mating period, gross lesions were recorded. The testes and epididymides were removed, weighed, and examined histopathologically. One of the cauda epididymides from each male was sampled for sperm motility, concentration, and morphology. The liver and thyroid gland were weighed and fixated.

There were no mortalities associated with TMC278. All pairs except one (male dosed with 100 mg/kg/day) mated within 4 days after pairing. There were no relevant clinical signs and no relevant effects on body weight, food consumption, gross or histopathological lesions, weights of epididymides or testes; and no adverse effects on the motility, concentration, or morphology of the sperm. There was no effect on fertility up to 1600 mg/kg/day. Weights of liver and thyroid gland showed a dose-related increase (absolute and/or relative to body weight) in all groups receiving TMC278.

The NOAEL for male fertility is at least 1600 mg/kg/day.

6.2.3. Embryo-Fetal Development Studies in Rats (with TMC278 Base)

The doses for the rat embryo-fetal development study were selected on the basis of the results of a pilot study (m2.6.7, Section 11.2, TMC278-NC127) in which no significant effects occurred. TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, from GDs 6 to 17 to groups of 30 (6 satellite animals for toxicokinetic sampling) pregnant Sprague Dawley rats at 0 (vehicle, 24 animals), 40, 120, or 400 mg/kg/day at a dose volume of 10 mL/kg (m2.6.7, Section 13.3, TMC278-NC105). Regular observations were made for clinical signs, morbidity, mortality, body weight, and food intake. Blood samples from satellite animals (3 time points over a 24-hour period from 3 females per group) were collected on GDs 6 and 16. The satellite animals were killed after the last sampling. The dams from the main study were sacrificed on GD 21 and gross lesions and the weight of thyroid gland were recorded. Pregnancy status and the numbers of corpora lutea, implantations, resorptions, and live and dead fetuses were noted. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no mortalities associated with TMC278. There were no relevant clinical signs. Reduced body weight gain (maximally 7%) and food consumption (maximally 4%) were noted in dams given 120 and 400 mg/kg/day. Weight of thyroid gland showed a dose-related increase (maximally 24%), which was statistically significant in the animals dosed with 120 and 400 mg/kg/day. There were no effects on gross pathology or pregnancy parameters; numbers of corpora lutea; implantations; early and late resorptions; live or dead fetuses; or on the pre- or postimplantation losses. There were also no effects on fetal body weight or sex ratio.

Visceral examinations showed a slight increase in dilated renal pelvis in 5 out of 149 and 7 out of 149 fetuses from the groups treated with 120 and 400 mg/kg/day, respectively. The dilated renal pelvis is considered a minor variation. Historical control incidences of dilated renal pelvis from negative control animals in 17 embryo-fetal development studies in rats, conducted at the Janssen Research and Development site in Beerse from [redacted] up to [redacted] are available ([redacted]. Tabulated HCD incidences (rat) of dilated renal pelvis from reproductive studies with study start dates between [redacted] and [redacted].
Janssen Research and Development, a division of Janssen Pharmaceutica NV, Beerse, Belgium. [redacted]). The rat embryo-fetal development study with RPV (TMC278-NC105) was conducted at this site in [redacted]. The average incidence of dilated renal pelvis in the historical studies was 30/2447 (1.2%) fetuses, with a standard deviation (SD) of 2.0%. The maximum incidence encountered in a single study was 11/155 (7.1%). The incidence of dilated renal pelvis at the low dose in the rat embryo-fetal development study with RPV was 2/155 (1.3%). This incidence falls well within the historical control range (0%-7.1%). For this reason, it is concluded that the incidence at the low dose in the rat embryo-fetal development study is not toxicologically relevant. The incidences of dilated renal pelvis at the mid and high dose of RPV showed a statistically significant increase when compared with control animals within the study. The incidences (5/149 [3.4%] and 7/149 [4.7%], respectively) were well below the maximum value of the historical control range (7.1%). Maximally 2 fetuses per litter presented dilated renal pelvis in these groups. The mechanism of action underlying the observed dilated renal pelvis might be related to the specific development of the rat kidney during late gestation, when it undergoes marked changes in size, weight, and histological structure. While the renal papilla is slowly and steadily increasing in length, the renal parenchyma is rapidly and almost exponentially increasing in weight. The disparity in growth rates frequently results in a kidney that has an enlarged renal pelvis, in late gestation. This is called an “apparent hydronephrosis” {17315}. “True hydronephrosis,” which can be encountered in all species including humans, is usually persistent when caused by partial urinary tract obstruction or chronic overfilling of the urinary tract. In contrast, the “apparent hydronephrosis” is a normal aspect of renal development in rats, disappears shortly after birth, and does not impair normal development of rat pups. For these reasons it is categorized as a minor variation. Therefore, the incidence of dilated renal pelvis or “apparent hydronephrosis” which is well below the maximum value of the historical control range, in the mid- and high-dose groups, is not toxicologically relevant.

The occurrences of the 2 malformations in 2 different litters of dams treated with 400 mg/kg/day noted upon external examinations were isolated and dissimilar in nature, and bore no relationship with treatment. In view of the isolated nature and the possible occurrence within a normal control population, these were not considered to be test article related.

The maternal and embryo fetal NOAEL was 40 mg/kg/day based on the changes on the body weight, food consumption, and the increase of dilated renal pelvis seen at higher doses. Systemic exposure expressed as C_{max} and AUC values are given in Table 32 below.

Table 32. Exposure to TMC278 (C_{max} and AUC) in an Oral Embryo-Fetal Developmental Study with TMC278 Base in Rats

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
		F (n = 6)	
40	GD 6	4.9	33
	GD 16	5.6	37
120	GD 6	6.0	65
	GD 16	7.2	63
400	GD 6	14	182
	GD 16	13	152

F: females

6.2.4. Embryo-Fetal Development Studies in Rabbits (with TMC278 Base)

The doses for the main rabbit embryo-fetal development study were selected on the basis of the results of 2 pilot studies. In the first study (m2.6.7, Section 11.2, TMC278-NC128), 75 and 150 mg of TMC278 base/kg/day from GDs 6 to 19 incurred complete resorption of litters. In the second study (m2.6.7, Section 11.2, TMC278-NC129), animals were dosed with 0 (0.5% aqueous HPMC), 5, 20, or 60 mg of TMC278 base/kg/day from GDs 6 to 19. The dose of 60 mg/kg/day resulted in reduced fetal survival and postimplantation loss without external fetal abnormalities.

In the main study, TMC278 base suspended in aqueous 0.5% (m/v) HPMC was administered once daily, by oral gavage, from GDs 6 to 19 to groups of 20 pregnant New Zealand white rabbits at 0 (vehicle), 5, 10, or 20 mg/kg/day in a dose volume of 5 mL/kg (m2.6.7, Section 13.4, TMC278-NC130). Blood samples for toxicokinetic analysis were collected from 3 animals per group on GDs 6 and 19 at 7 time points during the 24 hours after dosing. Clinical signs, body weight, and food consumption performance were recorded. The females were killed on GD 28 and a necropsy was performed. The females were examined for gross lesions, pregnancy status, the numbers of corpora lutea of pregnancy, implantations, resorptions, and live and dead fetuses. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no mortalities. There were no relevant clinical signs, no effects on body weight or food consumption, and no gross lesions at necropsy. Pregnancy parameters as assessed by the number of corpora lutea of pregnancy, implantations, resorptions, live and dead fetuses, or pre- or postimplantation loss were unaffected. Moreover, there were no effects on mean fetal body weight or on the sex ratio. There were no relevant changes at external, visceral, or skeletal fetal examination, except for a slight increase in numbers of fetuses exhibiting branches of the left subclavian artery originating from the aorta and hypoplastic interparietal bone which reached statistical significance in the group receiving 20 mg/kg/day. Since no clear dose-related increase in incidence of hypoplastic interparietal bones was observed, the latter finding was considered of minor toxicological importance. The aortic and pulmonary trunks and their respective branching are particularly ‘plastic’ in the fetus and are susceptible to modification, especially in the rabbit. As these changes are commonly seen in rabbits, they are considered to have little or no biological significance.

The maternal NOAEL was at least 20 mg/kg/day and the fetal NOAEL was 10 mg/kg/day. Systemic exposure expressed as C_{max} and AUC values are given in Table 33 below.

Table 33. Exposure to TMC278 (C_{max} and AUC) in an Oral Embryo-Fetal Development Study with TMC278 Base in Rabbits

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
		F (n = 3)	
5	GD 6	6.4	95
	GD 19	6.7	105
10	GD 6	9.7	162
	GD 19	10	170
20	GD 6	13	219
	GD 19	15	232

F: females

6.2.5. Dose Range Finding Peri- and Postnatal Development Study Including Juvenile Exposure in Rats with TMC278

The objectives of this pilot study were to investigate the tolerability of doses applied in the general and reproduction toxicity studies on pre- and postnatal development parameters (m2.6.7, Section 14.2, TMC278-NC168). Moreover, the effects of direct administration of TMC278 to pups for 2 weeks were evaluated in terms of exposure and postnatal development.

TMC278 suspended in 0.5% m/v aqueous HPMC was administered once daily by oral gavage to groups of 6 time-mated female Sprague Dawley rats from GD 6 to LD 7 (day of delivery is LD 0) at 0 (vehicle, 2 groups), 40, 120, and 400 mg/kg/day in a dose volume of 10 mL/kg. Regular observations were made for maternal clinical signs, body weight, and food consumption. The females were allowed to litter. Observations on nesting and nursing behavior were made. On LD 7, 8 male and 8 female pups of each group were selected for oral dosing by gavage with the same dose and at the same dose volume as their mothers. The selected pups from the second vehicle group were dosed with 400 mg/kg/day. Blood samples were taken from 12 nonselected male and 12 nonselected female pups of each group on LD 7. These pups were killed after the sampling. The selected pups were dosed from LD 12 up to and including LD 25. They were observed regularly for clinical signs, morbidity, and mortality, and their body weight was recorded. Blood samples for toxicokinetics were taken on LD 25. Necropsy was performed after the last sampling.

There were no mortalities among the dams. Moreover, the dams showed no abnormal clinical signs or effects on body weight or food consumption. There were no changes on pregnancy or litter parameters. Two pups from the group treated with 400 mg/kg/day were killed for humane reasons. There were no effects on the pups treated directly with TMC278 during the dosing period or at necropsy. Systemic exposure for pups expressed as C_{max} and AUC values are given in Table 34 below.

Table 34. Exposure to TMC278 (C_{max} and AUC) in Rat Pups via Lactation (LD7) and after 2 Weeks of Oral Dosing with TMC278

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		$AUC_{0–24h}$ (µg.h/mL)	
		M	F	M	F
40	LD7 [a]	0.05	0.07	0.62	0.74
	LD25 [b]	2.6	5.8	12	18
120	LD7	0.08	0.08	0.94	0.91
	LD25	3.7	3.6	34	28
400	LD7	0.16	0.14	1.9	1.8
	LD25	9.1	7.3	50	53
400[c]	LD25	6.4	4.9	43	39

M: males, F: females, LD: lactation day,
a. pups from dams dosed as indicated exposed in utero and via lactation (n = 12/sex),
b. pups from dams as indicated exposed in utero and via lactation and dosed by gavage from LD12 to LD25 (n = 8/sex),
c. pups from control dams dosed by gavage from LD12 – LD25 (n = 8/sex)

6.2.6. Rilpivirine: Peri- and Postnatal Developmental Toxicity Study in Rats with TMC278

TMC278 suspended in 0.5% (m/v) aqueous HPMC was administered once daily by oral gavage to groups of 25 time-mated female Sprague Dawley rats from GD6 to LD 20 at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (m2.6.7, Section 14.3, TMC278-NC131). Regular observations were made for maternal clinical signs, morbidity, and mortality. Weekly body weight and food consumption were determined. The females were allowed to litter. Litter size and sex ratio were determined. Pups weight was determined regularly. Developmental landmarks, including number of ears open, static righting reflex, eyes open, startle response, and pupillary light reflex were recorded on LDs 3, 5, 15, and 21. At necropsy of the dams after weaning of their litters, the number of implantation scars in uterus was determined. From the F_1 generation, 20 males and 20 females per group were kept and their growth, postweaning development, behavior, and reproductive performance were assessed.

There were no mortalities among the dams. The dams showed no relevant clinical signs and no effects on body weight, food consumption, gestation, or parturition. There were no effects on the numbers of pups born, the sex ratio, pup survival, or physical condition. There were no effects on pre- or postweaning body weight and gain, or development and behavior parameters. Sexual development, fertility, mating performance, and pregnancy parameters of the F_1 generation were unaffected by treatment of the F_0 generation. There were no effects on postmortem parameters at necropsy of the F_0 and F_1 generation.

The NOAEL for both the F_0 and F_1 generation was at least 400 mg/kg/day.

6.3. TAF

6.3.1. Fertility and Early Embryonic Development

6.3.1.1. Oral Fertility and General Reproduction Toxicity Study of TAF in Sprague-Dawley Rats

The purpose of this study was to evaluate effects of GS-7340-03 on mating and fertility when administered daily via oral gavage to male and female rats prior to mating and until termination (males) or through early gestation (females) (m2.6.7, Section 12.5, TX-120-2012). This included assessment of potential effects on gonadal function, mating behavior, implantation, female estrous cyclicity and general fertility of male and female rats. Male and female Crl:CD(SD) rats were assigned to 4 groups (22 animals/sex/group). Each group received dose preparations containing the vehicle control article or GS-7340-03 at 20, 80, or 160 mg f.b.e./kg/day (22, 90, 180 mg GS-7340-03/kg/day) at a dose volume of 10 mL/kg. Males were necropsied after at least 10 weeks of dosing, the reproductive organs were weighed followed by assessment of sperm motility and total concentration. Male and female reproductive performance was evaluated based on results of confirmation of mating and pregnancy.

No TAF-related mortality occurred. No adverse change in clinical condition was attributed to TAF. For males, dose-dependent decreased mean body weight was noted at 80 and 160 mg/kg/day throughout the study. At 160 mg/kg/day mean body weights were significantly less than the control group on Premating (PM) Day 28, Pairing Days 3, 7, and 10, and on postpairing (PP) Day 0 through the end of the study when the 160-mg/kg group mean body weight was 12% less than the control group. At 160 mg/kg/day, mean female body weight on PM Day 17 was slightly but significantly less (6%) than the control group. Mean gestational body weight gain was significantly reduced at 160 mg/kg/day between GD 0-7 (19%). Mean food consumption was significantly reduced in the male 160 mg/kg/day group during much of the premating phase. Female food consumption was slightly reduced premating at 80 and 160 mg/kg/day and significantly during gestation at 160 mg/kg/day.

There were no differences in premating estrous cycles. There were no test article-related differences in male or female reproductive parameters. Mating/fecundity/fertility indices were 100/95/95, 95/100/95, 91/95/86, and 95/95/91 in the control, 20, 80, and 160 mg/kg/day group males, respectively and 100/95/95, 100/100/100, 100/95/95, and 100/95/95 in the control, 20, 80, and 160 mg/kg/day group females. There were no test article-related effects on the uterine and fetal parameters and no significant differences in female reproductive organ weights.

In males, there was a slight increase in absolute testis weight (significant increase (9%) in the adjusted mean of the left testis only) at 160 mg/kg/day. This was considered test article-related but not adverse, as there were no other reproductive organ weight or functional reproductive effects. There were no test article-related effects observed on mean epididymal sperm motility or on sperm concentration. Mean sperm motility in the control, 20, 80, and 160 mg/kg/day groups was 91.8%, 95.2%, 95.5%, and 94.0%, respectively. Mean sperm concentration in the control, 20, 80, and 160 mg/kg/day groups was 942.2, 833.2, 966.1, and 928.5 millions/gram of caudal epididymal weight, respectively.

In conclusion, TAF was administered by oral gavage to male and female rats at 20, 80, or 160 mg f.b.e./kg/day prior to mating and until termination (males) or through early gestation (females). The TAF NOAEL for male and female toxicity was 80 mg/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day (the highest dose administered).

6.3.2. Embryo-Fetal Development

6.3.2.1. Oral Range Finding Embryo-Fetal Development Study of TAF in Rats

The purpose of this study was to provide information for selection of oral dosages to be used in a subsequent embryo-fetal development study in female rats and to provide a preliminary evaluation of the effects of GS-7340-02 on pregnancy and embryo-fetal development (m2.6.7, Section 11.3, TX-120-2001). In addition, the toxicokinetic characteristics of GS-7340-02 in pregnant rats were determined.

Sixty-two time-mated female Crl:CD(SD) rats were assigned to 4 study groups (n = 11, 17, 17, and 17) and received daily doses of either the vehicle control or 5, 100, or 200 mg/kg GS-7340-02 in the vehicle (4, 80, 160 mg f.b.e./kg/day). The dose volume was 5 mL/kg. Animals were dosed on Days 6 to 17 of gestation, inclusive.

All animals survived to scheduled necropsy, and there were no TAF-related clinical signs of toxicity. At 200 mg/kg/day, body weight gains were significantly lower between GD 8 and 10 when compared to controls, with marked differences also seen from GD10 to 12 and 16 to 18. Corrected body weights (body weight gains minus gravid uterus weights) at 200 mg/kg/day on GD 21 were 5% lower than controls, and mean body weight gain was 18% lower from GD 6 to 18, compared to the control group.

Food consumption was slightly decreased (-10%) in animals administered 200 mg/kg/day, during the treatment period. There were no maternal macroscopic findings attributed to TAF. There were no TAF-related effects noted on the number of corpora lutea, implantation sites, sex ratio, incidence of live/dead fetuses, resorptions, preimplantation and postimplantation losses, or gravid uterus weights.

The mean fetal weight was statistically significantly lower (-7.7%) in the 200 mg/kg/day group, compared to the control group. However, as these fetal weights were within historical control range, this was considered to be non-adverse. There were no major fetal malformations or minor anomalies noted.

Following oral gavage, toxicokinetic parameters for TAF could not be calculated as most concentration values of TAF were below the lower limit of quantitation (< 10.0 ng/mL). TAF was extensively converted to TFV. Concentrations of TFV readily appeared in plasma. Exposure to TFV increased with the increase in dose level from 5 to 200 mg/kg. The increases in TFV C_{max} and AUC_{0-t} were generally greater than dose proportional between the 5 to 200 mg/kg/day dose levels, with the exception of the increase in C_{max} from 5 to 200 mg/kg/day dose level on Day 17. Accumulation of TFV was observed after multiple dosing of TAF in pregnant rats.

In conclusion, treatment of female rats by oral gavage during GD 6 to 17, inclusive, with TAF at dose levels of 5, 100, and 200 mg/kg/day resulted in decreased body weight gains (-18%) and food consumption (-10%) at 200 mg/kg/day. Based on these results, the TAF NOAEL for maternal toxicity was considered to be 100 mg/kg/day and the TAF NOAEL for embryo-fetal development was 200 mg/kg/day.

Table 35. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rats on Gestation Days (GD) 6 and 17

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} µg·h/mL	C_{max} (µg/mL)	AUC_{0-t} µg·h/mL
5	6	0.109	0.17	NC	NC
	17	0.218	0.41	NC	NC
100	6	4.36	12.90	0.209	NC
	17	5.71	20.6	0.773	NC
200	6	6.94	26.9	0.215	NC
	17	5.92	35.7	0.397	0.649

NC: not calculated due to insufficient data

6.3.2.2. Oral Embryo-Fetal Development Study of TAF in Rats

The purpose of this study was to provide an evaluation of the effects of GS-7340-02 on pregnancy and embryo-fetal development (m2.6.7, Section 13.5, TX-120-2002). This study evaluated ICH Harmonised Tripartite Guideline stages C to D of the reproductive process. In addition, the toxicokinetic characteristics of GS-7340-02 in pregnant rats were determined.

Four groups of 25 pregnant female Crl:CD(SD) rats were administered TAF once daily, by oral gavage, from GD 6 to 17, inclusive. Targeted dose levels were 0 (vehicle control), 25, 100 and 250 mg/kg/day (targeted formulation concentrations of 0, 5, 20 and 50 mg/mL, respectively) and the dose volume was 5 mL/kg. A satellite group of pregnant animals (3, 9, 9, and 9 respectively) were dosed and used for toxicokinetic sampling.

Dose formulation analysis showed that each 5 mg/kg/day animal was administered a GS-7340-02 concentration of 3.85 mg/mL instead of 5 mg/mL for 5 to 8 days between GD 10 and 17, providing a daily dose of 19.3 mg/kg/day (77% of targeted dose) on these days. Additionally dose formulation analysis showed that each 20 mg/kg/day animal was administered a GS-7340-02 concentration of 12.9 mg/mL instead of 20 mg/mL for 4 to 7 days between GD 6 and 12, providing a daily dose of 64.6 mg/kg/day (65% of targeted dose) on these days.

The only potentially TAF-related clinical sign was transient salivation, which was noted in 7 of 25 animals in the 250 mg/kg/day group. For the 250 mg/kg/day group body weights, body weight gains and food consumption were significantly decreased during the treatment period. On GD 21, the mean body weight of the 250 mg/kg/day group was 10 % lower than that of the control group. Mean corrected body weights (body weight on GD 21 minus gravid uterus

weight) and mean corrected body weight gains (body weight gain on GD 6 to 21 minus gravid uterus weight) were also lower in animals administered 250 mg/kg/day, with the corrected mean body weights also 10% lower than controls on GD 21.

No TAF-related gross findings were noted. No effects were observed on the total number of corpora lutea, implantation sites, male fetuses, female fetuses, live fetuses, dead fetuses, or resorptions. Pre and post implantation loss was considered comparable to controls.

Fetal weights (males, females and sexes combined) were decreased in a dose dependent manner and remained within the range of historical control data, however fetal weights at 250 mg/kg/day were at the lower extreme of this range. The incidences of fetal major malformations, minor external, visceral and skeletal anomalies and were not affected by GS-7340-02. Sternebrae variants (1 to 4 and 5 and 6) were increased at 250 mg/kg/day.

Plasma concentrations of TAF were all below the lower limit of quantitation at a dose level 25 mg/kg/day. Exposure to TAF increased with the increase in TAF dose level from 25 to 250 mg/kg/day. Exposure to TFV increased with the increase in TAF dose level from 25 to 250 mg/kg/day. No accumulation of TFV was observed after multiple dosing of GS-7340-02 in rats. GS-7340 was extensively converted to TFV in rats following oral administration of GS-7340-02.

In conclusion, administration of TAF by once daily oral gavage in rats at levels of 25 (mean overall achieved dose 22 mg/kg/day), 100 (mean overall achieved dose of 84 mg/kg/day), and 250 mg/kg/day resulted in decreased body weights and food consumption at 250 mg/kg/day. At 250 mg/kg/day, there was decreased fetal body weight associated with some minor transitory delays in the rate of ossification. There was no evidence of embryolethality or teratogenicity attributed to TAF in this study. Based on these results, the maternal TAF NOAEL and the TAF NOAEL for embryo-fetal development were both considered to be the mid dose level, which resulted in GD17 AUC_{0-t} values of 17.4 and 0.2 µg·hr/mL for TFV and TAF, respectively.

Table 36. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rats on Gestation Days (GD) 6 and 17

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
25	6	1.11	2.72	NC	NC
	17	0.870	2.80	NC	NC
100	6	2.77	9.45	0.038	NC
	17	4.13	17.4	0.149	0.242
250	6	9.86	54.2	0.756	1.38
	17	7.35	55.7	0.597	1.38

NC: not calculated due to insufficient data

6.3.2.3. Oral Range Finding Embryo-Fetal Development Study of TAF in Rabbits

The objectives of this study were to provide information for selection of dosages to be used in subsequent embryo-fetal development studies in rabbits and to provide a preliminary evaluation of the effects of GS-7340-02 on pregnancy and embryo-fetal development (m2.6.7, Section 11.3, TX-120-2004). In addition, the toxicokinetic characteristics of TAF were determined. Thirty timed-mated New Zealand White rabbits were assigned to 5 groups (6 animals/group) and were dosed once daily, by oral gavage, from Day 7 to 20 of gestation. Dose levels were 0 (vehicle control), or GS-7340-02 at dose levels of 5, 25, 50, or 100 mg/kg/day at a dose volume of 10 mL/kg.

All animals survived to their scheduled termination. Decreased fecal output was noted on a few occasions throughout the study in all groups. At 100 mg/kg/day, 4 of 6 animals were noted as being thin. At 100 mg/kg/day, a slight mean (2.5%) weight loss was observed between GD 7 to 21. A decrease (8.5%) compared to controls for corrected mean body weight (body weight from GD 7 to 29 minus gravid uterus weight) was noted at 100 mg/kg/day. Decreased food intake was noted at 100 mg/kg/day during the treatment period which persisted to termination.

There were no TAF-related gross pathology findings for the dams. The pregnancy rate was 100% in all groups. The numbers of corpora lutea, implantation sites, live and dead fetuses and resorptions, the sex ratio, and the preimplantation and postimplantation losses were unaffected by TAF administration. The fetal weights were comparable to controls at each dose level. There were no major external malformations or minor external anomalies attributed to the test article administration.

Exposure to TAF and TFV increased with the increase in dose level from 5 to 100 mg/kg/day. Between the 5 to 100 mg/kg/day dose levels, the increases in C_{max} and AUC_{0-t} were greater than dose proportional for TAF and were approximately proportional for TFV. No accumulation of TFV was observed. TAF was extensively converted to TFV in rabbits following oral administration of GS-7340-02.

In conclusion, administration of TAF to pregnant rabbits by oral gavage at dose levels of 5, 25, 50 and 100 mg/kg/day on GD 7 to 20, inclusive, resulted in maternal effects evidenced by slightly decreased body weights and food intake at 100 mg/kg/day. There was no evidence of embryolethality, fetotoxicity or teratogenicity at any dose level. Based on this study, the TAF NOEL for maternal toxicity was considered to be 50 mg/kg/day (AUC_{0-t} = 11.8 and 5.7 μg hr/mL for TFV and TAF, respectively) and the TAF NOEL for embryo-fetal development was 100 mg/kg/day (AUC_{0-t} = 23.5 and 10.7 μg·hr/mL for TFV and TAF, respectively).

Table 37. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rabbits on Gestation Days (GD) 7 and 20

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
5	7	0.227	1.23	0.009	NC
	20	0.187	1.56	0.109	0.173
25	7	0.922	5.23	0.104	0.125
	20	0.907	6.01	1.01	1.14
50	7	1.93	8.85	0.349	0.390
	20	1.95	11.8	4.81	5.66
100	7	3.92	20.9	2.04	2.15
	20	3.09	23.5	8.88	10.7

NC= not calculated due to insufficient data

6.3.2.4. Oral Embryo-Fetal Development Study of TAF in Rabbits

The objective of this study was to detect potential adverse effects of GS-7340-02 on pregnant New Zealand White female rabbits and development of the embryo and fetus from implantation to closure of the hard palate, GD 7 to 20 (m2.6.7, Section 13.6, TX-120-2005). This study evaluated ICH Harmonised Tripartite Guideline stages C to D of the reproductive process. In addition, the toxicokinetic characteristics of GS-7340-02 were determined. GS-7430-02 was administered by oral gavage to time-mated F_0 generation female rabbits (20 main study females per group and 3 toxicokinetic females per group) at 0 (vehicle control), 10, 30 and 100 mg/kg/day in a dose volume in a dose volume of 10 mL/kg.

There was no mortality during the study. An increased incidence of decreased fecal output was noted at 100 mg/kg/day, compared to the vehicle control group and was associated with decreased food intake. Yellow staining of the fur was also observed more often in animals administered 30 and 100 mg/kg/day.

Lower body weight gains were noted at 100 mg/kg/day for the first week following treatment initiation. Lower food intake was noted at 100 mg/kg/day from GD 8 to 24. Three animals at this dose consumed less than 30 g for at least 4 days during the dosing period. There were no TAF-related macroscopic changes. The number of corpora lutea, implantation sites, live fetuses, dead fetuses, resorptions, the sex ratio and the pre and post implantation losses were unaffected by treatment with TAF. There was no effect of TAF upon fetal weights. The incidence of major malformations, minor external, visceral, skeletal anomalies and common skeletal variants were unaffected by TAF.

Exposure to TAF increased with the increase in GS-7340-02 dose level from 10 to 100 mg/kg/day. The increases in C_{max} were greater than proportional between the 10 to 100 mg/kg/day dose levels and the increases in AUC_{0-t} were greater than proportional between the 30 to 100 mg/kg/day dose level on GD 20.

Exposure to TFV increased with the increase in TAF dose level from 10 to 100 mg/kg/day. The increases in C_{max} and AUC_{0-t} were roughly proportional between the 10 to 100 mg/kg/day dose levels. Accumulation of TFV was observed after multiple dosing of TAF in rabbits. Concentrations of TFV were much higher than concentrations of TAF, therefore indicating that TAF was extensively converted to TFV in rabbits following oral administration of TAF.

In conclusion, TAF was administered to pregnant rabbits by daily oral gavage at dose levels of 0 (vehicle control), 10, 30 and 100 mg/kg/day on GD 7 to 20, inclusive. Treatment resulted in reduced maternal body weights and food consumption at 100 mg/kg/day compared to the control group. There was no evidence of embryolethality, fetotoxicity, or teratogenicity at any dose level.

The TAF NOAEL for maternal toxicity was 30 mg/kg/day (AUC_{0-t} = 1.1 and 5.0 µg·h/mL for TAF and TFV, respectively) and the TAF NOEL for embryo-fetal development was 100 mg/kg/day (AUC_{0-t} = 11.0 and 27.3 µg·h/mL for TAF and TFV, respectively), the highest dose tested.

Table 38. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rabbits on Gestation Days (GD) 7 and 20

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
10	7	0.261	1.94	0.008	NC
	20	0.260	2.02	0.155	NC
30	7	0.398	3.01	0.077	NC
	20	0.676	5.01	0.937	1.14
100	7	2.19	13.4	0.846	0.918
	20	2.97	27.3	9.19	11.0

NC= not calculated due to insufficient data

6.3.3. Prenatal and Postnatal Development

Per separate agreements with the FDA and CHMP, [redacted] As with the carcinogenicity study rationale, there is an inability to measure plasma concentrations of TAF in rats, and TFV exposure after TAF administration was less than that already tested in the TDF peri/postnatal study. The peri/postnatal studies with TDF clearly characterized the potential postnatal toxicity of TFV.The peri/postnatal study conducted with TDF is summarized below.

6.3.3.1. Oral Developmental and Perinatal/Postnatal Reproduction Toxicity Study of TDF in Rats, Including a Postnatal Behavioral/Functional Evaluation

One hundred twenty-five presumed pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats were randomly assigned to 5 dose groups, 25 rats per group (F_0 generation rats; m2.6.7, Section 14.4, R990202). An additional 12 rats were assigned to each of Groups II through V for satellite toxicokinetic evaluations. Suspensions of the test article, TDF (GS-4331-05), or the suspension vehicle were administered orally once daily to these naturally-bred female rats from GD 7 through LD 20, or GD 24 (rats that did not deliver a litter), at dosages of 0 (vehicle), 50, 150, 450, and 600 mg/kg/day. The dosage volume was 10 mL/kg, adjusted daily on the basis of the individual body weights recorded immediately before administration. Rats were observed for viability at least twice each day of the study. Observations for clinical signs of effects of the test article, abortions, premature deliveries, and deaths were made daily before dosage and approximately 60 ± 10 minutes after dosage administration. Body weights were recorded GD 0, daily during the dosage period, and at sacrifice. Feed consumption values were recorded on GDs 0, 7, 10, 12, 15, 18, 20, and 25 (if necessary) and LDs 1, 4, 7, 10, and 14. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, and pup viability at birth. Maternal behavior was evaluated on LDs 1, 4, 7, 14, and 21. On GD 7 and LD 20, blood samples were collected predose, at 15 and 40 minutes, and at 1, 2, 4, 6, 8, 12, and 24 hours postdose. Milk samples were collected from rats assigned to the satellite portion of the study and from 1 vehicle control group rat approximately 1 hour postdose on LD 11. After completion of the 21-day postpartum period, female rats were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Kidneys were excised, weighed (paired weight), and retained for possible histologic examination. The number and distribution of implantation sites was recorded. Gross lesions were retained for possible future evaluation.

Each F_1 generation litter was evaluated for viability at least twice each day. The pups in each litter were counted once each day. Clinical observations were recorded once daily during the preweaning period. Pup body weights were recorded on LDs 1, 4, 7, 14, and 21. On LD 21, all pups not selected for continued evaluation were sacrificed and examined for gross lesions. The following tissues were excised and retained for possible histologic evaluation: kidneys (paired weight recorded); sternum (with costochondral junction); and femur. Necropsy of the pups included a single cut at the suture of the frontal and parietal bones of the skull, and the cross-sectioned brain was examined for hydrocephaly. Tissues with gross lesions were retained for possible future evaluation.

All F_1 generation male and female rats were observed for viability at least twice each day of the study. These rats were also examined for clinical observations and general appearance once weekly during the postweaning period. Body weights for male rats were recorded weekly during the postweaning period and at sacrifice. Body weights for female rats were recorded weekly during the postweaning period and on GDs 0, 7, 10, 14, 17, and 20. Feed consumption values for male rats were recorded weekly, except during cohabitation. Feed consumption values for female rats were recorded weekly during the postweaning period, except during cohabitation and on GDs 0, 7, 10, 14, 17, and 20.

Female rats were evaluated for the age of vaginal patency, beginning on Day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on Day 39 postpartum. Beginning at 24 ± 1 day postpartum, 1 male rat and 1 female rat from each litter, where possible, were evaluated in a passive avoidance test for learning, short-term retention, and long-term retention. Beginning at approximately 70-days postpartum, 1 male rat and 1 female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory. At approximately 90 days of age, the F_1 generation rats were assigned to cohabitation.

Male rats were sacrificed after completion of the 21-day cohabitation period. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Testes and epididymides of male rats were excised and paired organ weights were recorded. The epididymides were retained in neutral buffered 10% formalin. The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin. Female rats were sacrificed on GD 20, cesarean-sectioned, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The rats were examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. Each fetus was weighed and examined for sex and gross external alterations. All fetuses were retained in alcohol.

No deaths related to TDF occurred in the F_0 generation rats. One rat in the 150 mg/kg/day dosage group was found dead. One rat in each of the 0 (vehicle), 450, and 600 mg/kg/day dosage groups were moribund sacrificed. All of these deaths were considered related to intubation errors, with the exception of the vehicle control group rat, which appeared to have dystocia. All other rats survived until scheduled sacrifice. Clinical observations of red perioral substance and excess salivation occurred in an increased or statistically significantly ($p \leq 0.05$ to $p \leq 0.01$) increased number of rats in the 150, 450, and 600 mg/kg/day dosage groups during the gestation period. Excess salivation and perioral substance were not considered related to treatment and probably did not reflect systemic toxicity of the test article. Test article-related clinical observations during gestation and urine-stained abdominal fur was observed in statistically significantly ($p \leq 0.05$ to $p \leq 0.01$) increased numbers of rats in the 450 and 600 mg/kg/day dosage groups, and ungroomed coat occurred in a statistically significantly ($p \leq 0.01$) increased number of rats in the 600 mg/kg/day dosage group. Two rats in each of the 450 and 600 mg/kg/day dosage groups had soft or liquid feces during gestation. Observations of urine-stained abdominal fur, red perioral substance, and ungroomed coat continued in statistically significantly increased numbers of rats in the 450 and 600 mg/kg/day dosage groups during the lactation period. Additionally, dehydration and emaciation occurred in increased or statistically significantly increased numbers of rats in the 450 and 600 mg/kg/day dosage groups, and cold to touch occurred in a significant number of 600 mg/kg/day dosage group rats during lactation. Soft or liquid feces occurred in 4 rats in each of the 450 and 600 mg/kg/day dosage groups during lactation. All other clinical observations were considered unrelated to the test article.

All necropsy observations were considered unrelated to administration of the test article. Terminal body weights, paired kidney weights, and the ratios of the kidney weights to the terminal body weights were comparable among the 5 dosage groups and did not statistically significantly differ. Body weight gains for the entire gestation dosage period (GDs 7 to 20) and the entire gestation period (GDs 0 to 20) were statistically significantly reduced in the 150, 450,

and 600 mg/kg/day dosage groups. Gestation body weights were statistically significantly reduced in the 150 mg/kg/day dosage group on GDs 19 and 20; in the 450 mg/kg/day dosage group on GDs 16 through 20; and in the 600 mg/kg/day dosage group on GDs 10 through 20. During the lactation period, maternal body weight gains tended to be increased over or comparable to the control group values in the 150, 450, and 600 mg/kg/day dosage groups and were statistically significantly increased in the 450 and 600 mg/kg/day dosage groups for the entire lactation period (LDs 1 to 21). Reflecting the reductions in body weight gain that occurred during the gestation period, lactation body weights continued to be statistically significantly reduced in the 450 mg/kg/day dosage group on LDs 1 through 6 and 8 and in the 600 mg/kg/day dosage group on LDs 1 through 5 and 7 through 9. Absolute (g/day) and relative (g/kg/day) feed consumption values during gestation were statistically significantly reduced in the 450 and 600 mg/kg/day dosage groups for the entire gestation dosage period (GDs 7 to 20) and the entire gestation period (GDs 0 to 20). During lactation, significant reductions in absolute and relative feed consumption values occurred in the 450 (absolute only) and 600 mg/kg/day dosage groups for the entire tabulated lactation period (LDs 1 to 14). Peripartum/postpartum pup mortality and stillbirths were increased in the 450 and 600 mg/kg/day dosage groups, and there was a concomitant significant reduction in the percentage of live born pups in the 600 mg/kg/day dosage group. The percentages of pups found dead or presumed cannibalized were increased or statistically significantly increased on LDs 1 and 2 to 4 in the 450 and 600 mg/kg/day dosage groups; and a significant number of dams had no surviving pups on LDs 2, 3, or 4 in these 2 dosage groups (5 and 10, respectively). Additionally, one dam in the 600 mg/kg/day dosage group had no surviving pups on LD 5. As a result of these pup deaths, the Viability Index was reduced in the 450 mg/kg/day dosage group and statistically significantly reduced in the 600 mg/kg/day dosage group. A significant number of pups were found dead or presumed cannibalized on LDs 8 to 14 in the 450 mg/kg/day dosage group and there was a corresponding slight reduction in the Lactation Index. Pup weights were statistically significantly reduced on LDs 1, 4, 7, 14, and 21 in the 450 and 600 mg/kg/day dosage groups. Increased or statistically significant increased numbers (compared to the control group values) of F_1 generation litters had pups that were cold to touch, pale, and had umbilical hernias in the 450 and 600 mg/kg/day dosage groups. A statistically significant increased number of litters had dams and pups that were not nesting in the 600 mg/kg/day dosage group. An increased number of pups that were found dead and necropsied in the 450 and 600 mg/kg/day dosage groups had no milk in the stomach. Weights of the kidneys were statistically significantly reduced in the 450 and 600 mg/kg/day dosage groups, reflecting the statistically significantly reduced average body weights in these groups on LD 21. Ratios of the kidney weights to the terminal body weights were comparable among the 5 dosage groups. All other natural delivery and litter observations were unaffected by dosages of the test article as high as 600 mg/kg/day.

Of the F_1 generation rats, 1 male rat in the 450-mg/kg/day group and 2 male rats in the 600 mg/kg/day dosage group, respectively, were found dead. One male rat in the 600 mg/kg/day dosage group was moribund sacrificed. Two female rats in each of the 450 and 600 mg/kg/day dosage groups were found dead. One female rat in the 450 mg/kg/day dosage group was moribund sacrificed. The F_1 male death in the 150 mg/kg/day dosage group was not considered related to maternal test-article exposure. The deaths in the 450 and 600 mg/kg/day dosage groups were considered related to the test article because: (1) they occurred in F_1 generation rats at maternally toxic dosages of 450 and 600 mg/kg/day; and (2) the incidences were

dosage-dependent. These rats are discussed below. All other F_1 generation rats survived until scheduled sacrifice. There were no adverse clinical observations in the F_1 generation male and female rats that were considered to be test article related. Necropsy observations of empty stomach, small and large intestines distended with gas, dark red area on the glandular mucosal surface of the stomach, and tan spleen were considered to be associated with dead or moribund sacrificed F_1 generation male and female rats. Dosage-dependent reduced to statistically significantly reduced terminal body weights of the F_1 generation male rats were observed at all dosage levels. Paired testes weights were reduced and statistically significantly reduced in the 450 and 600 mg/kg/day dosage groups, and the paired epididymides weight was statistically significantly reduced for the 600 mg/kg/day dosage group. Paired testes weights were also reduced, although not statistically significantly reduced in the 50 and 150 mg/kg/day dosage groups. The result was statistically significantly increased epididymides to body weight ratio for the 150, 450, and 600 mg/kg/day dosage groups. The significant reduction in the weight of the epididymides in the 600 mg/kg/day dosage group was not considered biologically important because the mating and fertility of the male rats in this dosage group were unaffected. Reflecting the reductions in pup body weights during the preweaning period, body weights and body weight gain of the F_1 generation rats in all treated groups were reduced or statistically significantly reduced during the postweaning period (Days 1 and 71 postweaning). Gestation body weights for the F_1 generation female rats were statistically significantly reduced in all treated groups. Gestation body weight gains were comparable for the 5 groups of the F_1 generation female rats. Absolute feed consumption values during the postweaning period for the F_1 generation males were statistically significantly reduced in all treated groups. Absolute feed consumption values during the postweaning period for the F_1 generation females were statistically significantly reduced over the postweaning period Days 8 to 15 by maternal dosages of 600 mg/kg/day of the test article. Absolute feed consumption values during the postweaning period for the F_1 generation females were comparable for all 5 groups, with the exception noted above. Relative feed consumption values during the postweaning period for the F_1 generation females statistically significantly increased for the 50 mg/kg/day dosage group and above from Days 1 to 71 postweaning. These changes were not considered biologically significant for the 50 mg/kg/day dosage group. The average day on which preputial separation was evident in male rats was statistically significantly increased and was dosage-dependent for F_1 generation male rats whose dams were administered dosages of the test article of 450 and 600 mg/kg/day. The time to reach vaginal patency in female rats was statistically significantly prolonged (450 mg/kg/day group) or prolonged (600 mg/kg/day group) for F_1 generation females whose dams were administered 450 or 600 mg/kg/day, respectively.

In the passive avoidance paradigm, F_1 male and female rats the 450 mg/kg/day dosage group had first trial latencies that were statistically significant increased. This effect was not dosage-dependent, probably because of the few animals tested in the 600 mg/kg/day dosage group. No other biologically important differences occurred for short- or long-term memory or learning in this paradigm. There were no biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F_1 generation male or female rats, as evaluated by performance in a water maze swim task. The biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F_1 generation males and females from the 450 and 600 mg/kg/day groups were considered improved performance for the passive avoidance paradigm compared to

the vehicle group. Mating performance of the F_1 generation male and female rats was unaffected by administration of the test article to the F_0 generation dams at dosages as high as 600 mg/kg/day. Cesarean-sectioning and litter parameters in the F_1 generation female rats were unaffected by administration of dosages of the test article as high as 600 mg/kg/day to the F_0 generation dams.

Tenofovir concentrations were determined in plasma and milk from pregnant female rats administered TDF by oral gavage for approximately 27 days at 0, 50, 150, 450, or 600 mg/kg/day. Blood samples were taken twice, once on Day 7 of presumed gestation (the first day of dosing) and once on Day 20 postpartum (the last day of dosing). Milk samples were collected at approximately 1 hour postdose on Day 11 postpartum.

Results for Day 7 median TFV plasma concentration verses time profiles showed rapid absorption of TFV followed by biexponential elimination for all dose groups. Plasma pharmacokinetic T_{max} values varied from 0.25 to 0.67 hours across the dose range. Median C_{max} and AUC_{inf} values showed a general increasing trend with dose that was not proportional, but this may be misleading due to the high intersubject variability and the short sampling time (12 to 24 hours).

Results for Day 20 median TFV plasma concentration verses time profiles showed rapid absorption of TFV followed by biexponential elimination for all dose groups. The 600-mg/kg/day dose group showed an almost linear decline from 0.67 to 12 hours. T_{max} values varied from 0.67 to 1.0 hour across the dose range.

Comparison of single dose Day 7 data with steady state Day 20 data revealed overall similar T_{max} and apparent terminal $t_{1/2}$ values. Also, both showed generally increasing values with dose for C_{max} and AUC and some apparent accumulation with multiple dosing, although this evaluation is confounded by the high variability and the limited data from the highest dose groups. Results for Day 11 median TFV concentrations from rat milk were 154, 249, 532, and 730 ng/mL for the 50-, 150-, 450-, and 600-mg/kg/day dose groups, respectively; representing 13.5%, 11.0%, 20.8%, and 23.5% of the TFV plasma concentration at similar times. Although there is a trend toward increasing percent in milk with increasing dose, the data is weak due to the comparison of data from different rats and the different numbers of observations between plasma data (n = 3 to 4) and milk (n = 6 to 11).

Therefore, all rats in dose groups that received TDF demonstrated exposure based on the measurable levels of TFV in plasma on Days 7 and 20. Both Day 7 and Day 20 postpartum pharmacokinetic rat plasma profiles demonstrated increased exposure of TFV with increasing oral doses of TDF. Day 11 postpartum data confirmed the presence of TFV in rat milk, with the results suggesting that TFV was not concentrated in milk.

Table 39. Mean Toxicokinetic Parameters of TFV in Rats on Gestation Day (GD) 7 and Lactation Day (LD) 20

TDF (mg/kg/day)	Day	TFV	
		C_{max} (µg/mL)	$AUC_{0-\infty}$ (µg·h/mL)
50	GD7	1.27	3.81
	LD20	1.46	5.88
150	GD7	2.53	7.84
	LD20	2.26	11.7
450	GD7	3.43	54.4
	LD20	4.93	26.8
600	GD7	4.71	29.0
	LD20	2.89	27.2

In conclusion, the maternal NOEL of TDF was 50 mg/kg/day. The 150, 450, and 600 mg/kg/day dosages caused adverse clinical observations, reductions in body weight gain during gestation, and increases in body weight gain during lactation; and the 450 and 600 mg/kg/day dosages reduced gestation and lactation feed consumption values. The developmental NOEL was 150 mg/kg/day. The 450 and 600 mg/kg/day dosages resulted in increased peri/postpartum pup mortality, reduced pup survival, and reduced pup body weights. The NOEL for general toxicity of TDF in the F_1 generation was 50 mg/kg/day (mortality occurred or body weights and/or feed consumption were reduced in the 150-, 450-, and 600-mg/kg/day dosage groups). The F_1 generation male and female NOEL for behavioral, reproductive, and developmental toxicity of TDF was 150 mg/kg/day (effects on sexual maturation were observed at dosages of 450 and/or 600 mg/kg/day).

6.4. FTC/RPV/TAF

The reproductive toxicity studies did not demonstrate any effects on male or female fertility, or fecundity, from rats treated with FTC, RPV or TAF prior to and during pregnancy. The results of the embryo-fetal developmental studies in rats and rabbits with FTC, RPV and TAF demonstrated the absence of a potential for teratogenicity at high exposures to the rabbit dams. The exposure to the rat dams to RPV elicited effects at the level of the thyroid gland, which were also found in general toxicity studies. The developmental toxicity studies did not demonstrate any effects on maternal behavior after parturition and during weaning, or development of offspring from dams administered RPV during pregnancy and lactation. In dams administered FTC, slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses of TDF.

In RPV toxicity studies with juvenile rats or with dogs and cynomolgus monkeys completed prior to the onset of adolescence/adulthood, no additional targets were noted and no increased sensitivity to the effects found in adult animals was evident.

With no expected toxicologic interactions with the FTC/RPV/TAF combination, further studies with FTC/RPV/TAF combination are not considered necessary. No cause for concern has been identified and studies with the FTC/RPV/TAF combination are unlikely to show new effects.

7. LOCAL TOLERANCE

7.1. FTC

No specific local tolerance studies have been conducted with FTC. In repeat dose oral toxicity studies, there were no FTC-related changes in the GI tract of mice, rats and monkeys.

7.2. RPV

7.2.1. Eye Irritation – Bovine Corneal Opacity-Permeability Assay

The ocular irritation potential of TMC278 was assessed in the bovine corneal opacity permeability (BCOP) assay. TMC278 was applied for 4 hours as a 20% w/w suspension in saline to the corneal epithelium (m2.6.7, Section 16.1, TMC278-NC202).

An increase in corneal opacity was noted, but no effect on permeability occurred. The score of 32.5 ± 3.0 classified TMC278 as a moderate eye irritant.

7.2.2. Skin Irritation – In Vivo

The skin irritation potential of TMC278 was evaluated in 3 New Zealand white rabbits. TMC278 (0.5 g moistened with 0.5 mL water on a gauze) was applied to 4 × 4 cm on the intact clipped skin for 4 hours covered by a semi-occlusive dressing. The skin reactions were scored 1, 24, 48 and 72 hours after removal of the dressing (m2.6.7, Section 16.1, TMC278-NC159).

All animals survived and there were no noteworthy clinical observations or effects on body weight. No skin reactions, staining of skin, or corrosive effects were observed. TMC278 was classified as “nonirritant” to rabbit skin.

7.2.3. Phototoxicity – Balb/c Mouse 3T3 Fibroblast Assay

The in vitro Balb/c mouse 3T3 fibroblast assay was used to assess the phototoxic and cytotoxic potential of TMC278 base. Cells were exposed to TMC278 base dissolved in DMSO in bath concentrations ranging from 0.001 to 2.16 mg/L, in the presence and the absence of UVA radiation. The final concentration of DMSO in Dulbecco’s phosphate-buffered saline was 1% (m2.6.7, Section 16.1, TMC278-NC188).

As the highest concentration had no effect on the Neutral Red uptake by these cells in the presence of UVA, TMC278 was considered to be nonphototoxic.

7.3. TAF

7.3.1. Ocular Irritation Study in Bovine Corneas

The Bovine Corneal Opacity and Permeability Assay (BCOP) was performed to assess the ocular irritancy potential of GS-7340-03 in vitro (m2.6.7, Section 16.2, TX-120-2013). Imidazole was tested in parallel as a positive control. The assay uses isolated bovine corneas as a means of

assessing the ocular corrosivity or severe irritancy potential of test substances in vitro. The isolated corneas were obtained as a by-product of the meat production industry. Two endpoints, corneal opacity and permeability, were measured and combined to give an In Vitro Irritancy Score which can be used to classify and rank test substances as potential eye irritants according to OECD guideline 437. TAF elicited an In Vitro Irritancy Score of 21.0 ± 8.7 with a 4-hour incubation and was predicted to be a noncorrosive/nonsevere eye irritant.

7.3.2. Dermal Irritation Study in Rabbits

Three female New Zealand White rabbits received a single 4 hour, semi-occlusive, dermal administration of approximately 0.5 g of GS-7340-03 as supplied moistened with water and were observed for 4 days (m2.6.7, Section 16.2, TX-120-2011). No local dermal reaction was observed in any animal throughout the duration of the study. The Primary Irritation Index was calculated to be 0.0; TAF was classified as a 'non-irritant'.

7.3.3. Phototoxicity Assessment

Phototoxicity studies are not considered necessary for TAF. The ultraviolet absorption spectrum was obtained with a TAF fumarate solution in 20 mM potassium phosphate buffer at pH 6.0. The absorption maxima (λ_{max}) were observed at 205 and 262 nm and the measured molar extinction coefficient at the characteristic peak at 262nm was $1.5 \times 10^4\ M^{-1}cm^{-1}$. Because TAF fumarate does not absorb light within the range of natural sunlight, no phototoxicity evaluation is deemed appropriate.

7.4. FTC/RPV/TAF

The FTC/RPV/TAF tablet is intended for oral use. No local tolerance studies were conducted for the FTC/RPV/TAF combination. Evaluation of local tolerance to the GI tract has been conducted during repeat-dose oral toxicity studies with each of the individual agents. No GI toxicity was observed in the FTC, RPV, or TAF toxicity studies. Clinical trials indicate that the individual agents, FTC/RPV/TDF, other FTC/TDF-containing regimens, and FTC/TAF-containing regimens are generally well tolerated; the incidences of GI disorders were consistent with those expected in the subject population and the known safety profiles of the study drugs. The potential for additive effects between the three agents in the FTC/RPV/TAF FDC is minimal.

8. OTHER TOXICITY STUDIES

8.1. Antigenicity

8.1.1. FTC

In view of the absence of indications from repeat-dose toxicity studies (see Section 3) that FTC might elicit allergic reactions, no specific antigenicity studies have been conducted.

8.1.2. RPV: Hypersensitivity – In Vivo

A murine local lymph node assay was conducted to establish the sensitization potential of TMC278 (m2.6.7, Section 17.2, TMC278-NC199). The compound was formulated in an acetone:olive oil (4:1) mixture at 5%, 10%, and 35%. Twenty-five microliters of each concentration was applied for 3 consecutive days on the dorsal surface of both ears of 4 female CBA/Ca/Ola/Hsd mice per group. The negative control group received the vehicle. The positive control group was treated with 2.5% streptozocin. Three days later, all animals received an intravenous injection of [^{3}H]methyl thymidine. Five hours later they were killed and the radioactivity in the auricular lymph nodes was measured.

The test to control ratio of radioactivity from thymidine incorporated in the draining lymph node ranged from 0.6 to 1.7, indicating that TMC278 was unlikely to cause delayed-type sensitization.

In view of the absence of indications from repeat dose toxicity studies (see Section 3) that TMC278 might elicit allergic reactions, no specific antigenicity studies have been conducted.

8.1.3. TAF: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)

Three groups, each comprising 5 female mice were administered GS-7340-03 at concentrations of 10, 25 or 50% w/v (m2.6.7, Section 17.3, TX-120-2014). Similarly constituted groups received the vehicle (acetone: olive oil (4:1 v/v)) or positive control substance (25% v/v hexyl cinnamic aldehyde). The mice were administered TAF by daily application of 25 µL of the appropriate concentration or control (vehicle or positive), to the dorsal surface of both ears for 3 consecutive days. The proliferative response of the lymph node cells (LNCs) from the draining auricular lymph nodes was assessed 5 days following the initial application, by measurement of the incorporation of ^{3}H-methyl thymidine (^{3}HTdR) by β-scintillation counting of LNC suspensions. The response was expressed as radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of ^{3}HTdR incorporation into LNC of test nodes relative to that recorded for control nodes (test/control ratio), termed as SI. The test substance is regarded as a sensitizer if at least one concentration of the chemical has a SI of 3 or more.

The SI obtained for 10%, 25%, and 50% w/v were 0.9, 1.0, and 1.0, respectively, which indicates that TAF did not show the potential to induce skin sensitization. The EC3 value (the “estimated concentration of 3” is the concentration of test substance which would result in a SI of 3 in the LLNA) was determined to be higher than 50% w/v. The SI for the positive control substance hexyl cinnamic aldehyde was 6.3, which demonstrates the validity of this study.

8.1.4. FTC/RPV/TAF

Studies with RPV and TAF have not indicated that antigenicity is of concern. No studies were deemed necessary with the FTC/RPV/TAF combination.

8.2. Immunotoxicity

8.2.1. FTC: TP-0006 – 28 Day Oral (Gavage) Immunotoxicity Study in Rats

The immunotoxicity of FTC was evaluated in rats administered once daily oral doses of FTC for 28 consecutive days (m2.6.7, Section 17.1, TOX146). Emtricitabine was suspended in 0.5% methylcellulose and administered by gavage to groups of 5 male and 5 female Sprague-Dawley rats at 0, 60, 240, and 1000 mg/kg/day. Following the final dose, each rat was administered an intravenous dose of 2 × 108 sheep RBCs. Five days later, blood samples were collected and the serum was analyzed by ELISA to detect IgM antibodies specific to sheep RBCs. There were no adverse effects of FTC during the dose period. Body weights were unaffected by treatment with FTC. Emtricitabine did not affect the titers to sheep RBCs at any of the doses administered. It was concluded that 1000 mg/kg/day FTC was a no-effect dose for immunotoxic potential.

8.2.2. FTC/RPV/TAF

Data from repeat-dose toxicity studies with FTC, RPV or TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) and specialized immunotoxicity studies with FTC do not suggest immunotoxic potential for FTC, RPV or TAF. The lack of effects on the PFC-assay in the 1-month rat study (TMC278-TOX5692; see Section 3.2.5) indicated that RPV had no immunotoxic potential.There were no notable effects of the combination of FTC/TDF on immunophenotyping or natural killer cell assay values in a 4-week dog study (TX-164-2004, see Section 3.4.2). No further studies were deemed necessary with the FTC/TAF combination.

8.3. Mechanistic Studies

8.3.1. FTC

For FTC, specific endpoints were included in repeat dose toxicity studies in mice and cynomolgus monkeys to evaluate the potential for mitochondrial toxicity.

8.3.2. RPV

8.3.2.1. Studies to Evaluate Endocrine Effects

8.3.2.1.1. Inhibition of ACTH-stimulated Cortisol Biosynthesis in Primary Cultures of Guinea Pig Adrenal Cells

TMC278 was assessed for its ability to inhibit cortisol biosynthesis in primary cultures of guinea pig adrenal cells (m2.6.7, Section 17.2, TMC278-Exp5653). The effects of TMC278 base dissolved in DMSO (final bath concentration 1%) in concentrations ranging from 1 to 10 µM upon the cortisol synthesis stimulated by $ACTH_{1-24}$ were determined in 2 independent repeats. Etomidate served as positive control.

TMC278 inhibited cortisol synthesis in one experiment with a median inhibitory concentration (IC_{50}) of 6 µM, but showed no significant inhibition in the second experiment. Etomidate rendered an IC_{50} value of 0.5 nM.

8.3.2.1.2. Evaluation of the Cortisol Biosynthesis in Crude Subcellular Fractions of Dog Adrenal Cortex

The effect of TMC278 base on adrenal corticosteroid biosynthesis was studied in vitro using cell free adrenal gland homogenate (E-fraction) from beagle dog (m2.6.7, Section 17.2, TMC278-FK4790). Nominal concentrations of TMC278 ranged from 0.03 to 75 µM (0.011 to 27.75 µg/mL). Etomidate (0.28 µM) served as positive control.

TMC278 at a concentration of 75 µM (27.75 µg/mL) caused 39% inhibition of the metabolism of pregnenolone compared to control. A concentration-dependent increase in progesterone and 17α-hydroxyprogesterone concentrations was noted concomitant with decreases of 11-deoxycorticosterone, 11-deoxycortisol, and corticosterone concentrations. Etomidate showed a 38% inhibition of the corticosteroid synthesis at 0.28 µM.

8.3.2.1.3. Single Dose Oral Endocrinology Study in Dogs

TMC278 base dissolved in PEG400 + CA was administered once, by oral gavage, at 0, 20, or 80 mg/kg in a dose volume of up to 10 mL/kg to groups of 6 male beagle dogs (m2.6.7, Section 17.2, TMC278-Exp5627). Mortality, clinical observations, body weight, and body weight gain were evaluated. Cortisol, ACTH, and aldosterone levels were measured (1 and 6 days after dosing). Pituitary-adrenocortical function was assessed by a challenge with corticotrophin releasing factor (CRF) (1 and 6 days after dosing). Toxicokinetic evaluation on blood samples taken on the day of dosing was also included.

There were no mortalities associated with TMC278. There were no relevant clinical signs, or effects on body weight or body weight gain. No relevant changes in plasma cortisol, aldosterone, or ACTH were recorded. At the CRF challenge, no relevant changes were seen in plasma cortisol levels. Systemic exposure expressed as C_{max} and AUC values are given in Table 40 below.

Table 40. Exposure of TMC278 (C_{max} and AUC) in a Single Dose Oral Endocrinology Study in Dogs with TMC278 Base

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC_{inf} (µg.h/mL)
		M (n = 6)	
20	Day 1	2.8	87
80	Day 1	2.8	105

M: males

8.3.3. TAF

8.3.3.1. Bone Turnover

Three nonGLP rat studies and one GLP dog study were conducted to evaluate the potential for TAF to alter bone metabolism.

8.3.3.1.1. Rats

A preliminary study in Sprague Dawley rats was conducted by oral daily dosing male and female rats 0 (vehicle), 6.25, 25, 100 or 400 mg/kg/day of GS-7340-02 for 6 days (m2.6.7, Section 6.3, R990139). However, the results are confounded due to administration of another compound in addition to GS-7340-02 on study day 3. Effects observed in all treatment groups included a dose dependent increase in PTH and a non-dose dependent reduction in 1,25-dihydroxycholecalciferol. The NOEL for this study was determined to be < 6.25 mg GS-7340-02/kg.

Changes in bone markers were assessed after daily oral administration of GS-7340-02 to Sprague-Dawley rats. Male rats were administered 0 (2 males; vehicle only) or 400 mg/kg GS-7430-02 (4 males) by oral gavage daily for 6 days (m2.6.7, Section 17.3, R990177). Urine was collected continuously over a 24-hour period while the animals were housed in the metabolic cages at the beginning and end of dosing. The treatment and control group animals were euthanized after 6 days of treatment. Blood samples were collected for clinical chemistry. No observable toxicities were observed over the duration of the study. One animal was euthanized due to non-drug related injury. Serum parathyroid hormone (PTH) concentrations were unchanged in animals administered TAF compared to controls on Day 6. Serum 1,25-dihydroxycholcalciferol was decreased by 80% in animals administered TAF compared to control rats. Urinary deoxypyridinoline (Dpd) was decreased by 38% in rats administered GS-7340-02 compared to control rats. The urinary calcium/creatinine and phosphorus/creatinine ratios of rats administered GS-7340-02 were increased 444% and 202%, respectively, compared to controls by Day 6. The results of this study demonstrated that TAF had effects on selected urinary and serum parameters. However, lack of strong statistical comparison to control animals and lack of correlation between data prevented any interpretation on the overall toxicity.

In the second study (m2.6.7, Section 17.3, R2000044), an additional dose was evaluated. This study examined changes in markers of calcium homeostasis in male Sprague-Dawley rats after once daily oral administration of TAF. Male Sprague-Dawley rats were administered 100 or 400 mg/kg GS-7340-02 by oral gavage on Days 0, 1, 2, 3, 4, and 5. The animals were housed in metabolic cages throughout the study. The animals were euthanized on Day 6. Blood samples were collected for clinical chemistry on Days 0, 1, and 6. Urine was collected on Days 0 to 6.

Rats administered 400 mg/kg/day had statistically significant decreases in rates of percent change of body weight from Day 0 on Days 2 through 6. No changes in plasma calcium, phosphorus, or ionized calcium were observed during the first day of the study in either treatment group. No changes in serum 25-hydroxyvitamin D_3, parathyroid hormone, or total carbon dioxide (CO_2) were observed in either treatment group. Significant dose-dependent reductions of 35% and 46% were observed in 1,25-dihydroxyvitamin D_3 on Day 6 from rats

administered 100 or 400 mg/kg/day TAF, respectively. Significant non-dose-dependent reductions of 15% and 14% were observed in serum calcium on Day 6 from rats administered 100 or 400 mg/kg/day TAF, respectively. Significant dose-dependent decreases of 20% and 31% in serum phosphate were observed in rats administered 100 or 400 mg/kg/day TAF, respectively, on Day 6. Urinary calcium: creatinine ratio values were significantly decreased by approximately 71% and 53% on Days 1 and 2, respectively, in rats administered 100 mg/kg/day TAF. Urinary calcium: creatinine ratio values were significantly decreased by 42% (Day 1) then significantly increased by 213% (Day 6) in rats administered 400 mg/kg/day TAF. No changes in urinary phosphorus: creatinine ratio, N-acetyl-ß-glucosaminidase (NAG):creatinine, cyclic adenosine monophosphate (cAMP):creatinine, pH, protein, urobilinogen, or specific gravity were observed. Daily administration of TAF to rats for 6 days caused significant and dose-dependent reductions in 1,25-dihydroxyvitamin D_3 and serum phosphate. Slight, significant decreases in serum calcium were observed in animals administered 400 mg/kg/day TAF on Day 6. Urinary excretion of calcium was initially decreased on Day 1 for both treatment groups. By Day 6, a significant increase in urinary calcium was observed in rats administered 400 mg/kg/day TAF. These data indicated that daily administration of TAF to rats for 6 days altered plasma vitamin D, calcium and phosphate levels as well as urinary excretion of calcium. The mechanistic basis of these changes and their biological relationships in terms of cause and effect are unknown.

8.3.3.1.2. Dogs

Oral doses of 37.5 or 75 mg/kg/day of GS-7340-02 or vehicle were administered to male beagle dogs (3/group) (m2.6.7, Section 17.3, D990142). Doses were administered by oral gavage once each day for 5 days. Emesis, excessive salivation and diarrhea were observed and weight loss and reduced food intake occurred.

On day 6, dogs had decreased WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts with a left shift in the myeloid series with under representation of mature segmented neutrophils as the predominant bone marrow finding. Dogs also had increased total protein, globulin, triglyceride, cholesterol, AST, ALP, and creatinine kinase (CK) values. A trend toward proteinuria was observed in the urinalysis data.

There were no biologically important changes observed in mean values for ionized calcium or N telopeptide. The lack of the expected age-related reduction in mean serum bone ALP (B-ALP) after GS-7340-02 administration suggested increased bone remodeling. The mean serum calcitriol (1, 25 dihydroxycholecalciferol) concentration was reduced by approximate 68% for both dose groups administered TAF. The mean serum 25-hydroxyvitamin D (25 hydroxycholecalciferol) concentration was reduced approximately 35% and 33% for the 37.5 and 75 mg/kg/day dose groups.

Gastrointestinal lesions characterized by epithelial cell necrosis, regenerative hyperplasia, and cyst formation were most prevalent in dogs administered 5 daily doses of 75 mg/kg/day TAF. Lymphoid cell depletion was noted in the lymph nodes, thymus and tonsil, also observed only in the 75 mg/kg/day animals. Bile duct hyperplasia and periportal inflammation was observed after administration of TAF.

8.3.3.2. Renal Function

The objective of this GLP study was to evaluate the pharmacological effects of GS-7340-02 on the renal system after oral gavage of 0 (vehicle), 100 or 1000 mg/kg/day GS-7340-02 to male Sprague-Dawley CD (Crl:CD®(SD)BR) rats (m2.6.7, Section 17.3, R990186). Thirty minutes after volume loading by oral gavage with 10 mL/kg saline, rats were administered a single oral gavage dose of the test or vehicle article (10 mL/kg). No adverse effects on renal function were observed. Although urinary output of calcium was increased at 1000 mg/kg/day, this correlated with an increase in serum calcium concentration and indicated that the kidneys were functioning in order to reduce the serum calcium load.

8.4. Dependence

No specific studies have been conducted to evaluate dependency of FTC, RPV, or TAF. Nucleoside analogs, as a class, have no known properties that would suggest development of dependence. There was no evidence of development of dependence in preclinical studies with FTC, RPV or TAF. In the tissue distribution studies using [^{14}C]TAF, little or no radioactivity was observed in brain tissues from dog (m2.6.4, Section 4.2.3.3, D990173-BP) and rat (m2.6.4, Section 4.2.3.2, AD-120-2020), while low levels of radioactivity was detected in mouse brain tissues suggesting low permeability across the blood brain barrier in mouse (m2.6.4, Section 4.2.3.1, AD-120-2011). Consequently, dependency studies were not considered warranted.

8.5. Metabolites

No specific toxicology studies with FTC, RPV or TAF metabolites were conducted as there were no unique human metabolites. The hydrolytic pathway of TAF to TFV via TFV monoamidate is similar to that observed in vivo; monophenyl PMPA, phenol, and PMPA are known metabolites. Studies have shown that metabolite levels in nonclinical species used for toxicology studies exceed those observed clinically (see m2.6.4, Section 5.3.3).

The by-products formed during conversion of TAF to TFV are [redacted], l-alanine, and phenol and are formed at nontoxic levels. The by-products formed during conversion of TDF to TFV are [redacted], carbon dioxide and [redacted].

Clinical data from Study GS-US-120-0104 demonstrated that the mean intracellular concentrations of TFV-DP ranged 0.677-1.49 µM in PBMCs in the patients dosed once daily at 25 mg TAF (PC-120-2008). Since the predominant PBMC metabolite of TAF is TFV-DP (AD-120-2035), the total TAF-related intracellular metabolites in PBMCs should not exceed 5 µM (conservative estimate). Therefore, the intracellular concentrations of each by-product formed during conversion of TAF to TFV should not exceed 5 µM. The intracellular concentrations of Metabolite X (alaninyl TFV) or Metabolite Y (isopropylalaninyl TFV) should be significantly lower than 5 µM {7415}. No prodrug was found in PBMCs. Tenofovir alafenamide showed low cytotoxicity in resting and dividing PBMCs, T-lymphoblastoid cells, and hepatocellular carcinoma (HepG2) cells, and provided > 1997-fold selectivity or greater relative to antiviral activity in T-lymphoblastoid cell lines (m2.6.2, Section 6.3).

L-Alanine is a naturally occurring nonessential amino acid which is found in a wide variety of foods. [redacted] accounts for ~12.6% of the total molecular weight of GS-7340 (free base). Therefore the total amount of [redacted] being administered at a 10 mg dose is ~ 1.26 mg, 0.025 mg/kg for a 50 kg person. Doses of more than 5g/kg have been reported to cause adverse effects (http://chem.sis.nlm.nih.gov/chemidplus/rn/67-63-0; accessed [redacted])

Phenol accounts for ~ 20% of the total molecular weight of GS-7340. Therefore, the total amount of phenol being administered at a 10 mg dose is ~2 mg; 0.04 mg/kg for a 50 kg person. The probable oral lethal dose to humans is estimated at 50-500 mg/kg (http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~CtJ8Sm:1; accessed [redacted].) IARC has determined that phenol is not classifiable as to its carcinogenicity to humans (Group 3) {29103}. Phenol is an inactive ingredient in the US Food and Drug Administration (FDA) approved products delivered intravenously, intramuscularly or subcutaneously at levels up to 1.3%. (http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm; accessed [redacted]). Examples of approved prescription drugs used chronically (eg, glycemic control, acromegaly) that contain phenol are presented in Table 41.

Table 41. Examples of Approved Prescription Drugs Containing Phenol

Product	Phenol concentration	Estimated daily dose of drug and phenol
LEVEMIR® - insulin detemir [rDNA origin] injection: 3mL PenFill 100 U insulin detemir/mL	1.8 mg/mL SC	Assuming 1.2 U/kg BID use and 60 kg, 144 U/day is 1.44 mL which would give 2.5 mg phenol/day
Victoza® - liraglutide [rDNA origin] injection (GLP-1) ; 6 mg/mL	1.8 mg/mL C	The max recommended dose is 1.8 mg/day (0.3 mL) which equals 0.54 mg phenol/day
Sandostatin® -octreotide acetate Injection, 200 and 1000 mcg/mL	5 mg/mL SC	The maximum recommended dose is 500 µg TID which would be 0.5 mL TID or 7.5 mg phenol/day

BID = twice daily; TID = 3 times a day

In conclusion, the by-products formed with the conversion of TAF to TFV are at low levels, showed low cytotoxicity, were evaluated in the TAF toxicology studies and of little toxicological concern.

8.6. Impurities

8.6.1. FTC

8.6.1.1. 1-Month Mouse Oral Qualification Study of FTC and TP-0296 (Degradant)

The toxicity of FTC in combination with 2% (by weight) TP-0296 (不純物FA*); FTC degradant) when administered orally by gavage to mice for 28 days was evaluated in order to qualify the degradant, TP-0296 (m2.6.7, Section 17.1, TOX153). Four groups of mice (10/sex/group) were administered 0.5% aqueous methylcellulose vehicle or 50/1, 150/3, or 450/9 mg/kg/day of the combination of FTC/TP-0296, respectively. Each dose level represented the desired FTC dosage in combination with 2% (by weight) dosage of TP-0296. The standard toxicological endpoints were recorded at regular intervals during the study.

All mice survived to the scheduled necropsy. There were no observations related to treatment with the combination of FTC and TP-0296 at any dose level. Body weight, food consumption, hematology, serum biochemistry values, and organ weights were unaffected by administration of the test article combination at any dose level. There were no gross or microscopic findings attributed to the test article combination. The NOEL for oral administration of FTC in combination with TP-0296 was 450/9 mg/kg/day FTC/TP-0296, the highest dose combination tested.

8.6.1.2. A 1-Month Oral Qualification Study of FTC Produced by the [redacted] Process in CD-1 Mice

The objective of the study was to evaluate the toxicity of FTC produced by the [redacted] Process when administered orally by gavage to mice for a minimum of 28 days to qualify potential impurities and/or degradants in FTC produced by this process (m2.6.7, Section 17.1, TX-162-2001). The test article, FTC (GS-9019) in the vehicle, 0.5% aqueous methylcellulose, was administered orally by gavage once daily for a minimum of 28 consecutive days to 3 groups (Groups 2 to 4) of Crl:CD-1®(ICR) BR mice. Dosage levels were 50, 150, and 450 mg/kg/day of the FTC test article. A concurrent control group (Group 1) received the vehicle on a comparable regimen. The dose volume was 10 mL/kg for all groups. Each group consisted of 10 animals/sex.

All animals were observed BID for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Clinical pathology evaluations (hematology or serum chemistry) were performed for all mice at the scheduled necropsy (study Week 4). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals in the control and 450-mg/kg/day groups; gross lesions were examined microscopically for all animals.

All animals survived to the scheduled necropsy. There were no test article-related clinical observations. No test article-related effects on body weights, food consumption, hematology, serum chemistry parameters, and organ weights were noted. There were no test article-related macroscopic or microscopic findings at any dose level of the FTC test article.

Based on the results of this study, the NOEL for oral (gavage) administration of the FTC test article (produced by the [redacted] chemistry method of synthesis) to mice for 28 consecutive days was 450 mg/kg/day, the highest dose tested.

8.6.1.3. Bacterial Reverse Mutation Assay of FTC to Qualify Degradant TP-0296

Emtricitabine in combination with 1% (by weight) TP-0296 (不純物 FA*, degradant), was tested in the Bacterial Reverse Mutation Assay to qualify degradant TP-0296 using *Salmonella typhimurium* tester strains TA98, TA 100, TA 1535, and TA 1537 and *Escherichia coli* tester strain WP2*uvr*A in the presence and absence of S9 metabolic activation (m2.6.7, Section 17.1, TOX151). The dose solutions were prepared separately in distilled water and both test articles were soluble at all concentrations used in the study. The assay was performed in 2 phases, using the plate incorporation method. The first phase, an initial toxicity-mutation assay, was used to establish

the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article combination.

In the initial toxicity-mutation assay, dose levels of FTC tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate, and the highest dose of TP-0296 tested was 50 µg/plate. No toxicity and no positive mutagenic response were observed at any dose. Based on these findings, the maximum doses plated in the confirmatory mutagenicity assay were 5000 µg/plate for FTC and 50 µg/plate for TP-0296.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed. The dose levels of FTC tested were 50, 150, 500, 1500, and 5000 µg/plate. The dose levels of TP-0296 tested were 0.50, 1.5, 5.0, 15, and 50 µg/plate. Neither precipitate nor toxicity was observed. The combination of FTC and 1% TP-0296 was concluded to be negative.

8.6.1.4. In Vitro Mammalian Chromosome Aberration Study of FTC to Qualify Degradant TP-0296

The chromosome aberration assay was used to evaluate the clastogenic potential of the combination of FTC and 1% TP-0296 (不純物FA*, degradant) (m2.6.7, Section 17.1, TOX152). This GLP-compliant study of FTC was conducted to qualify TP-0296 using Chinese Hamster ovary (CHO) cells in the absence and presence of S9 metabolic activation. Both FTC and TP-0296 were soluble in water at all concentrations used in the study.

Based on the lack of cytotoxicity in a preliminary study, doses of FTC chosen for the chromosome aberration assay ranged from 313 to 5000 µg/mL for both the activated and nonmetabolically activated treatment groups. The cells were treated for 4 and 20 hours in the nonactivated test system and for 4 hours in the S9 activated test system. All cells were harvested 20 hours after treatment initiation. The percentage of cells with structural or numerical aberrations in the FTC/TP-0296 groups was not significantly increased above that of the solvent control at any dose level ($p > 0.056$, Fisher's exact test).

Based on the results of this study, it was concluded that FTC with 1% TP-0296 was negative for the induction of structural and numerical chromosome aberrations in CHO cells.

8.6.2. RPV

8.6.2.1. In Vitro Bacterial Reverse Mutation (Ames) Test with 不純物A* and 不純物B*

不純物A* and 不純物B* (drug substance impurities, Figure 2), and TMC278 were dissolved in DMSO (m2.6.7, Section 17.2, TMC278-NC308 and TMC278-NC309). Each impurity was spiked at 4% by mixing a solution of the impurity with a solution of TMC278 at a ratio of 4:96. Each spiked solution was tested separately in 2 independent repeats of a bacterial reverse mutation assay (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537) up to 250 µg TMC278 per plate, in the presence or the absence of metabolic activation (Aroclor-induced rat liver S9 mix).

Figure 2. Structural Formulas of 不純物A* (Top) and 不純物B* (Bottom)



Precipitation was noted at 62.5 µg TMC278/plate and above. No biologically significant induction of the incidence of mutations frequency was noted in the absence or the presence of a metabolic activation system for each of the mixtures compared to their negative (vehicle) control. The appropriate response of the positive control articles confirmed the adequacy of the test system.

It was concluded that neither 不純物A* nor 不純物B* spiked to TMC278 at 4% was mutagenic in this test.

8.6.2.2. In Vitro Mammalian Forward Mutation Assay with Mouse Lymphoma Cells with 不純物A* and 不純物B*

不純物A* and 不純物B* (drug substance impurities [Figure 2]; m2.6.7, Section 17.2, TMC278-NC311 and TMC278-NC312), and TMC278, were dissolved in DMSO. Each impurity was spiked at 4% by mixing of a solution of the impurity with a solution of TMC278 at a ratio of 4:96. Each spiked solution was tested separately in 2 independent repeats of an in vitro mammalian forward mutation assay at the TK locus in mouse lymphoma L5178Y cells. Each mixture was incubated at a range of concentrations up to 75 µg TMC278/mL, the limit of toxicity in the absence of metabolic activation (Aroclor-induced rat liver S9 mix) for 3 or 24 hours; and up to 15 µg TMC278/mL, the limit of toxicity in the presence of S9 for 3 hours.

No biologically significant induction of the incidence of mutations frequency was noted in the absence or the presence of a metabolic activation system for each of the mixtures compared to their negative (vehicle) control. The appropriate response of the positive control articles confirmed the adequacy of the test system.

It was concluded that neither 不純物A* nor 不純物B* spiked to TMC278 at 4% induced an increased mutation frequency at the TK locus of L5178Y mouse lymphoma cells.

8.6.2.3. 1-Month Oral Rat Toxicity Study with 不純物A* and 不純物B*

不純物A* and 不純物B* (drug substance impurities [Figure 2]) were each spiked at 4% to TMC278 (m2.6.7, Section 17.2, TMC278-NC314). The spiked batches and TMC278 were suspended in 0.5 % (m/v) aqueous HPMC and administered by daily gavage for 1 month to

groups of 10 male and 10 female Sprague Dawley rats, at one dose of 10 mg/kg/day corresponding to the NOAEL of TMC278 in that species. The dosing volume was 10 mL/kg. The control group of 10 male and 10 female Sprague Dawley rats received the vehicle. Regular observations were made for clinical signs, morbidity, and mortality. Weekly body weight and food consumption were determined. Ophthalmic examination was conducted before the start of dosing and at the end of the dosing period. At the end of the study clinical laboratory investigations (hematology, blood chemistry, and urinalysis) were done. At necropsy gross lesions and organ weight were recorded and the target organs and tissues for TMC278 were sampled for histological examination.

There were no mortalities in the study and there were no relevant clinical signs, or effects on body weight, food consumption, or ophthalmoscopy. Hematological, serum chemistry, and urinalysis parameters were within normal ranges. No gross lesions were detected and organ weights and histopathological examination indicated no relevant changes that could be attributable to treatment.

It was concluded that 不純物A* and 不純物B* spiked to TMC278 at 4% did not induce any effects.

8.6.2.4. In Vitro Bacterial Reverse Mutation Test with 不純物H*

不純物H* (HCl salt of 不純物I*, Figure 3) dissolved in DMSO was evaluated in 3 bacterial reverse mutation studies (Ames test) (m2.6.7, Section 17.2, TMC278-NC179, TMC278-NC165, and TMC278-1646_0016451) with histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537. Concentrations of 78.13, 156.25, 312.5, 625, 1250, 2500, and 5000 µg per plate were tested, in the absence or the presence of metabolic activation (Aroclor-induced rat liver S9 mix).

Figure 3. Structural Formula 不純物G*



In one study (TMC278-NC179 [TOX6835]), 不純物H* induced an increase in the incidence of mutations in the strains TA98 and TA100 in the presence of rat liver S9-mix. In the other study (TMC278-NC165 [TOX6616]), the incidence of mutations of TA1537, in the absence of metabolic activation, and of TA98, in the presence of S-9 mix, were increased. In the third study (TMC278-1646_0016315 [TOX9643]) that served as a head to head comparison with 不純物F* and 不純物E* (see Sections 8.6.2.6 and 8.6.2.7), 不純物H* induced an increase in the incidence of mutations in the strains TA98 and TA1537, both in the presence and the absence of rat liver S9-mix; and in TA100 in the presence of rat liver S9-mix only.

These results confirmed the genotoxic potential of 不純物H*.

8.6.2.5. In Vitro Mammalian Chromosomal Aberration Assay with 不純物H*

不純物H* (HCl salt of 不純物I*, Figure 3) dissolved in DMSO was tested in an in vitro mammalian chromosome aberration screening test with human lymphocytes (m2.6.7, Section 17.2, TMC278-NC184). Incubation for 4 hours followed by a 20-hour expression period in the presence or the absence of a metabolic activation (Aroclor-induced rat liver S9 mix) was performed at a range of concentrations up to 521.75 µg/mL. Incubation for 24 hours in the absence of S9-mix occurred at a range of concentrations up to 391.32 µg/mL.

不純物H* induced a statistically significant increase in the frequency of cells with structural aberrations in the absence of metabolic activation (S9) after 4 hours of exposure at the maximum concentration tested, 521.75 µg/mL. 不純物H* was therefore considered to be clastogenic to human lymphocytes in vitro.

8.6.2.6. In Vitro Bacterial Reverse Mutation (Ames) Test with 不純物E*

不純物E* (trace impurity, Figure 4) dissolved in DMSO was evaluated in a bacterial reverse mutation study (Ames test) (m2.6.7, Section 17.2, TMC278-1646_0015298) with histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537. In each independent repeat, 7 concentrations ranging from 78.13 to 5000 µg per plate were tested, in the absence or the presence of metabolic activation (Aroclor-induced rat liver S9 mix).

Figure 4. Structural Formulas of 不純物E* (Left) and 不純物F* (Right)



不純物E* up to 5000 µg/plate did not induce an increased frequency of mutations in any of the tested strains, in the presence or the absence of rat liver S9-mix.

8.6.2.7. In Vitro Bacterial Reverse Mutation (Ames) Test with 不純物F*

不純物F* (trace impurity, Figure 4) dissolved in DMSO was evaluated in a bacterial reverse mutation study (Ames test) (m2.6.7, Section 17.2, TMC278-1646_0015299) with histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537. In each independent repeat, 7 concentrations ranging from 78.13 to 5000 µg per plate were tested, in the absence or the presence of metabolic activation (Aroclor-induced rat liver S9 mix).

The first test showed no increased frequency of mutations in any of the tested strains, in the presence or the absence of rat liver S9-mix. The second test showed the same results as the first test, except for TA98 in the presence of S6-mix. Almost all concentrations of 不純物F* showed a slightly more than 2-fold increase of the frequency of mutations compared to vehicle control,

which showed a low mutation frequency. A third test with TA98 in the presence of S9-mix showed no increased frequency of mutations up to 5000 µg/plate. It was concluded that 不純物F* has no mutagenic potential.

8.6.3. TAF

8.6.3.1. 2-Week Oral Toxicity Impurity Qualification Study in Rats with GS-7340-02

The purpose of this study was to evaluate the toxicity and to qualify potential impurities of TAF when administered daily via oral gavage to male rats for at least 14 days (m2.6.7, Section 17.3, TX-120-2008). Male Hsd:Sprague Dawley®™SD®™ rats were assigned to 5 groups (10 animals/toxicity group) and received either the vehicle [0.1% (v/v) Tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (Dow Chemical K100LV) prepared in reverse osmosis water] or GS-7340-02 at 5 or 50 mg/kg/day (10 mL/kg) from 2 different purity lots (Lot No. 1 - 97.7% and Lot No. 2 - 83.1%)] at a volume of 10 mL/kg. The impurities, including 13% GS-7339, were added to the more pure lot. Additional animals were assigned for toxicokinetic evaluation on Days 1 and 14.

Administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day. No test article-related findings were noted, and no differences were found between the 2 lots tested. The NOAEL for both lots is 50 mg kg/day (40 mg f.b.e./kg/day). In males administered 50 mg/kg/day on Day 14, the TFV C_{max} was 218 ng/mL and the AUC_{0-t} was 1965 ng·hr/mL for 97.7% pure GS-7340-02 (Lot No. 1: [redacted]) and the TFV C_{max} was 395 ng/mL and the AUC_{0-t} was 2481 ng·hr/mL for 83.1% pure GS-7340-02 (Lot No. 2: [redacted]).

8.6.3.2. 4-Week Oral Toxicity Impurity Qualification Study in Rats with GS-7340-03

The purpose of this study was to evaluate the toxicity and to qualify potential impurities of TAF when administered daily via oral gavage to male and female rats (10/sex/toxicity group) for at least 28 days (m2.6.7, Section 17.3, TX-120-2021). Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (free base equivalents). Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked impurities. Vehicle control animals were administered the vehicle control article (0.1% [w/v] hydroxypropylmethylcellulose K100LV and 0.1% [v/v] Tween® 20 in 99.8% [v/v]) reverse osmosis water). Doses were administered via oral gavage once daily for 29 days at a dose volume of 10 mL/kg. Additional animals were assigned for toxicokinetic evaluation on Days 1 and 28.

Administration of GS-7340-03 drug substance with 3 different impurity profiles by oral gavage for 28 days to rats was well tolerated at dose levels of 25 and 50 mg/kg/day. There were no significant clinical or histopathological differences between the 3 lots tested. The NOAEL for all 3 lots is 50 mg f.b.e./kg/day.

8.6.4. FTC/TDF: Degraded Tablets

Two qualification studies to investigate the potential toxicity of nondegraded and degraded FTC/TDF tablets following daily oral administration to the rat for a minimum of 14 days have been conducted. The second qualification study was conducted to verify the qualification of 不純物FB*(不純物FB* ; GS-9237) and 不純物FC* (GS-492127) due to the development of a new analytical assay for these two degradants.

8.6.4.1. FTC/TDF: 14-Day Oral Gavage Toxicity Study Comparing Non-Degraded and Degraded FTC/TDF in Sprague-Dawley Rats

The purpose of this study was to investigate the potential toxicity of non-degraded and degraded FTC/TDF following daily oral administration to the rat for a minimum of 14 days (m2.6.7, Section 17.4, TX-164-2001). Animals (10 males/group) were administered doses of 20/30, 67/100, or 200/300 mg/kg/day of non-degraded or degraded FTC/TDF in suspension vehicle at a dose volume of 10 mL/kg. The following parameters were measured during the study: mortality, clinical signs, detailed physical examination, body weights, food consumption, laboratory investigations (hematology, biochemistry, and urinalysis), gross pathology, organ weight analysis, and histopathology.

In this study, there were no deaths related to treatment with FTC/TDF (degraded or nondegraded). Clinical signs were limited to dose-related postdosing salivation. There were no effects on body weight or food consumption considered to be related to treatment with FTC/TDF (non-degraded and degraded). There were no toxicologically significant treatment-related changes in hematology, biochemistry, or urine parameters. Organ weight evaluation revealed marginal increases in the weights of adrenal glands (noted in all treated groups but those receiving degraded FTC/TDF at the lowest dose). No gross or histological changes were identified which might account for this increased adrenal weight. No treatment-related gross changes were observed at necropsy. Microscopic evaluation revealed hyperplasia of the anterior duodenal mucosa overlying Brunner's glands. It was seen in 7/10 animals treated at the high-dose level with nondegraded FTC/TDF and in 2/10 animals receiving degraded FTC/TDF and was considered to be treatment-related.

Daily oral administration of non-degraded and degraded FTC/TDF at dose levels of 20/30, 67/100, and 200/300 mg/kg/day for 14 consecutive days to male Sprague-Dawley rats was well tolerated. There were no toxicologically significant differences between groups treated with non-degraded and degraded FTC/TDF. A minor increase in adrenal weights at necropsy (all dose levels, except those receiving degraded FTC/TDF at the lowest dose) and a hyperplasia of the anterior duodenal mucosa overlying Brunner's glands was seen in animals treated at the high-dose level with non-degraded and degraded FTC/TDF.

In conclusion, the NOAEL in this study was considered to be 67/100 mg/kg/day FTC/TDF.

8.6.4.2. FTC/TDF: 14-Day Study of Non-degraded and Degraded FTC/TDF by Oral Gavage in Rats

The objective of this study was to determine the potential toxicity of degraded FTC/TDF when compared to non-degraded material administered by daily oral gavage for 14 days to rats. In addition, the toxicokinetic characteristics of non-degraded and degraded FTC/TDF were compared (m2.6.7, Section 17.4, TX-164-2005).

Male Sprague-Dawley rats were assigned to seven toxicity groups (10 animals/group) and received dose preparations containing the vehicle control article [Suspension vehicle (carboxymethylcellulose, 0.5% (w/v), benzyl alcohol, 0.9% (w/v), polysorbate 20, 0.5% (w/v) and sterile water for injection, USP] or non-degraded or degraded FTC/TDF at 20/30, 67/100 or 200/300 mg/kg/day.

Assessment of toxicity was based on clinical signs, body weights, body weight changes, food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations. Blood samples were collected from satellite animals for toxicokinetic evaluations of non-degraded and degraded TDF/FTC on Days 1 and 14.

There were no preterminal deaths, or non-degraded or degraded TDF/FTC-related clinical observations or changes in body weights, food consumption, coagulation or urinalysis parameters. There were no gross necropsy findings or changes in organ weights.

Administration of non-degraded or degraded FTC/TDF at 200/300 mg/kg/day was associated with minimal, non-adverse, decreases in red cell mass parameters (Hb and HCT). These changes were associated with minimal, non-adverse, red cell indices (MCV, MCH and RDW) changes indicating microcytosis. Findings observed in animals administered non-degraded FTC/TDF or degraded FTC/TDF regarding red cell mass parameters or indices were considered to be of comparable magnitude and had no microscopic correlates. Non-adverse, minimal, dose level-dependent increases in ALT activity were observed in rats administered non-degraded FTC/TDF at ≥ 67/100 mg/kg/day and degraded FTC/TDF at 200/300 mg/kg/day. Increased ALT activity was not associated with histological findings.

In the duodenum, there was minimal cryptal epithelium hyperplasia and minimal single cell necrosis with both degraded and non-degraded formulations of FTC/TDF at 200/300 mg/kg/day.

In conclusion, administration of non-degraded or degraded FTC/TDF by once daily oral gavage was well tolerated in rats at levels of 20/30, 67/100 and 200/300 mg/kg/day. Microscopically, minimal cryptal epithelium hyperplasia and minimal single cell necrosis were observed in the duodenum of animals administered both FTC/TDF formulations at 200/300 mg/kg/day. Based on these minor findings, the NOAEL was considered to be 200/300 mg/kg/day for both non-degraded and degraded TDF/FTC. No differences in toxicity were observed between non-degraded and degraded material. At all dose levels tested, exposure of FTC and tenofovir was similar for both non-degraded and degraded material (Day 14 FTC AUC_{0-24} 163.3 and 132.6 µg·h/mL; TFV AUC_{0-24} 13.6 and 13.7 µg·h/mL, for non-degraded and degraded materials, respectively).

8.7. Qualification of Impurities

8.7.1. FTC

The impurity profiles for batches of test article used in nonclinical toxicology studies are provided in m2.6.7, Sections 4.1.

Two 4-week oral toxicity studies have been conducted in mice to qualify impurities and degradants in the GMP material (m2.6.7, Section 17.1, TOX153 and m2.6.7, Section 17.1, TX-162-2001). Further, the FTC degradant, 不純物FA*, has also been qualified in genotoxicity studies (m2.6.7, Section 17.1, TOX151 and m2.6.7, Section 17.1, TOX152).

Based on their impurity profiles, the multiple GLP batches of FTC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

8.7.2. RPV

The impurity profiles for batches of test article used in nonclinical toxicology studies are provided in m2.6.7, Section 4.2.

不純物H* is the HCl salt of 不純物G* (Figure 3), a starting material in an earlier synthesis route of TMC278 drug substance. On the basis of the presence of [redacted] and [redacted] moieties in 不純物G*, its genotoxic potential was evaluated in 3 independent repeats of a bacterial reverse mutation assay (Ames) and an in vitro mammalian chromosomal aberration assay (see Section 4.2. of this document). The possible impact on human safety of the presence of 不純物H* in drug substance and drug product is evaluated in the Nonclinical Overview (m2.4).

Traces of 不純物E* and 不純物F* (Figure 4) may be present in TMC278 drug substance. These compounds having an [redacted] moiety, but no [redacted] share structural similarities with 不純物H*. The mutagenic potentials of the HCl salts of 不純物G*, 不純物H*, and of 不純物E* and 不純物F* were tested in an Ames test. Only 不純物H* showed a mutagenic potential. 不純物G* is considered a genotoxic impurity. The maximum allowable level of 不純物G* in a daily dose of 25 mg TMC278 was calculated to be [redacted] ppm, on the basis of the Threshold of Toxicological Concern (TTC) approach with daily treatment for 1-10 years per ICH M7 (Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 2014). The level of 不純物G* in drug substance and drug product has been controlled at less than [redacted] ppm.

Based on their impurity profiles, the multiple GLP batches of RPV tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

8.7.3. TAF

The impurity profiles for batches of test article used in nonclinical toxicology studies are provided in m2.6.7, Section 4.3.

Fourteen-day and 28-day oral toxicity studies were conducted in rats to qualify impurities and potential impurities in the GMP material (m2.6.7, Section 17.3, TX-120-2008, and m2.6.7, Section 17.3, TX-162-2021). The impurities spiked into the drug substance did not alter the NOAEL in rats (40 and 50 mg free base equivalents/kg/day).

Based on their impurity profiles, the multiple GLP batches of TAF tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

8.7.4. Qualification of Degradation Products in FTC/RPV/TAF Tablets

FTC/RPV/TAF tablets are bilayer tablets. The degradation products of TAF observed in the FTC/RPV/TAF tablets are consistent with those in the TAF drug substance. There are no unique impurities or degradants in the FTC/RPV/TAF tablets.

The impurities and degradation products present in FTC, RPV and TAF and in FTC/RPV/TAF tablets have been qualified through toxicology studies which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, or samples subjected to forced degradation conditions (high heat and humidity) (m3.2.P.5.5, Characterization of Impurities [FTC/RPV/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [FTC/RPV/TAF Tablets]).

8.8. Other Studies

8.8.1. Mutagenicity Test with TFV in the *Salmonella-Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay

Tenofovir was tested at 4610, 3070, 922, 615, 307, and 92.2 µg per plate in both the presence and absence of S9 mix on Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* tester strain WP2uvrA (m2.6.7, Section 17.3, 95-TOX-1278-006). The doses tested in an additional trial were 5000, 3330, 1000, 667, 333, and 100 µg per plate with tester strains TAl00 and TA1535 only in the presence of S9 mix.

Tenofovir caused positive (6.2- and 6.8-fold) increases in the mean number of revertants per plate with tester strain TA1535 only in the presence of S9 mix. No other increase in the mean number of revertants per plate was observed with any of the remaining tester strain/activation condition combinations either in the presence or absence of S9.

8.8.2. Mutagenicity Test on TFV in the L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay

The ability of TFV to induce forward mutations at the TK locus in the mouse lymphoma L5178Y cell line was evaluated (m2.6.7, Section 17.3, 95-TOX-1278-007). Tenofovir was soluble in water at 50.0 mg/mL.

In the preliminary assay, the test article was shown to be nontoxic with and without S9 metabolic activation at concentrations of 9.77 to 5000 µg/mL. In the main assay, TFV was negative at concentrations of 156 to 5000 µg/mL without metabolic activation, and in the presence of S9 metabolic activation the 5000 µg/mL treatment induced a small (approximately 2.5-fold) increase in the mutant frequency that exceeded the minimum criterion. Since only a single small increase was observed, TFV was considered equivocal with activation in this trial. The test article, TFV, was therefore considered negative without activation and equivocal with activation at the TK locus in L5178Y mouse lymphoma cells under the conditions used in this study.

8.8.3. Toxicological Findings of TFV in SIV Efficacy Models

Investigators from the California Regional Primate Research Center have reported results of antiviral efficacy studies of TFV in simian immunodeficiency virus (SIV)-infected and non-infected rhesus monkeys {1787}, {7311}, {12968}. These studies included administration of TFV to 12 gravid rhesus macaques, and to more than 85 infant and juvenile rhesus macaques from 1 day to 7.5 years of age at initiation of dosing. This age range covers the human equivalent of prenatal, infant, juvenile and adolescent phases of growth (see Table 42). The duration of treatment has ranged from 12 weeks to 13 years. Clinically relevant renal and bone pathology occurred only in rhesus monkeys in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Exposure levels (AUC_{ss} 140 µg·h/mL) at this dose were more than 30-fold higher than those of adult subjects (4.4 µg·h/mL) after a 300 mg/day dose of TDF. Effects in rhesus monkeys were reversible by decreasing or stopping exposure. Administration of lower doses of TFV did not cause renal dysfunction or abnormal bone density or growth.

The published studies (see Table 42) were conducted in collaboration with Gilead Sciences and published in peer review journals. All drug substance for these studies was provided by Gilead Sciences.

Table 42. Nonclinical Pharmacology/Toxicology Studies with TFV

API/Route/n	Age at Start of Treatment[a]	Dose (mg/kg/day)	Duration of Daily Treatment	Reference
Tenofovir/SC/n=12	Gestation Day 80; Infants Day 2	30	Gestation Days 80-157 (n=12); Infants: up to 9 months (n=9)	{1787}
Tenofovir/SC/n=54	1 day to 20 months	4, 10, 30	Up to 12 weeks (n=39) At least 8 months (n=13) At least 5 years (n=2)	{7311}
Tenofovir/SC/n=32[b]	1 day to 33 months	1.25 to 30	1 year to 13 years	{12968}

SC = subcutaneous

a. A general comparison of growth phases between human and rhesus monkeys is as follows {17656}:

Infant:	Human = 1-24 months;	Rhesus = 1-12 months
Child/Juvenile:	Human = 2-11 years;	Rhesus = 1-2 years
Adolescent:	Human = 12-16/18 years	Rhesus = 2-4 years

b. 15 animals previously reported in Van Rompay 2004, but observations extended.

Studies were conducted to assess the safety, efficacy, and placental transfer of TFV when administered once daily subcutaneously to gravid rhesus monkeys during the second and third trimesters and to their offspring (30 mg/kg/day) {1787}. Fetuses (SIV-infected, N = 6;

noninfected; N = 6) were monitored sonographically and maternal/fetal blood samples were collected at select time points for hematologic, clinical chemical, virologic, immunologic, and pharmacologic assessments. Newborns were delivered by cesarean section at term and nursery reared for postnatal studies. Infants were administered TFV once daily beginning on Day 2 of life until 9-months postnatal age. Results of these studies have shown significant placental transport of TFV, with peak fetal levels at 1 to 3 hours postmaternal administration; a significant and sustained reduction in viral load in SIV-infected fetuses and infants; and marked improvements in outcome (eg, survival, growth, health) in SIV-infected offspring. However, decreased infant body weights and alterations of select serum biochemical parameters (eg, decreased phosphorus levels, elevated ALP) have been shown to occur in 67% of TFV-treated infants, with severe growth restriction and bone-related toxicity in approximately 25% of animals studied. These data suggest that there is the potential for bone-related toxicity at chronic, high dosages, particularly in infants.

Daily subcutaneous administration of TFV at 10 mg/kg/day to 2 newborn rhesus monkeys for more than 5 years had no observed adverse effect on their bone density or growth {7311} , {12968}. The steady state AUC for these 2 rhesus monkeys was approximately 19 µg·h/mL (~4.3-fold exposure in adult human populations). Although data were variable, no drug-related changes occurred in the urinary excretion of phosphorus, calcium, potassium, protein, magnesium, or chloride. Radiographs and DXA scans of the animals were collected regularly from birth to 5 years of age. There was no radiological evidence of decreased bone opacity, changes in growth plates, bone formation, or bone resorption compared to control data. Bone biopsies at 3.5 years of age showed no significant differences in bone structure measurements.

Subcutaneous administration of TFV from 4 to 30 mg/kg/day to 39 infant rhesus monkeys for up to 3 months had no observed adverse effect on their health or growth {7311}. No proximal renal tubular dysfunction (PRTD) or growth restriction or bone abnormalities were observed after 12 weeks of dosing or after 3.5 months to 14 months after the end of treatment.

Subcutaneous administration of TFV at 30 mg/kg/day to 13 rhesus monkeys for at least 8 months resulted in PRTD with glucosuria, aminoaciduria, hypophosphatemia, growth restriction, osteomalacia and reduced clearance of TFV {7311}. Bone lesions were only observed in animals that also had PRTD. The long bone metaphyses and the physes and metaphyses of the vertebral bodies were incompletely ossified and there was overall decreased bone opacity. In 4 of the more severely affected animals, there were bone deformations and/or pathological fractures in the long bones or ribs. Serum phosphorus levels were markedly decreased and bone-ALP isoenzymes levels were increased. Urine samples collected by cystocentesis generally showed increased glucose and amino acid levels. Pharmacokinetic data collected after 11 to 24 months of TFV administration from 4 of the rhesus monkeys with moderate to severe bone disease had a mean AUC of 140 µg·h/mL (range 101 to 222 µg·h/mL), more than 30-fold that in adult subjects. The adverse effects were partially or totally reversible following either complete withdrawal of TFV or reduction of the daily regimen from 30 mg/kg/day to 2.5 or 10 mg/kg/day.

Disposition of the 13 rhesus monkeys chronically treated with TFV at 30 mg/kg/day included euthanasia (n = 6), a reduction in TFV dose (n = 6), or discontinuation of dosing (n = 1) {26057}. In the animals euthanized with severe skeletal lesions, the bone histology revealed irregular

hyperplastic growth plates and trabecular hyperplasia with widened osteoid seams. In the animal with severe bone disease and in which administration of TFV was discontinued, the serum phosphorus and ALP concentrations returned to normal in about 6 and 8 months, and the metaphyseal osteopenia resolved by 18 months. For the other 6 animals whose TFV dosage was decreased to either 30 mg/kg twice weekly (n = 1) or 10 mg/kg once daily (n = 5), there was improvement in serum phosphorus levels and radiological bone opacity, and a resumption of growth within approximately 6 months.

Van Rompay et. al. extended their observations and reported on the longer-term safety and pharmacokinetics of TFV in 32 infant and juvenile rhesus monkeys {12968}. As reported earlier, the primary toxicity of prolonged subcutaneous TFV administration was PRTD, characterized by glucosuria, decreased serum phosphorus, and increased serum ALP levels. The effect of daily subcutaneous administration of TFV at 10 to 30 mg/kg/day for 7 weeks to 25 months was described for 29 animals. Seven animals dosed with 10 to 30 mg/kg/day TFV had minimal to no evidence of persistent PRTD which was associated with a relatively high TFV clearance. Twenty two animals with relatively reduced TFV clearance had evidence of PRTD based on urine and serum markers. Five of these animals dosed at 10 to 30 mg/kg/day with PRTD had DXA scans performed. DXA confirmed low BMD in 3 of these animals with PRTD that had growth retardation and severe bone lesions. Two animals with PRTD that had their doses reduced and dietary supplements provided had normal BMD or reduced BMD. Animals that received low-dose regimens of TFV and had no persistent PRTD had relatively normal BMD.

9. DISCUSSION AND CONCLUSIONS

Comprehensive programs of nonclinical studies with FTC, RPV, TDF and TAF have been conducted. The nonclinical data indicates administration of the FTC/RPV/TAF FDC is unlikely to exacerbate known toxicities of the individual agents.

9.1. Target Organ Toxicity

9.1.1. FTC

The only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia.

9.1.2. RPV

The single dose toxicity of RPV was low. The repeat-dose toxicity studies identified a number of targets. The majority of the effects induced in the highest dose tested were reversible. The increase in APTT and PT in male rats and the follicular hypertrophy and incidence of small follicles in thyroid gland of male and female rats showed incomplete recovery after a 1-month postdosing period. In dogs, microscopical lesions in liver and increased serum concentration of ALP were not completely reversed. The targets identified are described below.

9.1.2.1. Liver

Liver effects in rats and mice differed qualitatively from those in dogs. In the rodent species, predominantly hepatocellular hypertrophy occurred accompanied by effects on liver-associated serum parameters. Liver enzyme activities in serum, ALT and ALP, were more consistently increased in the mouse studies than in the studies in rats. Moreover, serum total protein concentrations, mainly due to serum albumin, were increased. However, serum triglycerides and total bilirubin (rats only) concentrations were reduced.

Mice showed some additional or different effects compared to rats. It cannot be excluded that these differences are caused by the fact that in mice much higher exposures were achieved than in rats. The hepatocellular hypertrophy in mice was accompanied by an increase in vacuolization and single cell necrosis. Electron microscopy indicated that this hypertrophy was associated with peroxisome proliferation. By contrast, the incidence of these additional effects was lower in rats. In addition, mice showed pigmentation and proliferation of Kupffer cells and increased serum concentrations of cholesterol, calcium, and inorganic phosphorus. The interrelationship of the serum chemistry effects is doubtful and an association with the Kupffer cell effects is not very likely.

The increased serum urea concentration of female mice in the high dose group dosed with 320 mg/kg/day is in line with the increased food intake in that group.

In dogs, the liver effects changed with the duration of treatment. In the 1-month study, perivascular inflammatory reactions together with multifocal perivascular fibrosis and single cell necrosis were noted in the central part of the lobules. Moreover, increased MPS aggregates occurred and multifocal bile duct proliferation. These effects seem to be associated with increased serum concentrations of cholesterol and total bilirubin and increased activities of ALP and ALT. In the 6-month study, pigment-laden perivascular macrophages were noted; and in males only, prominent brown pigment in the gall bladder epithelium. The pigment in the macrophages was not stainable with Perl's stain, indicating that it probably was of lipogenic origin. This pigmentation was the only liver effect observed after 3 months of dosing (in high dose males only), indicating the relatively slow development of this effect, which also applies for the pigmentation in the gall bladder. In the 12-month dog study, pigmentation of hepatocytes and canaliculi was noted together with prominent brown pigment in gall bladder epithelium, both in males and females. The histopathological effects were associated with an increased serum concentration of total bilirubin and with an increase in serum ALP activity.

In the studied species, liver effects did not occur at the lowest dose tested.

9.1.2.2. Thyroid gland

Effects on the thyroid gland in rats were characterized by increased organ weight, hypertrophy of follicular epithelium, and reduced serum concentrations of T_4. The effects occurred similarly in males and females and were noticed after 7 days of treatment, even at the low dose of 40 mg/kg/day. Upon longer duration of treatment, increased serum concentrations of TSH were recorded and also a differential effect on T_3. The serum concentrations of this thyroid hormone (T_3) were less reduced than those of T_4, and in some studies were not affected or even increased. The effects on thyroid gland are thought to be caused by an increased clearance of T_4. A well-known and frequent cause of the enhanced T_4 clearance in rats is induction of T_4-uridine diphosphate glucuronosyltransferase (UDPGT) in liver. *Ex vivo* enzyme induction determinations in liver homogenate from rats treated with RPV for 6 months showed only a 20% higher UDPGT activity of the high-dose group dosed with 400 mg/kg/day compared to controls. There are no indications that RPV has a direct effect on thyroid gland or a particular affinity to thyroidal tissues. The thyroid gland effects occurred in all studies at the low dose and therefore prevented establishment of a NOAEL in rats.

The minimal follicular cell hypertrophy that was noted in thyroid gland of immature female cynomolgus monkeys in the 8-week study is probably associated with the young age of these animals at the start of the study. However, an effect by RPV cannot be excluded in view of the higher incidence of the effect in the groups treated with the test article compared to the control group. In this study, no levels of thyroid hormones or TSH were determined. Microscopical evaluation of other organs did not show any cause for the thyroidal effect.

9.1.2.3. Pituitary gland

Effects on the pituitary gland occurred only in rats and comprised an increase of swollen and vacuolated cells in the pars distalis. These effects are considered secondary to the thyroid gland effects. The indicated cells produce TSH. The low concentration of T_4 in the circulation is the feedback signal for the pituitary gland to produce more TSH. The effects on the pituitary gland were noted in males treated with the low dose in the 6-month rat study and hence prevented establishment of a NOAEL.

9.1.2.4. Kidneys

Kidney changes were seen in dogs in the 12-month study dosed with the high dose of 40 mg/kg/day, and in the 1-month study with transgenic mice and the 3-month study with CD-1 mice, both at the high dose of 320 mg/kg/day (and in the transgenic mice also at 80 mg/kg/day). The changes in the dog comprised moderate acute interstitial nephritis in 2 high dose males and minimal to slight corticomedullar mineralization in 3 out of 4 high dose females.

In the 1-month study with transgenic mice, all females, 3 out of 10 males treated with 320 mg/kg/day, and 1 out of 10 males at 80 mg/kg/day showed minimal to moderate degenerative or necrotic nephropathy. Minimal to moderate nephropathy was noted in half the number of female mice dosed with 320 mg/kg/day in the 3-month study. In the carcinogenicity study with CD-1 mice, no increased incidence of nephropathy, mortality associated with renal effects, or renal neoplasia was noted.

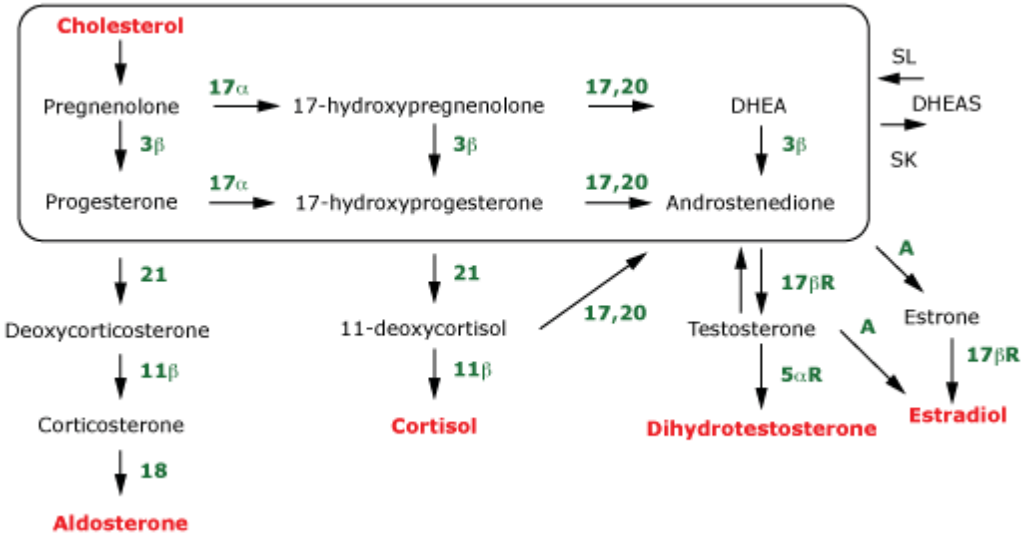
For the nephritis in dogs, a test article treatment relationship cannot be excluded. However in mice, such a relationship seems to be clear. The mechanism of these kidney effects is not clear taking into account the low percentage of the dose excreted via urine (see m2.6.4, Section 6.1.2).

9.1.2.5. Adrenal glands

In all species, except rabbits, involved in nonclinical safety studies with RPV, an effect on adrenal gland has been demonstrated. The levels of adrenal hormones or their precursors and ACTH in serum of rats and dogs, and the results of the in vitro studies with guinea pig primary cell cultures and homogenates of adrenal gland cortex of dog indicate that RPV partially inhibits cytochrome P450 isozyme 21 (CYP21) (see Figure 5). Cytochrome P450 isozyme 21 catalyzes the conversion from progesterone and 17α-hydroxyprogesterone to 11-deoxycorticosterone and 11-deoxycortisol, respectively; the direct precursors of respectively corticosterone and cortisol. A partial block of this pathway will lead to a reduced production of downstream hormones of which cortisol and corticosterone are the most important. Moreover, an accumulation of the substrates of CYP21, progesterone and 17α-hydroxyprogesterone and more upstream hormones may occur. In vivo, the reduced serum levels of cortisol and corticosterone due to inhibition of CYP21 will trigger an ACTH-response from the pituitary. The increased stimulus of the adrenal cortex by ACTH will partially counteract the reduction of the cortisol and corticosterone synthesis, but at the same time will increase the accumulation of the CYP21 substrates.

An additional effect of the CYP21 inhibition, notably in species that have a significant androgenic adrenal pathway, is that the increased ACTH stimulus and subsequent accumulation of progesterone and 17α-hydroxyprogesterone may lead to an increased androgen production. 17α-hydroxypregnenolone and 17α-hydroxyprogesterone are converted to DHEA and androstenedione, respectively; catalyzed by 17, 20 lyase (CYP17). To investigate the impact of the stimulation of the androgenic pathway, a study was conducted in cynomolgus monkeys. This monkey strain and all nonhuman primates share with man a significant androgenic adrenal pathway. The 8-week study in cynomolgus monkeys showed that RPV inhibits both CYP21 and CYP17. This dual effect led to a clear accumulation of progesterone and 17α-hydroxyprogesterone, both being substrates for both CYP21 and CYP17, and a reduction of the unchallenged serum levels of androstenedione and DHEA, and of the response of these androgens to ACTH challenge.

Figure 5. Synthetic Pathways for Adrenal Steroid Synthesis in Nonhuman Primates and Man



The first step in adrenal steroid synthesis is the combination of acetyl CoA and squalene to form cholesterol, which is then converted into pregnenolone. The enclosed area contains the core steroidogenic pathway utilized by the adrenal glands and gonads.
17α: 17α-hydroxylase (CYP17, P450c17); 17,20: 17,20 lyase (also mediated by CYP17); 3β: 3β-hydroxysteroid dehydrogenase; 21: 21-hydroxylase (CYP21A2, P450c21); 11β: 11β-hydroxylase; (CYP11B1, P450c11); 18 refers to the two-step process of aldosterone synthase (CYP11B2, P450c11as), resulting in the addition of an hydroxyl group that is then oxidized to an aldehyde group at the 18-carbon position; 17βR: 17β-reductase; 5αR: 5α-reductase; DHEA: dehydroepiandrostenedione; DHEAS: DHEA sulfate; A: aromatase (CYP19).

The effects of RPV on adrenal gland and its hormonal biosynthetic pathways are more prominent than the effects on gonads and their pathways (see below in more detail). This may be explained by the affinity of RPV for adrenal gland in repeat-dose studies in dogs and of radiolabel following the rat single-dose quantitative whole body autoradiography (QWBA) studies with [^{14}C]RPV (see m2.6.4, Section 4.2.2). In the QWBA studies, no particular affinity for gonads was noted.

Effects on adrenal hormones were noted in all repeat-dose dog studies with treatment duration of at least 7 days. Apparently, the effect needs some time to become manifested. This may explain why no effects were noted in the single oral dog study with a CRF challenge 24 hours after dosing. The fact that effects on adrenal pathways were already detected at the lowest dose levels in the dog and cynomolgus studies prevented a NOAEL to be established.

9.1.2.6. Ovaries

The effects in ovaries of dogs after at least 1 month of treatment with RPV were characterized by increased numbers of tertiary follicles, (cystic) luteinized follicles, and in some dogs, corpora lutea. These effects did not intensify over time after 3 months of dosing. The age of the dogs at the start of dosing in the 1-month studies was between 6.5 and 8 months. Probably, the still preadolescent age at the end of the 1-month studies has contributed to the more prominent effect in the test article-treated female dogs, compared to the controls that appeared still sexually

immature, than in the longer duration studies. In the 1-month studies, the ovarian effects were associated with changes in the other parts of the female genital tract and with the prominent activation of the mammary glands. It is conceivable that the mechanism of action of the effects on the ovaries in dogs is associated with the inhibition of CYP21 and CYP17 by RPV. But the exact mechanism of action of the observed early maturation (1-month studies) or activation of ovaries in the dog could not be evaluated from the general toxicity studies. Effects of RPV at the level of serum concentrations of progesterone and estradiol were difficult to evaluate due to the estrous cycle of the dogs.

The absence of ovarian effects in rats and immature cynomolgus monkeys provided no contribution to the establishment of the mechanism of the ovarian effect in dogs. The absence of the ovarian effects in the cynomolgus monkeys cannot be explained by lack of sensitivity of that species for the endocrine effects of RPV. The compound had a clear effect on adrenal CYP21 and CYP17 in that species concluded from the increases in serum progesterone and 17α-hydroxyprogesterone and from the reduction of DHEA and androstenedione. The more early stage of development of the monkeys (approximately 20 months of age at necropsy, at least 1 year prior to the onset of puberty) compared to the dogs (approximately 7 months of age at necropsy, a few months prior to the onset of puberty) may have contributed to the difference in response. However, a dog-specific effect cannot be excluded.

9.1.2.7. Testes

The Leydig cell changes seen in dogs only were characterized by bilateral diffuse swelling or hypertrophy noted at the end of the 1 and 12 months of treatment, and hyperplasia and hypertrophy after 3 and 6 months of treatment. The effects were minimal in the 1-month study, most prominent after 3 months of treatment, and again minimal after 6 and 12 months. It cannot be excluded that the minimal Leydig cell hypertrophy is associated with the effects of RPV on CYP21 and CYP17. However, the exact mechanism of action could not be evaluated in these general toxicity studies in which there were no effects on serum testosterone or LH. The absence of similar effects in mice and rats seems to indicate that the Leydig cell hypertrophy may be a dog-specific effect.

In addition to the Leydig cell effects, marginal findings were noted in the seminiferous tubules and secondary in the epididymides. The marginally increased focal unilateral atrophic tubules seen at the high dose after 3 and 6 months of treatment was associated with marginal cellular debris in the epididymides, in the mid- and the high-dose groups. Reviewing all the data on testes after completion of the development program, this effect and the reduced spermiogenesis leading to reduced numbers of spermatozoa in the epididymides of a single animal in the mid- and high-dose groups after 6 months of treatment are not considered of toxicological relevance. The main reasons for this conclusion are the similarity with background data and the lack of effects on spermiogenesis in the 12-month study upon staging of the spermatogenic cycles.

9.1.2.8. Red blood cells

In mice, rats, and dogs, RPV caused a small (hardly ever exceeding 10%) reversible reduction of red blood parameters in combination with regenerative signs. The effects usually occurred only in the high-dose groups in each of the studies with the indicated species. There were no clinical

signs or symptoms of anemia noted in any of the species. Since exposures at the high doses in the various species differed considerably, it seems that the effect on RBCs is not directly associated with exposure. Regeneration was evidenced by extramedullar hematopoiesis leading to increases in reticulocyte counts. The mechanism of action of the effect on red blood parameters could not be established in any species. However, there were no signs indicative of bone marrow suppression. The increased cellularity in bone marrow of female mice at the high dose is likely due to an increase of erythroid elements compensatory to the decrease of RBCs in the circulation. The increased myeloid/erythroid ratio in bone marrow of male mice is assumed due to an increase in myeloid precursor cells compensating the decrease of lymphocytes in the circulation. Moreover, in the mouse and rat carcinogenicity studies no effects were noted on bone marrow and hemopoietic cells.

9.1.2.9. Coagulation

In male rats, increased values for APTT and PT were noted in the 2-week pilot study with RPV at doses of 1500 mg/kg/day and higher, and in the 6-month study at all doses of RPV from 40 up to 400 mg/kg/day. The effects were not observed in the 1-month study with RPV at doses up to 160 mg/kg/day. The cause of this discrepancy is not known. However, it cannot be excluded that the development of the effect at lower doses takes more time than at higher doses. In other rat studies, APTT and PT were not determined. Increased values of intrinsic (APTT) and extrinsic (PT) coagulation times indicate a reduced efficacy of these major coagulation pathways. The relevance of this limited effect (less than a 30% increase) that was not associated with any bleeding, did not demonstrate a dose effect relationship, and which occurred in males only is questionable. Since the effect occurred also in the low dose, it contributes to the factors preventing the establishment of a NOAEL in rats.

9.1.3. TAF

The oral toxicity of TAF has been studied in mice, (TX-120-2006, TX-120-2007), rats (R990185, R990182, TOX-120-001), dogs (D990181, D990142, D990175, TOX-120-002), and monkeys (P2000114) for treatment periods up to 39 weeks. In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy vitamin D_3 and 25-hydroxyvitamin D_3 at doses in rats and dogs.

9.1.3.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs

administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

9.1.3.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1, 25 dihydroxy vitamin D_3 and 25-hydroxyvitamin D_3 in rats (≥25 mg/kg/day) and dogs (≥ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss but these changes were accompanied by a slight but significant decrease in serum 1, 25 dihydroxy vitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

9.1.3.3. Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

TAF had no discernible electrocardiograph effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%) which was associated with significant decreases in T3{29101}, {29104}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study.

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. These infiltrates were seen primarily in perivascular regions in the ciliary body, and less frequently in the choroid layer subjacent to the border of the peripheral retina with the ciliary body at the level of the ora ciliaris retinae (equivalent to the ora serrata in humans). Based on their bilateral nature and typical locations in perivascular regions of the ciliary body and junction of the choroid layer with the margin of the peripheral retina, these infiltrates were considered to be consistent with an increased incidence and/or size of minor, random perivascular infiltrates of mononuclear cells commonly associated with maintenance of the blood-eye barrier. No mononuclear cell infiltrates were observed in dogs following the 13 week recovery period, suggesting these are minor, dose-dependent, reversible changes in dogs associated with maintenance of the blood-eye barrier during dosing with no functional consequence. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. Distribution of 14[C]TAF to eyes has been assessed in mice, rats, and dogs (AD-120-2011,

AD-120-2020, and D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [^{14}C]-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes <8% that observed in plasma). Transient exposure to low levels of [^{14}C]-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [^{14}C]-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [^{14}C]-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The minimal infiltration of histiocytes observed in the eye of dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mitochondrial DNA levels up to 1 μM (approximately 2-fold higher than C_{max} after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (PC-120-2006). The active metabolite of TAF, tenofovir diphosphate is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, ATP (>10,000 fold) {4923}. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

9.1.4. FTC/RPV/TAF

Emtricitabine, RPV and TAF exhibit different patterns of target organ toxicity (see also Section 9.7). The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH in monkeys at large multiples of clinical exposure; therefore, these hematological findings are not considered relevant to clinical use. The principal target organs of toxicity following administration of RPV were the adrenal glands and associated steroid biosynthesis (mouse, rat, dog, and cynomolgus monkey), liver (mouse, rat, and dog), thyroid and pituitary glands (rat), kidney (mouse, dog), male and female reproductive organs (dog), the hematopoietic system (mouse, rat, and dog), and the coagulation system (rat). In general, effects were reversible. Effects on the liver, kidney, hematopoietic system, and coagulation system were mild and occurred at higher doses and exposures. The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone. Given that kidney effects (nephropathy) with RPV have been observed in mice and dogs at high doses, and that the routes of excretion differ for RPV (via feces) and TFV (renal excretion), renal toxicity is not anticipated to be an issue with the combination product. Given that pharmacokinetic interactions are unlikely based

on nonclinical (m2.6.4) and clinical (m2.7.2) data, and that the target organ profiles are different, administration of the combination product is unlikely to exacerbate known toxicities of the individual agents.

9.2. Genetic Toxicology

9.2.1. FTC

Emtricitabine was negative in the standard genotoxicity battery. There was no evidence of exacerbation of mutagenic potential in in vitro genotoxicity tests (Ames test and mouse lymphoma assay) with the combination of FTC and TDF as compared to the agents alone.

9.2.2. RPV

The potential of RPV to induce mutagenesis has been investigated in vitro in the Ames test and the mouse lymphoma assay, and in vivo in the mouse bone marrow micronucleus test. In the test systems, RPV as base or as the HCl salt was not able to induce mutations or chromosome damage at the highest feasible concentration or dose tested. The following is considered relevant for the interpretation of the in vitro genetic toxicity data.

Metabolism studies indicated that rat S9 mix used as metabolism enhancer in the Ames test and mouse lymphoma assay had a poor capacity to generate metabolites M30 and M31. These metabolites occur in man and are the result of a Michael addition to the [redacted] moiety of RPV. The metabolic pathway to M30 and M31 encompasses at least a reactive epoxide. Due to the poor capacity of rat S9 to generate M30 or M31, the reactive intermediate was not present in the Ames or mouse lymphoma assay; and therefore its potential to induce genotoxic effects was not tested. Since some batches of human S9 appeared to generate at least M30, an additional Ames test was done with human S9 as metabolism enhancer. The test results were optimized by selecting a batch of human S9 that yielded M30 in the Ames test at least at the approximate level of M30 detected in feces in the human metabolism study. Rilpivirine did not induce mutations under these test conditions. Moreover, RPV did not demonstrate a clastogenic or aneuploidic effect in bone marrow of mice and the concentration of M30 in mice was similar to that in man. This also indicates that the metabolic pathway with the potentially reactive intermediates was involved in this in vivo genotoxicity study.

The structures of metabolites M30 and M31 do not comprise any alerts for genotoxicity. In view of the absence of structural alerts and the absence of indications for genotoxicity in the Ames test with human S9 and the bone marrow micronucleus study in mice, neat genotoxicity testing of M30 and M31 was not considered necessary.

The [redacted] moiety of RPV generates a mutagenic alert. The drug substance impurities, 不純物G*, 不純物E*, and 不純物F*, contain the same moiety and were for that reason evaluated for their mutagenic potential. Only 不純物G*, tested as its HCl salt 不純物H*, increased the mutation frequency in the Ames test. 不純物G* contains also a [redacted], whereas the others do not. Therefore, it is concluded that the mutagenic potential of the [redacted] moiety is low and that the mutagenicity of 不純物G* is due to its [redacted] moiety.

The probability that genotoxic impurity 不純物G* could contribute significantly to an increased incidence of tumors in man upon administration of RPV is discussed in detail in the Nonclinical Overview (m2.4).

9.2.3. TAF

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli*), and a L5178Y gene mutation assay in mouse lymphoma cells. The in vivo evaluation consisted of a mouse bone marrow micronucleus study (at oral doses of 500, 1000, and 2000 mg/kg).

9.2.4. FTC/RPV/TAF

Emtricitabine, RPV and TAF were negative in genotoxicity studies. Therefore, the combination of the 3 components is not expected to have an altered genotoxicity profile as compared with that of the individual agents.

9.3. Carcinogenicity

9.3.1. FTC

Emtricitabine demonstrated low carcinogenic potential in conventional 2-year carcinogenicity studies at exposures that far exceeded (23- to 28-fold for FTC) human exposures at the therapeutic dose. It is considered unlikely that combination dosing would change this profile, as no exposure difference would be expected and no exacerbation of toxicity is expected.

9.3.2. RPV

The carcinogenic potential of RPV has been studied with the HCl salt by means of 2-year carcinogenicity studies in rats and in mice.

The exposures to RPV in the mouse study were as expected on the basis of the previous studies in that species. However, in the rat carcinogenicity, the exposure on Day 1 of the study was lower than anticipated on the basis of the results of the bridging study with the base and the HCl salt. The toxicokinetic data generated after 6 months of dosing in the rat study showed that exposure had declined during the study. In consultation with the US Food and Drug Administration (FDA) it was decided to continue the study and to keep the satellite animals for an additional sampling after 9 months of dosing. That sampling showed that exposure after 9 months of dosing was not different from that after 6 months of dosing. The outcome of the study in terms of a dose-related increase in neoplastic lesions illustrates that the study objectives were met. For that reason, the study is considered valid.

Hepatocellular adenomas and carcinomas are common spontaneous liver neoplasms in rodents {10217}. In general, hepatocarcinogens are divided into genotoxic and nongenotoxic agents {15694}. Since RPV is not genotoxic in a battery of in vitro and in vivo assays, the neoplastic lesions in liver observed in the mouse and rat carcinogenicity studies are considered a

consequence of a nongenotoxic mechanism of action of RPV, rather than an expression of a direct carcinogenic potential of the compound. For nongenotoxic carcinogens, several mechanisms of action have been reported for rodent liver neoplasm development, including phenobarbital-like cytochrome P450 induction {15695}. The increased liver weight, associated with signs of enzyme induction, caused by RPV was already evident in the repeat dose general toxicity studies. *Ex vivo* hepatic enzyme activity evaluated in the 3-month mouse study and the 6-month rat study showed that RPV caused strong induction of the CYP4A family in male and female mice and male rats; and induction of the CYP3A family in female rats (m2.6.4, Section 5.2.2.3). Electron microscopy of mouse liver noted peroxisome proliferation, a lesion commonly associated with CYP4A induction {15696}. This pattern of liver enzyme induction correlates well with the incidence of hepatocellular adenomas and carcinomas {15696}.

The repeat-dose toxicity studies in rats have demonstrated, without exception, effects on the thyroid gland often associated with effects in the pituitary gland, and on the thyroid hormones and TSH. All data point towards a mechanism by which RPV causes an increased clearance of thyroid hormones and a subsequently activated feedback on the thyroid gland by TSH synthesized in the pars distalis of the pituitary gland, rather than having a direct effect on thyroid hormone synthesis. Furthermore in rat liver, RPV caused induction of UDPGT, the most important enzyme involved in the metabolism and clearance of thyroid hormones. UDPGT induction is a well-known cause of increased thyroid hormone clearance and upon life-long occurrence associated with the development of follicular adenomas and carcinomas{15703}.

No neoplastic lesions were observed in adrenal glands in spite of the high affinity of RPV for this organ.

Taking into account the results of the genotoxicity and the carcinogenicity studies, it is concluded that RPV has not shown any potential to induce direct DNA-related effects. The relevance for man of the epigenetic carcinogenic effects on mouse and rat liver and rat thyroid gland is discussed in the Nonclinical Overview (m2.4).

9.3.3. TAF

No carcinogenicity studies were conducted with TAF. Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at TFV exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV-1 infection. At the high dose in female mice, liver adenomas were increased at exposures 16 times those observed in humans. In rats, the study was negative for carcinogenic findings at exposures up to 5 times those observed in humans at the therapeutic dose.

9.3.4. FTC/RPV/TAF

FTC and TDF have demonstrated low carcinogenic potential in conventional 2-year bioassays. Combination dosing with FTC/RPV/TAF is unlikely to alter this profile. Rilpivirine does not show any potential for direct DNA-related effects, and the relevance of the epigenetic carcinogenic effects on mouse and rat liver and rat thyroid are not considered relevant for humans.

Based on the overall lack of genotoxicity and the results of the 2-year carcinogenicity studies with the individual agents, the conduct of carcinogenicity studies with the FTC/RPV/TAF combination is not considered necessary nor would they add to the safety evaluation.

9.4. Reproductive Toxicity

9.4.1. FTC

There were no effects on fertility or embryo-fetal development when FTC was tested. Slightly longer oestrous cycles were observed in F_1 generation rats after exposure to high doses of FTC. No other significant effects were noted and NOELs were at exposures above human exposures.

9.4.2. RPV

The male and female rat fertility studies and peri- and postnatal developmental studies were conducted after the HCl-salt was chosen for further development. In view of the minor testicular effects of TMC278 in dogs characterized by marginal to limited Leydig cell hypertrophy, a separate male rat fertility study was conducted with up to the highest feasible dose of TMC278 (1500 mg/kg/day).

The reproductive and developmental toxicity studies did not demonstrate any effects on male or female fertility, fecundity, maternal behavior after parturition and during weaning, or development of offspring from dams treated with TMC278 during pregnancy and lactation. The results of the embryo-fetal developmental studies in rats and rabbits with the highest feasible exposures to TMC278 demonstrated the absence of a potential for teratogenicity at high exposures to the rabbit dams. The exposure to the rat dams elicited effects at the level of the thyroid gland, which were also found in general toxicity studies.

In toxicity studies with juvenile rats or with dogs and cynomolgus monkeys completed prior to the onset of adolescence/adulthood, no additional targets were noted and no increased sensitivity to the effects found in adult animals was evident.

9.4.3. TAF

Reproductive tissues were examined in repeat-dose toxicology studies in the rat, dog, and monkey. There were no treatment-related histologic alterations or changes in organ weights in the rat and the dog following chronic daily dosing, or in the monkey following 28 days of daily oral administration.

The TAF fumarate (GS-7340-03) oral rat fertility study (TX-120-2012) data indicate dose related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of GS-7340-02 up to 250 mg/kg/day (TX-120-2002), or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day (TX-120-2005). The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, $AUC_{0-\tau}$ values for TAF and TFV on Day 17 were 0.2 and 17.4 µg·h/mL, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, $AUC_{0-\tau}$ values for TAF and TFV on Day 20 were 11.0 and 27.3 µg·h/mL, respectively.

Tenofovir DF, but not TAF, has been tested in a peri/postnatal study. A perinatal-postnatal study of TDF showed increased pup mortality at maternally toxic doses ≥ 450 mg/kg/day (R990202). No TDF-related deaths occurred. The maternal NOEL of TDF was 50 mg/kg/day. The 150, 450, and 600 mg/kg/day dosages caused adverse clinical observations, reductions in body weight gain during gestation and increases in body weight gain during lactation and the 450 and 600 mg/kg/day dosages reduced gestation and lactation feed consumption values. The developmental NOEL was 150 mg/kg/day. The 450- and 600-mg/kg/day dosages resulted in increased peri/postparturn pup mortality, reduced pup survival and reduced pup body weights. The NOEL for general toxicity of TDF in the F_1 generation was 50 mg/kg/day (mortality occurred or body weights and/or feed consumption were reduced in the 150-, 450-, and 600-mg/kg/day dosage groups). The F_1 generation male and female NOEL for behavioral, reproductive, and developmental toxicity of TDF was 150 mg/kg/day (effects on sexual maturation were observed at dosages of 450 and/or 600 mg/kg/day).

No peri/postnatal study with TAF in rats is planned. Toxicokinetic data obtained following dosing of TAF to the rat clearly demonstrate an inability to measure plasma concentrations of the prodrug, TAF. Although significant plasma levels of the active drug, TFV, were obtained in the rat following chronic dosing with TAF, the TFV exposure was less than that already tested in the TDF peri/postnatal study (up to 15534 ng·h/mL via TAF compared to 27.3 µg·h/mL via TDF). As summarized above, TFV, delivered via TDF, has previously been assessed for peri/postnatal effects at exposures 100-times human exposure of TFV via TAF (0.273 µg·h/mL). The peri/postnatal study with TDF clearly characterized the potential postnatal toxicity of TFV.

9.4.4. FTC/RPV/TAF

Emtricitabine, RPV, TAF and TDF have not shown significant adverse effects in reproductive and developmental toxicity studies. The combination of the 3 components is not expected to have an altered reproductive toxicity profile compared with that of the individual agents. Slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses of TDF. The NOELs/NOAELs were at exposures above human exposures.

9.5. Local Tolerance

The FTC/RPV/TAF tablet is intended for oral use. No local tolerance studies were conducted for the FTC/RPV/TAF combination. Evaluation of local tolerance to the GI tract has been conducted during the repeat-dose oral toxicity studies with each of the individual agents. No GI toxicity was observed in the FTC, RPV, or TAF toxicity studies. Clinical trials indicate that the individual

agents, FTC/RPV/TDF, FTC/TDF-containing regimens, and FTC/TAF-containing regimens are generally well tolerated; the incidences of GI disorders were consistent with those expected in the subject population and the known safety profiles of the study drugs. The potential for additive effects between the three agents in the FTC/RPV/TAF FDC is minimal.

Local toxicity studies concluded that RPV was a moderately irritating to bovine eyes and nonirritating to rabbit skin. TAF fumarate was a noncorrosive/nonsevere eye irritant and nonirriating/noncorrosive to rabbit skin under semi-occluded conditions.

9.6. Other Toxicity Studies

9.6.1. FTC

There are no major human metabolites of FTC that require independent study. The identified metabolites have been assessed as part of the routine toxicology and qualification (of impurities) studies.

9.6.2. RPV

Rilpivirine did not show the potential to cause skin sensitization with repeated application to the dorsal surface of mouse ears. The RPV drug substance contains 3 impurities at levels that need qualification according to ICH Q3A. Two impurities, 不純物A* and 不純物B*, were qualified in an Ames test and a mouse lymphoma assay and in a 1-month oral rat study using RPV spiked with 4% of each of the impurities. None of the impurities induced a difference from the effects caused by TMC278.

The third impurity, 不純物C*, is the [redacted] isomer of RPV. This isomer was present in all drug substance batches involved in pivotal nonclinical studies at the level of minimally 0.61%. In view of the close structure relationship with RPV and the overage between the lowest nonclinical dose (5 mg/kg/day) and the clinical dose of 25 mg once daily, it is concluded that 不純物C* up to 7.44% of the daily dose of RPV is unlikely to incur untoward effects in man (average body weight 60 kg).

Three low level drug substance impurities, 不純物G*, 不純物E*, and 不純物F*, containing an [redacted] moiety were evaluated for their mutagenic potential. Only 不純物G*, tested as its HCl salt 不純物H*, induced a mutagenic response and a clastogenic response. It is concluded that the mutagenic potential of the [redacted] moiety is low and that the genotoxicity of 不純物G* is due to its [redacted]. The genotoxic impurity 不純物G* is controlled at a level well below that associated with the threshold for toxicological concern (TTC) {15703} for a daily intake of more than 12 months. The TTC is 1.5 µg/day for adults. With a daily dose of 25 mg RPV, the maximally allowable level of the impurity should be lower than [redacted] ppm. The actual level is well below [redacted] ppm.

9.6.3. TAF

TAF fumarate did not show the potential to cause skin sensitization with repeated application to the dorsal surface of mouse ears. Investigative toxicology studies were conducted in rats to examine the potential bone toxicity to TAF. The studies support the conclusion that TAF is not a direct bone toxin and that the bone loss observed may be secondary to treatment related perturbations in phosphate homeostasis.

No specific studies with TAF metabolites were conducted as there were no unique human metabolites in humans with TAF. The by-products formed during conversion of TAF to TFV are [redacted], L-alanine, and phenol and are formed at nontoxic levels.

Two 1-month impurity qualification studies were conducted in rats to evaluate potential drug substance impurities. In the first study, administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day. No test article related findings were noted, and no differences were found in the 2 lots tested. The NOAEL was considered 50 mg/kg/day (40 mg f.b.e./kg/day). In the second study, administration of 98% pure GS-7340-03 containing 11 spiked impurities and 97.8% pure GS-7340-03 containing 4 spiked impurities by oral gavage for 29 days to male and female rats resulted in no significant clinical or histopathological differences compared to administration of 99.3% pure GS-7340-03. The NOAEL was 50 mg f.b.e./kg/day.

9.6.4. FTC/RPV/TAF

Rilipivirine and TAF show low potential to cause contact sensitization. There are no new impurities or new degradants that require further qualification in the FTC/RPV/TAF bilayer tablet.

9.7. Comparative Exposures and Exposure Margins

Cross-species comparisons of exposure, relative safety margins, and the relevance of these effects for human health are discussed in m2.4, Section 4.9.2.

9.8. Conclusions

Comprehensive nonclinical toxicology programs were conducted with FTC, RPV, and TAF. The data are adequate to support the safety of the FTC/RPV/TAF FDC based on the following considerations.

Administration of TAF in combination with FTC and RPV is unlikely to exacerbate known toxicities of FTC and RPV. FTC, RPV and TAF have no clinically-significant overlapping toxicity. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH in monkeys at large multiples of clinical exposure (137-fold in mice; 20-fold in monkeys). Slight but statistically significant decreases (-11%) in several red blood cell parameters were also noted in male and female rats administered TAF at a dose level of 400 mg/kg/day for 28 days. Because hematological changes were slight and observed at high multiples of the clinical dose, effects are

not considered relevant to clinical use. The principal target organs of toxicity following administration of RPV were the adrenal glands and associated steroid biosynthesis (mouse, rat, dog, and cynomolgus monkey), liver (mouse, rat, and dog), thyroid and pituitary glands (rat), kidney (mouse, dog), male and female reproductive organs (dog), the hematopoietic system (mouse, rat, and dog), and the coagulation system (rat). In general, effects were reversible. Effects on the liver, kidney, hematopoietic system and coagulation system were mild and occurred at higher doses and exposures.

Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT (TQT) study in healthy subjects (see m2.6.2, Section 4.2.2). At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the cardiovascular effect seen with RPV alone. Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with bone and renal toxicity and included significant decreases in T3 {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.8, D2000006) or in the thorough QT study (m2.7.2, Section 2.3.2.2.1 [Study GS-US-120-0107]). No additional studies are considered necessary with the FTC/RPV/TAF combination.

The principal target organs of toxicity following oral administration of TAF were the kidney (karyomegaly, tubular degeneration) and bone. Given that kidney effects with RPV have been observed in mice and dogs at high doses, and that the routes of excretion differ for RPV and TAF, (RPV is primarily excreted via veses; TAF in metabolized intracellularly; TFV is excreted unchanged in the urine via glomerular filtration and tubular sectetion) renal toxicity is not anticipated to be an issue with the combination product. Given that pharmacokinetic interactions are unlikely and that the target organ profiles are different, administration of the combination product is unlikely to exacerbate known toxicities of the individual agents.

Adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa was observed after administration of TAF to mice. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is considered low. A minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in dogs administered the highest dose of TAF in the 39-week dog toxicity study. The ocular infiltrates were considered to be consistent with an increased incidence and/or size of minor, random perivascular infiltrates of mononuclear cells commonly associated with maintenance of the blood-eye barrier. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

NRTIs currently carry a class labeling for mitochondrial toxicity; however, both FTC and TAF have shown a low potential for mitochondrial toxicity in in vitro studies and in repeat-dose toxicity studies up to 39 weeks in duration. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinically relevant conditions. The potential for mitochondrial toxicity with the FDC is low.

Emtricitabine, RPV and TAF had no positive findings in genotoxicity assays. The FTC/RPV/TAF combination is not anticipated to alter the genotoxicity profile of the individual agents. FTC and TDF have demonstrated low carcinogenic potential in conventional 2-year bioassays. Combination dosing with FTC/RPV/TAF is unlikely to alter this profile. Rilpivirine does not show any potential for direct DNA-related effects, and the relevance of the epigenetic carcinogenic effects on mouse and rat liver and rat thyroid are not considered relevant for humans.

Emtricitabine, RPV, and TAF have not shown significant adverse effects in reproductive and developmental toxicity studies, and the combination of the 3 components is not expected to have an altered reproductive toxicity profile compared with that of the individual agents. Slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses of TDF. The NOEL and NOAEL have been clearly identified. Neither TDF nor TAF showed an effect on rabbit fetuses. FTC has not shown any adverse effects in reproductive and developmental toxicity studies. No additive effects are anticipated with the three-drug combination.

The FTC/RPV/TAF FDC is not anticipated to produce any new human metabolites. Identified impurities and degradants have been assessed as part of the routine toxicology or qualification studies with the individual agents.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008) and with the United States Food and Drug Administration (FDA) Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the FTC/RPV/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases on these drugs strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the extensive clinical safety data available from the clinical trials with the FTC/RPV/TDF FDC, and FTC/TAF containing regimens support the safety of the FTC/RPV/TAF FDC for the treatment of HIV-1 infection.

10. TABLES AND FIGURES

The tables and figure have been incorporated into the text.

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