Janssen Research & Development

Pharmacokinetics Written Summary

MODULE 2.6.4

Rilpivirine Long-Acting

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＊新薬承認情報提供時に置換

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LIST OF ABBREVIATIONS

ARV	antiretroviral
AUC_{0-t}	area under the plasma concentration versus time curve from time 0 to time 't'
BSA	bovine serum albumin
CA	citric acid
CHO	Chinese Hamster ovary
cDNA	complementary deoxyribonucleic acid
Cl_b	blood clearance
Cl_p	plasma clearance
C_{max}	maximum plasma concentration
CN-	nitrile
[redacted]	[redacted]
CYP	cytochrome P450
D50	diameter 50
DMSO	dimethyl sulfoxide
DOSS	dioctyl sodium sulfosuccinate
EC_{50}	50% effective concentration values
EDTA	ethylenediamine tetra-acetic acid
EMA	European Medicines Agency
F004	clinical formulation containing 100 mg/mL RPV LA in P338
F006	clinical formulation containing 300 mg/mL RPV LA in PS80
F_{abs}	absolute bioavailability
FDA	Food and Drug Administration
F_{rel}	relative bioavailability
G001	clinical formulation containing 300 mg/mL RPV LA in P338
GD	gestation day
GLP	good laboratory practices
GST	glutathione S-transferase
HCl	hydrochloride
HIV-1	human immunodeficiency virus type 1
HLM	human liver microsomes
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl-methylcellulose
IC_{50}	concentration resulting in 50% of maximum inhibition
IM	intramuscular
IV	intravenous
K_i	inhibition constant
K_m	substrate concentration
LA	long-acting
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LLOQ	lower limit of quantification
LSC	liquid scintillation counting
MATE	multi-antimicrobial extrusion protein
MBI	mechanism-based inhibition
MRM	multiple reaction monitoring
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate

NMR	nuclear magnetic resonance
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRS	NADPH regenerating system
NZW	New Zealand white
OCT2	organic cation transporter 2
OECD	Organization for Economic Co-operation and Development
P338	poloxamer 338
PE	polyethylene
PEG400	polyethylene glycol 400
P-gp	P-glycoprotein
PI	pre-incubation
PopPK	population pharmacokinetic
PS80	polysorbate 80
PVP	polyvinylpyrrolidine
QC	quality control
QWBA	quantitative whole-body autoradiography
RLG	radioluminography
RPV	rilpivirine
SC	subcutaneous
SCN-	thiocyanate
SPE	solid phase extraction
TEA	tetra ethyl ammonium
t_{max}	time to reach the maximum plasma concentration
TPGS	D-α-tocopheryl Polyethyleneglycol 1000 succinate
TR	total radioactivity
UDP-GT	uridine diphosphate-glucuronosyltransferase
ULOQ	upper limit of quantification
US	United States
Vd_{ss}	volume of distribution at steady-state
Vit E	vitamin E
V_{max}	maximum rate achieved

1. BRIEF SUMMARY

Rilpivirine (RPV, previously known as TMC278, JNJ-16150108 or R278474), a diarylpyrimidine derivative, is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) with *in vitro* activity against wild-type human immunodeficiency virus type 1 (HIV-1) and NNRTI-resistant mutants. Rilpivirine is available as a 25 mg oral tablet, which has been approved for the treatment of HIV-1 infection in antiretroviral (ARV) treatment-naïve adult patients in multiple countries including Europe (EMEA/H/C/002264), the United States of America, Canada, and Japan as EDURANT®.

Janssen Sciences Ireland UC in partnership with ViiV Healthcare Company are developing the RPV Long-Acting (LA) + Cabotegravir (CAB) LA injectable regimen for the treatment of HIV-1 infection. The overall objective of the CAB + RPV clinical program is to develop a novel, highly effective, well tolerated 2-drug intramuscular (IM) injectable regimen LA administration for the treatment of HIV-1 infection. This Marketing Authorization Application (MAA) focuses on the RPV LA component of this 2-drug regimen. CAB will be the subject of a separate, parallel, MAA, submitted by ViiV Healthcare. Janssen is the sponsor of the RPV LA development and manufacturing program; ViiV Healthcare is the sponsor of the CAB + RPV clinical program.

The present summary reviews the available data of RPV after administration as an IM injection. In addition, all the relevant nonclinical pharmacokinetic studies of RPV on distribution, metabolism and excretion performed for the EDURANT® registration (i.e., oral tablet) are also included.

After IM injection of RPV LA, with focus on the final clinical formulation G001 containing 300 mg RPV base/mL suspension and poloxamer 338 (P338; JNJ-4360418; 50 mg/mL), the pharmacokinetics of RPV has been studied in rabbits, dogs and minipigs. Distribution studies were conducted in rats and rabbits, and a pharmacokinetic study after administration of RPV LA and CAB LA was performed in rats. During the development of RPV LA, two other formulations were also administered in clinic i.e., the P338 (25 mg/mL)-containing formulation F004 (100 mg RPV base/mL suspension), and the polysorbate 80 (PS80)-containing formulation F006 (300 mg RPV base/mL suspension). Other formulations including F004 and F006 were also tested in animals after IM or subcutaneous (SC) administration and some studies were performed on the genotoxic impurity related substance D* of RPV. In addition, supportive pharmacokinetic/toxicokinetic studies on P338 were conducted after IM or oral administration of P338 or IM administration of RPV LA; these studies are described in Section 8.

For the registration of EDURANT®, RPV has been examined in both in vitro and in vivo test systems. The relevant information on the distribution, metabolism and excretion are included in this summary to support overall conclusions on RPV LA.

All pharmacokinetic studies were conducted in accordance with best scientific principles. Pivotal studies were conducted in compliance with the United States (US) Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations (21 CFR Part 58) and Organization for Economic Co-operation and Development (OECD) principles of GLP

(Directive 2004/10/EC) in OECD-adherent countries. All conducted studies are listed in Mod2.6.5.1/Pharmacokinetics Overview Table.

The following convention is applied throughout this Module: reference is made to 'RPV' when the hydrochloride (HCl) salt was administered and to 'RPV base' when the free base was administered. The RPV LA formulation contains the RPV base form. The dose or concentration is always given as base equivalent and the appropriate correction factor was used when the HCl salt was administered.

- **RPV LA**

In rabbits and minipigs, after a single IM administration of RPV LA as the P338-containing formulation (G001), the RPV release was fast, after which mean plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after 3 months. The absolute bioavailability (F_{abs} after 3 months) is 67% in rabbits at 150 mg/kg and ranges between 35 and 62% in minipigs at 600 mg, indicating the release from the depot was still incomplete after 3 months.

Several studies were performed in rabbits and minipigs, mainly comparing different P338 containing formulations and the final selected G001 formulation. No relevant changes in plasma profiles across studies were observed.

After repeated oral administration of RPV in various animal species used in the toxicology studies compared to IM administration of RPV LA in patients, the highest maximum plasma concentration (C_{max}) ratio (animal/human) of RPV was around 406 in female mice and 252 in male mice, 82 in female rats and 39 male rats, 123 in pregnant rabbits, 33 in dogs, and 2 in female monkeys. The highest $AUC_{0\text{-day}28}$ ratio (animal/human) of RPV was around 258 in female mice, 170 in male mice, 82 in female rats, 25 in male rats, 78 in pregnant rabbits, 22 in dogs, and 2 in female monkeys. After IM administration of RPV LA in minipigs and dogs compared to IM administration of RPV LA in patients, the C_{max} ratios (animal/human) were around 2 and 10 and $AUC_{0\text{-day}28}$ (animal/human) ratios were around 0.6 and 5, respectively.

In rabbits, at the administration site after a single IM administration of RPV LA (150 mg/kg; G001) at the end of a 1-month follow-up period, the RPV concentrations were high (1456-fold) compared those in the contralateral side. In the lymph nodes, the RPV concentrations were similar between the injection and contralateral site except for one rabbit for which higher concentrations were seen in the accessory popliteal lymph nodes of the injection side. In rats, after a single IM administration of RPV LA (60 mg/kg; G001), the highest exposures of RPV were measured in the left popliteal and medial iliac lymph nodes adjacent to the injection site with tissue/plasma $AUC_{0\text{-day}42}$ ratios of 12,203 and 2256, respectively. In the contralateral right popliteal and medial iliac lymph nodes, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 6.7 and 2.6, respectively. In the kidney, adrenal glands, lungs, liver, and pancreas, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 3.7, 3.2, 1.5, 1.5 and 1.2, respectively. The tissue/plasma $AUC_{0\text{-day}42}$ ratios were lower than 1 in brain (0.4), heart (0.8), spleen (0.97) and thymus (0.87).

In rats, following single IM administration at 60 mg/kg of RPV LA (G001) alone or in combination with a LA injectable suspension of GSK1265744A (CAB) at 10 mg/kg, the plasma concentrations of RPV were comparable for the 2 groups and the mean C_{max} and $AUC_{0\text{-}1444h \text{ or } 2months}$ values of RPV were similar.

In addition, other studies were performed after administration of RPV LA at lower concentration of RPV containing P338 or with PS80 after IM or SC in different species. A faster increase of the RPV concentrations were observed after administration of a P338 containing formulation compared to a PS80 containing formulation.

Some studies were performed in rats on the in vitro metabolism or in vivo after single IM injection of related substance C, a genotoxic impurity of RPV. It could be concluded that in the rat, related substance D is rapidly metabolically cleared and that the main metabolite pathways are sulfate conjugation and loss of the nitrile (CN-) function. Some pharmacokinetic and toxicokinetic studies were conducted after oral or IM administration of P338 in rats, rabbits and after IM administration of RPV LA in minipigs. Measurement of P338 was also performed in plasma samples from a clinical study (Mod5.3.1.2/TMC278LAHTX1001) after single IM administration of RPV LA (300 mg/mL in 50 mg/mL P338; 2-mL injection). After oral administration in rats and rabbits, no or very limited absorption of P388 was observed. After IM administration of P338 or RPV LA (G001) in rabbits, minipigs and human, the P338 release was fast, after which plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after at least 672 h.

- **From RPV oral (EDURANT®)**

In rats, tissue distribution of ^{14}C-RPV and its metabolites after single oral dose was rapid and extensive. The highest concentrations of radioactivity were measured in the liver, adrenal gland, brown fat and kidney. There was no evidence of undue retention and there were no indications of irreversible binding of RPV and its metabolites to melanin. In pregnant rats, there was distribution of ^{14}C-RPV to the placenta and the fetus. Total radioactivity (TR) exposure values in the placenta and in whole fetus were 0.94- and 0.64-fold those of maternal blood, respectively.

Rilpivirine is highly bound to plasma proteins and this is independent of the concentration and species. In the various animal species and human, plasma protein binding ranged from 99.08% to 99.97%. Rilpivirine is highly bound to human albumin and to a much lesser extent to α_1-acid glycoprotein. The distribution of RPV to red blood cells is limited in all species.

Some differences were seen in clearance across species. In rats, blood clearance (Cl_b) of RPV is moderate whereas in rabbits, dogs and monkeys it is low compared to the hepatic blood flow. The volume of distribution at steady-state (Vd_{ss}) was larger in rats, dogs and monkeys and very low in rabbits.

Rilpivirine is metabolized by Phase I and Phase II pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation and CN- split-off followed by reduction/oxidation, whether or not in combination with secondary pathways such as

glucuronidation, dehydration and catabolism of the glutathione conjugate. In mice, oxidation of RPV and to a lesser extent glutathione conjugation were the predominant pathways. In rats, the glutathione conjugation pathway was the predominant pathway whereas in dog and man, oxidation of RPV was the predominant one. No unique human metabolites were observed. In plasma of animals and human, unchanged RPV was more abundant than any metabolite. After repeated oral administration of RPV for 11 days in healthy subjects at 75 and 300 mg q.d, there was no disproportionate increase in exposure of any of the relevant metabolites compared to the parent compound exposure.

In all animal species and human, the predominant route of excretion was via feces (>85%). Renal excretion of TR was very limited (0.45% to 6.1% of the dose) in all animal species and human and the amount of unchanged RPV in urine was negligible. In rats, biliary excretion was limited (18%-25% of the dose) and the amount of unchanged RPV in bile was negligible. In rats, there was indication that RPV was excreted in milk.

In vitro, the cytochrome P450 (CYP) 3A4 isoenzyme plays a major role in the biotransformation of RPV. Rilpivirine might be a very weak inducer of CYP1A2 and CYP2B6 and a moderate inducer of CYP2C19 and CYP3A4. Ex-vivo induction studies in rodents showed that RPV is an inducer of the CYP3A-family (up to 1.7-fold in mice at 320 mg/kg and up to 6-fold in rats at 400 mg/kg) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, RPV induced uridine diphosphate-glucuronosyltransferase (UDP-GT) activity in mice (up to 2.3-fold at 320 mg/kg) and to a lesser extent in rats (up to 1.3-fold only at 400 mg/kg in males). In dogs, treatment with RPV did not result in any enzyme induction.

Rilpivirine is an inhibitor of CYP2C8 (inhibition constant (K_i) = 10μM) and CYP2C9 (K_i = 1.7 μM) in vitro whereas no inhibition is expected in vivo. In human liver microsomes (HLM), the limited mechanism-based inhibition (MBI) of CYP2C9 is unlikely to have clinical relevance at therapeutic doses of RPV.

Rilpivirine was shown to have P-glycoprotein (P-gp) inhibitor properties with an apparent concentration resulting in 50% of maximum inhibition (IC_{50}) value of 9.2 μM (3.4 μg/mL). Inhibition of the organic cation transporter 2 (OCT2) by RPV was evaluated in vitro. The in vitro IC_{50} for inhibition of OCT2 by RPV was 5.46 μM (2.0 μg/mL). The inhibition of multi-antimicrobial extrusion protein (MATE)-mediated transport by RPV was investigated in vitro in Chinese Hamster ovary (CHO) cells overexpressing MATE-1 and MATE-2K. The uptake of ^{14}C-Tetra Ethyl Ammonium (TEA) was inhibited by Rilpivirine with an IC_{50} value of 7.51 μM (2.75 μg/mL) for MATE-1 and of <0.05 μM (<0.018 μg/mL) for MATE-2K. In conclusion, the effect of RPV on MATE-1 is unlikely to be clinically relevant, but it cannot be excluded that RPV would inhibit MATE-2K at clinically relevant concentrations.

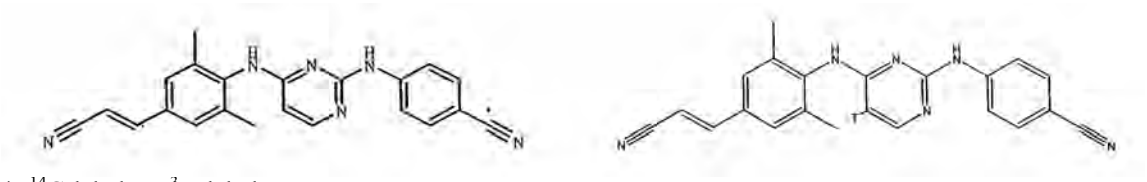
2. METHODS OF ANALYSIS

2.1. Rilpivirine

2.1.1. Radiolabeled Rilpivirine

A large number of studies, originally conducted to suppport the registration of EDURANT® and rediscussed in this summary for RPV LA, were conducted with radiolabeled RPV. Two radiolabeled (^{14}C and ^{3}H) RPV compounds were used but most of the studies were performed with ^{14}C. The ^{14}C atom was on the CN- carbon of the benzonitrile of the RPV molecule (Figure 1). The original material had a radiochemical purity of 98.2% and a specific activity of 2.03 GBq/mmol. The ^{3}H atom was in the pyrimidine moiety of RPV molecule. The radiochemical purity was 99.7% and the specific activity was 8.07 TBq/mmol (Figure 1), and was used in one study to investigate plasma protein binding and distribution in blood.

Figure 1: Structural Formula of ^{14}C-RPV (Left) and ^{3}H-RPV (Right)



*: ^{14}C-label; T: ^{3}H-label

The metabolic stability of the ^{14}C label of RPV was investigated following a single oral dose of ^{14}C-RPV at 10 mg/kg in Sprague-Dawley rats (Mod4.2.2.1/R278474-FK4686). The recovery of $^{14}CO_2$ from the expired air collected for 25 hours after administration was negligible. This indicates that the ^{14}C label is metabolically stable.

2.1.2. Radiochemical Methods

The following techniques were used:
- Tissue distribution of TR was studied by quantitative whole-body autoradiography (QWBA) in male pigmented rats and pregnant female Sprague-Dawley rats (see Section 4.1.1.1). The concentration of radioactivity in the different tissues was determined by radioluminography (RLG), whereas the concentration of radioactivity in the eye and in biological fluids (blood and plasma) was determined by liquid scintillation counting (LSC).
- TR in biological samples was measured by LSC, using appropriate scintillation cocktails. Aliquots of biological fluids were counted directly (plasma, urine and bile) following extraction or combustion (blood and feces residues).

In metabolism and/or mass balance studies, unchanged compound and/or its major metabolites were determined in various biological samples (plasma, urine, bile and feces). Mass balance was based on the recovery of radioactivity from various samples or pools of samples. In in vitro and in vivo studies with ^{14}C-labeled RPV, metabolite profiles were determined by radio-high performance liquid chromatography (HPLC). Metabolite identification was done by a combination of liquid chromatography with tandem mass spectrometry (LC-MS/MS) and co-chromatography with synthesized metabolites (see Sections 5.1 and 5.4.1).

2.1.3. Bioanalytical Methods

Bioanalytical methods were developed to support the RPV and RPV LA toxicokinetic and pharmacokinetic program. Methods were all based on the same detection technique, i.e. tandem mass spectrometry.

An LC-MS/MS method was validated for the determination of RPV in mouse, rat, rabbit, dog, minipigs and monkey ethylenediamine tetra-acetic acid (EDTA) plasma and dog heparin plasma. Tissue samples were analyzed with qualified research methods based on the validated plasma methods. The validation data for LC-MS/MS methods (heparin and EDTA plasma) are summarized below and details are outlined in the respective method validation reports.

In-study validation was conducted for nonclinical GLP studies. These validation data are appended to the individual preclinical study reports (Mod4.2.2.1/R278474-FK4240, Mod4.2.2.1/R278474-FK4170, Mod4.2.2.1/R278474-BA104, Mod4.2.2.1/R278474-FK4169, Mod4.2.2.1/TMC278-NC298 [BA1061], Mod4.2.2.1/TMC278-NC273 [BA1062]).

LC-MS/MS Methods

The LC-MS/MS method for mouse, rat, rabbit and dog plasma consisted of a solid phase extraction (SPE) followed by reversed phase HPLC coupled to tandem mass spectrometry. Plasma aliquots of calibration standard, quality control (QC) and unknown samples were buffered with 1 M [redacted], spiked with internal standard and applied onto the SPE column. After a washing procedure with [redacted], 1 M [redacted] and [redacted], the analyte was eluted with [redacted]% (98:2, v/v). The extract was evaporated to dryness under nitrogen and the residue was reconstituted in the mobile phase.

For mouse, rat and dog plasma, the LC-MS/MS assay was performed on a C18-[redacted] µm ([redacted] mm I.D. x [redacted] mm) column at a flow rate of [redacted] mL/min; the mobile phase being a mixture of 0.01 M [redacted] (pH [redacted] with [redacted]) in [redacted]/[redacted]. Quantitative LC-MS/MS analysis was carried out on an [redacted] MS/MS instrument with [redacted] ionization (positive ion mode). RPV was monitored at transitions m/z [redacted] to [redacted] and the stable isotope labeled internal standard [redacted] at transitions m/z [redacted] to [redacted].

For rabbit plasma isocratic chromatographic separation was achieved on a [redacted] µm C18 [redacted] ([redacted] mm I.D.x [redacted] mm) column at a flow rate of [redacted] mL/min; the mobile phase being a mixture of 0.01 M [redacted] (pH [redacted] with [redacted]) in [redacted]/[redacted]. Quantitative LC-MS/MS analysis was carried out on an [redacted] MS/MS instrument with [redacted] ionization (positive ion mode). The following transitions were monitored m/z [redacted] to [redacted] and m/z [redacted] to [redacted] for RPV and the internal standard [redacted] (structure analogue), respectively.

The LC-MS/MS assay for minipig plasma consisted of protein precipitation, followed by reversed phase HPLC coupled to tandem mass spectrometry. Plasma aliquots of calibration standard, QC and unknown samples were spiked with internal standard and precipitated with [redacted]. The assay was performed on a C18-[redacted] µm ([redacted] mm I.D. x [redacted] mm)

column; the mobile phase being a mixture of 0.01 M [redacted] (pH [redacted] with [redacted]) in [redacted]. Quantitative LC-MS/MS analysis was carried out on an [redacted] MS/MS instrument with [redacted] ionization (positive ion mode). Rilpivirine was monitored at transitions m/z [redacted] to [redacted] and the stable isotope labeled internal standard [redacted] at transitions m/z [redacted] to [redacted].

The LC-MS/MS assay for monkey plasma was based on purification by protein precipitation with [redacted]. The LC-MS/MS assay was performed on a C18- [redacted] µm ([redacted] mm I.D. x [redacted] mm) column; the mobile phase being a mixture of 0.01 M [redacted] (pH [redacted] with [redacted]) in [redacted]. Detection occurred on an [redacted] MS/MS instrument with [redacted] ionization (positive ion mode). The following multiple reaction monitoring (MRM) transitions were monitored: m/z [redacted] to [redacted] for RPV and m/z [redacted] to [redacted] for the stable isotope labeled internal standard [redacted].

For the LC-MS/MS assays a fixed calibration range was defined for each species. Table 1 presents the anticoagulant, sample volume and effective linear range for the analysis of animal heparin or EDTA plasma.

Table 1: Concentration Range, Anticoagulant and Sample Volume for Validated LC-MS/MS Methods

Species	Anticoagulant	Volume (mL)	RPV LLOQ-ULOQ (ng/mL)
Mouse	EDTA[b]	0.05	2.00 - 4000
Rat	EDTA[a]	0.1	1.00 - 2000
Rabbit	EDTA[b]	0.1	1.00 - 2000
Dog	EDTA[a]	0.1	1.00 - 2000
	Heparin[b]	0.1	1.00 - 2000
Minipig	EDTA[b]	0.05	1.00 - 2000
Monkey	EDTA[b]	0.05	1.00 - 2000

[a] Full validation; [b] Partial validation
LLOQ: lower limit of quantification; ULOQ: upper limit of quantification

The selectivity of the LC-MS/MS assay towards endogenous compounds was proven in six different batches of non-pooled blank EDTA or heparin plasma.

The inter-batch accuracy and inter-batch precision were calculated by comparing the theoretical concentration with the mean measured concentration for sets of QC samples at 4 concentrations (lower limit of quantification (LLOQ), Low, Medium and High). The accuracy was within the criteria of 80 to 120% at the LLOQ QC level and 85 to 115% at the other levels. The inter-batch precision of the LLOQ QC was ≤20% and ≤15% at the other levels. Detailed information on the accuracy and precision can be found in the method validation reports.

Stability

The stability of RPV was assessed in the stock solution solvent (methanol) and in biological matrices (both heparin and EDTA blood and plasma at several temperatures). The test article was found not to be stable in daylight. When RPV is exposed to daylight, the drug is transformed to

the Z-isomeric form. Therefore, each assay was carried out under yellow light conditions and samples were protected from light.

RPV was stable in methanol for at least 6 months after storage in a freezer (-18°C), for 1 month in a refrigerator (4°C ± 2°C) and for 3 days at room temperature (Mod4.2.2.1/R278474-FK4170) (yellow light conditions).

All the conditions in which RPV was found to be stable in blood, plasma and processed QC samples, are detailed in Table 2.

Table 2: Long- and Short-Term Conditions Under Which RPV is Found to be Stable

Species	Short-term storage		Long-term storage	Processed QC samples
	Blood	Plasma	Plasma	
Mouse (EDTA)	2 h at refrigerator temp. 2 h at room temp. 2 h at 37°C	24 h at RT 3 freeze/thaw cycles	914 days in freezer	3 days
Rat (EDTA)			581 days in freezer	2 days
Dog (EDTA or heparin)			1085 days in freezer	5 days
Rabbit (EDTA)	2 h at refrigerator temp. 4 h at room temp. 2 h at 37°C		1119 days in freezer	6 days
Minipig (EDTA)	2 h on melting ice 4 h at room temp. 2 h at 37°C		343 days in freezer	6 days
Monkey (EDTA)	2 h on melting ice 4 h at room temp. 2 h at 37°C		361 days in freezer	2 days

EDTA: ethylene diamine tetra-acetic acid; RT: room temperature

2.2. Poloxamer 338

Bioanalytical methods were developed to support the P338 toxicokinetic and pharmacokinetic studies. Plasma assays for P338, used to support GLP studies were validated according to US FDA guidance and European Medicines Agency (EMA) guideline on bioanalytical method validation [1, 2]. It was necessary to expand the acceptance criteria for this assay by 5% due to the complexity of this analyte and the challenges encountered during assay development. The analyte is a polymer that undergoes in source fragmentation and multiple transitions are monitored and summed to obtain the required sensitivity, which increases assay variability. An LC-MS/MS method was validated for the determination of P338 in rat (Mod4.2.2.1/BA13148 [304191]) and rabbit (Mod4.2.2.1/BA13146 [304196]) EDTA plasma at [redacted] ([redacted], [redacted], UK).

Detailed information on the method validations for the analysis of P338 is described in the individual validation reports which are referenced in Mod2.6.5.1.

The validated LC-MS/MS method for rat and rabbit plasma consisted of a protein precipitation step followed by reversed phase HPLC coupled to tandem mass spectrometry. Plasma aliquots of calibration standard, QC and unknown samples were spiked with internal standard and precipitated with acetonitrile.

Chromatography was on an [redacted] ([redacted] x [redacted] mm, [redacted] µm) column at a flow rate of [redacted] mL/min; the aqueous mobile phase being 0.01M [redacted] + [redacted] % [redacted] and [redacted] (10/90, v/v) as organic mobile phase. Mass spectrometric detection was on an [redacted] MS/MS instrument with [redacted] ionization (positive ion mode).

Table 3 presents the anticoagulant, sample volume and assay range for rat and rabbit.

Table 3: Calibration Range, Anticoagulant and Sample Volume for Validated LC-MS/MS Methods for P338

Species	Anticoagulant	Volume (mL)	LLOQ-ULOQ (µg/mL)
Rat	EDTA[a]	0.025	1.00 - 100
Rabbit	EDTA[a]	0.025	1.00 - 100

[a] Full validation
LLOQ: lower limit of quantification; ULOQ: upper limit of quantification

The inter-batch accuracy and inter-batch precision were calculated by comparing the theoretical concentration with the mean measured concentration for sets of QC samples at 4 concentrations (LLOQ, Low, Medium and High). The accuracy was within the criteria of 75 to 125% at the LLOQ QC level and 80 to 120% at the other levels. The inter-batch precision of the LLOQ QC was ≤25% and ≤20% at the other levels. Freeze/thaw-, benchtop- and long-term stability was proven. Detailed information on all validation experiments can be found in the method validation reports.

For non-GLP studies, qualified LC-MS/MS methods were used for the determination of P338 at Janssen's internal Bioanalysis lab. These are scientifically sound methods with documented preset acceptance criteria and QC samples for batch acceptance. These methods used the same principle for quantification of P338 as the assays validated at [redacted]. In-study validation data are maintained in the raw data. In one study, the same qualified assay was used to document exposure in human plasma. The range for each assay is documented in the respective study data. The LLOQ in plasma ranged between 0.075 – 1.00 µg/mL. A qualified method was also used for tissues and the LLOQ ranged between 1.00 - 5.00 µg/g.

3. ABSORPTION

3.1. Absorption

Studies in which the final clinical formulation for IM administration (i.e. RPV 300 mg/mL, P338 50 mg/mL (G001)) was used, are described below.

3.1.1. Studies in Mice

No studies were performed in mice with the G001 formulation. Studies performed with other formulations are described in Section 8.1.1.

3.1.2. Studies in Rats

No studies were performed in rats with the G001 formulation. Studies performed with other formulations are described in Section 8.1.2.

3.1.3. Studies in Rabbits

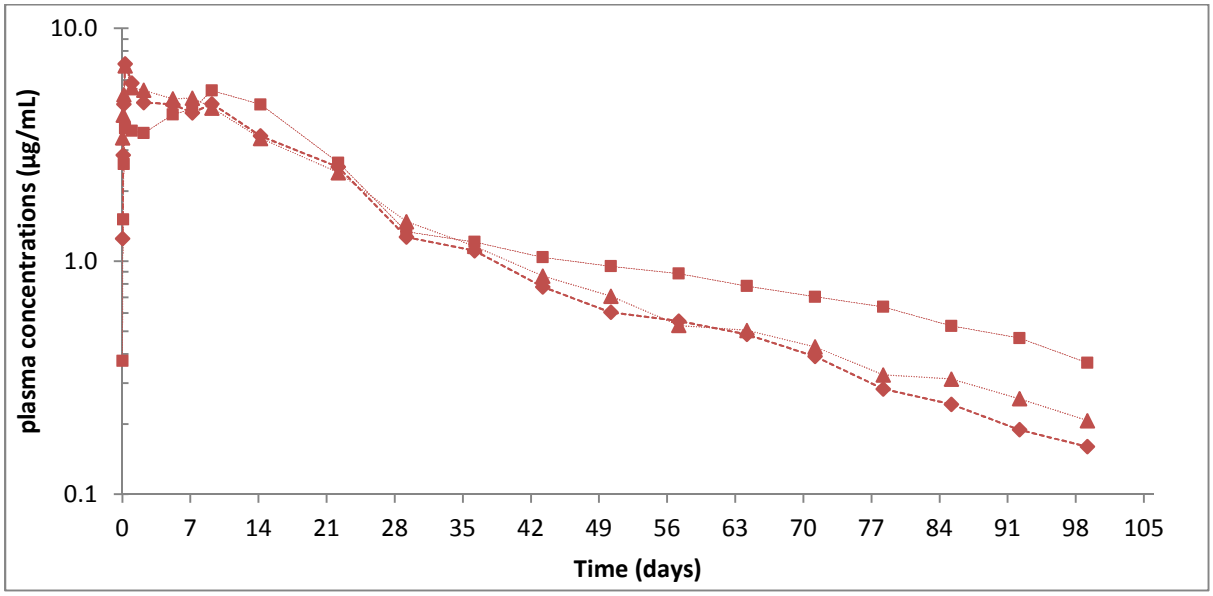
3.1.3.1. Single Dose Administration

Two IM studies were performed in female New Zealand white (NZW) rabbits (n=3 per formulation) with RPV LA (150 mg/kg; 0.5 mL/kg) in P338 (G001; used as reference control). In the first study (Mod4.2.2.2/2683_14278 [FK7491]), 2 other formulations were administered IM with a follow-up of 1 month: the first formulation tested contained an aged (i.e., larger particle size) RPV batch of the G001 formulation and the second formulation was a 3-fold dilution of the G001 formulation containing 100 mg/mL RPV in P338 (16.7 mg/mL) (Mod2.6.5.3A). In the second study (Mod4.2.2.2/2683_14279 [FK7521]), 3 other formulations were administered IM with a follow-up of 3 months: each formulation consisted of 300 mg/mL RPV, and was milled to smaller diameter (D50), or was an aged G001 formulation stored at 40°C or was freshly milled to edge of specification (Mod2.6.5.3B).

After a single IM administration of RPV LA as G001 in female rabbits, the initial release was rapid in both studies with high plasma concentrations being reached within 24 h after administration. After the high plasma concentrations on the first day after dosing, the concentrations declined until Day 3; then they slowly increased again up to 9 or 14 days after administration. After this second peak, plasma levels declined slowly and remained quantifiable up to the last sampling point (1 or 3 months; Figure 2). After 1 and 3 months of follow-up, the C_{max} and the $AUC_{0\text{-}1or3month}$ values were 4.6 and 6.5 µg/mL and 2383 and 3561 µg.h/mL, respectively.

In the study with 1 month of follow-up, the highest $AUC_{0\text{-}1month}$ value was obtained with the 3-fold diluted G001 formulation taking into account the dose difference, whereas the exposure after the formulation containing an aged RPV batch was similar to that of the G001 formulation. In the study with 3 months of follow-up, the $AUC_{0\text{-}3months}$ values were similar between the freshly milled to smaller D50 formulation, aged clinical batch and G001 formulation whereas it was slightly lower (12%) with the aged clinical batch stored at 40°C.

Figure 2: Individual Plasma Profiles of RPV After Single IM Administration of RPV LA (G001) in Rabbits After 3 Months of Follow-up at 150 mg/kg (Mod4.2.2.2/2683_14279 [FK7521])



An additional study was performed in female NZW rabbits (n=3 per formulation) after IM administration of G001 formulation (150 mg/kg; 0.5 mL/kg) with different particle sizes (Table 4) with a follow-up of 3 weeks (Mod4.2.2.2/FK12066 and Mod2.6.5.3C).

Table 4: The Particle Sizes of Different Formulations Administered in Rabbits

Particle Size	D_v10 (µm)	D_v50 (µm)	D_v90 (µm)	D_v99 (µm)
Target	0.081	0.218	0.953	2.596
Aged at higher temperature	0.082	0.226	1.964	3.888
Smaller particle size, close to target	0.082	0.202	0.576	2.173

The $AUC_{0\text{-}3weeks}$ values were the highest when administering the formulation with the smallest particle size, followed by the targeted one and the aged one at higher temperature: the relative bioavailability (F_{rel}), as compared to the targeted particle size formulation, was 128% and 88%, respectively.

There was one study performed with another formulation (non-G001); this is described in Section 8.1.3.

3.1.4. Studies in Dogs

3.1.4.1. Single Dose Administration

No single dose studies were performed in dogs with the G001 formulation. Studies performed with other formulations are described in Section 8.1.4.

3.1.4.2. Multiple Dose Administration

G001 formulation was administered IM in a 4-week GLP toxicity study in male and female beagle dogs (Mod4.2.3.2/TOX10759 and Mod2.6.5.4A). The dose of RPV was 150 mg/dog (18-21 mg/kg; 0.5 mL) and 1200 mg/dog (143-160 mg/kg: 4 x 1 mL) at Days 1 and 15.

Systemic exposure to RPV increased generally in a sub-proportional manner in males and females on Days 1 and 15 between doses and was generally comparable between Days 1 and 15 at 150 and 1200 mg/dog in both sexes. A trend to slightly higher C_{max} in females than males was noted at 150 mg/dog on both days. In general, exposure (AUC) was comparable between sexes at 150 and 1200 mg/dog on both days (Table 5).

Table 5: Mean RPV Toxicokinetic Parameters Following Repeated Intramuscular Administration of RPV LA (G001) in Dogs

Dose	Number Per Group/ Sex	Sampling period	C_{max} (µg/mL)	t_{max} (h)	AUC_{0-264h} [a] (µg.h/mL)	AUC_{0-336h} [b] (µg.h/mL)	AUC_{0-600h} [c] (µg.h/mL)
150 mg/dog	3/M	Day 1	0.12	264	22	29	-
		Day 15	0.18	24	35	-	63
	3/F	Day 1	0.25	24	39	46	-
		Day 15	0.40	24	49	-	94
1200 mg/dog	5/M	Day 1	1.2	24	185	218	-
		Day 15	1.4	24	217	-	435
	5/F	Day 1	1.2	24	175	204	-
		Day 15	1.2	24	206	-	410

[a] $AUC_{0-264h\ (11\ days)}$; [b] $AUC_{0-336h\ (14\ days)}$; [c] $AUC_{0-600h\ (25\ days)}$
IM Administration on Day 1 and Day 15

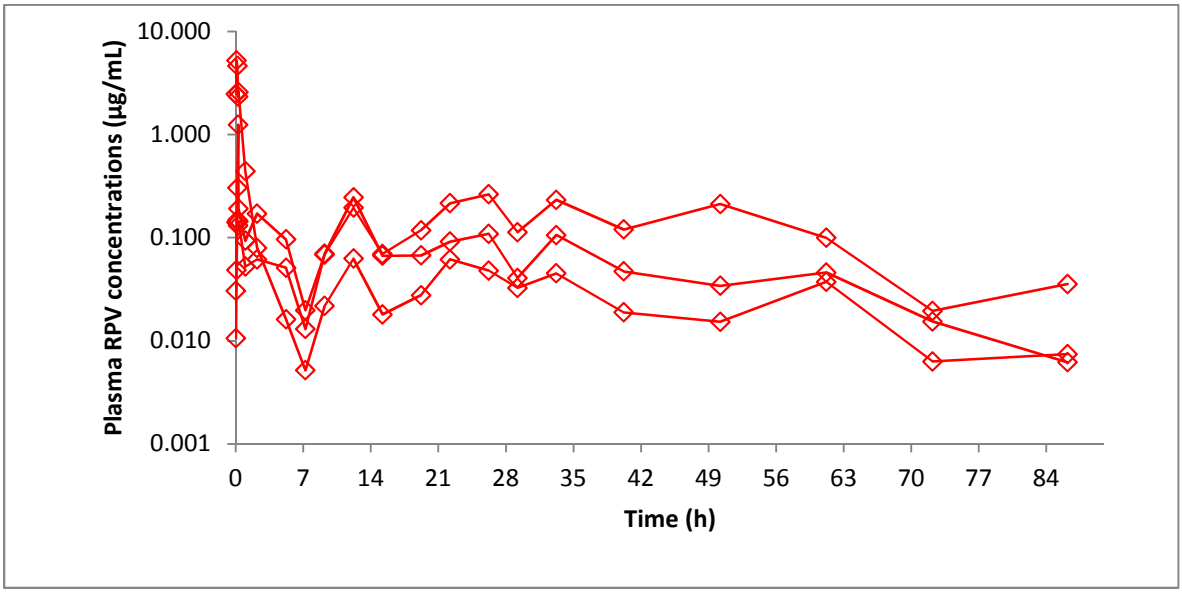
3.1.5. Studies in Minipigs

3.1.5.1. Single Dose Administration

In the first study, G001 formulation was administered IM to male Göttingen minipigs (n=3) with a follow-up period of 3 months at 600 mg/minipig (~60 mg/kg; 2 mL) (Mod4.2.3.6/TMC278-NC359 [TOX9403]). In addition, 5 other formulations containing 300 mg/mL of RPV with P338 (50 mg/mL) and Na-deoxycholate (2 mg/mL) or polyethylene polyethylene glycol (PE PEG) 350 (1.5 mg/mL) or dioctyl sodium sulfosuccinate (DOSS) (2 mg/mL) or 200 mg/mL of RPV base with P338 at 50 mg/mL with and without Na-deoxycholate were also administered (Mod2.6.5.3D).

After IM administration of G001 formulation in male minipigs, mean RPV plasma concentrations fluctuated or remained constant until a drop in the plasma concentrations occurred between Day 1 and 7. Afterwards, relatively flat profiles were observed between Day 9 and 86 (Figure 3). The mean C_{max} and $AUC_{0-3months}$ values of RPV at 600 mg/minipig (G001) were 2.2 µg/mL and 152 µg.h/mL, respectively. The plasma profiles were very similar across the different formulations tested. The highest $AUC_{0-3month}$ values were obtained at 600 mg/minipig with the G001 formulation and 300 mg/mL of RPV with P338 and DOSS. Based on this study, the G001 formulation was selected to be further evaluated in the clinic and became the final formulation.

Figure 3: Individual Plasma Profiles of RPV After Single IM Administration of RPV LA (G001) in Male Minipigs After 3 Months of Follow-up (Mod4.2.3.6/TMC278-NC359 [TOX9403]) at 600 mg/Minipig



In two other studies, G001 formulation was administered as reference in male minipigs. In the first study (Mod4.2.2.2/2683_14277 [FK7490]) with a follow-up of 1 month, 2 other formulations were administered IM: the first formulation tested contained an aged (i.e., larger particle size) RPV batch of the G001 formulation and the second one was a 3-fold dilution of the G001 formulation containing 100 mg/mL RPV in P338 (Mod2.6.5.3E). In the second study (Mod4.2.2.2/2683_14125 [FK7520]) with a follow-up of 3 months, 3 other formulations were administered IM: one formulation consisted of 300 mg/mL RPV LA, milled to smaller D50, a second formulation consisted of the aged G001 formulation, stored at 40°C, and the last one contained 300 mg/mL of RPV LA, freshly milled to edge of specification (Mod2.6.5.3F).

In the first study, after 1 month of follow-up, the C_{max} and $AUC_{0\text{-}1month}$ values of the 3-fold diluted G001 formulation taking into account the dose difference appeared slightly higher than those of the clinical formulation G001 and similar to the formulation containing the aged RPV batch. In the second study, after 3 months of follow-up, the $AUC_{0\text{-}3months}$ values from the formulation consisting of 300 mg/mL RPV LA, milled to smaller D50, were higher than the other 3 formulations (fresh and aged G001 formulation and the formulation freshly milled to edge of specification).

The G001 formulation was also administered IM to male minipigs with a follow-up period of 3 months at 600 mg/minipig and compared with 3 other formulations at 300 mg/mL of RPV contained sodium metabisulfite or low or high concentration of polyvinylpyrrolidone (PVP) (Mod4.2.2.2/2683_0040908 (FK10294) and Mod2.6.5.3G). The mean $AUC_{0\text{-}3months}$ value after G001 administration was comparable to that after low PVP administration while it was lower after administration of the formulation containing sodium metabisulfite (F_{rel} = 80%) or high PVP (58%).

In addition, RPV was also given after intravenous (IV) administration (slow bolus) at 2 mg/kg in 20% Captisol in male minipigs (Mod4.2.2.2/2683_14125 (FK7520) and Mod2.6.5.3F). The Vd_{ss} was large and total plasma clearance (Cl_p) was low (Table 6).

Table 6: Mean RPV Pharmacokinetic Parameters Following Intravenous Administration of RPV in Minipigs (n=3)

$AUC_{0\text{-}48\ h}$ (µg.h/mL)	$AUC_{0\text{-}\infty}$ (µg.h/mL)	$t_{1/2}$ (h)	Cl_p (mL/h/kg)	Vd_{ss} (L/kg)
2.97	2.80	8	753	4.9

There was one study performed with another formulation (non-G001); this is described in Section 8.1.5.

3.1.5.2. Multiple Dose Administration

The G001 formulation was administered IM in a 6-week (Mod4.2.3.2/TMC278-NC368 [TOX9508] and Mod2.6.5.4B), and a 9-month toxicity study (Mod4.2.3.2/TMC278-NC349 [TOX9517] and Mod2.6.5.4C) in male and female Göttingen minipigs. In the 6-week study, RPV LA was dosed every 2 weeks at 600 mg/injection (75-83 mg/kg; 4 injections at days 0, 14, 28 and 42). In the 9-month study minipigs received once monthly injections at 600 mg/injection (66-85 mg/kg, 10 injections at days 0, 28, 56, 84, 112, 140, 168, 196, 224 and 252).

The release from the injection site started fast (t_{max} between 2 and 7 h after injection) in the 2 studies. In the 6-week study, plateau concentrations were reached approximately 24 h post-dose which remained fairly constant up to 2 weeks. Corresponding $AUC_{0\text{-}336h}$ ($AUC_{0\text{-}day14}$) values in female minipigs after the first injection tended to be somewhat higher than in males. This difference disappeared after the third dose although the difference of weight between males and females was maintained. In the 9-month study, a plateau phase was reached at 24 h after dosing after which concentrations declined slowly or were maintained up to 28 days after dosing. Exposure, represented by mean C_{max} and AUC values, after repeated dosing was similar or 2.4-fold higher (AUC in males) than that after the single dose. In general, after single dosing, exposure was higher in females than in males. Values were comparable between males and females after repeated dosing (Table 7).

Table 7: Mean RPV Toxicokinetic Parameters Following Repeated Intramuscular Administration of RPV LA (G001) in Minipigs

Dose	Number Per Group/ Sex	Sampling period	C_{max} (µg/mL)	t_{max} (h)	AUC[e] (µg.h/mL)
600 mg[a] Every 2 weeks	3/M	Day 1	0.58	4.3	18
		Day 28[c]	0.72	5	51
	3/F	Day 1	1.0	5.3	33
		Day 28[c]	1.2	4.3	53
600 mg[b] Every month	3/M	Day 1	0.19	7	21
		Day 224[d]	0.35	6	50
	3/F	Day 1	0.64	4.3	35
		Day 224[d]	0.40	26	44

[a] Each 600 mg dose was administered IM once every 2 weeks for a total of 6 weeks (4 injections); [b] Each 600 mg dose was administered IM once every month for a total of 9 months (10 injections); [c] Third administration; [d] Ninth administration; [e] For 6-week study $AUC_{0\text{-}336h}$; For 9-month study $AUC_{0\text{-}672h}$
AUC: area under the plasma concentration-time curve; C_{max}: maximum plasma concentration; F: female; h: hour; M: male; t_{max}: time to reach the maximum plasma concentration

3.2. Kinetic Parameters, Bioequivalence and/or Bioavailability

Total clearance of RPV is low compared to the hepatic blood flow in rabbits and minipigs. Furthermore, Vd_{ss} is very low in rabbits but large in minipigs. Half-lives are comparable after IV dosing. After IM administration of RPV LA after single administration of G001 with a follow-up of 3 months, the F_{abs} is 67% in rabbits at 150 mg/kg and ranges between 35-62% in minipigs at 600 mg (Table 8).

Table 8: Mean Plasma Pharmacokinetic Parameters of RPV After IV Administration of RPV or IM Administration of G001 Formulation

Species	Route	Formulation	Dose	C_{max} (µg/mL)	t_{max} (h)	$AUC_{0\text{-}\infty}$ (µg.h/ mL)	$t_{1/2}$ (h)	Cl_p (Cl_b) (L/h/kg)	Vd_{ss} (L/kg)	F_{abs} (%)
Female rabbits	IV[a]	PEG400/ water (25%)	1.25 mg/kg	8.5[f]	NA	44	12	0.03 (0.049)	0.32	-
	IM[b]	G001	150 mg/kg	6.5	78.3	3,562[g]	-	-	-	67
Male minipigs	IV[c]	20% Captisol	2 mg/kg	1.7[f]	NA	2.8	8	0.75	4.9	-
	IM[d]	G001	600 mg (~67 mg/kg[f])	2.2	100	152	-	-	-	62
	IM[c]	G001	600 mg (69 mg/kg)	0.23	2.67	25	-	-	-	35[h]
	IM[e]	G001	600 mg (22-30 mg/kg)	0.12	0.5-24	16				43[i]

[a] Mod4.2.2.2/TMC278-FK4293; [b] Mod4.2.2.2/2683_14279 (FK7521); [c] Mod4.2.2.2/2683_14125 (FK7520); [d] Mod4.2.3.6/TMC278-NC359 (TOX9403); [e] Mod4.2.2.2/2683_0040908 (FK10294); [f] C_0; [g] $AUC_{0\text{-}Day99}$; [h] calculated using the minipig receiving IV and RPV LA (G001) after a wash out of 1 week; [i] the mean dose 26 mg/kg was used for calculation
$AUC_{0\text{-}\infty}$: area under the plasma concentration versus time curve from time 0 to infinity; Cl_b: blood clearance; Cl_p: plasma clearance; C_{max}: maximum plasma concentration; F_{abs}: absolute bioavailability; IM: intramuscular; IV: intravenous; NA: not applicable; PEG: polyethylene glycol; $t_{1/2}$: half-life; t_{max}: time at C_{max}; Vd_{ss}: volume of distribution at steady-state

3.3. Comparison of Exposure in Animals and Man

The C_{max} and AUC values of RPV after repeated oral administration in various animal species used in the toxicology studies and after repeated IM administration of RPV LA in dogs and minipigs are summarized in Table 9. These exposures were compared with the human predicted exposure (C_{max} = 0.14 µg/mL and $AUC_{0\text{-}day28}$ = 83 µg.h/mL) obtained in HIV-1 infected patients

at steady-state after 600 mg of RPV LA given every 4 weeks (G001) (Mod5.3.5.3/RPV LA PopPK report) using the population pharmacokinetic (PopPK) model.

After repeated oral administration of RPV in various animal species used in the toxicology studies compared to IM administration of RPV LA in patients, the highest C_{max} ratio (animal/human) of RPV was around 406 in female mice and 252 in male mice, 82 in female rats and 39 male rats, 123 in pregnant rabbits, 33 in dogs, and 2 in female monkeys. The highest $AUC_{0\text{-day}28}$ ratio (animal/human) of RPV was around 258 in female mice, 170 in male mice, 82 in female rats, 25 in male rats, 78 in pregnant rabbits, 22 in dogs, and 2 in female monkeys. After IM administration of RPV LA in minipigs and dogs compared to IM administration of RPV LA in patients, the C_{max} ratios (animal/human) of RPV were around 2 and 10 and $AUC_{0\text{-day}28}$ (animal/human) ratios of RPV were around 0.6 and 5, respectively.

Table 9: RPV Exposure in Animals (Oral/IM Administration) Relative to Human (IM Administration)

Species	RPV formulation	Sampling Time	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	C_{max} ratio	AUC_{0-24h} (µg.h/mL)	$AUC_{0-day28}$ ratio[b]
Mouse	PO: RPV in HPMC (0.5% w/v)	Week 28	M/9	20	9.8	69	76	26
			M/9	60	22	154	230	78
			M/9	160	36	252	505	170
			F/9	20	9.9	69	51	17
			F/9	60	29	203	278	94
			F/9	160	58	406	766	258
Rat	PO: RPV base in PEG400/CA (10%)	Day 28	M/4	10	0.88	6.3	7.2	2.4
			M/4	40	2.6	19	27	9.1
			M/4	160	6.7	48	51	17
			F/4	10	1.6	11	14	4.7
			F/4	40	5.8	41	42	14
			F/4	160	8.7	62	89	30
	PO: RPV base in PEG400/CA (10%)	Day 175[a]	M/3	40	1.7	12	12	4
			M/3	120	3.0	21	35	12
			M/3	400	6.2	43	73	25
			F/5	40	6.6	46	50	17
			F/5	120	8.8	62	116	39
			F/6	400	16	112	244	82
	PO: RPV in HPMC (0.5% w/v)	Week 39	M/9	40	0.82	6	6.3	2
			M/9	200	1.3	9	8.2	3
			M/9	500	1.8	13	14	5
			M/9	1500	2.2	15	18	6
			F/9	40	2.1	15	14	5
			F/9	200	4.7	33	41	14
			F/8	500	8.5	59	46	16
			F/9	1500	9.4	66	84	28
Pregnant rat	PO: RPV base in PEG400/CA (10%)	Day 11 (GD 16)	F/4	40	5.6	39	37	12
			F/4	120	7.2	50	63	21
			F/6	400	13	91	152	51
Juvenile rat (aged 25 days)	PO: RPV in HPMC (0.5% w/v)	Day 14	M/8	40	2.6	18	12	4
			M/7	120	3.7	26	34	11
			M/7	400	9.1	64	50	17
			F/8	40	5.8	41	18	6
			F/8	120	3.6	25	28	9
			F/7	400	7.3	51	53	18
Pregnant rabbit	PO: RPV base in HPMC (0.5% w/v)	Day 14 (GD 19)	F/3	5	6.7	47	105	35
			F/3	10	10	70	170	57
			F/3	20	15	105	232	78
Dog	PO: RPV base in PEG400/CA (10%)	Day 363	M/4	5	1.1	8	17	6
			M/2	10	1.3	9	24	8
			M/4	40	4.1	29	65	22
			F/4	5	1.5	10	19	6
			F/4	10	2.2	15	36	12
			F/3	40	5.5	38	61	21
Monkey	PO: RPV in HPMC (1%)/Tween 20	Day 55	F/8	100 b.i.d	0.14	1	2.7	0.9
			F/7	250 b.i.d	0.31	2	4.6	1.6
Minipig	IM: RPV LA (G001)	Day 224 (after 8 injections)	M/3	600 mg/ 4weeks	0.35	2	50[c]	0.6
			F/3	600 mg/ 4weeks	0.41	3	44[c]	0.5
Dog	IM: RPV LA (G001)	Day 224 (after 8 injections)	M/3	1200 mg/ 2 weeks	1.4	10	435[d]	5
			F/3	1200 mg/ 2 weeks	1.2	9	410[d]	5

[a] Total dosing volume of 10 mL/kg was changed after Day 83 to two administrations of 5 mL/kg with 1.5 hours between the two administrations. The underlined dose is the no observed adverse effect level dose when it is determined; [b] animal/human AUC ratio was calculated as follows: AUC_{0-24h} at steady-state multiplied by 28 days in animals divided by $AUC_{0-day28}$ obtained in HIV infected patients at steady-state after 600 mg every 4 weeks; [c] $AUC_{0-672h\ (28days)}$; [d] $AUC_{0-600h\ (25days)}$; CA: citric acid; HPMC: hydroxypropyl-methylcellulose; IM: intramuscular; PO: oral

4. DISTRIBUTION

A single IM dose of RPV LA (as the G001 formulation) has been administered in rats and rabbits to determine the distribution of RPV in certain tissues/organs (see Section 4.1).

For RPV oral, tissue distribution has been studied in pigmented Long Evans rats and pregnant female Sprague-Dawley rats after a single oral administration of ^{14}C-RPV by means of QWBA, as well as in a single oral dose pharmacokinetic study administering RPV in rats. Protein binding and/or distribution in blood cells of RPV has been studied in vitro in mouse, rat, rabbit, guinea pig, dog, monkey and human.

4.1. Tissue Distribution

4.1.1. Rilpivirine LA

4.1.1.1. Studies in Rats

A single IM dose of RPV LA (G001; 60 mg/kg; 0.2 mL/kg) was administered to male Sprague-Dawley rats in the left hind leg (n=3; Mod4.2.2.3/ADME_58575 and Mod2.6.5.5A). The concentrations of RPV were determined in different tissues/organs and in blood and plasma with a follow-up of 42 days. All individual RPV plasma concentration-time profiles showed two distinct concentration peaks or shoulders within 216 h post-dose, the first one occurring between 4 and 24 h, and the second one between 120 and 216 h. Subsequently, a slow first-order decline in plasma concentrations was observed, lasting till the last observation time point of 42 days post-dose (Table 10). The highest exposures of RPV were measured in left popliteal and medial iliac lymph nodes adjacent to injection site with tissue/plasma $AUC_{0\text{-day}42}$ ratios of 12,203 and 2256, respectively. In the contralateral right popliteal and medial iliac lymph nodes, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 6.7 and 2.6, respectively. In the kidney, adrenal gland, lungs, liver, and pancreas, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 3.7, 3.2, 1.5, 1.5 and 1.2, respectively. The tissue/plasma $AUC_{0\text{-day}42}$ ratios were lower than 1 in brain (0.4), heart (0.8), spleen (0.97) and thymus (0.87) (Table 10).

Table 10: Tissue, Plasma and Blood Concentrations of RPV and Tissue/Plasma or Blood Ratios of RPV After IM Administration of RPV LA(G001) in Rats

Tissue/organs	C_{max} (µg/mL or g)	t_{max} (h)	$AUC_{0\text{-day}42}$ (µg.h/mL or g)	Tissue-to-plasma $AUC_{0\text{-day}42}$ ratio	Tissue to blood $AUC_{0\text{-day}42}$ ratio
Blood	0.061	2	15.1	0.64	1
Plasma	0.129	2	23.7	1	1.6
Adrenal gland	0.16	168	73.5	3.2	4.9
Brain	0.023	168	2.7[a]	0.4	0.7
Eye	BLQ	-	-	-	-
Heart	0.046	24	12.5[b]	0.8	1.2
Kidney	0.16	168	84.8	3.7	5.6
Liver	0.069	24	24[b]	1.5	2.3
Lung	0.075	24	33.9	1.5	2.2
Lymph Node (Medial iliac left)	409	168	51,200	2256	3391
Lymph Node (Medial iliac right)	0.088	24	58.2	2.6	3.9
Lymph Node (Popliteal left)	1100	504	277,000	12,203	18,344
Lymph Node (Popliteal right)	1.2	24	153	6.7	10
Pancreas	0.058	168	28.1	1.2	1.9
Spleen	0.048	168	22.1	0.97	1.5
Thymus	0.094	24	19.7	0.87	1.3
Thyroid	0.050	168	-	-	-

[a] $AUC_{0\text{-day}7}$; [b] $AUC_{0\text{-day}21}$
BLQ: below the limit of quantification <10.0 ng/g

4.1.1.2. Studies in Rabbits

A single IM dose of RPV LA (G001) was administered in the musculus biceps femoris to female NZW rabbits at 150 mg/kg (0.5 mL/kg; n=3) (Mod4.2.2.2/2683_14278 [FK7491] and Mod2.6.5.5B). RPV concentrations at the administration and contralateral sides were measured at the end of a 1-month follow-up period (Table 11). At the administration site, the RPV concentrations were high (1456-fold) compared those in the contralateral side. In the lymph nodes, the RPV concentrations were similar between the injection and contralateral side except for one rabbit for which higher concentrations were seen in the accessory popliteal lymph nodes of the injection side.

Table 11: Mean Tissue Concentrations (Range) of RPV in the Administration Site and Lymph Nodes at the Administration and Contralateral Sides and Plasma Concentrations (Range) in Female Rabbits (n=3) After Single Intramuscular Dosing at 150 mg/kg at 1-Month Post-Dosing

Tissue/organs	C (µg/mL or g)	Tissue-to-Plasma Concentration Ratio
Plasma	1.43 (1.3-1.5)	-
Administration site (injection side)	5390 (5000-5830)	3769 (3560-3887)
Administration site (contralateral side)	3.7 (2.1-6.4)	2.6 (1.4-4.2)
Lymph Node, accessory axillary (injection side)	0.45[a] (0.37-0.53)	0.31[a] (0.29-0.35)
Lymph Node, accessory axillary (contralateral side)	0.61 (0.57-0.66)	0.43[a] (0.38-0.50)
Lymph Node, medial iliac (injection side)	0.86 (0.49-1.15)	0.60 (0.33-0.77)
Lymph Node, medial iliac (contralateral side)	0.59 (0.52-0.65)	0.41 (0.35-0.46)
Lymph Node, accessory popliteal (injection side)	13 (1.0-37)	9.1 (0.66-28)
Lymph Node, accessory popliteal (contralateral side)	1.2 (0.34-2.87)	0.84 (0.23-2.2)

[a] n=2

C: concentration

4.1.2. Rilpivirine

4.1.2.1. Studies in Rats

The tissue distribution of RPV and its metabolites was studied in male pigmented Long Evans rats and pregnant female Sprague-Dawley rats by QWBA, following a single oral dose of ^{14}C-RPV base in polyethylene glycol 400 (PEG400)/citric acid (CA) (10%) at 40 mg/kg (Mod4.2.2.3/TMC278-NC108 [FK4951]), (Mod4.2.2.3/TMC278-NC109 [FK4950]) (Mod2.6.5.5C and Mod2.6.5.5D). The tissue distribution of RPV was assessed in a limited set of tissues obtained in a pharmacokinetic study in male Sprague-Dawley rats given a single oral dose of RPV at 40 mg/kg (PEG400/CA (10%)) (Mod4.2.2.3/FK4195 and Mod2.6.5.5E).

In pigmented rats, highest TR levels were observed at 4 hours post-dose in non-pigmented and most pigmented tissues, indicating a rapid distribution of ^{14}C-RPV-related radioactivity. Only in the pigmented parts of the eye and the uveal tract, the highest concentration of radioactivity was measured at 24 hours after dosing.

In most non-pigmented tissues, radioactivity levels were only quantifiable by RLG until 4 hours after dosing. The highest concentration of radioactivity was measured in the liver and the exposure (AUC_{0-4h}) was 12-fold higher than the AUC_{0-4h} observed in blood (Figure 4). In the adrenal gland, brown fat and kidney AUC_{0-4h} values were about 4- to 5-fold those in blood. In pancreas and white fat, the AUC_{0-4h} values were almost 3-fold that in blood. In spleen, based on C_{4h}, the radioactivity level was almost 3-fold that in blood. Tissue to blood AUC_{0-4h} ratios in lung, heart, white skin and thyroid were about 2. AUC_{0-4h} values in prostate gland, bone marrow, muscle, testis and brain were similar or a bit lower than those in blood.

In pigmented tissues, the radioactivity decreased more slowly than in the other tissues and was still quantifiable by RLG 14 days post-dose. Tissue to blood AUC_{0-336h} ratios were 146 (uveal tract), 18 (brain meninges) and 15 (pigmented skin). Although levels in pigmented tissues at 14 days post-dose still represented about 20% of corresponding peak levels, radioactivity levels

decreased from 4 or 24 hours onwards. Therefore, no undue retention of ^{14}C-RPV derived material is expected.

The QWBA study in non-pigmented pregnant rats showed a similar tissue distribution profile as the one observed in the male rat (Figure 5). In most tissues, highest radioactivity levels were observed at 4 hours post-dose, indicating rapid distribution into the tissues. Only in uterine epithelium and to a lesser extent in adrenal gland, radioactivity concentrations declined more slowly than in blood from 8 to 24 hours.

Some specific procreative tissues were evaluated. The $AUC_{0\text{-}8h}$ values of uterine epithelium, mammary gland and ovary were 4-, 3- and 2-fold higher than the corresponding blood $AUC_{0\text{-}8h}$ values, respectively. The $AUC_{0\text{-}8h}$ values in uterus, placenta and vagina were similar to slightly lower than those in blood. The $AUC_{0\text{-}8h}$ in whole fetus was 0.64-fold that in maternal blood, suggesting that the placenta presents a partial barrier for RPV and/or its metabolites.

In a pharmacokinetic study after administration of non-radiolabeled RPV base in male Sprague-Dawley rats, plasma and tissue samples from the adrenal glands, brain, liver, and muscle were collected. Maximum tissue concentrations were observed within 20 to 60 min after administration. Tissue levels declined in parallel with plasma concentrations. Highest tissue concentrations were observed in the liver and the adrenal gland reaching tissue-to-plasma $AUC_{0\text{-}24h}$ ratios of 3.4 and 2.7, respectively. In brain and muscle, the tissue-to-plasma ratios were 0.49 and 0.45, respectively. These results were similar to the data obtained with radiolabeled material showing high distribution to the liver and adrenal glands and lower distribution to the muscles and brain.

Figure 4: Tissue to Blood AUC_{0-4h} Ratios of Total Radioactivity, as Determined by Radioluminography of Whole-Body Sections of Tissues in the Male Pigmented Long Evans Rats After a Single Oral Administration of ^{14}C-RPV Base at 40 mg/kg

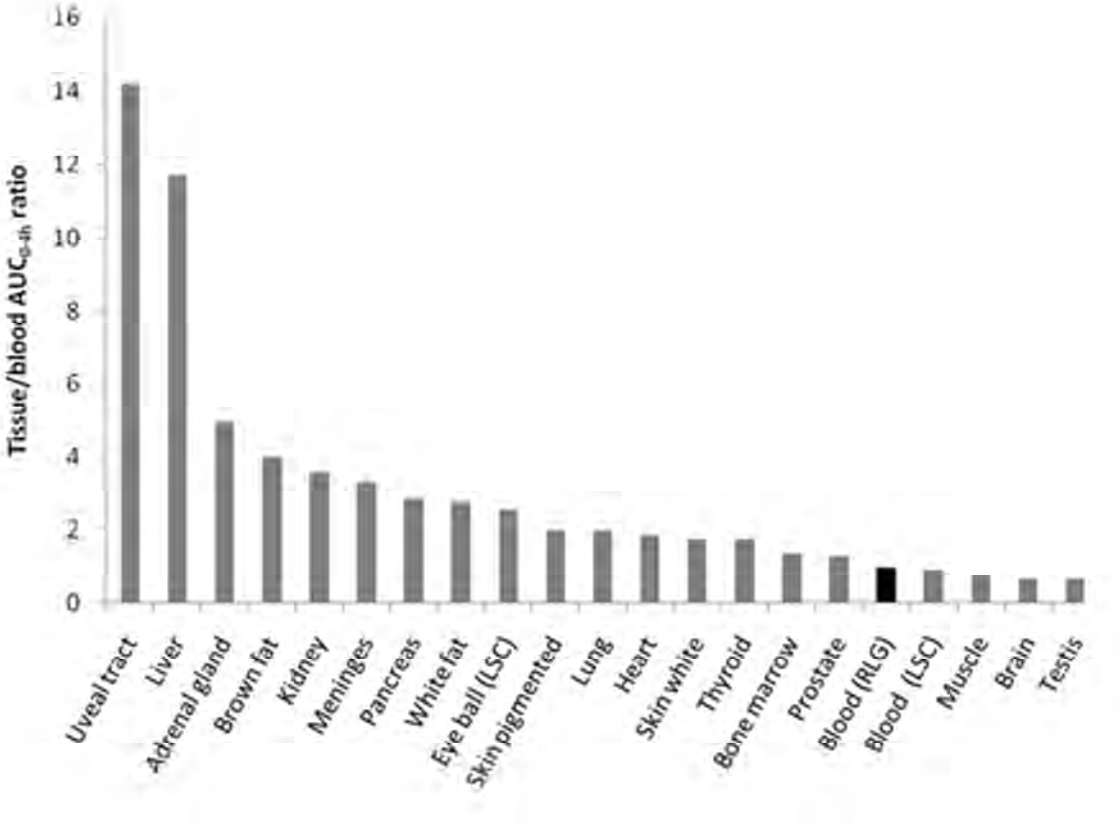
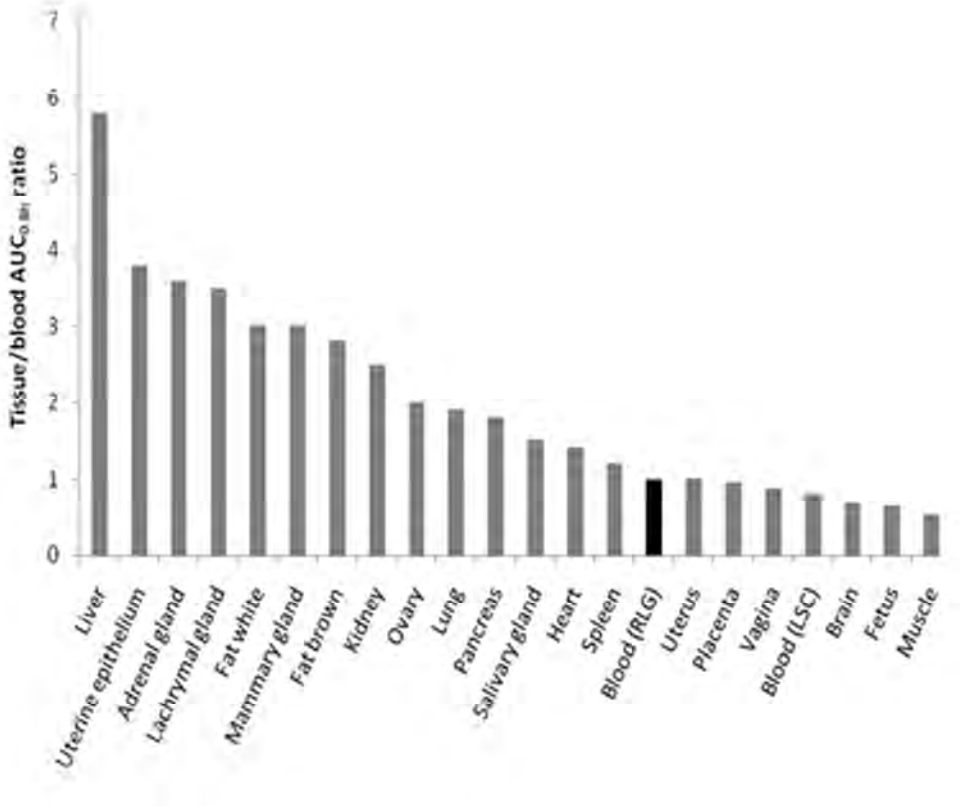


Figure 5: Tissue to Blood AUC_{0-8h} Ratios of Total Radioactivity, as Determined by Radioluminography of Whole-Body Sections of Tissues in the Pregnant Female Sprague-Dawley Rats After a Single Oral Administration of ^{14}C-RPV Base at 40 mg/kg



4.1.2.2. Studies in Dogs

Rilpivirine concentrations were measured in some tissues collected during the 1- and 6-month toxicology studies in dogs. Plasma and adrenal glands were obtained from dogs (3 animals/sex/group) following a 1-month toxicity study (Mod4.2.3.2/TMC278-Exp.5650 and Mod2.6.5.5E). Plasma, adrenal gland and liver were also collected from dogs (3 animals/sex/group) following a 6-month study (Mod4.2.3.2/TMC278-NC115 [TOX6110] and Mod2.6.5.5F). In both studies, animals received RPV base (PEG400/CA (10%)) oral doses of 5, 10, and 40 mg/kg/day. Plasma and tissues were collected at autopsy on Day 28/29 and Day 56 (recovery period) in the 1-month study or on Day 93 and Day 184/185 in the 6-month study.

In both studies, the adrenal gland concentrations were higher than the corresponding plasma concentrations with mean tissue-to-plasma concentration ratios ranging from 1.3 to 8.1. After 1 month of recovery, however, the adrenal gland levels as well as the plasma levels decreased below the limit of quantification. In the liver, RPV concentrations were also higher than in plasma, with tissues to plasma concentration ratios ranging from 7.7 to 13. In the 6-month study, adrenal and liver tissue-to-plasma ratios were similar between Day 93 and Day 184/185 of the study.

4.2. Protein Binding and Distribution in Blood Cells

The plasma protein binding of RPV was studied in vitro by equilibrium dialysis. Plasma samples from male and female CD-1 mice, male and female Sprague-Dawley rats, female NZW rabbits, male beagle dogs and healthy male adult subjects were fortified with ^{3}H-RPV at concentrations ranging from 0.01 to 100 µg/mL (animals) and from 0.01 to 3.0 µg/mL (human) (Mod4.2.2.3/TMC278-NC112 [FK5273]). The distribution of RPV to various compartments of blood and the binding of RPV to purified human serum albumin and α_1-acid glycoprotein were also studied (Mod2.6.5.6A). The distribution of ^{14}C-RPV in blood and the protein binding of ^{14}C-RPV in plasma were also investigated in samples from female guinea pigs at 2.5 and 8 µg/mL and from female monkeys at 2.5 and 5 µg/mL (Mod4.2.2.3/TMC278-NC332 [FK6820]) (Mod2.6.5.6B). In addition, the protein binding of unlabeled RPV was determined in plasma samples from male and female CD-1 mice, male and female Sprague-Dawley rats, male beagle dogs and healthy male subjects at concentrations of 0.03, 0.3 or 1 µg/mL (animals) and 0.01, 0.03, 0.1, 0.3, 1 or 3 µg/mL (human) (Mod4.2.2.3/TMC278-FK4217).

Rilpivirine was highly bound to plasma proteins in all species and the plasma protein binding was found to be concentration independent. Plasma protein binding values ranged between 99.08% and 99.97%. RPV was highly bound to human albumin (99.5% at a physiological concentration of 4.3% and irrespective of the RPV concentration) and to a much lesser extent to α_1-acid glycoprotein (48.8% at a physiological concentration of 0.07% and an RPV concentration of 1 µg/mL). The rank order of blood to plasma concentration ratio in all species was monkey >dog >rat >man >guinea pig >rabbit >mouse and ranged from 0.96 to 0.58. In all species, irrespective of the concentration, a very limited percentage of RPV distributed to the plasma water compartment and the values ranged from 0.06 to 0.5%. In guinea pig, male and female rats, male dog, monkey and man, the percentage of RPV distributed to plasma proteins ranged from 61.8 to 94.1%, and the percentage of RPV distributed to blood cells ranged from 5.8 to 37.6%. In mouse and rabbit, RPV distributed almost completely to plasma proteins (see Table 12).

The protein binding results of unlabeled RPV were in line with the ones obtained with radiolabeled compound at the same range of concentrations. No data were obtained at 0.01 and 0.03 µg/mL due to the fact that concentrations were below the limit of quantification.

Table 12: Plasma Protein Binding and Blood Distribution of RPV at 1 µg/mL (Various Species) or 2.5 µg/mL (Guinea pig and Monkey)

	Mouse		Rat		Guinea pig	Rabbit	Dog	Monkey	Human
	M	F	M	F	F	F	M	F	M
Plasma protein binding (%)	99.93	99.94	99.84	99.86	99.87	99.97	99.35	99.14	99.67
Free drug (%)	0.07	0.06	0.16	0.14	0.13	0.03	0.66	0.86	0.33
Blood to plasma ratio	0.60	0.58	0.67	0.67	0.64	0.61	0.68	0.96	0.66
Distribution to									
Plasma water (%)	0.07	0.07	0.15	0.14	0.11	0.03	0.50	0.53	0.20
Plasma proteins (%)	100	>100	93.0	94.1	84.7	>100	73.4	61.8	78.1
Blood cells (%)	<0	<0	6.8	5.8	15.2	<0	26.2	37.6	21.7

4.3. Placental Transfer

The placental transfer of RPV was studied in pregnant Sprague-Dawley rats, by QWBA, after a single oral dose (gavage) of ^{14}C-RPV base in PEG400/CA (10%), at 40 mg/kg (Mod4.2.2.3/TMC278-NC109 [FK4950] and Mod2.6.5.5D). The $AUC_{0\text{-}8h}$ values in the placenta and in whole fetuses were 0.95- and 0.64-fold the $AUC_{0\text{-}8h}$ value of maternal blood, respectively. This suggests that the placenta is only a partial barrier for RPV and its metabolites.

5. METABOLISM

In vivo and *in vitro* metabolism studies with RPV, as well as the enzymes involved in the metabolism of RPV in the human hepatocytes and enzyme induction and inhibition studies have been conducted to support the registration of oral RPV (EDURANT®) submission. Relevant studies that support the RPV LA MAA are rediscussed below.

5.1. Chemical Structures and Quantities of Metabolites in Biological Samples

5.1.1. Studies in Mice

Male and female CD-1 mice were dosed orally, by gavage, with a single dose of ^{14}C-RPV base in PEG400/CA (10%) at 20 or 320 mg/kg (Mod4.2.2.4/TMC278-NC190 [FK5621]) (Mod2.6.5.9A). Plasma samples were collected up to 24 hours after dosing and urine and feces samples up to 96 hours after dosing. Radioactivity was determined by scintillation counting and samples were analyzed by radio-HPLC. Plasma concentrations of RPV were measured by LC-MS/MS and the metabolite profiling and identification were done by radio-HPLC and LC-MS/MS analyses.

After oral administration of ^{14}C-RPV base, 87% to 96% of the TR was eliminated in feces and 1.8% to 4.2% was excreted in urine. Unchanged RPV accounted for 7.9-8.8% and 33-34% of the administered dose in the overall pooled 0-48-hour feces at 20 and 320 mg/kg, respectively. Renal elimination was limited, only traces of unchanged RPV were excreted in urine (0.02-0.63% of the dose) at the two dose levels.

Rilpivirine was extensively metabolized in the mouse, as a large number of metabolites were detected (Figure 6). Metabolic pathways included oxidative pathways (aliphatic and aromatic hydroxylation) and glutathione conjugation, followed by secondary metabolism (metabolism of the glutathione conjugate, glucuronidation, dehydration).

In feces, by far the predominant metabolite fraction was composed of M41 (hydroxy metabolite of S-methyl conjugate of RPV) and M42 (aromatic hydroxylation at the 5-position of the pyrimidinyl moiety), accounting for 18-26% of the 20 mg/kg dose and for 9-13% of the 320 mg/kg dose. In this fraction, M42 was the most abundant metabolite for both genders and at the two dose levels, estimated at about 14% and 17% of the 20 mg/kg dose, and at 5.9% and 8.0% of the 320 mg/kg dose in male and female mice, respectively. The cysteinyl conjugates (M13 co-eluted with M14) and the mercapturic acids (M17 co-eluted with M18) accounted for 6.2-9.2% of the dose in total (two dose levels, both genders). M24 (hydroxylation on the cyanoethenyl moiety) co-eluted with M25 (oxidation followed by glucuronidation of RPV) and

accounted for less than 3.4% in total (two dose levels, both genders). M30 (carboxylic acid metabolite on the cyanoethenyl moiety) accounted for about 1.6-3.1% of the 20-mg/kg dose and about 1.2-1.5% of the 320-mg/kg dose. Several minor metabolites in both male and female mice were present: M38 (hydroxylation of RPV) and M43 (Z isomer of RPV) that co-eluted with M45 (S-methyl conjugate) accounted for less than 2.2%. M21 (hydroxylated methyl sulphonyl conjugate) and M33 (hydroxylation at the methyl group of RPV) accounted for less than 1.4%. M27 (tricyclic metabolite) co-eluted with M28 (tricyclic metabolite of S-methyl conjugate of RPV) and M29 (sulphoxidation of M45), and M47 (dimer) accounted for less than 0.8%. M35 and M46 (unknown structures) accounted for less than 0.7%.

In urine, M25 (oxidation in combination with glucuronidation) was the most abundant metabolite at both dose levels and accounted for 0.4-1.6% of the dose. M13, M14, M17 and M18 were more formed in female mice than in male mice and accounted for 1.2% and 1.1% of the dose in females (at 20 and 320 mg/kg, respectively) and for 0.54% and 0.27% of the dose in males (at 20 and 320 mg/kg, respectively). M42 was also excreted in urine (0.06-0.37% of the dose).

In plasma, unchanged RPV was by far the main circulating compound at both dose levels. At all time points, M33 (hydroxymethyl-RPV) was the most abundant plasma metabolite, it accounted for 1.5-6.6% of the plasma radioactivity. Other metabolites (M13 co-eluted with M14, M27, M30 and M36) accounted for less than 1.3% of the sample radioactivity. Traces of several other metabolites (M17, M18, M31 (hydroxyl metabolite on the cyanoethenyl moiety), M42 and M43) were detected.

The metabolite profile of RPV was qualitatively comparable in male and female mice.

5.1.2. Studies in Rats

5.1.2.1. Single Dose Administration

Male and female Sprague-Dawley rats were dosed orally, by gavage, with a single dose of ^{14}C-RPV base in PEG400/CA (10%) at 40 mg/kg (Mod4.2.2.4/TMC278-NC113 [FK4933]) (Mod2.6.5.9B). Plasma samples were collected up to 24 hours after dosing and urine and feces samples up to 96 hours after dosing. Plasma concentrations of RPV were measured by LC-MS/MS. The biliary metabolite profile of RPV was also investigated in male Sprague-Dawley rats after a single dose of ^{14}C-RPV base in PEG400/CA (10%) at 40 mg/kg (Mod4.2.2.4/TMC278-NC145 [FK5525]) (Mod2.6.5.9C). In both studies, the TR was measured by scintillation counting. Metabolite profiles were determined by reversed-phase radio-HPLC and identification of the metabolites was carried out using co-chromatography with synthesized metabolites, enzymatic hydrolysis and LC-MS/MS analysis.

After oral administration of ^{14}C-RPV base, the radioactivity was predominantly excreted in feces (93% of the dose) and 0.45% (males) to 1.8% (females) was excreted in urine. Unchanged RPV accounted for 47% and 43% of the administered dose in the overall pooled 0-48-hour feces in males and females, respectively. Urinary excretion of unchanged RPV was negligible.

Rilpivirine was metabolized to a moderate extent. Metabolic pathways included oxidative pathways (aliphatic and aromatic hydroxylation), glutathione conjugation and metabolites derived thereof (Figure 6).

In feces, dimerization of a thiol intermediate resulting from glutathione conjugation (M47), was the most predominant metabolic pathway in male and female rats (4.0% and 3.8% of the dose, respectively). M41/M42 and M43/M45, which co-eluted pairwise, resulted from hydroxylation, isomerization, or glutathione conjugation and accounted on average for 2.4% to 3.6% of the dose in both genders. A variety of minor metabolites (M21, M24/M27/M28/M29, M33, M38 and M46) were also observed in rat feces; each represented less than 2% of the administered dose. M30 accounted for 0.47% and 0.05% of the dose in male and female rats, respectively.

In urine, only the mercapturic acids (M17 and M18) were detected and accounted for 1.1% and 0.45% in female rats and for 0.02% and 0.03% of the dose in male rats, respectively. Several unknown metabolites were also present in urine but accounted in total for <0.4% of the dose.

In plasma, unchanged RPV accounted for the largest fraction of the circulating radioactivity. Only two minor metabolites, M12, a cysteinylglycine-S-conjugate, which co-eluted with M14, a cysteinyl-S-conjugate, were present in plasma. They accounted for 4-14% of the plasma radioactivity in total, at all time points.

The metabolic profile of RPV is qualitatively comparable in male and female rats.

In bile, the amount of radioactivity excreted within 24 hours after a dose of ^{14}C-RPV base was rather low i.e. 18% and 25% of the radioactive dose, in restrained and nonrestrained rats, respectively. The percentage of unchanged RPV excreted in the bile during this time period was negligible (~0.2%). The most important biotransformation pathway involved conjugation of glutathione to RPV to form M10 followed by formation of the cysteinylglycine-S-conjugate M12 and the cysteine-S-conjugate M14. They accounted for 6.4% in total. From this pathway, other metabolites were generated. The most abundant ones were M9 (thiol glucuronide conjugate) and M18 (mercapturic acid S-conjugate of RPV) and each accounted for less than 2.8%. The minor one was M1 (oxidation and glucuronidation of M14) and accounted for 0.63%. Other minor metabolites were M25 (1.4%) and M30 (0.4%) (see Mod2.6.5.11C).

5.1.2.2. Multiple Dose Administration

In an attempt to understand the time-dependent decrease in exposure in male rats in the carcinogenicity study, the metabolic profiles of plasma samples obtained after oral administration of RPV at 1500 mg/kg/day in male and female rats were assessed by liquid chromatography with ultraviolet detector and LC/MS (Mod4.2.3.4.1/TMC278-NC123 [TOX7221]), (Mod4.2.2.4/TMC278-NC290 [FK6376]) (see Section 3.3).

In all samples, unchanged RPV was by far the major circulating compound. The comparison of the plasma profiles from male and female rats at Day 1, Week 27 and Week 39 did not show a relevant increase in metabolites after repeated administration.

5.1.3. Studies in Dogs

Male beagle dogs were dosed orally with a single dose of ^{14}C-RPV base in PEG400/CA (10%) at 5 mg/kg (Mod4.2.2.4/TMC278-NC114 [FK5143]) (Mod2.6.5.9D). Plasma, urine and feces were collected up to 168 hours (one week) after dosing. Plasma concentrations of RPV were measured by LC-MS/MS. Radioactivity levels were determined by LSC and metabolite profiles were investigated by radio-HPLC and LC-MS/MS.

After oral administration of ^{14}C-RPV base radioactivity was excreted predominantly in feces (95% of the dose), while a very low amount of radioactivity was excreted in urine (1.7% of the dose). Unchanged drug represented 45% of the administered dose in the overall pooled 0-72-hour feces. Unchanged RPV was not detected in urine.

RPV was not extensively metabolized in dogs. The most important biotransformation pathway of RPV in dogs was oxidation at various positions of the molecule. In addition, but less significant, direct N-glucuronidation of RPV and further metabolism of the oxidized metabolites via dehydration (ring closure), glucuronidation, and sulfation occurred (Figure 6). The most abundant fecal metabolites included M33 (hydroxymethyl-RPV), M42 (hydroxyl metabolite at the 5-position of the pyrimidinyl moiety of RPV), and M44 (monooxygenated-RPV) and represented 8.7%, 5.3% and 4.3% of the dose, respectively. In addition, M30 (carboxylic acid) and M48 (unknown), which co-eluted, represented 3.1% of the dose. Other minor fecal metabolites (M23 co-eluted with M27, M37, M40, M46, and M49) were also detected and individually these metabolites did not represent more than 2% of the administered dose.

In urine, several minor metabolites (M3, M12, M14, M19, M25, M30, and M36) were identified, none of which represented more than 0.08% of the radioactive dose.

In plasma, unchanged RPV was the only radioactive component detected. Minor metabolites that were present in trace amounts and were detected only by LC-MS included M15 (*N*-glucuronide), M19 (glucuronide), M27, M30 and M33.

5.1.4. Studies in Humans

Six healthy male subjects received a single oral dose of 150 mg ^{14}C-RPV base (administered as a PEG400 formulation) (Mod4.2.2.4/TMC278-NC157 [FK5344]) (Mod2.6.5.9E). Urine and plasma were collected for up to one week after dosing, feces for up to two weeks after dosing. Plasma concentrations of RPV were measured by LC-MS/MS. Radioactivity levels were determined by LSC. Metabolite profiles were determined by reversed-phase radio-HPLC and identification of the metabolites was carried out using co-chromatography with synthesized metabolites, LC-MS/MS and nuclear magnetic resonance (NMR) analysis. In plasma, the more sensitive method liquid chromatography-accurate radioisotope counting was used for metabolite profiles.

After oral administration radioactivity was mainly excreted in feces (85% of the dose over the 14-day period) while in urine a low amount of radioactivity was excreted (6.1% of the dose over the 7-day period). Unchanged RPV represented on average 26% of the administered dose in feces. Unchanged RPV was not detected in urine.

Rilpivirine was extensively metabolized. The most important biotransformation pathway of RPV was oxidation. The most abundant fecal metabolite was M42 which accounted on average for 16% of the dose. M33 (hydroxymethyl-RPV) accounted for 3.0% of the administered dose, M30 (carboxylic acid derivative) for 2.7% and a metabolite of unknown structure (M35) accounted for 2.2% of the administered dose. Some minor metabolites resulting from further biotransformation of M33 (M27, M11 and M23) were also detected (each <1.6%).

In urine, apart from M30 (0.03%), metabolites were phase II metabolites (glucuronides (0.9% of the administered dose) or glutathione-derived (1.2% of the administered dose) conjugates).

In plasma unchanged drug accounted for the major part of the TR. Several minor metabolites were detected namely, the glucuronide of RPV (M15), the tricyclic metabolite (M27), and hydroxymethyl-RPV (M33), others (glucuronide of hydroxymethyl-RPV, and of hydroxylated RPV) were only present in trace amounts.

In general, all identified metabolites in human matrices were also detected in at least 1 animal species (see Figure 6).

Identification and estimation of the abundance of circulating metabolites of RPV were determined in pooled plasma (n=6/timepoint) from male and female healthy subjects received repeat once daily oral doses of RPV at 75 and 300 mg collected on Day 1 and 11 (i.e. at steady-state) at pre-dose (only on Day 1), 0.5, 3, 6, 12 and 24 h. (Mod4.2.2.4/1646_0027483 [FK10104] and Mod2.6.5.9F). HPLC conditions were identical to those used in the human excretion mass balance study discussed above.

Rilpivirine was by far the major circulating entity in plasma at all time points for both dose groups on Day 1 and 11. M15 (N-glucuronide of RPV), M27 (tricyclic metabolite), M30 (loss of cyanide and carboxylic acid formation), M33 (hydroxymethyl-RPV) and M43 (cis RPV) were identified as the most important circulating metabolites on Day 1 and 11. Seven additional minor RPV metabolites have been identified. In addition, the most relevant metabolites detected in this study were already detected in previous studies in human or in animals. Metabolites of RPV at steady-state showed a pharmacokinetic profile comparable in shape to that of the parent drug. At the 75 mg dose, the increase in exposure of metabolites between Day 1 and Day 11 was in the same order of magnitude as for RPV. Rilpivirine has an increase in exposure by a factor of 2.45. For the 300 mg dose, a similar exposure between Day 1 and 11 was found for the metabolites M15, M30, M33 and M43. M27 had a slightly higher increase of exposure of 1.68 between Day 1 and 11. Rilpivirine had an increase of exposure of 2.10 between Day 1 and 11. The estimated abundance (AUC) of all metabolites on Day 11 was similar or higher in the 75 mg dose group compared to the 300 mg dose group except for metabolite M43 of which the estimated abundance (AUC) was slightly higher in the 300 mg dose group.

In summary, no disproportionate increase in exposure of the relevant metabolites were observed after repeated dosing for 11 days at 75 and 300 mg, compared to RPV exposure. In addition, the concentrations of all metabolites were approximately similar at Day 11 between the 2 doses.

The antiviral activities of M33 and M42 were tested on a panel of wild-type and mutant HIV-1 virus strains to determine their in vitro antiviral activity. The 50% effective concentration values (EC_{50}) for the wild-type virus were 0.4 nM for M33, 18 nM for M42 and 0.5 nM for RPV.

5.1.5. Metabolite Plasma Profile of Rilpivirine Across Species

In a pilot study the comparative metabolite profile of RPV was investigated in mouse, rat, rabbit, dog and human plasma after single and/or repeated oral administration of cold RPV base (Mod4.2.2.4/TMC278-NC155 [BA45]). A modified LC-MS/MS method was used for the quantification (when the authentic substance was available) and comparative metabolite profiling, by analytical responses, of RPV.

In mouse plasma after single dose administration of RPV base at 2000 mg/kg (micronucleus test) only one metabolite (a metabolite with a molecular mass 18 higher) was detected. In rat plasma after single and repeated administration of RPV base the cysteinyl glycine conjugate, the cysteine conjugate, the N-acetyl-cysteine conjugate and a metabolite with a molecular mass 18 higher were detected. In rabbit plasma after repeated oral administration of RPV one metabolite with a molecular mass 18 amu higher and traces of *N*-glucuronide were detected. No metabolites were found in dog plasma. In human plasma after single and repeated administration of RPV base, *N*-glucuronide (probably M15), cysteinyl glycine (probably M12) and cysteine conjugates (probably M14) and an unidentified metabolite at that time (probably M27) were detected.

5.2. Possible Metabolic Pathways

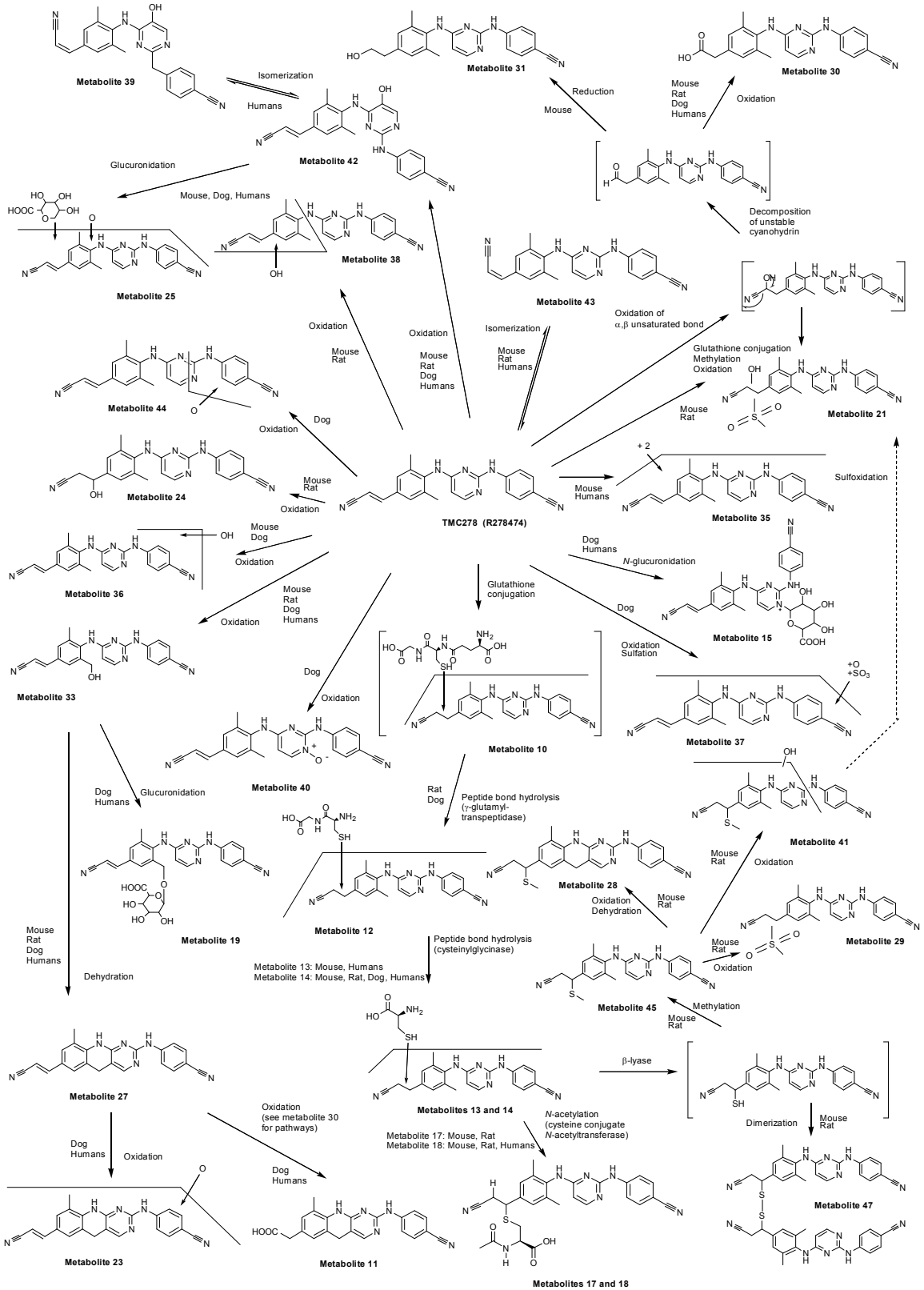
A number of RPV metabolites was identified in the in vivo studies in mice (Mod4.2.2.4/TMC278-NC190 [FK5621]), rats (Mod4.2.2.4/TMC278-NC113 [FK4933]), dogs (Mod4.2.2.4/TMC278-NC114 [FK5143]) and humans (Mod4.2.2.4/TMC278-NC157 [FK5344]) (Mod2.6.5.9A, Mod2.6.5.9B, Mod2.6.5.9D and Mod2.6.5.9E). The structures of these metabolites and the in vivo metabolic pathways are represented in Figure 6. Rilpivirine is metabolized via Phase I and Phase II reactions and the most important pathways are hydroxylation and glutathione conjugation. The contribution of the different metabolic pathways to the overall disposition of RPV is represented in Table 13.

Table 13: Total Percentage of the Administered Dose Metabolized per Major Pathways in Man and its Corresponding Percentages in Mice, Rats and Dogs After Oral Administration of ^{14}C-RPV

Metabolites	Mice		Rats	Dogs	Man
	20 mg/kg	320 mg/kg	40 mg/kg	5 mg/kg	150 mg
5-Hydroxyl RPV at the pyrimidinyl moiety (M42)	18 – 26 [a]	9.2 – 13[a]	2.8-3.6[b]	5.3	16
Hydroxymethyl of RPV (M33)	0.5 – 0.7	1.3 – 1.0	0.54-0.54	8.7 (traces in plasma)	3.0 (seen in plasma)
Carboxylic acid metabolite of the cyanoethenyl moiety (M30)	1.6 – 3.1	1.5 – 1.2	0.47 - 0.05	3.1[c]	2.7
Unknown (M35)	< 0.2	< 0.2	-	-	2.2
Tricyclic metabolite (M27) and carboxylic metabolite of M27 (M11)	0.3 - <0.2 [d]	<0.2 – 0.1 [d]	0.99–1.60 [f]	3.1[g] (traces of M27 in plasma)	2.2 (M27 seen in plasma)
Glutathione-derived conjugates (M13, M14, and M18)	9.6 – 7.9[e]	8.7 – 7.3 [e]	0.03 - 0.46 [i]	< 0.08 [h]	1.2
Unchanged compound	8.8 – 7.9	33 – 34	47 – 43	45	26
N-glucuronide of RPV (M15)	-	-	-	traces in plasma	0.6 (seen in plasma)

[a] Co-eluted with M41, M42 was estimated at 13.9-16.6% (20 mg/kg) and at 5.9-8.0% (320 mg/kg); [b] Co-eluted with M41; [c] Co-eluted with M48; [d] Co-eluted with M28 and M29; [e] includes M17; [f] Co-eluted with M24, M28 and M29; [g] Including M23; [h] Each of them; [i] M14 co-eluted with M12; In mice and rats, the first number is male data

Figure 6: In Vivo Metabolic Pathways of RPV in Animals and Humans (Excluding Rat Bile)



5.3. Presystemic Metabolism (Glycemic Index/Hepatic First-Pass Effects)

Across species, the first-pass effect on RPV is limited and appears to be higher in mice and in rats than in dogs.

5.4. In Vitro Metabolism, Including P450 Studies

5.4.1. In Vitro Metabolic Pathways

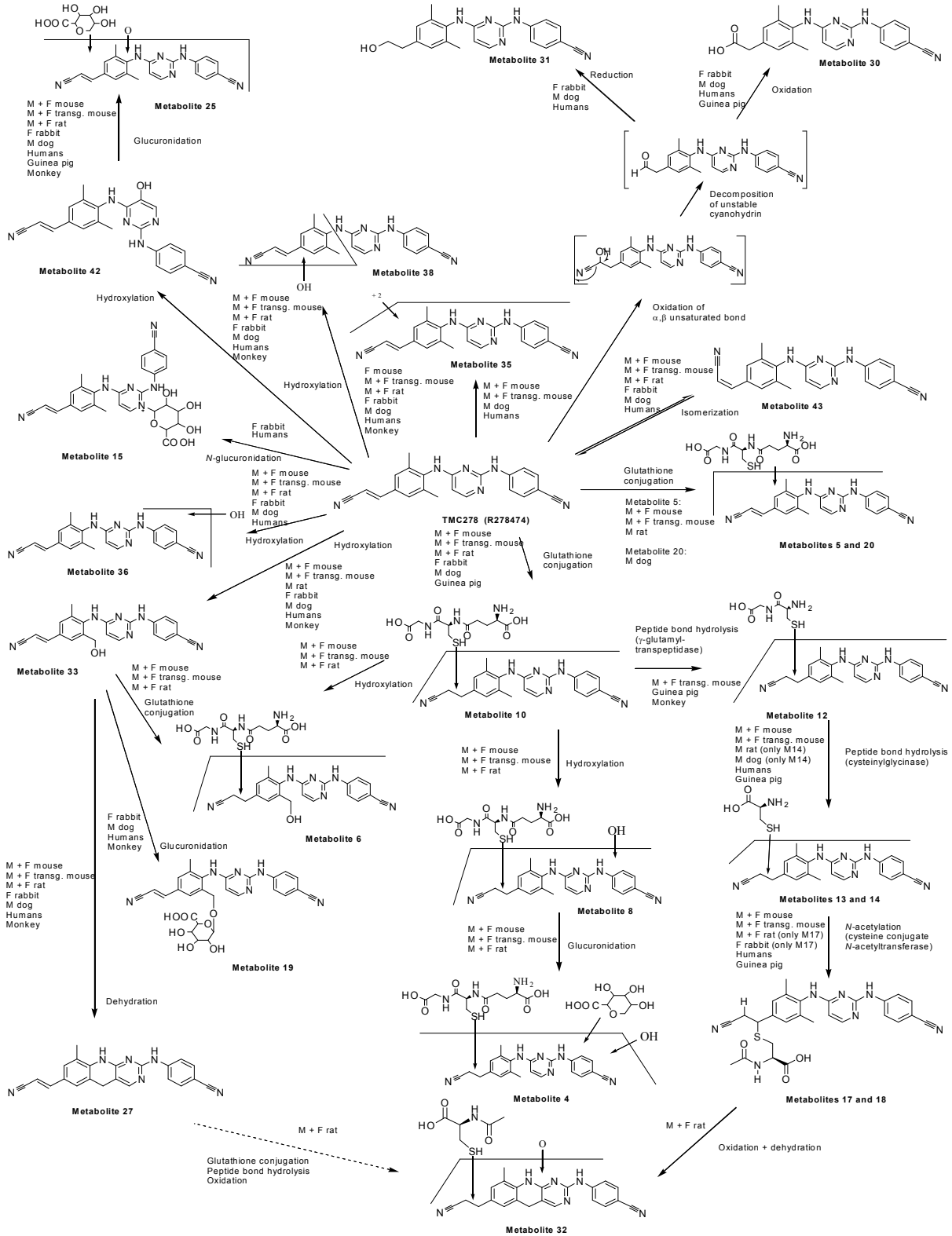
The in vitro metabolism of ^{14}C-RPV was studied in hepatocytes (suspensions and primary cultures) and liver subcellular fractions (microsomes and 12,000 x g supernatant fractions) of male and female Swiss albino mice, male and female black agouti ras H2 microinjected mice, male and female Sprague-Dawley rats, female NZW rabbits, male beagle dogs and man (Mod4.2.2.4/TMC278-NC102 [FK4728]) (Mod2.6.5.10A). In addition, in vitro metabolism was also studied in hepatocyte primary cultures and 12,000 x g liver supernatant fractions from female Dunking Hartley guinea pigs and female or male cynomolgus monkeys (Mod4.2.2.4/TMC278-NC333 [FK6818]) (Mod2.6.5.10B). Rilpivirine (5 μM) was incubated in the above systems at 37^{o}C for various time periods. Incubates were analyzed for metabolites by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for the identification of metabolites.

In each species, a large number of metabolites was detected (see Figure 7). Overall, RPV was metabolized via different metabolic pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation, CN- release followed by reduction/oxidation, and isomerization. Aromatic hydroxylation at the pyrimidinyl moiety (M42) subsequently followed by glucuronidation (M25) was an important metabolic pathway in all the species, and it was the most important in vitro biotransformation route in human, dog and rabbit. Aliphatic hydroxylation at one of the methyl groups of the cyanoethenyl-2,6-dimethylphenyl moiety (M33) subsequently followed by dehydration to form a tricyclic metabolite (M27), proved to be an important metabolic pathway in human, monkey and rabbit, but was less important in the other animal species. The combination of aliphatic hydroxylation with glutathione conjugation (M6) occurred in the mouse strains and in male and female rats, but not in the other species. Aliphatic hydroxylation in combination with glucuronidation (M19), on the contrary, was observed in rabbit, dog, monkey and human, but not in mouse, rat and guinea pig. Glutathione conjugation subsequently followed by conversions leading to mercapturic acid metabolites (M17 and M18) was a main metabolic route in mouse, rat and guinea pig. In the other species, the mercapturic acid biosynthesis route proved to be a minor pathway, and not all intermediary metabolites were detected. Hydroxylation of the glutathione conjugate (M8), subsequently followed by glucuronidation was also solely observed in mouse and rat. The release of the CN- group followed by reduction/oxidation, resulting in the formation of an alcohol metabolite (M31) and a carboxylic acid metabolite (M30), was a minor metabolic pathway in rabbit, guinea pig, dog and human, and could not be detected in mouse, rat and monkey. N-glucuronidation at the pyrimidinyl moiety of RPV (M15) was an important biotransformation pathway in rabbit and could also be detected in human but not in the other species.

All identified RPV metabolites that were detected in human in vitro systems were also detected in at least one animal species.

In a previous in vitro metabolism study with cold RPV (Mod4.2.2.4/TMC278-FK4152), glutathione conjugation of RPV was identified as the most important metabolic pathway in man and rodents. This was not confirmed in the in vitro study with ^{14}C-RPV and in vivo where hydroxylation was the most important metabolic pathway in man.

Figure 7: In Vitro Metabolic Pathways of RPV in the Liver of Swiss Albino Mouse, Sprague-Dawley Rat, Guinea Pig, Rabbit, Dog, Monkey, Man and Black Agouti ras H2 Mouse



5.4.2. Isozymes Involved in the Metabolism of Rilpivirine in Human Liver

5.4.2.1. CYP450 Isozymes Involved in Rilpivirine Metabolism

The in vitro metabolism of ^{14}C-RPV was studied in HLM in the presence of a nicotinamide adenine dinucleotide phosphate (NADPH)-generating system (Mod4.2.2.4/TMC278-NC141 [FK5300]) (Mod2.6.5.10C). The CYP reaction phenotyping of RPV metabolism was performed by different approaches including effect of CYP diagnostic inhibitors on RPV metabolism, metabolism in expressed CYP systems (*E. coli* cells and Supersomes®) and correlation analysis of metabolism rate in a panel of 10 batches of characterized HLM. Incubations were conducted at various RPV concentrations (0.5-50 µM) for 15 minutes with a protein concentration of 0.25 mg/mL. In a preceding nonradiolabeled pilot metabolism study, the identification of CYP isoenzymes was based on inhibitor and metabolism experiments with heterologous expression systems (Mod4.2.2.4/TMC278-FK4151).

In the radiolabeled study, one primary RPV metabolite, M42, and 4 minor metabolites i.e. M33, M27 and the co-eluting metabolites M35 and M36 were formed. The apparent Michaelis-Menten constant substrate concentration ($K_{m)}$ and maximum rate achieved ($V_{max)}$ values for the metabolism of RPV in HLM were 4.17 µM and 381 pmol/mg/min, respectively. The use of different CYP diagnostic inhibitors showed that RPV metabolism was markedly inhibited by the different CYP3A diagnostic inhibitors. Formation of M33 was moderately inhibited with the CYP2C8/9/10 inhibitor sulphaphenazole. Metabolism experiments in expressed CYP450 *E. coli* and Supersomes® systems clearly indicated the involvement of CYP3A isoforms and to some extent of the CYP1A2 isoform. Correlation analysis showed involvement of CYP3A and CYP2C19 in the formation of several metabolites, though for CYP2C19 this was not confirmed in the other phenotyping experiments. CYP1A2 might also play a role in the formation of M33. During some of the experiments in this study, the recovery of the TR was around 70% in the presence of NADPH and cofactor. Addition of glutathione to the incubation mixture resulted in a 50% decrease of bound radioactivity. This suggests that glutathione was able to scavenge hypothetical reactive intermediates. Comparison of the metabolic profile of RPV after incubation in HLM in the absence and presence of glutathione confirmed the formation of several glutathione conjugates.
In conclusion, overall RPV metabolism as well as formation of all its metabolites were mainly catalyzed by CYP3A4. Additionally, it was observed that formation of certain metabolites could also be catalyzed to a lesser extent by CYP2C19, CYP1A2 and CYP2C8/9/10.

In the nonradiolabeled metabolism study, CYP3A4 was clearly involved in the metabolism of RPV based on both inhibition and metabolism data in heterologous expression systems. Metabolism experiments with heterologous expression systems also indicated the possible involvement of CYP1A1, CYP1B1, CYP2C18 and CYP3A5 in the metabolism of RPV.

In an earlier study, using cold compound, a K_m value of 4.94 µM and a V_{max} value of 0.84 nmol/mg/min was calculated. Based on these kinetic parameters, a human in vivo intrinsic Cl of 1410 L/h and a hepatic clearance of 0.042 L/h/kg were predicted (Mod4.2.2.4/TMC278-FK4288).

5.4.2.2. GST Isoforms Involved in RPV Metabolism

The identification of the glutathione S-transferase (GST) isoforms (alpha, mu and pi) involved in the metabolism of ^{14}C-RPV was studied in vitro using heterologous expressed GST (Mod4.2.2.4/TMC278-FK4789). ^{14}C-RPV was tested at 5 and 200 μM using reduced glutathione (GSH, 1 mM) as co-substrate. In addition, incubations were performed in the absence of GST to estimate the amount of non-enzymatic conjugation.

Conjugation with glutathione was more dependent on the mu than the pi isoform of GST, although both isoforms were involved.

5.5. Enzyme Induction and Inhibition

5.5.1. In Vitro Study Measuring CYP Activity and CYP Messenger Ribonucleic Acid (MRNA) Induction in Human Hepatocytes

The potential of RPV to induce CYP450 activities was determined in primary cultures from cryopreserved human hepatocytes originating from 3 different donors and compared to the data obtained with the positive controls omeprazole, rifampicin and ethanol (Mod4.2.2.4/TMC278-NC186 [FK5720]) (Mod2.6.5.12A). Cells were treated for 2 consecutive days either with vehicle (dimethyl sulfoxide (DMSO)), with RPV (2.5, 10 and 25 μM) or with the CYP inducers, i.e. omeprazole (CYP1A2), rifampicin (CYP2B6/2C19/3A4) or ethanol (CYP2E1). Induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP2E1 and CYP3A4) was assessed at the end of the 48-hour treatment period, using corresponding probe substrates (phenacetin (CYP1A2), S-mephenytoin (CYP2B6 and CYP2C19), chlorzoxazone (CYP2E1) and testosterone (CYP3A4)). LC-MS/MS was used to measure the products of the probe substrates in order to determine the CYP activity of the hepatocytes. In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time reverse transcription-polymerase chain reaction.

Most of the batches responded well to the treatment of positive inducers in all assays, except for the CYP2E1 assay. However, the inhibition control (positive control + 25 μM RPV) revealed that RPV seemed to mask the induction of all investigated CYPs. Based on the observed fold-changes of mRNA expression and fold-induction of CYP activities, it can be concluded that RPV might be a very weak inducer of CYP1A2 (6-fold less than omeprazole) and CYP2B6 (4.5-fold less than rifampicin) in human hepatocytes. In addition, the results indicate that RPV appears to be a moderate inducer of CYP2C19 (1.4-fold less than rifampicin) and CYP3A4 (2-fold less than rifampicin) in human hepatocytes. No conclusion could be drawn for CYP2E1.

5.5.2. In Vitro Study Measuring GST Activity Induction in Human Hepatocytes

The potential of RPV to induce GST was evaluated in one batch of primary human hepatocytes in the presence of 3 concentrations of RPV (1, 10, and 30 μM), incubated for 3 consecutive days (Mod4.2.2.4/TMC278-FK4824).

RPV had a low or no effect on GST activity or GST-alpha and GST-mu immunoreactive protein levels when compared with enzyme rates or levels observed in hepatocytes treated with the

vehicle control DMSO. However, the positive controls (phenobarbital, rifampin or 2,3,7,8-tetrachlorodibenzo-p-dioxin) did not result in induction of GST in human hepatocytes and therefore no conclusions can be drawn on the inducing properties of RPV on the GST activity and expression from this study.

5.5.3. Ex-vivo Studies Measuring Enzyme Activities in Mouse, Rat and Dog Liver

Rilpivirine in aqueous hydroxypropyl-methylcellulose (HPMC) (0.5%) was administered for 3 months to male and female CD-1 mice at doses of 20, 80 and 320 mg/kg/day (Mod4.2.2.4/TMC278-NC192 [FK5563]) (Mod2.6.5.12B). Rilpivirine base in PEG400/CA (10%) was administered to male and female Sprague-Dawley rats at doses of 40, 120 and 400 mg/kg/day and to male and female beagle dogs at doses of 5, 10 and 40 mg/kg/day for 6 months (Mod4.2.2.4/TMC278-NC193 [FK5564]), (Mod4.2.2.4/TMC278-NC140 [FK5518]) (Mod2.6.5.12C and Mod2.6.5.12D).

To examine the effect of RPV on some hepatic enzyme activities, microsomal fractions of livers from the above mentioned RPV treated animals were assayed for protein and total CYP content, and for the activities of 7-ethoxyresorufin O-deethylase, 7-pentoxyresorufin O-depentylase, 4-nitrophenol hydroxylase, testosterone 6β-hydroxylase and lauric acid 12-hydroxylase. These enzyme activities are well known markers for the induction of CYP1A, CYP2B, CYP2E, CYP3A and CYP4A forms, respectively. Microsomes were also assayed for lauric acid 11-hydroxylase activity, which is largely catalyzed by CYP2E1 and for thyroxine UDP-GT activity. Additionally, liver cytosolic fractions were assayed for protein content and GST activity towards 1-chloro-2, 4 dinitrobenzene as a substrate. In addition, in rats, the effect of RPV on some hepatic enzyme activities were also examined in liver samples from a 2-week study (RPV base in PEG400/CA (10%) at 40, 120 and 400 mg/kg/day) (Mod4.2.2.4/TMC278-FK4247). In this study, the liver microsomes were assayed for protein and for 7-ethoxyresorufin *O*-deethylase (CYP1A1, CYP1A2), 7-pentoxyresorufin-dealkylase (CYP2B), aniline hydroxylase (CYP2E1), *N*-ethyl morphine *N*-demethylase (CYP3A1, CYP3A2), lauric acid hydroxylase (CYP4A1) and thyroxine (T4) glucuronosyltransferase activities.

In mice, RPV was an inducer of the CYP4A forms in both male and female animals (up to 25- and 20-fold, respectively) (see Table 14). Some induction was also seen with the CYP3A forms (up to 1.7-fold in both males and females). RPV treatment induced UDP-GT activity in male and female mice (up to 2.1- and 2.3-fold, respectively) and decreased GST activity in male mice to 44% at 320 mg/kg/day.

In rats, RPV was an inducer of CYP4A forms in male rats (4.7-fold) whereas in female rats RPV was an inducer of CYP3A forms (6-fold) and possibly also of CYP2B and CYP4A forms (see Table 14). Rilpivirine treatment had some effect on UDP-GT activity in male rats (induction of 1.3-fold only at high dose level) and on GST activity in female rats (induction of 1.5-fold). In the 2-week study, the results were similar.

In dogs, treatment with RPV did not result in any induction of CYP1A1, CYP2B, CYP2E, and CYP4A, UDP-GT or GST activity. Rilpivirine produced some decrease in microsomal CYP3A-

dependent testosterone 6β-hydroxylase activity but this effect was confined to the two highest dose levels and was not dose-dependent (see Table 14).

Table 14: Percentage of Testosterone 6β-hydroxylase, Lauric Acid 12-hydroxylase and UDP-GT Activities Relative to Control Values in Hepatic Microsomal Fractions of CD-1 Mouse, Sprague-Dawley Rat and Beagle Dog After Repeated Administration of RPV or RPV Base

Species	Dose (mg/kg/day)	Testosterone 6β-hydroxylase (CYP3A)		Lauric acid 12-hydroxylase (CYP4A)		Thyroxine UDP glucuronosyltransferase (UDP-GT)	
		Male	Female	Male	Female	Male	Female
Mouse (Mod4.2.2.4/TMC278-NC192 [FK5563])	20	111	153**	147*	126	108	138*
	80	156***	174***	525***	521***	150**	164***
	320	174***	175***	2499***	1966***	210***	229***
Rat (Mod4.2.2.4/TMC278-NC193 [FK5564])	40	95	120	140	75	65**	127
	120	125	300***	262**	93	77*	98
	400	120	600***	466***	127*	125*	134
Dog (Mod4.2.2.4/TMC278-NC140 [FK5518])	5	85		100		82	
	10	57**		102		75	
	40	74*		113		68	

* p<0.05; ** p<0.01; *** p<0.001

5.5.4. In Vitro Inhibition of Human CYP450 Enzymes by Rilpivirine

Rilpivirine was tested for its inhibitory effect on the metabolism of various human CYP450 probe substrates to gain information about the possibility of clinically relevant interactions with other drugs (Mod4.2.2.4/TMC278-FK4123), (Mod4.2.2.4/TMC278-NC283 [FK6443]). Incubations with P450 probe substrates, selective towards CYP1A2, CYP2A6, CYP2C8/9/10, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4A and CYP3A4/5 were performed in HLM in the absence and presence of RPV at 8 different concentrations ranging between 0.03 and 400 µM (Table 15).

Table 15: Interaction of RPV With Human CYP450 in Vitro

Substrate	CYP involved	IC_{50}-value (µM)
Phenacetin	CYP1A2	34.0
Coumarin	CYP2A6	>100 (15.7)[a]
Tolbutamide	CYP2C8/9/10	3.99
Dextromethorphan	CYP2D6	3.88
Bufuralol	CYP2D6	12.0
Testosterone	CYP3A4	6.29
Cyclosporin A	CYP3A4	16.8
Midazolam	CYP3A**4**/5	4.20
	CYP3A4/**5**	18.3
Lauric acid	CYP4A	>100 (15.9) [a]
	CYP2E1	9.79

[a] % inhibition at 100 µM

Under these conditions, RPV was a potent inhibitor of CYP2C19 and CYP2E1. CYP2C19 activity was blocked 70% at a concentration of 0.06 µM (0.02 µg/mL) and 86% of CYP2E1 activity was inhibited at a concentration of 0.03 µM (0.01 µg/mL). However, an in vitro study with cultured hepatocytes (see Section 5.5.1) indicated a moderate induction of CYP2C19 by

RPV. For CYP2E1, there were some discrepancies in this in vitro study: Rilpivirine seemed to be a strong inhibitor of CYP2E1 with chlorzoxazone as a substrate and not with lauric acid as a substrate. However, in the in vitro drug-drug interaction study (see Section 7.2), no interaction was observed between RPV and chlorzoxazone. Therefore, as they were not confirmed by subsequent studies, the inhibition of CYP2C19 and CYP2E1 by RPV are considered not relevant. For the other CYPs, taking into account a mean C_{max} value of about 0.13 µg/mL for oral administration of RPV and 0.14 µg/mL for IM administration of RPV LA in human at steady-state, inhibition in vivo is unlikely.

Inhibition of CYP2C8-mediated paclitaxel 6α-hydroxylation and CYP2C9-mediated S-warfarin-7-hydroxylation by RPV (0.1 - 300 or 200 µM, respectively) was also investigated in HLM (Mod4.2.2.4/TMC278-NC283 [FK6443]). RPV is an inhibitor of CYP2C8 and CYP2C9 with a K_i of 10 and 1.7 µM, respectively. Taking into account a mean C_{max} value of about 0.13 or 0.14 µg/mL for RPV after oral or IM in human, inhibition of CYP2C8 and CYP2C9 by RPV is not expected.

Furthermore, MBI of CYP2C9 by RPV (0.1-100 µM) was investigated in HLM with tolbutamide as a probe substrate (Mod4.2.2.4/1646_0030536 [FK10162] and Mod2.6.5.12E). Tienilic acid was used as positive reference inhibitor. The MBI potential was evaluated as the % decrease in CYP2C9 activity in the presence of NADPH regenerating system (NRS) relative to samples without NRS, after 20-fold dilution. A decrease in CYP activity with pre-incubation (PI) + NRS compared to PI – NRS is regarded as potential MBI involvement.

Inhibition data up to 3 µM demonstrated only a small decrease in CYP2C9 activity during PI with NRS versus without NRS, and the annual percentage rate of charge of 288 indicated only a limited MBI potential. From 10 µM RPV onwards, CYP2C9 activity decreased similarly after PI of HLM with RPV both with and without NRS. This means that from 10 µM RPV onwards there is no additional effect due to MBI of RPV on top of the % effect already seen due to reversible CYP2C9 inhibition and/or due to direct CYP2C9 inactivation without metabolic activation. The clinical relevance of these findings should always be considered in the context of expected free RPV concentrations. Therefore, although in a clinical setting the low-end concentrations (C_{max} = 0.13 or 0.14 µg/mL at steady-state) are probably the most relevant, the limited MBI of CYP2C9 is unlikely to have clinical relevance at therapeutic doses of RPV.

5.5.5. Effect of Rilpivirine on Adrenal Gland

The effect of RPV on cortisol biosynthesis in dog adrenal cortex cell-free extracts was determined (Mod4.2.2.4/TMC278-FK4790) (see also Mod2.6.6/Section 8.3).

Rilpivirine at a nominal 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.

6. EXCRETION

Excretion studies after oral administration of ^{14}C-RPV in mice, rats, dogs, and humans have been conducted to support the registration of oral RPV (EDURANT®) submission. These studies that support the RPV LA MAA are rediscussed below.

6.1. Routes and Extent of Excretion

The excretion of RPV was studied after single oral administration of ^{14}C-RPV base in male and female CD-1 mice at 20 and 320 mg/kg (Mod4.2.2.4/TMC278-NC190 [FK5621]), in Sprague-Dawley rats at 40 mg/kg (Mod4.2.2.4/TMC278-NC113 [FK4933]), (Mod4.2.2.4/TMC278-NC145 [FK5525]) and in male beagle dogs at 5 mg/kg (Mod4.2.2.4/TMC278-NC114 [FK5143]) (Mod2.6.5.9A, Mod2.6.5.9B, Mod2.6.5.9C and Mod2.6.5.9D). Healthy male subjects were dosed orally with 150 mg ^{14}C-RPV base (Mod4.2.2.4/TMC278-NC157 [FK5344]) (Mod2.6.5.9E). Urine and feces samples were collected up to 96 hours after dosing in rodents, up to 168 hours after dosing in dogs and humans (only urine) and up to 336 hours in humans (only feces). TR was measured by scintillation counting.

In rodents, the TR was rapidly excreted with 90% to 94% (at 20 mg/kg) and 69% to 74% (at 320 mg/kg) of the radioactive dose eliminated in mice and 79% to 84% eliminated in rats within the first 24 hours after dosing. In dogs, excretion was relatively slow with 54% of the radioactive dose eliminated within the first 24 hours. In mice, rats and dogs, the predominant route of excretion of ^{14}C-RPV was via the feces. The majority of the TR was eliminated in feces as unchanged RPV in mice (33-34% at 320 mg/kg), in rats (43-47%) and in dogs (43%) at 48 hours after dosing. Only, in mice at 20 mg/kg, one metabolite M42 was the most abundant in feces. Renal excretion was very limited (0.45 to 4.2% of the radioactivity dose) in all animal species and the amount of unchanged RPV in urine was negligible. The excretion was virtually complete at 96 hours after dosing in rodents and at 168 hours after dosing in dogs (Table 16). In a biliary excretion study in male Sprague-Dawley rats, the amount of radioactivity excreted in bile within 24 hours after dosing was rather low, only 18% and 25% of the administered radioactivity, in restrained and nonrestrained animals, respectively. The amount of unchanged RPV excreted in bile during this time period was negligible (about 0.2%). The biliary excretion study demonstrated that the major part of unchanged RPV excreted in feces in rats had not been absorbed.

The excretion of RPV in humans was similar to that seen in the nonclinical species. 85% of the dose was excreted in feces and excretion was virtually complete at 336 hours after dosing. Unchanged RPV represented on average 26% of the administered dose in feces. In humans, the amount of TR recovered in urine was somewhat higher (6.1% of the administered dose over the 7-day period) than in animals. Unchanged RPV in urine was negligible.

Table 16: Urinary and Fecal Excretion of the Radioactivity Following a Single Oral Dose of ^{14}C-RPV Base in Mouse and Rat at 96 Hours After Dosing and in Dog and Human at 168 Hours After Dosing

% of administered dose	Mouse 20 mg/kg		Mouse 320 mg/kg		Rat 40 mg/kg		Dog 5 mg/kg	Human 150 mg
	Male	Female	Male	Female	Male	Female	Male	Male
Urine	3.51	4.19	1.84	3.62	0.45	1.77	1.73	6.13
Feces	87.8	87.1	95.8	88.8	93.3	92.6	94.7	85.1
Cage washings	3.61	3.79	1.18	3.37	0.12	0.68	0.38	-
Total Recovered	**94.9**	**95.1**	**98.9**	**95.8**	**93.9**	**95.1**	**96.8**	**91.2**[a]

[a] Expressed as percent of the administered dose in the 0-168h urine and 0-336h feces

6.2. Excretion in Milk

No studies have been conducted to assess directly the excretion of RPV into milk. In the QWBA study in pregnant Sprague-Dawley rats (see Section 4.1.1.1), some radioactivity was seen in the mammary glands (tissue/blood $AUC_{0\text{-}8h}$ ratio = 3), which indicates the potential for excretion of RPV-related radioactivity via the milk.

In a dose range finding study for a pre- and postnatal developmental study (Mod4.2.3.5.3/TMC278-NC168 [TOX6847]) it was found that pups were exposed to RPV through the milk of the dams dosed with RPV (40, 120 and 400 mg/kg/day). On Day 7 of lactation, exposure ($AUC_{0\text{-}24h}$) in pups was 0.62 and 0.74 µg.h/mL at 40 mg/kg, 0.94 and 0.91 µg.h/mL at 120 mg/kg and 1.9 and 1.8 µg.h/mL at 400 mg/kg in males and females, respectively. Exposure in pups dosed through milk on Day 7 of lactation was approximately 20- to 35-fold lower than in pups directly dosed by oral gavage on Day 25 of age.

7. PHARMACOKINETIC DRUG INTERACTIONS

7.1. Transporter Studies

The inhibition of transport of the OCT2 substrate ^{14}C-metformin by RPV was evaluated in CHO cells stably transfected with complementary deoxyribonucleic acid (cDNA) encoding for this transporter (Mod4.2.2.6/1646_0025128 [FK10042] and Mod2.6.5.15A). Quinidine and cimetidine were used as positive control inhibitors. Transport, and inhibition thereof, was tested in the presence of 1% bovine serum albumin (BSA).

The IC_{50} value for inhibition of OCT2 by RPV was 5.46 µM (2.0 µg/mL).

The inhibition of transport of the MATE-1 and MATE-2K substrate ^{14}C-TEA by RPV was evaluated in CHO cells stably transfected with cDNA encoding for this transporter (Mod4.2.2.6/1646_0035314 [FK10420] and Mod2.6.5.15B). Quinidine and pyrimethamine were used as positive control inhibitors. Transport, and inhibition thereof, was tested in the presence of 1% BSA.

The uptake of ^{14}C-TEA was inhibited by RPV with an IC_{50} value of 7.51 µM (2.75 µg/mL) for MATE-1 and of <0.05 µM (<0.018 µg/mL) for MATE-2K. In conclusion, the effect of RPV on

MATE-1 is unlikely to be clinically relevant, but it cannot be excluded that RPV would inhibit MATE-2K at clinically relevant concentrations.

In the Nonclinical Overview (Mod2.4/Sec3.7.1 and 5.2) these results are compared to the clinical data.

7.2. Drug-Drug Interactions

The in vitro interaction of RPV with the metabolism of sertraline (substrate of multiple CYPs, Monoamine oxidase and UDP-GT), paroxetine (CYP2D6), clarithromycin (CYP3A4), sildenafil (CYP3A4), omeprazole (CYP2C19 and CYP3A4), chlorzoxazone (CYP2E1), 17α-ethinylestradiol (phase II metabolism), S-mephenytoin (CYP2C19) and norethindrone (different isoenzymes) was investigated in a pooled batch of HLM and the same was done for abacavir (alcohol dehydrogenase) in a pooled batch of human liver cytosol (Mod4.2.2.6/TMC278-NC194 (FK5568]) (Mod2.6.5.15C).

Rilpivirine seemed to have a significant inhibitory effect (IC_{50} <5 μM) on the metabolism of clarithromycin, sildenafil, S-mephenytoin and norethindrone and a moderate effect (5 μM <IC_{50} <10 μM) on sertraline, paroxetine and 17α-ethinylestradiol. Omeprazole metabolism was only poorly inhibited by RPV, displaying an IC_{50}-value of 12 μM. RPV has under these conditions no measurable effect on the metabolism of abacavir or chlorzoxazone, as metabolite formation of the latter compounds was not inhibited (IC_{50} >30 μM).

These in vitro data indicate a possible effect of RPV on the in vivo metabolism of clarithromycin, sildenafil, S-mephenytoin, and norethindrone and also albeit somewhat less likely, with sertraline, paroxetine and 17α-ethinylestradiol. No inhibition is expected for omeprazole, abacavir, and chlorzoxazone.

In the Nonclinical Overview (Mod2.4/Sec3.5) these results are compared to the clinical data.

7.3. Combination of RPV LA With CAB LA in Rats

The pharmacokinetics of RPV was determined following single IM administration at 60 mg/kg (0.2 mL/kg) of RPV LA (G001) alone or in combination with a LA injectable suspension of GSK1265744A (CAB) at 10 mg/kg (0.05 mL/kg) in male Sprague-Dawley rats with a follow-up of 2 months (Mod4.2.2.6/1955_0018187 [FK7565] and Mod2.6.5.15D). The plasma concentrations of RPV were comparable for the 2 groups. Initial release was quick for both groups, with peak concentrations that were reached within 2-6 h after administration. After the peak, the levels showed a biphasic decline consisting of a quick decline during up to 2 days after dose administration, followed by a slower decrease up to the last sampling point, 2 months after dose administration. The mean C_{max} and $AUC_{0\text{-}1444h\ or\ 2months}$ values of RPV were similar between the 2 groups (Table 17).

Table 17: Mean (n=3) Pharmacokinetic Parameters of RPV in Male Rats After Single IM Administration at 60 mg/kg, Dosed Alone as RPV LA (G001) in Combination with GSK1265744A (CAB) at 10 mg/kg

Dose (mg/kg)	C_{max} (µg/mL)	t_{max} (h)	$AUC_{0\text{-}1444h}$ (µg.h/mL)	$AUC_{0\text{-}\infty}$ (µg.h/mL)
60 (RPV LA)	0.11	4.7	23	26
60 (RPV LA) + 10 (CAB)	0.11	2.0	24	27

8. OTHER PHARMACOKINETIC STUDIES

8.1. After Administration of Other non-G001 Rilpivirine LA Formulations

8.1.1. Studies in Mice

A single SC administration of 25 mg/mL RPV LA in P338 (3.8 mg/mL) at 2.5, 5, 10 and 20 mg/kg or in Vitamin E-D-α-tocopheryl polyethyleneglycol 1000 succinate (Vit E-TPGS) at 20 mg/kg was given to male and female Swiss mice (Mod4.2.3.7.7/TMC278-NC196 [TOX7354]), followed by a period of 4 and 18 days. At 20 mg/kg of RPV LA in P338, C_{max} and $AUC_{0\text{-}\infty}$ values of RPV were 1.6 µg/mL and 60 µg.h/mL in males and 2.2 µg/mL and 74 µg.h/mL in females, respectively. $AUC_{0\text{-}\infty}$ values increased in a fairly dose-proportional fashion. The exposure to RPV (both C_{max} and AUC) was slightly higher in females than in males.

Eighteen days post-dosing, the concentrations of RPV in the spleen and thymus were below the quantification limit (<0.017 µg/g) in both sexes and at all dose levels studied (Mod2.6.5.16A). On the other hand, after RPV LA in P338, the skin concentrations of RPV at the injection site were high and amounted to 0.033, 0.16, 0.11 and 67 µg/g in male mice and to 0.68, 0.53, 0.57 and 48 µg/g in female mice at 2.5, 5, 10 and 20 mg/kg, respectively. Taking into account the high variability, the concentrations of RPV at the injection site were much higher than those in plasma. At 20 mg/kg the skin-to-plasma ratio was on average 1599, an indication that a substantial part of the compound remained at the injection site.

8.1.2. Studies in Rats

In male Sprague-Dawley rats (Mod4.2.3.7.7/TMC278-NC244 [TOX7896]), after single IM or SC administration of F004 (100 mg RPV base/mL and 25 mg/mL of P338) at doses of 5 and 20 mg/kg with an 8-week follow-up period, RPV plasma concentrations increased up to 7 h post-dose and declined thereafter (slightly faster after IM than after SC administration). Mean C_{max} values of RPV were approximately 2-fold higher after IM than after SC administration, but mean $AUC_{0\text{-}day56}$ values were comparable for the 2 routes (15 versus 16 µg.h/mL for IM and SC at 20 mg/kg, respectively). A dose-proportional increase in $AUC_{0\text{-}day56}$ and a less than dose-proportional increase in C_{max} were observed.

After a follow-up period of 8 weeks, mean concentrations of RPV in thymus and spleen were below the detection limit (0.005 µg/g) (Mod2.6.5.16B). Mean concentrations of RPV in muscle at the injection site (IM) amounted to on average 2.0 µg/g and 8.3 µg/g at 5 and 20 mg/kg, respectively.

8.1.3. Studies in Rabbits

In female NZW rabbits (Mod4.2.2.7/TMC278-NC356 [FK6993] and Mod2.6.5.16C), after single IM administration, the pharmacokinetics of F004 at 50 mg/kg was compared with 300 mg/mL RPV LA in PS80 (F006) at 150 mg/kg after a 1-month follow-up period. The release profile of F004 was different from that of F006, particularly for the first phase of the plasma concentration-time profile. Dosing of F004 led to a rapid onset of drug availability with t_{max} between 8 and 24 h post-dose, while dosing of F006 resulted in a gradual release until Day 14. Plasma C_{max} and $AUC_{0\text{-}day36}$ values of RPV were 7.5 µg/mL and 1423 µg.h/mL after 50 mg/kg of F004 and 5.8 µg/mL and 2786 µg.h/mL at 150 mg/kg of F006, respectively.

8.1.4. Studies in Dogs

In a first study (Mod4.2.2.7/Innovation-NC114 [FK5458]) in male beagle dogs (n=2), a suspension of RPV base or RPV.HCl at 25 mg/mL in P338 at 10 mg/mL was administered IM at 5 mg eq./kg (Mod2.6.5.16D). IM administration of RPV LA resulted in a rapid onset of drug release over a period of 2 days, with mean RPV C_{max} values of 0.17 µg/mL observed within 48 h. Thereafter plasma concentrations slowly declined to undetectable concentrations from 3 months (Day 94) onwards after dosing. The mean $AUC_{0\text{-}3months}$ value was 39 µg.h/mL. Such an initial drug release was not observed after IM dosing of RPV.HCl, where C_{max} values of 0.095 µg/mL were reached at 48 h after dosing, and after which concentrations remained constant for about 10 days. Thereafter plasma concentrations declined slowly. $AUC_{0\text{-}3months}$ values were 34 µg.h/mL.
In addition, selected tissues (liver, muscle [at the injection and non-injection site], adrenal gland, abdominal fat, spleen, lymph nodes [iliac, popliteal, auxiliary, mandibulary], skin and thymus) were collected on Days 94 and 184 at necropsy of each animal. In addition, at Day 29, biopsies of iliac and popliteal lymph nodes were performed. The RPV concentrations were measured in all these tissues. After IM administration of RPV LA at 5 mg/kg in P338 (25 mg/mL) at Day 29, high concentrations of RPV were found in the iliac (8.6-30 µg/g) and popliteal (4.6-8.4 µg/g) lymph nodes adjacent to the injection site, being 100 times higher than those in plasma. Three and six months after dosing, concentrations in these adjacent lymph nodes decreased to concentrations similar to those observed in more distant lymphoid tissues and lymph nodes (0.01-0.03 µg/g). The concentrations in tissues (adrenal gland, abdominal fat, liver, muscle, skin, spleen, thymus and several lymph nodes) sampled at sacrifice (3 or 6 months post-dose) ranged between 0.010 and 0.085 µg/g at 3 months post-dose, and between 0.06 and 0.031 µg/g at 6 months post-dose, demonstrating a decrease between the 2 sacrifice time points.

In a second study (Mod4.2.2.7/TMC278-NC238 [FK5998] and Mod2.6.5.16E), 5 mg/kg of RPV (25 mg/mL) formulated in P338 (3.7 mg/mL) was given IM or SC to male beagle dogs with a follow-up of 6 months. Similar concentration-time profiles were observed as described for the first study. C_{max} and $AUC_{0\text{-}6months}$ values of RPV after IM dosing were 0.62 µg/mL and 23 µg.h/mL, respectively.

In male and female beagle dogs (Mod4.2.3.7.7/TMC278-NC234 [TOX7781] and Mod2.6.5.16F), F004 at a single dose of 200 and 400 mg/animal was given as 2 subsequent IM injections separated by 24 h, followed by a 3-month follow-up period. In general, exposure to

RPV (both C_{max} and $AUC_{0\text{-}1or\ 3\ month}$) showed an increase proportional with dose in the dose range 200-400 mg/animal and there were no overall gender differences noted. $AUC_{0\text{-}1month}$ values in males and females dosed at 400 mg/animal were 188 and 204 µg.h/mL, while $AUC_{0\text{-}3months}$ values were 281 and 265 µg.h/mL, respectively.
In addition, selected tissues (brain, spleen, thymus, testis) were collected on Days 29/30 and 92/93 at necropsy. After IM administration at 200 or 400 mg/animal at Day29/30 and Day 92/93, the mean RPV tissue-to-plasma concentration ratios in males and females ranged between 1.5 and 2.3 in brain, 2.8 and 2.9 in spleen and 1.8 and 6.0 in thymus. In testes, the tissue-to-plasma concentration ratios ranged between 0.54 and 0.88 in males.

In another study, single SC dosing of RPV LA at 5 or 20 mg/kg, formulated in Vit-E-TPGS, was performed in male beagle dogs (Mod4.2.2.7/TMC278-NC203 [FK5821] and Mod2.6.5.16G). Suspensions containing RPV at 25 mg/mL with a particle size of 400 nm and at 100 mg/mL with particle sizes of 400 nm or 800 nm were administered. Taking into consideration the inter-individual variability in plasma concentrations, the release profiles were very similar between the 3 treatment groups. Furthermore, the exposures as well as the plasma profiles were hardly influenced by changing the size of the particles from 400 to 800 nm at 20 mg/kg. $AUC_{0\text{-}3months}$ values were 14, 38 and 30 µg.h/mL after 5 mg/kg (particle size of 400 nm), 20 mg/kg with particle sizes of 400 nm or 800 nm, respectively.
In addition, selected tissues (adrenal gland, abdominal fat, liver, axillary and mandibular lymph node, muscle, skin (injection and non-injection sites), spleen, thymus) were collected on Day 93 at necropsy for each group. The concentrations of RPV in skin at the injection site were very high (> 500.000 times higher than those in plasma) 3-months after administration, indicating that a substantial part of the compound remained at the injection site after SC administration. The highest tissue-to-plasma RPV concentration ratios were obtained in the auxiliary lymph node (10-206), liver (12-15), abdominal fat (9-12), and skin (6.3-11) at non-injection site. For adrenal gland, mandibular lymph node, muscle, spleen and thymus, the mean tissue-to-plasma ratios ranged from about 1.6 to 8.

8.1.5. Studies in Minipigs

In male Göttingen minipigs, the pharmacokinetics of RPV LA (F004) at a single IM dose of 400 mg (~20 mg/kg) was compared with 300 mg/mL RPV LA in PS80 (F006) at a single IM dose of 450 mg (~22.5 mg/kg). A 1- and 3-month follow-up period was included (Mod4.2.2.7/TMC278-NC295 [FK6407] and Mod2.6.5.16H). The plasma profiles were different particularly during the first month. A faster increase in plasma concentrations was seen with F004 (t_{max} = 8h) than with F006 (t_{max} = 160-360 h). Furthermore, mean C_{max} and $AUC_{0\text{-}3months}$ values of RPV were about 2.3-times higher with F004 than with F006 with C_{max} values of 0.049 versus 0.015 µg/mL and $AUC_{0\text{-}3months}$ values 20 and 8.7 µg.h/mL, respectively.

In male Göttingen minipigs, after IM administration, the pharmacokinetics of 100 and 200 mg/mL RPV LA (P338) at doses of 200 and 400 mg (~20 and 40 mg/kg) was compared with 100, 200, 250 and 300 mg/mL RPV LA (PS80) at 200, 400, 500 and 600 mg (~20, 40, 50 and 60 mg/kg). A 1-month follow-up period was included (Mod4.2.2.7/TMC278-NC344 [FK7034] and Mod2.6.5.16I). At a dose of 400 mg, the mean $AUC_{0\text{-}1month}$ value of RPV was 15 µg.h/mL

when RPV LA was formulated in P338 and was 9.8 µg.h/mL when RPV LA was formulated in PS80. When comparing plasma profiles (100 and 200 mg/mL) between the 2 formulations, different plasma profiles were observed from the start of dosing until 144 h (6 days) post-dose. Plasma concentrations for the P338 formulations (100 and 200 mg/mL) increased up to 6 h, declined from 6 to 72 h and then remained fairly constant from 72 to 648 h. Plasma concentrations for the PS80 formulations (100-300 mg/mL) dropped almost immediately (t_{max} = 0.5-2 h) after dosing, increased slowly up to 144 h and then remained fairly constant until 27 days. Higher mean $AUC_{0\text{-}1month}$ values (approximately 30 to 50%) were obtained for the P338 formulations, compared to the same concentration of PS80 formulations.

Toxicokinetics was also determined in a 39-week (with 13-week interim kill) IM study in minipigs. The test article was administered at 75 mg/kg on Days 1, 30, 60 and 90, and at 600 mg/animal on Days 120, 150, 180, 210, 240 and 270. In addition, injections were performed at 300 mg/animal on Days 120, 150, 180 and 210. On Day 93 (i.e. after the 4th dose administration) an interim sacrifice was performed involving half of the number of animals of each sex in each group. The other animals were maintained in the study for another 6 months (Mod4.2.3.7.7/ TMC278-NC296 (TOX8580) and Mod2.6.5.16J).

C_{max} and AUC values of RPV were comparable to somewhat higher after repeated dosing on Day 60/240 than after single dosing on Day 1. C_{max} and AUC were comparable to somewhat higher in females than in males after single and repeated dosing. At Day 240, $AUC_{day240\text{-}269}$ values were 43 µg.h/mL in males and 35 µg.h/mL in females.

8.2. Rilpivirine Impurity related substance D*

Some in vitro or in vivo studies were performed focusing on the related substance Dgenotoxic impurity of RPV.

8.2.1. In Vitro Metabolism Study

The in vitro metabolism of ^{14}C-related substance D*(JNJ-19376123), a genotoxic impurity of RPV, was explored in rat and human liver subcellular fractions (microsomes and S9 supernatant) and in rat and human hepatocytes (Mod4.2.2.7/2683_0038991 [FK10250]). In subcellular liver fractions of rat and human, the major metabolites of related substance D*originated from oxidation and release of thiocyanate (SCN-). In hepatocytes, direct sulfate conjugation was by far the major metabolite in the rat and was one of the major metabolites in human. The metabolic pathways that have been detected in vitro in rat and human liver models represent the major in vivo clearance pathways in the rat, viz. sulfate conjugation and loss of the CN- function, which gets further metabolized to SCN-. It is thus most likely that also in human this impurity is efficiently cleared via metabolic degradation.

8.2.2. In Vivo Metabolism Studies

The pharmacokinetics of RPV and its genotoxic impurity (related substance) was determined in male rats 1 week after dosing (Mod4.2.2.7/2683_0038991 [FK10250]). The rats were administered a single IM injection of RPV LA with spiked levels of related substance Dat 60 mg RPV/rat and 925 ppm related substance/rat (original batch contained 1-2 ppm related substance). Plasma concentrations of related substance Dwere low

but could be measured up to 24 h (n=1), 52 h (n=2) or 100 h (n=1) after dosing. C_{max} value was reached at 2 h after dosing (first sampling time point). $AUC_{0-24\ h}$ value of related substance D was only about 0.1% compared to the $AUC_{0-24\ h}$ value of RPV.

Following a single IM injection of RPV LA (G001) at 60 mg/rat with spiked levels of ^{14}C-related substance D* (185 µg/rat) (Mod4.2.2.7/2683_0039002 [FK10345]), about 44% of the administered radioactive dose was excreted via urine within 96 h (of which 68% was excreted within 0-7 h) and about 22% was excreted via feces (of which 59% was excreted within the first 24 h). A small amount of the radioactive dose was present in the muscle of the injection site (1.4%), but 28% of the radioactivity was still present in the carcass (n=2) at 96 h after dosing. The total recovery of the administered radioactivity was 96% (n=2). TR in plasma decreased very slowly as a function of time. The plasma metabolism profiles of the radioactivity showed that the total amount of the radioactivity was predominantly accounted for by SCN-.

The plasma concentration of related substance D rapidly decreased after dosing and reached levels of only 0.1% of the C_{max} (t_{max} = 0.5 h) after 24 h.

In urine, most of the administered radioactivity excreted (21% of the dose) was the sulfate metabolite. About 9% of the administered radioactivity was SCN- and only 0.65% of the dose was identified as the impurity related substance B in the first collection interval (0-7 h). In feces, 2.6% of the dose was the impurity related substance B and 1.2% of the dose was SCN- (0-24 h) and in later time intervals, hardly any related substance D, SCN- or other entities could be detected.

In conclusion, in rat, related substance D is rapidly metabolically cleared and that the main metabolite pathways were sulfate conjugation and loss of the CN- function.

8.3. Poloxamer 338

Some studies were conducted after measuring in plasma or other matrices P338, excipient of the G001 formulation.

8.3.1. Pharmacokinetic Studies

The plasma concentrations of P338, were measured after a single oral administration of P338 (solution in water) at 1600 mg/kg to female Sprague-Dawley rats and to female NZW rabbits, using the same experimental conditions as the GLP embryo-fetal development studies (Mod4.2.3.7.7/TMC278-NC348 [TOX9680] and Mod4.2.3.7.7/TMC278-NC347 [TOX9679]). The plasma concentrations of P338 were below the quantification limit in all samples, i.e., below 0.1 µg/mL (Mod4.2.2.7/FK13157) in female rats. In female rabbits, some variabilities were observed in the plasma profiles (Mod4.2.2.7/FK13159 and Mod2.6.5.16K). In general, P338 concentrations were quantifiable, i.e., above the LLOQ of 0.075 µg/mL, starting from 2 h to 8 h. C_{max} values were reached between 48 to 72 h after dosing, followed by a mono-phasic declined. Concentrations were still above the LLOQ at 336 h in 3 rabbits (Table 18).

In female Göttingen minipigs, after single IM administration of RPV LA (G001) at 600 mg using the same experimental conditions as in the 9-month GLP study (Mod4.2.3.2/TMC278-NC349 [TOX9517]), concentrations of P338, were measured (Mod4.2.2.7/FK13161 and Mod2.6.5.16L).

The corresponding dose of P338 was 100 mg (4.6 mg/kg). The time to reach peak plasma concentration of P338 ranged from 24 h to 72 h. P338 concentrations were still above the limit of quantification (0.375 µg/mL) at 672 h after injection. The minipig plasma pharmacokinetic parameters of P338 are described in Table 18.

P338 plasma concentrations were also measured in human samples from a clinical study (Mod5.3.1.2/TMC278LAHTX1001) in healthy volunteers after single IM administration of RPV LA (G001; 2-mL injection). Pharmacokinetic parameters of P338 were determined in human plasma (Mod4.2.2.7/FK13131 and Mod2.6.5.16M). C_{max} values were reached within 48 h to 72 h, followed by an initial fast decrease until 672 h and a slower decrease afterwards, with concentrations still detected up to 4032 h after injection.

Table 18: Mean Pharmacokinetic Parameters of P338 After Single Oral or Intramuscular Administration of P338

Species	Route	Dose of P338	Number per group/ Sex	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
Rabbit	oral	1600 mg/kg	4/F	0.42	48	24	4.1
						264-336	69
Minipig	IM	100 mg	4/F	52.3	24	24	841
						672	13,600
Human	IM	100 mg	6	3.91	48	24	34
						336	580
						672	717

[a] Median value
F: female; IM: intramuscular

A pharmacokinetic study was conducted after single IM injection of P338 in male rats (n=3) at 10 mg/kg (Mod4.2.2.7/FK13409 and Mod2.6.5.16N). P338 concentrations were measured in plasma, liver, kidney, feces and urine. The samples were collected up to Day 22. The LLOQ in plasma, liver, kidney, feces, and urine were 0.2 µg/mL, 5 µg/g, 1 µg/g, 1 µg/g and 0.5 µg/mL, respectively.

The C_{max} of P338 (61 µg/mL) was achieved at 7 h and the corresponding $AUC_{0\text{-}529h\ (\sim 22\ days)}$ was equal to 2170 µg.h/mL. The tissue-to-plasma $AUC_{0\text{-}529h}$ ratios were 1.1 in kidneys and 5 in the liver. In urine, 9.41% of the dose was excreted as unchanged P338 in the 0-144h interval. Since all concentrations were below quantification limit in feces, only ~10% of the administered dose was eliminated at the end of the experiment, and via the urine. At the last time point 529h after dosing, P338 is still detected in plasma, liver and kidney, showing the slow elimination of P338 in rats.

8.3.2. Toxicokinetic Studies

8.3.2.1. Rats

A non-GLP, single and repeat-dose IM toxicity/toxicokinetic study was conducted after administration of P338 in female Sprague-Dawley rats (4/group), followed by a 1-month follow-up period (Mod4.2.3.7.7/TOX13295 and Mod2.6.5.16O). P338 (25 or 50 mg/mL as a sterile

aqueous solution) was administered at 5 mg/kg on Day 0 in the single dose phase, and at 5, 10 mg/kg/day on Days 0, 3, 6 and 9, and at 2x5 mg/kg/dose on Days 0 and 7 in the repeat-dose phase. A GLP combined male and female fertility and embryo-fetal toxicity study was conducted in rats after IM administration of P338 (Mod4.2.3.7.7/TOX13391 and Mod2.6.5.16P). P338 was administered every 3 days at 2.5, 5 and 10 mg/kg from 28 days before mating up to 3 days before necropsy (at least 21 administrations) and from 14 days before mating to gestation day (GD) 15 (at least 11 administration). A GLP peri- and postnatal development study was conducted in rats after IM administration of P338 (Mod.4.2.3.3.7/TOX13546 and Mod2.6.5.16Q). P338 was administered every 3 days at 2.5, 5 and 10 mg/kg/dose, to female Sprague-Dawley rats at G6 till weaning (13 administrations). The toxicokinetic parameters of P338 are described in Table 19.

In general, exposure (C_{max} and $AUC_{0\text{-}72h}$) values of P338 increased less than dose-proportional or close to dose-proportional. Comparing the first and last day of dosing, C_{max} and $AUC_{0\text{-}72h}$ values were similar or slightly higher (up to 1.6-fold) after repeated IM administration every 3 days or every week. No clear difference in exposure (C_{max} and $AUC_{0\text{-}72h}$) was seen between males and females.

The exposure (C_{max} and $AUC_{0\text{-}72h}$) values of P338 was similar after the single dose of 10 mg/kg every 3 days compared to 2x5 mg/kg every week.

Table 19: Mean Toxicokinetic Parameters of P338 After Intramuscular Administration of P338 in Rats

Study	Dose of P338 (mg/kg)	Number per group/ Sex	Day	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
Mod4.2.3.7.7/ TOX13295	5	4/F	0	25	3-7	72	679
						∞	810
	5 every 3 Days	4/F	0	25	7	72	705
						∞	752
			9	38	2-7	72	987
						168	1170
			-	-	-	288 (12 days)	3235[b]
	10 every 3 Days	4/F	0	49	7-12	72	1570
						∞	1690
			9	65	7-12	72	1950
						168	2390
			-	-	-	288 (12 days)	7063[b]
	10 (2x5) every week	4/F	0	49	7-12	72	1470
						∞	1770
			7	57	7	72	1550
						168	1790
			-	-	-	336 (14 days)	3737[b,c]
Mod4.2.3.7.7/ TOX13391	2.5 every 3 Days	3/M	0 (1st dose)	-	-	-	-
			54 (19th dose)	10	7	72	386
			-	-	-	1512 (63 days)	7870[b]
		3/F	0 (1st dose)	-	-	-	-
			30 (GD 15)	-	-	-	-
	5 every 3 Days	3/M	0 (1st dose)	29	7	72	746
			54 (19th dose)	23	7	72	825
			-	-	-	1512 (63 days)	16,000[b]
		3/F	0 (1st dose)	18	4.5	72	503
			30 (GD 15)	17	7	72	491
			-	-	-	792 (33 days)	4960[b]
	10 every 3 Days	3/M	0 (1st dose)	33	7	72	944
			54 (19th dose)	34	7	72	1480
			-	-	-	1512 (63 days)	26,000[b]
		3/F	0 (1st dose)	26	4.5	72	666
			30 (GD 15)	26	7	72	879
			-	-	-	792 (33 days)	6220[b]
Mod4.2.3.7.7/ TOX13546	2.5 every 3 Days	3/F	0 (GD6)	-	-	-	-
			30 (LD17)	7.8	7	72	260
	5 every 3 Days	3/F	0 (GD6)	14	7	72	413
			30 (LD17)	14	39.5[a] (7-72)	72	686
			-	-	-	792 (33 days)	4860
	10 every 3 Days	3/F	0 (GD6)	26	7	72	807
			30 (LD17)	25	7	72	990
			-	-	-	792 (33 days)	8410

[a] Median value; [b] $AUC_{0\text{-}cumulativetlast}$ corresponds to the AUC during the overall experiment; [c] n=3
-: no toxicokinetic evaluation possible; F: female; M: male; GD: gestation day; LD: lactation day

8.3.2.1. Rabbits

A non-GLP, single and repeat-dose IM toxicity/toxicokinetic study was conducted in female NZW rabbits (4/group), followed by a 1-month follow-up period (Mod4.2.3.7.7/TOX13296 and Mod2.6.5.16R). P338 (as a sterile aqueous solution) was administered at 5 mg/kg on Day 0 in the single dose phase, and at 2.5 and 5 mg/kg on Days 0 and 7 and at 2.5 mg/kg on Days 0, 4 and 8 in the repeat-dose phase. A GLP embryo-fetal toxicity study of P338 was conducted in female rabbits after IM administration of P338 (Mod4.2.3.7.7/TOX13376 and Mod2.6.5.16S). P338 was administered at 2.5 and 5 mg/kg on GD 6 and GD 12. The toxicokinetic parameters of P338 are described in Table 20.

In general, exposure (C_{max} and $AUC_{0\text{-}tlast}$) values of P338 increased in close to dose-proportional. Comparing the first and last day of dosing, C_{max} and $AUC_{0\text{-}72h}$ values were similar or slightly higher (up to 1.4-fold) after repeated IM administration every 4 or 6 days or every week.

Table 20: Mean Toxicokinetic Parameters of P338 After Intramuscular Administration of P338 in Rabbits

Study	Dose of P338 (mg/kg)	Number per group/ Sex	Day	C_{max} (µg/mL)	t_{max} [a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
Mod4.2.3.7.7/ TOX13296	5 SD	4/F	0	50	24	96	2760
						168	3290
						∞	3740
	2.5 RD every 4 Days	4/F	0	20	12-24	96	1140
						∞	1330
			8	27	12-24	96	1620
						168	1970
			-	-	-	288 (12 days)	4137 [b]
	2.5 RD every week	4/F	0	30	7-24	96	1580
						96-168	1860
						∞	2070
			7	28	12-24	96	1650
						168	2010
			-	-	-	336 (14 days)	4423 [b]
	5 RD every week	4/F	0	49	12-24	96	2800
						168	3350
						∞	3610
			7	55	24	96	3180
						168	3880
			-	-	-	336 (14 days)	7228 [b]
Mod4.2.3.7.7/ TOX13376	2.5 RD every week	4/F	0 (GD 6)	24	24	144	1490
			6 (GD 12)	25	24	144	1330
			-	-	-	288 (12 days)	2820 [b]
	5 RD every week	4/F	0 (GD 6)	40	24	144	2410
			6 (GD 12)	49	24	144	2670
			-	-	-	288 (12 days)	5080 [b]

[a] Median value; [b] $AUC_{0\text{-}cumulativetlast}$ corresponds to the AUC during the overall experiment
-: no toxicokinetic evaluation possible; F: female; GD: gestation day; IM: intramuscular; SD: single dose; RD: repeat-dose

9. DISCUSSION AND CONCLUSIONS

9.1. Rilpivirine LA

In rabbits and minipigs, after a single IM administration of RPV LA as the P338-containing formulation (G001), the RPV release was fast, after which mean plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after 3 months. After a follow up of 3 months, the F_{abs} is 67% in rabbits at 150 mg/kg and ranges between 35 and 62% in minipigs at 600 mg, indicating the release from the depot was still incomplete after 3 months.

Several studies were performed in rabbits and minipigs, mainly comparing different formulations containing P338 to the G001 formulation. No relevant changes in plasma profiles across studies were observed.

After repeated oral administration of RPV in various animal species used in the toxicology studies compared to IM administration of RPV LA in patients, the highest C_{max} ratio (animal/human) of RPV was around 406 in female mice and 252 in male mice, 82 in female rats and 39 male rats, 123 in pregnant rabbits, 33 in dogs, and 2 in female monkeys. The highest $AUC_{0\text{-day}28}$ ratio (animal/human) of RPV was around 258 in female mice, 170 in male mice, 82 in female rats, 25 in male rats, 78 in pregnant rabbits, 22 in dogs, and 2 in female monkeys. After IM administration of RPV LA in minipigs and dogs compared to IM administration of RPV LA in patients, the C_{max} ratios (animal/human) of RPV were around 2 and 10 and $AUC_{0\text{-day}28}$ (animal/human) ratios were around 0.6 and 5, respectively.

In rabbits, at the administration site after a single IM administration of RPV LA (150 mg/kg; G001) at the end of a 1-month follow-up period, the RPV concentrations were high (1456-fold) compared those in the contralateral side. In the lymph nodes, the RPV concentrations were similar between the injection and contralateral site except for one rabbit for which higher concentrations were seen in the accessory popliteal lymph nodes of the injection side. In rats, after a single IM administration of RPV LA (60 mg/kg; G001), the highest exposures of RPV were measured in the left popliteal and medial iliac lymph nodes adjacent to the injection site with tissue/plasma $AUC_{0\text{-day}42}$ ratios of 12,203 and 2256, respectively. In the contralateral right popliteal and medial iliac lymph nodes, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 6.7 and 2.6, respectively. In the kidney, adrenal glands, lungs, liver, and pancreas, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 3.7, 3.2, 1.5, 1.5 and 1.2, respectively. The tissue/plasma $AUC_{0\text{-day}42}$ ratios were lower than 1 in brain (0.4), heart (0.8), spleen (0.97) and thymus (0.87).

In rats, following single IM administration at 60 mg/kg of RPV LA (G001) alone or in combination with a LA injectable suspension of GSK1265744A (CAB) at 10 mg/kg, the plasma concentrations of RPV were comparable for the 2 groups and the mean C_{max} and $AUC_{0\text{-1444h or 2months}}$ values of RPV were similar.

In addition, other studies were performed after administration of RPV LA at lower concentration of RPV containing P338 or with PS80 after IM or SC in different species. A faster increase of the RPV concentrations were observed after administration of a P338 containing formulation compared to a PS80 containing formulation.

Few studies were performed in rats on the in vitro metabolism or in vivo after single IM injection of related substance C, a genotoxic impurity of RPV. It could be concluded that in the rat, related substance D is rapidly metabolically cleared and that the main metabolite pathways are sulfate conjugation and loss of the CN- function. Some pharmacokinetic and toxicokinetic studies were conducted after oral or IM administration of P338 in rats, rabbits and after IM administration of RPV LA in minipigs. Measurement of P338 was also performed in plasma samples from a clinical study (Mod5.3.1.2/TMC278LAHTX1001) after single IM administration of RPV LA (300 mg/mL in 50 mg/mL P338; 2-mL injection). After oral administration in rats and rabbits, no or very limited absorption of P388 was observed. After IM administration of P338 or RPV LA (G001) in rabbits, minipigs and human, the P338 release was fast, after which plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after at least 672 h.

9.2. From RPV oral (EDURANT®)

In rats, tissue distribution of ^{14}C-RPV and its metabolites after single oral dose was rapid and extensive. The highest concentrations of radioactivity were measured in the liver, adrenal gland, brown fat and kidney. There was no evidence of undue retention and there were no indications of irreversible binding of RPV and its metabolites to melanin. In pregnant rats, there was distribution of ^{14}C-RPV to the placenta and the fetus. TR exposure values in the placenta and in whole fetus were 0.94- and 0.64-fold those of maternal blood, respectively.

Rilpivirine is highly bound to plasma proteins and this is independent of the concentration and species. In the various animal species and human, plasma protein binding ranged from 99.08% to 99.97%. Rilpivirine is highly bound to human albumin and to a much lesser extent to α_1-acid glycoprotein. The distribution of RPV to red blood cells is limited in all species.

Some differences were seen in clearance across species. In rats, Cl_b of RPV is moderate whereas in rabbits, dogs and monkeys it is low compared to the hepatic blood flow. The Vd_{ss} was larger in rats, dogs and monkeys and very low in rabbits.

Rilpivirine is metabolized by Phase I and Phase II pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation and CN- split-off followed by reduction/oxidation, whether or not in combination with secondary pathways such as glucuronidation, dehydration and catabolism of the glutathione conjugate. In mice, oxidation of RPV and to a lesser extent glutathione conjugation were the predominant pathways. In rats, the glutathione conjugation pathway was the predominant pathway whereas in dog and man, oxidation of RPV was the predominant one. No unique human metabolites were observed. In plasma of animals and human, unchanged RPV was more abundant than any metabolite. After repeated oral administration of RPV for 11 days in healthy subjects at 75 and 300 mg q.d, there was no disproportionate increase in exposure of any of the relevant metabolites compared to the parent compound exposure.

In all animal species and human, the predominant route of excretion was via feces (>85%). Renal excretion of TR was very limited (0.45% to 6.1% of the dose) in all animal species and human and the amount of unchanged RPV in urine was negligible. In rats, biliary excretion was limited

(18%-25% of the dose) and the amount of unchanged RPV in bile was negligible. In rats, there was indication that RPV was excreted in milk.

In vitro, the CYP3A4 isoenzyme plays a major role in the biotransformation of RPV. Rilpivirine might be a very weak inducer of CYP1A2 and CYP2B6 and a moderate inducer of CYP2C19 and CYP3A4. Ex-vivo induction studies in rodents showed that RPV is an inducer of the CYP3A-family (up to 1.7-fold in mice at 320 mg/kg and up to 6-fold in rats at 400 mg/kg) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, RPV induced UDP-GT activity in mice (up to 2.3-fold at 320 mg/kg) and to a lesser extent in rats (up to 1.3-fold only at 400 mg/kg in males). In dogs, treatment with RPV did not result in any enzyme induction.

Rilpivirine is an inhibitor of CYP2C8 (K_i = 10μM) and CYP2C9 (K_i = 1.7 μM) in vitro whereas no inhibition is expected in vivo. In HLM, the limited MBI of CYP2C9 is unlikely to have clinical relevance at therapeutic doses of RPV.

Rilpivirine was shown to have P-gp inhibitor properties with an apparent IC_{50} value of 9.2 μM (3.4 μg/mL). Inhibition of the OCT2 transporter by RPV was evaluated in vitro. The in vitro IC_{50} for inhibition of OCT2 by RPV was 5.46 μM (2.0 μg/mL). The inhibition of MATE-mediated transport by RPV was investigated in vitro in CHO cells overexpressing MATE-1 and MATE-2K. The uptake of ^{14}C-TEA was inhibited by Rilpivirine with an IC_{50} value of 7.51 μM (2.75 μg/mL) for MATE-1 and of <0.05 μM (<0.018 μg/mL) for MATE-2K. In conclusion, the effect of RPV on MATE-1 is unlikely to be clinically relevant, but it cannot be excluded that RPV would inhibit MATE-2K at clinically relevant concentrations.

10. TABLES AND FIGURES

Supplemental tables and figures are included at appropriate points throughout the summary within the text; additional information is provided within the Pharmacokinetic Tabulated Summaries, located in Mod2.6.5.

11. LIST OF LITERATURE CITATIONS

1. Bioanalytical Method Validation, Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, 2018.
2. Guideline on Bioanalytical Method Validation. EMEA, 21 July 2011.

Janssen Research & Development

Pharmacokinetics Tabulated Summary

MODULE 2.6.5

Rilpivirine Long-Acting

Issue Date: July 3, 2019
Document No.: EDMS-ERI-167266903

2.6.5.1 Pharmacokinetics: Overview

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Analytical Methods					
	SPF Sprague-Dawley rat	oral ^{14}C-RPV	No	Janssen R&D	R278474-FK4686/ 4.2.2.1
	Mouse EDTA plasma	In vitro RPV	No	Janssen R&D	R278474-FK4240/ 4.2.2.1
	Rat EDTA plasma	In vitro RPV	No	Janssen R&D	R278474-FK4170/ 4.2.2.1
	Rabbit EDTA plasma	In vitro RPV	No	Janssen R&D	R278474-BA104/ 4.2.2.1
	Dog EDTA plasma	In vitro RPV	No	Janssen R&D	R278474-FK4169/ 4.2.2.1
	Minipig EDTA plasma	In vitro RPV	No	Janssen R&D	TMC278-NC298 (BA1061)/ 4.2.2.1
	Cynomolgus EDTA plasma	In vitro RPV	No	Janssen R&D	TMC278-NC273 (BA1062)/ 4.2.2.1
	Rat plasma	In vitro P338	Yes	[redacted] UK	BA13148 (304191)/ 4.2.2.1
	Rabbit plasma	In vitro P338	Yes	[redacted], UK	BA13146 (304196)/ 4.2.2.1

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Absorption					
Absorption- Tissue Distribution- Single dose + 1-month follow up	NZW rabbit	IM (0.5 mL/kg) Formulation A: P338 nanosuspension (G001) Formulation B: P338 nanosuspension (old API batch) Formulation C: P338 nanosuspension (3-fold diluted G001)	No	Janssen R&D	2683_14278 (FK7491)/ 4.2.2.2
Absorption- Single dose + 3-month follow up	NZW rabbit	IM (0.5 mL/kg) Formulation B: P338 nanosuspension freshly milled to smaller d_{50} Formulation C: P338 nanosuspension (fresh G001 at 5°C) Formulation D: P338 nanosuspension (aged G001 at 40°C) Formulation E: P338, nanosuspension freshly milled to edge of specification	No	Janssen R&D	2683_14279 (FK7521)/ 4.2.2.2
Absorption – Single dose + 3-week follow-up	NZW rabbit	IM (0.5 mL/kg; G001) Nanosuspensions with different particle sizes	No	Janssen R&D	FK12066/ 4.2.2.2
4-week RD toxicity + 2-week recovery	Beagle dog	IM injection RPV (G001) 150mg (0.5mL) 1200mg (4x1mL)	Yes	[redacted]	TOX10759/ Mod4.2.3.2
Absorption- Single dose + 3-month follow-up	Göttingen minipig	IM injection (2 mL) P338 (G001) P338/Na-deoxycholate P338/PE PEG350 P338/DOSS *[Nanosuspension]*	No	Janssen R&D	TMC278-NC359 (TOX9403)/ 4.2.3.6

(Continued)

2.6.5.1 Pharmacokinetics: Overview

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Absorption (Continued)					
Absorption- Single dose + 1-month follow-up	Göttingen minipig	IM (2 mL) Formulation A: P338 nanosuspension (G001) Formulation B: P338 nanosuspension (old API batch) Formulation C: P338 nanosuspension (3-fold diluted G001)	No	Janssen R&D	2683_14277 (FK7490)/ 4.2.2.2
Absorption- Single dose + 3-month follow-up	Göttingen minipig	IV (1 mL/kg) or IM (2 mL) Formulation A: 20% Captisol (for IV dosing) Formulation B: P338 nanosuspension freshly milled to smaller d_{50} Formulation C: P338 nanosuspension (fresh G001 at 5°C) Formulation D: P338 nanosuspension (aged G001 at 40°C) Formulation E: P338, nanosuspension freshly milled to edge of specification	No	Janssen R&D	2683_14125 (FK7520)/ 4.2.2.2
Absorption- Single dose + 3-month follow-up	Göttingen minipig	IM (2mL) Nanosuspensions A = control (G001) B = with sodium metabisulfite C = low PVP concentration D = high PVP concentration	No	Janssen R&D	2683_0040908 (FK10294)/ 4.2.2.2
Absorption- 6-Week Repeat Dose	Göttingen minipig	IM injection (2 mL) Negative control V1 = P338 (50 mg/mL) V2 = P338 (160 mg/mL)*/* Formulation A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 (= G001)	Yes	Janssen R&D	TMC278-NC368 (TOX9508)/ 4.2.3.2
Absorption- 9-Months Repeat Dose	Göttingen minipig	IM injection (2 mL) Control solution Vehicle: P338 Formulation A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 (= G001)	Yes	Janssen R&D	TMC278-NC349 (TOX9517)/ 4.2.3.2

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Absorption (Continued)					
Absorption- Single dose	NZW rabbit	IV Base in PEG400/sterile water (25%)	No	Janssen R&D	TMC278-FK4293/ 4.2.2.2
Distribution					
Absorption- Tissue Distribution- Single dose + 1-month follow up	Sprague Dawley rat	IM (0.20 mL/kg) G001	No	Janssen R&D	ADME_58575 4.2.2.3
Absorption- Tissue Distribution- Single dose + 1-month follow up	NZW rabbit	IM (0.5 mL/kg) Formulation A: P338 nanosuspension (G001) Formulation B: P338 nanosuspension (old API batch) Formulation C: P338 nanosuspension (3-fold diluted G001)	No	Janssen R&D	2683_14278 (FK7491)/ 4.2.2.2
Tissue Distribution (Single dose)	Rat/ pigmented Long Evans	Oral/Gavage (^{14}C-RPV in PEG400/CA (10%))	No	Janssen R&D	TMC278-NC108 (FK4951)/ 4.2.2.3
Tissue Distribution (Single dose)	Pregnant rat/ Sprague Dawley	Oral/Gavage (^{14}C-RPV in PEG400/CA (10%))	No	Janssen R&D	TMC278-NC109 (FK4950)/ 4.2.2.3
Absorption, Single Dose	Rat/ Sprague Dawley	Intravenous (base in PEG400/sterile water (25%) Oral/Gavage (base in PEG400 or PEG400/CA (10%))	No	J&J PRD	TMC278-FK4195/ 4.2.2.3
Tissue Distribution (Repeat dose)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	Janssen R&D	TMC278-Exp.5650/ 4.2.3.2
Tissue Distribution (Repeat dose)	Dog/ beagle	Oral/Gavage (base in PEG400/CA (10%))	Yes	Janssen R&D	TMC278-NC115 (TOX6110)/ 4.2.3.2
Protein Binding Blood Distribution	Mouse, rat, rabbit, dog, human	In vitro (^{3}H-RPV)	No	Janssen R&D	TMC278-NC112 (FK5273)/ 4.2.2.3

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Distribution (Continued)					
Protein Binding Blood Distribution	Guinea pig, monkey	In vitro (^{14}C-RPV)	No	Janssen R&D	TMC278-NC332 (FK6820)/ 4.2.2.3
Protein Binding	Mouse, rat, dog, human	In vitro (unlabeled RPV)	No	Janssen R&D	TMC278-FK4217/ 4.2.2.3
Metabolism					
Metabolism Excretion (Single dose)	Mouse/ CD-1	Oral/Gavage (^{14}C-RPV in PEG400/CA (10%)	No	Janssen R&D	TMC278-NC190 (FK5621)/ 4.2.2.4
Metabolism Excretion (Single dose)	Rat/ Sprague Dawley	Oral/Gavage (^{14}C-RPV in PEG400/CA (10%)	No	Janssen R&D	TMC278-NC113 (FK4933)/ 4.2.2.4
Metabolism Excretion in Bile (Single dose)	Rat/ Sprague Dawley	Oral/Gavage (^{14}C-RPV in PEG400/CA (10%)	No	Janssen R&D	TMC278-NC145 (FK5525)/ 4.2.2.4
Metabolism (Repeated dose)	Rat/ Sprague Dawley	Oral/Gavage (HCl salt in HPMC (0.5%))	No	Janssen R&D	TMC278-NC123 (TOX7221)/ 4.2.3.4.1 - TMC278-NC290 (FK6376)/ 4.2.2.4
Metabolism (Single dose)	Dog/ beagle	Oral/Capsule (^{14}C-RPV in PEG400/CA (10%)	No	Janssen R&D	TMC278-NC114 (FK5143)/ 4.2.2.4
Metabolism (Single dose)	Human	Oral/Gavage (^{14}C-RPV in PEG400)	No	Janssen R&D	TMC278-NC157 (FK5344)/ 4.2.2.4
Metabolism In vitro	Human	In vitro: (RPV)	No	Janssen R&D	1646_0027483 (FK10104)/ 4.2.2.4

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Metabolism In vitro (Continued)					
Metabolism In vitro	Mouse, rat, rabbit, dog, human	In vitro: (Plasma samples from different studies)	No	Janssen R&D	TMC278-NC155 (BA45)/ 4.2.2.4
Metabolism In vitro	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions (^{14}C-RPV)	No	Janssen R&D	TMC278-NC102 (FK4728)/ 4.2.2.4
Metabolism In vitro	Guinea pig, monkey	In vitro: hepatocytes, subcellular liver fractions (^{14}C-RPV)	No	Janssen R&D	TMC278-NC333 (FK6818)/ 4.2.2.4
Metabolism In vitro	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions (RPV)	No	Janssen R&D	TMC278-FK4152/ 4.2.2.4
Metabolism In vitro	Human	In vitro: human liver microsomes, E. coli expressed CYP isoforms, supersomes	No	Janssen R&D	TMC278-NC141 (FK5300)/ 4.2.2.4
Metabolism In vitro	Human	In vitro: HLM, E. Coli expressed CYP isoforms (RPV)	No	Janssen R&D	TMC278-FK4151/ 4.2.2.4
Metabolism In vitro	Human	In vitro: HLM (RPV)	No	Janssen R&D	TMC278-FK4288/ 4.2.2.4
Metabolism In vitro		In vitro: heterologous expressed GST isoforms (^{14}C-RPV)	No	Janssen R&D	TMC278-FK4789/ 4.2.2.4
Induction/Inhibition					
Induction	Human	In vitro: cryopreserved human hepatocytes (CYP activity and mRNA level)	No	Janssen R&D	TMC278-NC186 (FK5720)/ 4.2.2.4
Induction	Human	In vitro: human hepatocytes (GST activity)	No	[redacted]	TMC278-FK4824/ 4.2.2.4

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Induction/Inhibition (Continued)					
Metabolism Induction/Inhibition (3 months)	Mouse/ CD-1	Ex vivo: hepatic microsomes	Yes	[redacted]	TMC278-NC192 (FK5563)/ 4.2.2.4
Metabolism Induction/Inhibition (6 months)	Rat/ Sprague Dawley	Ex vivo: hepatic microsomes	Yes	[redacted]	TMC278-NC193 (FK5564)/ 4.2.2.4
Metabolism Induction/Inhibition (6 months)	Dog/ beagle	Ex vivo: hepatic microsomes	Yes	[redacted]	TMC278-NC140 (FK5518)/ 4.2.2.4
Metabolism Induction/Inhibition (2 weeks)	Rat/Sprague Dawley	Ex vivo: hepatic microsomes	No	Janssen R&D	TMC278-FK4247/ 4.2.2.4
In Vitro Inhibition	HLM	In vitro CYP1A2, CYP2A6, CYP2C8,9,10, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5 and CYP4A	No	Janssen R&D	TMC278-FK4123/ 4.2.2.4
In Vitro Inhibition	HLM	In Vitro CYP2C8 and CYP2C9-mediated	No	Janssen R&D	TMC278-NC283 (FK6443)/ 4.2.2.4
Inhibition	HLM	In vitro: MBI of CYP2C9 by TMC278	No	Janssen R&D	1646_0030536 (FK10162)/ 4.2.2.4
Effect Adrenal Gland	Dog	In vitro: adrenal cortex cell-free extracts	No	Janssen R&D	TMC278-FK4790/ 4.2.2.4

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Pharmacokinetic Drug Interactions					
Inhibition of transport OCT2 substrate	CHO cell lines	In vitro: ^{14}C-metformin by RPV	No	Janssen R&D	1646_0025128 (FK10042)/ 4.2.2.6
Inhibition of transport of the MATE-1 and MATE-2K substrate	CHO cells	In vitro: ^{14}C-tetra ethyl ammonium by RPV	No	Janssen R&D	1646_0035314 (FK10420)/ 4.2.2.6
Drug-drug Interactions	Human	In vitro: human liver microsomes	No	Janssen R&D	TMC278-NC194 (FK5568)/ 4.2.2.6
Pharmacokinetics of RPV and/or cabotegravir	Sprague Dawley rat	IM RPV (fresh clinical G001 batch) with or without cabotegravir (200 mg/mL GSK1265744LAP injectable suspension)	No	Janssen R&D	1955_0018187 (FK7565)/ Mod4.2.2.6
Other					
Absorption- Tissue Distribution- Single dose + 4- or 18-days follow-up	Albino Swiss mouse	SC injection V1 = P338 V2 = RPV LA (Vit E-TPGS)	No	Janssen R&D	TMC278-NC196 (TOX7354)/ 4.2.3.7.7
Absorption- Tissue Distribution- Single dose + 8-week follow-up	Sprague-Dawley rat	SC (0.2 mL) or IM (0.2 mL) or IV (1 mL) injection SC or IM: RPV LA with P338 (non-G001) IV: PEG 400/25% sterile water	No	Janssen R&D	TMC278-NC244 (TOX7896)/ 4.2.3.7.7
Absorption- Single dose + 1-month follow up	NZW rabbit	IM (0.5 mL or 2x0.16 mL) P338 nanosuspension of RPV (F004) PS80 nanosuspension of RPV (F006)	No	Janssen R&D	TMC278-NC356 (FK6993)/ 4.2.2.7
Absorption- Tissue Distribution- Single dose + 6-month follow-up	Beagle dog	IM or SC (0.2 mL/kg) Group A (IM) and C (SC): P338 nanosuspension (for RPV; non-G001) Group B (IM): P338 nanosuspension (for HCl salt of RPV)	No	Janssen R&D	Innovation-NC114 (FK5458)/ 4.2.2.7

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Other (Continued)					
Absorption- Single dose + 6-month follow-up	Beagle dog	IV (1.25 mg/mL): 75% PEG400/25% sterile water solution of RPV SC or IM: nanosuspension of RPV in Pluronic F108 (25 mg/mL; non-G001)	No	Janssen R&D	TMC278-NC238 (FK5998)/ 4.2.2.7
Absorption- Tissue Distribution- Single dose + 3-month follow-up	Beagle dog	SC or IM injection (1 mL) F004 formulation	Yes	Janssen R&D	TMC278-NC234 (TOX7781)/ 4.2.3.7.7
Absorption- Tissue Distribution- Single dose + 3-month follow-up	Beagle dog	SC (0.1 mL/kg/site) RPV LA (Vit-E TPGS)	No	Janssen R&D	TMC278-NC203 (FK5821)/ 4.2.2.7
Absorption- Single dose + 3-month follow-up	Göttingen minipig	IM RPV LA F006 (1 x 1.5mL) RPV LA F004 (4 x 1 mL)	No	[redacted]	TMC278-NC295 (FK6407)/ 4.2.2.7
Absorption- Single dose + 1-month follow-up	Göttingen minipig	IM (2 mL) RPV LA (P338 (non G001) or PS80)	No	[redacted]	TMC278-NC344 (FK7034)/ 4.2.2.7
13/39-week toxicity (monthly doses)	Göttingen Minipig	IM (2 mL) RPV LA (P338 (non G001) or PS80)	Yes	[redacted]France	TMC278-NC296 (TOX8580))/ 4.2.3.7.7
Absorption – Single dose + 1-week follow-up[a]	Sprague-Dawley rat	IM Nanosuspension; related substance D*	No	Janssen R&D	2683_0038991 (FK10250)/ 4.2.2.7
Excretion – Single dose[a]	Sprague-Dawley rat	Nanosuspension; ^{14}C-related substance D*	No	Janssen R&D	2683_0039002 (FK10345)/ 4.2.2.7

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Other (Continued)					
Single-Dose	NZW rabbit	Oral gavage of P338 demineralised water *[aqueous solution]*	Yes	Janssen R&D	TMC278-NC348 (TOX9680)/ 4.2.3.7.7
Single-Dose	Sprague-Dawley rat	Oral gavage of P338 demineralised water [aqueous solution]	Yes	Janssen R&D	TMC278_NC347 (TOX9679)/ 4.2.3.7.7
Single-Dose	Sprague-Dawley rat	Oral P338 + demineralized water	No	Janssen R&D	FK13157/ 4.2.2.7
Single-Dose	NZW rabbit	Oral P338 + demineralized water[b]	No	Janssen R&D	FK13159/ 4.2.2.7
Absorption- 9-Months Repeat Dose	Göttingen minipig	IM injection (2 mL) Control solution Vehicle: P338	Yes	Janssen R&D	TMC278-NC349 (TOX9517)/ 4.2.3.2
Single-Dose	Göttingen minipig	IM P338 and RPV LA	No	Janssen R&D	FK13161/ 4.2.2.7
Single-Dose	Human plasma	IM P338 and RPV LA	No	Janssen R&D	FK13131/ 4.2.2.7
Single-Dose	Sprague-Dawley rat	IM P338[b]	No	Janssen R&D	FK13409/ 4.2.2.7
SD + RD followed by 1-month follow-up	Sprague-Dawley rat	IM P338[b]	No	Janssen R&D	TOX13295/ 4.2.3.7.7

(Continued)

2.6.5.1 Pharmacokinetics: Overview (Continued)

Test Article: rilpivirine/rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Test Facility	Study No. Location in CTD
Other (Continued)					
Embryo-fetal toxicity (Seg I & II)	Sprague-Dawley rat	IM P338[b]	Yes	[redacted] France	TOX13391/ 4.2.3.7.7
Pre-and Postnatal Development	Sprague-Dawley rat	IM (bolus) P338[b]	Yes	[redacted] France	TOX13546/ 4.2.3.7.7
SD + RD followed by a 1-month follow-up	NZW rabbit	IM P338 (sterile aqueous solution)[b]	No	Janssen R&D	TOX13296/ 4.2.3.7.7
Embryo-fetal toxicity (Seg II)	NZW rabbit	IM P338 (sterile aqueous solution)[b]	Yes	[redacted] France	TOX13376/ 4.2.3.7.7

[a] For these studies, no individual tabulated summaries are prepared since these studies do not deal with RPV but with its genotoxic impurity related substance D*
[b] In these studies poloxamer was studied

API = active pharmaceutical ingredient; CA = citric acid; CHO: Chinese hamster ovary; [redacted] = [redacted]; CYP = cytochrome P450; DOSS = dioctyl sodium sulfosuccinate; d_{50} = diameter 50; EDTA = ethylenediaminetetraacetic acid; F004 = clinical formulation containing 100 mg/mL TMC278 LA in P338; F006 = clinical formulation containing 300 mg/mL TMC278 LA in PS80; GST = glutathione S-transferase; G001 = clinical formulation containing 300 mg/mL TMC278 LA in P338; HCl = hydrogen chloride; HLM = human liver microsomes; [redacted] = [redacted]; IM = intramuscular; IV = intravenous; LA = long acting; MATE = multidrug and toxic extrusion; MBI = mechanism based inhibition; mRNA = messenger ribonucleic acid; NZW = New Zealand white; OCT-2 = organic cation transporter 2; PE = phosphatidylethanolamine PEG = polyethylene glycol; PVP = polyvinylpyrrolidone; P338 = Poloxamer 338 (Pluronics F108); PS80 = polysorbate 80; RPV = rilpivirine; RD = repeated-dose; R&D = Research and Development; SC = subcutaneous; SD = single-dose; TPGS = D-α-Tocopheryl Polyethylene glycol 1000 Succinate; V = vehicle; Vit E = vitamin E

Note: The available studies of RPV after administration of RPV LA are listed. In addition, all the relevant nonclinical pharmacokinetic studies of RPV on distribution, metabolism and excretion performed for the EDURANT® submission (i.e., oral tablet) are also included. In addition, supportive poloxamer studies were conducted as well and are included in the file.

＊新薬承認情報提供時に置換

2.6.5.2 Analytical Methods and Validation Reports

Test Article: rilpivirine/rilpivirine LA

The bioanalytical methods to support the toxicokinetic and pharmacokinetic program of Rilpivirine, as well as the use of radio-labeled RPV (^{14}C and ^{3}H) and the radiochemical methods, have been described in Mod2.6.4/Sec2.

2.6.5.3A Pharmacokinetics: Absorption after a Single Intramuscular Dose in Rabbits

Test Article: rilpivirine LA

Study No.	**2683_14278 (FK7491)**		
Location in CTD	**4.2.2.2**		
Species	New Zealand white rabbit		
Feeding Condition	Fed		
Vehicle/Formulation	Formulation A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 buffer (= G001 formulation) Formulation B: nanosuspension containing 300 mg/mL RPV LA (old API batch) in 50 mg/mL P338 buffer Formulation C: 3-fold dilution of clinical batch G001 containing 100 mg/mL RPV LA in 16.7 mg/mL P338, glucose, NaH_2PO, citric acid, NaOH buffer		
Route	IM (0.5 mL/kg)		
Gender (M/F)/Number of Animals	Female/3 per group		
Compound	RPV LA		
Dose	Formulation A and B: 150 mg/kg Formulation C: 50 mg/kg		
Follow-up period	1 month		
Sample	plasma		
Analyte	RPV (TMC278)		
Assay	LC-MS/MS		
Pharmacokinetic Parameters	**Formulation A (G001): 150 mg/kg**	**Formulation B: 150 mg/kg**	**Formulation C: 50 mg/kg**
C_{max} (ng/mL)	4550	5490	3307
t_{max} (h)	84	124	90
$AUC_{0\text{-}864h\ (\sim 1\ month)}$ (ng.h/mL)	2,383,421	2,639,606	1,150,113
$AUC_{0\text{-}\infty}$ (ng.h/mL)	3,138,909	3,519,438	1,292,412

API = active pharmaceutical ingredient; G001 = final clinical formulation; IM = intramuscular; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; P338 = poloxamer 338; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.3B Pharmacokinetics: Absorption after a Single Intramuscular Dose in Rabbits

Test Article: rilpivirine LA

Study No.	**2683_14279 (FK7521)**			
Location in CTD	**4.2.2.2**			
Species	New Zealand white rabbit			
Feeding Condition	Fed			
Vehicle/Formulation	Formulation B: nanosuspension containing 300 mg/mL RPV LA, fresh batch milled to smaller d50 Formulation C: nanosuspension containing 300 mg/mL RPV LA, fresh clinical batch G001 stored at 5 °C Formulation D: nanosuspension containing 300 mg/mL RPV LA, aged clinical batch G001 stored at 40 °C Formulation E: nanosuspension containing 300 mg/mL RPV LA, fresh batch milled to edge of specification			
Route	IM (0.5 mL/kg)			
Gender (M/F)/Number of Animals	Female/3 per group			
Compound	RPV LA			
Dose	150 mg/kg			
Follow-up period	3-month			
Sample	plasma			
Analyte	RPV (TMC278)			
Assay	HPLC-MS/MS			
Pharmacokinetic Parameters	**Formulation B**	**Formulation C (G001)**	**Formulation D**	**Formulation E**
C_{max} (ng/mL)	11,287	6453	3903	5030
t_{max} (h)	12.7	78.3	221	12.7
$AUC_{0\text{-day}99\ (3\ months)}$ (ng.h/mL)	3,401,630	3,561,529	3,073,349	3,484,029

G001 = final clinical formulation; HPLC-MS/MS = high performance liquid chromatography coupled to tandem mass spectroscopy; IM = intramuscular; LA = long acting; P338 = poloxamer 338; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.3C Pharmacokinetics: Absorption after a Single IM Dose in Rabbits

Test Article: rilpivirine LA

Study No.	**FK12066**		
Location in CTD	**4.2.2.2**		
Species	New Zealand white Rabbit		
Feeding Condition	Fed		
Vehicle/Formulation	RPV LA 300 mg/mL in poloxamer 338 50 mg/mL (G001 formulation) with Formulation A: smaller particle size, close to target; Formulation B: target; Formulation E: aged at higher temperature		
Route	IM (0.5 mL/kg)		
Gender (M/F)/Number of Animals	Female/6 per group		
Compound	RPV LA		
Dose	150 mg/kg		
Follow up period	3 weeks		
Sample	plasma		
Analyte	RPV (TMC278)		
Assay	HPLC-MS/MS		
Pharmacokinetic Parameters	**Formulation A**	**Formulation B**	**Formulation E**
$C_{max}1$ (ng/mL)	10,200	6210	35,400
$T_{max}1$[a] (h)	7-24	7-24	24
$C_{max}2$ (ng/mL)	7840	6300	5390
$T_{max}2$[a] (h)	53-341	53-221	221-341
$AUC_{0-533h\ (3\ weeks)}$ (ng·h/mL)	3,080,000	2,400,000	2,120,000

[a] Median (Min – Max)

F = female; G001 = final clinical formulation; HPLC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; IM = intramuscular; NA = Not available; NZW = New Zealand White; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.3D Pharmacokinetics: Absorption after a Single Intramuscular Dose in Minipig

Test Article: rilpivirine LA

Study No.	**TMC278-NC359 (TOX9403)**					
Location in CTD	**4.2.3.6**					
Species	Göttingen minipig					
Feeding Condition	Fed					
Vehicle/Formulation	Group A: nanosuspension containing 200 mg/mL RPV LA in 50 mg/mL P338 Group B: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 (= G001) Group D: nanosuspension containing 200 mg/mL RPV LA in 50 mg/mL P338 + 2 mg/mL Na-deoxycholate Group E: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 + 2 mg/mL Na-deoxycholate Group F: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 + 1.5 mg/mL PE PEG350 Group G: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 + 2 mg/mL DOSS					
Route	IM (2 mL)					
Gender (M/F)/Number of Animals	Male/3 per group					
Compound	RPV LA					
Dose	Group A and D: 400 mg Group B, E and F: 600 mg					
Follow-up period	3-month					
Sample	plasma					
Analyte	RPV (TMC278)					
Assay	HPLC-MS/MS					
Pharmacokinetic Parameters	**Group A**	**Group B (G001)**	**Group D**	**Group E**	**Group F**	**Group G**
Dose (mg)	**400**	**600**	**400**	**600**	**600**	**600**
C_{max} (ng/mL)	317	2245	1781	1079	465	681
t_{max} (h)	4.3	100.3[a]	2.2	2.3	4.0	2.7
$AUC_{0\text{-}day29}$ (ng.h/mL)	42,918	77,473	31,369	45,118	34,398[b]	66,845
$AUC_{0\text{-}day86}$ (ng.h/mL)	95,131	152,011	68,970	100,614	101,399[b]	152,243

[a] Individual t_{max} values: 2.0, 6.0 and 293.0 h

[b] n = 2

DOSS = dioctyl sodium sulfosuccinate; G001 = final clinical formulation; HPLC-MS/MS = high performance liquid chromatography coupled to tandem mass spectroscopy; IM = intramuscular; LA = long acting; P338 = poloxamer 338; PE = phosphatidylethanolamine; PEG = polyethylene glycol; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.3E Pharmacokinetics: Absorption after a Single Intramuscular Dose in Minipigs

Test Article: rilpivirine LA

Study No.		**2683_14277 (FK7490)**	
Location in CTD		**4.2.2.2**	
Species		Göttingen minipig	
Feeding Condition		Fed	
Vehicle/Formulation		Formulation A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 buffer (= G001 formulation) Formulation B: nanosuspension containing 300 mg/mL RPV LA (old API batch) in 50 mg/mL P338 buffer Formulation C: 3-fold dilution of clinical batch G001 containing 100 mg/mL RPV LA in 16.7 mg/mL P338, glucose, NaH2PO0, citric acid, NaOH buffer	
Route		IM (2 mL)	
Gender (M/F)/Number of Animals		Male/3 per group	
Compound		RPV LA	
Dose		Formulation A and B: 600 mg Formulation C: 200 mg	
Follow-up period		1 month	
Sample		plasma	
Analyte		RPV (TMC278)	
Assay		LC-MS/MS	
Pharmacokinetic Parameters	**Formulation A (G001): 600 mg**	**Formulation B: 600 mg**	**Formulation C: 200 mg**
C_{max} (ng/mL)	73.8	75.0	51.8
t_{max} (h)	3.67	3.67	2.00
$AUC_{0\text{-}696h(\sim 1\ month)}$ (ng.h/mL)	10,160	12,919	4319
$AUC_{0\text{-}\infty}$ (ng.h/mL)	10,293	14,634	5009

G001 = final clinical formulation; IM = intramuscular; LA = long acting; P338 = poloxamer 338; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.3F Pharmacokinetics: Absorption after a Single Intravenous or Intramuscular Dose in Minipigs

Test Article: rilpivirine LA

Study No.	**2683_14125 (FK7520)**				
Location in CTD	**4.2.2.2**				
Species	Göttingen minipig				
Feeding Condition	Fed				
Vehicle/Formulation	Formulation A: 2 mg/mL RPV LA solution in 20% Captisol at pH 3.68 Formulation B: nanosuspension containing 300 mg/mL RPV LA, fresh batch milled to smaller d50 Formulation C: nanosuspension containing 300 mg/mL RPV LA, fresh clinical batch G001 stored at 5 °C Formulation D: nanosuspension containing 300 mg/mL RPV LA, aged clinical batch G001 stored at 40 °C Formulation E: nanosuspension containing 300 mg/mL RPV LA, fresh batch milled to edge of specification				
Route	Formulation A: slow bolus IV (1 mL/kg) Formulation B, C, D, E: IM (2 mL)				
Gender (M/F)/Number of Animals	Male/ (IV: 4; IM: 3 per group)				
Compound	RPV LA				
Dose	IV: 2 mg/kg IM: 600 mg				
Sample	plasma				
Analyte	RPV (TMC278)				
Assay	HPLC-MS/MS				
Pharmacokinetic Parameters	**Formulation A:**	**Formulation B:**	**Formulation C (G001)**	**Formulation D**	**Formulation E**
	2 mg/mL IV	**600 mg IM**	**600 mg IM**	**600 mg IM**	**600 mg IM**
C_0 (ng/mL)	1701	-	-	-	-
$t_{1/2}$ (h)	8	-	-	-	-
$AUC_{0\text{-}48h}$ (ng.h/mL)	2974	-	-	-	-
$AUC_{0\text{-}\infty}$ (ng.h/mL)	2797	-	-	-	-
CL_p (mL/h/kg)	753	-	-	-	-
Vd_{SS} (mL/kg)	4,904	-	-	-	-
C_{max} (ng/mL)	-	708	234	164	92.2
t_{max} (h)	-	3.33	2.67	10.00	3.83
$AUC_{0\text{-}day106}$ (ng.h/mL)	-	58,022	24,806	24,769	24,815

G001 = final clinical formulation; HPLC-MS/MS = high performance liquid chromatography coupled to tandem mass spectroscopy; IM = intramuscular; IV = intravenous; LA = long acting; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.3G Pharmacokinetics: Absorption after a Single IM Dose in Minipigs

Test Article: rilpivirine LA

Study No.	**2683_0040908 (FK10294)**			
Location in CTD	**4.2.2.2**			
Species	Minipig (Gottingen)			
Feeding Condition	Fed			
Vehicle/Formulation	Formulation A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 buffer (= G001 formulation) Formulation B: nanosuspension containing 300 mg/mL RPV LA with sodium metabisulfite containing formulation Formulation C: nanosuspension containing 300 mg/mL RPV LA, lyo formulation with low PVP concentration Formulation D: nanosuspension containing 300 mg/mL RPV LA, lyo formulation with high PVP concentration			
Route	IM (2 mL)			
Sample	Plasma			
Assay	HPLC-MS/MS			
LLOQ	1.00 ng/mL			
Compound	RPV (TMC278)			
Dose	600 mg			
Gender (M/F)/Number of Animals	M:3	M:3	M:3	M:3
Pharmacokinetic Parameters	**Formulation A (G001)**	**Formulation B**	**Formulation C**	**Formulation D**
C_{max} (ng/mL)	120	19.9	38.6	19.8
T_{max} (h)	0.5-24	6-24	6-384	24
$AUC_{0\text{-day }85}$ (~3 months) (ng·h/mL)	15,662	12,580	15,623	9131
Frel (vs A; $AUC_{0\text{-day }85}$)	-	80	100	58

G001 = final clinical formulation; HPLC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; IM = intramuscular; LLOQ = lower limit of quantification; M = male; NA = Not available; PVP = polyvinylpyrrolidone; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.4A Pharmacokinetics: Absorption after Repeated Intramuscular Doses in Dogs

Test Article: rilpivirine LA

Study No.	**TOX10759**					
Location in CTD	**4.2.3.2**					
Species	Beagle dog					
Feeding Condition	Fed					
Vehicle/Formulation	Control: G002, 0 mg/mL Dose groups: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 (= G001)					
Route	IM (0.5 mL [150 mg]; 4 mL [control and 1200 mg])					
Gender (M/F)/Number of Animals	Male /Female; 5/sex/group					
Compound	RPV LA					
Duration of Dosing	Dosing on Days 1 and 15, followed by a 2-week recovery period					
Sample	Plasma					
Analyte	RPV (TMC278)					
Assay	LC-MS/MS					
Dose (mg/dog)	**0 (Control)**		**150**		**1200**	
Pharmacokinetic Parameters						
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Day 1						
C_{max} (ng/mL)	-	-	116	245	1220	1220
t_{max} (h)	-	-	264	24	24	24
$AUC_{0\text{-}336h}$ (ng.h/mL)	-	-	28,800	45,700	218,000	204,000
Day 15	-	-				
C_{max} (ng/mL)	-	-	177	394	1440	1230
t_{max} (h)	-	-	24	24	24	24
$AUC_{0\text{-}264h}$ (ng.h/mL)	-	-	34,500	48,500	217,000	206,000
$AUC_{0\text{-}600h}$	-	-	63,400	94,200	435,000	410,000

F = female; IM = intramuscular; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; M = male; RPV LA = rilpivirine long acting (TMC278 base)

G001 = final clinical formulation; G002 = control

2.6.5.4B Pharmacokinetics: Absorption after Repeated Intramuscular Doses in Minipigs

Test Article: rilpivirine LA

Study No.	**TMC278-NC368 (TOX9508)**			
Location in CTD	**4.2.3.2**			
Species	Göttingen minipig			
Feeding Condition	Fed			
Vehicle/Formulation	Group C (control): 50 mg/mL P338 buffer Group V1: 50 mg/mL P338 Group V2: 160 mg/mL P338 Group A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 (= G001)			
Route	IM (2 mL)			
Gender (M/F)/Number of Animals	Male /Female; 3/sex/group			
Compound	RPV LA			
Dose (mg/injection)	600 mg/injection			
Duration of Dosing	Dosing on Days 0, 14, 28 and 42 (terminal kill 3 or 4 days after last injection)			
Sample	Plasma			
Analyte	RPV (TMC278)			
Assay	LC-MS/MS			
Pharmacokinetic Parameters	**Day 0 (after single dose)**		**Day 28 (after 3rd dose)**	
Sex	**M**	**F**	**M**	**F**
C_{max} (ng/mL)	580	1004	715	1232
t_{max} (h)	4.33	5.33	5.00	4.33
$AUC_{0\text{-}336h}$ (ng.h/mL)	18,378	33,153	51,359	52,751

G001 = final clinical formulation; IM = intramuscular; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; P338 = poloxamer 338; V = vehicle; RPV LA = rilpivirine long acting (TMC278 base)

2.6.5.4C Pharmacokinetics: Absorption after Repeated Intramuscular Doses in Minipigs

Test Article: rilpivirine LA

Study No.	**TMC278-NC349 (TOX9517)**			
Location in CTD	**4.2.3.2**			
Species	Göttingen minipig			
Feeding Condition	Fed			
Vehicle/Formulation	Group C (control): 50 mg/mL P338 buffer Group V: 50 mg/mL P338 Group A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 (= G001)			
Route	IM (2 mL)			
Gender (M/F)/Number of Animals	Male /Female (3/sex/group)			
Compound	RPV LA			
Dose (mg/injection)	600 mg/injection			
Duration of Dosing	once monthly (dosing on Days 0, 28, 56, 84, 112, 140, 168, 196, 224 and 252) for 9 months (terminal kill 6 or 7 days after last injection)			
Sample	Plasma			
Analyte	RPV (TMC278)			
Assay	LC-MS/MS			
Pharmacokinetic Parameters	**Day 0 (after single dosing)**		**Day 224**	
Sex	**M**	**F**	**M**	**F**
C_{max} (ng/mL)	189	637	349	402
t_{max} (h)	7.0	4.3	6.0	26.0
AUC_{0-672h} (ng.h/mL)	20,696	34,964	50,071	44,342

G001 = final clinical formulation; IM = intramuscular; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; P338 = poloxamer 338; RPV LA = rilpivirine long acting (TMC278 base); V = vehicle

2.6.5.5A Pharmacokinetics: Organ Distribution in Rat

Test Article: rilpivirine LA

Study No.	**ADME_58575**		
Location in CTD	**4.2.2.3**		
Species	Sprague-Dawley rat		
Feeding Condition	Fed		
Vehicle/Formulation	RPV LA 300 mg/mL in Poloxamer 338 50 mg/mL (G001 formulation)		
Route	Intramuscular		
Gender (M/F)/Number of Animals	M/3 or 6 per timepoint		
Dose	Group 1: 60 mg/kg – Group 2: 120 mg/kg		
Duration of Dosing	Single dose		
Sampling Times (h)	24, 72, 168, 504 and 1008 h post-dosing		
Dose (mg/kg)	60[a]		120[b]
	Full profiles	Selected time points only (24, 72, 168, 504 and 1008 h)	Full profiles
C_{max} (ng/mL)	76.5 (±61.1)	57.1 (±32.3)	93.9
t_{max} (h)	15.5 [4, 24]	24 [24, 24]	24
$t_{1/2}$ (h)	679 (± 248)	NC	920
$AUC_{0\text{-}24h}$ (ng·h/mL)	1340 (± 944)	685 (± 387)	1610
$AUC_{0\text{-}1008h\ (\sim Day\ 42)}$ (ng·h/mL)	19,100 (± 5680)	18,200 (± 5160)	41,500
$AUC_{0\text{-}\infty}$ (ng·h/mL)	25,700 (± NA)[c]	NC	68,500

(Continued)

2.6.5.5A Pharmacokinetics: Organ Distribution in Rat (Continued)

Test Article: rilpivirine LA

Sampling Times (h)	**Group 1: 60mg/kg**			
Tissues/Organs	**Mean C_{max} tissue** (µg/mL or µg/g)	**Mean $AUC_{0\text{-}1008h\ (\sim Day\ 42)}$** (µg·h/mL or µg·h/g)	**Mean tissue: plasma $AUC_{0\text{-}1008h\ (\sim Day\ 42)}$ ratio** (-)	**Mean tissue: blood $AUC_{0\text{-}1008h\ (\sim Day\ 42)}$ ratio**
Plasma	0.13	23.7	1.0	1.6
Blood	0.061	15.1	0.64	1.0
Adrenal gland, left	0.16	73.5	3.2	4.9
Brain	0.023	6.59[d]	0.29	0.44
Eye, left	BQL	NC	NC	NC
Heart	0.046	15.7[d]	0.69	1.0
Kidney, left	0.16	84.8	3.7	5.6
Liver	0.069	32.2[d]	1.4	2.1
Lung	0.075	33.9	1.5	2.2
Medial iliac lymph node, left	409	51,200	2256	3391
Medial iliac lymph node, right	0.088	58.2	2.6	3.9
Pancreas	0.058	28.1	1.2	1.9
Popliteal lymph node, left	1100	277,000	12,203	18,344
Popliteal lymph node, right	1.2	153	6.7	10
Spleen	0.048	22.1	0.97	1.5
Thymus	0.094	19.7	0.87	1.3
Thyroid gland, left	0.050	NC	NC	NC

[a] NCA of individual full plasma concentration-time profiles and individual plasma concentration-time profiles using selected 24, 72, 168, 504 and 1008h time points only; mean of n = 6 (± SD), except for T_{max}: median [min, max]
[b] NCA of mean pooled plasma concentration-time profiles
[c] Mean of two individual values. The extrapolation for the calculation of the AUC∞ exceeded 25% in 4 out of 6 animals and were, therefore, excluded from the mean calculation
[d] Extrapolated values as a result of mean BQL tissue concentrations, with mean BQL value equated to zero
BQL = Below Quantification Limit varying between <8.93 ng/g and <10.0 ng/g; G001 = final clinical formulation; LA= long acting; M = male; NC = Not calculated; RPV LA = rilpivirine long-acting (TMC278 base)

2.6.5.5B Pharmacokinetics: Organ Distribution in Rabbit

Test Article: rilpivirine LA

Study No.	**2683_14278 (FK7491)**					
Location in CTD	**4.2.2.2**					
Species	New Zealand white rabbit					
Feeding Condition	Fed					
Vehicle/Formulation	Formulation A: nanosuspension containing 300 mg/mL RPV LA in 50 mg/mL P338 buffer (= G001 formulation) Formulation B: nanosuspension containing 300 mg/mL RPV LA (old API batch) in 50 mg/mL P338 buffer Formulation C: 3-fold dilution of clinical batch G001 containing 100 mg/mL RPV LA in 16.7 mg/mL P338, glucose, NaH2PO, citric acid, NaOH buffer					
Route	IM					
Compound	RPV LA					
Duration of Dosing	Single dose					
Dose	Formulation A and B: 150 mg/kg Formulation C: 50 mg/kg					
Dosing period	Single dose					
Gender (M/F)/Number of Animals	Female/3 per group					
Analyte	RPV (TMC278)					
Assay	LC-MS/MS					
Sampling Time Tissues	only in Group A (G001), at 1-month post-dosing					
Concentration (µg/mL or g))	**Animal A1**		**Animal A2**		**Animal A3**	
Plasma	1.29		1.50		1.49	
Administration side	**no**	**yes**	**no**	**yes**	**no**	**yes**
Administration site	2.58	5,000	6.37	5,340	2.1	5,830
Lymph nodes, accessory axillary	0.656	0.373	-	0.53	0.565	-
Lymph nodes, medial iliac	0.599	0.839	0.648	1.15	0.524	0.489
Lymph nodes, popliteal	2.87	36.5	0.385	0.996	0.339	2.44
Lymph nodes, mandibular	-	-	-	-	0.623	0.693

API = active pharmaceutical ingredient; G001 = final clinical formulation; IM = intramuscular; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; P338 = poloxamer 338; RPV LA = rilpivirine long-acting (TMC278 base); - = not applicable

2.6.5.5C Pharmacokinetics: Organ Distribution in Rat

Test Article: rilpivirine

Study No.	**TMC278–NC108 (FK4951)**									
Location in CTD	**4.2.2.3**									
Species	Rat (pigmented Long Evans)									
Feeding Condition	Not fasted									
Vehicle/Formulation	RPV base in PEG400/CA (10%)									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/5									
Dose (mg/kg)	40									
Radionuclide	^{14}C-RPV									
Specific Activity (kBq/mg)	233									
Sampling Times (h)	**1**		**4**		**24**		**96**		**336**	
Tissues/Organs	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**	**Conc. (µg eq./g)**	**Tissue/ Blood**
Adrenal gland	3.29	4.64	7.93	5.13	1.21	8.66	0.351	6.15	BLQ	-
Blood (LSC)	0.710	1.00	1.34	0.867	0.139	1.00	0.057	1.00	0.026	1.00
Blood (RLG)	0.708	1.00	1.55	1.00	BLQ	-	BLQ	-	BLQ	-
Bone	BLQ	-	0.348	0.225	BLQ	-	BLQ	-	BLQ	-
Bone marrow	1.10	1.55	2.00	1.29	BLQ	-	BLQ	-	BLQ	-
Brain	0.506	0.715	0.981	0.634	BLQ	-	BLQ	-	BLQ	-
Brown fat	2.95	4.17	6.00	3.88	BLQ	-	BLQ	-	BLQ	-
Eye ball (LSC)	0.967	1.37	5.08	3.29	3.73	26.8	1.78	31.2	1.03	39.8
Heart	1.42	2.00	2.77	1.79	BLQ	-	BLQ	-	BLQ	-
Kidney	2.78	3.93	5.27	3.41	0.707	5.07	BLQ	-	BLQ	-
Liver	9.52	13.4	16.6	10.7	2.28	16.4	0.390	6.82	BLQ	-
Lung	1.03	1.45	3.54	2.29	BLQ	-	BLQ	-	BLQ	-
Meninges	1.84	2.60	5.80	3.75	1.73	12.4	1.57	27.5	1.21	46.6
Muscle	0.555	0.783	1.23	0.792	BLQ	-	BLQ	-	BLQ	-
Pancreas	2.20	3.11	4.23	2.73	BLQ	-	BLQ	-	BLQ	-
Prostate	0.865	1.22	2.07	1.34	BLQ	-	BLQ	-	BLQ	-
Skin pigmented	0.915	1.29	3.73	2.41	1.60	11.5	1.67	29.3	0.657	-
Skin white	1.10	1.55	2.93	1.89	0.761	5.47	0.470	8.24	BLQ	-
Spleen	NA	-	4.18	2.70	BLQ	-	BLQ	-	BLQ	-
Testis	0.306	0.433	1.25	0.807	BLQ	-	BLQ	-	BLQ	-
Thyroid	1.58	2.23	2.26	1.46	BLQ	-	BLQ	-	BLQ	-
Uveal tract	5.45	7.70	28.2	18.2	38.3	275	10.9	191	6.86	265
White fat	0.945	1.33	5.63	3.64	BLQ	-	BLQ	-	BLQ	-
Additional Information	Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 4 hours after dosing and as determined with LSC from 24 hours onwards.									

(Continued)

2.6.5.5C Pharmacokinetics: Organ Distribution in Rat (Continued)

Test Article: rilpivirine

Sampling Times (h)	**1, 4, 24, 96 and 336**	
Tissues/Organs	**AUC_{0-4h} (µg.h/g)**	**Tissue/Blood AUC_{0-4h} ratio**
Adrenal gland	18.5 (140[a])	4.95 (6.76[b])
Blood (LSC)	3.43[c]	0.92
Blood (RLG)	3.74	1.00
Bone	NC[d]	-
Bone marrow	5.20	1.39
Brain	2.48	0.66
Brown fat	14.9	3.98
Eye ball (LSC)	9.55 (616[e])	2.55 (20.5[f])
Heart	6.98	1.87
Kidney	13.5 (58.9[g])	3.61 (4.21[h])
Liver	43.9 (265[a])	11.7 (12.8[b])
Lung	7.38	1.97
Meninges	12.4 (530[e])	3.32 (17.6[f])
Muscle	2.95	0.79
Pancreas	10.7	2.86
Prostate	4.83	1.29
Skin pigmented	7.42 (436[e])	1.98 (14.5[f])
Skin white	6.59 (82.2[a])	1.76 (3.97[b])
Spleen	-	-
Testis	2.48	0.66
Thyroid	6.55	1.75
Uveal tract	53.2 (4380[e])	14.2 (146[f])
White fat	10.3	2.75

[a] AUC_{0-96h}
[b] Calculated with AUC_{0-96h}
[c] AUC_{0-24h} = 14.0 µg.h/g, AUC_{0-96h} = 20.7 µg.h/g and AUC_{0-336h} = 30.1 µg.h/g
[d] NC: not calculated, too limited data
[e] AUC_{0-336h}
[f] Calculated with AUC_{0-336h}
[g] AUC_{0-24h}
[h] Calculated with AUC_{0-24h}

- = not applicable; BLQ = below limit of quantification; CA = citric acid; LSC = liquid scintillation counting; M = male; NA = not analyzed; PEG400 = polyethylene glycol 400; RLG = radioluminography; RPV: rilpivirine

2.6.5.5D Pharmacokinetics: Organ Distribution in Rat

Test Article: rilpivirine

Study No.	**TMC278–NC109 (FK4950)**							
Location in CTD	**4.2.2.3**							
Species	Rat (Sprague Dawley, pregnant)							
Feeding Condition	Not fasted							
Vehicle/Formulation	RPV base in PEG400/CA (10%)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	F/4							
Dose (mg/kg)	40							
Radionuclide	^{14}C-RPV							
Specific Activity (kBq/mg)	233							
Sampling Times (h)	**1**		**4**		**8**		**24**	
Tissues/Organs	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**	**Conc. (µg eq./g)**	**Tissue/Blood**
Adrenal gland	4.52	3.59	9.76	3.47	7.37	4.05	1.75	17.0
Blood (LSC)	1.01	0.802	2.32	0.83	1.40	0.769	0.102	1.00
Blood (RLG)	1.26	1.00	2.81	1.00	1.82	1.00	BLQ	-
Brain	0.849	0.674	1.92	0.683	1.24	0.681	BLQ	-
Fat brown	3.55	2.82	7.29	2.59	6.22	3.42	BLQ	-
Fat white	1.37	1.09	8.18	2.91	8.12	4.46	BLQ	-
Fetus	0.627	0.498	1.87	0.665	1.19	0.654	BLQ	-
Heart	1.85	1.47	3.76	1.34	2.73	1.50	BLQ	-
Kidney	3.91	3.10	5.78	2.06	5.76	3.17	0.582	5.70
Lachrymal gland	3.87	3.07	9.56[a]	3.40	7.48	4.11	BLQ	-
Liver	7.73	6.14	14.4	5.13	13.3	7.31	0.800	7.84
Lung	2.42	1.92	5.19	1.85	3.46	1.90	BLQ	-
Mammary gland	2.46	1.95	7.70	2.74	7.72	4.24	0.400	3.92
Muscle	0.643	0.510	1.39	0.495	1.14	0.626	BLQ	-
Ovary	NA[b]	-	5.90	2.10	4.03	2.21	BLQ	-
Pancreas	2.59	2.06	4.34	1.54	4.08	2.24	BLQ	-
Placenta	1.10	0.87	2.63	0.936	1.88	1.03	BLQ	-
Salivary gland	1.96	1.56	4.00	1.42	2.94	1.62	BLQ	-
Spleen	1.84	1.46	2.82	1.00	2.37	1.30	BLQ	-
Uterine epithelium	1.49	1.18	10.6	3.77	10.6	5.82	5.51	54.0
Uterus	1.18[a]	0.937	2.63[a]	0.936	2.36	1.30	0.303[c]	3.00
Vagina	0.857	0.680	2.36	0.840	1.95	1.07	0.249[a]	2.44
Additional Information	Tissue/ blood concentration ratios were calculated preferably with blood total radioactivity levels as determined with RLG till 8 hours after dosing and as determined with LSC at 24 hours.							

(Continued)

2.6.5.5D Pharmacokinetics: Organ Distribution in Rat (Continued)

Test Article: rilpivirine

Sampling Times (h)	**1, 4, 8 and 24**	
Tissues/Organs	**AUC_{0-8h} (µg.eq.h/g)**	**Tissue/Blood AUC_{0-8h} ratio**
Adrenal gland	57.7 (120[d])	3.6 (5.8[f])
Blood (LSC)	12.8 (20.7[d])	0.81
Blood (RLG)	15.9	1.0
Brain	10.8	0.68
Fat brown	45.0	2.8
Fat white	47.6	3.0
Fetus	10.1	0.64
Heart	22.2	1.4
Kidney	39.6 (75.7[d])	2.5 (3.7[f])
Lachrymal gland	56.0	3.5
Liver	92.3 (163[d])	5.8 (7.9[f])
Lung	29.7	1.9
Mammary gland	47.3 (86.9[d])	3.0 (4.2[f])
Muscle	8.41	0.53
Ovary	31.4[e]	2.0
Pancreas	28.5	1.8
Placenta	15.1	0.95
Salivary gland	23.7	1.5
Spleen	18.3	1.2
Uterine epithelium	61.2 (186[d])	3.8 (9.0[f])
Uterus	16.3 (32.3[d])	1.0 (1.6[f])
Vagina	13.9 (27.1[d])	0.87 (1.3[f])
Additional Information	-	

[a] n=2

[b] NA = not analyzed due to flare effect of the high radioactive concentration in the formulation in stomach

[c] n=1

[d] AUC_{0-24h}

[e] AUC was calculated with concentration versus time data at 4 and 8 hours. For this tissue, no data were available at 1-hour post dose

[f] AUC_{0-24h} ratio calculated with blood (LSC) = 20.7 µg eq.h/g

- = not applicable; BLQ = below limit of quantification (0.196 µg eq./g); CA = citric acid; F = female; LSC = liquid scintillation counting; PEG400 = polyethylene glycol 400; RLG = radioluminography; RPV = rilpivirine

2.6.5.5E Pharmacokinetics: Organ Distribution in Rat

Test Article: rilpivirine

Study No.	**TMC278–FK4195**				
Location in CTD	**4.2.2.3**				
Species	Rat (Sprague-Dawley)				
Feeding Condition	-				
Vehicle/Formulation	RPV base in PEG400/CA (10%)				
Sample	plasma				
Analyte	RPV (TMC278)				
Assay	LC-MS/MS				
Vehicle/Formulation	RPV base in PEG400/sterile water (25%)	RPV base in PEG400	RPV base in PEG400/CA (10%)		
Route	IV (slow bolus injection)	Oral (gavage)	Oral (gavage)		
Gender (M/F)/Number of Animals	M/3	F/3	M/3	F/3	M/3
Dose (mg/kg/day)	4	40	40	160	400
Concentration (mg/mL)	2	4	4	16	40
Pharmacokinetic Parameters					
C_{max} (ng/mL)	5.3 [a]	1.3	1.7	3.3	6.6
t_{max} (h)	NA	1.0	1.0	8.0	8.0
AUC (ng.h/mL)	3.1	9.8	12	48	64
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	4.4	2.8	4.6	5.7	3.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)
Bioavailability (Fabs %)	NA	32	39	39	21
Clearance (L/h/kg)	1.3	NA	NA	NA	NA
Vd_{ss} (L/kg)	4.1	NA	NA	NA	NA
Additional Information	The sampling times were 7 min (iv only), 20 min, 1, 3, 8 and 24 hours after dose administration. Tissue samples from the adrenal gland, brain, liver and muscle were collected as well in this study. Maximum tissue concentrations were observed within 20 to 60 min after administration. Tissue levels declined in parallel with plasma concentrations. Tissue to plasma concentration (AUC0-24h) ratios were 3.4, 2.7, 0.49 and 0.45 for liver, adrenal gland, brain and muscle, respectively.				

[a] C_0 extrapolated value at 0 h.

CA = citric acid; LC-MS/MS = liquid chromatography with tandem mass spectrometry; NA = not applicable; PEG400 = polyethylene glycol 400; Vd_{ss} = volume of distribution at steady state

2.6.5.5F Pharmacokinetics: Organ Distribution in Dog

Test Article: rilpivirine

Study No.	**TMC278–Exp.5650**											
Location in CTD	**4.2.3.2**											
Species	Dog (beagle)											
Feeding Condition	Not fasted											
Vehicle/Formulation	RPV base in PEG400/CA (10%)											
Route	Oral (gavage)											
Sample	plasma											
Analyte	RPV (TMC278)											
Assay	LC-MS/MS											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose (mg/kg/day)	5				10				40			
Duration of Dosing (day)	1	28	1	28	1	28	1	28	1	28	1	28
Pharmacokinetic Parameters												
C_{max} (ng/mL)	939	1465	1345	1985	1311	5620	1304	2519	2791	11632	2432	9543
t_{max} (h)	3.0	2.0	3.0	11	6.0	8.0	2.0	5.0	19	5.0	12	8.0
AUC (ng.h/mL)	13,374[a]	27,110	18,628	36,791	21,977[a]	102,621	14,059[a]	46,570	51,188[a]	204,202	40,036[a]	160,201
(Time for calculation –h)	(0-24)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	33	29	11	181	28	33	21	43	21	48	17	50
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)
Plasma (ng/mL)		1085		1927		2497		2484		4137		3589
Adrenal gland (ng/g)		1875		2583		4787		5525		14,746		6033
Tissue to plasma ratio		1.7		1.3		1.9		2.2		3.6		1.7
Additional Information	The sampling times were 0, 0.5, 1, 2, 4, 8 and 24 h after dosing on Day 1 and Day 28. On Day 30, 31, 35, 44, 49 and 56 at 8:00. During the 1-month recovery period the mean through levels decreased from Day 30 to Day 56. The TMC278 levels were measured in the adrenal gland and plasma at autopsy.											

[a] $AUC_{0-\infty}$ extrapolation > 45%.

CA = citric acid; LC-MS/MS = liquid chromatography with tandem mass spectrometry; PEG400 = polyethylene glycol.

2.6.5.5G Pharmacokinetics: Organ Distribution in Dog

Test Article: rilpivirine

Study No.	**TMC278–NC115 (TOX6110)**					
Location in CTD	**4.2.3.2**					
Species	Dog (beagle)					
Feeding Condition	Not fasted					
Vehicle/Formulation	RPV base in PEG400/CA (10%)					
Route	Oral (gavage)					
Sample	Plasma					
Analyte	RPV (TMC278)					
Assay	LC-MS/MS					
Pharmacokinetic Parameters						
Dose (mg/kg)	5		10		40	
No. of Animals	M:6	F:6	M:6	F:6	M:6	F:6
Toxicokinetics:	M:3	F:3	M:3	F:3	M:3	F:3
Day 0						
C_{max} (ng/mL)	1028	702	1288	1234	1483	818
t_{max} (h)	2.0	2.3	6.0	2.7	12	9.3
$AUC_{0\text{-}24h}$ (ng.h/mL)	10,587	9179	21,786	20,002	22,584	10,173
Day 85						
C_{max} (ng/mL)	1390	1132	2009	2193	2492	3101
t_{max} (h)	2.3	3.3	2.3	2.0	2.0	2.7
$AUC_{0\text{-}24h}$ (ng.h/mL)	21,261	17,591	27,789	27,222	41,306	51,454
Day 176						
C_{max} (ng/mL)	1512	1376	1951	1933	3948	2875
t_{max} (h)	1.7	1.7	4.0	4.0	2.7	2.0
$AUC_{0\text{-}24h}$ (ng.h/mL)	21,101	17,352	25,821	31,912	68,263	43,089

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2.6.5.5G Pharmacokinetics: Organ Distribution in Dog (Continued)

Test Article: rilpivirine

Pharmacokinetic Parameters						
Dose (mg/kg)	5		10		40	
Gender (M/F)/Number of Animals	M:6	F:6	M:6	F:6	M:6	F:6
Toxicokinetics:	M:3	F:3	M:3	F:3	M:3	F:3
Tissue concentration (ng/g)						
Liver						
- Day 92	10,885	7795	22,450	15,950	50,650	40,500
- Day 183 or 184	10,127	7427	13,897	12,247	25,157	25,167
Adrenal						
- Day 92	5460	3250	18,550	8080	35,300	30,150
- Day 183 or 184	5950	2360	6127	4273	17,673	15,427
Plasma (ng/ml)						
- Day 92	1347	603	2303	1751	6547	4072
- Day 183 or 184	1143	582	1310	1009	2615	2688
Additional Information	6 months repeated-dose toxicity with a 3-month interim kill. The sampling times were 0, 1, 2, 4, 8 and 24 h after dosing at Day 1, Day 86 and Day 177. The TMC278 levels were measured in the liver, adrenal gland and plasma at autopsy at Day 93 and Day 184(males)/185(females). Tissue to plasma ratios for the liver were 8.1/8.9 and 13/13 at 5 mg/kg, 9.7/11 and 9.1/12 at 10 mg/kg and 7.7/9.6 and 9.9/9.4 at 40 mg/kg in males and females on Day93/Day184 or 185, respectively. Tissue to plasma ratios for the adrenal gland were 4.1/5.2 and 5.4/4.1 at 5 mg/kg, 8.1/4.7 and 4.6/4.2 at 10 mg/kg and 5.4/6.8 and 7.4/5.7 at 40 mg/kg in males and females on Day93/Day184 or 185, respectively.					

CA = citric acid; LC-MS/MS = liquid chromatography with tandem mass spectrometry; ND = not determined; PEG400 = polyethylene glycol

2.6.5.6A Pharmacokinetics: Plasma Protein Binding and Blood Distribution

Test Article: rilpivirine

Study No.	**TMC278–NC112 (FK5273)**													
Location in CTD	**4.2.2.3**													
Method	The plasma protein binding of RPV was studied by equilibrium dialysis of plasma samples after fortification with ^{3}H-labeled RPV. Plasma was subjected to equilibrium dialysis against a 0.067 M phosphate buffer, pH 7.17, at 37°C for 3 hours. Concentration of ^{3}H –RPV in dialysis compartments was determined by liquid scintillation counting. The binding of RPV to purified human serum albumin and α_1-acid glycoprotein was also investigated by equilibrium dialysis. In blood distribution studies, samples of whole blood were combusted in an oxidizer and $^{3}H_2O$ captured and counted by liquid scintillation.													
Species	Swiss CD-1 Mouse[a] (Male)		Swiss CD-1 Mouse[a] (Female)		Sprague Dawley Rat[a] (Male)		Sprague Dawley Rat[a] (Female)		New Zealand White Rabbit[b] (Female)		Beagle Dog[b] (Male)		Human[b] (Male)	
Parameters Measured														
Concentration Tested in Plasma (µg/mL)	0.01-100		0.01-100		0.01-100		0.01-100		0.01-100		0.01-100		0.01-3	
Plasma Bound % at 1 µg/mL[c]	99.93		99.94		99.84		99.86		99.97		99.35		99.67	
Concentration Tested in Blood (µg/mL)	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1
Blood-to-Plasma Ratio	0.60	0.60	0.58	0.58	0.69	0.67	0.67	0.67	0.61	0.61	0.69	0.68	0.67	0.66
Distribution to: Plasma Water (%)	0.12	0.07	0.06	0.07	0.16	0.15	0.13	0.14	0.02	0.03	0.5	0.5	0.3	0.2
Plasma Proteins (%)	101	100	102	103	90.5	93.0	94.1	94.1	102	103	72.6	73.4	77.3	78.1
Blood Cells (%)	-0.9	-0.2	-1.7	-3.0	9.3	6.8	5.8	5.8	-1.8	-2.8	26.9	26.2	22.4	21.7
Hematocrit (%)	40		41		38		38		38		51		48	
Additional Information	Preliminary studies evaluated: (a) length of time needed to reach equilibrium in the dialysis cells (3 hours was deemed sufficient); (b) the effect of pH (as pH increased from 5.1 to 8.4, the percentage bound increased from 99.59 to 99.80, so pH was standardized at 7.4 for human plasma); and (c) binding to purified human plasma protein (at physiological concentrations, RPV (0.01 to 3 µg/mL) bound to serum albumin (99.50%) and α_1-acid glycoprotein (between 25.9% and 55.0%).													

[a] For rat and mouse, each value represents a mean of 4 observations (2 pools x 2)
[b] For rabbit, dog and man, each value represents a mean of 10 observations (5 individual samples x 2)
[c] Over the concentration range tested the fraction bound to plasma remained nearly the same
RPV = rilpivirine

2.6.5.6B Pharmacokinetics: Plasma Protein Binding and Blood Distribution

Test Article: rilpivirine

Study No.	**TMC278–NC332 (FK6820)**			
Location in CTD	**4.2.2.3**			
Method	The plasma protein binding of RPV was studied by equilibrium dialysis of plasma samples after fortification with ^{14}C-labeled RPV. Plasma was subjected to equilibrium dialysis against a 0.067 M phosphate buffer, pH 7.17, at 37°C for 4 hours. Concentration of ^{14}C –RPV in dialysis compartments was determined by liquid scintillation counting. In blood distribution studies, samples of whole blood were combusted in an oxidizer and $^{14}CO_2$ captured and counted by liquid scintillation.			
Species	Dunkin Hartley Guinea Pig		Cynomolgus Monkey	
Parameters Measured				
Concentration Tested in Plasma (µg/mL)	2.5	8	2.5	5
Plasma Bound %	99.87	99.87	99.14	99.08
Concentration Tested in Blood (µg/mL)	2.5	8	2.5	5
Blood-to-Plasma Ratio	0.64	0.63	0.96	0.94
Distribution to: Plasma Water (%)	0.11	0.11	0.53	0.59
Plasma Proteins (%)	84.71	85.23	61.84	63.46
Blood Cells (%)	15.18	14.66	37.63	35.96
Hematocrit (%)	46		40	
Additional Information	-			

RPV = rilpivirine

2.6.5.7A Pharmacokinetics: Study in Pregnant or Nursing Rats

Test Article: rilpivirine/rilpivirine LA

Not Applicable

2.6.5.8 Pharmacokinetics: Other Distribution Study

Test Article: rilpivirine/rilpivirine LA

No other distribution studies have been performed.

2.6.5.9A Pharmacokinetics: Metabolism In Vivo in Mice

Test Article: rilpivirine

Study No.		**TMC278-NC190 (FK5621)**						
Location in CTD		**4.2.2.4**						
Species		**Mouse (CD-1)**						
Gender (M/F)/Number of Animals		**M12/F12 (plasma profile) - M12/F12 (excretion mass balance)**						
Feeding Condition		**Not fasted**						
Vehicle/Formulation		**PEG400/CA (10%)**						
Route		**Oral**						
Dose of RPV base (mg/kg)		**20 and 320**						
Radionuclide		**^{14}C**						
Specific Activity (kBq/mg)		**345 and 23**						
Dose (mg/kg)		**20**				**320**		
Sample		**Urine**		**Fecal Extract**		**Urine**		**Fecal Extract**
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Time (h)		**0-24**	**0-24**	**0-48**	**0-48**	**0-24**	**0-24**	**0-48**	**0-48**
% of Administered Radioactivity in Excreta		**2.82**	**3.46**	**64.05**	**65.38**	**1.27**	**2.88**	**75.2**	**71.3**
Parent (UD)		0.63	0.08	8.2	7.8	0.11	0.02	33	34
M13	Cysteine-S-conjugate	0.14	0.39			0.09	0.31		
M14	Cysteinyl-S-conjugate	0.08	0.58			0.06	0.55		
M13+M14				5.0	4.4			5.1	2.8
M17	Mercapturic acid conjugate	0.19	0.15			0.08	0.14		
M18	Mercapturic acid conjugate	0.13	0.07			0.04	0.10		
M17+M18				4.2	2.3			3.3	3.4
M21	Hydroxylated sulfonyl conjugate			1.4	1.0			0.7	0.3
M25	Oxidation combined with glucuronidation	0.42	1.6			0.42	1.6		
M24+M25	Hydroxylation on the cyanoethenyl moiety, Oxidation combined with glucuronidation			3.4	2.7			1.6	1.6
M27+M28+M29	Dehydration of M33; Aliphatic hydroxylation and dehydration of M45; Sulphoxidation of M45			0.3	<0.2			<0.2	0.1
M30	Carboxylic acid metabolite			1.6	3.1			1.5	1.2
M33	Aliphatic hydroxylation			0.5	0.7			1.3	1.0
M35	Unknown structure			<0.2	<0.2			<0.2	<0.2
M38	Hydroxylation			1.4	1.4			1.0	0.8
M41+M42[a]	Hydroxylation of M45; Aromatic hydroxylation			18	26			9.2	13
M42	Aromatic hydroxylation	0.37	0.20			0.11	0.06		
M43+M45	Z-isomerization , S-Methyl conjugate			2.2	1.8			1.3	1.2

(Continued)

2.6.5.9A Pharmacokinetics: Metabolism In Vivo in Mice (Continued)

Test Article: rilpivirine

Dose (mg/kg)		**20**				**320**			
Sample		**Urine**		**Fecal Extract**		**Urine**		**Fecal Extract**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Time (h)		**0-24**	**0-24**	**0-48**	**0-48**	**0-24**	**0-24**	**0-48**	**0-48**
% of Administered Radioactivity in Excreta		**2.82**	**3.46**	**64.05**	**65.38**	**1.27**	**2.88**	**75.2**	**71.3**
M46	Unknown structure			0.5	0.7			0.5	0.6
M47	Dimerization			0.3	<0.2			0.8	0.2

Sample		**Plasma**											
		Male			**Female**			**Male**			**Female**		
Time (h)		**1**	**3**	**8**	**1**	**3**	**8**	**1**	**3**	**8**	**1**	**3**	**8**
% of Plasma radioactivity		**11.1[b]**	**6.85[b]**	**9.46[b]**	**8.28[b]**	**5.70[b]**	**12.5[b]**	**30.7[b]**	**26.3[b]**	**42.5[b]**	**26.8[b]**	**32.2[b]**	**49.4[b]**
Parent (UD)		105	98	97	99	97	95	93	86	95	95	95	91
M13+M14	Cysteine-S-conjugate; Cysteinyl-S-conjugate	0.5	0.5	0.6	0.5	<0.4	0.6	<0.8	0.9	1.0	<1.2	1.8	<0.8
M27	Dehydration of M33	0.6	0.8	0.8	0.6	0.9	0.6	<0.8	1.2	<0.8	<1.2	<0.7	<0.8
M30	Carboxylic acid metabolite	0.6	0.5	0.6	0.7	0.8	1.0	<0.8	0.9	1.4	<1.2	1.3	1.5
M33	Aliphatic hydroxylation	4.5	3.1	3.5	3.4	6.6	4.3	2.7	2.1	1.5	2.8	4.1	2.2
M36	Hydroxylation of RPV	0.3	0.8	0.4	0.6	1.3	0.5	<0.8	<0.9	<0.8	1.2	<0.7	<0.8

Additional Information

96 h after administration of RPV, 88% and 87% of the dose was excreted in feces and 3.5% and 4.2% in urine at 20 mg/kg and 96% and 89% of the dose was excreted in feces and 1.84% and 3.6% in urine at 320 mg/kg, in males and females, respectively. Over the 4-day collection period, more than 95% of the administered dose was recovered.

[a] Metabolite fraction mainly composed of M42, estimated at about 14% and 17% of the 20 mg/kg dose, and at 5.9% and 8.0% of the 320 mg/kg dose in male and female mice, respectively.

[b] Total radioactivity levels in µg-eq./mL.

CA = citric acid; F = female; M = male; PEG400 = polyethylene glycol; UD = unchanged drug; RPV = rilpivirine

2.6.5.9B Pharmacokinetics: Metabolism In Vivo in Rats

Test Article: rilpivirine

Study No.					**TMC278-NC113 (FK4933)**						
Location in CTD					**4.2.2.4**						
Species					**Rat (Sprague Dawley)**						
Gender (M/F)/Number of Animals					**M12/F12 (plasma profile) – M5/F5 (excretion mass balance)**						
Feeding Condition					**Not fasted**						
Vehicle/Formulation					**PEG400/CA (10%)**						
Route					**Oral**						
Dose of RPV base (mg/kg)					**40**						
Radionuclide					**^{14}C**						
Specific Activity (kBq/mg base eq.)					**37**						
Sample		**Urine**		**Fecal Extract**		**Plasma**					
		Male	**Female**	**Male**	**Female**		**Male**			**Female**	
Time (h)		**0-24**	**0-24**	**0-48**	**0-48**	**1**	**4**	**8**	**1**	**4**	**8**
% of Administered Radioactivity in Excreta or µg-eq./mL in Plasma		**0.39[a]**	**1.6[a]**	**74[a]**	**65[a]**	**1.4**	**1.1**	**0.88**	**4.0**	**3.7**	**2.0**
Parent (UD)		n.d.	0.01	47	43	60[b]	72[b]	71[b]	83[b]	78[b]	64[b]
M12+M14	Cysteinylglycine-S-conjugate; Cysteinyl-S-conjugate	n.d.	n.d.	n.d.	n.d.	7.0[c]	3.8[c]	5.6[c]	14[c]	13[c]	10[c]
M17	Mercapturic acid conjugate	0.02	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M18	Mercapturic acid conjugate	0.03	0.45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M21	Hydroxylated sulfonyl conjugate	n.d.	n.d.	0.20	0.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M24+M27+ M28+M29	Hydroxylation on cyanoethenyl moiety; Dehydration of M33; Aliphatic hydroxylation and dehydration of M45; Oxidation of M45	n.d.	n.d.	0.99[d]	1.6[d]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M30	Carboxylic acid metabolite	n.d.	n.d.	0.47	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M33	Aliphatic hydroxylation	n.d.	n.d.	0.54	0.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M38	Hydroxylation	n.d.	n.d.	0.70	0.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M41+M42	Hydroxylation of M45; Aromatic hydroxylation	n.d.	n.d.	2.8[e]	3.6[e]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M43+M45	Isomerization; S-Methyl conjugate	n.d.	n.d.	2.4[f]	2.9[f]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M46	Unknown	n.d.	n.d.	0.99	0.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M47	Dimerization	n.d.	n.d.	4.0	3.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Additional Information

96 h after administration of RPV, 93% of the dose was excreted in feces and 0.45% and 1.8% in urine in males and females, respectively. Over the 4-day collection period, 94% and 95% of the administered dose was recovered in males and females, respectively.

[a] Percentage of administered dose.
[b] Percentage of injected sample radioactivity.
[c] Sum of % of administered dose of M12 and M14, which co-eluted together in rat plasma samples.
[d] Sum of % of administered dose of M24, M27, M28 and M29, which co-eluted together in rat feces samples.
[e] Sum of % of administered dose of M41 and M42, which co-eluted together in rat feces samples.
[f] Sum of % of administered dose of M43 and M45, which co-eluted together in rat feces samples.

CA = citric acid; F = female; M = male; n.d. = Not detected by radiometric detector; PEG400 = polyethylene glycol; UD = unchanged drug; RPV = rilpivirine

2.6.5.9C Pharmacokinetics: Metabolism In Vivo in Rats (Biliary Excretion)

Test Article: rilpivirine

Study No.		**TMC278-NC145 (FK5525)**	
Location in CTD		**4.2.2.4**	
Species		**Rat (Sprague Dawley)**	
Gender (M/F)/Number of Animals		**M/3 (restrained); M/3 (non-restrained)**	
Feeding Condition		**Not fasted**	
Vehicle/Formulation		**PEG400/CA (10%)**	
Route		**Oral**	
Dose of RPV base (mg/kg)		**40**	
Radionuclide		**^{14}C**	
Specific Activity (kBq/mg)		**37**	
Sample		**Bile (Restrained Rats)**	**Bile (Non-Restrained Rats)**
Time (h)		**0-24**	**0-24**
% of Administered Radioactivity in Bile		**18[a]**	**25**
Metabolite Code		**Metabolite Profile - % Dose Radioactivity in 0-24 h Bile**	**Samples Not Profiled**
Parent (UD)		0.19	
M1	Glucuronidation and oxidation of M14	0.63	
M9	Thiol glucuronide conjugate	2.8	
M10+M12+ M14	Cysteinylglycine-S-conjugate; Cysteinyl-S-conjugate	6.4	
M18	Mercapturic acid conjugate	2.7	
M25	Oxidation combined with glucuronidation	1.4	
M30	Carboxylic acid metabolite	0.4	
	Sum	**14[a]**	

[a] The calculated total % dose value (14%) varies from the actual value (18%) due to an error arising from integration of noise in a radiochromatogram.

CA = citric acid; M = male; PEG 400 = polyethylene glycol; UD = unchanged drug

2.6.5.9D Pharmacokinetics: Metabolism In Vivo in Dogs

Test Article: rilpivirine

Study No.					**TMC278-NC114 (FK5143)**						
Location in CTD					**4.2.2.4**						
Species					**Dog (beagle)**						
Gender (M/F)/Number of Animals					**3M**						
Feeding Condition					**Fed**						
Vehicle/Formulation					**PEG400/CA (10%)**						
Route					**Oral (Capsule)**						
Dose of RPV base (mg/kg)					**5**						
Radionuclide					**^{14}C**						
Specific Activity (kBq/mg)					**99**						
Sample		**Urine**	**Feces**				**Plasma (mean)**				
Time (h)		**0-168h**	**0-72**	**0.25**	**0.5**	**1**	**2**	**4**	**6**	**8**	**24**
% of Administered Radioactivity in Excreta or µg-eq./mL in Plasma		**1.73**	**81**	**0.06**	**0.21**	**0.37**	**0.57**	**0.65**	**0.54**	**0.46**	**0.40**
Pooled Sample Results: % Dose Recovered		**0-24 h**	**0-72 h**	**Pooled plasma[a]**		**1h**		**4h**		**8h**	
Parent (UD)		n.d	45			94		73		91	
M11	Carboxylic acid metabolite on the cyanoethenyl moiety of M27	n.d	0.98			n.d.		n.d.		n.d.	
M23+M27	Monooxy-M27; Tricyclic metabolite, originating from oxidation and dehydration most probably of M33	n.d	2.1			n.d.		n.d.		n.d.	
M30+M48	Carboxylic acid metabolite; Unknown	0.03, n.d.	3.1			n.d.		n.d.		n.d.	
M33	Aliphatic hydroxylation	n.d	8.7			n.d.		n.d.		n.d.	
M37	Sulfate conjugate of monooxygenated-RPV	n.d	0.31			n.d.		n.d.		n.d.	
M40	N-oxide-RPV	n.d	0.50			n.d.		n.d.		n.d.	
M42	Aromatic hydroxylation	n.d	5.3			n.d.		n.d.		n.d.	
M44	Monooxygenated RPV	n.d	4.3			n.d.		n.d.		n.d.	
M46	Unknown structure	n.d	0.09			n.d.		n.d.		n.d.	
M49	Unknown structure	n.d	1.4			n.d.		n.d.		n.d.	

Additional Information

In urine, minor metabolites (M3, M12, M14, M19, M25 and M36) were detected which accounted for less than 0.08% of the dose. In plasma, minor metabolites were present in trace amounts and included M15, M19, M27, M30, and M33.

168 h after administration of RPV, 95% and 1.7% of the administered dose was excreted in feces and urine, respectively. Over the 7-day collection period, 97% of the administered dose was recovered.

[a] Percentage of injected sample radioactivity.

CA = citric acid; M = male; n.d. = Not detected by radiometric detector; PEG 400 = polyethylene glycol; UD = unchanged drug; RPV = rilpivirine

2.6.5.9E Pharmacokinetics: Metabolism In Vivo in Humans

Test Article: rilpivirine

Study No.				**TMC278-NC157 (FK5344)/TMC278-C119**					
Location in CTD				**4.2.2.4**					
Species				**Male Subjects**					
Gender (M/F)/Number of Subjects				**6M**					
Feeding Condition				**Fed (Breakfast)**					
Vehicle/Formulation				**PEG400 (25 mg/mL)**					
Route				**Oral**					
Dose of RPV base (mg)				**150**					
Radionuclide				**^{14}C**					
Specific Activity (kBq/mg)				**11.8**					
Sample		**Urine**	**Feces**	**Plasma Total Radioactivity (% of Sample Radioactivity in Plasma)**					
Time (h)		**0-168h**	**0-168h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**
% of Administered Radioactivity or Conc. (µg-eq./mL)		**6.13**	**76**	**0.40**	**0.57**	**0.77**	**0.56**	**0.36**	**0.32**
Average Sample Results: % Dose Recovered			**Selection of Samples**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**
Parent (UD)		Traces	25.5	58.8	55.7	65.7	47.2	45.7	44.1
M3	Unknown structure		0.3						
M11	Carboxylic Acid Metabolite of M27		1.6						
M13+M14 and M18	Glycine conjugates of RPV; mercapturic acid conjugate of RPV	1.2							
M15	*N*-Glucuronide of RPV	0.6		4.2	4.1	5.3	8.7	9.5	6.2
M19	Glucuronide of M33	0.3							
M23	Oxidized Metabolite of M27		0.7						
M25	Glucuronide of M42	0.6							
M27	Tricyclic Metabolite		0.6	6.6	6.6	9.7	6.5	5.7	8.0
M30	Carboxylic Acid Metabolite	0.03	2.7						
M33	Hydroxymethyl RPV		3.0	LOQ	1.8	2.9	3.4	3.5	5.1
M35	Unknown structure		2.2						
M39	Cis 5-Hydroxy Pyrimidinyl (Cis of M42)		0.4						
M42	5-Hydroxyl Pyrimidinyl		16.1						
M43	Cis RPV		0.6						
M46	Unknown structure		0.5						

Additional Information

168 h after administration of RPV, 76% and 6.1% of the administered dose was excreted in feces and urine, respectively. Over the 14-day collection period, 91% of the administered dose was recovered.

LOQ = Below the limit of quantification; M = male; PEG 400 = polyethylene glycol; UD = unchanged drug; RPV = rilpivirine

2.6.5.9F Pharmacokinetics: Metabolism In Vivo in human plasma

Test Article: rilpivirine

Study No.		**1646_0027483 (FK10104)**											
Location in CTD		**4.2.2.4**											
Species		**Human Plasma (M&F)**											
Route		**Oral**											
Analysis		**LC/UV/MS**											
Sample		**Plasma samples (pooled; n=6)**											
		DAY 1											
Dose of RPV base (mg q.d.)		**75 mg**						**300 mg**					
Time (h)		**0**	**0.5**	**3**	**6**	**12**	**24**	**0**	**0.5**	**3**	**6**	**12**	**24**
RPV*		≤1.13	6.82	238	214	117	103	NQ	21.5	679	629	324	298
M15**	N-glucuronide at the N1-position of the pyrimidinyl moiety of RPV	LOD	0.26	17.9	24.9	18.2	14.1	LOD	0.42	20.6	23.5	18.2	12.6
M27^{+}	Tricyclic metabolite	0.10	0.12	7.96	11.7	8.24	9.78	0.07	0.20	11.4	14.8	10.3	11.9
X1^{+}	M30 + 2O-2H	LOD	0.01	LOD	0.01	0.02	0.04	LOD	LOD	0.002	0.004	0.01	0.03
X2^{+}	UD + O + Glucuronide	LOD	LOD	0.02	0.02	0.01	0.02	LOD	LOD	0.03	0.02	0.02	0.02
M19^{+}	Glucuronide of M33	LOD	LOD	0.08	0.12	0.10	0.07	LOD	LOD	0.10	0.13	0.10	0.06
M25^{+}	Glucuronide of M42	LOD	0.01	0.27	0.19	0.09	0.11	LOD	0.03	0.42	0.25	0.12	0.14
M30^{+}	Loss of cyanide and carboxylic acid formation	LOD	0.04	2.13	1.74	0.92	1.02	LOD	0.08	3.04	2.18	1.09	1.27
X4^{+}	UD + 2O-2H	LOD	LOD	LOD	LOD	0.02	0.03	LOD	LOD	0.03	0.04	0.03	0.03
M33^{+}	Hydroxymethyl RPV (R419763)	LOD	LOD	0.61	0.86	0.59	0.64	LOD	0.01	0.87	0.94	0.59	0.62
M36^{+}	UD + O	0.01	LOD	0.53	0.43	0.22	0.17	LOD	0.01	0.69	0.54	0.26	0.20
M38^{+}	UD + O	LOD	LOD	0.09	0.09	0.05	0.05	LOD	LOD	0.16	0.15	0.07	0.06
M43^{+}	Cis RPV	0.09	0.20	3.29	4.68	2.05	2.72	0.03	0.73	10.9	12.6	4.79	7.27

(Continued)

2.6.5.9F Pharmacokinetics: Metabolism In Vivo in human plasma (Continued)

Test Article: rilpivirine

		DAY 11									
Dose of RPV base (mg q.d.)		**75 mg**					**300 mg**				
Time (h)		**0.5**	**3**	**6**	**12**	**24**	**0.5**	**3**	**6**	**12**	**24**
RPV*		247	459	449	307	320	529	1302	1183	715	696
M15**	N-glucuronide at the N1-position of the pyrimidinyl moiety of RPV	28.3	38.4	39.0	28.2	21.5	19.1	21.6	22.0	18.1	10.5
M27+	Tricyclic metabolite	31.0	33.2	32.3	22.6	28.1	26.2	25.8	22.7	16.4	18.7
X1+	M30 + 2O-2H	0.33	0.31	0.30	0.24	0.32	0.27	0.21	0.25	0.19	0.18
X2+	UD + O + Glucuronide	0.05	0.06	0.07	0.04	0.05	0.04	0.04	0.05	0.03	0.03
M19+	Glucuronide of M33	0.17	0.19	0.21	0.18	0.10	0.08	0.12	0.14	0.12	0.05
M25+	Glucuronide of M42	0.24	0.44	0.40	0.21	0.29	0.27	0.50	0.39	0.20	0.25
M30+	Loss of cyanide and carboxylic acid formation	1.85	3.44	3.00	1.67	1.99	2.09	3.41	2.87	1.38	1.74
X4+	UD + 2O-2H	0.15	0.15	0.16	0.08	0.14	0.13	0.16	0.18	0.08	0.14
M33+	Hydroxymethyl RPV (R419763)	0.96	1.43	1.44	0.95	1.03	0.72	1.18	0.92	0.61	0.65
M36+	UD + O	0.30	0.55	0.53	0.38	0.33	0.24	0.54	0.43	0.18	0.21
M38+	UD + O	0.09	0.21	0.18	0.10	0.09	0.13	0.26	0.24	0.10	0.11
M43+	Cis RPV	3.74	4.91	5.23	3.09	4.72	5.58	8.96	6.68	5.10	6.23

*Mean data for RPV obtained using a validated LC/MS/MS method

**LC/UV data were used, assuming the LC/UV response for M15 and RPV was similar. Knowing the LC/UV peak area for RPV and the corresponding concentration determined by a validated LC/MS/MS method, the concentration of M15 was estimated using a rule of three

+ LC/MS data were used, assuming the LC/MS response for these metabolites and RPV were similar. Knowing the LC/MS peak are for RPV and the corresponding concentration determined by a validated LC/MS/MS method, the estimated concentration of metabolites was calculated using a rule of three.

F = female; LC/UV/MS = liquid chromatography coupled to tandem mass spectrometry and UV detection; LOD = limit of detection; M = Male; NQ = Not quantifiable (<1 ng/mL); RPV = rilpivirine

2.6.5.10A Pharmacokinetics: Metabolism In Vitro

Test Article: rilpivirine

Study No.	**TMC278-NC102 (FK4728)**
Location in CTD	**4.2.2.4**
Type of Study	In vitro metabolism of RPV in hepatocytes and liver subcellular fractions in different species and in man.
Methodology	^{14}C-RPV (5 μM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for identification of the metabolites.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	**Mouse (male, Swiss albino)**				**Mouse (female, Swiss albino)**				**Mouse (male, black Agouti rasH2)**				**Mouse (female, black Agouti rasH2)**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
Parent (UD)	11.8	3.9	57.1	85.3	3.2	4.4	71.9	81.6	13.1	3.9	49.1	72.4	6.1	1.9	57.7	77.3
2	-	0.5	-	0.6	-	0.7	-	1.0	-	-	-	0.9	-	-	0.7	0.6
3	0.6	1.0	-	-	1.0	0.6	-	-	-	-	-	-	-	-	-	-
4+5	4.1	2.0	-	0.5	7.2	5.2	-	-	6.6	4.6	0.7	-	8.6	4.6	0.6	-
6	4.8	2.9	0.7	-	3.5	2.2	-	0.7	8.2	3.1	0.8	-	4.1	3.6	0.7	-
7	2.8	1.4	1.1	-	1.0	-	0.6	-	-	-	-	-	-	-	-	-
8	1.8	2.9	1.4	-	3.3	6.7	0.8	-	3.0	3.5	2.3	-	4.5	5.3	4.0	-
10	61.9	49.7	35.9	-	7.6	12.6	17.1	-	-	-	-	-	-	-	-	-
10 (+12* or +14)**									28.3	43.9	36.0*	-	15.0**	36.4	23.8*	-
13	-	-	-	0.9	-	-	-	1.3	-	-	3.3	0.8	-	-	0.7	0.5
14	-	18.4	-	-	6.0	9.2	-	-	5.3	18.0	-	-	-	15.3	-	-
17	1.5	5.3	-	0.7	3.2	8.9	-	-	5.2	7.9	-	0.9	5.7	6.7	-	0.6
18	-	10.5	-	-	3.0	0.6	-	-	-	11.1	-	-	-	5.2	-	-
22	-	-	0.9	2.4	-	-	0.6	3.9	-	-	-	5.5	-	-	-	3.2
25	13.3	7.3	-	-	49.3	46.9	-	-	25.5	6.5	-	-	45.2	23.7	-	-
27	1.8	-	0.9	1.1	-	-	1.1	2.5	2.2	1.0	0.8	2.4	1.2	-	1.3	2.0
33	0.8	-	0.9	2.2	1.8	-	1.2	2.8	2.4	-	0.8	2.6	3.5	-	1.5	1.9
35+36	0.9	-	0.7	2.6	1.8	-	1.2	4.1	1.4	-	0.5	1.9	1.8	-	2.3	2.9
38	-	-	-	-	0.9	0.7	0.8	0.6	-	-	0.6	-	-	-	1.3	-
42	-	0.5	1.3	1.4	0.9	1.6	5.4	2.2	-	0.7	2.9	2.1	0.9	0.7	9.6	1.5
43	0.9	1.5	0.8	0.5	-	-	1.6	0.4	0.7	0.7	1.9	0.9	0.4	-	2.7	0.4
Sum	**107.0**	**107.8**	**101.7**	**98.2**	**93.7**	**100.3**	**102.3**	**101.1**	**101.9**	**104.9**	**99.7**	**90.4**	**97.0**	**103.4**	**106.9**	**90.9**

(Continued)

2.6.5.10A Pharmacokinetics: Metabolism In Vitro (Continued)

Test Article: rilpivirine

Study No.	**TMC278-NC102 (FK4728)**
Location in CTD	**4.2.2.4**
Type of Study	In vitro metabolism of RPV in hepatocytes and liver subcellular fractions in different species and in man.
Methodology	^{14}C-RPV (5 μM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for identification of the metabolites.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	**Rat (male, Sprague-Dawley)**				**Rat (female, Sprague-Dawley)**				**Rabbit (female, New Zealand)**				**Dog (male, Beagle)**				**Man**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
Parent (UD)	59.5	8.2	42.2	90.2	47.7	18.2	64.0	96.4	20.2	1.0	28.7	87.4	72.3	25.4	91.5	90.7	75.8	23.1	34.8	43.5
2	-	-	-	-	-	-	-	-	1.3	-	1.3	-	-	0.9	-	-	-	-	4.8	1.6
3	-	-	-	-	-	-	-	-	0.8	-	1.1	-	-	0.5	-	-	0.2	1.5	-	-
4	0.6	2.1	-	-	0.3	1.4		-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	5.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0.5	1.0	3.5	-	-	0.5	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	1.5	0.7	3.9	-	0.7	2.0	-	-	0.4	1.8	2.3	1.3
8	0.5	1.6	4.3	-	0.7	2.1	1.5	-				-	-	-	-	-	-	-	-	-
10	35.6	56.4	40.7	-	46.7	58.7	25.7	-	3.8	3.0	2.1	1.0	1.2	6.4	1.4	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.2	4.8	-	-
14	-	5.6	-	-	-	-	-	-	-	-	-	-	1.5	4.2	-	-	1.5	4.9	-	-
15	-	-	-	-	-	-	-	-	15.8	11.3	-	-	-	-	-	-	1.1	4.6	-	-
17	0.8	7.1	0.6	1.1	3.2	22.5	-	-	5.3	8.8	-	-	-	-	-	-	0.5	6.8	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7	-	-
19	-	-	-	-	-	-	-	-	7.6	11.9	4.8	1.3	3.7	9.4	-	-	0.2	2.1	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	0.9	-	-	-	-
22	-	-	-	-	-	-	0.4	0.7	-	-	-	-	-	-	-	-	-	-	3.6	7.8
25	0.8	1.1	-	-	1.7	3.5	-	-	38.8	46.2	5.4	-	8.6	22.0	-	-	11.8	31.0	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	2.6	6.7	-	-	-	-	-	-
27	0.4	0.7	0.9	0.7	0.6	-	-	-	-	-	11.8	0.4	-	-	2.0	0.8	2.4	7.2	5.6	4.9

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2.6.5.10A Pharmacokinetics: Metabolism In Vitro (Continued)

Test Article: rilpivirine

Study No.	**TMC278-NC102 (FK4728)**
Location in CTD	**4.2.2.4**
Type of Study	In vitro metabolism of RPV in hepatocytes and liver subcellular fractions in different species and in man.
Methodology	^{14}C-RPV (5 μM) was incubated for various time periods with hepatocytes (suspensions and primary cultures) and with liver subcellular fractions (microsomes and 12,000 x g supernatant fractions). Samples were analyzed by radio-HPLC. Co-chromatography, enzyme hydrolysis, LC-MS/MS and NMR techniques were used for identification of the metabolites.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	**Rat (male, Sprague-Dawley)**				**Rat (female, Sprague-Dawley)**				**Rabbit (female, New Zealand)**				**Dog (male, Beagle)**				**Man**			
Metabolites	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**	**SK**	**PCK**	**12,000g**	**MICR**
30+31	-	-	-	-	-	-	-	-	-	-	4.6	1.2	-	-	0.5+0.8	-	0.9***	1.6*	3.6	0.5***
32	-	1.3	-	-	-	6.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	1.0	1.1	-	-	-	-	-	-	4.6	1.2	-	-	2.3	1.0	1.4	1.1	2.5	2.9
34	-	-	-	-	-	-	-	-	-	-	-	-	1.7	6.0	-	-	-	-	-	-
35+36	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	0.5	1.2	0.4		9.8	7.9
36	-	-	1.1	2.1	-	-	0.5	1.2	2.5		6.0	1.4	-	-	-	-	-	-	-	-
38	-	-	0.7	2.0	-	-	-	0.5	-	-	6.9	0.4	-	-	0.6	-	-	-	3.8	-
42	-	-	1.1	2.0	-	-	1.2	1.1	-	-	5.1	0.8	1.7	2.6	3.5	1.2	0.5	1.0	6.6	6.0
43	2.8	-	3.8	0.9	2.0	0.8	3.8	1.4	-	-	1.2	1.4	-	0.6	0.9	0.9	1.8	0.6	Trace	-
Sum	**101.5**	**85.1**	**105.3**	**101.4**	**102.9**	**113.8**	**97.7**	**101.3**	**97.6**	**82.9**	**87.5**	**96.5**	**94.0**	**88.0**	**105.0**	**96.7**	**100.9**	**93.6**	**73.8**	**76.4**

- = not detected; 12,000g = 12,000 x g supernatant fractions; HPLC = high performance liquid chromatography; LC-MS/MS = liquid chromatography with tandem mass spectrometry; MICR = microsomes; NMR = nuclear magnetic resonance; PCK = primary culture; SK = suspension culture; UD = unchanged drug; RPV = rilpivirine

* The figures represent the sum of the % of M10 and M12

** The figures represent the sum of the % of M10 and M14

*** Figure represents only M30

2.6.5.10B Pharmacokinetics: Metabolism In Vitro

Test Article: rilpivirine

Study No. **Location in CTD**	**TMC278-NC333 (FK6818)** **4.2.2.4**
Type of study	In vitro metabolism of RPV in hepatocytes (primary cell cultures) and 12000 x g liver supernatant fractions of monkey and Guinea pigs.
Methodology	^{14}C-RPV (5 μM) was incubated with hepatocytes (primary cultures) for approximately 24 hours and with liver subcellular fractions (12,000 x g supernatant fractions) for 120 minutes. Samples were analyzed by radio-HPLC and metabolites identified by LC-MS/MS.

Percentage of injected sample radioactivity for unchanged compound and its metabolites

Study System	**Guinea pig (female, Dunking Hartley)**		**Monkey (male, Cynomolgus)**	**Monkey (female, Cynomolgus)**
Metabolites	**PCK**	**12,000g**	**PCK**	**12,000g**
Parent (UD)	2.5	30.9	9.1	12.6
M10	ND	55.0	ND	ND
M12	45.8	ND	10.7	ND
M13+M14	19.1	ND	ND	ND
M17+M18	11.1	ND	ND	ND
M19	ND	ND	7.4	ND
M22	ND	ND	ND	8.3
M25	7.1	ND	53.5	ND
M27	ND	ND	ND	10.6
M30	2.6	2.8	ND	ND
M33	ND	ND	ND	12.7
M38	ND	ND	ND	9.2
M42	ND	ND	ND	9.4
Sum	**88.2**	**88.7**	**80.6**	**62.7**

12,000g = 12,000 x g supernatant fractions; HPLC = high performance liquid chromatography; LC-MS/MS = liquid chromatography with tandem mass spectrometry; ND = not detected; PCK = primary culture; UD = unchanged drug; RPV = rilpivirine

2.6.5.10C Pharmacokinetics: Metabolism In Vitro

Test Article: rilpivirine

Study No.	**TMC278-NC141 (FK5300)**
Location in CTD	**4.2.2.4**
Type of study	Enzyme kinetics of RPV metabolism in human liver microsomes.
Method	^{14}C-RPV was incubated at various concentrations in human liver microsomes for 15 minutes at a protein concentration of 0.25 mg/ml. The amount of unchanged RPV remained in the samples was determined by radio-HPLC and the % metabolized (substrate turnover rate) was calculated. The kinetic parameters were calculated by a Michaelis-Menten equation using validated Winnonlin software (Pharsight, Winnonlin 4.0.1).
Results	
I. Protein concentration: 0.25 mg/ml	
Substrate Conc. (µM)	**Product rate (pmol/mg/min)[a]**
0.5	32.2, 36.8, 28.5
1	60.7, 57.2, 56.2
3	146, 124, 150
5	206, 222, 248
7.5	281, 199, 205
10	327, 420, 223
15	287, 299, 231
20	270, 308, 350
30	317, 341, 429
50	356, 396, 222
K_m (± std error)	**4.17 (± 1.06) µM**
V_{max} (± std error)	**381 (± 26) pmol/min/mg protein**

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2.6.5.10C Pharmacokinetics: Metabolism In Vitro (Continued)

Test Article: rilpivirine

Study No.	**TMC278-NC141 (FK5300)**
Location in CTD	**4.2.2.4**
Type of study	CYP reaction phenotyping – Effect of diagnostic CYP inhibitors on the metabolism of RPV
Method	Inhibition of the metabolism of RPV in human liver microsomes by diagnostic inhibitors was carried out with ^{14}C-RPV (5 µM) for 15 minutes at a protein concentration of 0.25 mg/ml. The amounts of unchanged RPV and its metabolites (M27, M33, M35+M36[b] and M42[b]) were determined by radio-HPLC. The values represent the percentage of inhibition obtained for each inhibitor in comparison to a control incubate (without inhibitor). Each value represents mean of three observations.

Results

		% Inhibition of Metabolism[c]				
Diagnostic Inhibitor	**CYP P450 Form**	**Overall[d]**	**M27[d]**	**M33[d]**	**M35+M36[b]**	**M42[b,c]**
Furafylline (10 µM)	CYP1A2	-10.9	-10.9	-13.9	66.7	-22.3
Coumarin (100 µM)	CYP2A6	-9.3	-25.0	-44.4	75.0	-13.4
Sulphaphenazole (10 µM)	CYP2C8/9/10	-3.0	0.0	30.6	86.1	-25.1
Quinidine (10 µM)	CYP2D6	4.4	1.56	-5.56	69.4	-3.24
4-methylpyrazole (20 µM)	CYP2E1	-6.5	1.56	-8.33	63.9	-21.1
Ticlopidine (5 µM)	CYP2C19/D6	-3.1	-2.94	-27.8	25.0	-7.91
Ketoconazole (1 µM)	CYP3A4	107	100	100	100	100
Troleandomycin (200 µM)	CYP3A4	107	100	100	100	100
Clarithromycin (15 µM)	CYP3A	57.2	45.3	38.9	58.3	57.1
Ritonavir (0.15 µM)	CYP3A	91.3	100	100	100	80.2
1-aminobenzotriazole	CYP P450	104	100	100	100	100

(Continued)

2.6.5.10C Pharmacokinetics: Metabolism In Vitro (Continued)

Test Article: rilpivirine

Study No.	**TMC278-NC141 (FK5300)**
Location in CTD	**4.2.2.4**
Type of study	CYP reaction phenotyping – Metabolism of ^{14}C-RPV in *E. coli* expressed CYP isoforms.
Method	The metabolism of RPV in *E. coli* expressed CYP systems (prepared in-house) was carried out with ^{14}C-RPV (5 µM) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged RPV and its metabolites (M50, M2, M22[b], M27, M33[b], M35+M36[b], M51, and M42[b]) were determined by radio-HPLC. Each value represents mean ± S.D of three observations.

Results

Cytochrome P-450 Form (100 pmol/ml)	**Overall % Metabolism[e]**	**Product formation rate (pmol/min. 100 pmol P450)**							
		M50	**M2**	**M22**	**M27**	**M33**	**M35+M36**	**M51**	**M42**
CYP1A2	1.40 ± 0.26	-	-	-	-	-	-	-	-
CYP2A6	1.07 ± 1.44	-	-	-	-	-	-	-	-
CYP2B6	1.30 ± 1.76	-	-	-	-	-	-	-	-
CYP2C8	0.43 ± 0.38	-	-	-	-	-	-	-	-
CYP2C9	0.47 ± 0.42	-	-	-	-	-	-	-	-
CYP2C19	0.37 ± 0.32	-	-	-	-	-	-	-	-
CYP2D6	1.07 ± 1.85	-	-	-	-	-	-	-	-
CYP2E1	0.00 ± 0.00	-	-	-	-	-	-	-	-
CYP3A4	86.87 ± 1.40	4.11 ± 0.35	9.03 ± 0.71	15.6 ± 1.30	2.58 ± 0.95	3.00 ± 0.58	20.4 ± 2.95	-	6.28 ± 0.75
CYP3A5	0.53 ± 0.68	-	-	-	-	-	-	-	0.31 ± 0.34

(Continued)

2.6.5.10C Pharmacokinetics: Metabolism In Vitro (Continued)

Test Article: rilpivirine

Study No.	**TMC278-NC141 (FK5300)**
Location in CTD	**4.2.2.4**
Type of study	CYP reaction phenotyping – Metabolism of ^{14}C-RPV in CYP isoforms (Supersomes®).
Method	The metabolism of RPV in expressed CYP systems (Supersomes®) was carried out with ^{14}C-RPV (5 µM) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged RPV and its metabolites (M52, M2, M22[b], M27, M33 [b], M35+M36[b], M51[b] and M42[b]) were determined by radio-HPLC. Each value represents mean ± S.D of three observations.

Results

Cytochrome P-450 Form (100 pmol/ml)	**Overall Metabolism[e]**	**Product formation rate (pmol/min. 100 pmol P450)**							
		M52	**M2**	**M22**	**M27**	**M33**	**M35+M36**	**M51**	**M42**
CYP1A2	1.17 ± 0.64	-	-	-	-	-	-	-	-
CYP2A6	0.37 ± 0.32	-	-	-	-	-	-	-	-
CYP2B6	0.50 ± 0.44	-	-	-	-	-	-	-	-
CYP2C8	0.00 ± 0.00	-	-	-	-	-	-	-	-
CYP2C9	0.43 ± 0.38	-	-	-	-	-	-	-	-
CYP2C19	0.43 ± 0.40	-	-	-	-	-	-	-	-
CYP2D6	2.37 ± 2.45	-	-	-	-	-	-	-	-
CYP2E1	0.23 ± 0.40	-	-	-	-	-	-	-	-
CYP3A4	39.5 ± 2.1	0.97 ± 0.84	4.44 ± 0.86	3.39 ± 0.32	4.14 ± 0.68	4.00 ± 0.46	7.72 ± 1.73	-	5.19 ± 1.13
CYP3A5	28.3 ± 2.7	0.61 ± 0.21	1.64 ± 0.21	5.00 ± 0.52	0.25 ± 0.43	5.08 ± 0.36	2.56 ± 0.21	2.61 ± 0.91	3.81 ± 1.14
CYP3A7	26.3 ± 2.3	-	1.17 ± 0.14	3.50 ± 1.39	-	4.33 ± 1.15	6.14 ± 0.60	-	6.39 ± 0.59

(Continued)

2.6.5.10C Pharmacokinetics: Metabolism In Vitro (Continued)

Test Article: rilpivirine

Study No.	**TMC278-NC141 (FK5300)**
Location in CTD	**4.2.2.4**
Type of study	CYP reaction phenotyping – Correlation analysis of RPV metabolites with CYP activities.
Methodology	The metabolism of ^{14}C-RPV (5 µM) was examined with a characterized panel of 10 human liver microsomal preparations. The protein concentration of the samples was 0.25 mg/ml and the time of incubation was 15 min. The amounts of unchanged RPV and its metabolites (M27, M33 [b], M35 + M36 [b] and M42 [b]) were determined by radio-HPLC. The rate of product formation was calculated for RPV metabolites and were correlated (pair-wise) with the CYP isoform dependent enzyme activities of corresponding batches of human liver microsomes.

Results

Enzyme activities (CYP isoform)	**Overall RPV metabolism Correlation (r^2)**	**RPV metabolite correlation coefficient (r^2)**			
		M27	**M33**	**M35 + M36**	**M42**
7-ethoxyresorufine *O*-deethylase **(1A2)**	0.119	-0.050	0.523	0.093	0.073
Phenacetin *O*-deethylase **(1A2)**	0.052	-0.186	0.347	0.102	0.047
Coumarin 7-hydroxylase **(2A6)**	-0.071	0.055	-0.297	0.201	-0.080
Taxol 6-α-hydroxylase **(2C8)**	-0.437	-0.613	-0.324	-0.660	-0.289
Tolbutamide methyl hydroxylase **(2C9, 10)**	-0.711	-0.842	-0.530	-0.468	-0.608
S-mephenytoin 4-hydroxylase **(2C19)**	**0.748**	**0.704**	**0.878**	0.107	**0.790**
Dextromethorphan *O*-demethylase **(2D6)**	-0.413	-0.538	-0.310	-0.611	-0.306
Bufuralol hydroxylase **(2D6)**	-0.442	-0.578	-0.348	-0.659	-0.325
Chlorozoxazone 6-hydroxylase **(2E1)**	0.030	-0.098	-0.303	-0.389	0.215
Lauric acid ω-1 hydroxylase **(2E1)**	-0.543	-0.709	-0.393	-0.450	-0.424
Testosterone 6-β-hydroxylase **(3A4)**	**0.819**	**0.749**	0.485	-0.003	**0.881**
Cyclosporine oxidase **(3A)**	**0.716**	**0.744**	0.336	0.015	**0.746**
Taxol 3'- hydroxylase **(3A4)**	**0.889**	**0.938**	**0.503**	0.383	**0.872**
Midazolam 4-hydroxylase **(3A4/A5)**	**0.864**	**0.817**	**0.611**	0.055	**0.876**
Midazolam 1'- hydroxylase **(3A5/A4)**	**0.577**	**0.594**	0.329	-0.310	**0.638**
Lauric acid ω- hydroxylase **(4A)**	-0.001	-0.207	-0.185	-0.499	0.173

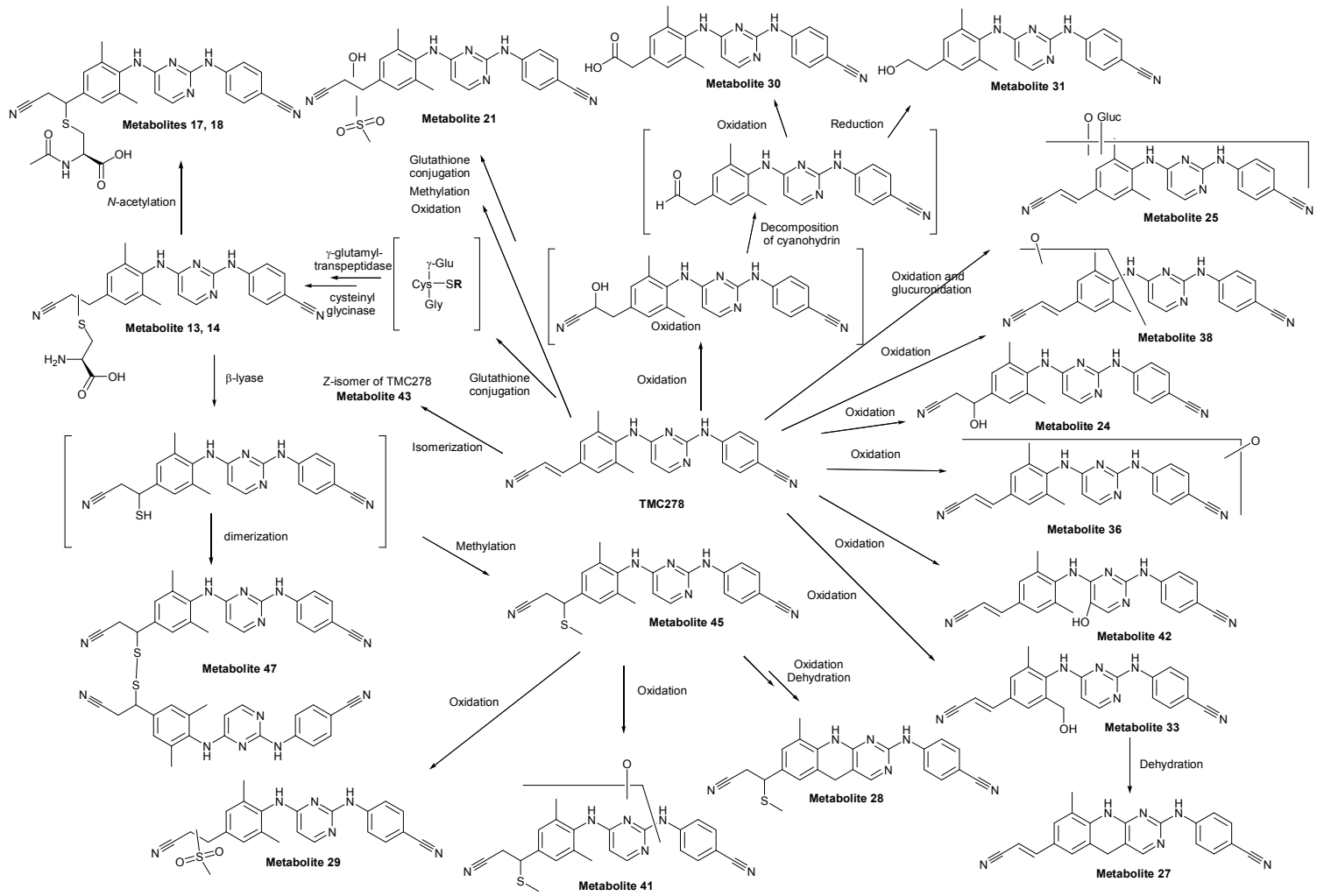
[a] Triplicate value is used in the determination of K_m and V_{max}
[b] Major metabolite in human liver microsomes (> 5 % of the sample radioactivity)
[c] Calculated from control incubation (without inhibitor); higher the positive value and higher the extent of inhibition
[d] Negative values indicates higher % product formation in test sample compared to the control. This was more prominent with the minor metabolites. For all qualitative purposes, all negative values were considered as no inhibition
[e] Overall % metabolism of RPVcalculated from % drug that remained in the sample at the end of the incubation

CYP = cytochrome P450; HPLC = high performance liquid chromatography; S.D. = standard deviation; K_m = substrate concentration; V_{max} = maximum rate achieved; - No measurable product observed in radio-HPLC profile (LLOQ = 211 dpm)
Bolded numbers = Positive correlations higher than 0.500; RPV = rilpivirine

2.6.5.11A Pharmacokinetics: Metabolism in Vivo – Proposed Metabolic Pathway in Mice

Test Article: rilpivirine

Study No.	**TMC278–NC190 (FK5621)[a]**
Location in CTD	**4.2.2.4**
Species	**Mouse**

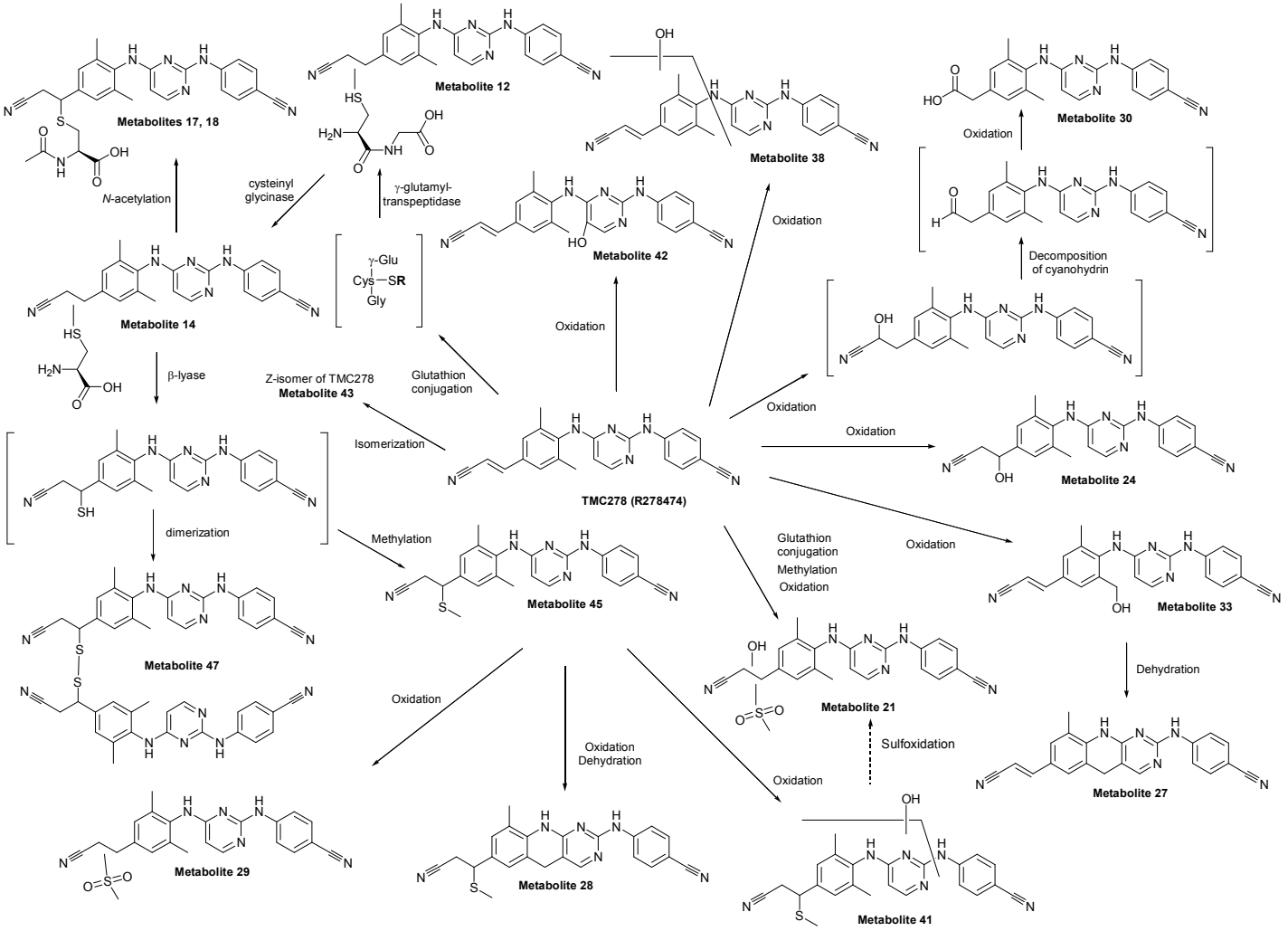


[a] See Tabulated Summary 2.6.5.9A

2.6.5.11B Pharmacokinetics: Metabolism in Vivo – Proposed Metabolic Pathway in Rats

Test Article: rilpivirine

Study No.	**TMC278–NC113 (FK4933)[a]**
Location in CTD	**4.2.2.4**
Species	**Rat**

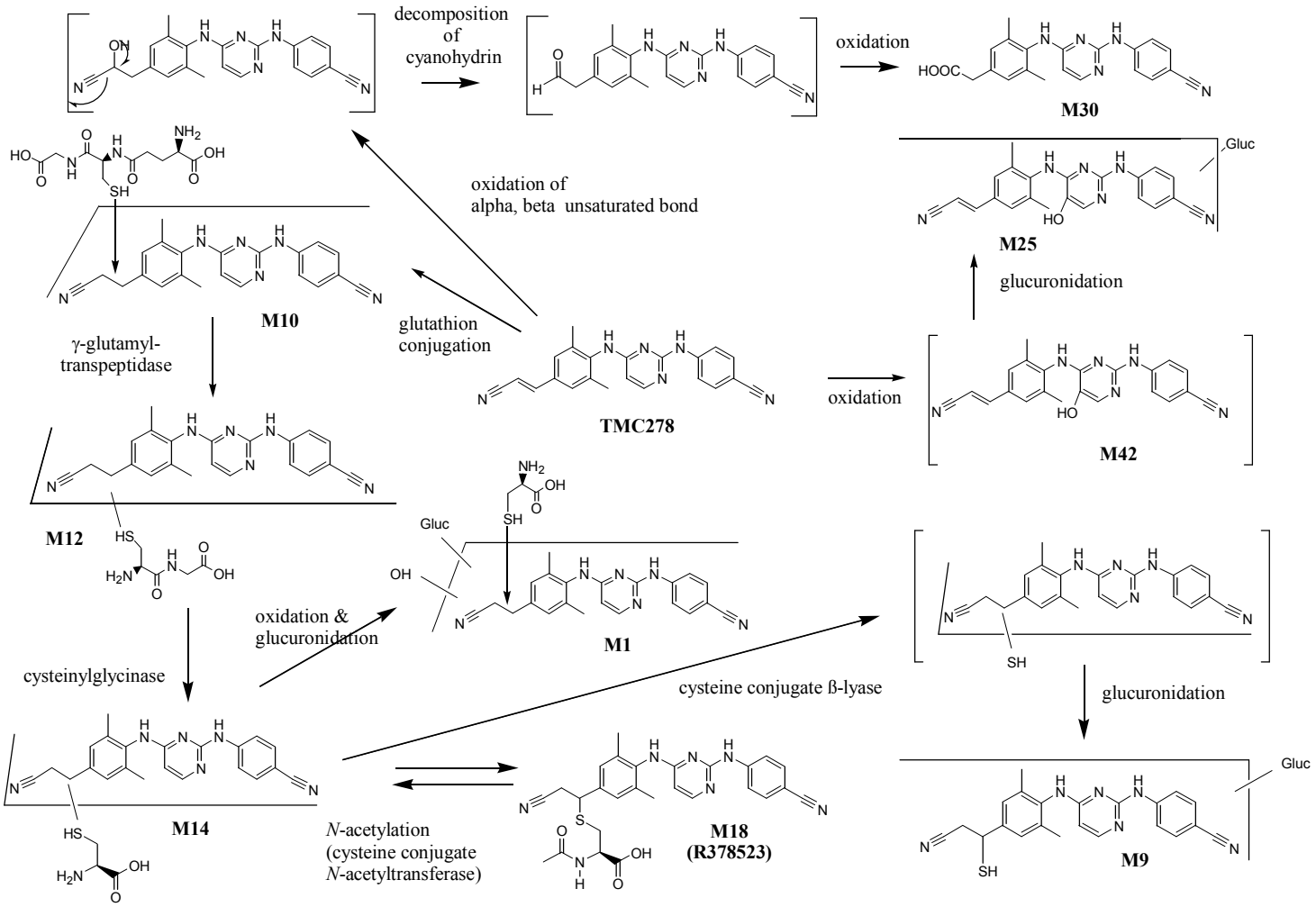


[a] See Tabulated Summary 2.6.5.9B

2.6.5.11C Pharmacokinetics: Metabolism in Vivo – Proposed Metabolic Pathway in Rat Bile

Test Article: rilpivirine

Study No.	**TMC278–NC145 (FK5525)[a]**
Location in CTD	**4.2.2.4**
Species	**Rat**

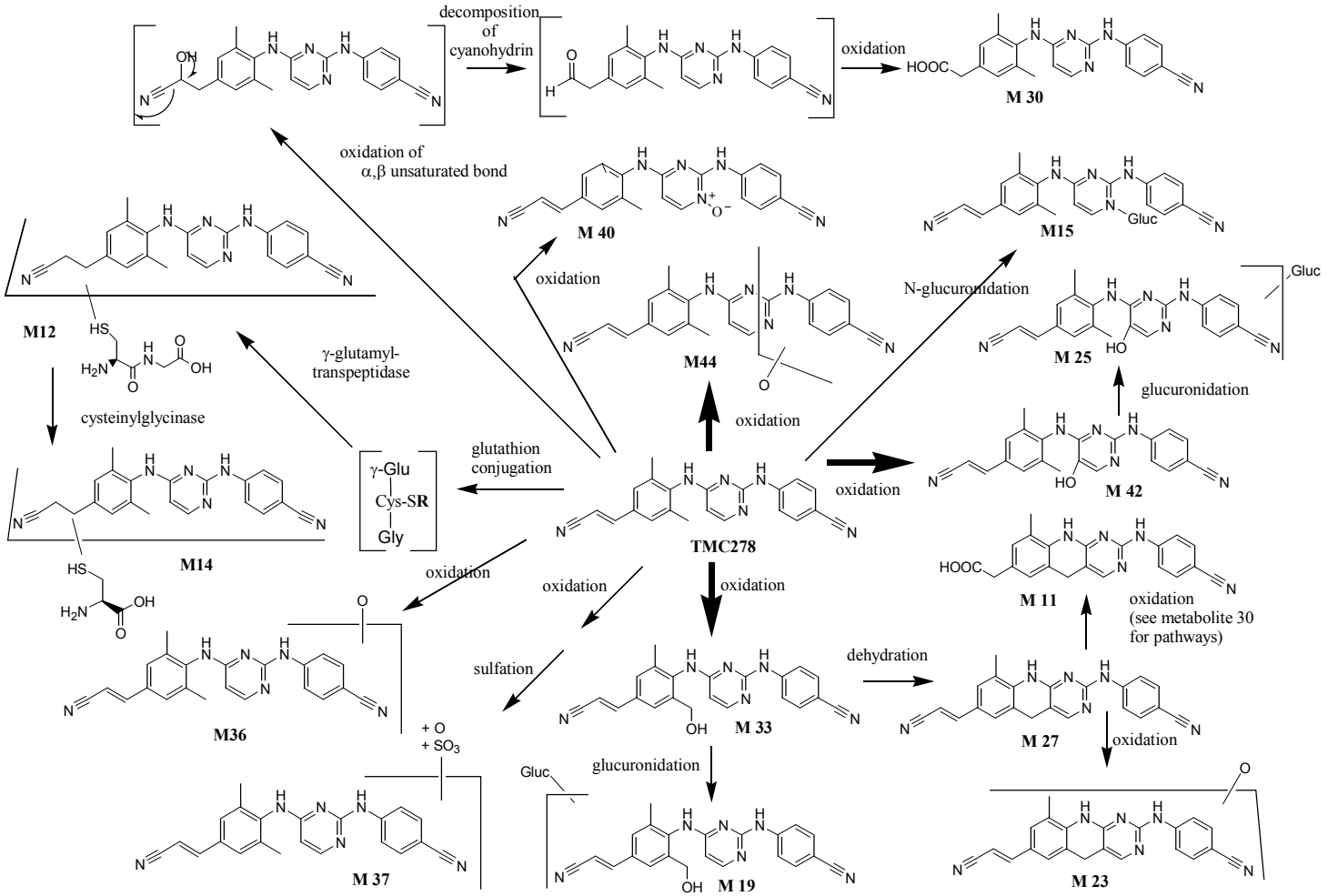


[a] See Tabulated Summary 2.6.5.9C

2.6.5.11D Pharmacokinetics: Metabolism in Vivo – Proposed Metabolic Pathway in Dogs

Test Article: rilpivirine

Study No.	**TMC278–NC114 (FK5143)[a]**
Location in CTD	**4.2.2.4**
Species	**Dog (beagle)**

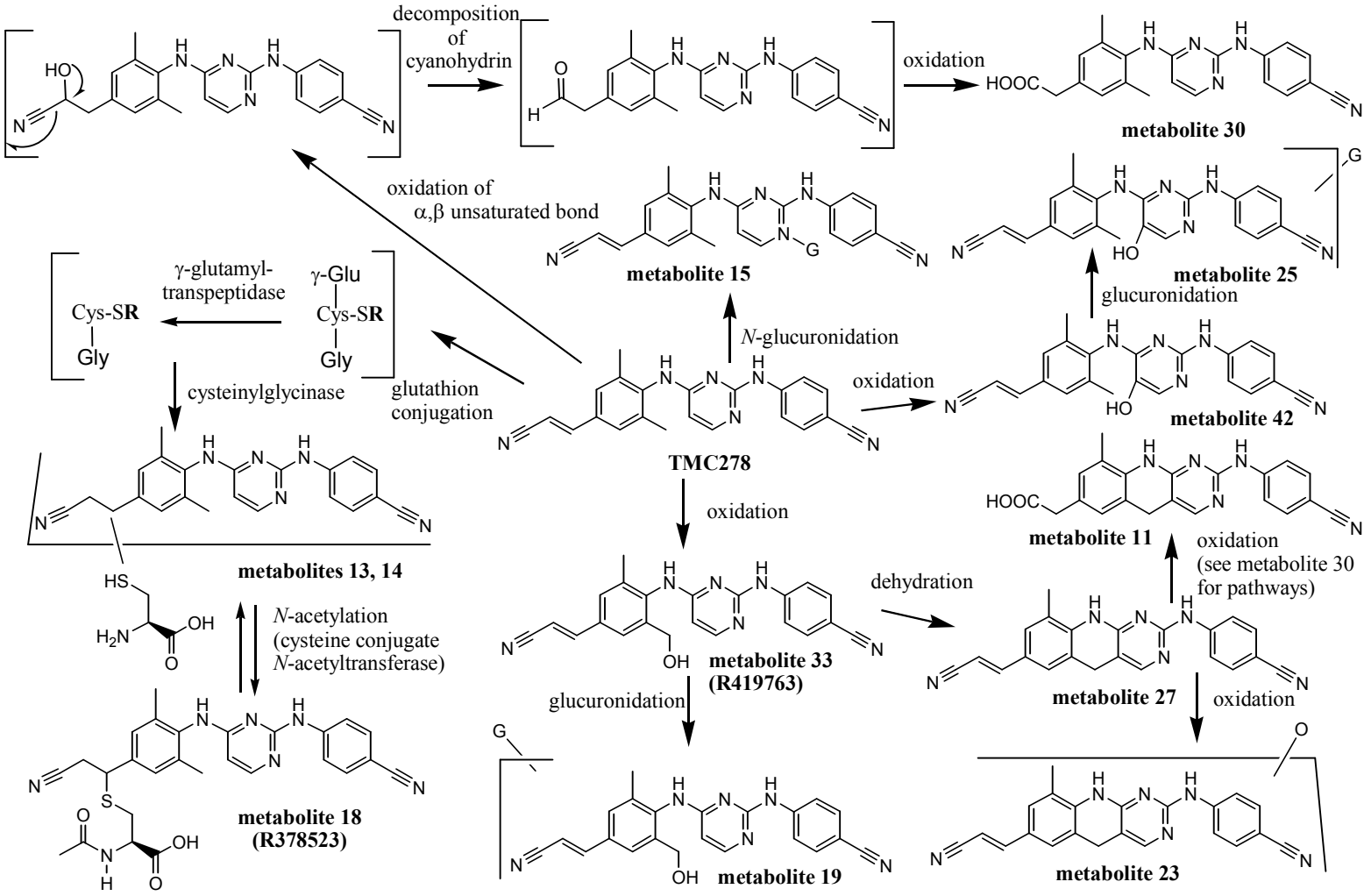


[a] See Tabulated Summary 2.6.5.9D

2.6.5.11E Pharmacokinetics: Metabolism in Vivo – Proposed Metabolic Pathway in Humans

Test Article: rilpivirine

Study No.	**TMC278–NC157 (FK5344)[a]**
Location in CTD	**4.2.2.4**
Species	**Human**



[a] See Tabulated Summary 2.6.5.9E
TMC278: RPV

2.6.5.12A Pharmacokinetics: Induction/Inhibition in Human

Test Article: rilpivirine

Study No.	**TMC278-NC186 (FK5720)**
Location in CTD	**4.2.2.4**
Type of Study	An in vitro study to assess the potential of RPV to induce CYP enzyme activities in cryopreserved human hepatocytes
Method	After establishment of the hepatocyte cultures, human hepatocytes were treated either with vehicle (DMSO), with various concentrations of RPV or with the positive control compounds, omeprazole, rifampicin, or ethanol for 48 h. At the end of the treatment period, induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP2E1, CYP3A4) was measured based on the probe substrate metabolism. Mean fold induction of the different CYP-isoforms in cryopreserved human hepatocytes treated with RPV and positive controls was expressed against the vehicle control. In addition, induction of CYP activities was also determined by measurement of mRNA expression levels by TaqMan real-time RT-PCR. In total, three different individual batches of cryopreserved human hepatocytes were used in this study. The results are tabulated in the table below and each value is mean of three observations.

Test Condition	**Mean fold induction in enzyme activity levels**				
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP2E1**	**CYP3A4**
Control (Vehicle)	1.00	1.00	1.00	1.00	1.00
RPV (2.5 µM)	1.06	1.28	1.44	0.98	0.24
RPV (10 µM)	0.62	0.71	1.20	1.37	0.04
RPV (25 µM)	0.51	0.58	1.25	0.93	0.05
Rifampicin (50 µM)	NA	2.60	3.07	NA	14.43
Rifampicin (50 µM) + RPV (25 µM)[a]	NA	0.43	0.28	NA	0.10
Omeprazole (25 µM)	4.95	NA	NA	NA	NA
Omeprazole (25 µM) + RPV (25 µM)[a]	2.35	NA	NA	NA	NA
Ethanol (100 mM)	NA	NA	NA	1.24	NA
Ethanol (100 mM) + RPV (25 µM)[a]	NA	NA	NA	0.76	NA

Test Condition	**Mean fold change in mRNA expression levels**				
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP2E1**	**CYP3A4**
Control (Vehicle)	1.00	1.00	1.00	1.00	1.00
RPV (2.5 µM)	2.55	2.89	1.19	0.81	27.12
RPV (10 µM)	3.17	2.96	1.12	0. 56	25.95
RPV (25 µM)	3.58	1.18	0.60	1.12	5.08
Rifampicin (50 µM)	NA	6.80	1.69	NA	54.88
Omeprazole (25 µM)	15.07	NA	NA	NA	NA
Ethanol (100 mM)	NA	NA	NA	1.01	NA

Additional Information	-

[a] Inhibition control to investigate interference of RPV with measurement of CYP activities

CYP = cytochrome 450; DMSO = dimethylsulfoxide; mRNA = messenger ribonucleic acid; NA = not applicable; RT-PCR = reverse transcriptase-polymerase chain reaction; RPV = rilpivirine

2.6.5.12B Pharmacokinetics: Induction/Inhibition in Mice

Test Article: rilpivirine

Study No.	**TMC278-NC192 (FK5563)**
Location in CTD	**4.2.2.4**
Type of Study	Possible induction and/or inhibition of drug metabolizing enzymes by RPV evaluated ex vivo in liver microsomes.
Method	Microsomal fractions of livers from RPV HCl treated animals were isolated. Swiss albino CD1 mice were treated with 0 (0.5% (w/v) HPMC, vehicle), 20, 80 and 320 mg.base eq./kg/day RPV for three months. Liver microsomal fractions were analyzed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Liver cytosolic fractions were analyzed for protein and GSH *S*-transferase activity towards CDNB as substrate. Results are presented for groups of 5 mouse liver pools, each pool being prepared from the livers of 2 mice.

Results	Control (vehicle only)		RPV (mg.base.eq/kg/day)					
			20		80		320	
Gender (M/F)	M	F	M	F	M	F	M	F
Microsomal protein[a]	17	16	18	16	19*	19**	25***	23***
CYP content[b]	1.2	0.83	1.2	0.99*	1.4*	1.0**	1.6***	1.2***
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	315	223	378	284	362	322**	273	187
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	86	150	103	168	92	191**	88	135
4-Nitrophenol hydroxylase (CYP2E)[d]	1.9	2.4	2.1	2.3	2.3**	2.6	1.8	2.1
Testosterone 6β-hydroxylase (CYP3A)[d]	2.7	2.1	3.0	3.2**	4.2***	3.6***	4.7***	3.6***
Lauric acid 11-hydroxylase[d]	1.1	1.2	1.1	1.4	1.4	2.6***	3.4***	4.3***
Lauric acid 12-hydroxylase (CYP4A)[d]	0.83	1.5	1.2*	1.9	4.4***	7.8***	21***	30***
UDPglucuronosyltransferase (substrate thyroxine)[c]	16	11	17	16*	23**	19***	33***	26***
Cytosolic protein[a]	97	106	93	102	97	108	115**	133***
GSH *S*-transferase (substrate CDNB)[e]	8.1	2.0	8.1	2.3	6.3*	2.2	3.6***	1.7
Additional Information	Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$							

[a] Units : mg protein/g liver
[b] Units : nmol/mg protein
[c] Units : pmol/min/mg protein
[d] Units : nmol/min/mg protein
[e] Units : µmol/min/mg protein

CDNB = 1-chloro2,4-dinitrobenzene; CYP = cytochrome 450; F = female; GSH = glutathione; HPMC = hydroxypropyl methylcellulose; M = male; UDP = uridine diphosphate; RPV = rilpivirine

2.6.5.12C Pharmacokinetics: Induction/Inhibition in Rats

Test Article: rilpivirine

Study No.	**TMC278-NC193 (FK5564)**							
Location in CTD	**4.2.2.4**							
Type of Study	Possible induction and/or inhibition of drug metabolizing enzymes by RPV evaluated ex vivo in liver microsomes							
Method	Microsomal fractions of livers from RPV base treated animals were isolated. Sprague Dawley rats were treated with 0 (100 mg/ml citric acid in PEG400, vehicle), 40, 120 and 400 mg/kg/day RPV for 6 months. Liver microsomal fraction were analyzed for protein and GSH *S*-transferase activity towards CDNB as substrate. Results are presented for groups of 5 rats.							
Results	Control (vehicle only)		RPV (mg/kg/day)					
			40		120		400	
Gender (M/F)	M	F	M	F	M	F	M	F
Microsomal protein[a]	61	45	65	45	66	47	72**	54***
CYP content[b]	0.84	0.56	0.83	0.56	0.81	0.63*	0.87	0.69***
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	28	24	24	26	21	32*	18*	29
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	50	11	43	11	52	14*	37	13*
4-Nitrophenol hydroxylase (CYP2E)[d]	0.64	0.73	0.62	0.69	0.60	0.77	0.46*	0.69
Testosterone 6β-hydroxylase (CYP3A)[d]	0.64	0.05	0.61	0.06	0.80	0.15***	0.77	0.30***
Lauric acid 11-hydroxylase[d]	0.42	0.34	0.47	0.30	0.57*	0.37	0.77***	0.42*
Lauric acid 12-hydroxylase (CYP4A)[d]	0.47	0.44	0.66	0.33	1.2**	0.41	2.2***	0.56*
UDPglucuronosyltransferase (substrate thyroxine)[c]	5.7	5.6	3.7**	7.1	4.4*	5.5	7.1*	7.5
Cytosolic protein[a]	130	12	132	112***	132	111***	134	117*
GSH *S*-transferase[e]	2.6	1.2	2.5	1.7*	2.4	1.6	2.3	1.9**
Additional Information	Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$							

[a] Units : mg protein/g liver
[b] Units : nmol/mg protein
[c] Units : pmol/min/mg protein
[d] Units : nmol/min/mg protein
[e] Units : µmol/min/mg protein

CDNB = 1-chloro2,4-dinitrobenzene; CYP = cytochrome 450; F = female; GSH = glutathione; HPMC = hydroxypropyl methylcellulose; M = male; UDP = uridine diphosphate; RPV = rilpivirine

2.6.5.12D Pharmacokinetics: Induction/Inhibition in Dogs

Test Article: rilpivirine

Study No.	**TMC278-NC140 (FK5518)**			
Location in CTD	**4.2.2.4**			
Type of Study	Possible induction and/or inhibition of drug metabolizing enzymes by RPV evaluated ex vivo in liver microsomes			
Method	Microsomal and cytosolic fractions of livers from RPV base treated animals were isolated. Male and female Beagle dogs were treated with 0 (citric acid (100 mg/ml) in PEG400, vehicle solution) 5, 10 and 40 mg/kg/day RPVfor six months. Liver microsomes were analysed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Cytosolic fractions were analyzed for protein content and GSH *S*-transferase activity. Groups of 2 male and 2 female control and RPV treated beagle dogs were combined for statistical analysis of data.			
Results	Control (vehicle only)	RPV (mg/kg/day)		
		5	10	40
Microsomal protein[a]	40.7	42.3	44.4	43.2
CYP content[b]	0.72	0.72	0.75	0.70
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	183	169	217	162
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	76	84	96	87
4-Nitrophenol hydroxylase (CYP2E)[d]	0.50	0.59	0.53	0.51
Testosterone 6β-hydroxylase (CYP3A)[d]	0.58	0.49	0.33**	0.43*
Lauric acid 11-hydroxylase[d]	0.21	0.22	0.22	0.22
Lauric acid 12-hydroxylase (CYP4A)[d]	0.83	0.83	0.85	0.94
UDPglucuronosyltransferase (substrate thyroxine)[c]	2.8	2.3	2.1	1.9
Cytosolic protein[a]	128	129	124	124
GSH *S*-transferase (substrate CDNB)[e]	1.24	1.02	1.04	1.00
Additional Information	Values significantly different from control are: * $p < 0.05$; ** $p < 0.01$			

[a] Units : mg protein/g liver
[b] Units : nmol/mg protein
[c] Units : pmol/min/mg protein
[d] Units : nmol/min/mg protein
[e] Units : µmol/min/mg protein

CDNB = 1-chloro2,4-dinitrobenzene; CYP = cytochrome 450; GSH = glutathione; UDP = uridine diphosphate; RPV = rilpivirine

2.6.5.12E Pharmacokinetics: Induction/Inhibition in human liver microsomes

Test Article: rilpivirine

Study No.	**1646_0030536 (FK10162)**
Location in CTD	**4.2.2.4**
Type of Study Method	Mechanism based inhibition (MBI) potential of RPV towards CYP2C9 activity was investigated in human liver microsomes. The MBI was evaluated as the % decrease in CYP2C9 activity in the presence of NADPH regenerating system (NRS) relative to samples without NRS,after 15 min incubation.

CYP involved	Substrate	CYP activity	**RPV (0.1-100µM during PI)**			**Tienilic acid (0.005-5µM during PI) Reference inhibitor**		
			% Total Inactivation at 100 µM (+NRS)	% Mechanism based effect at 100 µM (-NRS *versus* + NRS)	APR	% Total Inactivation at 100 µM (+NRS)	% Mechanism based effect at 5 µM (-NRS *versus* + NRS)	APR
CYP2C9	Tolbutamide	4-methyl hydroxylation	80.8	1.40	288	70.2	50.9	37.1

APR = apparent partition ratio; MBI = Mechanism based inhibition; NRS = NADPH regenerating system; PI = pre-incubation; RPV = rilpivirine

2.6.5.13 Pharmacokinetics: Excretion

Test Article: rilpivirine

The excretion study results in mice, rats, dogs and humans are described in the Tabulated Summaries 2.6.5.9A, 2.6.5.9B, 2.6.5.9D and 2.6.5.9E, respectively.

2.6.5.14 Pharmacokinetics: Excretion into Bile

Test Article: rilpivirine

The excretion study results in rats are described in the Tabulated Summary 2.6.5.9C.

2.6.5.15A Pharmacokinetics: Drug-Drug Interactions

Test Article: rilpivirine

Study No.	**1646_0025128 (FK10042)**
Location in CTD:	**4.2.2.6**
Type of Study:	Inhibition of transport of a prototypical substrate of OCT2 (^{14}C-metformin)
Method	CHO-cell lines stably transfected with OCT2 (SLC22A2) and parental cells. Untransfected CHO cells were used as controls (CHO-Parent). Transport and inhibition were tested in the presence of 1% BSA.
Uptake of metformin in CHO-parental cells	4.56 ± 1.24 pmol/mg/min
Update of metformin in CHO-OCT2 cells (without inhibitor	352 ± 25 pmol/mg/min

			IC_{50} (µM)			Max. inhibition (%)			At conc. (µM)
Substrate	**Inhibitor**	**Protein**	**avg**	**±**	**sd**	**avg**	**±**	**sd**	
Metformin	RPV	1% BSA	5.46	±	0.50	90.6	±	0.6	50

BSA = bovine serum albumin; CHO = Chinese hamster ovary; OCT = organic cation transporter; RPV: rilpivirine

2.6.5.15B Pharmacokinetics: Drug-Drug Interactions

Test Article: rilpivirine

Study No.	**1646_0035314 (FK10420)**
Location in CTD:	**4.2.2.6**
Type of Study:	Inhibition of MATE-1 (SLC47A1) and MATE-2K (SLC47A2) by RPV
Method	CHO cell lines stably transfected with MATE-1 (SLC47A1) and MATE-2K (SLC47A2), and parental cell lines. The inhibition by RPV of MATE-1 and MATE-2K transport was investigated in transfected CHO cells in the presence of 1% BSA.

	CHO-parent			**CHO-MATE-1**			
	average (pmol/mg protein.min)		**st.dev** (pmol/mg protein.min)	**average** (pmol/mg protein.min)		**st.dev** (pmol/mg protein.min)	**Maximal activity (%)**
TEA (MATE-1 and MATE-2K substrate)	1.85	±	0.12	43.7	±	2.72	100
TEA + 0.05 µM RPV	2.58	±	1.26	50.1	±	4.98	114
TEA + 0.03 µM RPV	3.51	±	1.89	38.9	±	3.19	84.7
TEA + 1.5 µM RPV	2.20	±	0.43	40.4	±	5.38	89.5
TEA + 10 µM RPV	1.91	±	0.29	19.7	±	1.26	42.4
TEA + 50 µM RPV	1.91	±	0.71	6.60	±	2.13	11.2
TEA + 1 µM Quininidine	2.09	±	0.57	34.8	±	3.73	78.3
TEA + 50 µM Quininidine	2.69	±	0.58	10.1	±	2.45	17.6

	CHO-parent			**CHO-MATE-2K**			
	average (pmol/mg protein.min)		**st.dev** (pmol/mg protein.min)	**average** (pmol/mg protein.min)		**st.dev** (pmol/mg protein.min)	**Maximal activity (%)**
TEA	1.31	±	0.53	7.47	±	0.34	100
TEA + 0.05 µM RPV	1.83	±	0.18	3.98	±	0.91	35.0
TEA + 0.3 µM RPV	1.31	±	0.53	3.61	±	0.51	37.4
TEA + 1.5 µM RPV	1.38	±	0.35	3.35	±	0.73	32.1
TEA + 10 µM RPV	1.13	±	0.44	2.75	±	0.43	26.4
TEA + 50 µM RPV	1.72	±	0.17	1.91	±	0.05	3.1
TEA + 0.03 µM Pyrimethamine	1.22	±	0.1	3.50	±	0.51	37.0
TEA + 3 µM Pyrimethamine	1.34	±	0.39	2.44	±	0.40	17.8

Additional Information: quinidine and pyrimethamine are positive control inhibitors

BSA = bovine serum albumin; CHO = Chinese hamster ovary; MATE= Multi-antimicrobial extrusion protein; TEA = Tetra Ethyl Ammonium; RPV = rilpivirine

2.6.5.15C Pharmacokinetics: Drug-Drug Interactions

Test Article: rilpivirine

Study No.	**TMC278–NC194 (FK5568)**				
Location in CTD	**4.2.2.6**				
Type of Study	Inhibition of metabolism by RPV of interacting drugs was investigated				
Method	The interaction of RPV with the metabolism of interacting drugs was investigated in a pooled batch of human liver microsomes. The inhibitory potential of RPV on the overall metabolism and/or the formation of their major metabolites is shown. The IC_{50}-values represent the concentration in µM or µg-base-eq/mL of RPV inhibiting the metabolism by 50%.				
	IC_{50} (95% confidence interval)		**Positive control**		
Interacting drugs	**µM**	**µg-base-eq/mL**	**µM**	**Inhibitor**	**% Inhibition**
S-mephenytoin[a]	1.3[a] (0.74 - 1.8)	0.46[a] (0.27 - 0.65)	1	**3-benzyl-phenobarbital**	81
Sildenafil	1.4 (-0.13 - 3.0)	0.53 (-0.047 - 1.1)	1	**ketoconazole**	125
Clarithromycin	2.0 (0.042 - 4.0)	0.74 (0.015 - 1.46)	1	**ketoconazole**	93
Norethindron	3.9 (2.6 - 5.3)	1.44 (0.93 - 1.95)	1	**ketoconazole**	84
Sertraline	5.2 (-3.1 - 14)	1.9 (-1.1 - 4.9)	10[3]	**1-aminobenzotriazole**	167[b]
Paroxetine	6.6 (-1.2 - 14)	2.4 (-0.42 - 5.3)	3	**quinidine**	91
17α-Ethinyloestradiol[c]	6.5[c] (4.2 - 8.7)	2.4[c] (1.5 - 3.2)	1	**ketoconazole**	56/59[d]
Omeprazole	12.0 (7.0 - 17)	4.4 (2.6 - 6.2)	1/1	**3-benzyl-pheno-barbital/ketoconazole**	92
Abacavir[e,f]	>30[f]	>11[f]	600	**4-methylpyrazole**	95
Chlorzoxazone[g]	>30[g]	>11[g]	100	**diethyldithiocarbamate**	-184[h]
Additional Information	-				

[a] As determined by the formation of the 4-hydroxy metabolite only.
[b] This inhibition is not significantly different from the boiled fraction.
[c] As determined by the formation of a hydroxy metabolite.
[d] 56 / 59 % inhibition of metabolism of unchanged drug and inhibition of formation of a hydroxy metabolite, respectively.
[e] Tested in cytosol fractions, not in microsomes.
[f] As determined by disappearance from the unchanged abacavir, as well as the formation of its carboxylic acid metabolite.
[g] As determined by disappearance from the unchanged chlorzoxazone, as well as the formation of its 6-hydroxy metabolite.
[h] No inhibition was observed.

RPV = rilpivirine

2.6.5.15D Pharmacokinetics: Drug-Drug Interactions

Test Article: rilpivirine

Study No.	**1955_0018187 (FK7565)**	
Location in CTD	**4.2.2.6**	
Species	Rat Crl:CD(SD) IGS (M)	
Feeding Condition	Fed	
Sample	Plasma	
Analyte	RPV (TMC278)	
Assay	HPLC-MS/MS	
LLOQ	100 ng/mL	
Vehicle/Formulation	RPV LA: 300 mg/mL RPV nanosuspension (G001 batch); GSK1265744LAP injectable suspension	
Dose	60 mg/kg RPV LA alone or in combination with 10 mg/kg GSK1265744LAP	
Duration of Dosing	Single-Dose	
Route	IM (RPV LA: 0.2 mL/kg; GSK 1265744LAP: 0.05 mL/kg)	
Study Design	RPV was given as single agent or combined with GSK1265744A, and a third group (GSK1265744LAP only) was included as well	
	RPV (60 mg/kg)	RPV (60 mg/kg) + GSK1265744LAP (10 mg/kg)
Pharmacokinetic Parameters		
C_{max} (ng/mL)	112	108
T_{max} (h)	4.67	2
$AUC_{0\text{-}1444h \text{ or } 0\text{-}2 \text{ months}}$ (ng·h/mL)	23,225	24,105
$AUC_{0\text{-}\infty}$ (ng.h/mL)	25,640	26,870
Additional Information: GSK1265744LAP is a long acting injectable suspension, that was combined in this study with RPV LA;		

HPLC-MS/MS = high-performance liquid chromatography with mass spectrometry; IM = Intramuscular; LLOQ = lower limit of quantification; M = male; RPV = rilpivirine

2.6.5.16A Pharmacokinetics: Other – Pharmacokinetics and Organ Distribution in Mice

Test Article: rilpivirine LA

Study No.	**TMC278-NC196 (TOX7354)**									
Location in CTD	**4.2.3.7.7**									
Species	Albino Swiss mouse									
Feeding Condition	Fed									
Vehicle/Formulation	V1 = nanocrystal suspension of RPV LA (25 mg/mL) with 3.8 mg/mL P338 V2 = nanocrystal suspension of RPV LA (25 mg/mL) in Vit E-TPGS									
Route	SC (varying from 0.1 to 0.8 mL/kg)									
Compound	RPV LA									
Dose (mg/kg)	0, 2.5, 5, 10, 20 (in V1) 0, 20 (in V2)									
Dosing period	Single-dose, followed by an 18-day observation period									
Gender (M/F)/Number of Animals	Male/Female/(6/sex/vehicle + 12/sex/dose group)									
Analyte	RPV									
Assay	LC-MS/MS									
Plasma										
	2.5 mg/kg in V1		**5 mg/kg in V1**		**10 mg/kg in V1**		**20 mg/kg in V1**		**20 mg/kg in V2**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
C_{max} (ng/mL)	708	1020	635	2040	1430	2770	1610	2160	962	2850
t_{max} (h)	2.2	1.0	4.0	2.0	4.0	4.0	2.0	4.0	4.0	2.0
$AUC_{0-\infty}$ (ng.h/mL)	9330	10,700	16,800	22,600	34,000	41,800	60,300	74,200	44,900	65,400
Sampling Times	**Day 18**									
Tissues/Organs (µg/g)	**2.5 mg/kg (V1)**		**5 mg/kg (V1)**		**10 mg/kg (V1)**		**20 mg/kg (V1)**		**20 mg/kg (V2)**	
MALES										
Spleen	<0.005		<0.005		<0.005		<0.00795		<0.00825	
Thymus	<0.005		<0.005		<0.005		<0.0168		<0.0129	
Skin	0.0332		0.163 (23.9[a])		0.114		66.9 (2,096)		56.1 (1,453)	
FEMALES										
Spleen	<0.005		<0.005		<0.005		<0.0113 (0.16[a])		<0.00675 (0.15[a])	
Thymus	<0.005		<0.005		<0.005		< 0.00985		<0.00955 (0.22[a])	
Skin	0.675		0.526		0.569 (48.7[a])		47.9 (1,769)		26.4 (1,078)	

[a] n = 1

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F = female; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; M = male; P338 = poloxamer 338; RPV = rilpivirine (TMC278 base); SC = subcutaneous; t_{max} = time to reach the maximum plasma concentration; V = vehicle; Vit E-TPGS = Vitamin E D-α-Tocopheryl Polyethyleneglycol 1000 Succinate

( ) = tissue/plasma ratio

2.6.5.16B Pharmacokinetics: Other – Pharmacokinetics and Organ Distribution in Rat

Test Article: rilpivirine LA

Study No.			**TMC278-NC244 (TOX7896)**		
Location in CTD			**4.2.3.7.7**		
Species			Sprague-Dawley rat		
Feeding Condition			Fed		
Vehicle/Formulation			Formulation B, D, E and F: nanosuspension containing 25 or 100 mg/mL RPV LA in P338 Formulation A: RPV at 1.25 mg/mL as a 75% PEG400/25% sterile water solution		
Route			Group B and D: SC (0.2 mL/kg) Group E and F: IM (0.2 mL/kg) Group A: IV (1 mL/kg) → no distribution data		
Compound			RPV LA		
Dose (mg/kg)			SC and IM: 5 and 20 IV: 1.25 mg/kg		
Dosing period			Single-dose, followed by an 8-week observation period		
Gender (M/F)/Number of Animals			Male; 6 per group		
Analyte			RPV		
Assay			LC-MS/MS		
Plasma			**Day 0**		
	Group A: 1.25 mg/kg IV	**Group B: 5 mg/kg SC**	**Group D: 20 mg/kg SC**	**Group E: 5 mg/kg IM**	**Group F: 20 mg/kg IM**
C_{max} (ng/mL)	1120[c]	42.0	72.9	70.6	158
t_{max} (h)	-	3	7	7	7
$AUC_{0\text{-}56days}$ (ng.h/mL)	1230 [c]	3540	15,500	3840	15,300
F_{abs}	-	72	79	78	78
Sampling Times			**Day 56**		
Tissues/Organs (µg/g or µg/mL)		**Group B: 5 mg/kg SC**	**Group D: 20 mg/kg SC**	**Group E: 5 mg/kg IM**	**Group F: 20 mg/kg IM**
Thymus		<0.005	<0.005[b]	<0.005	<0.005
Spleen		<0.005	<0.005	<0.005	<0.005
Muscle		<0.00819[b]	102	1.98	8.34[a]
Plasma		<0.002	<0.002[b]	<0.004[b]	<0.002[b]

Remark: n = 6 for thymus, spleen and plasma and n = 3 for skin at injection site

[a] n = 1

[b] Median value

[c] C_0 (ng/mL) and $AUC_{0\text{-}inf}$ (ng.h/mL) for Group A

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F_{abs} = absolute bioavailability; IM = intramuscular; IV = intravenous; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; P338 = poloxamer 338; RPV = rilpivirine (TMC278 base); SC = subcutaneous; t_{max} = time to reach the maximum plasma concentration

2.6.5.16C Pharmacokinetics: Other- Pharmacokinetics in Rabbit

Test Article: rilpivirine LA

Study No.	**TMC278-NC356 (FK6993)**		
Location in CTD	**4.2.2.7**		
Species	New Zealand White Rabbit		
Feeding Condition	Fed		
Vehicle/Formulation	Formulation A – surfactant: Poloxamer 338 (Batch ID: [redacted] (reference)) Formulation B – surfactant: Tween/Lipoid (Batch ID: [redacted])		
Route	IM		
Compound	RPV LA		
Dosing period	Single-dose with a 1-month follow-up period		
Gender (M/F)/Number of Animals	Female; 3 per group		
Analyte	RPV		
Assay	LC-MS/MS, HPLC-MS/MS		
Pharmacokinetic Parameters			
Formulation	F004	F006	F006
Dose (mg/kg)	50	150	150
Dose volume (mL/kg)	0.5	0.5	3 x 0.16 = 0.5
C_{max} (ng/mL)	7547	5763	4877
t_{max} (h)	18.67	348	348
$AUC_{0\text{-}852h(day36)}$ (ng.h/mL)	1,423,661	2,786,141	2,469,268
$AUC_{0\text{-}\infty}$ (ng.h/mL)	1,663,280	3,583,889[a]	3,452,088[b]

[a] n = 1
[b] n = 2

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; (HP)LC-MS/MS = (high performance) liquid chromatography coupled to tandem mass spectrometry; IM = intramuscular; LA = long acting; RPV LA = rilpivirine long-acting (TMC278 base); t_{max} = time to reach the maximum plasma concentration

2.6.5.16D Pharmacokinetics: Other – Pharmacokinetics and Organ Distribution in Dog

Test Article: rilpivirine

Study No.	**Innovation-NC114 (FK5458)**		
Location in CTD	**4.2.2.7**		
Species	Beagle dog		
Feeding Condition	Fasted overnight		
Vehicle/Formulation	Group A (IM) and C (SC): 10 mg/mL P338 nanosuspension (for RPV base at 25 mg/mL) Group B (IM): 10 mg/mL P338 nanosuspension (for HCl salt of TMC278 at 25 mg/mL)		
Route	IM or SC (0.1 mL/kg/site, ie 0.2 mL/kg)		
Compound	TMC278 base or TMC278.HCl		
Dose (mg/kg)	5 mg/kg		
Dosing period	Single-dose with a 6-month follow-up period		
Gender (M/F)/Number of Animals	Male/2 per group		
Analyte	RPV		
Assay	LC-MS/MS		
Plasma	**Group A**	**Group B**	**Group C**
C_{max} (ng/mL)	173	95.3	38.4
t_{max} (h)	24	48	144
$AUC_{0\text{-}13\ days}$ (ng.h/mL)	27,000	18,000	8400
$AUC_{0\text{-}29\ days}$ (ng.h/mL)	33,900	25,400	16,300
$AUC_{0\text{-}92\ days\ (3months)}$ (ng.h/mL)	39,400	33,600	24,400
$AUC_{0\text{-}120\ days}$ (ng.h/mL)	41,400 (n=1)	29,100 (n=1)	28,100 (n=1)
$AUC_{0\text{-}184\ days}$ (ng.h/mL)	-	31,800 (n=1)	30,700 (n=1)
Sampling Times	Day 29 (only iliac and popliteal lymph nodes), 94 and 184		

Tissues/Organs (µg/g or µg/mL)	**Day 94**			**Day 184**		
	Group A	**Group B**	**Group C**	**Group A**	**Group B**	**Group C**
Plasma	< 0.001	0.0024	0.0033	< 0.001	0.0019	0.0013
Liver	0.021	0.052 (21)	0.046 (14)	0.012	0.034 (18)	0.031 (24)
Muscle (injection site)	142	176 (72,727)	0.47 (144)	11.5	83.1 (44,919)	0.048 (38)
Muscle (non-injection site)	0.085	0.082 (34)	0.019 (5.7)	< 0.005	0.021 (12)	< 0.005 (NC)
Adrenal gland	0.0098	0.023 (9.3)	0.022 (6.9)	< 0.005	0.012 (6.3)	0.012 (9.5)
Abdominal fat	0.027	0.059 (24)	0.049 (15)	0.011	0.023 (13)	0.022 (17)
Spleen	< 0.005	0.016 (6.7)	0.010 (3.0)	< 0.005	0.0078 (4.2)	0.0071 (5.5)
Iliac lymph node	0.015	0.14 (59)	0.017 (5.2)	0.011	0.012 (6.3)	0.0099 (7.7)
Popliteal lymph node	0.014	0.056 (23)	0.027 (8.2)	0.031	0.012 (6.4)	0.0055 (4.3)
Auxiliary lymph node	0.026	0.023 (10)	0.022 (6.8)	0.012	< 0.005 (NC)	0.0086 (6.7)
Mandibular lymph node	0.010	0.024 (10)	0.013 (4.0)	0.0069	0.011 (5.9)	0.0078 (6.1)
Skin (injection site)	0.017	1.14 (471)	223 (68,615)	0.0095	0.022 (12)	143 (111,719)
Skin (non-injection site)	0.012	0.034 (14)	0.024 (7.4)	0.0067	0.022 (12)	0.013 (10)
Thymus	0.015	0.083 (34)	0.044 (13)	0.0072	0.024 (13)	0.020 (16)

(Continued)

2.6.5.16D Pharmacokinetics: Other – Pharmacokinetics and Organ Distribution in Dog (Continued)

Test Article: rilpivirine

Sampling Times	Day 29 (only iliac and popliteal lymph nodes), 94 and 184					
Tissues/Organs (µg/g)	**Iliac lymph node**			**Popliteal lymph node**		
Treatment/Dog	**Day 29**	**Day 94**	**Day 184**	**Day 29**	**Day 94**	**Day 184**
A/1	8.57	0.015		4.57	0.014	
A/2	29.5		0.011	8.42		0.031
B/1	0.46	0.14		0.063	0.056	
B/2	0.19		0.012	0.045		0.012
C/1	0.016	0.017		0.054	0.027	
C/2	0.11		0.010	0.082		0.0055

Single Cell Suspensions on Day 94 (ng/g)	**Iliac lymph node**	**Thymus**
A/1	1.14	<1.0
B/1	<1.0	<1.0
C/1	<1.0	<1.0

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; IM = intramuscular; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; NC = not calculated; P338 = poloxamer 338; RPV LA = rilpivirine long-acting (TMC278 base); SC = subcutaneous; t_{max} = time to reach the maximum plasma concentration

( ) = tissue to plasma ratio; for Group A = not calculated since plasma concentrations were below the lower limit of quantification (<1.00 ng/mL)

2.6.5.16E Pharmacokinetics: Other- Pharmacokinetics in Dog

Test Article: rilpivirine LA

Study No.	**TMC278-NC238 (FK5998)**		
Location in CTD	**4.2.2.7**		
Species	Beagle Dog		
Feeding Condition	Fed		
Vehicle/Formulation	Formulation A: 75% PEG400/25 % sterile water solution of RPV LA at 1.25 mg/mL Formulation B: Nanosuspension of RPV LA in Pluronics F108 (3.7 mg/mL) at 25 mg/mL (particle size: 200 nm) Formulation C: Nanosuspension of RPV LA in Pluronics F108 (3.7 mg/mL) at 25 mg/mL (particle size: 200 nm)		
Route	Formulation A: IV (1 mL/kg) Formulation B: IM (0.1 mL/kg/site, ie 0.2 mL/kg) Formulation C: SC (0.1 mL/kg/site, ie 0.2 mL/kg)		
Compound	RPV LA		
Dose	Formulation A: 1.25 mg/kg Formulation B and C: 5 mg/kg		
Dosing Period	Single dose with a 6-month follow-up period		
Sample	Plasma		
Analyte	RPV		
Assay	LC-MS/MS		
Pharmacokinetic Parameters	**Formulation A: 1.25 mg/kg IV**	**Formulation B: 5 mg/kg IM**	**Formulation C: 5 mg/kg SC**
Gender (M/F)/Number of Animals	M: 6	M: 3	M: 3
C_o (ng/mL)	570	-	-
C_{max} (ng/mL)	-	619	31.4
$t_{1/2, 24\text{-}96h}$ (h)	15	-	-
t_{max} (h)	-	0.5	288
$AUC_{0\text{-}92d}$ (ng.h/mL)	6210	23,200	17,800
$AUC_{0\text{-}176d\ (6\ months)}$ (ng.h/mL)		23,200	19,700
F_{abs}	-	102	88

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F_{abs} = absolute bioavailability; IM = intramuscular; M = male; IV = intravenous; PEG = polyethylene glycol; SC = subcutaneous; t_{max} = time to reach the maximum plasma concentration
RPV LA = rilpivirine long-acting (TMC278 base)

2.6.5.16F Pharmacokinetics: Pharmacokinetics and Organ Distribution in Dog

Test Article: rilpivirine LA

Study No.	**TMC278-NC234 (TOX7781)**							
Location in CTD	**4.2.3.7.7**							
Species	Beagle dog							
Feeding Condition	Fed							
Vehicle/Formulation	Group V: P338 in pyrogen free water Other groups: nanosuspension containing 100 mg/mL RPV LA in P338 (25 mg/mL)							
Route	Group V: SC and IM (1 mL/injection) Groups A and D: SC (1 mL/injection) Groups B and E: IM (1 mL/injection)							
Frequency of dosing	Groups A and B: 1 injection Groups V, D and E: 2 injections							
Compound	RPV LA							
Dose (mg/kg)	200 or 400 mg/total dose/dog							
Dosing period	Single dose							
Gender (M/F)/Number of Animals	Male + Female/ 6/sex/group							
Analyte	RPV							
Assay	LC-MS/MS							
Plasma	**After 1 month of single dosing**							
	Subcutaneous				**Intramuscular**			
Dose (mg)	**200**		**400**		**200**		**400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
C_{max} (ng/mL)	119	185	210	210	2290	2170	2300	6190
t_{max} (h)	280	220	440	450	0.33	4.6	17	8.3
$AUC_{0\text{-}696h}$ (ng.h/mL)	56,800	70,600	105,000	109,000	117,000	109,000	188,000	204,000
Plasma	**After 3 months of single dosing**							
	Subcutaneous				**Intramuscular**			
Dose (mg)	**200**		**400**		**200**		**400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
C_{max} (ng/mL)	107	191	222	225	2130	1440	2170	5260
t_{max} (h)	360	250	300	480	0.33	0.55	17	16
$AUC_{0\text{-}2040h}$ (ng.h/mL)	97,800	134,000	219,000	234,000	148,000	121,000	281,000	265,000

(Continued)

2.6.5.16F Pharmacokinetics: Pharmacokinetics and Organ Distribution in Dog (Continued)

Test Article: rilpivirine LA

Study No.	**TMC278-NC234 (TOX7781)**							
Location in CTD	**4.2.3.7.7**							
Sampling Time	**Day 29/30**							
Tissues/Organs (ng/mL or ng/g)	**Subcutaneous**				**Intramuscular**			
Dose (mg)	**200**		**400**		**200**		**400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Brain	169 (1.8)	131 (1.8)	694 (1.9)	430 (1.9)	115 (1.5)	112 (1.8)	261 (1.6)	263 (2.2)
Spleen	189 (2.0)	145 (2.0)	569 (2.7)	536 (2.5)	133 (1.8)	142 (2.3)	347 (2.2)	311 (2.6)
Thymus	576 (6.0)	285 (3.9)	606 (2.9)	1204 (5.0)	234 (3.2)	353 (5.7)	634 (4.0)	676 (6.0)
Testis	59.0 (0.63)	-	143 (0.69)	-	44.0 (0.57)	-	82.0 (0.54)	-
Plasma	95.2	71.8	209	227	74.5	61.2	158	118
Sampling Time	**Day 92/93**							
Tissues/Organs (ng/mL or ng/g)	**Subcutaneous**				**Intramuscular**			
Dose (mg)	**200**		**400**		**200**		**400**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Brain	29.2 (1.9)	42.1 (1.6)	84.0 (1.7)	55.8 (1.6)	18.1 (2.0)	19.3 (1.9)	46.6 (2.3)	33.4 (1.6)
Spleen	36.2 (2.5)	49.5 (1.9)	113 (2.3)	67.5 (1.9)	23.8 (2.7)	22.1 (2.2)	63.5 (2.9)	46.1 (2.3)
Thymus	84.2 (5.9)	105 (4.0)	173 (3.5)	101 (2.9)	34.4 (3.8)	27.4 (2.8)	76.0 (3.9)	109 (4.9)
Testis	12.1 (0.80)	-	34.0 (0.69)	-	6.42[a] (0.64)	-	18.3 (0.88)	-
Plasma	15.2	26.8	48.4	36.0	9.18	10.1	22.1	20.3

[a] median

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F = female; IM = intramuscular; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; M = male; P338 = poloxamer 338; RPV LA = rilpivirine long-acting (TMC278 base); SC = single dose; t_{max} = time to reach the maximum plasma concentration; V = vehicle

( ) tissue/plasma ratio's

2.6.5.16G Pharmacokinetics: Pharmacokinetics and Organ Distribution in Dog

Test Article: rilpivirine LA

Study No.	**TMC278-NC203 (FK5821)**		
Location in CTD	**4.2.2.7**		
Species	Beagle dog		
Feeding Condition	Fed		
Vehicle/Formulation	Treatment A: nanosuspension containing 125 mg/mL RPV LA in Vit E-TPGS at 25 mg/mL with particle size of 400 nm Treatment B: nanosuspension containing 125 mg/mL RPV LA in Vit E-TPGS at 100 mg/mL with particle size of 400 nm Treatment C: nanosuspension containing 125 mg/mL RPV LA in Vit E-TPGS at 100 mg/mL with particle size of 800 nm		
Route	SC (0.1 mL/kg/site, ie 0.2 mL/kg)		
Compound	RPV LA		
Dose (mg/kg)	Treatment A: 5 mg/kg Treatment B and C: 20 mg/kg		
Dosing period	Single dose with 3-month follow-up period		
Gender (M/F)/Number of Animals	Male/3 per group		
Analyte	RPV		
Assay	LC-MS/MS		
Plasma	**Group A: 5 mg/kg**	**Group B: 20 mg/kg**	**Group C: 20 mg/kg**
C_{max} (ng/mL)	19.6	39.4	38.8
t_{max} (h)	256	408	528
$AUC_{0\text{-}92days}$ (ng.h/mL)	14,400	38,200	29,600
Sampling Time		**Day 93**	
Tissues/Organs (ng/mL or ng/g)	**Group A: 5 mg/kg**	**Group B: 20 mg/kg**	**Group C: 20 mg/kg**
Adrenal gland	9.06[a] (3.9)	60.6 (6.0)	25.7 (4.7)
Abdominal fat	21.4 (9.1)	96.2 (10)	63.8 (12)
Liver	30.4 (13)	117 (12)	83.5 (15)
Axillary lymph node	159 (68)	2080 (206)	54.3 (10)
Mandibular lymph node	6.16[a] (2.6)	73.6 (7.3)	16.8 (3.1)
Muscle	6.08[a] (2.6)	16.0 (1.6)	10.9 (2.0)
Skin (injection site)	2,553,333 (>1,000,000)	6,436,667 (>500,000)	5,796,667 (>1,000,000)
Skin (non-injection site)	26.3[b] (11)	63.8 (6.3)	61.9 (11)
Spleen	6.52[b] (2.8)	22.1 (2.2)	18.0 (3.3)
Thymus	8.85 (3.8)	77.2 (7.6)	36.2 (6.7)
Plasma	2.34	10.1	5.44

[a] Median value
[b] n = 2

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; LA = long acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectroscopy; RPV LA = rilpivirine long-acting (TMC278 base); SC = single dose; t_{max} = time to reach the maximum plasma concentration; Vit E-TPGS = Vitamin E D-α-Tocopheryl Polyethyleneglycol 1000 Succinate
( ) tissue/plasma ratio's

2.6.5.16H Pharmacokinetics: Other- Pharmacokinetics in Minipig

Test Article: rilpivirine LA

Study No.	**TMC278-NC295 (FK6407)**		
Location in CTD	**4.2.2.7**		
Species	Göttingen Minipig		
Feeding Condition	Fed		
Vehicle/Formulation	Formulation A: 300 mg/mL RPV LA injectable suspension in P338 (25 mg/mL; F006) (1 x 1.5 mL) Formulation B: 300 mg/mL RPV LA injectable suspension in P338 (25 mg/mL; F006) (1 x 1.5 mL) Formulation C: 100 mg/mL RPV LA injectable suspension in P338 (25 mg/mL; F004) (4 x 1 mL)		
Route	IM		
Compound	RPV LA		
Dose	Formulation A and B: 450 mg Formulation C: 400 mg		
Dosing Period	Single dose		
Follow-up period	Formulation A: 1-month Formulations B and C: 3 months		
Sample	Plasma		
Analyte	RPV		
Assay	LC-MS/MS		
Pharmacokinetic Parameters	**Formulation A: 450 mg**	**Formulation B: 450 mg**	**Formulation C: 400 mg**
Gender (M/F)/Number of Animals	M: 2	M: 2	M: 2
C_{max} (ng/mL)	16.7	15.3	49.4
t_{max} (h)	300	264	8.0
$AUC_{0\text{-}696h}$ (ng.h/mL)	7485	5279	13,886
$AUC_{0\text{-}2040h\ (\sim 3\ months)}$ (ng.h/mL)	-	8716	19,632
$AUC_{0\text{-}\infty}$ (ng.h/mL)	-[a]	9926	28,656[b]

[a] Not calculated because of increasing plasma concentrations

[b] n = 1

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; IM = intramuscular; LA: long-acting; LC-MS/MS: liquid chromatography coupled to tandem mass spectrometry; P338 = poloxamer 338 = JNJ-46360418-AAA; RPV LA = rilpivirine long-acting (TMC278 base); t_{max} = time to reach the maximum plasma concentration

2.6.5.16l Pharmacokinetics: Other- Pharmacokinetics in Minipig

Test Article: rilpivirine LA

Study No.	**TMC278-NC344 (FK7034)**					
Location in CTD	**4.2.2.7**					
Species	Göttingen Minipig					
Feeding Condition	Fed					
Vehicle/Formulation	P338 or PS80					
Route	IM (2 mL)					
Compound	RPV LA					
Dosing Period	Single dose					
Sample	Plasma					
Analyte	RPV					
Assay	LC-MS/MS					
Pharmacokinetic Parameters						
Dose (mg)	**400 (P338)**	**200 (P338)**	**600 (PS80)**	**500 (PS80)**	**400 (PS80)**	**200 (PS80)**
Gender (M/F)/Number of Animals	**M/3**	**M/3**	**M/3**	**M/3**	**M/3**	**M/3**
C_{max} (ng/mL)	97.97	49.57	33.80	29.87	30.53	13.40
t_{max} (h)[a]	6.0	4.0	384	144	312	144
$AUC_{0\text{-}648h}$ (ng.h/mL)	14,461	5717	11,335	8148	9777	4309

[a] Median value

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; IM = intramuscular; LA = long-acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; M = male; P338 = poloxamer 338 = JNJ-46360418-AAA; PS80 = polysorbate 80; RPV LA = rilpivirine long-acting (TMC278 base); t_{max} = time to reach the maximum plasma concentration

2.6.5.16J Pharmacokinetics: Other- Pharmacokinetics in Minipig

Test Article: P338

Study No.	**TMC278-NC296 (TOX8580)**	
Location in CTD	**4.2.2.7**	
Species	Göttingen Minipig	
Feeding Condition	Fed	
Vehicle/Formulation	PS80	
Route	IM	
Compound	RPV LA	
Dosing Period	13/39 weeks	
Sample	plasma	
Assay	LC-MS/MS	
Pharmacokinetic Parameters		
Dose (mg/kg)	75	
Gender (M/F)/Number of Animals	M: 6	F: 6
Day 1 (1st administration)		
C_{max} (ng/mL)	34.4	74.9
$AUC_{day1-30}$ (ng.h/mL)	10058	26,484
$AUC_{0-\infty}$ (ng.h/mL)	16,943[a]	33,720[b]
D60 (3th administration)		
C_{max} (ng/mL)	52.7[a]	105[a]
$AUC_{Day60-89}$ (ng.h/mL)	20,620[a]	25,951[a]
Day 240 (9th - last administration)		
C_{max} (ng/mL)	108[a]	105[a]
$AUC_{day240-269}$ (ng.h/mL)	43,410[a]	35,474[a]

[a] n = 3
[b] n = 4

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; IM = Intramuscular; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; NZW = New Zealand White; P338 = poloxamer 338

2.6.5.16K Pharmacokinetics: Other- Pharmacokinetics in Rabbit

Test Article: P338

Study No.	**FK13159**
Location in CTD	**4.2.2.7**
Species	NZW Rabbit
Feeding Condition	Fed
Vehicle/Formulation	P338 + demineralized water
Route	Oral
Compound	P338
Dosing Period	Single dose
Sample	plasma
Assay	(HP)LC-MS/MS
Pharmacokinetic Parameters	
Dose (mg/kg)	1600
Gender (M/F)/Number of Animals	F:4
C_{max} (ng/mL)	417
t_{max} (h)[a]	48.00 (48.00 – 72.00)
t_{last} (h)[a]	336 (264 – 336)
AUC_{0-24h} (ng.h/mL)	4100
AUC_{last} (ng.h/mL)	68,900

[a] Median value (min-max)

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F= female; (HP)LC-MS/MS = (high performance) liquid chromatography coupled to tandem mass spectrometry; NZW = New Zealand White; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration

2.6.5.16L Pharmacokinetics: Other- Pharmacokinetics in Minipig

Test Article: P338

Study No.	**FK13161**	
Location in CTD	**4.2.2.7**	
Species	Göttingen Minipig	
Feeding Condition	Fed	
Vehicle/Formulation	RPV LA	
Route	IM	
Compound	P338 and RPV	
Dosing Period	Single dose	
Sample	Plasma	
Assay	(HP)LC-MS/MS	
Pharmacokinetic Parameters	P338	RPV
Dose (mg)	100	600
Gender (M/F)/Number of Animals	F: 4	F: 4
C_{max} (ng/mL)	52,300	32.2
t_{max} (h)[a]	24 (24 – 72)	16 (4 – 336)
AUC_{0-24h} (ng.h/mL)	841,000	406
AUC_{0-672h} (ng.h/mL)	13,600,000	6070

[a] Median value (min-max)

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F = female; (HP)LC-MS/MS = (high performance) liquid chromatography coupled to tandem mass spectrometry; LA = long-acting; P338 = Poloxamer 338; RPV = rilpivirine; t_{max} = time to reach the maximum plasma concentration

2.6.5.16M Pharmacokinetics: Other- Pharmacokinetics in Human plasma

Test Article: P338

Study No.	**FK13131**
Location in CTD	**4.2.2.7**
Species	Human plasma
Feeding Condition	Fed
Vehicle/Formulation	RPV LA + demineralized water
Route	IM
Compound	P338
Dosing Period	Single dose
Sample	Plasma
Assay	(HP)LC-MS/MS
Pharmacokinetic Parameters	
Dose (mg)	100
Gender (M/F)/Number of Animals	6 subjects
C_{max} (ng/mL)	3907
t_{max} (h)[a]	48 (48-72)
t_{last} (h)[a]	3024 (672 – 4032)
AUC_{0-24h} (ng.h/mL)	34,202
AUC_{0-336h} (ng.h/mL)	580,175
AUC_{0-672h} (ng.h/mL)	717,091
$t_{1/2}$	8499

[a] median value (min-max)

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; (HP)LC-MS/MS = (high performance) liquid chromatography coupled to tandem mass spectrometry; IM = intramuscular; LA = long-acting; RPV = rilpivirine; t_{max} = time to reach the maximum plasma concentration; $t_{1/2}$: half-life

2.6.5.16N Pharmacokinetics: Other- Pharmacokinetics in Rat

Test Article: P338

Study No.	**FK13409**			
Location in CTD	**4.2.2.7**			
Species	Sprague-Dawley Rat			
Feeding Condition	Fed			
Vehicle/Formulation	P338			
Route	IM			
Compound	P338			
Dosing Period	Single dose			
Sample	Plasma, liver and kidney tissue, urine, feces			
Assay	(HP)LC-MS/MS			
Pharmacokinetic Parameters	Plasma	Kidney left	Kidney right	Liver
Dose (mg/kg)	10	10	10	10
Gender (M/F)/Number of Animals	M:1	M:1	M:1	M:1
C_{max} (ng/mL)	61,300	7930	7300	37,900
t_{max} (h)[a]	7.00 (7.00 – 7.00)	24.00 (24.00 – 24.00)	24.00 (24.00 – 24.00)	72.00 (72.00 – 72.00)
t_{last}[a]	529 (529 – 529)	529 (529 – 529)	529 (529 – 529)	529 (529 – 529)
AUC_{0-24h} (ng.h/mL)	1,130,000	95,200	87,600	315,000
AUC_{0-72h} (ng.h/mL)	1,710,000	427,000	412,000	1,860,000
AUC_{last}(ng.h/mL)	2,170,000	2,280,000	2,290,000	10,900,000
$t_{1/2}$ (h)	210	620	870	170

Mean cumulative amount excreted into urine and cumulative % dose excreted as unchanged drug in urine

Time h	**Cumulative amount Excreted (ug)**	**Cumulative % dose excreted As unchanged drug into urine**
0-8h	98.32	2.54
0-24h	269.20	6.94
0-48h	331.40	8.54
0-72h	341.01	8.79
0-96h	352.38	9.09
0-120h	358.47	9.24
0-144h	365.07	9.41

All concentration of P338 in feces were below the quantification limit (limit of quantification = 1 µg/g)

[a] Median value (min-max)

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; (HP)LC-MS/MS = (high performance) liquid chromatography coupled to tandem mass spectrometry; IM = Intramuscular; M = male; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration; $t_{1/2}$: half-life

2.6.5.16O Pharmacokinetics: Other- Pharmacokinetics in Rat

Test Article: P338

Study No.	**TOX13295**			
Location in CTD	**4.2.3.7.7**			
Species	Sprague-Dawley Rat			
Feeding Condition	Fed			
Vehicle/Formulation	P338			
Route	IM			
Compound	P338			
Dosing Period	Single dose/Repeated dose (every three days or every week with a 1-month follow-up			
Sample	Plasma			
Assay	LC-MS/MS			
Pharmacokinetic Parameters				
	SD		RD	
Dose (mg/kg)	5	5	10	10 (2 sites of injection at 5 mg/kg)
Gender (M/F)/Number of Animals	F:4	F:4	F:4	F:4
1st dose				
C_{max} (ng/mL)	24,500	24,600	49,200	48,700
t_{max} (h)	3 – 7	7	7 - 12	7 - 12
t_{last} (h)	96 – 168	72	72	96 - 168
AUC_{0-72h} (ng.h/mL)	679,000	705,000	1,570,000	1,470,000
2nd dose				
C_{max} (ng/mL)		38,400	67,100	
t_{max} (h)		2 - 7	7	
AUC_{0-72h} (ng.h/mL)		872,000	1,760,000	
3th dose				
C_{max} (ng/mL)		20,800	58,400	
t_{max} (h)		7	7	
AUC_{0-72h} (ng.h/mL)		673,000	1,790,000	
Last dose				
C_{max} (ng/mL)		38,000	64,500	57,400
t_{max} (h)		2 - 7	7 – 12	7
t_{last} (h)		336 – 456	456	96 - 336
AUC_{0-72h} (ng.h/mL)		987,000	1,950,000	1,550,000
AUC_{0-168h} (ng.h/mL)		1,170,000	2,390,000	1,790,000[a]

[a] AUC was extrapolated for one rat

AUC = area under the plasma concentration versus time curve; C_{max} = maximum plasma concentration; F = female; IM = Intramuscular; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; RD = repeated dose; SD = single-dose; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration

2.6.5.16P Pharmacokinetics: Other- Pharmacokinetics in Rat

Test Article: P338

Study No.	**TOX13391**					
Location in CTD	**4.2.3.7.7**					
Species	Sprague-Dawley Rat					
Feeding Condition	Fed					
Vehicle/Formulation	Sterile aqueous solution containing Glucose (anhydrous), Citric acid monohydrate, Sodium dihydrogen phosphate monohydrate, NaOH until pH = 7.0 ± 0.1 in water for injection / Solution					
Route	IM					
Compound	P338					
Dosing Period	M: one administration every three days from 28 days before mating, throughout mating and up to 3 days before necropsy (21 administrations) F: one administration every three days from 14 days before mating, throughout mating and throughout organogenesis (last administration on G15 for the applicable females – 11 to 15 administrations depending to the day of mating)					
Sample	Plasma					
Assay	LC-MS/MS					
Pharmacokinetic Parameters						
Dose (mg/kg)	2.5		5		10	
Gender (M/F)/Number of Animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
1st dose						
C_{max} (ng/mL)	-	-	29,200	18,300	33,100	25,600
t_{max} (h)	-	-	7	4.5 (2-7)[a]	7	4.5 (2-7)[a]
AUC_{0-72h} (ng.h/mL)	-	-	746,000	503,000	944,000	666,000
AUC_{inf} (ng.h/mL)	-	-	NA	477,000[b]	NA	775,000[b]
4th dose						
C_{max} (ng/mL)	8860[b]	10,800	19,900	14,100	28,900	27,800
t_{max} (h)	7[b]	7	7	7	7	7
AUC_{0-72h} (ng.h/mL)	276[b]	333,000	555,000	437,000	922,000	779,000
7th dose (M)/G3 (F)						
C_{max} (ng/mL)	14,100	-	24,000	13,800[b]	37,400	4380
t_{max} (h)	7	-	7	7[b]	7	7 (0-7)[a]
AUC_{0-72h} (ng.h/mL)	456,000	-	742,000	412,000[b]	1,360,000	192,000
10th dose (M)/G9 (F)						
C_{max} (ng/mL)	8030	-	22,600	16,000[b]	39,600	9040
t_{max} (h)	7	-	7	7[b]	7	7
AUC_{0-72h} (ng.h/mL)	300,000	-	716,000	439,000[b]	1,410,000	378,000

(Continued)

2.6.5.16P Pharmacokinetics: Other- Pharmacokinetics in Rat (Continued)

Test Article: P338

Study No.	**TOX13391**					
Location in CTD	**4.2.3.7.7**					
Pharmacokinetic Parameters						
Dose (mg/kg)	2.5		5		10	
Gender (M/F)/Number of Animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
13th dose (M)/G15 (F)						
C_{max} (ng/mL)	11,300	-	25,900	16,700	19,800	26,200
t_{max} (h)	7	-	7	7	7 (0-7)[a]	7
AUC_{0-72h} (ng.h/mL)	401,000	-	990,000	491,000	838,000	879,000
16th dose						
C_{max} (ng/mL)	14,500		15,800		39,000	
t_{max} (h)	7		7		7	
AUC_{0-72h} (ng.h/mL)	492,000		729,000		1,540,000	
19th dose						
C_{max} (ng/mL)	10,100		23,300		34,100	
t_{max} (h)	7		7		7	
AUC_{0-72h} (ng.h/mL)	386,000		825,000		1480,000	
$AUC_{0-cummulative\ tlast}$ (ng.h/mL)	7,870,000	3,660,000	16,000,000	4,960,000	26,000,000	6,220,000

[a] median range; [b] based on a single animal

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F = female; IM = Intramuscular; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; M = male; NA = not applicable; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration

2.6.5.16Q Pharmacokinetics: Other- Pharmacokinetics in Rat - PPN

Test Article: P338

Study No.	**TOX13546**		
Location in CTD	**4.2.3.7.7**		
Species	Sprague-Dawley Rat		
Feeding Condition	Fed		
Vehicle/Formulation	Sterile aqueous solution containing Glucose (anhydrous), 1 mg/ml Citric acid monohydrate, 2 mg/ml Sodium dihydrogen phosphate monohydrate, NaOH until pH = 7.0 ± 0.1 in water for injection / Solution		
Route	IM		
Compound	P338		
Dosing Period	F: one administration every three days from GD6 to day before necropsy = LD20		
Sample	Plasma		
Pharmacokinetic Parameters			
Dose (mg/kg)	**2.5**	**5**	**10**
Gender (M/F)/Number of Animals	F: 3	F: 3	F: 3
GD6 - 1st dose			
C_{max} (ng/mL)	n/c	14,200	26,400
t_{max} (h)	n/c	7	7
AUC_{0-72h} (ng.h/mL)[a]	n/c	414,000	807,000
GD12 3th dose			
C_{max} (ng/mL)	n/c	8780[a]	2350
t_{max} (h)	n/c	7[a]	7
AUC_{0-72h} (ng.h/mL)[a]	n/c	274,000[a]	684,000
GD18 5th dose			
C_{max} (ng/mL)	n/c	14,300	6870
t_{max} (h)	n/c	7	7
AUC_{0-72h} (ng.h/mL)[a]	n/c	470,000	301,000
LD2 - 7th dose			
C_{max} (ng/mL)	n/c	8840	21,900
t_{max} (h)	n/c	7	7
AUC_{0-72h} (ng.h/mL)[a]	n/c	318,000	766,000

(Continued)

2.6.5.16Q Pharmacokinetics: Other- Pharmacokinetics in Rat – PPN (Continued)

Test Article: P338

Study No.		**TOX13546**	
Location in CTD		**4.2.3.7.7**	
Pharmacokinetic Parameters			
Dose (mg/kg)	**2.5**	**5**	**10**
Gender (M/F)/Number of Animals	F: 3	F: 3	F: 3
LD8 - 9th dose			
C_{max} (ng/mL)	n/c	8180	18,300
t_{max} (h)	n/c	7	7
AUC_{0-72h} (ng.h/mL)[a]	n/c	299,000	688,000
LD17 - 12th dose			
C_{max} (ng/mL)	7,830	14,100	25,200
t_{max} (h)	7	39.5 (7-72)[b]	7
AUC_{0-72h} (ng.h/mL)[a]	260,000	686,000	990,000
GD6 – L17[b]			
$AUC_{0\text{-cummulative tlast}}$ (ng.h/mL)	n/c	4,860,000	8,410,000

[a] based on a single animal; [b] median range

b PN D4, 5, 20 and 21 (pups from treated dams): plasma concentrations of P338 were all below LLOQ

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; F = female; GD = gestation day; IM = Intramuscular; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; LD = lactation day; n/c = could not be calculated; LLOQ = lower limit of quantification; PPN = pre- and postnatal development; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration

2.6.5.16R Pharmacokinetics: Other- Pharmacokinetics in Rabbit

Test Article: P338

Study No.	**TOX13296**			
Location in CTD	**4.2.3.7.7**			
Species	New-Zealand White rabbit			
Feeding Condition	Fed			
Vehicle/Formulation	Sterile aqueous solution containing Glucose (anhydrous), Citric acid monohydrate Sodium dihydrogen phosphate monohydrate, NaOH until pH = 7.0 ± 0.1			
Route	IM			
Compound	P338			
Dosing Period	Single dose/Repeated dose (every three days or every week with a 1-month follow-up			
Sample	Plasma			
Assay	LC-MS/MS			
Pharmacokinetic Parameters				
	SD		RD	
Dose (mg/kg)	5	2.5	2.5	5
	D0	D 0, 4 and 8	D 0 and 7	D 0 and 7
Gender (M/F)/Number of Animals	F:4	F:4	F:4	F:4
1st dose				
C_{max} (ng/mL)	50,200	19,700	30,000	48.900
t_{max} (h)	24	12 - 24	7 – 24	12 - 24
t_{last} (h)	168 - 336	96	96 – 168	168
AUC_{0-96h} (ng.h/mL)	2,760,000	1,140,000	1,580,000	2,800,000
AUC_{0-168h} (ng.h/mL)	3,290,000	1,140,000	1,860,000	3,350,000
AUC_{0-inf} (ng.h/mL)	3,740,000	1,330,000	2,070,000	3,610,000
2nd dose				
C_{max} (ng/mL)		23,800		
t_{max} (h)		24		
AUC0-72h (ng.h/mL)		1,380,000		
Last dose				
C_{max} (ng/mL)		27,000	28,400	55,100
t_{max} (h)		12 - 24	12 – 24	24
t_{last} (h)		96 – 336	168 – 504	168 - 504
AUC_{0-72h} (ng.h/mL)		1,620,000	1,650,000	3,180,000
AUC_{0-168h} (ng.h/mL)		1,970,000	2,010,000	3,880,000
AUC_{0-last} (ng.h/mL)		2,160,000	2,260,000	4,410,000

[a] at 96h

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; D = day; IM = Intramuscular; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; NC = Not calculated; SD = single-dose; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration

2.6.5.16S Pharmacokinetics: Other- Pharmacokinetics in Rabbit

Test Article: P338

Study No.	**TOX13376**	
Location in CTD	**4.2.3.7.7**	
Species	New-Zealand White rabbit	
Feeding Condition	Fed	
Vehicle/Formulation	Sterile aqueous solution containing Glucose (anhydrous), Citric acid monohydrate, Sodium dihydrogen phosphate monohydrate, NaOH until pH = 7.0 ± 0.1 in water for injection/ Solution	
Route	IM	
Compound	P338	
Dosing Period	GD 6 and GD 12	
Sample	Plasma	
Assay	LC-MS/MS	
Pharmacokinetic Parameters		
Dose (mg/kg)	2.5	5.0
Gender (M/F)/Number of Animals	F: 4	F: 4
GD6		
C_{max} (ng/mL)	24,300	40,400
t_{max} (h)	24	24
$AUC_{(0\text{-}144h)}$ (ng.h/mL)	1,490,000	2,410,000
GD12		
C_{max} (ng/mL)	24.8	49,200
t_{max} (h)	24	24
$AUC_{0\text{-}144h}$ (ng.h/mL)	1,330,000	2,670,000
GD6 + GD12		
$AUC_{(0\text{-cumulative tlast})}$ (ng.h/mL)	2,820,000	5,080,000[a]

[a] $AUC_{(0\text{-cumulative tlast})}$ is similar to $AUC_{(0\text{-}288h)}$. For animal 58 $AUC_{(0\text{-}288h)}$ also was used although for this animal $AUC_{(0\text{-}480h)}$ was available

AUC = area under the plasma concentration versus time curve; C_{max} maximum plasma concentration; GD = gestation day; IM = Intramuscular; LA = long-acting; LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; P338 = poloxamer 338; t_{max} = time to reach the maximum plasma concentration