Janssen Research & Development

Toxicology Written Summary

MODULE 2.6.6

Rilpivirine Long-Acting

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TABLE OF CONTENTS

TABLE OF CONTENTS **2**

LIST OF IN-TEXT TABLES **5**

LIST OF IN-TEXT FIGURES **6**

LIST OF ABBREVIATIONS **7**

1. BRIEF SUMMARY **9**

2. SINGLE-DOSE TOXICITY **13**
2.1. RPV (studies supporting the registration of oral RPV (EDURANT®)) 13
2.1.1. Mouse Study 13
2.1.2. Rat Study 13
2.1.3. Dog Study 14
2.1.4. Overall Conclusion 14
2.2. RPV LA 14

3. REPEAT-DOSE TOXICITY **15**
3.1. RPV (studies supporting the registration of oral RPV (EDURANT®)) 15
3.1.1. Studies in Mice 15
3.1.1.1. Non-Pivotal Pilot Oral Carcinogenicity Dose-Range Finding Studies in Mice 15
3.1.1.1.1. 2-Week Study with CD-1 Mice 15
3.1.1.1.2. 1-Month Study with Transgenic Mice 16
3.1.1.2. Pivotal 3-Month Oral Toxicity and Carcinogenicity Dose-Range Finding Study with RPV in Mice 17
3.1.2. Studies in Rats 19
3.1.2.1. Non-Pivotal Pilot Oral Toxicity and Carcinogenicity Dose-Range Finding, and RPV Base – RPV Bridging Studies in Rats 19
3.1.2.1.1. 5-Day Oral Tolerance Study with RPV Base 19
3.1.2.1.2. 2-Week Oral Toxicity Study with RPV Base 20
3.1.2.1.3. 2-Week Oral Study with RPV Base and RPV by Gavage and via Diet in Rats 20
3.1.2.1.4. 2-Week Pilot Oral Carcinogenicity Dose-Range Finding Study with RPV in Rats 21
3.1.2.1.5. 1-Month Bridging Study with RPV Base and RPV in Rats 22
3.1.2.2. Pivotal Oral Toxicity Studies with RPV Base in Rats 23
3.1.2.2.1. 1-Month Oral Toxicity Study Including Immunotoxicity Evaluation in Rats 23
3.1.2.2.2. 6-Month Oral Toxicity Study Including 1-Month Recovery in Rats 24
3.1.3. Study in Rabbits 27
3.1.3.1. 5-Day Oral Dose-Range Finding Toxicity Study with RPV Base in Rabbits 27
3.1.4. Studies in Dogs 27
3.1.4.1. Non-Pivotal Pilot Oral Toxicity with RPV Base and RPV Base – RPV Bridging Studies 27
3.1.4.1.1. 5-Day Pilot Oral Toxicity Study with RPV Base 27
3.1.4.1.2. 7-Day Pilot Oral Toxicity Study with RPV Base 28
3.1.4.1.3. 1-Month Bridging Study with RPV Base and RPV in Dogs 28
3.1.4.2. Pivotal Oral Toxicity Studies with RPV Base in Dogs 29
3.1.4.2.1. 1-Month Toxicity Study Including 1-Month Recovery in Dogs 29
3.1.4.2.2. 6-Month Toxicity Study Including 3-Month Interim Evaluation in Dogs 31
3.1.4.2.3. 12-Month Toxicity Study in Dogs 34
3.1.5. Studies in Non-Human Primates 36
3.1.5.1. 8-Week Oral Toxicity Study with RPV in Juvenile Female Cynomolgus Monkeys 37
3.1.6. Overall Conclusion 38
3.2. RPV LA 38
3.2.1. Dog 38
3.2.2. Minipig 40
3.2.2.1. 6-Week Toxicity Study 40
3.2.2.2. 9-Month Toxicity Study 41

3.2.3. Overall Conclusion 42

4. GENOTOXICITY 42
4.1. In Vitro Nonmammalian Cell System 42
4.1.1. Ames Tests with Rat Liver S9 42
4.1.2. Ames Test with Human Liver S9 43
4.2. In Vitro Mammalian Cell System 43
4.3. In Vivo Mammalian System 44
4.4. Other Systems 44
4.5. Overall Conclusion 44

5. CARCINOGENICITY 45
5.1. Long-Term Studies 45
5.1.1. 2-Year Carcinogenicity Study in Mice 45
5.1.2. 2-Year Carcinogenicity Study in Rats 47
5.2. Other Studies 51
5.3. Overall Conclusion 51

6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY 51
6.1. Fertility and Early Embryonic Development 51
6.1.1. Male Rat Fertility Study with RPV 51
6.1.2. Female Rat Fertility and Early Embryonic Developmental Study with RPV 52
6.2. Embryofetal Development 52
6.2.1. Studies in Rats with RPV Base 52
6.2.2. Studies in Rabbits with RPV Base 53
6.3. Prenatal and Postnatal Development, Including Maternal Function 54
6.3.1. Dose-Range Finding Pre- and Postnatal Development Study Including Juvenile Exposure in Rats with RPV 54
6.3.2. Pre- and Postnatal Developmental Toxicity Study in Rats with RPV 55
6.4. Overall Conclusion 56

7. LOCAL TOLERANCE 56
7.1. RPV (studies supporting the registration of oral RPV (EDURANT®) 56
7.1.1. In Vitro Studies 56
7.1.1.1. Eye Irritation - Bovine Corneal Opacity-Permeability Assay 56
7.1.1.2. Phototoxicity - Balb/c Mouse 3T3 Fibroblast Assay 57
7.1.2. In Vivo Studies 57
7.1.2.1. Skin Irritation 57
7.1.2.2. Hypersensitivity 57
7.2. RPV LA 57
7.2.1. *In Vitro* Hen's Egg Chorioallantoic Membrane Irritation Test (HET-CAM) 57
7.2.2. Local Lymph Node Assay 58
7.2.3. Acute Intramuscular and Subcutaneous Irritation of Formulations including P338 in Rabbits 58
7.2.4. Acute Intramuscular Irritation of Formulations including P338 in Minipigs 59
7.3. Overall Conclusion 59

8. OTHER TOXICITY STUDIES 59
8.1. Antigenicity 60
8.2. Immunotoxicity 60
8.3. Mechanistic Studies with RPV 60
8.3.1. Single Dose Oral Endocrinology Study in Dogs 60
8.3.2. Inhibition of ACTH-stimulated Cortisol Biosynthesis in Primary Cultures of Guinea Pig Adrenal Cells 60
8.3.3. Effect of RPV Base on the Cortisol Biosynthesis in Crude Subcellular Fractions of Dog Adrenal Cortex 61
8.4. Dependence 61
8.5. Studies on Metabolites 61
8.6. Studies on Impurities 61

8.6.1. Qualification According to ICH Q3A 61
8.6.1.1. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance A* and related substance B* 62
8.6.1.2. In Vitro Mammalian Forward Mutation Assay with Mouse Lymphoma Cells with related substance A* and related substance B* 62
8.6.1.3. 1-Month Oral Toxicity Study in Rats with related substance A* and related substance B* 63
8.6.1.4. Summary 63
8.6.2. Impurities with a Genotoxic Alert 63
8.6.2.1. related substance G* 63
8.6.2.1.1. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance G* 64
8.6.2.1.2. In Vitro Mammalian Chromosomal Aberration Assay with related substance G* 64
8.6.2.2. related substance E* and related substance F* 64
8.6.2.2.1. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance E* 65
8.6.2.2.2. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance F* 65
8.6.3. Residual Solvent N-methylpyrrolidone 65
8.6.4. Overall Conclusion 66
8.7. Studies Done With RPV LA Formulations Other Than Selected G001 Clinical Formulation 66
8.7.1. Single Dose Toxicity Studies 66
8.7.1.1. Mouse 66
8.7.1.2. Rat 67
8.7.1.3. Dog 67
8.7.1.4. Minipig 68
8.7.2. Repeat-Dose Toxicity Study 68
8.7.2.1. Minipig 68
8.8. Acute Intramuscular Irritation of Formulations (Not Including P338) in Rabbits 69
8.9. Studies with Poloxamer 338 69
8.9.1. Genotoxicity 69
8.9.1.1. In Vitro Nonmammalian Cell System 69
8.9.1.2. In Vitro Mammalian System 70
8.9.2. Reproductive and Developmental Toxicity 70
8.9.2.1. Rat Embryofetal Development (Oral Gavage) 70
8.9.2.2. Rat Fertility and Embryofetal Development (Intramuscular) 71
8.9.2.3. Rabbit Embryofetal Development (Oral Gavage) 73
8.9.2.4. Rabbit Embryofetal Development (Intramuscular) 74
8.9.2.5. Rat Pre- and Postnatal Development (Intramuscular) 75

9. DISCUSSION AND CONCLUSIONS 76
9.1. General Toxicity 77
9.2. Genotoxicity 82
9.3. Carcinogenicity 83
9.4. Reproductive and Developmental Toxicity 84
9.5. Juvenile Toxicity 85
9.6. Other Toxicity 85
9.7. RPV LA 85

10. TABLES AND FIGURES 87

11. LIST OF LITERATURE CITATIONS 87

＊新薬承認情報提供時に置換

LIST OF IN-TEXT TABLES

Table 1: Exposure to RPV base (C_{max} and AUC) in an Oral Single Dose Study with RPV Base in Dogs 14
Table 2: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study with RPV in Mice 16
Table 3: Exposure to RPV base (C_{max} and AUC) in a 1-Month Oral Study with RPV in Mice 17
Table 4: Exposure to RPV base (C_{max} and AUC) in a 3-Month Oral Study with RPV in Mice 19
Table 5: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study with RPV Base in Rats 20
Table 6: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study in Rats with RPV Base and RPV 21
Table 7: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study in Rats with RPV 22
Table 8: Exposure to RPV base (C_{max} and AUC) in a 1-Month Oral Bridging Study with RPV Base and RPV in Rats 23
Table 9: Exposure to RPV base (C_{max} and AUC) in a 1-Month Toxicity Study of RPV Base in Rats 24
Table 10: Exposure to RPV base (C_{max} and AUC) in a 6-Month Oral Toxicity Study with RPV Base in Rats 26
Table 11: Exposure to RPV base (C_{max} and AUC) in a 7-Day Oral Toxicity Study with RPV Base in Dogs 28
Table 12: Exposure to RPV base (C_{max} and AUC) in a 1-Month Oral Bridging Study with RPV Base and RPV in Dogs 29
Table 13: Exposure to RPV base (C_{max} and AUC) in a 1-Month Toxicity Study Including 1-Month Recovery with RPV Base in Dogs 31
Table 14: Exposure to RPV base (C_{max} and AUC) in a 6-Month Toxicity Study with RPV Base in Dogs 34
Table 15: Exposure to RPV base (C_{max} and AUC) in a 12-Month Toxicity Study of RPV Base in Dogs 36
Table 16: Exposure to RPV base (C_{max} and AUC) in an 8-Week Oral Study with RPV in Juvenile Female Cynomolgus Monkeys 38
Table 17: Mean Toxicokinetic Parameters of RPV After Two Intramuscular Injections of RPV LA in Male and Female Beagle Dogs Given 2 Weeks Apart 40
Table 18: Mean Toxicokinetic Parameters of RPV After Intramuscular Injections of RPV LA Given 2 Weeks Apart to Male and Female Minipigs Apart For 6 Weeks 41
Table 19: Mean Toxicokinetic Parameters of RPV After Intramuscular Injections of RPV LA Given 2 Weeks Apart to Male and Female Minipigs For 9 Months 41
Table 20: Exposure to RPV base (C_{1h} and AUC) in a Single Oral Dose Bone Marrow Micronucleus Study with RPV Base in Mice 44
Table 21: Mortality in a Mouse Carcinogenicity Study with RPV 46
Table 22: Neoplastic Liver Changes in a Mouse Carcinogenicity Study with RPV 47
Table 23: Exposure to RPV (C_{max} and AUC) in a Mouse Carcinogenicity Study with RPV 47
Table 24: Mortality in the Rat Carcinogenicity Study with RPV 48
Table 25: Neoplastic Liver and Thyroid gland Changes in a Rat Carcinogenicity Study with RPV 50
Table 26: Exposure to RPV (C_{max} and AUC Values) in a Rat Carcinogenicity Study with RPV 50
Table 27: Exposure to RPV base (C_{max} and AUC) in an Oral Embryofetal Developmental Study with RPV Base in Rats 53
Table 28: Exposure to RPV base (C_{max} and AUC) in an Oral Embryofetal Development Study with RPV Base in Rabbits 54
Table 29: Exposure to RPV base (C_{max} and AUC) in Rat Pups via Lactation (LD7) and after 2 Weeks of Oral Dosing with RPV 55
Table 30: Exposure of RPV base (C_{max} and AUC) in a Single Dose Oral Endocrinology Study in Dogs with RPV Base 60
Table 31: Mean Toxicokinetic Parameters after Repeated Intramuscular Dosing of RPV LA in the F006 Formulation in Minipigs 69
Table 32: Mean Toxicokinetic Parameters in Female Rats After Single And Repeat IM Dosing of P338 72
Table 33: Mean Toxicokinetic Parameters in Male and Female Rats After IM Dosing of P338 in a Fertility and Embryofetal Development Study 73
Table 34: Mean Toxicokinetic Parameters in Female Rabbits After Single And Repeat IM Dosing of 74

Table 35: Summary of Poloxamer 338 Toxicokinetic Parameters in Pregnant New Zealand White Rabbit Plasma Following Intramuscular Administration of Poloxamer 338 on GD6 and GD12 75
Table 36: Summary of Poloxamer 338 Toxicokinetic Parameters in a Pre- and Postnatal Development Study in Time-Mated Female Rats Following Intramuscular Administration of Poloxamer 338 from GD 6 until Weaning 76

LIST OF IN-TEXT FIGURES

Figure 1: Metabolites M30 (Left) and M31 (Right) 43
Figure 2: Structural Formulas of related substance A* (Top) and related substance B* (Bottom) 61
Figure 3: Structural Formula related substance D* 64
Figure 4: Structural Formulas of related substance E* (Left) and related substance F* (Right) 65
Figure 5: Synthetic Pathways for Adrenal Steroid Synthesis in Non-human Primates and Man 80

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
A/G ratio	albumin/globulin ratio
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
$AUC_{0-\infty}$	area under the time vs concentration curve from the moment of administration to infinity
BCOP	bovine corneal opacity-permeability
b.i.d.	bis in diem, twice daily
C_{1h}	concentration determined 1 hour after dosing
CA	citric acid
CAC	carcinogenicity assessment committee
C_{max}	maximum concentration
CRF	corticotrophin releasing factor
CYPxx	cytochrome P450 isozyme xx
DHEA	dehydroepiandrosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRF	dose-range finding
ECG	electrocardiogram
F	female
F1	first generation
FDA	United States Food and Drug Administration
GD	gestation day
γGT	γ-glutamyltransferase
GLP	good laboratory practices
HCl	hydrochloride
HIV	human immunodeficiency virus
HPMC	hydroxypropylmethylcellulose
IC_{50}	median inhibitory concentration
ICH	international conference on harmonization
LD	lactation day
LH	luteinizing hormone
M	male
m/v	mass per volume
MPS	mononuclear phagocytic system
MTD	maximum tolerated dose
(N)NRTI	(non-)nucleoside reverse transcriptase inhibitor

NOAEL	no observed adverse effect level
NOEL	no observed effect level
NZW	New Zealand white
OECD	organization for economic co-operation and development
PEG400	polyethylene glycol with molar mass between 380 and 420
PFC	plaque forming cells
ppm	parts per million = 10^{-6}
PR	interval between the peak of the P wave and the peak of the R wave on ECG
PT	prothrombin time
q.d.	quaque dies = once daily
QRS	part of the ECG complex comprising the Q, R, and S-waves
QT	interval between the start of the Q wave and the end of the T-wave on ECG
QWBA	quantitative whole-body autoradiography
RR	interval between the peak of R waves of 2 consecutive ECG complexes
T_3	triiodothyronine
T_4	tetraiodothyronine/thyroxine
tk	thymidine kinase
TSH	thyroid stimulating hormone
Tween 20	polyoxyethylenesorbitan monooleate 20
UDPGT	T_4-uridine diphosphate glucuronosyltransferase

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 as EDURANT® for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients in multiple countries including Europe (EMEA/H/C/002264), the United States of America, Canada, and Japan.

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.

This summary provides an overview of the studies conducted with RPV, both for the oral (EDURANT®) as well as the LA indication.

Focus is on the final selected RPV LA clinical formulation G001 containing 300 mg RPV base/mL suspension and poloxamer 338 (P338; 50 mg/mL). Any use of RPV LA further in this document refers to this G001/P338 formulation unless specified otherwise. Two other RPV LA formulations were also used in some early clinical studies, however were not selected as the formulation to be commercialized 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). Toxicity studies to support these other formulations are discussed in separate sections in this document.

The **oral RPV formulation** has been examined extensively in single and repeat-dose toxicity studies, genotoxicity, carcinogenicity, fertility, reproductive and embryo-fetal development, and pre-and postnatal development studies. Mechanism of action studies were conducted to elucidate the effects observed. In addition, local tolerance was evaluated, and impurities were assessed and qualified according to ICH Q3A. All these studies are being resubmitted as part of this MAA.

After **IM injection of RPV LA**, repeat-dose toxicity in dogs and minipigs was assessed. A 4-week dog study was conducted to bridge with the oral RPV program. For this purpose, the dog was considered the most appropriate species as it was more sensitive to RPV-related changes compared to the rodent, and it demonstrated a metabolism closer to human. The minipig was used in 6-week and 9-month studies, as it is a well-established animal model to study local tolerability of IM administered drugs. In addition, single and repeat-dose toxicity and/or local tolerance studies examining various formulations of RPV LA were conducted in mouse, rat, rabbit, dog and minipig. Also, the *in vitro* irritating properties and *in vivo* skin sensitizing potential of RPV LA formulation were evaluated. Impurity qualification is described as well.

Poloxamer 338, the excipient in which the RPV LA formulation is prepared, is currently not registered for IM administration. Available bridging data from non-GLP P338 studies conducted by the supplier, the general toxicity profile of poloxamers, as well as data from a similar poloxamer, poloxamer 188 (P188), are discussed in the nonclinical overview (Mod2.4/Sec4.8.9). Additional supportive kinetic (see Mod2.6.4/Sec8.3), genotoxicity, fertility and embryofetal development and pre- and postnatal development studies have been conducted and are described in this document.

All toxicology 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. Information (GLP status, location of test facilities, …) on studies is listed in Mod2.6.7.1 and Mod2.6.7.2. The dates of study conduct, and location of the raw data are mentioned in the individual study reports. Detailed toxicokinetic data for the studies with the G001 formulation are provided in Mod2.6.7.3. Drug substance information can be found in Mod2.6.7.4.

The following convention is applied throughout this document: 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. The analyte in bio-analytical determinations is referred to as ‘RPV base’.

RPV (studies supporting the registration of oral RPV (EDURANT®)

All in vivo studies were done by oral administration, with the exception of the sensitization and dermal irritation studies. For oral dosing, RPV base was dissolved in polyethylene glycol 400 (PEG400) usually with 100 mg/mL citric acid (CA) to improve exposure, with the exception of the rabbit studies in which RPV base was suspended in 0.5% (m/v) aqueous hydroxypropylmethylcellulose (HPMC). RPV was suspended in 0.5% (m/v) aqueous HPMC in the mouse, rat, and dog studies. In the studies with cynomolgus monkeys, the vehicle was 1% (m/v) aqueous HPMC with 0.5% Tween 20.

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

Repeat-dose toxicity studies were done in mice as (preparation for) a 3-month dose-range finding carcinogenicity study, in rats for up to 6 months, in non-pregnant rabbits for 5 days as preparation for the dose-range finding early embryonic development studies, in dogs up to 12 months, and in immature female cynomolgus monkeys up to 8 weeks, the latter as part of the assessment of the effects of RPV on juvenile animals. Juvenile rats were involved in a 2-week oral dosing study starting on lactation day (LD) 12. The dogs in studies up to 1-month duration

are also considered immature. Since the animals were 6.5 to 8 months old at the start of these studies they were not yet sexually mature at the end of the dosing period. Reversibility (after a 1-month recovery period) of the effects of RPV base on the high dose group was investigated in the 6-month rat study and the 1-month dog study.

The targets of toxicity of RPV (base) identified in the repeat-dose studies were: red blood cells (mouse, rat, and dog), coagulation (rat), liver (rat and dog), kidneys (mouse and dog), thyroid gland with secondary effects on the pituitary gland (rat), adrenal glands (mouse, rat, dog, and cynomolgus monkey), testes (dog), and ovaries (dog, in immature females with secondary effects on other tissues of the genital tract and on mammary glands). The majority of the induced effects appeared to be completely reversible after a 1-month post-dosing period. The effects on thyroid gland and coagulation in rats and on liver and serum alkaline phosphatase (ALP) in dogs showed signs of recovery but this was not complete at the end of the 1-month post-dosing period. A number of targets were affected at the low dose tested in dogs and cynomolgus monkeys preventing establishment of No Observed Adverse Effect Levels (NOAEL).

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

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

RPV (base) had similar targets or induced the same type of adverse effects in juvenile or sexually immature rats, dogs, and cynomolgus monkeys as in adult animals. The RPV base-related effects on the female genital tract and mammary glands in dogs at the end of the 1-month studies differed from the effects in longer duration studies with sexually mature animals at the end of the dosing period. The activation of ovaries in dogs treated with RPV base in the 1-month studies led to secondary activation noticed in the other parts of the genital tract and mammary glands. These secondary effects occur normally in sexually mature animals during estrous cycle. Therefore, they are not noted as a difference between test article-treated and control animals in longer duration studies. However, in the 1-month study the control animals were still dormant. In the evaluation relative to control animals, the secondary effects were noted. Activation of ovaries was not induced during an 8-week study in immature cynomolgus monkeys at the age of approximately 18 months at the start of the study.

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

The drug substance contains 3 impurities that need to be qualified according to Guideline Q3A of the International Conference on Harmonization (ICH), entitled: "Impurities in New Drug Substances". Two of these, related substance A*, and related substance B*, have been evaluated upon spiking to the drug substance at a level of 4%. Qualification comprised a bacterial reverse mutation Ames test, a mouse lymphoma assay, and a 1-month oral rat study. The presence of the impurities at 4% did not modify the effects of RPV in any of the tests. The third impurity, related substance C*, is the [redacted]-isomer of RPV. This isomer was present in all drug substance batches involved in pivotal nonclinical studies at the level of minimally 0.61%. The qualified dose level of related substance C* in this study was 0.03 mg/kg/day.

Three further (potential) impurities, related substance D*, related substance E*, and related substance F*, which contain moieties with a mutagenic alert, are present in the drug substance at levels that do not warrant qualification according to ICH Q3A. The mutagenic potential of the [redacted] salt of related substance D*, related substance G*, and of related substance E* and related substance F* was tested in an Ames test. Only related substance G* showed a mutagenic potential. So, it is concluded that related substance D* is a genotoxic impurity.

Assessments of these impurities in relation to clinical dose regimens are discussed in Mod2.4/Sec4.8.8.

RPV LA

RPV LA, was well tolerated in dogs at 1200 mg once weekly for 4 weeks and in minipigs at 600 mg every 2 weeks for 6 weeks and once monthly for 9 months. Main findings comprised of local effects noted at the injection sites. Slight, short-lasting erythema was observed, and white deposits were noted at the injection sites at necropsy, accompanied by swelling and discoloration of draining lymph nodes. Microscopic examination showed macrophage infiltration and eosinophilic deposits of/at the injection sites. A macrophage infiltration response was also noted in the draining/regional lymph nodes. Additionally, findings in the 4-week bridging dog study were in line with the toxicological profile as described for the oral RPV formulation.

Also, IM and/or subcutaneous (SC) single or repeat-dose toxicity studies in mice, rats, rabbits, dogs and minipigs with various RPV LA formulations showed good tolerability. Similar findings at the injection sites and the draining/regional lymph nodes were noted in these studies. The extent and occurrence of these effects was depending on the time between administration and post-mortem evaluation.

Assessment of a safety margin for the IM LA formulation relative to the oral RPV formulation is described in Mod2.4/Sec4.8.7.

RPV LA did not show irritating properties *in vitro*. RPV LA was not a skin sensitizer. There were no signs of delayed-type sensitization in a mouse local lymph node assay.

The genotoxic impurity related substance D*, the specified impurities related substance H*, related substance I* and related substance C* as well as the residual solvent N-methylpyrrolidone (NMP) were toxicologically qualified. Assessment in related to clinical dosing regimens is discussed in Mod2.4/Sec4.8.8.

The excipient P338 was evaluated as a vehicle (compared to control) in the 9-month minipig study mentioned previously and in fertility, embryo-fetal development and pre-and postnatal development studies in rats and rabbits. No adverse findings were noted in these studies. Safety margins for P338 exposure are satisfactory and are discussed in Mod2.4/Sec4.8.9. In *in vitro* bacterial and mammalian genotoxicity tests with P338, there was no indication for mutagenic potential or induction of chromosome aberrations.

Studies supporting other RPV IM formulations included: single dose local tolerability studies in mice, rats, dogs and minipigs, a 9-month repeated dose toxicity study in minipigs and an acute irritation study in rabbits.

2. SINGLE-DOSE TOXICITY

2.1. RPV (studies supporting the registration of oral RPV (EDURANT®)

Single dose evaluations were part of the initial oral dose-range finding studies in rats and dogs. The single dose toxicity of RPV base on mice was evaluated in the bone marrow micronucleus study (see Section 4.3).

2.1.1. Mouse Study

For a bone marrow micronucleus study (Mod4.2.3.3.2/TMC278-Exp.5538, Mod4.2.3.3.2/TMC278-Exp.5538 (FK4259) and Mod2.6.7.9), a single oral dose of RPV base dissolved in polyethylene glycol (PEG)400 with CA was administered by gavage to 10 male and 10 female CD-1 mice per group. The doses were 0 (vehicle), 100, 400, and 1600 mg/kg given in a volume of 20 mL/kg. The dose of 1600 mg/kg was the maximally feasible dose on the basis of the maximally feasible volume for a single dose study, 20 mL/kg, and a supersaturated and highly viscous solution of 800 mg/mL. A limited number of samples for confirmation of exposure were taken from the animals over a period of 6 hours after dosing. Five animals of each sex per group were killed 24 and 48 hours after dosing.

RPV base did not cause any mortality. There were no abnormalities in clinical observations or relevant effects on body weight. At necropsy, no test article-related gross lesions were recorded. At 1600 mg/kg, mean plasma concentration determined 1 h after dosing ($C_{1h)}$ was 60 and 58 µg/mL and the area under the time-(for 6 hours after dosing)-versus-concentration curve (AUC_{0-6h}) was 307 and 287 µg.h/mL in males and females, respectively. These values were essentially similar to those at 400 mg/kg, indicating saturation of absorption.

2.1.2. Rat Study

The highest feasible concentration of an oversaturated solution of RPV base in PEG400 was 80 mg/mL, in terms of viscosity for administration by gavage to rats. This concentration was administered by oral gavage at a volume of 10 mL/kg, to 6 male and 6 female Sprague-Dawley rats (Mod4.2.3.1/TMC278-Exp.5559, Mod4.2.3.1/TMC278-Exp.5559 (FK4278) and

Mod2.6.7.5), rendering a dose of 800 mg/kg. Mortality and clinical observations were evaluated for 1 day following the administration. Four males and 4 females were sampled to assess exposure.

RPV base did not cause any mortality. All males and 2 out of 6 females showed salivation during the day of dosing. At necropsy, no test article-related gross lesions were recorded. The mean maximum plasma concretion (C_{max}) was 8.0 and 18.0 µg/mL and the $AUC_{0-\infty}$ was 86 and 233 µg.h/mL in males and females, respectively.

2.1.3. Dog Study

For dogs, the highest feasible concentration of an oversaturated solution of RPV base in PEG400 with or without CA was 100 mg/mL in terms of viscosity for the administration by gavage. The dogs were pretreated with the vehicles applied in this study. PEG400 was administered by oral gavage at 0.8 mL/kg to two groups of 1 male and 1 female beagle dog and PEG400 + CA to another group (Mod4.2.3.2/TMC278-Exp.5461, Mod4.2.3.2/TMC278-Exp.5461 (FK4102) and Mod2.6.7.5). The next day, the PEG400-pretreated animals were dosed with RPV base dissolved in PEG400 at a single dose of 40 or 80 mg/kg. The PEG400 + CA pretreated animals received RPV base dissolved in PEG400 + CA at a single dose of 80 mg/kg. The animals were observed for 4 days after dosing. No clinical pathology or necropsy was performed on these animals.

RPV base did not cause any mortality. Only in the group dosed with 80 mg/kg dissolved in PEG400 a higher incidence of vomiting occurred for 2 days after dosing. Slightly soft feces were recorded for the male at Day 3 after dosing and for the female at Days 2 and 3 after dosing. The animals dosed with 40 mg/kg were transferred to the repeat-dose arm of the study, 3 weeks after the end of the single dose observation period. Exposure data are presented in Table 1.

Table 1: Exposure to RPV base (C_{max} and AUC) in an Oral Single Dose Study with RPV Base in Dogs

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

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

2.1.4. Overall Conclusion

Single dose toxicity of RPV base is minimal. At an absorption-saturating dose (mice) and at the maximal feasible dose in rats and dogs, no meaningful toxicity was induced.

2.2. RPV LA

For RPV LA, no single dose toxicity studies were conducted with the selected clinical G001formulation. Single dose toxicity studies with other surfactants in the LA formulations, or examining varying concentrations and particle sizes of RPV LA, and various routes of administration have been conducted. These studies are described in Section 8.7.1.

3. REPEAT-DOSE TOXICITY

3.1. RPV (studies supporting the registration of oral RPV (EDURANT®)

Systemic toxicity of RPV base or RPV after repeat dosing was studied in mice, rats, rabbits, dogs and cynomolgus monkeys. Pivotal repeat-dose studies were conducted in mice (3 months), rats (1 and 6 months), dogs (1, 3, 6, and 12 months), and cynomolgus monkeys (8 weeks). All pivotal studies were preceded by pilot or dose-range-finding studies.

The mouse study served as dose-range finder for the carcinogenicity study in that species. The 5-day rabbit study was a pilot for the rabbit embryofetal toxicity study. The studies in rats and dogs were designed to investigate the toxicity profile of RPV (base) and to support clinical studies and marketing authorization. The reversibility upon repeat dosing was investigated in rats and dogs.

All pivotal studies in rats and dogs were conducted with RPV base dissolved in PEG400, usually with CA. In the rabbit study, RPV base was suspended in 0.5% (m/v) hydroxypropyl-methylcellulose (HPMC) in water as rabbits do not tolerate PEG400. Following the selection of the HCl salt as the chemical form to be marketed, 1-month studies in rats and dogs compared the kinetics and toxicity of RPV base and RPV. The studies in mice and cynomolgus monkeys were conducted with RPV suspended in aqueous HPMC. For an overview of all repeat-dose toxicity studies (see Mod2.6.7.1). Quantitative mean effects are expressed as percentage or fold change from values of the vehicle control group unless stated otherwise.

3.1.1. Studies in Mice

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

3.1.1.1. Non-Pivotal Pilot Oral Carcinogenicity Dose-Range Finding Studies in Mice

3.1.1.1.1. 2-Week Study with CD-1 Mice

In a 2-week study with CD-1 mice (Mod4.2.3.2/TMC278-NC118 (TOX6479) and Mod2.6.7.6), RPV was administered by gavage or via diet in doses up to 2000 and 5000 mg/kg/day, respectively.

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

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

Hematology showed reduced red blood cell count, hemoglobin, and hematocrit (all 8%) and increased reticulocyte count (23%) and reduced eosinophil counts in females when administered by diet (also at 40 mg/kg/day).

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

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

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

Systemic exposure expressed as C_{max} and AUC values determined at the end of the dosing period are given in Table 2 below.

Table 2: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study with RPV in Mice

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

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

3.1.1.1.2. 1-Month Study with Transgenic Mice

In a 1-month study, RPV was administered orally by gavage to transgenic CB6F1-nonTgrasH2 mice at 0 (vehicle), 20, 80, or 320 mg/kg/day (Mod4.2.3.2/TMC278-NC121 (TOX6740) and Mod2.6.7.6).

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

Hematology showed reduced red blood cell count, hemoglobin, and hematocrit (all up to 6%), reticulocyte count (56%, females only) and increased number of platelets (up to 24%).

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

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

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

Systemic exposure expressed as C_{max} and AUC values are given in Table 3 below.

Table 3: Exposure to RPV base (C_{max} and AUC) in a 1-Month Oral Study with RPV in Mice

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

[a]: $AUC_{0-\infty:}$ on Day 1 otherwise AUC_{0-24h}, M: males, F: females

3.1.1.2. Pivotal 3-Month Oral Toxicity and Carcinogenicity Dose-Range Finding Study with RPV in Mice

RPV was administered once daily by oral gavage for 3 months to CD-1 mice. Groups of 25 animals per sex (10 for the main study and 15 satellite animals for toxicokinetics) were given 0 (vehicle; 12 satellite animals per sex), 20, 80, or 320 mg/kg/day in 10 mL/kg (Mod4.2.3.2/TMC278-NC119 (TOX6739) and Mod2.6.7.7A). All animals were observed regularly for clinical signs, morbidity, and mortality. Body weight of all animals was recorded but assessed for main animals only. For main animals, food consumption and body weight gain were determined. Main animals were sampled for hematology and serum chemistry and necropsied and inspected for gross lesions, at the end of the study. A range of organs was weighed, and a complete set of organs and tissues was preserved for microscopical examination. For possible electron microscopical evaluation, liver samples from 5 male and 5 female mice of the control group and the group treated with 320 mg/kg/day were fixated in phosphate buffer containing 3% glutaraldehyde. Blood samples for toxicokinetic analysis were drawn from 3 satellite animals per gender per time point on 5 time points within 24 hours on Days 1, 31 and 87. After the last sampling these animals were killed.

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

Body weight at the end of the dosing period was increased up to 11% in the males and females dosed with 320 mg/kg/day. This increase was associated with a body weight gain over the whole

dosing period of slightly more than 150%. The effects on body weight and body weight gain were in line with a 20% higher food consumption essentially throughout the dosing period in males and females dosed with 320 mg/kg/day.

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

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

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

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

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

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

In females treated with the high dose, a marginal decrease of the number of corpora lutea and generations of corpora lutea was noted in ovaries. Also, granulocyte infiltration in the

endometrium was marginally decreased in this group. It cannot be excluded that these gonadal effects indicate a reduced cyclic activity.

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

Based on the findings in animals with 80 mg/kg/day in liver, the NOAEL was considered to be 20 mg/kg/day. Systemic exposure expressed as C_{max} and AUC values are given in Table 4 below.

Table 4: Exposure to RPV base (C_{max} and AUC) in a 3-Month Oral Study with RPV in Mice

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

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

3.1.2. Studies in Rats

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

3.1.2.1. Non-Pivotal Pilot Oral Toxicity and Carcinogenicity Dose-Range Finding, and RPV Base – RPV Bridging Studies in Rats

3.1.2.1.1. 5-Day Oral Tolerance Study with RPV Base

RPV base dissolved in PEG400 was administered once daily by oral gavage for 5 days at 0 (vehicle), 40 or 400 mg/kg/day in 8 mL/kg to 5 male Sprague-Dawley rats/group (Mod4.2.3.2/TMC278-Exp.5463, Mod4.2.3.2/TMC278-Exp.5463 (FK4103) and Mod2.6.7.6).

There was no mortality and the treatment was well tolerated. There were no relevant clinical signs or effects on body weight or on hematology. There were no relevant histopathological changes in the examined tissues. Systemic exposure after repeated oral dosing of 400 mg/kg/day was shown by a C_{max} of 2 µg/mL and an $AUC_{0-\infty}$ of 28 µg.h/mL.

3.1.2.1.2. 2-Week Oral Toxicity Study with RPV Base

RPV base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 2 weeks to Sprague-Dawley rats at 0 (water), 0 (vehicle), 40, 120 or 400 mg/kg/day in 10 mL/kg (Mod4.2.3.2/TMC278-Exp.5535, Mod4.2.3.2/TMC278-Exp.5535 (FK4243) and Mod2.6.7.6). All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with RPV base had additionally 4 males and 4 females for toxicokinetic analysis.

There were no mortalities in this study.

Increased serum concentrations of total protein (3%, males) and albumin (7%, males) were noted in animals dosed with 400 mg/kg/day. Moreover, decreased concentrations of thyroxine (T_4, maximally 66%) were determined at all doses in males and/or females associated with increased TSH (up to 100%) in animals dosed with 120 and 400 mg/kg/day.

At necropsy, increased thyroid gland weight (19%) in females given 400 mg/kg/day and hypertrophy of the thyroid follicular epithelium in male and/or female rats at all doses were noted.

Systemic exposure expressed as C_{max} and AUC values are given in Table 5 below.

Table 5: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study with RPV Base in Rats

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

[a]: $AUC_{0-\infty}$: on Day 1 otherwise AUC_{0-24h}, M: males, F: females

3.1.2.1.3. 2-Week Oral Study with RPV Base and RPV by Gavage and via Diet in Rats

RPV was administered once daily to Sprague-Dawley rats by oral gavage suspended in 0.5% (m/v) aqueous HPMC at a dose of 400 mg/kg/day or by dietary administration at 400 or 1200 mg/kg/day (Mod4.2.3.2/TMC278-NC136 (TOX6417) and Mod2.6.7.6). RPV base was administered by diet at 400 mg/kg/day. The control group received unmedicated diet. All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with RPV base had additionally 6 males and 6 females for toxicokinetic analysis.

No mortality was noted during this study. There were no toxicologically relevant differences between groups dosed with RPV base or RPV at 400 mg/kg/day by gavage or through the diet.

With both forms, decreased white blood cell count (maximally 23%) and hemoglobin, and hematocrit (maximally 6%) occurred.

Increased ALT activity in serum (up to 44%) in males treated with 400 mg/kg/day and decreased concentration of cholesterol (29%) in males given RPV by gavage were determined.

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

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

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

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

M: males, F: females

3.1.2.1.4. 2-Week Pilot Oral Carcinogenicity Dose-Range Finding Study with RPV in Rats

RPV suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage, for 2 weeks to Sprague-Dawley rats at 0 (vehicle), 400, 1500, or 2000 mg/kg/day in 10 mL/kg (Mod4.2.3.2/TMC278-NC177 (TOX6813) and Mod2.6.7.6). All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with RPV had additionally 3 males and 3 females for toxicokinetic analysis.

There were no mortalities in this study.

Increased activated partial thromboplastin time (APTT) and prothrombin time (PT, both up to 20%) were determined in males from 1500 mg/kg/day onward.

Serum chemistry showed decreased serum concentrations of chloride (3%), glucose (up to 18%), urea nitrogen (33%), and creatinine (maximally 23%) in females given 1500 or 2000 mg/kg/day. In the latter group, males had decreased concentrations of cholesterol (30%).

Increased urinary volume (up to 100%) was recorded in animals given 1500 and 2000 mg/kg/day.

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

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

Table 7: Exposure to RPV base (C_{max} and AUC) in a 2-Week Oral Study in Rats with RPV

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

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

3.1.2.1.5. 1-Month Bridging Study with RPV Base and RPV in Rats

This study served to bridge the toxicity and toxicokinetics after administration of RPV base with those after RPV (Mod4.2.3.2/TMC278-NC117 (TOX6385) and Mod2.6.7.6). Both compounds were administered once daily, by oral gavage to Sprague-Dawley rats for 4 weeks at 0 (vehicle), 10, or 400 mg/kg/day in 10 mL/kg. RPV base was dissolved in PEG400 + CA and RPV was suspended in 0.5% (m/v) aqueous HPMC. All groups comprised 10 male and 10 female rats for the toxicological evaluations and the groups treated with RPV base and RPV had additionally 6 males and 6 females for toxicokinetic analysis.

No compound-related mortality was noted. No effects were noted in either group dosed with 10 mg/kg/day. There were no significant differences in the toxicity parameters of both forms dosed at 400 mg/kg/day.

Hematology showed decreased hematocrit (3%) and increased red blood cell count (4%).

Increased serum concentrations of albumin (up to 7%, males) and total protein (5%, males) and decreased levels of cholesterol (up to 22%, females), triglycerides (35%, females), urea (up to 18%), potassium (13%, females), and chloride (2%) were determined. Moreover, serum concentration of T_4 was decreased up to 61% whereas those of triiodothyronin (T_3) and TSH were increased up to 57% (males only) and up to 51%, respectively.

Urinary volume was increased up to 98%.

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

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

Table 8: Exposure to RPV base (C_{max} and AUC) in a 1-Month Oral Bridging Study with RPV Base and RPV in Rats

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

[a]: $AUC_{0-\infty:}$ on Day1 otherwise AUC_{0-24h}, M: males, F: Females

3.1.2.2. Pivotal Oral Toxicity Studies with RPV Base in Rats

3.1.2.2.1. 1-Month Oral Toxicity Study Including Immunotoxicity Evaluation in Rats

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 4 weeks to groups of 18 male and 18 female Sprague-Dawley rats at 0 (water, negative control), 0 (vehicle), 10, 40 or 160 mg/kg/day in 10 mL/kg (Mod4.2.3.2/TMC278-Exp.5692 and Mod2.6.7.7B); 8 male and 8 female satellite animals per group were used for the plaque forming cells (PFC) assay. Test article-treated groups had 4 more males and females for toxicokinetic analysis. Regular observations were made for clinical signs, morbidity and, mortality. Body weight, food consumption and body weight gain were determined throughout the dosing period. Ophthalmic examinations were performed prior to dosing and at the end of the dosing period (negative control, vehicle, and 160 mg/kg/day animals, only). All main animals were sampled for hematological, serum chemistry and urinalysis parameters at the end of the dosing period. The PFC satellites received on 4 consecutive days an intravenous injection with sheep red blood cells. Thereafter, they were killed, and a suspension of spleen cells was prepared and incubated with sheep red blood cells and Guinea pig complement. The resulting plaques are considered to be antibody-producing cells. At necropsy, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology. Blood samples were drawn for toxicokinetics on Days 1 and 29.

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

In view of the absence of findings of toxicological relevance in the group treated with 10 mg/kg/day, this dose was the NOAEL. Moreover, the absence of effects in the PFC assay indicated that RPV base has no immunotoxic potential. Systemic exposure expressed as C_{max} and AUC values is given in Table 9 below.

Table 9: Exposure to RPV base (C_{max} and AUC) in a 1-Month Toxicity Study of RPV Base in Rats

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

[a]: $AUC_{0-\infty}$: on Day 1 otherwise AUC_{0-24h}, M: males, F: females

3.1.2.2.2. 6-Month Oral Toxicity Study Including 1-Month Recovery in Rats

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 6 months to groups of 20 male and 20 female Sprague-Dawley rats (Mod4.2.3.2/TMC278-NC101 (TOX6142)) at 0 (vehicle), 40, 120 or 400 mg/kg/day in 10 mL/kg (Mod2.6.7.7C). There were 6 satellite rats of each sex per RPV base-treated group for toxicokinetic analysis, and 10 rats of each sex in the control and high dose group for assessment of reversibility of the findings at one-month post-dose. Regular observations were made for clinical signs, morbidity and, mortality. Body weight, food consumption and body weight gain were determined throughout the dosing and recovery periods. Ophthalmic examinations were performed prior to dosing and at the end of the dosing and recovery. Samples for hematological, serum chemistry and urinalysis parameters were taken after 3 months of treatment, and at the end of dosing and recovery periods. In addition, serum concentrations of TSH, T_3, T_4, adrenocorticotropic hormone (ACTH), corticosterone, and progesterone were determined in samples taken prior to the autopsies. At necropsy, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology. Blood samples were drawn for toxicokinetics on Days 1, 31 and 87. At necropsy, blood and liver samples were taken for determination of RPV base.

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

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

Compound-related clinical signs included salivation in females at all dose levels and in males at 400 mg/kg/day and wet urogenital region in both genders at 400 mg/kg/day. There were no relevant effects on ophthalmic examinations, body weight or food consumption.

In hematology, increases in APTT (up to 23%) and PT (up to 27%) were noted in RPV base-treated males of all groups without a dose-related trend, at all sampling times including the end of the recovery period. At the end of the dosing period, red blood cell count, hemoglobin, and hematocrit of males dosed with 400 mg/kg/day were reduced by 5%. The eosinophil count in RPV base-treated females were 25 – 33% lower than control values without a clear dose-related trend. Red blood cell parameters and eosinophil counts showed complete recovery after termination of dosing.

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

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

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

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

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

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

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

In view of the effects on coagulation parameters and the thyroid and pituitary glands observed in the low dose group treated with 40 mg/kg/day, a NOAEL could not be established in this study. Systemic exposure expressed as C_{max} and AUC values are given in Table 10 below.

Table 10: Exposure to RPV base (C_{max} and AUC) in a 6-Month Oral Toxicity Study with RPV Base in Rats

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

[a]: $AUC_{0-\infty:}$ on Day 1 otherwise AUC_{0-24h}, M: male, F: female

3.1.3. Study in Rabbits

3.1.3.1. 5-Day Oral Dose-Range Finding Toxicity Study with RPV Base in Rabbits

This non-pivotal study was part of the dose-range finding studies for the embryofetal development study with RPV base in rabbits. For studies with pregnant rabbits see Section 6.2.2.

RPV base suspended in 0.5% (m/v) aqueous HPMC was administered by oral gavage for 5 days to New Zealand white (NZW) rabbits (Mod4.2.3.2/TMC278-NC126 (TOX6146) and Mod2.6.7.6). Groups of 5 non-pregnant females received 0 (vehicle), 100, 300, or 1000 mg/kg in 10 mL/kg. Daily observations were made for clinical signs, morbidity and mortality. Body weight and food consumption were determined at the end of the dosing period. Blood samples for hematology, serum chemistry, and toxicokinetic analyses were taken at the end of the dosing period. At necropsy, gross lesions of external features and body cavities were recorded but no organs were weighed, and no organs or tissues were taken for histopathology.

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

3.1.4. Studies in Dogs

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

3.1.4.1. Non-Pivotal Pilot Oral Toxicity with RPV Base and RPV Base – RPV Bridging Studies

3.1.4.1.1. 5-Day Pilot Oral Toxicity Study with RPV Base

RPV base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 5 days to groups of beagle dogs each comprising 1 male and 1 female at 0 (vehicle) and 80 mg/kg/day in 0.8 mL/kg, after a wash-out period of 7 days following a similar single dose (Mod4.2.3.2/TMC278-Exp.5461, Mod4.2.3.2/TMC278-Exp.5461 (FK4102) and Mod2.6.7.5).

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

3.1.4.1.2. 7-Day Pilot Oral Toxicity Study with RPV Base

RPV base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 7 days to groups of 4 male beagle dogs at 0 (negative control, water), 0 (vehicle), 20, 40, or 80 mg/kg/day in 1 mL/kg (Mod4.2.3.2/TMC278-Exp.5534, Mod4.2.3.2/TMC278-Exp.5534 (FK4244) and Mod2.6.7.6). There were no mortalities. Serum levels of total bilirubin (111% and 108% at 40 and 80 mg/kg/day, respectively), glucose, and cholesterol (11% and 10%, respectively, both at 80 mg/kg/day) were increased. AUCs of cortisol concentrations in serum were decreased 56 to 75% in all groups treated with RPV. Multifocal inflammatory cell infiltration and multifocal vacuolated cells in the zona fasciculata of the adrenal glands were noted in all treated groups. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 11 below.

Table 11: Exposure to RPV base (C_{max} and AUC) in a 7-Day Oral Toxicity Study with RPV Base in Dogs

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

M: males

3.1.4.1.3. 1-Month Bridging Study with RPV Base and RPV in Dogs

This study served to bridge the toxicity and toxicokinetics of RPV base dissolved in PEG400 + CA with those of RPV suspended in 0.5% (m/v) aqueous HPMC. Both forms were administered once daily by oral gavage to groups of 3 male and 3 female beagle dogs for 1 month at 0 (vehicle), 5, or 40 mg/kg/day in 1 mL/kg (Mod4.2.3.2/TMC278-NC116 (TOX6334) and Mod2.6.7.6).

No mortalities occurred in this study. There were no significant differences in the toxicity parameters. Effects were almost exclusively observed in the groups dosed with both forms at 40 mg/kg/day.

Reduced red blood cell (7%, females) and reticulocyte (up to 61%, males) counts and hematocrit (15%) were noted.

Increased serum concentrations of bilirubin (up to 2-fold) and increased activities of ALT (up to 6-fold), AST (14%, females), ALP (up to 2-fold), and γ-glutamyltransferase (γGT; up to 6-fold) were recorded. Moreover, serum concentration of triglycerides (up to 53%) and inorganic phosphate (up to 11%) were decreased.

Endocrinology showed increased serum concentrations of ACTH (92%), progesterone (up to 11-fold, males) and 17α-hydroxyprogesterone (also in males given 5 mg/kg/day; up to 34-fold in males dosed with 40 mg/kg/day) and decreased levels of cortisol (up to 54%).

At necropsy, increased weights of liver (up to 19%) and adrenal glands (up to 32%) were recorded. Histopathology lesions comprised swollen cells with densely stained cytoplasm in zona

reticularis/fasciculata and presence of foamy cells in the zona fasciculata of adrenal glands. Moreover, an increase in the number of cystic luteinized follicles or tertiary follicles in the ovaries was noted, with a tendency towards an increase in glandular development in the uterus. In testes, prominent Leydig cells were noted in 1 animal. Males and females showed centrilobular/periportal inflammation, brown pigmented macrophages, single cell death, and oval cell proliferation in liver.

Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 12 below.

Table 12: Exposure to RPV base (C_{max} and AUC) in a 1-Month Oral Bridging Study with RPV Base and RPV in Dogs

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

M: males, F: females

3.1.4.2. Pivotal Oral Toxicity Studies with RPV Base in Dogs

3.1.4.2.1. 1-Month Toxicity Study Including 1-Month Recovery in Dogs

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to groups of 3 male and 3 female beagle dogs at 0 (water, negative control), 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg (Mod4.2.3.2/TMC278-Exp.5650 and Mod2.6.7.7D).To evaluate reversibility of the effects of RPV in a 1-month post-dosing period, 2 males and 2 females were added to the negative control group and the group treated with 40 mg/kg/day. Regular observations were made for clinical signs, morbidity and mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations and ECG recordings were performed and samples for hematology, serum chemistry, and urinalysis were taken prior to dosing, at the end of the 1-month dosing and post-dosing periods. Serum concentrations of progesterone, cortisol, aldosterone, ACTH, and estradiol (females, only) were determined in samples taken on Days 1 and 16, and towards the end of the dosing and post-dosing periods. For the determination of AUCs for ACTH, cortisol, and aldosterone, 4 samples were taken over a 24-hour period. Progesterone and estradiol were determined in pre-dosing and 4-h samples. Blood samples for toxicokinetics were taken on Day 1 and towards the end of the dosing and post-dosing periods. Single blood samples for trough level and elimination determinations were taken regularly during the dosing and recovery periods. At necropsy, blood and adrenal gland samples were taken for determination of RPV base. Moreover, gross lesions and organ weight were recorded, and tissues and organs were sampled for histopathology. Adrenal gland samples of the

dogs of the negative and vehicle groups and the group dosed with 40 mg/kg/day were examined by electron microscopy.

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

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

Progesterone concentrations were increased in a more or less dose-related fashion, both in the trough level samples and more pronounced in the samples taken 4 hours after dosing on Day 16, and even more towards the end of the dosing period, in males and females treated with RPV base. The increases were almost 30- and 10-fold at the end of the dosing period in males and females dosed with 40 mg/kg/day, respectively. The AUCs of ACTH were increased at the end of the dosing period, without a clear dose relationship and not attaining statistical significance. The AUCs of cortisol showed a tendency for decrease in the animals dosed with 10 and 40 mg/kg/day. After a 1-month post-dosing period, all parameters showed similar values in the control group and the group treated with 40 mg/kg/day.

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

In the adrenal cortex, histopathology showed an increase in the number of swollen cells with dense cytoplasm and reduced Oil red O-staining indicative for a reduction of neutral fat content in the animals dosed with 10 and 40 mg/kg/day compared with the vehicle group. The ultrastructural evaluation of adrenal gland samples showed that tissue from males and females treated with 40 mg/kg/day showed a reduction in the number of cytoplasmic lipid vacuoles in endocrine cells. This explains and confirms the light microscopy observations of less Oil red O-staining and dense cytoplasm. In addition to these findings, an increase of macrophage-like cells containing many small- and large-sized lipid vacuoles and secondary lysosomes with lipid and

lamellar material was noted in the zona fasciculata. The higher number of these cells containing material representing membrane detritus indicates that RPV base may have a mild degenerative effect in this zone. The female genital tract showed increased activation in the groups treated with 10 and 40 mg/kg/day. In the ovaries, corpora lutea were detected in 2 animals dosed with 10 mg/kg/day and in 1 animal that received 40 mg/kg/day. This finding was associated with prominent high epithelium in the oviduct, increased glandular development in the uterus, cornification with increased thickness of the epithelium of the cervix and vagina and with active alveolar development in mammary glands. More prominent tertiary follicles were noted in all test article-treated animals that had not ovulated at the end of dosing period. In liver, minimal to moderate centrilobular perivascular inflammatory reaction was observed in males. Minimal increase in the number of multifocally dispersed centrilobular hepatocytes with a clear appearance was noted in the groups treated with 10 and 40 mg/kg/day. Moreover, a slight to moderate increase of MPS aggregates, slight to minimal centrilobular hepatocellular single cell necrosis and multifocal centrilobular perivascular fibrosis and minimal multifocal bile duct proliferation occurred in males and females dosed with 10 and 40 mg/kg/day.

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

Given the dose-related trend in endocrinology results and ovaries weight already evident in the group treated with the low dose, 5 mg/kg/day, a No Observed Effect Level (NOEL) was not established. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 13 below. The mean adrenal gland to plasma ratios of the concentrations of RPV base ranged from 1.3 to 3.6 without clear dose relationship. After 1 month of recovery, the concentration of RPV base in adrenal gland as well as in plasma was below the limit of quantification.

Table 13: Exposure to RPV base (C_{max} and AUC) in a 1-Month Toxicity Study Including 1-Month Recovery with RPV Base in Dogs

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

M: males, F: females

3.1.4.2.2. 6-Month Toxicity Study Including 3-Month Interim Evaluation in Dogs

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 6 months to groups of 6 male and 6 female beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg (Mod4.2.3.2/TMC278-NC115 (TOX6110) and Mod2.6.7.7E). Regular observations were made for clinical signs, morbidity and mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations, ECG recordings, and samples for clinical pathology were taken prior to dosing, prior to 3-month interim evaluation, and at the end of the dosing

period. Clinical pathology comprised a standard set of hematological, serum chemistry and urinalysis parameters. In addition, serum concentrations of 17α-hydroxyprogesterone, progesterone, cortisol, and ACTH were determined. For the determination of AUCs for ACTH and cortisol, 4 samples were taken over a 24-hour period from the first 3 male and 3 female animals in each group. Blood samples for toxicokinetics were taken towards the 3-month interim evaluation, and towards the end of the dosing period. In addition, on the day of necropsy, just prior to the sacrifice, a single blood sample and at necropsy samples of liver and adrenal gland were taken for the determination of RPV base. After 3 months of treatment, the last 2 males and 2 females of each group were killed for the interim evaluation. The remaining animals were killed at the end of the 6-month treatment period. At both necropsies, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology.

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

Serum chemistry towards 3 months of dosing showed 40 and 23% increase in cholesterol concentrations of males and females, respectively, dosed with 40 mg/kg/day. Total bilirubin was increased 46% in females treated with 10 mg/kg/day and 31 and 77% in males and females, respectively, dosed with 40 mg/kg/day. ALP activity was increased 85% in the females dosed with 5 and 10 mg/kg/day and almost 3.5-fold in females dosed with 40 mg/kg/day. The increase was 28% and 72% in males treated with respectively 10 and 40 mg/kg/day. The same parameters were increased at the end of the 6-month treatment period to an almost similar extent as after 3 months of treatment, except ALP. The enzyme activity in males and females dosed with 40 mg/kg/day had increased 130% in males and almost 5.5-fold in females.

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

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

Given the changes seen in adrenals and ovaries at the lowest dose of 5 mg/kg/day, no NOAEL was established. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 14 below. The mean adrenal gland to plasma ratios of the concentrations of RPV base ranged from 4.1 to 8.1 over the groups without a clear dose relationship. Liver to plasma ratios of the concentrations of RPV base ranged from 7.7 to 13. Adrenal and liver to plasma ratios did not change between Day 93 and Day 184/185 of treatment.

Table 14: Exposure to RPV base (C_{max} and AUC) in a 6-Month Toxicity Study with RPV Base in Dogs

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

M: males, F: females

3.1.4.2.3. 12-Month Toxicity Study in Dogs

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 12 months to groups of 4 male and 4 female beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg (Mod4.2.3.2/TMC278-NC107 (TOX6314) and Mod2.6.7.7F). Regular observations were made of clinical signs, morbidity and, mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations and ECG recordings were made and samples for hematological, serum chemistry and urinalysis were taken prior to dosing, after 1 week (ECGs only), 1, 3, 6, and 9 months of treatment and at the end of the dosing period. Serum concentrations of 17α-hydroxyprogesterone, progesterone, estradiol, testosterone (in males only), and luteinizing hormone (LH, in males only) were determined in samples taken prior to the first administration (no samples for testosterone and LH), and prior to dosing after 3 (no samples for testosterone and LH), 6, and 9 months of treatment, and at the end of the treatment period. For the determination of AUCs for ACTH and cortisol, 4 samples were taken over a 24-hour period before the first administration and after 3, 6, 9, and 12 months of treatment at 1, 2, 4, and 24 hours after a daily dose of RPV base. Blood samples for toxicokinetics were taken on Day 1 and after 3 and 6 months of treatment and prior to necropsy. At necropsy, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology. The female animal dosed with 10 mg/kg/day that was killed for humane reasons following a gavage error on Day 21 was replaced.

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

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

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

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

Terminal body weight showed a dose-related reduction in males notably in all groups treated with RPV base. Mean adrenal gland weight was increased in animals treated with 40 mg/kg/day. The absolute increase was 23% in both males and females. The increase relative to body weight was 50% in males and 46% in females. This increased organ weight is probably associated with the (slight to moderate) increase in the size of the zona fasciculata in the adrenal cortex of one male and 3 females treated with 40 mg/kg/day and the bilateral focal hypertrophy of cells in this zone in one male of the same group. Moreover, histopathology showed dense cytoplasm of the cells in the zona fasciculata and/or reticularis of all females treated with RPV base, including the animals killed intercurrently, and of a single male dosed with 10 mg/kg/day, and the majority of the males treated with 40 mg/kg/day. This effect was usually associated with a minimal reduction of cellular neutral fat deposits. In the high dose group, half of the males and females showed pigment deposits in the adrenal cortex. The weight of ovaries showed a dose-related increase in females dosed with 10 and 40 mg/kg/day. The absolute increase was respectively 40 and 120% of control values. The increase relative to body weight was respectively 48 and 170% of control values. These weight effects are likely associated with the increase in the number of antral follicles and the prominent corpora lutea in the animals dosed with 10 and 40 mg/kg/day. In liver, yellow pigmentation in hepatocytes and canaliculi was noted in all males and females given 40 mg/kg/day and in one female given 10 mg/kg/day. Prominent brown pigment in the epithelium of the gall bladder was noted in males given 40 mg/kg/day. In testes, minimal

hypertrophy of the Leydig cells was recorded in two males given 40 mg/kg/day. However, this effect had no impact on Sertoli cell functioning or spermatogenesis as an evaluation of the main stages of the spermatogenic cycles did not indicate any changes. The kidney changes were only seen in the group treated with 40 mg/kg/day and included acute interstitial nephritis in two males and minimal to slight corticomedullary mineralization in all terminally killed females.

As a consequence of the adrenal changes at the low dose group, no NOAEL was established. Systemic exposure expressed as C_{max} and AUC_{0-24h} values are given in Table 15 below.

Table 15: Exposure to RPV base (C_{max} and AUC) in a 12-Month Toxicity Study of RPV Base in Dogs

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

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

3.1.5. Studies in Non-Human Primates

Non-human primates like man have a significant adrenal androgenic pathway which is not present in rodents and dogs. For that reason, the effects of RPV on that pathway were evaluated in cynomolgus monkeys. Estrous cycle physiology in cynomolgus monkeys resembles that of man better than dogs. Consequently, this non-human primate species at an age prior to puberty was used to evaluate also the early ovarian maturation observed in immature dogs.

The doses for the toxicity study in cynomolgus monkeys were selected on the basis of a toxicokinetic study. For the repeat-dose part of that toxicokinetic study (Mod4.2.3.2/TMC278-TiDP38-NC326 (FK6541) and Mod2.6.7.6), the animals were habituated to restraining chairs for ECG recordings, and cortisol, progesterone and ACTH levels were determined. RPV was administered for 15 days to 3 groups of 3 young mature female cynomolgus monkeys at 0 (vehicle), 200 (as 2 x 100) or 500 (as 2 x 250) mg/kg/day. Blood for hormone analysis was taken at pre-dose and on Days 1 and 14 at 0, 1, 2, 4, 8, 9, 10, 12, 16 and 24h. ECG and blood pressure were recorded on Days 2 and 15. No clear test article-related changes were noted for progesterone and ACTH. A decrease in cortisol levels was seen at the end of the dosing period in the high dose group only. No abnormalities were noted on RR, PR, or QT (also corrected according to Fridericia) intervals, on the form of the QRS complex or the T-wave. Moreover, no treatment-related effects were noted on the systolic, diastolic, or mean arterial blood pressure. Mean heart rate at all times was similar in all groups, ranging from 226 to 248 beats/min.

3.1.5.1. 8-Week Oral Toxicity Study with RPV in Juvenile Female Cynomolgus Monkeys

RPV suspended in 1% m/v aqueous HPMC with 0.5% Tween 20 was administered for 8 weeks to three groups of eight immature female cynomolgus monkeys at 0 (vehicle), 200 or 500 mg/kg/day. Animals were dosed 0, 100, or 250 mg/kg twice daily (b.i.d.), with a 6-hour interval, at a volume of 5 mL/kg (Mod4.2.3.2/TMC278-TiDP38-NC248 (TOX8862) and Mod2.6.7.7G). Regular observations of clinical signs, menses, morbidity and mortality were made. Weekly, body weights were determined. Blood samples were taken for clinical pathology including hematology, clinical chemistry, and urinalysis prior to the start of dosing and towards the end of the dosing period. Blood samples for endocrinology (progesterone, estradiol, and LH) were taken prior to the start of the dosing period and weekly prior to the RPV administration (at approximately the same time as the samples were taken during the dosing period). ACTH stimulation tests including determination of serum levels of ACTH (only prior to challenge), cortisol, 17α-hydroxyprogesterone, progesterone, androstenedione, and dehydroepiandrosterone (DHEA) prior to ACTH challenge and 15, 30, and 60 minutes after challenge were done prior to the start of the dosing period and 2, 4, and 6 weeks after the start of dosing. Samples for toxicokinetics were taken the day before necropsy at 10 time points over a 24-hour period. At necropsy at the end of the dosing period, gross lesions were recorded. A range of organs was weighed, and a complete set of organs and tissues was examined microscopically.

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

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

Only incidental vaginal bleedings not indicative of menses were recorded. Moreover, no follicular or ovulatory effects were noted on serum levels of progesterone, estradiol, or LH. Microscopic evaluation of ovaries did not show any indication of activation. Minimal follicular

cell hypertrophy in the thyroid gland was scored in 1 control animal, 3 animals dosed with 200 mg/kg/day, and 4 animals dosed with 500 mg/kg/day.

Since at the lowest dose tested several hormonal effects were evident, no NOAEL was established. Systemic exposure expressed as C_{max} and AUC values are given in Table 16 below.

Table 16: Exposure to RPV base (C_{max} and AUC) in an 8-Week Oral Study with RPV in Juvenile Female Cynomolgus Monkeys

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

C_{max1} = C_{max} after 1st daily dose, C_{max2} = C_{max} after 2nd daily dose

3.1.6. Overall Conclusion

The repeat-dose toxicity studies have identified a series of targets of toxicity of RPV (base): red blood cells in mouse, rat, and dog, coagulation in rat, liver in rat and dog, kidneys in mouse and dog, thyroid gland with secondary effects on the pituitary gland in rat, adrenal glands in mouse, rat, dog, and cynomolgus monkey, testes in dog, and ovaries in dog, in immature females with secondary effects on other parts of the genital tract and on mammary glands, compared to immature control animals.

The majority of the induced effects appeared to be completely reversible after a recovery period. The effects on thyroid gland and coagulation in rats showed signs of recovery but this was not complete at the end of the 1-month post-dosing period.

For mice and rats, a NOAEL could be established. However, dogs and cynomolgus monkeys showed effects at the level of the adrenal glands at the low dose tested. Moreover, dogs showed also effects on ovaries in almost all pivotal studies at the low dose given. These effects prevented establishment of NOAELs in these species.

3.2. RPV LA

Repeat-dose toxicity studies with RPV LA (in the G001 clinical formulation, being 300 mg/mL RPV LA suspension in the presence of 50 mg/mL P338) have been performed in beagle dogs and Göttingen minipigs and are described below. In addition, a 9-month toxicity study with 3-month interim sacrifice was conducted in minipig with the F006 formulation; as this study is considered supportive, it is described in Section 8.7.2.1.

3.2.1. Dog

In a 4-week GLP study, male and female beagle dogs (n=3/sex/group) received IM injections of RPV LA (clinical formulation G001) at 150 and 1200 mg/dog at 2-week intervals (i.e., injections on Days 1 and 15). Potential reversibility of any findings was evaluated during a 2-week recovery period (additional 2 dogs/sex/group for vehicle and high dose). Necropsies were performed on Day 29 (main dogs) and Day 43 (recovery dogs). Assessments included mortality, clinical and eye observations, dermal scoring, food consumption, body weight, electrocardiogram (ECG), hematology, clinical chemistry, coagulation, urinalysis, toxicokinetics

(Table 17), organ weights, and gross and microscopic pathology. In addition, cortisol, progesterone and 17α-(OH)-progesterone levels were determined in blood (Mod4.2.3.2/TOX10759 and Mod2.6.7.7H), to allow comparison of the systemic toxicity profile with the oral RPV formulation.

No mortality was observed in the study. No treatment-related changes in body weight or food consumption, and no eye, electrocardiology, hematology (at 150 mg), clinical chemistry, urinalysis and endocrinology (at 150 mg) findings were noted.

One dog at 150 mg and all dogs at 1200 mg exhibited moderate swelling/slight edema at the administration sites. Administration site swelling/edema was still evident at the end of the 2-week recovery period.

At 1200 mg, slightly higher eosinophils and fibrinogen counts, indicative of a minimal inflammatory response, were observed on Day 16. In males minimal to slight increases in progesterone and 17α-(OH)-progesterone were noted from Day 1 onwards up to the end of recovery. Cortisol levels did not change.

Liver weights were increased at 1200 mg, in females at the end of dosing and prostate weights were increased in males at the end of dosing and recovery, with no microscopic or clinical pathology correlates. Their significance is equivocal.

At 150 and 1200 mg, white deposits at the administration site correlated with amorphous eosinophilic deposits, macrophage aggregation and mononuclear cell infiltration seen at histopathology. Draining lymph nodes showed pale discoloration, and microscopically macrophage aggregation was observed.

In conclusion, IM administration of RPV LA once every 2 weeks for 4 weeks at 150 and 1200 mg/dog was well tolerated. Most findings were local to the administration site and progesterone related changes at the high dose were considered consistent with the toxicological profile of RPV after oral administration at the low dose in dogs (5 mg/kg/day). Corresponding exposure values at the 1200mg dose were 1.44 and 1.23 µg/mL (C_{max}) and 217 and 206 µg.h/mL (AUC_{0-264h}) for males and females, respectively. In addition, AUC_{0-600h} values were 435 and 410 µg.h/mL for males and females, respectively.

Table 17: Mean Toxicokinetic Parameters of RPV After Two Intramuscular Injections of RPV LA in Male and Female Beagle Dogs Given 2 Weeks Apart

Dose	Day	C_{max} (µg/mL)		AUC (µg.h/mL)[a]		AUC_{0-600h} (µg.h/mL)	
		M	F	M	F	M	F
150 mg	1	0.12	0.25	28.8	45.7	-	-
	15	0.18	0.39	34.5	48.5	63.4	94.2
1200 mg	1	1.22	1.22	218	204	-	-
	15	1.44	1.23	217	206	435	410

[a] AUC_{0-336h} on Day 1 and AUC_{0-264h} on Day 15

3.2.2. Minipig

3.2.2.1. 6-Week Toxicity Study

In a 6-week GLP study the systemic and local toxicity of 600 mg RPV LA (clinical formulation G001,) was evaluated in Göttingen minipigs (n=3/sex/group). RPV LA was administered IM (2 mL/injection) at alternating injection sites once every two weeks (Day 0, 14, 28 and 42). Three other groups were included: one control solution (buffer without P338) and two P338 containing groups (solution containing 50 mg/mL and 160 mg/mL [maximum feasible]). The animals were sacrificed 3 or 4 days after the last injection. Assessments included mortality, clinical observations, food consumption, body weight and weight gain, hematology, clinical chemistry, coagulation, biomarkers (C-reactive protein [CRP] and haptoglobin), urinalysis, toxicokinetics (Table 18), organ weights, and gross and microscopic pathology (Mod4.2.3.2/TMC278-NC368 (TOX9508) and Mod2.6.7.6).

No test article-related mortality was observed. No effects on body weight, weight gain, food consumption, hematology, coagulation, clinical chemistry, urinalysis, haptoglobin or organ weights were noted in any of the groups.

In serum, marked increases in CRP were seen in all treatment groups (i.e., RPV LA and vehicle groups) within 24 hours of injection, but were more pronounced and had an earlier onset in animals administered the formulation containing RPV LA.

In the negative control and vehicle groups, no relevant clinical signs were noted, and no relevant gross or histological changes were seen. In animals receiving the RPV LA formulation, slight, short-lasting erythema was occasionally observed in males the day after injection and in females 1 to up to 4 days after injection. At necropsy, white deposits (3-6 cm) at the injection sites, and white discoloration of draining lymph nodes were observed, which was associated with swelling of the medial iliac lymph node in one male. This correlated histologically with a granulomatous reaction consisting of macrophage infiltration (filled with eosinophilic material believed to be RPV), and extracellular amorphous pale eosinophilic deposits, accompanied by an inflammatory infiltrate in some females. The draining medial iliac and/or deep inguinal lymph nodes showed macrophage infiltration and multinucleated macrophages (with eosinophilic contents) with focal amorphous eosinophilic deposits in a deep inguinal lymph node of one female.

In conclusion, IM injections (every 2 weeks) of RPV LA, or P338 controls to minipigs for a period of 6 weeks were well tolerated.

Table 18: Mean Toxicokinetic Parameters of RPV After Intramuscular Injections of RPV LA Given 2 Weeks Apart to Male and Female Minipigs Apart For 6 Weeks

Dose	Day	C_{max} (µg/mL)		AUC_{0-336h} (µg.h/mL)	
		M	F	M	F
600	0	0.58	1.00	18.38	33.15
	28	0.72	1.23	51.36	52.75

3.2.2.2. 9-Month Toxicity Study

In a 9-month GLP study the systemic and local toxicity of the clinical formulation G001 were determined in Göttingen minipigs (n=3/sex/group). The formulation was administered once monthly by IM injection with a fixed injection volume of 2 mL resulting in a dose of 600 mg per injection. A control group only receiving the buffer as used in the G001 formulation, and a vehicle control group receiving that buffer in the presence of 50 mg/mL P338 were included. Assessments included mortality, clinical observations, food consumption, body weight and weight gain, hematology, clinical chemistry, coagulation, biomarker CRP, urinalysis, toxicokinetics (Table 19), organ weights, and gross and microscopic pathology (Mod4.2.3.2/TMC278-NC349 (TOX9517) and Mod2.6.7.7I)

No mortality occurred during the study. No adverse effects on body weight, weight gain, food consumption, clinical chemistry, urinalysis or organ weights were noted.

Hematology showed a minimal increase in white blood cell counts (lymphocytes, monocytes, eosinophils, basophils) and fibrinogen in single animals after the last dose. A tendency towards an increase in CRP relative to vehicles and controls was found in the RPV LA-dosed minipigs 24 hours after IM injection on Day 0 and Day 224. CRP values had returned to baseline 3 days after those injections.

After the 1st injection (Day 1), slight erythema (<2 cm) was observed in 2 males and 1 female dosed with RPV LA for 1 day. At necropsy, white deposits at the injection sites, and swollen and/or discolored regional lymph nodes (popliteal, medial and internal iliac) were seen in the RPV LA-dosed group. Microscopic examination showed macrophage infiltration at the injection site. The macrophage infiltration response was variable in the regional lymph nodes. Areas of eosinophilic deposits were seen at the last injection site (injection 3 days prior to autopsy) of the males, most likely corresponding to not-yet-phagocytosed formulation. No relevant in-life or post-mortem findings were associated with dosing the vehicle including P338.

In conclusion, 600 mg RPV LA (G001 formulation) was well tolerated when administered monthly via IM injection for 9 months.

Table 19: Mean Toxicokinetic Parameters of RPV After Intramuscular Injections of RPV LA Given 2 Weeks Apart to Male and Female Minipigs For 9 Months

Dose	Day	C_{max} (µg/mL)		$AUC_{Day0-30}$ (µg.h/mL)	
		M	F	M	F
600 mg	0	0.19	0.64	20.70	34.96
	224	0.35	0.40	50.07	44.34

3.2.3. Overall Conclusion

RPV LA was well tolerated when administered IM to dogs or minipigs with main findings related to local injection site effects and consisting of slight to moderate short-lasting erythema/swelling and white eosinophilic deposits at the injection site. Macrophage infiltration in these deposits and regional lymph nodes were accompanied by swelling and discoloration of the lymph nodes. The systemic toxicity related effects noted in dogs administered RPV LA were consistent with those after oral administration.

4. GENOTOXICITY

Genotoxicity testing of RPV base or RPV comprised 4 nonmammalian (bacterial) reverse gene mutation (Ames) tests, 2 mammalian (mouse lymphoma) forward mutation assays and 1 in vivo mouse bone marrow micronucleus assay. All in vitro studies, with the exception of one Ames test, were done in the absence and in the presence of a metabolic activation system (S9-mix) using liver S9 fraction from Aroclor-treated rats. One Ames test was conducted in the presence of human S9. Prior to the main in vitro studies, cytotoxicity range-finding experiments were performed. These studies were conducted in support of the registration of oral RPV (EDURANT®). As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), the conclusion on potential effects can be applied to the RPV LA formulation as well.

4.1. In Vitro Nonmammalian Cell System

4.1.1. Ames Tests with Rat Liver S9

Rilpivirine base and RPV dissolved in dimethyl sulfoxide (DMSO; final concentration 0.1%) were tested in bacterial reverse gene mutation tests (Ames test) (Mod4.2.3.3.1/TMC278-Exp.5540, Mod4.2.3.3.1/TMC278-Exp.5693, Mod4.2.3.3.1/TMC278-TiDP6-NC335 (TOX8918), and Mod2.6.7.8A, B, and C). The Ames tests were done, in triplicate, with 5 histidine-requiring strains of *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and TA102. On the basis of precipitation that limited scoring in range-finding studies with plate incorporation and concentrations up to 5000 µg/plate, RPV base was tested in two main studies. Each of the studies comprised 2 independent repeat assays using the plate incorporation method up to 500 µg/plate. RPV was studied in one experiment with 2 independent repeat assays, one using the plate incorporation method and the other the pre-incubation method, up to 250 µg/plate.

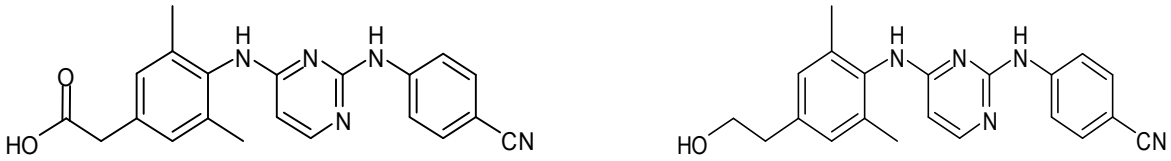
In none of these studies, RPV base or RPV tested at the highest scorable concentration[a] induced a biologically significant increase (more than 2 times the frequency of the vehicle) of the mutation frequency in any of the tested strains, irrespective of the presence of metabolic activation. In all these studies, the positive controls gave the anticipated results indicating the validity of the test system.

[a] In the 2nd assay of the study with RPV base (Mod4.2.3.3.1/TMC278-NC279 (TOX8086)), the pre-incubation method was used. Under these conditions, no precipitation occurred at the highest tested concentration in the absence of S9.

4.1.2. Ames Test with Human Liver S9

The metabolic pathway of RPV generating metabolites M30 and M31 (Figure 1) encompasses potentially reactive metabolites (epoxide) due to Michael addition at the α,β-unsaturated nitrile moiety [7].

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



Usually, metabolic pathways are evaluated in in vitro genotoxicity studies due to the presence of the S9 fraction of Aroclor-induced rat liver. However, rat liver S9 appeared not to yield either of these metabolites (Mod4.2.2.4/TMC278-NC102 (FK4728)) and for that reason, it was unclear whether the metabolic pathway with the potentially reactive metabolites had been adequately evaluated. In contrast, some batches of human liver S9 did yield M30 and M31. Moreover, M30 was detected in feces at 3% of the administered dose in the human metabolism study (Mod5.3.3.1/TMC278-C119). For these reasons, RPV base was tested in an Ames test in the presence of human liver S9 (Mod4.2.3.3.1/TMC278-NC279 (TOX8086) and Mod2.6.7.8D). RPV base dissolved in DMSO was tested in 4 independent assays up to 500 µg/plate: the highest scorable concentration due to precipitations. The concentration of M30 + M31 (incomplete separation in liquid chromatography) indicated that the human S9 metabolic enhancer was active. The validity of the test system was evaluated by the response of positive controls used in the absence of a metabolic enhancer upon plate incorporation or pre-incubation, separate from each of the 4 assays. In parallel, RPV metabolites M30 and M31, the carboxylic and hydroxyl metabolites of the α, β-unsaturated nitrile moiety of RPV, respectively, were determined by means of radiolabel LC-MS. In the first parallel assay, only the presence of M30 and M31 could be established due to the low radioactive dose. In the subsequent parallel incubations, a higher specific radioactivity of ^{14}C-RPV was applied allowing the quantitative determination of the sum of M30 and M31.

It is concluded that RPV, under conditions that it is metabolized via a pathway encompassing potentially reactive intermediates such as epoxide, did not induce a biologically significant increase of the mutation frequency. The experimental conditions in the Ames test yielded M30 + M31 at 1.4 – 2.6% of the dose applied (Mod2.6.7.8D).

4.2. In Vitro Mammalian Cell System

RPV base and RPV dissolved in DMSO were tested in mouse lymphoma L5178Y cells (Mod4.2.3.3.1/TMC278-Exp.5539, Mod4.2.3.3.1/TMC278-TiDP6-NC336 (TOX8919)) for their potential to induce forward mutations at the tk locus (5-trifluorothymidine resistance). Prior to the main studies, a cytotoxicity range-finding experiment was performed for both compounds.

The maximum tested concentration of RPV base on the basis of precipitation that limited scoring was 500 µg/mL for a 3-hour exposure period without metabolic activation, 35 µg/mL for a

3-hour exposure period with metabolic activation, and 100 µg/mL for a 24-hour exposure period without metabolic activation (Mod2.6.7.8E).

RPV was tested in one study with 2 independent repeat assays up to 50 µg/mL for a 3-hour exposure period without metabolic activation, maximally 20 µg/mL for a 3-hour exposure period with metabolic activation, and up to 50 µg/mL for a 24-hour exposure period without metabolic activation (Mod2.6.7.8F).

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

4.3. In Vivo Mammalian System

To evaluate the clastogenic and aneuploidic effect on early forms of erythrocytes in bone marrow, RPV base dissolved in PEG400 + CA was administered to mice in an in vivo micronucleus test (Mod4.2.3.3.2/TMC278-Exp.5538, Mod4.2.3.3.2/TMC278-Exp.5538 (FK4259) and Mod2.6.7.9). Single oral (gavage) doses of 0 (vehicle control), 100, 400 and 1600 mg/kg in 20 mL/kg were administered to 10 male and 10 female CD-1 mice per group. The dose of 1600 mg/kg was the maximally feasible dose on the basis of the maximally feasible volume for a single dose study, 20 mL/kg, and a supersaturated and highly viscous solution of 80°mg/mL. Bone marrow was sampled from 5 males and 5 females per group, 24 and 48 hours after dosing. Systemic exposure in terms of C_{1h} and AUC_{0-6h} was determined. A positive control group of 5 male and 5 female CD-1 mice received a single oral dose of 40 mg cyclophosphamide per kg body weight and was sampled for bone marrow 48 hours after dosing.

RPV base up to a single maximal feasible dose of 1600 mg/kg did not induce structural or numerical chromosome aberrations. At this dose, no bone marrow toxicity or signs of systemic toxicity were noted (Mod2.6.7.9). Exposure data are presented in Table 20.

Table 20: Exposure to RPV base (C_{1h} and AUC) in a Single Oral Dose Bone Marrow Micronucleus Study with RPV Base in Mice

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

M: males, F: females

4.4. Other Systems

No studies in other systems have been conducted.

4.5. Overall Conclusion

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

5. CARCINOGENICITY

These studies were conducted in support of the registration of oral RPV (EDURANT®). As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), the conclusion on potential effects can be applied to the RPV LA formulation as well.

5.1. Long-Term Studies

In view of the use of RPV, i.e. long treatment duration to suppress HIV-1 replication in treatment-naïve patients, in combination with other anti-retroviral compounds and in line with ICH guideline S1 A, entitled "Need for Carcinogenicity Studies of Pharmaceuticals", it was decided that the carcinogenicity potential of RPV should be investigated in animal experiments. Two preliminary range-finding studies in mice were performed; one in wild-type CD-1 mice (Mod4.2.3.2/TMC278-NC118 (TOX6479) and Mod2.6.7.6) and one in CB6F1-nonTgrasH2 mice (Mod4.2.3.2/TMC278-NC121 (TOX6740) and Mod2.6.7.6). For strategic reasons, it was decided to conduct a long-term carcinogenicity study in CD-1 mice.

The results of a 13-week carcinogenicity dose-range finding study in mice (Mod4.2.3.2/TMC278-NC119 (TOX6739) and Mod2.6.7.7A) and of the 6-month oral rat study (Mod4.2.3.2/TMC278-NC101 (TOX6142) and Mod2.6.7.7C) were used to design the long-term carcinogenicity studies. The designs of the main carcinogenicity studies were in line with recommendations of the FDA Carcinogenicity Assessment Committee (CAC) [1]. The CAC requested a much higher top dose in the rat carcinogenicity study than tested in previous pivotal rat repeat-dose studies. For that reason, it was decided to include a fourth dose group in the carcinogenicity study to ensure that the study would finish with at least three dose groups in case the top dose would lead to increased mortality and early termination of that group. As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), no carcinogenicity studies were done with RPV LA. The conclusion on carcinogenic potential can be applied to the RPV LA formulation as well.

5.1.1. 2-Year Carcinogenicity Study in Mice

RPV formulated in 0.5% m/v aqueous HPMC was administered once daily by oral gavage for 24 months (Mod4.2.3.4.1/TMC278-NC120 (TOX7222) and Mod2.6.7.10A). One control and 3 treated groups were given 0 (vehicle), 20, 60 or 160 mg/kg/day at a dose volume of 10 mL/kg. Each group consisted of 60 male and 60 female CD-1 mice (main animals) along with 24 male and 24 female satellite animals for toxicokinetic analysis (18 animals/sex in control group). The intended treatment duration was 104 weeks. Stop-dosing and termination rules communicated and agreed by FDA were applied due to increased mortality rates (See Table 21).

Regular observations were made of mortality, clinical signs, palpable masses, body weight, and food consumption. Sampling for hematology was done at the end of the treatment period. A full necropsy was performed on all animals, including those found dead or sacrificed moribund during the study, and all gross lesions were recorded. A selection of organs was weighed. Tissues and organs indicated by protocol were sampled and preserved for histological examination. Samples of any abnormal tissues were also retained and processed for examination. In those

cases where a lesion was not clearly delineated, contiguous tissue was fixed with the grossly affected region and sectioned as appropriate. In addition to any masses, the lymph nodes draining the regions adjacent to those masses were also preserved. Blood samples were collected for toxicokinetic analysis on Day 1 and during Week 28.

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

Table 21: Mortality in a Mouse Carcinogenicity Study with RPV

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

M: males, F: females, [§]: kept off dose till week 104.

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

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

No relevant changes in hematology were observed.

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

In the liver, a statistically significant dose-related increase in total hepatocellular tumors (adenomas and carcinomas combined) was seen in males dosed with RPV from 20 mg/kg/day onwards. The dose-related increase in liver adenomas, alone, reached statistical significance in males treated at 60 and 160 mg/kg/day. An increase in the males with liver carcinomas, that was not statistically significant, was seen in all groups treated with RPV (see Table 22). The incidences of carcinomas and of adenomas for all groups treated with RPV and for the groups

dosed with 60 and 160 mg/kg/day, respectively, were above the ranges expected from background data of the testing facility. The incidences increasing over background data are considered treatment-related. The incidences of liver adenomas and of adenomas and carcinomas combined were statistically significantly increased and above the background data range in female mice treated with 60 and 160 mg/kg/day (Table 22).

Table 22: Neoplastic Liver Changes in a Mouse Carcinogenicity Study with RPV

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

M: males, F: females

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

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

It was concluded that oral administration of RPV to CD-1 mice at doses of 20, 60, or 160 mg/kg/day produced a dose-related increase in total hepatocellular tumors, adenoma and carcinoma, in males dosed with 20 and in males and females that received 60 and 160 mg/kg/day. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as C_{max} and AUC values is presented in Table 23 below.

Table 23: Exposure to RPV (C_{max} and AUC) in a Mouse Carcinogenicity Study with RPV

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

[a]: $AUC_{0-\infty}$: on Day 1 otherwise AUC_{0-24h}, M: males, F: females

5.1.2. 2-Year Carcinogenicity Study in Rats

RPV formulated in 0.5% m/v aqueous HPMC was administered once daily by oral gavage for 24 months (Mod4.2.3.4.1/TMC278-NC123 (TOX7221) and Mod2.6.7.10B). One control and 4 treated groups were given 0 (vehicle), 40, 200, 500, or 1500 mg/kg/day at a dose volume of 10 mL/kg. Each group consisted of 65 male and 65 female Sprague-Dawley rats (main animals) along with 9 male and 9 female satellite animals for toxicokinetic analysis (6 animals/sex in

control group). The intended treatment duration was 104 weeks. Dosing of the females from group 2 was completed in Week 98. All female groups were terminated in Week 99 due to increased mortality rates according to stop-dosing and termination rules in agreement with FDA.

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

There was no effect of treatment on mortality (see Table 24). No treatment-related pathologies contributing to death and no adverse clinical signs were observed.

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

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

Table 24: Mortality in the Rat Carcinogenicity Study with RPV

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

M: males, F: females

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

controls from a dose of 200 mg/kg/day onwards. The lowering of α2-globulin was already statistically significant at a dose of 40 mg/kg/day.

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

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

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

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

In thyroid gland, when compared with the controls, a statistically significant increase of the number of follicular cell adenomas and of adenomas and carcinomas combined was seen in all groups of male and female rats treated with RPV with only a marginally apparent dose-related trend. The incidence of follicular cell adenomas was above background levels in the males and females at 200, 500, or 1500 mg/kg/day. The incidence of the carcinomas was above background levels in males only at 200 and 1500 mg/kg/day. These neoplastic changes presented in Table 25 are considered to be treatment-related. Non-neoplastic changes related to these tumors included

increased incidences of follicular cell hypertrophy and cystic follicular cell hyperplasia were seen in females of all groups treated with RPV. In males dosed with 200, 500, or 1500 mg/kg/day, the incidence of follicular cell hypertrophy was increased.

None of the liver or thyroid gland tumors was considered contributory to death in any rat.

Table 25: Neoplastic Liver and Thyroid gland Changes in a Rat Carcinogenicity Study with RPV

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

M: males, F: females

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

It is concluded that the oral administration of RPV to Sprague-Dawley rats at doses of 40, 200, 500, or 1500 mg/kg/day produced neoplastic changes in liver and thyroid gland. Increased incidences of liver adenomas were seen in males and females given 200 mg/kg/day or above and in females at 40 mg/kg/day without attaining statistical significance. Moreover, a statistically significant increase in follicular cell adenoma and/or carcinoma was seen in the thyroid gland among all groups of males and females treated with RPV, generally without a dose-related trend. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as C_{max} and AUC values is presented in Table 26 below.

Table 26: Exposure to RPV (C_{max} and AUC Values) in a Rat Carcinogenicity Study with RPV

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

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

5.2. Other Studies

No other carcinogenicity studies have been conducted.

5.3. Overall Conclusion

The carcinogenicity studies with RPV in mice and rats demonstrated in both species an increased incidence of hepatocellular tumors: adenomas and carcinomas in mice and adenomas in rats. In the latter species, also follicular cell adenomas and carcinomas in thyroid gland were noted. No neoplastic lesions were noted associated with the other targets of toxicity of RPV. The carcinogenic potential of RPV is discussed in Section 9.3. As the active moiety for the oral and LA formulation is essentially the same this conclusion is applicable to the RPV LA formulation as well.

6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive and developmental toxicity of RPV was evaluated in studies on fertility and early embryonic development with rats, studies on embryofetal development with rats and rabbits, and a pre- and postnatal developmental study in rats. The embryofetal development studies were conducted with RPV base; the other studies with RPV. These studies were conducted in support of the registration of oral RPV (EDURANT®). As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), no reproductive and developmental studies were done with RPV LA. The conclusion on potential effects can be applied to the RPV LA formulation as well.

6.1. Fertility and Early Embryonic Development

6.1.1. Male Rat Fertility Study with RPV

RPV suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage to groups of 25 male Sprague-Dawley rats at 0 (vehicle), 100, 400, or 1600 mg/kg/day in a dose volume of 10 mL/kg (Mod4.2.3.5.1/TMC278-NC124 (TOX6861) and Mod2.6.7.12A). Treatment started 10 weeks prior to mating, during mating and for 3-4 weeks after the mating period. The males were paired 1:1 for mating with untreated females. Successful mating was established on the basis of sperm detection in vaginal lavage. Regular observations were made for clinical signs, morbidity, and mortality. Male body weight was recorded weekly throughout the dosing period and food consumption during the pre-mating period. At necropsy of the males, 3 to 4 weeks after the end of the mating period, gross lesions were recorded. The testes and epididymides were removed, weighed and examined histopathologically. One of the cauda epididymides from each male was sampled for sperm motility, concentration, and morphology. The liver and thyroid gland were weighed and fixated.

There were no mortalities associated with RPV. All pairs except one (male dosed with 100 mg/kg/day) mated within 4 days after pairing. There were no relevant clinical signs and no relevant effects on body weight, food consumption, gross or histopathological lesions, weights of epididymides or testes, and no adverse effects on the motility, concentration, or morphology of the sperm. There was no effect on fertility up to 1600 mg/kg/day. Weights of liver and thyroid

gland showed a dose-related increase (absolute and/or relative to body weight) in all groups receiving RPV.

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

6.1.2. Female Rat Fertility and Early Embryonic Developmental Study with RPV

RPV suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage to groups of 24 female Sprague-Dawley rats at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (Mod4.2.3.5.1/TMC278-NC125 (TOX6708) and Mod2.6.7.12B). Treatment started 2 weeks prior to mating, during mating and till Day 7 of pregnancy. The females were paired 1:1 for mating with untreated males. Successful mating was established on the basis of sperm detection in vaginal smears. Regular observations were made for clinical signs, morbidity, and mortality. Female body weights were determined weekly till pregnancy and on pregnancy Days 0 (day of sperm detection), 8, and 14 and food consumption was determined weekly during the pre-mating period and pregnancy. Estrous cycle was determined on vaginal smears taken daily during the mating period and the pre-coital interval was calculated. The females were sacrificed on Day 14 of pregnancy for evaluation of pregnancy status and determination of copulation and fertility rates (e.g., number of corpora lutea, implantations and live fetuses).

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

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

6.2. Embryofetal Development

6.2.1. Studies in Rats with RPV Base

The doses for the rat embryofetal development study were selected on the basis of the results of a pilot study (Mod4.2.3.5.2/TMC278-NC127 (TOX6147) and Mod2.6.7.11) in which no significant effects occurred. RPV base dissolved in PEG400 + CA was administered once daily, by oral gavage, from Gestation Day (GD; day of sperm detection is GD 0) 6 to 17 to groups of 30 (6 satellite animals for toxicokinetic sampling) pregnant Sprague-Dawley rats at 0 (vehicle, 24 animals), 40, 120, or 400 mg/kg/day at a dose volume of 10 mL/kg (Mod4.2.3.5.2/TMC278-NC105 (TOX6268) and Mod2.6.7.13A). Regular observations were made for clinical signs, morbidity, mortality, body weight, and food intake. Blood samples from satellite animals (3 time points over a 24-hour period from 3 females per group) were collected on GD 6 and GD 16. The satellite animals were killed after the last sampling. The dams from the main study were sacrificed on GD 21 and gross lesions and the weight of thyroid gland were recorded. Pregnancy status and the numbers of corpora lutea, implantations, resorptions, and live and dead fetuses

were noted. The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no mortalities associated with RPV base. There were no relevant clinical signs. Reduced body weight gain (maximally 7%) and food consumption (maximally 4%) were noted in dams given 120 and 400 mg/kg/day. Weight of thyroid gland showed a dose-related increase (maximally 24%) which was statistically significant in the animals dosed with 120 and 400 mg/kg/day. There were no effects on gross pathology or pregnancy parameters, numbers of corpora lutea, implantations, early and late resorptions, live or dead fetuses, or on the pre- or post-implantation losses. There were also no effects on fetal body weight or sex ratio. Visceral examinations showed a slight increase in dilated renal pelvis in 5 out of 149 and 7 out of 149 fetuses from the groups treated with 120 and 400 mg/kg/day, respectively. The dilated renal pelvis is considered a minor variation. The occurrences of two malformations in two different litters of dams treated with 400 mg/kg/day noted upon external examinations were isolated and dissimilar in nature and bore no relationship with treatment. In view of the isolated nature and the possible occurrence within a normal control population, these were not considered to be test article-related.

The maternal and embryofetal NOAEL was 40 mg/kg/day based on the changes on the body weight, food consumption and the increase of dilated renal pelvis seen at higher doses. Systemic exposure expressed as C_{max} and AUC values are given in Table 27 below.

Table 27: Exposure to RPV base (C_{max} and AUC) in an Oral Embryofetal Developmental Study with RPV Base in Rats

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

F: females

6.2.2. Studies in Rabbits with RPV Base

The doses for the main rabbit embryofetal development study were selected on the basis of the results of 2 pilot studies. In the first study (Mod4.2.3.5.2/TMC278-NC128 (TOX6235)), 75 and 150 mg RPV base/kg/day from GD 6 to 19 incurred complete resorption of litters. In the second study (Mod4.2.3.5.2/TMC278-NC129 (TOX6280)), animals were dosed with 0 (0.5% aqueous HPMC), 5, 20, or 60 mg RPV base/kg/day from GD 6 to 19. The dose of 60 mg/kg/day resulted in reduced fetal survival and post-implantation loss without external fetal abnormalities (Mod2.6.7.11).

In the main study, RPV base suspended in aqueous 0.5% (m/v) HPMC was administered once daily, by oral gavage, from GD6 to 19 to groups of 20 pregnant NZW rabbits at 0 (vehicle), 5, 10, or 20 mg/kg/day in a dose volume of 5 mL/kg (Mod4.2.3.5.2/TMC278-NC130 (TOX6313) and Mod2.6.7.13B). Blood samples for toxicokinetic analysis were collected from 3 animals per

group on GD6 and GD19 at 7 time points during the 24 hours after dosing. Clinical signs, body weight, and food consumption performance were recorded. The females were killed on GD28 and a necropsy was performed. The females were examined for gross lesions, pregnancy status and the numbers of corpora lutea of pregnancy, implantations, resorptions, and live and dead fetuses. The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

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

The maternal NOAEL was at least 20 mg/kg/day and the fetal NOAEL was 10 mg/kg/day. Systemic exposure expressed as C_{max} and AUC values are given in Table 28 below.

Table 28: Exposure to RPV base (C_{max} and AUC) in an Oral Embryofetal Development Study with RPV Base in Rabbits

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

F: females

6.3. Prenatal and Postnatal Development, Including Maternal Function

6.3.1. Dose-Range Finding Pre- and Postnatal Development Study Including Juvenile Exposure in Rats with RPV

The objectives of this pilot study were to investigate the tolerability of doses applied in the general and reproduction toxicity studies on pre- and postnatal development parameters (Mod4.2.3.5.3/TMC278-NC168 (TOX6847) and Mod2.6.7.14A). Moreover, the effects of direct administration of RPV to pups for 2 weeks were evaluated in terms of exposure and postnatal development.

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

There were no mortalities among the dams. Moreover, the dams showed no abnormal clinical signs or effects on body weight or food consumption. There were no changes on pregnancy or litter parameters. Two pups from the group treated with 400 mg/kg/day were killed for humane reasons. There were no effects on the pups treated directly with RPV during the dosing period or at necropsy. Systemic exposure for pups expressed as C_{max} and AUC values are given in Table 29 below.

Table 29: Exposure to RPV base (C_{max} and AUC) in Rat Pups via Lactation (LD7) and after 2 Weeks of Oral Dosing with RPV

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

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

6.3.2. Pre- and Postnatal Developmental Toxicity Study in Rats with RPV

RPV suspended in 0.5% (m/v) aqueous HPMC was administered once daily by oral gavage to groups of 25 time-mated female Sprague-Dawley rats from GD6 to LD 20 at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg (Mod4.2.3.5.3/TMC278-NC131 (TOX6972) and Mod2.6.7.14B). Regular observations were made for maternal clinical signs, morbidity, and mortality. Weekly body weight and food consumption were determined. The females were allowed to litter. Litter size and sex ratio were determined. Pups weight was determined regularly. Developmental landmarks: number of ears open, static righting reflex, eyes open, startle response and pupillary light reflex were recorded on LD3, 5, 15, and 21. At necropsy of the dams after weaning of their litters, the number of implantation scars in uterus

was determined. From the F1 generation, 20 males and 20 females per group were kept and their growth, post-weaning development and behavior and reproductive performance were assessed.

There were no mortalities among the dams. The dams showed no relevant clinical signs, no effects on body weight, food consumption, gestation, or parturition. There were no effects on the numbers of pups born, the sex ratio, or pup survival or physical condition. There were no effects on pre or post-weaning body weight and gain or development and behavior parameters. Sexual development, fertility and mating performance, and pregnancy parameters of the F1-generation were unaffected by treatment of the F0-generation. There were no effects on post-mortem parameters at necropsy of the F0 and F1-generation.

The NOAEL for both the F0 and F1-generation was at least 400 mg/kg/day.

6.4. Overall Conclusion

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

Direct dosing of rat pups from LD 12 to LD 25 up to 400 mg/kg/day was well tolerated.

As the active moiety for the oral and LA formulation is essentially the same this conclusion is applicable to the RPV LA formulation as well.

7. LOCAL TOLERANCE

The potential of RPV was tested in local tolerance studies. Eye irritation and phototoxicity were evaluated in in vitro models and skin sensitization and irritation were in in vivo models.

In addition, an in vitro irritation study and a local lymph node assay with various RPV LA formulations have been conducted. Local tolerance of several RPV LA formulations containing P338 was assessed in rabbits and minipigs.

7.1. RPV (studies supporting the registration of oral RPV (EDURANT®)

7.1.1. In Vitro Studies

7.1.1.1. Eye Irritation - Bovine Corneal Opacity-Permeability Assay

The ocular irritation potential of RPV was assessed in the bovine corneal opacity-permeability (BCOP) assay. RPV was applied for 4 hours as a 20% w/w suspension in saline to the corneal epithelium (Mod4.2.3.6/TMC278-NC202 (TOX7619) and Mod2.6.7.16).

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

7.1.1.2. Phototoxicity - Balb/c Mouse 3T3 Fibroblast Assay

The in vitro Balb/c mouse 3T3 fibroblast assay was used to assess the phototoxic and cytotoxic potential of RPV. Cells were exposed to RPV dissolved in DMSO in bath concentrations ranging from 0.001 to 2.16 mg/L, in the presence and the absence of ultraviolet A radiation. The final concentration of DMSO in Dulbecco's phosphate-buffered saline was 1% (Mod4.2.3.6/TMC278-NC188 (TOX7218) and Mod2.6.7.16).

As the highest concentration had no effect on the Neutral Red uptake by these cells in the presence of ultraviolet A, RPV was considered to be non-phototoxic.

7.1.2. In Vivo Studies

7.1.2.1. Skin Irritation

The skin irritation potential of RPV was evaluated in 3 NZW rabbits. RPV (0.5 g moistened with 0.5 mL water on a gauze) was applied to 4 x 4 cm on the intact clipped skin for 4 hours covered by a semi-occlusive dressing. The skin reactions were scored 1, 24, 48 and 72 hours after removal of the dressing (Mod4.2.3.6/TMC278-NC159 (TOX7555) and Mod2.6.7.16).

All animals survived and there were no noteworthy clinical observations or effects on body weight. No skin reactions, staining of skin or corrosive effects were observed. RPV was classified as "non-irritant" to rabbit skin.

7.1.2.2. Hypersensitivity

A murine local lymph node assay was conducted to establish the sensitization potential of RPV (Mod4.2.3.6/TMC278-NC199 (TOX7453) and Mod2.6.7.16). In step 1 of the experiment, RPV was formulated in an acetone olive oil (4:1) mixture at 5, 10, and 35%. Twenty-five µL of each concentration was applied for 3 consecutive days on the dorsal surface of both ears of 4 female CBA/Ca/Ola/Hsd mice per group. The negative control group received the vehicle. The positive control group was treated with 2.5% streptozocin. Three days later, all animals received an intravenous injection of ^{3}H-methyl thymidine. Five hours later they were killed and the radioactivity in the auricular lymph nodes was measured.

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

7.2. RPV LA

7.2.1. *In Vitro* Hen's Egg Chorioallantoic Membrane Irritation Test (HET-CAM)

The irritation potential of RPV base formulated as a suspension at 200 and 300 mg/mL in different vehicles (all containing polysorbate 80, lipoid egg phosphatidylglycerol [E PG] and dextrose), was evaluated by determining the response times of irritant endpoints (hemorrhage, lysis and coagulation) observed on the chorioallantoic membrane (CAM) of fertilized chicken eggs. A standard treatment of 5 minutes and an extended test of 60 minutes (for detailed evaluation of blood vessels) were conducted. N,N-dimethylformamide was used as positive

control and 0.9% sodium chloride as negative control (Mod4.2.3.6/TMC278-NC301 (TOX8460) and Mod2.6.7.16).

When the CAM was treated for 5 minutes, all vehicle and test item formulation were classified as non-eye irritants under the conditions of the study. When the CAM was treated for 60 minutes, no to very mild effects were observed. The very mild effect could be related to the physicochemical characteristics of the formulation (i.e., viscous, requiring extensive rinsing to remove it from the CAM).

In conclusion, after the standard treatment and extended test, the three vehicles and the parenteral formulations did not induce significant irritation.

7.2.2. Local Lymph Node Assay

In order to assess the potential to cause skin sensitization using the mouse local lymph node assay, RPV was applied topically as 5%, 10% or 35% w/v preparations in acetone:olive oil (4:1) (Step 1, see 7.1.2.2). In addition (step 2), 3 suspension formulations of 2.5% w/v RPV base in 5% vitamin E-D-α-tocopheryl polyethyleneglycol 1000 succinate (VitE-TPGS), Tween 80 or P338 were assessed following topical application. To bypass the skin barrier (step 3), 2.5% RPV base in 2.5% P338 or VitE-TPGS, was also administered via SC injections (Day 1: dorsum of the ear; Day 2: nape of the neck). A positive control group (2.5% streptozotocin) via the SC route was included as well (Mod4.2.3.6/TMC278-NC199 (TOX7453) and Mod2.6.7.16).

In all formulations and routes of exposures the test substance did not show the capacity to cause skin sensitization.

7.2.3. Acute Intramuscular and Subcutaneous Irritation of Formulations including P338 in Rabbits

The acute local irritation of two different formulations of RPV LA was studied in male NZW rabbits (6/group). Aqueous suspension formulations containing 100 mg/mL RPV base (P338 or Vit E-TPGS) were studied after a single (1 mL) IM or SC injection in one non-GLP study (Mod4.2.3.6/TMC278-NC237 (TOX7774) and Mod2.6.7.16). P338- and Vit E-TPGS containing vehicle control groups were included. RPV LA in P338 caused a slightly more prominent acute inflammatory response at the injection site than when formulated in Vit E-TPGS, both by the SC and IM route. On the other hand, RPV base in Vit E-TPGS caused marginally more prominent necrosis than in P338 when injected by the SC route.

In another non-GLP study, six formulations of RPV LA suspensions and their vehicles were tested comparatively in female NZW rabbits (6/group) for acute irritation after (1 mL) IM injection (Mod4.2.3.6/TMC278-NC367 (TOX9445) and Mod2.6.7.16). The different formulations were: P338 (50 mg/mL) with RPV LA at 2 doses (200 or 300 mg), P338 (50 mg/mL)/Na-deoxycholate (2 mg/mL) with RPV LA at 2 doses (200 or 300 mg), P338 (50 mg/mL)/polyethylene (PEG) 350 phosphatidylethanolamine (PE) (1.5 mg/mL) with RPV LA at 300 mg/kg, and P338 (50 mg/mL)/(DOSS) (2 mg/mL) with RPV LA at 300 mg. The respective vehicle controls were included. The formulations containing 200 or 300 mg of RPV

LA and 50 mg/mL of P338 showed the most favorable characteristics based on local injection site reactions and interstitial distribution.

7.2.4. Acute Intramuscular Irritation of Formulations including P338 in Minipigs

In two non-GLP pharmacokinetic and local tolerance studies, six different formulations of RPV LA were compared in Göttingen minipigs (3/group).

In the first study (Mod4.2.2.7/TMC278-NC344 (FK7034) and Mod2.6.7.16), six formulations with different concentrations of RPV LA containing either P338 or Tween/lipoid, were tested. The doses of RPV LA varied between 200 and 600 mg, and the concentrations ranged from 100 to 300 mg/mL. All formulations were well tolerated. No clearly discernible differences were noted between the formulations in terms of local and systemic effects.

In the second study (Mod4.2.3.6/TMC278-NC359 (TOX9403) and Mod2.6.7.16), RPV LA formulations contained P338, P338/Na-deoxycholate, P338/PE PEG 350 or P338/DOSS. The doses of RPV base were 400 or 600 mg, and the concentrations were 200 or 300 mg/mL. All formulations were well tolerated when administered IM.

The highest plasma exposures achieved were at the 600 mg/minipig dose level with formulations of RPV LA with P338 (i.e., G001 formulation) and RPV LA with P338/DOSS (see Mod2.6.4/Sec3.1.5.1).

7.3. Overall Conclusion

RPV was classified as a moderate eye irritant in vitro but was not skin-irritating in an in vivo rabbit irritation test. RPV tested negative for the potential to cause phototoxicity, and did not induce delayed-type hypersensitivity or immunotoxic effects on the challenge of rats with sheep red blood cells.

RPV LA did not show irritating properties in vitro. RPV LA was not a skin sensitizer and there were no signs of delayed-type sensitization in a mouse local lymph node assay. Different formulations with RPV LA and P338 dosed once IM in rabbits or minipigs were well tolerated with limited local injection site effects.

8. OTHER TOXICITY STUDIES

Studies to investigate the mechanism of action of RPV (base) on adrenal hormones and the studies with relevant impurities of the drug substance or the drug product are presented in Sections 8.3 and 8.6, respectively. These studies were conducted in support of the registration of oral RPV (EDURANT®). As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), the conclusion on potential effects can be applied to the RPV LA formulation as well.

In addition, toxicology studies and/or local tolerance with RPV LA in other formulations than the selected clinical G001 formulation or not containing P338, are described in Sections 8.7 and 8.8.

Supportive genotoxicity, and reproductive and developmental toxicity studies with P338 have been conducted. These studies are described in Section 8.9.

8.1. Antigenicity

In view of the absence of any signs (see Section 3.2.2) that RPV (base) might elicit allergic reactions, no antigenicity studies have been conducted.

8.2. Immunotoxicity

The lack of effects on the PCF-assay in the 1-month rat study (Mod4.2.3.2/TMC278-Exp.5692) indicated that RPV base had no immunotoxic potential.

8.3. Mechanistic Studies with RPV

Studies were conducted to address the mechanism of action of the effects on the biosynthesis of adrenocortical hormones observed in general toxicity studies in rats and dogs and on the functioning of the pituitary-adrenal axis.

8.3.1. Single Dose Oral Endocrinology Study in Dogs

RPV base dissolved in PEG400 + CA was administered once, by oral gavage, at 0, 20, or 80 mg/kg in a dose volume of up to 10 mL/kg, to groups of 6 male beagle dogs (Mod4.2.3.7.3/TMC278-Exp.5627 and Mod2.6.7.17). Mortality, clinical observations, body weight and body weight gain were evaluated. Cortisol, ACTH and aldosterone levels were measured (1 and 6 days after dosing). Pituitary-adrenocortical function was assessed by a challenge with corticotrophin releasing factor (CRF) (1 and 6 days after dosing). Toxicokinetic evaluation on blood samples taken on the day of dosing was also included.

There were no mortalities associated with RPV base. There were no relevant clinical signs, or effects on body weight or body weight gain. No relevant changes in plasma cortisol, aldosterone or ACTH were recorded. At the CRF challenge, no relevant changes were seen in plasma cortisol levels. Systemic exposure expressed as C_{max} and AUC values are given in Table 30 below.

Table 30: Exposure of RPV base (C_{max} and AUC) in a Single Dose Oral Endocrinology Study in Dogs with RPV Base

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	$AUC_{0-\infty}$ (µg.h/mL)
		M (n = 6)	
20	Day 1	2.8	87
80	Day 1	2.8	105

M: males

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

RPV was assessed for its ability to inhibit cortisol biosynthesis in primary cultures of Guinea pig adrenal cells (Mod4.2.3.7.3/TMC278-Exp.5653 and Mod2.6.7.17). The effects of RPV base dissolved in DMSO (final bath concentration 1%) in concentrations ranging from 1 nM to 10 µM upon the cortisol synthesis stimulated by $ACTH_{1-24}$ were determined in 2 independent repeats. Etomidate served as positive control.

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

8.3.3. Effect of RPV Base on the Cortisol Biosynthesis in Crude Subcellular Fractions of Dog Adrenal Cortex

The effect of RPV base on adrenal corticosteroid biosynthesis was studied in vitro using cell free adrenal gland homogenate (E-fraction) from beagle dog (Mod4.2.2.4/TMC278-FK4790 and Mod2.6.7.17). Nominal concentrations of RPV base ranged from 0.03 to 75 µM (0.011 to 27.75 µg/mL). Etomidate (0.28 µM) served as positive control.

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

8.4. Dependence

In view of the primary pharmacodynamics of RPV, as NNRTI active against HIV-1, and the absence of relevant indications in the secondary pharmacodynamics profile, no dependence studies have been conducted.

8.5. Studies on Metabolites

In view of the absence of a unique human metabolite and of structural alerts in human metabolites identified (see Mod2.6.4/Sec5.1.4), no specific safety studies on metabolites have been conducted.

8.6. Studies on Impurities

8.6.1. Qualification According to ICH Q3A

related substance A* ([redacted] salt of related substance H*), related substance B* ([redacted] salt of related substance F) (Figure 2), and related substance C* ([redacted]-isomer of RPV) are drug substance impurities specified at a level equal to or exceeding 0.15% were qualified according to ICH Q3A.

Figure 2: Structural Formulas of related substance A* (Top) and related substance B* (Bottom)

[redacted]

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The first two impurities were tested by spiking at 4% of the drug substance, a fair multiple of the specified level of [redacted]% for related substance A* and [redacted]% for related substance B* (see Module 3.2.S.4.1/Impurities). The third impurity, related substance C*, was present in all drug substance batches involved in pivotal nonclinical studies at the level of minimally 0.61% and was qualified based on the oral 1-month repeated dose toxicity study in dogs. The qualified dose level of related substance C* in this study was 0.03 mg/kg/day. Evaluation of related substance C* in relation to RPV and RPV LA dosing schedules is described in Mod2.4/Sec4.8.8.

8.6.1.1. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance A* and related substance B*

related substance A* (Mod4.2.3.7.6/TMC278-TiDP6-NC308 (TOX8901) and Mod2.6.7.17), related substance B* (Mod4.2.3.7.6/TMC278-TiDP6-NC309 (TOX8900) and Mod2.6.7.17), and RPV were dissolved in DMSO. Each impurity was spiked at 4% by mixing a solution of the impurity with a solution of RPV at a ratio of 4:96. Each spiked solution was tested separately in 2 independent repeats of a bacterial reverse mutation assay (Ames test) with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) up to 250 µg RPV per plate, in the presence or the absence of metabolic activation (Aroclor-induced rat liver S9 mix).

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

It was concluded that neither related substance A* nor related substance B* spiked to RPV at 4% was mutagenic in this test.

8.6.1.2. In Vitro Mammalian Forward Mutation Assay with Mouse Lymphoma Cells with related substance A* and related substance B*

related substance A* (Mod4.2.3.7.6/TMC278-TiDP6-NC311 (TOX8899) and Mod2.6.7.17), related substance B* (Mod4.2.3.7.6/TMC278-TiDP6-NC312 (TOX8898) and Mod2.6.7.17), and RPV were dissolved in DMSO. Each impurity was spiked at 4% by mixing of a solution of the impurity with a solution of RPV at a ratio of 4:96. Each spiked solution was tested separately in 2 independent repeats of an in vitro mammalian forward mutation assay at the tk locus in mouse lymphoma L5178Y cells. Each mixture was incubated at a range of concentrations up to 75 µg RPV/mL, the limit of toxicity in the absence of metabolic activation (Aroclor-induced rat liver S9 mix) for 3 or 24 hours, and up to 15 µg RPV/mL, the limit of toxicity in the presence of S9 for 3 hours.

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

It was concluded that neither related substance A* nor related substance B* spiked to RPV at 4% induced an increased mutation frequency at the tk locus of L5178Y mouse lymphoma cells.

8.6.1.3. 1-Month Oral Toxicity Study in Rats with related substance A* and related substance B*

related substance A* and related substance B* were each spiked at 4% to RPV (Mod4.2.3.7.6/TMC278-TiDP6-NC314 (TOX8886) and Mod2.6.7.17). The spiked batches and RPV were suspended in 0.5 % (m/v) aqueous HPMC and administered by daily gavage for 1 month to groups of 10 male and 10 female Sprague-Dawley rats, at one dose of 10 mg/kg/day corresponding to the NOAEL of RPV in that species. The dosing volume was 10 mL/kg. The control group of 10 male and 10 female Sprague-Dawley rats received the vehicle. Regular observations were made for clinical signs, morbidity and mortality. Weekly body weight and food consumption were determined. Ophthalmic examination was conducted before the start of dosing and at the end of the dosing period. At the end of the study clinical laboratory investigations (hematology, blood chemistry, and urinalysis) were done. At necropsy gross lesions and organ weight were recorded and the target organs and tissues for RPV were sampled for histological examination.

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

It was concluded that related substance A* and related substance B* spiked to RPV at 4% did not induce any effects.

8.6.1.4. Summary

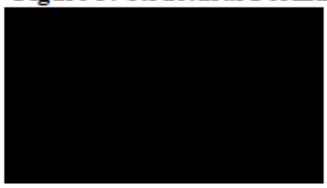
The two impurities, related substance A* and related substance B*, spiked at 4% showed no genotoxic potential. The three batches tested in the one-month rat study showed no relevant differences in toxicological profile. Consequently, for both impurities the qualified dose level is 0.4 mg/kg/day. As these impurities occur in the drug substance, they are applicable to oral RPV as well as RPV LA. Evaluation of these impurities in relation to RPV and RPV LA dosing schedules is described in Mod2.4/Sec4.8.8.

8.6.2. Impurities with a Genotoxic Alert

8.6.2.1. related substance G*

related substance G* is the [redacted] salt of related substance D* (Figure 3), a starting material in an earlier synthesis route of RPV drug substance (see Mod3.2.S.2.6) and is therefore applicable for oral RPV as well as RPV LA. The analytical method does not discriminate between the two isomers; therefore, the test results cover both impurities. On the basis of the presence of [redacted] and [redacted] moieties in related substance D*, its genotoxic potential (measured as related substance G*) was evaluated in 3 independent repeats of a bacterial reverse mutation assay (Ames) and an in vitro mammalian chromosomal aberration assay. The possible impact on human safety of the presence of related substance G* in drug substance and drug product is evaluated in Mod2.4/Sec4.8.8.

Figure 3: Structural Formula related substance D*



8.6.2.1.1. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance G*

related substance G* dissolved in DMSO was evaluated in three bacterial reverse mutation studies (Ames test) (Mod4.2.3.7.6/TMC278-NC179 (TOX6835), Mod4.2.3.7.6/TMC278-NC165 (TOX6616), Mod4.2.3.7.6/TMC278-1646_0016315 (TOX9643) and Mod2.6.7.17), with histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537. Concentrations of 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg per plate were tested, in the absence or the presence of metabolic activation (Aroclor-induced rat liver S9 mix).

In one study (Mod4.2.3.7.6/TMC278-NC179 (TOX6835) and Mod2.6.7.17), related substance G* induced an increase in the incidence of mutations in the strains TA98 and TA100 in the presence of rat liver S9-mix. In the other study (Mod4.2.3.7.6/TMC278-NC165 (TOX6616) and Mod2.6.7.17), the incidence of mutations of TA1537, in the absence of metabolic activation, and of TA98, in the presence of S-9 mix, was increased. In the third study (Mod4.2.3.7.6/TMC278-1646_0016315 (TOX9643) and Mod2.6.7.17) that served as a head to head comparison with related substance E* and related substance F* (see 8.6.2.2), related substance G* induced an increase in the incidence of mutations in the strains TA98 and TA1537 both in the presence and the absence of rat liver S9 -mix and in TA100 in the presence of rat liver S9-mix, only (Mod2.6.7.17).

These results confirmed the genotoxic potential of related substance G*.

8.6.2.1.2. In Vitro Mammalian Chromosomal Aberration Assay with related substance G*

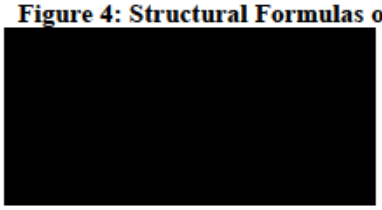
related substance G* dissolved in DMSO was tested in an in vitro mammalian chromosome aberration screening test with human lymphocytes Mod4.2.3.7.6/TMC278-TiDP6-NC184 (TOX7006) and Mod2.6.7.17). Incubation for 4 hours followed by a 20-hour expression period in the presence or the absence of a metabolic activation (Aroclor-induced rat liver S9 mix) was performed at a range of concentrations up to 521.75 µg/mL. Incubation for 24 hours in the absence of S9-mix occurred at a range of concentrations up to 391.32 µg/mL.

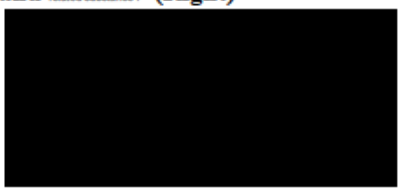
related substance G* induced a statistically significant increase in the frequency of cells with structural aberrations in the absence of metabolic activation (S9) after 4 hours of exposure at the maximum concentration tested, 521.75 µg/mL. related substance G* was therefore considered to be clastogenic to human lymphocytes in vitro (Mod2.6.7.17).

8.6.2.2. related substance E* and related substance F*

Traces of related substance E* and related substance F* (Figure 4) may be present in RPV drug substance. These compounds having an [redacted] moiety, but no [redacted] share structural similarities with related substance G*. Their genotoxic potential was evaluated in a bacterial reverse mutation test (Ames).

Figure 4: Structural Formulas of related substance E* (Left) and related substance F* (Right)





8.6.2.2.1. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance E*

related substance E* dissolved in DMSO was evaluated in a bacterial reverse mutation study (Ames test) (Mod4.2.3.7.6/TMC278-1646_0015298 (TOX9645) and Mod2.6.7.17) with histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537. In each independent repeat 7 concentrations ranging from 78.13 to 5000 µg per plate were tested, in the absence or the presence of metabolic activation (Aroclor-induced rat liver S9 mix).

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

8.6.2.2.2. In Vitro Bacterial Reverse Mutation (Ames) Test with related substance F*

related substance F* dissolved in DMSO was evaluated in a bacterial reverse mutation study (Ames test) (Mod4.2.3.7.6/TMC278-1646_0015299 (TOX9644) and Mod2.6.7.17) with histidine-requiring *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537. In each independent repeat 7 concentrations ranging from 78.13 to 5000 µg per plate were tested, in the absence or the presence of metabolic activation (Aroclor-induced rat liver S9 mix).

The first test showed no increased frequency of mutations in any of the tested strains, in the presence or the absence of rat liver S9-mix. The second test showed the same results as the first test, except for TA98 in the presence of S6-mix. Almost all concentrations of related substance F* showed a slightly more than 2-fold increase of the frequency of mutations compared to vehicle control which showed a low mutation frequency. A third test with TA98 in the presence of S9-mix showed no increased frequency of mutations up to 5000 µg/plate. It is concluded that related substance F* has no mutagenic potential.

8.6.3. Residual Solvent N-methylpyrrolidone

NMP is a specified residual solvent in the RPV LA drug product. It is an ICH Q3C (R5) Class 2 solvent; its permitted daily intake is 5.3 mg/day. The dosing scheme with a RPV LA initiation dose of 900 mg (followed by a once monthly continuation dose) represents the worst-case scenario in terms of exposure to NMP. The highest dose in this scenario presumably translates into a daily dose of 30 mg RPV base. Consequently, NMP is qualified up to 176,666 ppm. This limit also adequately covers the other treatment scenarios.

The actually measured concentration of NMP is 458 ppm in drug product. The specification limit is 2000 ppm (Mod3.2.S.3.2 Impurities).

8.6.4. Overall Conclusion

Three specified impurities (related substance H*, related substance I* and related substance C*) were found non-genotoxic in standard in vitro bacterial and mammalian cell assays. The hydrochloric acids of related substance H* and related substance I* were further evaluated and toxicologically qualified in a 1-month repeated dose rat study. The impurity related substance D* was genotoxic in vitro in both bacterial and mammalian cell assays. N-methylpyrrolidone, present in the RPV LA drug product, is a class 2 residual solvent, with a specification limit far below the acceptable daily intake. The relevance of each of these impurities is further discussed in the nonclinical overview (Mod 2.4/Sec4.8.8)

The impurities related substance E* and related substance F* were not genotoxic in standard genotoxicity tests and are not further discussed as they are present below the ICH Q3A qualification level.

8.7. Studies Done With RPV LA Formulations Other Than Selected G001 Clinical Formulation

8.7.1. Single Dose Toxicity Studies

8.7.1.1. Mouse

A single SC dose of RPV LA (25 mg/mL) was given SC to male and female Swiss CD1 mice at 2.5, 5, 10 and 20 mg/kg (12 animals/sex/group) as an aseptic aqueous suspension with 2.5% (w/v) P338, or at 20 mg/kg (12 animals/sex/group) as an aqueous suspension with Vit E-TPGS (Mod4.2.3.7.7/TMC278-NC196 (TOX7354) and Mod2.6.7.17). Vehicle control groups (6 animals/sex/group) were included for each formulation. Rats were followed up for 18 days.

Both RPV LA formulations were well tolerated. No adverse clinical signs or effects on body weight or food consumption were observed.

At 4 days post-dose the spleen weight was slightly increased in male mice at 10 and 20 mg/kg, and at all dose levels in female mice. Histologically moderate extramedullary hematopoiesis was noted in the spleen and liver on Day 18 post-dose at 20 mg/kg.

Macroscopically white deposits were noted at the injection site in a dose-dependent manner. At the Day 18 necropsy, the white deposits were still present at 20 mg/kg with both formulations. Histopathologically, four days after injection, local inflammatory changes were found at the injection site and were considered related to the vehicles (i.e., minimal hemorrhage, minimal multifocal infiltration of inflammatory cells in the subcutis or muscle; minimal to slight lymphohistiocytic infiltration and minimal focal granulocytic infiltration in brown adipose tissue) or to the administration of the test article itself (i.e. white deposits, minimal to slight (multi)focal subacute or granulomatous inflammation at all doses; minimal to slight central necrosis and/or cyst formation with granulocytic infiltration at doses ≥10 mg/kg, and minimal fibrosis at 20 mg/kg). Partial recovery was observed after 18 days.

No differences in effect were noted between the two tested surfactants.

8.7.1.2. Rat

RPV LA (25 or 100 mg/mL) was administered once, SC or IM to male Sprague-Dawley rats (6 rats/group) at 5 and 20 mg/kg as a colloidal suspension (average particle size 200 nm) with 2.5% (w/v) P338 as surfactant (Mod4.2.3.7.7/TMC278-NC244 (TOX7896) and Mod2.6.7.17). An intravenous arm (1.25 mg/mL RPV base in polyethylene glycol 400/25 % sterile water) was added in this study to calculate bioavailability. A follow-up period of 8 weeks was included.

At 5 (SC or IM) and 20 mg/kg (IM) no adverse general clinical signs, or clinical signs of irritation at the injection site were seen. At 20 mg/kg SC slight swelling of the injection sites was noted between Day 4 and Day 19 post-dose.

At the SC site of injection, white deposits were occasionally observed at 5 mg/kg, and in most animals at 20 mg/kg which were associated with minimal granulomatous inflammation, with minimal fibrosis or minimal mononuclear cell infiltration in one rat at 5 mg/kg, and two rats at 20 mg/kg. At the IM site of injection, white deposits were noted in one rat at 20 mg/kg. Minimal focal muscle fiber degeneration and slight multifocal granulomatous inflammation with minimal multifocal mononuclear cell infiltrate were noted microscopically.

8.7.1.3. Dog

Male and female beagle dogs (6/sex/group) (Mod4.2.3.7.7/TMC278-NC234 (TOX7781) and Mod2.6.7.17) received 200 or 400 mg of RPV LA (100 mg/mL; particle size 200 nm) over 2 administrations (SC or IM) as an aqueous suspension with 2.5% (w/v) P338. There was a 24-h time interval between the subsequent injections. The dogs (3/sex/group/time point) were sacrificed at 1- or 3-months post-dose.

No relevant clinical signs were noted except for changes at the injection sites, and no adverse changes in ocular parameters, ECG, heart rate, body weight (gain), food consumption, hematology, coagulation, serum and urinary parameters, and hormonal levels.

Both routes of administration were well tolerated. Locally, the IM route showed slightly less effects than the SC route as judged by the number of incidences of hardening or swelling at the injection site and the number of animals showing this finding. The findings were transient, with the exception of two IM injection sites at which a hardening remained present until necropsy (in 1 animal at interim kill and in 1 animal at terminal kill).

Examination of the injection sites showed effects similar to those seen previously in rodents; i.e. white deposits of a material presumed to be RPV, and diffuse (SC) or multifocal (IM) inflammatory reactions, which had lessened in severity by the terminal necropsy at 3 months. The reaction comprised granulomatous inflammation (swollen macrophages with eosinophilic vacuolated cytoplasm) with a lymphohistiocytic infiltrate. IM administration was slightly better tolerated than SC dosing.

IM dosing at 200 mg resulted in a slight increase in medial iliac lymph node weight. At 400 mg, an increased weight of the medial iliac lymph nodes (SC and IM), the popliteal lymph nodes

(IM), and axillary lymph nodes (SC) was observed. Histopathological examination of the lymph nodes revealed macrophages with eosinophilic vacuolated content at 1-month post-dose.

8.7.1.4. Minipig

Two groups of two male Göttingen minipigs received a single 1.5 mL IM injection of formulation F006 (300 mg/mL RPV LA suspension in the presence of PS80) providing a dose of 450 mg. This was followed by necropsy at 1- or 3-months post-dose. One additional group of two male minipigs received formulation F004 (100 mg/mL RPV LA suspension in the presence of P338) providing a dose of 400 mg. This was followed by necropsy at 3 months post-dose (Mod4.2.2.7/TMC278-NC295 (FK6407) and Mod2.6.7.17).

Treatment was generally well tolerated. There were no treatment-related effects in terms of clinical health, body weight gain or organ weights. White material was seen at macroscopic examination in all treated injection sites in animals sacrificed 3 months after receiving F004 or F006. Both formulations resulted in areas of foamy macrophages in at least a proportion of the treated sites. This finding was likely due to macrophages engulfing the deposited test material. The popliteal lymph nodes showed white areas only in animals that received the F004 formulation.

8.7.2. Repeat-Dose Toxicity Study

8.7.2.1. Minipig

In a 9-month GLP study with 3-month interim sacrifice the systemic and local toxicity of the formulation F006 (300 mg/mL RPV LA suspension in the presence of PS80; 200 nm particle size) was assessed following repeated IM injections once every 30 days in Göttingen minipigs (6 animals/sex) for 9 months (Mod4.2.3.7.7/TMC278-NC296 (TOX8580) and Mod2.6.7.17). A vehicle control group (4 animals/sex) and a saline control group (2 animals/sex) were added as well. The test article was administered at 75 mg/kg under a dosage volume of 250 µL/kg on Days 1, 30, 60 and 90, and at a fixed volume of 2 mL/injection (i.e., 600 mg/animal) on Days 120, 150, 180, 210, 240 and 270. The IM injections were performed alternately into the left and right thigh muscles with separate injection sites. In addition, injections were performed at a fixed volume of 1 mL/injection (i.e., 300 mg/animal) on Days 120, 150, 180 and 210 in the left or right m. longissimus dorsi. On Day 93 (i.e. after the 4^{th} 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.

No clinical signs were observed. No effects on body weight (gain), food consumption, ECG, ophthalmoscopy, clinical chemistry, urinalysis or organ weights were noted either in any of the groups. Local effects, consisting of generally slight or well-defined erythema and induration/swelling, were observed at the injection sites in animals from all groups. A higher incidence of erythema at the injection site was observed in the RPV LA-treated animals; the erythema extended to the whole thigh in several RPV LA-treated animals. Induration was noted more often in animals treated with RPV LA compared to the vehicle and saline control animals. In all groups the local signs generally were persistent for a few days.

At hematological investigations, slightly increased neutrophil counts were noted in Week 13 in some animals given RPV LA.

At macroscopic examination, white deposit was seen at the injection sites in animals given RPV LA, sometimes accompanied by induration of the muscle. This correlated with microscopic findings, which consisted of macrophage infiltration and/or amorphous pale eosinophilic deposit (probably deposited RPV LA) in the injection sites and occasionally in the draining lymph nodes. These changes were sometimes associated with lymphohistiocytic, neutrophilic infiltration, fibroplasia, and/or muscle necrosis. These findings are expected reactions towards a particulate formulation injected IM.

The mean C_{max} and AUC values of RPV after single (Day 1) and repeated (Day 60: 3rd injection; Day 240: 9th injection) administration are given in Table 31.

Table 31: Mean Toxicokinetic Parameters after Repeated Intramuscular Dosing of RPV LA in the F006 Formulation in Minipigs

Dosing Day	C_{max} (µg/mL)		AUC (µg.h/mL)[c]	
	M	F	M	F
1[a]	0.034	0.075	16.9	33.7[d]
60[a]	0.053	0.11	20.6	26.0
240[b]	0.11	0.11	43.4	35.5

[a] n = 6; [b] n = 3; [c] $AUC_{0-\infty}$ for Day 1; AUC_{0-696h} for Day 60 ($AUC_{day\ 60-89}$) and Day 240 ($AUC_{day\ 240-269}$); [d] n = 4
F006 = 300 mg/mL RPV LA suspension in the presence of PS80
M = male; F = female

8.8. Acute Intramuscular Irritation of Formulations (Not Including P338) in Rabbits

An aqueous suspension formulation containing 300 mg/mL RPV LA (PS80/lipoid E PG) was studied in a non-GLP study and administered via a single dose IM injection only (Mod4.2.3.7.7/TMC278-NC280 (TOX8482) and Mod2.6.7.17). Administration of the vehicle induced only minimal changes; findings were more pronounced, however, in rabbits treated with the RPV LA suspension, and were expected due to administration of a crystalline material.

8.9. Studies with Poloxamer 338

Genotoxicity and fertility, embryofetal development and pre- and postnatal development studies have been conducted with P338, the excipient used in the final clinical formulation G001 and previous clinical formulation F004.

8.9.1. Genotoxicity

8.9.1.1. In Vitro Nonmammalian Cell System

Bacterial reverse gene mutation tests with P338 (in sterile purified water) were carried out using five strains of *Salmonella (S.) typhimurium*, TA98, TA1537, TA100, TA1535 and TA102, in the absence and presence of rat liver S9 mix by means of the plate incorporation method (Mod4.2.3.7.7/TMC278-NC357 (TOX9535) and Mod 2.6.7.17). Two mutation experiments were

performed at a maximum concentration of 10,000 µg P338/plate. Several positive controls were tested as well.

P338 showed no mutagenic potential towards the various *S. typhimurium* strains in the absence and presence of S9-mix under the test conditions, up to the maximum concentration of 10,000 µg/plate.

The positive controls induced a biologically significant increase in the number of revertant colonies, demonstrating its mutagenic potential and validity of the study.

8.9.1.2. In Vitro Mammalian System

Human lymphocytes were exposed to P338 (in sterile purified water) both in the absence and presence of S9 mix derived from rat livers (Mod4.2.3.7.7/TMC278-NC358 (TOX9523) and Mod2.6.7.17). Cells were incubated for 3 hours (+ 18-hour recovery) and 21 hours in the absence of S9 mix at 3,600, 6,000 and 10,000 µg/mL of P338. In the presence of S9 mix, cells were treated for 3 hours followed by an 18- or 21-hour recovery at 3,600, 6,000 and 10,000 µg/mL. Several positive controls were tested as well.

In both the absence and presence of S9 mix, P338 caused no statistically significant increases in the proportion of metaphase figures containing chromosomal aberrations, at any concentration, when compared with the solvent control, under the experimental conditions described.

All positive control compounds caused statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system and the efficacy of the S9 mix.

8.9.2. Reproductive and Developmental Toxicity

The developmental toxicity of P338 was assessed in rats and rabbits upon oral dosing at the maximum feasible dose of 1,600 mg/kg/day. Based on two pharmacokinetics studies the exposure to P338 after oral administration in both species proved very limited (Mod2.6.4/Sec8.3.1). Therefore, studies were repeated with P338 administered via the IM route. In addition, a fertility study part was included in the rat embryo-fetal development study. Pre-and postnatal development was also evaluated in rats.

8.9.2.1. Rat Embryofetal Development (Oral Gavage)

A non-GLP 2-week dose-range finding (DRF) study with P338 (solution in demineralized water) was performed in six female Sprague-Dawley rats ((Mod4.2.3.7.7/1955_0016643 (TOX9648) and Mod2.6.7.17). The animals were dosed at the maximum feasible dose of 1,600 mg/kg by oral gavage for 14 days. Another group of six rats served as the vehicle control group. No treatment-related mortality, clinical observations or changes at necropsy occurred. Body weight gain was reduced during the first week of treatment but partially recovered during the second week. Food consumption was not affected.

Based on this DRF study, 1600 mg/kg/day of P338 (as a solution in demineralized water) was selected to be administered in the subsequent GLP embryofetal development study

(Mod4.2.3.7.7/TMC278-NC347 (TOX9679) and Mod2.6.7.17). Pregnant Sprague-Dawley rats (22/group) were dosed orally by gavage from GD 6 through 17. A vehicle control group comprising 22 female rats was included. The females were euthanized on Day 21 of pregnancy, a necropsy was performed, and macroscopic abnormalities and pregnancy and embryofetal development parameters were determined.

No deaths or premature sacrifices occurred during the study. Nineteen out of twenty-two rats were pregnant in both the control and 1600 mg/kg/day dosed groups. No treatment-related observations were recorded. At 1600 mg/kg/day, body weight performance was minimally reduced from the second week of dosing onwards, persisting into the post-dosing period (GD 14-20). The corrected mean maternal weight gain was comparable to that of the control group. Food consumption was unaltered. Pregnancy parameters as assessed by the numbers of corpora lutea of pregnancy, implantation, resorptions, live and dead fetuses and the extent of pre- and post-implantation losses, fetal weight and sex ratio were unaffected by treatment. Furthermore, there were no P338-related malformations or increased incidences of minor abnormalities observed on external, visceral or skeletal examination.

8.9.2.2. Rat Fertility and Embryofetal Development (Intramuscular)

As it was shown in a pharmacokinetic study in female rats that plasma concentrations of P338 after a single oral dose of 1600 mg/kg/day of P338 were undetectable (see Mod2.6.4/Sec8.3.1), the embryofetal development study needed to be repeated. Therefore, P338 was administered via the IM route.

A non-GLP, single and repeat-dose IM toxicity/toxicokinetic study was conducted in female Sprague-Dawley rats (4/group), followed by a 1-month follow-up period (Mod4.2.3.7.7/TOX13295 and Mod2.6.7.17). P338 (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.

No mortality was observed throughout the study. A transient body weight loss (3%) was observed after the single dose of 5 mg/kg, while no effects were observed after repeat dosing. Food consumption was unaffected in all groups. There were no P338-related findings at gross necropsy and histopathological examination.

Toxicokinetic parameters showed that acceptable exposure levels were reached for assessing embryofetal development via the IM route (Table 32).

Table 32: Mean Toxicokinetic Parameters in Female Rats After Single And Repeat IM Dosing of P338

Dose of P338 (mg/kg)	Day	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
5	0	25	3-7	72	679
				∞	810
5 Every 3 Days	0	25	7	72	705
				∞	752
	9	38	2-7	72	987
				168	1170
	-	-	-	288 (12 days)	3235[b]
10 Every 3 Days	0	49	7-12	72	1570
				∞	1690
	9	65	7-12	72	1950
				168	2390
	-	-	-	288 (12 days)	7063[b]
10 (2x5) Every week	0	49	7-12	72	1470
				∞	1770
	7	57	7	72	1550
				168	1790
	-	-	-	336 (14 days)	3737[b]

[a] Median value; [b] $AUC_{0\text{-}cumulativetlast}$ corresponds to the AUC during the overall experiment
-: no toxicokinetic evaluation possible

Based on this DRF study, a combined male and female fertility and embryofetal development study was conducted (Mod4.2.3.7.7/TOX13391 and Mod2.6.7.17). P338 was administered by IM injection at dose levels of 2.5, 5 and 10 mg/kg/occasion to groups of 22 male and 22 female Sprague-Dawley rats. The animals were treated every three calendar days before mating (4 weeks for males and 2 weeks for females). Treatment then continued throughout mating and up to 3 days before necropsy of the males or until GD 15 for the females. A control group of 22 males and 22 females received the vehicle (sterile aqueous solution containing glucose (anhydrous), 1 mg/mL CA monohydrate, 2 mg/ml sodium dihydrogen phosphate monohydrate, NaOH until pH = 7.0 ± 0.1 in water for injection) at the same dose volume (0.2 mL/kg). The females were euthanized on Day 21 of pregnancy, a necropsy was performed, and macroscopic abnormalities and pregnancy and embryofetal development parameters were determined. Males were necropsied after 9 weeks of treatment (21 administrations), following completion of the caesarean sections and gonadal organs were weighed.

No test article-related mortality or clinical signs were noted in males or females. No relevant effects were seen on mean body weight (gain) or food consumption during the dosing period (males) or during pre-mating and gestation period (females). There was no indication of effects related to fertility (estrous cycles, mating performance, mean pre-coital interval) and no post-mortem effects were noted on organ weights in males or females. All females had viable fetuses at terminal caesarean section. There was no test item-related effect on implantation, embryo-fetal survival, mean fetal weight or sex ratio in any group and no external, visceral or skeletal abnormalities were attributed to P338.

Exposure was confirmed in all P338 treated animals (**Table 33**). No clear sex difference was noted.

Table 33: Mean Toxicokinetic Parameters in Male and Female Rats After IM Dosing of P338 in a Fertility and Embryofetal Development Study

Dose of P338 (mg/kg)	Sex	Day	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
2.5 Every 3 Days	M	0 (1st dose)	-	-	-	-
		54 (19th dose)	10	7	72	386
		-	-	-	1512 (63 days)	7870[b]
	F	0 (1st dose)	-	-	-	-
		30 (GD 15)	-	-	-	-
5 Every 3 Days	M	0 (1st dose)	29	7	72	746
		54 (19th dose)	23	7	72	825
		-	-	-	1512 (63 days)	16,000[b]
	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	M	0 (1st dose)	33	7	72	944
		54 (19th dose)	34	7	72	1480
		-	-	-	1512 (63 days)	26,000[b]
	F	0 (1st dose)	26	4.5	72	666
		30 (GD 15)	26	7	72	879
		-	-	-	792 (33 days)	6220[b]

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

8.9.2.3. Rabbit Embryofetal Development (Oral Gavage)

A non-GLP 2-week DRF study with P338 (solution in demineralized water) was performed in five NZW rabbits (Mod4.2.3.7.7/TMC278-NC366 (TOX9649) and Mod2.6.7.17). The animals were dosed with P338 at 1,600 mg/kg by oral gavage for 14 days. Another group of 5 animals served as the vehicle control group. No treatment-related mortality or changes at necropsy occurred. Some animals showed soft feces. Body weight performance and food consumption were reduced during the entire treatment period. At the end of the study, body weight gain was 52% lower and total food consumption was 26% reduced compared to controls.

Based on this DRF study, 1600 mg/kg/day of P338 was selected to be administered in the subsequent GLP embryofetal development study. Pregnant NZW rabbits (20/group) were dosed orally by gavage from GD 6 through 19 (Mod4.2.3.7.7/TMC278-NC348 (TOX9680) and Mod2.6.7.17). A vehicle control group comprising 20 female rabbits was included. The females were euthanized on Day 28 of pregnancy, a necropsy was performed, and macroscopic abnormalities and pregnancy and embryofetal development parameters were determined.

There were no deaths or premature sacrifices during the study. Seventeen and nineteen out of twenty rabbits were pregnant in the control and 1600 mg/kg/day dosed groups. At 1600 mg/kg/day, fifteen out of twenty rabbits exhibited soft feces, and fecal output was reduced in six rabbits. The soft feces persisted in nine rabbits during the post-treatment period. Body weight performance was reduced from GD9 through GD19, after which body weight gain and food consumption partly recovered. Pregnancy parameters and embryofetal development were unaffected.

8.9.2.4. Rabbit Embryofetal Development (Intramuscular)

Similar to the rat, it was also shown in the rabbit in a pharmacokinetic study that plasma concentrations of P338 after a single oral dose of 1600 mg/kg/day of P338 were low (see Mod2.6.4/Sec8.3.1), and that the embryofetal development study needed to be repeated via the IM route.

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 Mod2.6.7.17). 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.

No mortality was observed throughout the study. A local reaction was observed after dosing in all groups. Body weight and food consumption were unaffected during the study. No toxicologically relevant gross or histopathological findings were noted at the injection sites.

Toxicokinetic parameters showed that acceptable exposure levels were reached for assessing embryofetal development via the IM route (Table 34).

Table 34: Mean Toxicokinetic Parameters in Female Rabbits After Single And Repeat IM Dosing of

Dose of P338 (mg/kg)	Day	C_{max} (µg/mL)	t_{max} [a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
5 SD	0	50	24	96	2760
				168	3290
				∞	3740
2.5 RD every 4 Days	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	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	0	49	12-24	96	2800
				168	3350
				∞	3610
	7	55	24	96	3180
				168	3880
	-	-	-	336 (14 days)	7228 [b]

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

Based on this DRF study, an embryofetal development study was conducted in pregnant NZW rabbits (20/group) (Mod4.2.3.7.7/TOX13376 and Mod2.6.7.17). Intramuscular injections were given at 2.5 and 5 mg/kg/occasion of P338 on GD 6 and 12. A control group of 20 pregnant females received the vehicle (sterile aqueous solution containing glucose (anhydrous), 1 mg/mL

CA monohydrate, 2 mg/mL sodium dihydrogen phosphate monohydrate, NaOH until pH = 7.0 ± 0.1 in water for injection) at the same dose volume (0.5 mL/kg).

No test article-related mortality or clinical signs were noted. No relevant effects were seen on mean body weight (gain), food consumption, macroscopic post-mortem examination or gravid uterus weight. All females had viable fetuses at terminal caesarean section. There was no test item-related effect on embryo-fetal survival, mean fetal weight or sex ratio in any group and no external, visceral or skeletal abnormalities were attributed to P338. Exposure was confirmed in all P338 dosed rabbits (Table 35).

Table 35: Summary of Poloxamer 338 Toxicokinetic Parameters in Pregnant New Zealand White Rabbit Plasma Following Intramuscular Administration of Poloxamer 338 on GD6 and GD12

Dose of P338 (mg/kg)	Day	C_{max} (µg/mL)	t_{max} [a] (h)	t_{last} (h)	$AUC_{0\text{-}tlast}$ (µg.h/mL)
2.5 RD every week	0 (GD 6)	24	24	144	1490
	6 (GD 12)	25	24	144	1330
	-	-	-	288 (12 days)	2820 [b]
5 RD every week	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; GD: gestation day; RD: repeat-dose

8.9.2.5. Rat Pre- and Postnatal Development (Intramuscular)

Based on the DRF study in female rats receiving P338 via IM injection (see Mod4.2.3.7.7/TOX13295 and Section 8.9.2.2), a pre- and postnatal development study was conducted in rats (Mod4.2.3.7.7/TOX13391). This DRF study is described in Section 8.9.2.2.

Poloxamer 338 was administered by IM injection to groups of 22 time-mated female Sprague-Dawley rats at dose levels of 2.5, 5 and 10 mg/kg/dose (0.2 mL/kg), every three calendar days, from GD6 till weaning (13 administrations) (Mod4.2.3.7.7/TOX13546 and Mod2.6.7.17). A vehicle group (sterile aqueous solution containing glucose (anhydrous), 1 mg/mL CA monohydrate, 2 mg/mL sodium dihydrogen phosphate monohydrate, NaOH to pH = 7.0 ± 0.1 in water for injection) was also included. Regular observations were made for maternal clinical signs, morbidity, mortality body weight and food consumption. The females were allowed to give birth. Partum and litter parameters, including duration of gestation, abnormalities at delivery, the number and sex of pups born, pup survival and pup weights were recorded. Developmental landmarks: static righting reflex, gripping reflex, pupillary reflex and auditory reflex were recorded from LD8, 17 and 21, respectively. At necropsy of the dams after weaning of their litters, the number of implantation scars in uterus was determined. From the F1 generation, 20 males and 20 females per group were kept and their growth, post-weaning development and behavior and reproductive performance were assessed.

There were no mortalities among the dams. The dams showed no relevant clinical signs, no effects on body weight, food consumption, gestation, or parturition. There were no effects on the

numbers of pups born, the sex ratio, or pup survival or physical condition. There were no effects on pre or post-weaning body weight and gain or development and behavior parameters. Sexual development, fertility and mating performance, and pregnancy parameters of the F1-generation were unaffected by treatment of the F0-generation. There were no effects on post-mortem parameters at necropsy of the F0 and F1-generation.

Exposure was confirmed in all animals in the P338-dosed groups with the peak plasma concentration, C_{max}, generally reached at 7 hours.

The NOAEL for both the F0 and F1-generation was at least 10 mg/kg/dose, administered every three days, corresponding to a mean C_{max} and $AUC_{(0\text{-tau})}$ of 25.2 µg/mL and 990 µg.h/mL, respectively at the end of the dosing period.

Table 36: Summary of Poloxamer 338 Toxicokinetic Parameters in a Pre- and Postnatal Development Study in Time-Mated Female Rats Following Intramuscular Administration of Poloxamer 338 from GD 6 until Weaning

Dose of P338 (mg/kg)	Day	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last} (h)	$AUC_{0\text{-tlast}}$ (µg.h/mL)
2.5 every 3 Days	0 (GD6)	-	-	-	-
	30 (LD17)	7.8	7	72	260
5 every 3 Days	0 (GD6)	14	7	72	413
	30 (LD17)	14	39.5[a] (7-72)	72	686
	-	-	-	792 (33 days)	4860
10 every 3 Days	0 (GD6)	26	7	72	807
	30 (LD17)	25	7	72	990
	-	-	-	792 (33 days)	8410

[a] Median value;
-: no toxicokinetic evaluation possible GD: gestation day; LD: lactation day

9. DISCUSSION AND CONCLUSIONS

In this section, the significance of any issues that arose in the toxicology studies with RPV (base) or RPV LA and their results are discussed. The relevance of the effects observed for human health and the margin of safety will be discussed in Module 2.4/Nonclinical Overview. The toxicology program in support of the registration of oral RPV (EDURANT®) comprised of oral single dose toxicity studies in mice, rats and dogs as part of the micronucleus study in mice and of the DRF studies for repeat dosing. Repeat-dose oral studies were performed in mice (up to 3 months), rats (up to 6 months), non-pregnant rabbits (up to 5 days), dogs (up to 12 months), and immature female cynomolgus monkeys (up to 8 weeks). A standard package of in vitro genotoxicity studies was conducted. A mouse oral in vivo bone marrow study was done. Two-year oral carcinogenicity studies were conducted in mice and rats. The reproductive and developmental toxicity studies comprised a male and a female fertility study in rats, embryofetal developmental studies in rats and rabbits, and a pre- and postnatal developmental study in rats. The comprehensive package of safety studies is completed with local tolerance studies, mechanistic studies, and safety studies with impurities encountered in drug substance or the drug product. As the active moiety for the oral and LA formulation is essentially the same (HCl salt

for the oral RPV formulation versus base for the LA formulation), the conclusion on potential effects can be applied to the RPV LA formulation as well. For RPV LA additional studies included repeated dose studies in dog and minipig and studies to specifically evaluate the safety of the excipient P338.

9.1. General Toxicity

The single dose toxicity of RPV (base) was minimal. The repeat-dose toxicity identified a number of targets including liver, thyroid and pituitary gland, kidneys, adrenal glands, ovaries and testes, red blood cells and coagulation. The majority of the effects induced in the highest dose tested were reversible. The increase in APTT and PT in male rats and the follicular hypertrophy and incidence of small follicles in thyroid gland of male and female rats showed incomplete recovery after a 1-month post-dosing period. In dogs, microscopical lesions in liver and increased serum concentration of ALP were not completely reversed. As the active moiety for the LA formulation is essentially the same as for the oral formulation (HCl salt for the oral formulation versus base for the LA formulation), the target organs identified are deemed relevant for RPV LA as well. The following targets were identified:

Liver

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

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

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

In dogs, the liver effects changed with the duration of treatment. In the 1-month study, perivascular inflammatory reactions together with multifocal perivascular fibrosis and single cell necrosis were noted in the central part of the lobules. Moreover, increased MPS aggregates occurred and multifocal bile duct proliferation. These effects seem to be associated with increased serum concentrations of cholesterol and total bilirubin and increased activities of ALP and ALT. In the 6-month study, pigment-laden perivascular macrophages were noted and in

males only, prominent brown pigment in the gall bladder epithelium. The pigment in the macrophages was not stainable with Perl's stain indicating that it probably was of lipogenic origin. This pigmentation was the only liver effect observed after 3 months of dosing (in high dose males only) indicating the relatively slow development of this effect which also applies for the pigmentation in the gall bladder. In the 12-month dog study, pigmentation of hepatocytes and canaliculi was noted together with prominent brown pigment in gall bladder epithelium, both in males and females. The histopathological effects were associated with an increased serum concentration of total bilirubin and with an increase in serum ALP activity.

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

Thyroid gland

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

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

Pituitary gland

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

Kidneys

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

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

For the nephritis in dogs, a test article treatment relationship cannot be excluded. However, in mice, such a relationship seems to be clear. The mechanism of these kidney effects is not clear taken into account the low percentage of the dose excreted via urine (see Mod2.6.4/Sec 6.1).

Adrenal glands

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

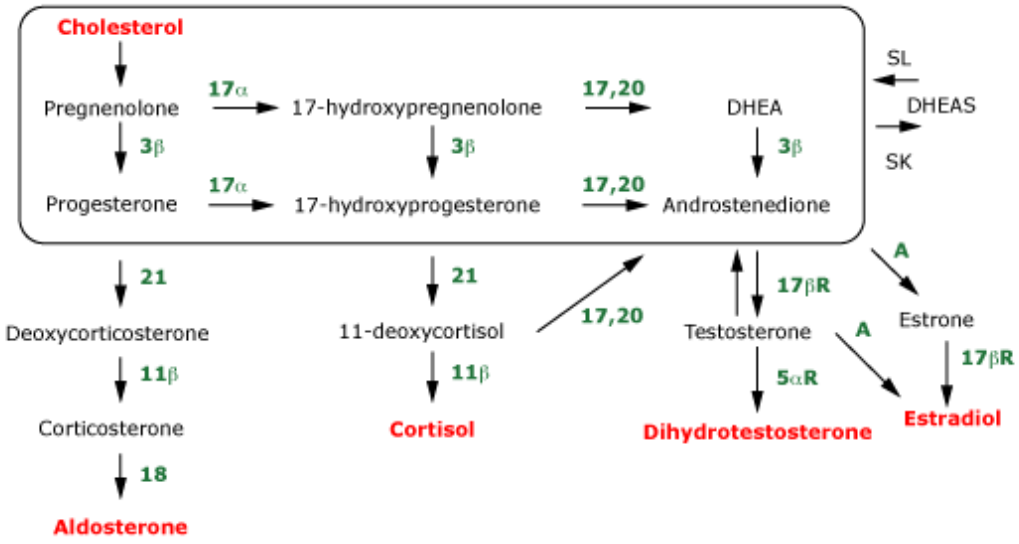
An additional effect of the CYP21 inhibition, notably in species that have a significant androgenic adrenal pathway, is that the increased ACTH stimulus and subsequent accumulation of progesterone and 17α-hydroxyprogesterone may lead to an increased androgen production. 17α-hydroxypregnenolone and 17α-hydroxyprogesterone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

unchallenged serum levels of androstenedione and DHEA and of the response of these androgens to ACTH challenge.

The effects of RPV (base) on adrenal gland and its hormonal biosynthetic pathways are more prominent than the effects on gonads and their pathways (see below in more detail). This may be explained by the affinity of RPV (base) for adrenal gland in repeat-dose studies in dogs and of radiolabel following the rat single dose quantitative whole-body autoradiography (QWBA) studies with ^{14}C-RPV base (see Mod2.6.4/Sec4.1.1). In the QWBA studies, no particular affinity for gonads was noted.

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

Figure 5: Synthetic Pathways for Adrenal Steroid Synthesis in Non-human Primates and Man



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

Ovaries

The effects in ovaries of dogs after at least 1 month of treatment with RPV base were characterized by increased numbers of tertiary follicles, (cystic) luteinized follicles, and in some dogs by corpora lutea. These effects did not intensify over time after 3 months of dosing. The

age of the dogs at the start of dosing in the 1-month studies was between 6.5 and 8 months. Probably, the still pre-adolescent age at the end of the 1-month studies has contributed to the more prominent effect in the test article-treated female dogs, compared to the controls that appeared still sexually immature, than in the longer duration studies. In the 1-month studies, the ovarian effects were associated with changes in the other parts of the female genital tract and with the prominent activation of the mammary glands. It is conceivable that the mechanism of action of the effects on the ovaries in dogs is associated with the inhibition of CYP21 and CYP17 by RPV base. But the exact mechanism of action of the observed early maturation (1-month studies) or activation of ovaries in the dog could not be evaluated from the general toxicity studies. Effects of RPV base at the level of serum concentrations of progesterone and estradiol were difficult to evaluate due to the estrous cycle of the dogs.

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

Testes

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

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

Red blood cells

In mice, rats, and dogs, RPV (base) caused a small (hardly ever exceeding 10%) reversible reduction of red blood parameters in combination with regenerative signs. The effects usually occurred only in the high dose groups in each of the studies with the indicated species. There were no clinical signs or symptoms of anemia noted in any of the species. Since exposures at the high doses in the various species differed considerably, it seems that the effect on red blood cells is not directly associated with exposure. Regeneration was evidenced by extramedullar hematopoiesis leading to increases in reticulocyte counts. The mechanism of action of the effect on red blood parameters could not be established in any species. However, there were no signs indicative of bone marrow suppression. The increased cellularity in bone marrow of female mice at the high dose is likely due to an increase of erythroid elements compensatory to the decrease of red blood cells in the circulation. The increased myeloid/erythroid ratio in bone marrow of male mice is assumed due to an increase in myeloid precursor cells compensating the decrease of lymphocytes in the circulation. Moreover, in the mouse and rat carcinogenicity no effects were noted on bone marrow and hemopoietic cells.

Coagulation

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

9.2. Genotoxicity

The potential of RPV (base) to induce mutagenesis has been investigated in vitro in the Ames test and the mouse lymphoma assay and in vivo in the mouse bone marrow micronucleus test. In none of the test systems, RPV as base or as HCl salt was able to induce mutations or chromosome damage, at the highest feasible concentration or dose tested. The following is considered relevant for the interpretation of the in vitro genetic toxicity data.

Metabolism studies indicated that rat S9 mix used as metabolism enhancer in the Ames test and mouse lymphoma assay had a poor capacity to generate metabolites M30 and M31. These metabolites occur in man and are the result of a Michael addition to the α, β-unsaturated nitrile moiety of RPV base. The metabolic pathway to M30 and M31 encompasses at least a reactive epoxide. Due to the poor capacity of rat S9 to generate M30 or M31, the reactive intermediate was not present in the Ames or mouse lymphoma assay and therefore, its potential to induce genotoxic effects was not tested. Since some batches of human S9 appeared to generate at least

M30, an additional Ames test was done with human S9 as metabolism enhancer. The test results were optimized by selecting a batch of human S9 that yielded M30 in the Ames test at least at the approximate level of M30 detected in feces in the human metabolism study. RPV (base) did not induce mutations under these test conditions. Moreover, RPV base did not demonstrate a clastogenic or aneuploidic effect in bone marrow of mice (Mod4.2.3.3.2/TMC278-Exp.5538, Mod4.2.3.3.2/TMC278-Exp.5538 (FK4259) and Mod2.6.7.9). The concentration of M30 in mice is similar to that in man (see Mod2.6.4/Sec5.2). So, the metabolic pathway with the potentially reactive intermediates was involved in this in vivo genotoxicity study.

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

The α, β-unsaturated nitrile moiety of RPV (base) generates a mutagenic alert. The drug substance impurities, related substance D*, related substance E*, and related substance F*, contain the same moiety and were for that reason evaluated for their mutagenic potential. Only related substance D*, tested as its [redacted] salt related substance G*, increased the mutation frequency in the Ames test. related substance D* contains also a [redacted] whereas the others do not. Therefore, it is concluded that the mutagenic potential of the [redacted] moiety is low and that the mutagenicity of related substance D* is due to its primary aromatic amine moiety.

The probability that genotoxic impurity related substance D* could contribute significantly to an increased incidence of tumors in man upon administration of RPV (base) is discussed in detail in Module 2.4/Nonclinical Overview.

9.3. Carcinogenicity

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

The exposures to RPV in the mouse study were as could be expected on the basis of the previous studies in that species. In the rat carcinogenicity, however, the exposure on Day 1 study was lower than was anticipated on the basis of the results of the bridging study with the base and the HCl salt. The toxicokinetic data generated after 6 months of dosing in the rat study showed that exposure had declined during the study. In consultation with FDA it was decided to continue the study and to keep the satellite animals for an additional sampling after 9 months of dosing. That sampling showed that exposure after 9 months of dosing was not different from that after 6 months of dosing. The outcome of the study in terms of a dose-related increase in neoplastic lesions illustrates that the study objectives were met. For that reason, the study is considered valid. No genicity studies in rats and in mice.

Hepatocellular adenomas and carcinomas are common spontaneous liver neoplasms in rodents [4]. In general, hepatocarcinogens are divided into genotoxic and non-genotoxic agents [3]. Since RPV (base) is not genotoxic in a battery of in vitro and in vivo assays, the neoplastic lesions in liver observed in the mouse and rat carcinogenicity studies are considered a

consequence of a non-genotoxic mechanism of the action of RPV base rather than an expression of a direct carcinogenic potential of the compound. For non-genotoxic carcinogens, several mechanisms of action have been reported for rodent liver neoplasm development including phenobarbital-like cytochrome P450 induction [5]. The increased liver weight associated with signs of enzyme induction caused by RPV base was already evident in the repeat-dose general toxicity studies. Ex vivo hepatic enzyme activity evaluated in the 3-month mouse study and the 6-month rats study showed that RPV base caused strong induction of the CYP4A family in male and female mice and male rats and induction of the CYP3A family in female rats. Electron microscopy of mouse liver noted peroxisome proliferation, a lesion commonly associated with CYP4A induction. This pattern of liver enzyme induction correlates well with the incidence of hepatocellular adenomas and carcinomas [6].

The repeat-dose toxicity studies in rats have demonstrated, without exception, effects on the thyroid gland often associated with effects in the pituitary gland and on the thyroid hormones and TSH. All data point towards a mechanism by which RPV base causes an increased clearance of thyroid hormones and a subsequently activated feedback on the thyroid gland by TSH synthesized in the pars distalis of the pituitary gland rather than having a direct effect on thyroid hormone synthesis. Furthermore, in rat liver, RPV base caused induction of UDPGT, the most important enzyme involved in the metabolism and clearance of thyroid hormones. UDPGT induction is a well-known cause of increased thyroid hormone clearance and upon life-long occurrence associated with the development of follicular adenomas and carcinomas [2].

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

Taking into account the results of the genotoxicity and the carcinogenicity studies, it is concluded that RPV (base) has not shown any potential to induce direct DNA-related effects. As mentioned previously these conclusions are deemed relevant for RPV LA as well. The relevance for man of the epigenetic carcinogenic effects on mouse and rat liver and rat thyroid gland is discussed in the nonclinical overview (Mod2.4).

9.4. Reproductive and Developmental Toxicity

The male and female rat fertility studies and pre- and postnatal developmental studies were conducted after the HCl salt was chosen for further development. In view of the minor testicular effects of RPV base in dogs characterized by marginal to limited Leydig cell hypertrophy, a separate male rat fertility study was conducted with up to the highest feasible dose of RPV, 1600 mg/kg/day.

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

As mentioned previously these conclusions are deemed relevant for RPV LA as well.

9.5. Juvenile Toxicity

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

9.6. Other Toxicity

RPV showed no potential for phototoxicity or sensitization and appeared to be non-irritant for skin and moderately irritant for eyes.

The drug substance contains three impurities at levels that need qualification according to ICH Q3A. Two impurities, related substance A*, and related substance B*, were qualified in an Ames test and a mouse lymphoma assay and in a 1-month oral rat study using RPV spiked with 4% of each of the impurities. None of the impurities induced a difference from the effects caused by RPV. The third impurity, related substance C*, is the [redacted]-isomer of RPV. This isomer was present in all drug substance batches involved in pivotal nonclinical studies at the level of minimally 0.61%. The qualified dose level of related substance C* in this study was 0.03 mg/kg/day.

Three low level drug substance impurities, related substance D*, related substance E*, and related substance F*, containing an [redacted] moiety were evaluated for their mutagenic potential. Only related substance D*, tested as its [redacted] salt related substance G*, induced a mutagenic response and a clastogenic response. It is concluded that the mutagenic potential of the [redacted] moiety is low and that the genotoxicity of related substance D* is due to its [redacted].

Assessments of these impurities in relation to clinical dose regimens are discussed in Mod2.4/Sec4.8.8.

9.7. RPV LA

In view of the RPV LA development, additional (dedicated) studies in dogs and minipigs were conducted in which the selected clinical formulation G001 (300 mg RPV/mL in 50 mg/mL P338) was tested. The dog study was conducted to bridge with the oral RPV program. Furthermore, the dog was considered the most appropriate species as it was more sensitive to RPV-related changes compared to the rodent, and it demonstrated a metabolism closer to human. The minipig was used as it is a well-established animal model to study local tolerability of IM administered drugs.

The compound was well tolerated in dogs at 1200 mg once weekly for 4 weeks and in minipigs at 600 mg once monthly for 9 months. Main findings comprised of local effects noted at the injection sites. Slight, short-lasting erythema was observed, and white deposits were noted at the injection sites at necropsy, accompanied by swelling and discoloration of draining lymph nodes. Microscopic examination showed macrophage infiltration and eosinophilic deposits of/at the injection sites. A macrophage infiltration response was also noted in the draining/regional lymph nodes. These findings were considered to be a reaction to the deposited material rather than a manifestation of local irritation. Effects related to systemic exposure to RPV LA in the dog study

were consisted with those seen after oral administration at similar exposures: increases in progesterone and 17α-(OH)-progesterone were noted up to the end of the study, however without changes in cortisol.

Various other RPV LA formulations were examined in single- and repeat-dose toxicology. All were well tolerated both systemically and locally at the IM or SC injection site. Local (inflammatory) reactions were observed at the injection sites but were generally mild and transient.

RPV LA showed no irritation potential *in vitro* and was devoid of skin sensitization potential in a mouse local lymph node assay.

The genotoxic impurity related substance D*, the specified impurities related substance H*, related substance I* and related substance C* as well as the residual solvent NMP were toxicologically qualified for the anticipated treatment regimens. Assessments of these impurities in relation to clinical dose regimens are discussed in Mod2.4/ Sec4.8.8.

The excipient poloxamer 338 was non-genotoxic in bacterial and mammalian cell assays, showed no relevant systemic or local toxicity in general toxicity studies in the minipig and did not induce any adverse findings on male or female fertility in the rat, embryofetal development in the rat and rabbit or pre- and postnatal development in the rat.

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 Toxicology Tabulated Summaries, located in Mod2.6.7.

11. LIST OF LITERATURE CITATIONS

Literation citations are located in Mod4.3.

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Janssen Research & Development

Toxicology Tabulated Summary

MODULE 2.6.7

Rilpivirine Long-Acting

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2.6.7.1 Toxicology: Overview

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Single-Dose Toxicity									
	Mouse/Albino Swiss	Oral/Gavage	Single dose	**Base**: 0 (vehicle), 100, 400, 1600 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-Exp.5538/ 4.2.3.3.2
	Rat/Sprague Dawley	Oral/Gavage	Single dose	**Base**: 800 (PEG400)	[redacted]	Yes	No	J&J PRD	TMC278-Exp.5559/ 4.2.3.1
	Dog/Beagle	Oral/Gavage	Single dose	**Base**: 40, 80 (PEG400) 80 (PEG400 + CA)	[redacted]	Yes	No	J&J PRD	TMC278-Exp.5461/ 4.2.3.2
Repeat-Dose Toxicity									
	Mouse/Albino Swiss	Oral/Gavage or diet	2 weeks	**Hydrochloride salt**: 0 (control diet), 40 (diet), 400 (diet), 5000 (diet), 400 (gavage), 2000 (gavage)	[redacted]	Yes	No	J&J PRD	TMC278-NC118 (TOX6479)/ 4.2.3.2
	Mouse/CB6F1-nonTgrasH2	Oral/Gavage	1 month	**Hydrochloride salt**: 0 (vehicle), 20, 80, 320 (0.5% HPMC)	[redacted]	Yes	Yes	[redacted]	TMC278-NC121 (TOX6740)/ 4.2.3.2
	Mouse/Albino Swiss	Oral/Gavage	3 months	**Hydrochloride salt**: 0 (vehicle), 20, 80, 320 (0.5% HPMC)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC119 (TOX6739)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	5 days	**Base**: 0 (water), 0 (vehicle), 40, 400 (PEG400)	[redacted]	Yes	No	J&J PRD	TMC278-Exp.5463/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	2 weeks	**Base**: 0 (water), 0 (vehicle), 40, 120, 400 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-Exp.5535/ 4.2.3.2

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Repeated-Dose Toxicity (Continued)									
	Rat/Sprague Dawley	Oral/Gavage or diet	2 weeks	**Hydrochloride salt**: 0 (control diet), 400 (diet), 1200 (diet), 400 (gavage) **Base**: 400 (diet)	[redacted]	Yes	No	J&J PRD	TMC278-NC136 (TOX6417)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	2 weeks	**Hydrochloride salt**: 0 (vehicle), 400, 1500, 2000 (0.5% HPMC)	[redacted]	Yes	No	J&J PRD	TMC278-NC177 (TOX6813)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	1 month	**Base**: 0 (vehicle),10, 400 (PEG400 + CA) **Hydrochloride salt**: 0 (vehicle), 10, 400 (0.5% HPMC)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC117 (TOX6385)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	1 month	**Base**: 0 (water), 0 (vehicle), 10, 40, 160 (PEG400 + CA)	[redacted]	Yes	Yes	[redacted]	TMC278-Exp.5692/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	6 months	**Base**: 0 (vehicle), 40, 120, 400 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC101 (TOX6142)/ 4.2.3.2
	Rabbit/New Zealand white	Oral/Gavage	5 days	**Base**: 0 (vehicle), 100, 300, 1000 (0.5% HPMC)	[redacted]	Yes	No	J&J PRD	TMC278-NC126 (TOX6146)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	5 days	**Base**: 40, 80 (PEG400) 80 (PEG400 + CA)	[redacted]	Yes	No	J&J PRD	TMC278-Exp.5461/ 4.2.3.2

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Repeated-Dose Toxicity (Continued)									
	Dog/Beagle	Oral/Gavage	7 days	**Base**: 0 (water), 0 (vehicle), 20, 40,80 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-Exp.5534/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	1 month	**Base**: 0 (vehicle), 5, 40 (PEG400 + CA) **Hydrochloride salt**: 0 (vehicle), 5, 40 (0.5% HPMC)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC116 (TOX6334)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	1 month	**Base**: 0 (water), 0 (vehicle), 5, 10, 40 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-Exp.5650/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	6 months	**Base**: 0 (vehicle), 5, 10, 40 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC115 (TOX6110)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	12 months	**Base**: 0 (vehicle), 5, 10, 40 (PEG400 + CA)	[redacted]	Yes	Yes	[redacted]	TMC278-NC107 (TOX6314)/ 4.2.3.2
	Monkey/ Cynomolgus	Oral/Gavage	2 weeks	**Hydrochloride salt:** 0 (vehicle), 100, 250 (1% HPMC 2910 5mPA + 0.5% TWEEN 20)	[redacted]	Yes	No	[redacted]	TMC278-TiDP38-NC326 (FK6541)/ 4.2.3.2
	Monkey/ Cynomolgus	Oral/Gavage	8 weeks	**Hydrochloride salt**: 0 (vehicle), 200, 500 bid (1% HPMC + 0.5% polysorbate 20)	[redacted]	Yes	Yes	[redacted]	TMC278-TiDP38-NC248 (TOX8862)/ 4.2.3.2

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Repeated-Dose Toxicity (Continued)									
	Dog/Beagle	IM injection JNJ-16150108-AAA – G002 0mg/mL 300mf/mL RPV LA in 50 mg/mL P338	every 2 weeks during 4 weeks	0, 150, 1200 mg/injection	[redacted]	Yes	Yes	[redacted]	TOX10759/ 4.2.3.2
	Göttingen minipig	IM injection (2 mL) Negative control V1 = P338 (50 mg/mL) V2 = P338 (160 mg/mL) *[Nanocrystal suspension with P338 at 50 mg/mL]*	every 2 weeks during 6 weeks	600 mg/injection @ 300 mg/mL	[redacted]	Yes	Yes	Janssen R&D	TMC278-NC368 (TOX9508)/ 4.2.3.2
	Göttingen minipig	IM injection (2 mL) Control solution Vehicle: P338 *[Nanocrystal suspension*]	once monthly for 9 months	600 mg/injection @ 300 mg/mL	[redacted]	Yes	Yes	Janssen R&D	TMC278-NC349 (TOX9517)/ 4.2.3.2
Genotoxicity – In Vitro									
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	**Base**: 0, 7.81, 15.63, 31.25, 62.50, 125, 250 and 500 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-Exp.5540/ 4.2.3.3.1
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	**Base**: 0, 7.81, 15.63, 31.25, 62.50, 125, 250 and 500 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-Exp.5693/ 4.2.3.3.1

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Genotoxicity – In Vitro (Continued)									
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	**Hydrochloride salt**: 0, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-TiDP6-NC335 (TOX8918)/ 4.2.3.3.1
	Hepatocytes, liver subcellular fractions	In vitro	---	/	[redacted]	No	No	J&J PRD	TMC278-NC102 (FK4728)/ 4.2.2.4
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	**Base**: 0, 3, 7.81, 10, 15.63, 25, 31.25, 50, 62.5, 100, 125, 200, 250, 400 and 500 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-NC279 (TOX8086)/ 4.2.3.3.1
	Mouse Lymphoma cells L5178Y	In vitro	---	**Base**: 0, 0.5, 1, 2, 2.5, 4, 5, 6, 8, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 250, 500 µg/mL	[redacted]	No	Yes	J&J PRD	TMC278-Exp.5539/ 4.2.3.3.1
	Mouse Lymphoma cells L5178Y	In vitro	---	**Hydrochloride salt**: 0, 2.5, 5, 10, 12.5, 15, 20, 25, 30, 35, 40 and 50 µg/mL	[redacted]	No	Yes	[redacted]	TMC278-TiDP6-NC336 (TOX8919)/ 4.2.3.3.1
Genotoxicity – In Vivo									
	Mouse/Albino Swiss	Oral/Gavage	Single dose	**Base**: 0 (vehicle), 100, 400, 1600 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-Exp5538/ 4.2.3.3.2
	Mouse/CD1	Oral/Gavage	2 years	**Hydrochloride salt**: 0 (vehicle), 20, 60, 160(0.5% HPMC)	[redacted]	Yes	Yes	[redacted]	TMC278-NC120 (TOX7222)/ 4.2.3.4.1

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Carcinogenicity									
	Mouse/CD1	Oral/Gavage	2 years	**Hydrochloride salt**: 0 (vehicle), 20, 60, 160 (0.5% HPMC)	[redacted]	Yes	Yes	[redacted]	TMC278-NC120 (TOX7222)/ 4.2.3.4.1
	Rat/Sprague Dawley	Oral/Gavage	2 years	**Hydrochloride salt**: 0 (vehicle), 40, 200, 500, 1500 (0.5% HPMC)	[redacted]	Yes	Yes	[redacted]	TMC278-NC123 (TOX7221)/ 4.2.3.4.1
Reproductive and Developmental Toxicity									
	Rat/Sprague Dawley	Oral/Gavage	10 weeks prior to mating and 4 weeks after the mating period	**Hydrochloride salt**: 0 (vehicle), 100, 400, 1600 (0.5% HPMC)	[redacted]	No	Yes	[redacted]	TMC278-NC124 (TOX6861)/ 4.2.3.5.1
	Rat/Sprague Dawley	Oral/Gavage	2 weeks prior to pairing and up to day 7 of pregnancy	**Hydrochloride salt**: 0 (vehicle), 40, 120, 400 (0.5% HPMC)	[redacted]	No	Yes	J&J PRD	TMC278-NC125 (TOX6708)/ 4.2.3.5.1
	Rat/Sprague Dawley	Oral/Gavage	GD6-17	**Base**: 0 (vehicle), 40, 120, 400 (PEG400 + CA)	[redacted]	No	No	J&J PRD	TMC278-NC127 (TOX6147)/ 4.2.3.5.2
	Rat/Sprague Dawley	Oral/Gavage	GD6-17	**Base**: 0 (vehicle), 40, 120, 400 (PEG400 + CA)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC105 (TOX6268)/ 4.2.3.5.2

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Reproductive and Developmental Toxicity (Continued)									
	Rabbit/New Zealand white	Oral/Gavage	GD6-19	**Base**: 0 (vehicle), 25, 75, 150 (0.5% HPMC)	[redacted]	Yes	No	J&J PRD	TMC278-NC128 (TOX6235)/ 4.2.3.5.2
	Rabbit/New Zealand white	Oral/Gavage	GD6-19	**Base**: 0 (vehicle), 5, 20, 60 (0.5% HPMC)	[redacted]	No	No	J&J PRD	TMC278-NC129 (TOX6280)/ 4.2.3.5.2
	Rabbit/New Zealand white	Oral/Gavage	GD6-19	**Base**: 0 (vehicle), 5, 10, 20 (0.5% HPMC)	[redacted]	Yes	Yes	J&J PRD	TMC278-NC130 (TOX6313)/ 4.2.3.5.2
	Rat/Sprague Dawley	Oral/Gavage	GD6-LD7	**Hydrochloride salt**: 0 (vehicle), 40, 120, 400 (0.5% HPMC)	[redacted]	Yes	No	[redacted]	TMC278-NC168 (TOX6847)/ 4.2.3.5.3
	Rat/Sprague Dawley	Oral/Gavage	GD6-LD20	**Hydrochloride salt**: 0 (vehicle), 40, 120, 400 (0.5% HPMC)	[redacted]	No	Yes	[redacted]	TMC278-NC131 (TOX6972)/ 4.2.3.5.3
Local Tolerance									
	Bovine corneas	In vitro	---	**Hydrochloride salt**: 20% w/w in 0.9% NaCl (vehicle)	[redacted]	No	No	J&J PRD	TMC278-NC202 (TOX7619)/ 4.2.3.6
	Balb/c mouse fibroblast	In vitro	---	**Hydrochloride salt**: 0.001 to 2.16 mg/L	[redacted]	No	No	J&J PRD	TMC278-NC188 (TOX7218)/ 4.2.3.6

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Local Tolerance (Continued)									
	Rabbit/New Zealand white	Dermal application	4 hours	**Hydrochloride salt**: 0.5 g	[redacted]	No	No	[redacted]	TMC278-NC159 (TOX7555)/ 4.2.3.6
	Mouse/CBA/CA	Dermal application	3 days	**Hydrochloride salt**: 5, 10 and 35% in Acetone/Olive oil (4:1)	[redacted]	No	No	[redacted]	TMC278-NC199 (TOX7453)/ 4.2.3.6
	CAM of fertilized chicken eggs	*In vitro* (nanosuspension in PS80)	-	@ 200 and 300 mg/mL	[redacted]	No	No	Janssen R&D	TMC278-NC301 (TOX8460)/ 4.2.3.6
	Rabbit/New Zealand White	IM or SC injection (1 mL/injection site) P338 or Vit E-TPGS	Single dose	0 (vehicle), @ 100 mg/mL	[redacted]	No	Yes	Janssen R&D	TMC278-NC237 (TOX7774)/ 4.2.3.6
	Rabbit/New Zealand White	IM injection (1 mL) P338 P338/Na-deoxycholate P338/PEG350 PE P338/DOSS *[aqueous nanosuspension]*	single + observation period of 48 or 72 h	@ 200-300 mg/mL @ 200-300 mg/mL @ 300 mg/mL @ 300 mg/mL	[redacted]	No	No	Janssen R&D	TMC278-NC367 (TOX9445)/ 4.2.3.6
	Göttingen minipig	IM injection (2 mL) P338 PS80 *[aqueous nanosuspension]*	Single dose	@ 100 or 200 mg/mL @ 100, 200, 250 or 300 mg/mL	[redacted]	Yes	No	[redacted]	TMC278-NC344 (FK7034)/ 4.2.2.7

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Local Tolerance (Continued)									
	Göttingen minipig	IM injection (2 mL) P338 P338 P338/Na-deoxycholate P338/Na-deoxycholate; P338/PE PEG350 P338/DOSS) *[Nanosuspension*]	Single + 3-month follow-up	@ 200 mg/mL @ 300 mg/mL @ 200 mg/mL @ 300 mg/mL @ 300 mg/mL @ 300 mg/mL	[redacted]	Yes	No	Janssen R&D	TMC278-NC359 (TOX9403)/ 4.2.3.6
Other Toxicity Studies									
Mechanistic	Dog/Beagle	Oral/Gavage	Single dose	**Base**: 0 (vehicle), 20, 80 (PEG400 + CA)	[redacted]	No	No	J&J PRD	TMC278-Exp.5627/ 4.2.3.7.3
	Guinea pig adrenal cells	In vitro	---	**Base**: 10^{-9} to 10^{-5}M	[redacted]	No	No	J&J PRD	TMC278-Exp.5653/ 4.2.3.7.3
	Dog adrenal cortex	In vitro	---	**Base**: 0.03 to 75 µM	[redacted]	No	No	J&J PRD	TMC278-FK4790/ 4.2.2.4
Impurities	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA102	In vitro	---	**Hydrochloride salt**: 0, 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 µg/plate spiked with 4% related substance A*	[redacted]	No	Yes	J&J PRD	TMC278-TiDP6-NC308 (TOX8901)/ 4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	**Hydrochloride salt**: 0, 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 µg/plate spiked with 4% related substance B*	[redacted]	No	Yes	J&J PRD	TMC278-TiDP6-NC309 (TOX8900)/ 4.2.3.7.6

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Other Toxicity Studies (Continued)									
Impurities	Mouse Lymphoma cells L5178Y	In vitro	---	**Hydrochloride salt**: up to 75 µg/mL spiked with 4% related substance A*	[redacted]	No	Yes	[redacted]	TMC278-TiDP6-NC311 (TOX8899)/ 4.2.3.7.6
	Mouse Lymphoma cells L5178Y	In vitro	---	**Hydrochloride salt**: up to 75 µg/mL spiked with 4% related substance B*	[redacted]	No	Yes	[redacted]	TMC278-TiDP6-NC312 (TOX8898)/ 4.2.3.7.6
	Rat/Sprague Dawley	Oral/Gavage	1-month	**Hydrochloride salt**: 0 (vehicle), 10, 10 spiked with 4% related substance A*, 10 spiked with 4% related substance B* (0.5% HPMC)	[redacted]	No	Yes	J&J PRD	TMC278-TiDP6-NC314 (TOX8886)/ 4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	related substance G* 0, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate	[redacted]	No	No	J&J PRD	TMC278-NC179 (TOX6835)/ 4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	related substance G* 0 , 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate	[redacted]	No	No	J&J PRD	TMC278-NC165 (TOX6616)/ 4.2.3.7.6

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Other Toxicity Studies (Continued)									
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	related substance G*: Exp 1 (2): 0 (vehicle), 78.13 (93.75), 156.25 (187.5), 312.5 (375), 625 (750), 1250 (1500), 2500 (3000) and 5000 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-1646_0016315 (TOX9643)/ 4.2.3.7.6
	Human lymphocytes	In vitro	4 or 24 hours	related substance G*: 0, 65.22, 130.44, 260.88 and 521.75 µg/mL	[redacted]	No	No	[redacted]	TMC278-TiDP6-NC184 (TOX7006)/ 4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	related substance E*: Exp 1 (2): 0 (vehicle), 78.13 (93.75), 156.25 (187.5), 312.5 (375), 625 (750), 1250 (1500), 2500 (3000) and 5000 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-1646_0015298 (TOX9645)/ 4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA102	In vitro	---	related substance E*: Exp 1+3 (2): 0 (vehicle), 78.13 (93.75), 156.25 (187.5), 312.5 (375), 625 (750), 1250 (1500), 2500 (3000) and 5000 µg/plate	[redacted]	No	Yes	J&J PRD	TMC278-1646_0015299 (TOX9644)/ 4.2.3.7.6
	Albino Swiss mouse	SC injection V1 = P338 V2 = Vit E-TPGS *[Nanocrystal suspension]*	Single dose	0, 2.5, 5, 10, 20 @ 25 mg/mL	[redacted]	Yes	No	Janssen R&D	TMC278-NC196 (TOX7354)/ 4.2.3.7.7

(Continued)

＊新薬承認情報提供時に置換

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Other Toxicity Studies (Continued)									
	Rat/Sprague Dawley	SC (0.2 mL) or IM (0.2 mL) or IV (1 mL) injection SC or IM: P338 IV: PEG 400/25% sterile water *[Nanocrystal suspension]*	Single dose	SC or IM: 5 or 20 @ 25 or 100 mg/mL IV: 1.25 @ 1.25 mg/mL	[redacted]	Yes	No	Janssen R&D	TMC278-NC244 (TOX7896)/ 4.2.3.7.7
	Dog/Beagle	SC or IM injection (1 mL) P338 *[Nanocrystal suspension]*	Single dose	200 or 400 mg/total dose/dog @ 100 mg/mL	[redacted]	Yes	Yes	Janssen R&D	TMC278-NC234 (TOX7781)/ 4.2.3.7.7
	Göttingen minipig	IM injection (1 or 1.5 mL) new: PS80 (=F006) current: P338 (=F004) *[Nanocrystal suspension]*	Single dose	new: 450 mg @ 300 mg/mL current: 400 mg @ 100 mg/mL	[redacted]	Yes	No	[redacted]	TMC278-NC295 (FK6407)/ 4.2.2.7
	Göttingen minipig	IM injection in thigh (250 µL/kg on days 1, 30, 60 and 90; 2 mL/inj. on days 120, 150, 180, 210, 240 and 270) + IM injection in m. long. dorsi (1 mL/inj. on days 120, 150, 180 and 210) Negative control (0.9% NaCl) V = PS80 + lipoid *[Nanocrystal suspension]*	Once monthly for 39 weeks (13-week interim sacrifice)	thigh: 75 mg/kg @ 300 mg/mL (up to week 13 [interim sacrifice]) + 600 mg/animal @ 300 mg/mL afterwards + m. long. dorsi: 300 mg/animal @ 300 mg/mL	[redacted]	Yes	Yes	[redacted]	TMC278-NC296 (TOX8580)/ 4.2.3.7.7

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Other Toxicity Studies (Continued)									
	Rabbit/New Zealand White	IM injection (1 mL/injection) (PS80) [*aqueous nanosuspension*]	Single dose	@ 300 mg/mL	[redacted]	No	No	Janssen R&D	TMC278-NC280 (TOX8482)/ 4.2.3.7.7
	Salmonella typhimurium	*In vitro* bacterial reverse mutation test with P338[b]	-	up to 10,000 µg/plate	[redacted]	No	Yes	Janssen R&D	TMC278-NC357 (TOX9535)/ 4.2.3.7.7
	Human lymphocytes	*In vitro* chromosome aberration test with P338[b]	3h incubation + 18h recovery	up to 10,000 µg/plate	[redacted]	No	Yes	[redacted]	TMC278-NC358 (TOX9523)/ 4.2.3.7.7
	Rat/Sprague Dawley	Oral gavage of P338 demineralised water *[aqueous solution]*	Single dose	1600	[redacted]	No	No	Janssen R&D	FK13157/ 4.2.2.7
	Rabbit/New Zealand White	Oral gavage of P338 demineralised water *[aqueous solution]*	Single dose	1600	[redacted]	Yes	No	Janssen R&D	FK13159/ 4.2.2.7
	Rat/Sprague Dawley	Oral gavage of P338 demineralised water *[aqueous solution]*	14 days	0 1600	[redacted]	No	No	Janssen R&D	1955_0016643 (TOX9648)/ 4.2.3.7.7
	Rat/Sprague Dawley	Oral gavage of P338 demineralised water *[aqueous solution]*	GD 6-17	0 1600	[redacted]	No	Yes	Janssen R&D	TMC278-NC347 (TOX9679)/ 4.2.3.7.7

(Continued)

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)[a]	Batch No.	TK	GLP	Test Facility	StudyNo./ Location in CTD
Other Toxicity Studies (Continued)									
	Rat/Sprague Dawley	IM injection of P338 Sterile aqueous solution	Single dose Repeated dosing	5, 10, 2x5	[redacted]	Yes	No	Janssen R&D	TOX13295/ 4.2.3.7.7
	Rat/Sprague Dawley	Oral gavage of P338 Sterile aqueous solution	Repeated dosing[c]	2.5, 5, 10	[redacted]	Yes	Yes	[redacted]	TOX13391/ 4.2.3.7.7
	Rabbit/New Zealand White	Oral gavage of P338 demineralised water *[aqueous solution]*	14 days	0 1600	[redacted]	No	No	Janssen R&D	TMC278-NC366 (TOX9649)/ 4.2.3.7.7
	Rabbit/New Zealand White	Oral gavage of P338 demineralised water *[aqueous solution]*	GD6-19	0 1600	[redacted]	No	Yes	Janssen R&D	TMC278-NC348 (TOX9680)/ 4.2.3.7.7
	Rabbit/New Zealand White	IM injection of P338 demineralised water *[aqueous solution]*	Single dose Repeated dosing	2.5, 5	[redacted]	Yes	No	Janssen R&D	TOX13296/ 4.2.3.7.7
	Rabbit/New Zealand White	IM injection of P338 demineralised water *[aqueous solution]*	GD6 and GD12	2.5, 5	[redacted]	Yes	Yes	[redacted]	TOX13376/ 4.2.3.7.7
	Rat/Sprague Dawley	IM injection of P338 demineralised water *[aqueous solution]*	One administration every three calendar days from GD 6 to weaning (13 administrations)	0, 2.5, 5, 10 mg/kg/dose	[redacted]	Yes	Yes	[redacted]	TOX13546/ 4.2.3.7.7

(Continued

2.6.7.1 Toxicology: Overview (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

[a] Unless otherwise specified
[b] Only Poloxamer 338 was administered (without TMC278).
[c] Males: one administration every three days from 28 days before mating, throughout mating and up to 3 days before necropsy (21 administrations), Females: 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)

bid = twice a day; CA = citric acid; CAM = chroioallantoic membrane; DOSS = dioctyl sodium sulfosuccinate; F004 = clinical formulation containing 100 mg/mL TMC278 LA in P338; F006 = clinical formulation containing 300 mg/mL TMC278 LA in PS80; G001 = clinical formulation containing 300 mg/mL TMC278 LA in P338; GD = gestation day; GLP: good laboratory practice; h = hour; [redacted] = [redacted]; HPMC = hydroxylpropyl methylcellulose; IM = intramuscular; IV = intravenous; J&JPRD = Johnson & Johnson Pharmaceutical Research and Development; LA = long acting; LD = lactation day; m. long. dorsi = musculus longissimus dorsi; NaCl = sodium chloride; PEG400 = polyethylene glycol 400; PEG350 PE = polyethylene glycol 350; P338 = Poloxamer 338 (Pluronics F108); PS80 = polysorbate 80; RPV = previously known as TMC278, JNJ-16150108 or R278474; R&D = Research and Development; SC = subcutaneous; TK = toxicokinetics; TPGS = D-α-Tocopheryl Polyethyleneglycol 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 toxicology studies of RPV 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.

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	Doses (mg/kg)[a]	GLP	Study No/ Location in CTD
Single Dose Toxicity					
	Mouse/Albino Swiss	Oral/Gavage	**Base**: 0 (vehicle), 100, 400, 1600 (PEG400 + CA)	Y	TMC278-Exp.5538 (FK4259)/ 4.2.3.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 800 (PEG400)	N	TMC278-Exp.5559 (FK4278)/ 4.2.3.1
	Dog/Beagle	Oral/Gavage	**Base**: 40, 80 (PEG400) 80 (PEG400 + CA)	N	TMC278-Exp.5461 (FK4102)/ 4.2.3.2
Repeat Dose Toxicity					
	Mouse/Albino Swiss	Oral/Gavage or diet	**Hydrochloride salt**: 0 (control diet), 40 (diet), 400 (diet), 5000 (diet), 400 (gavage), 2000 (gavage)	N	TMC278-NC118/ 4.2.3.2
	Mouse/CB6F1-nonTgrasH2	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 20, 80, 320 (0.5% HPMC)	Y	TMC278-NC121/ 4.2.3.2
	Mouse/Albino Swiss	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 20, 80, 320 (0.5% HPMC)	Y	TMC278-NC119/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 0 (water), 0 (vehicle), 40, 400 (PEG400)	N	TMC278-Exp.5463 (FK4103)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 0 (water), 0 (vehicle), 40, 120, 400 (PEG400 + CA)	Y	TMC278-Exp.5535 (FK4243)/ 4.2.3.2

(Continued)

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	Doses (mg/kg)[a]	GLP	Study No/ Location in CTD
Repeat Dose Toxicity (Continued)					
	Rat/Sprague Dawley	Oral/Gavage or diet	**Hydrochloride salt**: 0 (control diet), 400 (diet), 1200 (diet), 400 (gavage) **Base**: 400 (diet)	N	TMC278-NC136 (TOX6417)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 400, 1500, 2000 (0.5% HPMC)	N	TMC278-NC177 (TOX6813)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 0 (vehicle),10,400 (PEG+CA) **Hydrochloride salt**: 0 (vehicle), 10, 400 (0.5% HPMC)	Y	TMC278-NC117 (TOX6385)/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 0 (water), 0 (vehicle), 10, 40, 160 (PEG400 + CA)	Y	TMC278-Exp.5692/ 4.2.3.2
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 0 (vehicle), 40, 120, 400 (PEG400 + CA)	Y	TMC278-NC101 (TOX6142)/ 4.2.3.2
	Rabbit/New Zealand white	Oral/Gavage	**Base**: 0 (vehicle), 100, 300, 1000 (0.5% HPMC)	N	TMC278-NC126 (TOX6146)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	**Base**: 40, 80 (PEG400) 80 (PEG400 + CA)	N	TMC278-Exp.5461 (FK4102)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	**Base**: 0 (water), 0 (vehicle), 20, 40, 80 (PEG400 + CA)	Y	TMC278-Exp.5534 (FK4244)/ 4.2.3.2

(Continued)

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	Doses (mg/kg)[a]	GLP	Study No/ Location in CTD
Repeat Dose Toxicity (Continued)					
	Dog/Beagle	Oral/Gavage	**Base**: 0 (vehicle), 5, 40 (PEG400 + CA) **Hydrochloride salt**: 0 (vehicle), 5, 40 (0.5% HPMC)	Y	TMC278-NC116 (TOX6334)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	**Base**: 0 (water), 0 (vehicle), 5, 10, 40 (PEG400 + CA)	Y	TMC278-Exp.5650/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	**Base**: 0 (vehicle), 5, 10, 40 (PEG400 + CA)	Y	TMC278-NC115 (TOX6110)/ 4.2.3.2
	Dog/Beagle	Oral/Gavage	**Base**: 0 (vehicle), 5, 10, 40 (PEG400 + CA)	Y	TMC278-NC107 (TOX6314)/ 4.2.3.2
	Monkey/Cynomolgus	Oral/Gavage	**Hydrochloride salt:** 0 (vehicle), 100, 250 (1% HPMC 2910 5mPA + 0.5% TWEEN 20)	N	TMC278-TiDP38-NC326 (FK6541)/ 4.2.3.2
	Monkey/Cynomolgus	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 200, 500 bid (1% HPMC + 0.5% polysorbate 20)	Y	TMC278-TiDP38-NC248 (TOX8862)/ 4.2.3.2
4-week RD toxicity + 2-week recovery	Dog/Beagle	IM injection JNJ-16150108-AAA-G002 0 mg/mL 300 mg/mL RPV LA in 50 mg/mL P338	0, 150, 1200 mg/injection	Y	TOX10759 4.2.3.2

(Continued)

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	Doses (mg/kg)[a]	GLP	Study No/ Location in CTD
Repeat Dose Toxicity (Continued)					
	Göttingen minipig	IM injection (2 mL) Negative control V1 = P338 (50 mg/mL) V2 = P338 (160 mg/mL) *[Nanocrystal suspension with P338 at 50 mg/mL]*	600 mg/injection @ 300 mg/mL	Y	TMC278-NC368 (TOX9508)/ 4.2.3.2
	Göttingen minipig	IM injection (2 mL) Control solution Vehicle: P338 *[Nanocrystal suspension*]	600 mg/injection @ 300 mg/mL	Y	TMC278-NC349 (TOX9517)/ 4.2.3.2
Genotoxicity – In Vivo					
	Mouse/Albino Swiss	Oral/Gavage	**Base**: 0 (vehicle), 100, 400, 1600 (PEG400 + CA)	Y	TMC278-Exp.5538/ 4.2.3.3.2
Carcinogenicity					
	Mouse/CD-1	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 20, 60, 160 (0.5% HPMC)	Y	TMC278-NC120 (TOX7222)/ 4.2.3.4.1
	Rat/Sprague Dawley	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 40, 200, 500, 1500 (0.5% HPMC)	Y	TMC278-NC123 (TOX7221)/ 4.2.3.4.1
Reproductive and Developmental					
	Rat/Sprague Dawley	Oral/Gavage	**Base**: 0 (vehicle), 40, 120, 400 (PEG400 + CA)	Y	TMC278-NC105 (TOX6268)/ 4.2.3.5.2
	Rabbit/New Zealand white	Oral/Gavage	**Base**: 0 (vehicle), 25, 75, 150 (0.5% HPMC)	N	TMC278-NC128 (TOX6235)/ 4.2.3.5.2
	Rabbit/New Zealand white	Oral/Gavage	**Base**: 0 (vehicle), 5, 10, 20 (0.5% HPMC)	Y	TMC278-NC130 (TOX6313)/ 4.2.3.5.2
	Rat/Sprague Dawley	Oral/Gavage	**Hydrochloride salt**: 0 (vehicle), 40, 120, 400 (0.5% HPMC)	N	TMC278-NC168 (TOX6847)/ 4.2.3.5.3

(Continued)

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	Doses (mg/kg)[a]	GLP	Study No/ Location in CTD
Local Tolerance					
	Göttingen minipig	IM injection (2 mL) P338 PS80 *[aqueous nanosuspension*]	@ 100 or 200 mg/mL @ 100, 200, 250 or 300 mg/mL	N	TMC278-NC344 (FK7034)/ 4.2.2.7
	Göttingen minipig	IM injection (2 mL) P338 P338 P338/Na-deoxycholate P338/Na-deoxycholate; P338/PE PEG350 P338/DOSS) *[Nanosuspension*]	@ 200 mg/mL @ 300 mg/mL @ 200 mg/mL @ 300 mg/mL @ 300 mg/mL @ 300 mg/mL	N	TMC278-NC359 (TOX9403)/ 4.2.3.6
Other					
	Mouse/Albino Swiss	SC injection V1 = P338 V2 = Vit E-TPGS *[Nanocrystal suspension]*	0, 2.5, 5, 10, 20 @ 25 mg/mL	N	TMC278-NC196 (TOX7354)/ 4.2.3.7.7
	Rat/Sprague Dawley	SC (0.2 mL) or IM (0.2 mL) or IV (1 mL) injection SC or IM: P338 IV: PEG 400/25% sterile water *[Nanocrystal suspension]*	SC or IM: 5 or 20 @ 25 or 100 mg/mL IV: 1.25 @ 1.25 mg/mL	N	TMC278-NC244 (TOX7896)/ 4.2.3.7.7
	Dog/Beagle	SC or IM injection (1 mL) P338 *[Nanocrystal suspension]*	200 or 400 mg/total dose/dog @ 100 mg/mL	Y	TMC278-NC234 (TOX7781)/ 4.2.3.7.7
	Göttingen minipig	IM injection (1 or 1.5 mL) new: PS80 current: P338 *[Nanocrystal suspension]*	new: 450 mg @ 300 mg/mL current: 400 mg @ 100 mg/mL	N	TMC278-NC295 (FK6407)/ 4.2.2.7

(Continued)

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies

Test Article: rilpivirine, rilpivirine LA, P338

Type of Study	Test System	Route (Vehicle/Formulation)	Doses (mg/kg)[a]	GLP	Study No/ Location in CTD
Other (Continued)					
	Göttingen minipig	IM injection in thigh (250 µL/kg on days 1, 30, 60 and 90; 2 mL/inj. on days 120, 150, 180, 210, 240 and 270) + IM injection in m. long. dorsi (1 mL/inj. on days 120, 150, 180 and 210) Negative control (0.9% NaCl) V = PS80 + lipoid *[Nanocrystal suspension]*	thigh: 75 mg/kg @ 300 mg/mL (up to week 13 [interim sacrifice]) + 600 mg/animal @ 300 mg/mL afterwards + m.long. dorsi: 300 mg/animal @ 300 mg/mL	Y	TMC278-NC296 (TOX8580)/ 4.2.3.7.7
	Rabbit/New Zealand white	Oral gavage of P338 demineralised water *[aqueous solution]*	1600	N	FK13159/ 4.2.2.7
	Rat/Sprague Dawley	IM injection of P338 *Sterile aqueous solution*	5, 10, 2x5	N	TOX13295/ 4.2.3.7.7
	Rat/Sprague Dawley	Oral gavage of P338 *Sterile aqueous solution*	2.5, 5, 10	Y	TOX13391/ 4.2.3.7.7
	Rabbit/New Zealand white	IM injection of P338 demineralised water *[aqueous solution]*	5, 2.5	N	TOX13296/ 4.2.3.7.7
	Rabbit/New Zealand white	IM injection of P338 demineralised water *[aqueous solution]*	2.5, 5	Y	TOX13376/ 4.2.3.7.7

[a] Unless otherwise stated

bid = twice a day; CA = citric acid; DOSS = dioctyl sodium sulfosuccinate; GLP: good laboratory practice; HPMC = hydroxylpropyl methylcellulose; IM = intramuscular; inj. = injection; IV = intravenous; LA = long acting; m. long. dorsi = musculus longissimus dorsi; N = no; NaCl = sodium chloride; PEG400 = polyethylene glycol 400; PEG350 = polyethylene glycol 350; P338 = Poloxamer 338 (Pluronics F108); PS80 = polysorbate 80; RD = repeated dose; RPV = previously known as TMC278, JNJ-16150108 or R278474; SC = subcutaneous; TPGS = D-α-Tocopheryl Polyethyleneglycol 1000 Succinate; V = vehicle; Vit E = vitamin E; Y = yes

Note: The available studies of RPV after administration of RPV LA are listed. In addition, all the relevant nonclinical toxicology studies of RPV 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.

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: rilpivirine LA

Steady-State AUC (ng.h/mL)

Daily Dose (mg/kg)	Study Duration	Dog		Minipig	
		M	F	M	F
150	4-weeks	28,800	45,700		
600	6-weeks			18,378	33,153
600	9-months			20,696	34,964
1200	4-weeks	218,000	204,000		
150[a]	4-weeks	34,500	48,500		
600[b]	6-weeks			51,359	52,751
600[c]	9-months			50,071	44,342
1200[a]	4-weeks	217,000	206,000		

Source: Mod4.2.3.2/TOX10759, Mod4.2.3.2/TMC278-NC368 (TOX9508), Mod4.2.3.2/TMC278-NC349 (TOX9517)
[a] day 15; [b] day 28; [c] day 224

Note: The available studies of RPV LA are listed, exposure values originate from studies in which the clinical formulation G001 was applied.

(Continued)

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data

Test Article: rilpivirine LA

		C_{max} (ng/mL)			
		Dog		Minipig	
Daily Dose (mg/kg)	**Study Duration**	**M**	**F**	**M**	**F**
150	4-weeks	116	245		
600	6-weeks			580	1004
600	9-months			189	637
1200	4-weeks	1220	1220		
150[a]	4-weeks	177	394		
600[b]	6-weeks			715	1232
600[c]	9-months			349	402
1200[a]	4-weeks	1440	1230		

Source: Mod4.2.3.2/TOX10759, Mod4.2.3.2/TMC278-NC368 (TOX9508), Mod4.2.3.2/TMC278-NC349 (TOX9517)
[a] day 15; [b] day 28; [c] day 224

Note: The available studies of RPV LA are listed, exposure values originate from studies in which the clinical formulation G001 was applied.

2.6.7.4 Toxicology: Drug Substance

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
Proposed Specification: [redacted]	99.3	0.36	0.06	0.06	1-Month Repeated Dose Oral Toxicity Study in the Rat.	TMC278-NC117 (TOX6385)/ 4.2.3.2
					1-Month Repeated Dose Oral Toxicity Study in the Beagle Dog.	TMC278-NC116 (TOX6334)/ 4.2.3.2
					6-Month Repeated Dose Oral Toxicity Study with 1-Month Recovery in the Rat.	TMC278-NC101 (TOX6142)/ 4.2.3.2
					6-Month Repeated Dose Oral Toxicity Study with 3-Month Interim Kill in the Beagle Dog.	TMC278-NC115 (TOX6110)/ 4.2.3.2
					Oral Embryo-Fetal Toxicity Study in the Rat.	TMC278-NC105 (TOX6268)/ 4.2.3.5.2
					Pilot Oral Embryo-Fetal Toxicity Study in the Rabbit.	TMC278-NC128 (TOX6235)/ 4.2.3.5.2
					Oral Embryo-Fetal Toxicity Study in the Rabbit.	TMC278-NC130 (TOX6313)/ 4.2.3.5.2

(Continued)

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
Proposed Specification:						
[redacted]	98.0	0.62	0.06	ND	52-Week Oral (Gavage) Toxicity Study in the Beagle Dog.	TMC278-NC107 (TOX6314)/ 4.2.3.2
[redacted]	98.2	0.21	0.20	0.31	In Vitro Bacterial Reverse Mutation Test in Salmonella typhimurium with Human Liver S9-Mix.	TMC278-NC279 (TOX8086)/ 4.2.3.3.1
[redacted]	98.5	0.61	0.14	0.16	In Vitro Bacterial Reverse Mutation Test with Salmonella Typhimurium.	TMC278-Exp.5693/ 4.2.3.3.1
					1-month Oral Toxicity Study in the Rat with Immunotoxicity.	TMC278-Exp.5692/ 4.2.3.2
					1-month Repeated Dose Oral Toxicity Study in the Beagle Dog with 1-month Recovery.	TMC278-Exp.5650/ 4.2.3.2
[redacted]	96.8	1.4	1.56	ND	2-Week Repeated Dose Oral Toxicity Study in the Rat.	TMC278-Exp.5535/ 4.2.3.2

(Continued)

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
[redacted] (Continued)	96.8	1.4	1.56	ND	7-Day Repeated Dose Oral Toxicity Study in the Beagle Dog.	TMC278-Exp.5534/ 4.2.3.2
					In Vitro Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique.	TMC278-Exp.5539/ 4.2.3.3.1
					In Vitro Bacterial Reverse Mutation Test with Salmonella Typhimurium.	TMC278-Exp.5540/ 4.2.3.3.1
					In Vivo Micronucleus Test on Bone Marrow Cells in the Mouse.	TMC278-Exp.5538/ 4.2.3.3.2
[redacted]	100.3	0.37	0.09	0.05	1-Month Repeated Dose Oral Toxicity Study in the Rat.	TMC278-NC117 (TOX6385)/ 4.2.3.2
					1-Month Repeated Dose Oral Toxicity Study in the Beagle Dog.	TMC278-NC116 (TOX6334)/ 4.2.3.2

(Continued)

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
[redacted] (Continued)	100.3	0.37	0.09	0.05	1-Month Repeated Dose Oral Toxicity Study in the CB6F1-nonTgrasH2 Mouse.	TMC278-NC121 (TOX6740)/ 4.2.3.2
					3-Month Repeated Dose Oral Toxicity Study in the Mouse.	TMC278-NC119 (TOX6739)/ 4.2.3.2
					Oral Female Fertility Study in the Rat.	TMC278-NC125 (TOX6708)/ 4.2.3.5.1
					Oral Male Fertility Study in the Rat.	TMC278-NC124 (TOX6861)/ 4.2.3.5.1
[redacted]	97.8	0.27	ND	ND	Oral Male Fertility Study in the Rat.	TMC278-NC124 (TOX6861)/ 4.2.3.5.1
[redacted]	98.8	0.70	0.21	0.11	Oral Pre and Post-natal Developmental Toxicity Study in the Rat.	TMC278-NC131 (TOX6972)/ 4.2.3.5.3
[redacted]	99.7	0.17	0.32	0.22	Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice For 104 Weeks.	TMC278-NC120 (TOX7222)/ 4.2.3.4.1
					Carcinogenicity Study by Oral Gavage Administration to Sprague Dawley Rats for 104 weeks.	TMC278-NC123 (TOX7221)/ 4.2.3.4.1

(Continued)

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
Proposed Specification: [redacted]	99.6	0.18	0.12	ND	Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells.	TMC278-TiDP6-NC336 (TOX8919)/ 4.2.3.3.1
					Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells.	TMC278-TiDP6-NC311 (TOX8899)/ 4.2.3.7.6
					In Vitro Bacterial Reverse Mutation Test (Ames-test) in Salmonella typhimurium.	TMC278-TiDP6-NC309 (TOX8900)/ 4.2.3.7.6
					In Vitro Bacterial Reverse Mutation Test (Ames-test) in Salmonella typhimurium.	TMC278-TiDP6-NC308 (TOX8901)/ 4.2.3.7.6
					1-Month Repeated Dose Oral Impurity Qualification Study in the Rat.	TMC278-TiDP6-NC314 (TOX8886)/ 4.2.3.7.6

(Continued)

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
Proposed Specification:						
[redacted] (Cont'd)	99.6	0.18	0.12	ND	Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells.	TMC278-TiDP6-NC312 (TOX8898)/ 4.2.3.7.6
					In Vitro Bacterial Reverse Mutation Test in Salmonella typhimurium.	TMC278-TiDP6-NC335 (TOX8918)/ 4.2.3.3.1
[redacted]	98.8	0.42	0.28	0.14	Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice For 104 Weeks.	TMC278-NC120 (TOX7222)/ 4.2.3.4.1
					Carcinogenicity Study by Oral Gavage Administration to Sprague Dawley Rats for 104 weeks.	TMC278-NC123 (TOX7221)/ 4.2.3.4.1
[redacted]	98.6	0.88	0.23	0.15	Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice For 104 Weeks.	TMC278-NC120 (TOX7222)/ 4.2.3.4.1
					Carcinogenicity Study by Oral Gavage Administration to Sprague Dawley Rats for 104 weeks.	TMC278-NC123 (TOX7221)/ 4.2.3.4.1
[redacted]	100.4	ND	0.13	0.05	Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice For 104 Weeks.	TMC278-NC120 (TOX7222)/ 4.2.3.4.1
					Carcinogenicity Study by Oral Gavage Administration to Sprague Dawley Rats for 104 weeks.	TMC278-NC123 (TOX7221)/ 4.2.3.4.1
[redacted]	99.7	0.18	0.11	ND	8-Week Repeated Dose Oral Toxicity Study in the Juvenile Female Cynomolgus Monkey with Emphasis on Endocrinological Evaluation.	TMC278-TiDP38-NC248 (TOX8862)/ 4.2.3.2

(Continued)

2.6.7.4 Toxicology: Drug Substance (Continued)

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
Proposed Specification:						
[redacted]	96.9	97.0	97.0	96.7	Local tolerance study following subcutaneous and intramuscular injection in New-Zealand white Rabbits.	TMC278-NC237 (TOX7774)/ 4.2.3.6
					Single Dose IM/SC Toxicity Study in the Beagle Dog with a 3-month Follow-up Period.	TMC278-NC234 (TOX7781)/ 4.2.3.7.7
[redacted]	99.0	99.1	99.2	98.6	Local tolerance study following subcutaneous and intramuscular injection in New-Zealand white Rabbits.	TMC278-NC237 (TOX7774)/ 4.2.3.6
[redacted]	/	/	/	/	In Vitro Bacterial Reverse Mutation Test in Salmonella typhimurium.	TMC278-1646_0016315 (TOX9643)/ 4.2.3.7.6
[redacted]	/	/	/	/	In Vitro Bacterial Reverse Mutation Test in Salmonella typhimurium.	TMC278-1646_0015298 (TOX9645)/ 4.2.3.7.6
[redacted]	/	/	/	/	In Vitro Bacterial Reverse Mutation Test in Salmonella typhimurium.	TMC278-1646_0015299 (TOX9644)/ 4.2.3.7.6
[redacted]	96.9	/	/	/	13/39-week toxicity study by monthly IM injection in Göttingen minipigs.	TMC278-NC296 (TOX8580)/ 4.2.3.7.7
[redacted]	/	/	/	/	In Vitro Bacterial Reverse Mutation Test in Salmonella typhimurium.	TMC278-NC357 (TOX9535)/ 4.2.3.7.7
					In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes.	TMC278-NC358 (TOX9523)/ 4.2.3.7.7

(Continued)

2.6.7.4 Toxicology: Drug Substance

Test Article: rilpivirine, rilpivirine LA, P338

Batch No.	Purity (%)	Specified Impurities (%)			Type of Study	Study No./ Location in CTD
		A	B	C		
Proposed Specification:						
[redacted]	100.2	/	/	/	4-week RD toxicity + 2-week recovery	TOX10759/ Mod4.2.3.2
[redacted]	101.2	/	/	/	6-week RD toxicity	TMC278-NC368 (TOX9508)/ Mod4.2.3.2
					9-month RD toxicity	TMC278-NC349 (TOX9517)/ Mod4.2.3.2
[redacted]	99.4	/	/	/	9-month RD toxicity	TMC278-NC349 (TOX9517)/ Mod4.2.3.2
[redacted]	/	/	/	/	Oral Developmental Toxicity Study in the Rat.	TMC278-NC347 (TOX9679)/ 4.2.3.7.7
					Oral Developmental Toxicity Study of Poloxamer 338 in the Rabbit.	TMC278-NC348 (TOX9680)/ 4.2.3.7.7
[redacted]	/	/	/	/	Combined male and female fertility and embryo-foetal toxicity study by the IM route in the rat (Segment I & II).	TOX13391/ 4.2.3.7.7
					Embryo-foetal toxicity study by the IM route in the rabbit (Segment II).	TOX13376/ 4.2.3.7.7
					Pre- and post-natal development study by the intramuscular route (bolus) in the rat (Segment III)	TOX13546/ 4.2.3.7.7

IM = intramuscular; LA = long acting; ND = not detected or <0.05 w/w%; RD = repeated dose; RPV = previously known as TMC278, JNJ-16150108 or R278474; SC = subcutaneous

2.6.7.5 Single-Dose Toxicity

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mice/SPF Albino Swiss (CD-1) 5M + 5F (MNT)	Oral/Gavage (PEG400 + CA)	0, 100, 400, 1600 mg/kg	No mortality. No clinical abnormalities. **100mg/kg:** C_{max} (µg/mL) = 39 (M), 33 (F) and $AUC_{0\text{-}6h}$ (µg.h/mL) = 158 (M), 130 (F) **400mg/kg:** C_{max} (µg/mL) = 59 (M), 68 (F) and $AUC_{0\text{-}6h}$ (µg.h/mL) = 262 (M), 258 (F) **1600mg/kg:** C_{max} (µg/mL) = 60 (M), 58 (F) and $AUC_{0\text{-}6h}$ (µg.h/mL) = 307 (M), 287 (F)	TMC278-Exp.5538/ 4.2.3.3.2
Rat/Sprague Dawley 2M + 2F 4M + 4F (TK)	Oral/Gavage (PEG400)	800 mg/kg	No mortality. Clinical signs: Salivation in all males and 2/6 females. C_{max} = 18 (F) and 8.0 (M) µg/mL and $AUC_{0\text{-}\infty}$ = 233 (F) and 86 (M) µg.h/mL.	TMC278-Exp.5559/ 4.2.3.1
Dog/Beagle 1M + 1F/group	Oral/Gavage (PEG400) or (PEG400 + CA)	SD: 40 mg/kg (PEG400), 80 mg/kg (PEG400) 80 mg/kg (PEG400 + CA) RD (5-day): 80 mg/kg/ day (PEG400 + CA)	No mortality. **SD/PEG** **40 mg/kg**: No effects C_{max} = 1.1 (M) and 0.95 (F) µg/mL and $AUC_{0\text{-}24h}$ = 21 (M) and 19 (F) µg.h/mL. **80 mg/kg**: Vomiting (1 and 2 days after dosing, male), soft feces (3 days after dosing (male) and 2 or 3 days after dosing (female)). C_{max} = 0.88 (M) and 0.81 (F) µg/mL and $AUC_{0\text{-}24h}$ = 17 (M) and 16 (F) µg.h/mL **SD/PEG +CA** **80 mg/kg**: No effects C_{max} = 2.1 (M) and 2.2 (F) µg/mL and $AUC_{0\text{-}24h}$ = 42 (M) and 49 (F) µg.h/mL **RD/PEG + CA** **80 mg/kg**: Salivation 4th day (male), vomiting 3rd day (male), gastrointestinal irritation. On day 5, C_{max} = 13 (M) and 8.0 (F) µg/mL and $AUC_{0\text{-}24h}$ = 262 (M) and 154 (F) µg.h/mL.	TMC278-Exp.5461/ 4.2.3.2

CA = citric acid, F = female; M = male; PEG400 = polyethylene glycol 400, RD = repeated dose; SD = single dose; TK = toxicokinetics

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mouse/Swiss Main animals: 5M/5F Satellite animals: 3M/3F	Admixed to diet	2-week	0, 40, 400, 5000 mg/kg/day TMC278.HCl	**40 mg/kg:** C_{max}: 8.9-8.3 µg/mL; $AUC_{0\text{-}24h}$:150-148 µg.h/mL (M-F) **400 mg/kg:** C_{max}: 57-63 µg/mL; $AUC_{0\text{-}24h}$: 1066-1320 µg.h/mL (M-F) ↓ BWG (M: 22%, F: 17%), ↑ FC (M: 46%, F: 39%), ↓ RBC (M: 8%), Hb (M: 7%), Hct (M: 8%), eosinophils (F: 48%), ↑ reticulocytes (M: 11%, F: 23%), ↑ bilirubin (M: 3-fold, F: 3-fold), ALP (M: 2-fold, F: 3-fold), AST (M: 1-fold, F: 3-fold), albumin (M: 13%), cholesterol (F: 22%), ↓ triglycerides (M: 50%, F: 73%). **5000 mg/kg:** All animals died.	TMC278-NC118 (TOX6479)/ 4.2.3.2
	Oral gavage (0.5% w/v HPMC)		400, 2000 mg/kg/day TMC278.HCl	**400 mg/kg:** ↓ BWG (M:13%, F: 29%), ↑ reticulocytes (M: 17%, F: 23%), ↑ bilirubin (F: 2-fold), ALP (M: 3-fold, F: 2-fold), ALT (M: 1-fold, F: 3-fold), AST (F: 2-fold), albumin (M: 22%), cholesterol (F: 48%), calcium (M: 8%, F: 5%), protein (M: 13%), urea (M: 19%, F: 40%), ↓ triglycerides (F: 63%). C_{max}: 63-94 µg/mL; $AUC_{0\text{-}24h}$: 687-123 µg.h/mL (M-F) **2000 mg/kg:** All animals died.	
Mouse/CB6F1-nonTgrasH2 Main animals: 10M/10F Satellite animals: 15M/15F	Oral/gavage (0.5% W/V HPMC)	1-month	0, 20, 80, 320 mg/kg/day TMC278.HCl	**20 mg/kg:** Liver: hypertrophy, Uterus: atrophy C_{max}: 14-15 µg/mL; $AUC_{0\text{-}24h}$: 64-54 µg.h/mL (M-F) **80 mg/kg:** ↑ liver (M: 24%, F: 29%). Liver: hypertrophy, Kidney: degenerative/necrotic nephropathy. C_{max}: 30-37 µg/mL; $AUC_{0\text{-}24h}$: 250-240 µg.h/mL (M-F) **320 mg/kg**: 2 mortalities. ↓ BW and ↑ FC. ↓ RBC (M: 4%), Hb (M: 4%, F: 4%), Hct (F: 6%), reticulocytes (F: 56%), ↑ platelets (M: 14%, F: 24%), calcium (F: 72%), IP (F: 42%), bilirubin (F: 3-fold), urea (M: 72%, F: 58%), protein (M:9% ,F: 18%), albumin (M: 17%), cholesterol (M: 46%, F: 110%), ALP (M: 3-fold,F: 2-fold). ↑ liver (M: 2-fold, F: 2-fold), kidney (F: 14%), spleen (F: 40%), ↓ ovaries (50%), thymus (F: 2!%). Liver: hypertrophy, Kidney: degenerative/necrotic nephropathy, Thymus: lymphoid depletion, Bone marrow: myeloid cell hyperplasia, Spleen: erythro/myelopoiesis, Uterus: atrophy. C_{max}: 66-69 µg/mL; $AUC_{0\text{-}24h}$: 1090-942 µg.h/mL (M-F)	TMC278-NC121 (TOX6740)[b]/ 4.2.3.2

(Continued)

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague Dawley 5M/group	Oral/gavage (PEG400)	5-day	0 (water), 0 (vehicle), 40, 400 mg/ kg/day TMC278 base	**40 mg/kg:** No effects C_{max}: 1.4 µg/mL; AUC_{0-24h}: 14 µg.h/mL **400 mg/kg**: ↑ potassium (7%), No histopathology changes C_{max}: 1.8 µg/mL; AUC_{0-24h}: 28 µg.h/mL NOEL = 40 mg/kg/day	TMC278 - Exp.5463/ 4.2.3.2
Rat/Sprague Dawley Main groups: 5M/5F Satellite groups:4M/4F	Oral/gavage (PEG400 + CA)	14-day	0 (water), 0 (vehicle), 40, 120, 400 mg/kg/day TMC278 base	**40 mg/kg:** ↓ T_4 (M: 10%, F: 32%). Thyroid: hypertrophy (M). C_{max} = 2.2-5.7 µg/mL (M-F). AUC_{0-24h}= 16-42 µg.h/mL (M-F) **120 mg/kg**: ↑TSH (M: 94%, F: 40%); ↓ T_4 (M: 27%, F: 50%). Thyroid: hypertrophy C_{max} = 3.6-7.8 µg/mL (M-F); AUC_{0-24h}= 35-88 µg.h/mL (M-F) **400 mg/kg**: ↑ protein (M: 3%); ↑ albumin (M: 7%). ↑TSH (M: 120%, F: 51%); ↓ T_4 (M: 29%, F: 66%). ↑ thyroid weight (36%, F). Thyroid: hypertrophy. C_{max} = 8.4-15 µg/mL (M-F); AUC_{0-24h}= 84-152 µg.h/mL (M-F)	TMC278-Exp.5535[b]/ 4.2.3.2
Rat/Sprague Dawley Main groups: 5M/5F Satellite groups:6M/6F	Admixed to diet	14-day	0 (control diet), 400, 1200 mg/kg/day TMC278.HCl or 400 mg/kg/day TMC278 base	**400 mg/kg (TMC278.HCl-diet):** ↓WBC (F: 13%); ↑ ALT (M: 33%); ↑ thyroid weight (M: 20%, F: 54%). Thyroid: hypertrophy C_{max}: 5.2-7.2 µg/mL; AUC_{0-24h}= 82-137 µg.h/mL (M-F). **400 mg/kg (TMC278 base-diet):** ↓WBC (M: 18%, F: 23%); Hb (M: 6%); Hct (M: 6%); ↑ thyroid weight (M: 27%, F: 39%). Thyroid: hypertrophy C_{max}: 3.7-4.3 µg/mL; AUC_{0-24h}= 57-86 µg.h/mL (M-F) **1200 mg/kg (TMC278.HCl-diet):** ↓WBC (F: 9%); Hb (M: 6%); Hct (M: 6%); ↑ALT (M: 44%); ↑liver weight (M: 30%, F: 18%); ↑thyroid weight (M: 60%, F: 77%). Liver: hypertrophy, Thyroid: hypertrophy. C_{max}: 11-13 µg/mL; AUC_{0-24h}= 180-266 µg.h/mL (M-F)	TMC278-NC136 (TOX6417)/ 4.2.3.2
	Oral/gavage (0.5% w/v HPMC)		400 mg/kg/day TMC278.HCl	**400 mg/kg (TMC278.HCl-gavage):** ↓WBC (F: 19%); ↓cholesterol (M: 29%); ↑ALT (M: 19%); ↑ thyroid weight (M: 33%, F: 39%). Thyroid: hypertrophy C_{max}: 7.3-12 µg/mL; AUC_{0-24h} = 51-103 µg.h/mL (M-F)	

(Continued)

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague Dawley Main groups: 5M/5F Satellite groups:3M/3F	Oral/gavage (0.5% w/v HPMC)	2-week	0 (vehicle), 400, 1500, 2000 mg/kg/day TMC278.HCl	**400 mg/kg:** ↑ thyroid weight (F: 42%). Thyroid: hypertrophy (F). C_{max} = 5.2-12 µg/mL (M-F); AUC_{0-24h}= 42-96 µg.h/mL (M-F) **1500 mg/kg:**↑ APTT (M: 19%), PT (M: 20%); ↓ chloride (F: 3%); glucose (F: 18%); urea nitrogen (F: 33%); creatinine (F: 23%). ↑ thyroid weight (F: 62%). Thyroid: hypertrophy. C_{max} = 9.5-12 µg/mL (M-F); AUC_{0-24h}= 86-115 µg.h/mL (M-F) **2000 mg/kg:** ↑ APTT (M: 14%), PT (M: 15%); ↓ chloride (F: 3%); cholesterol (M: 30%); glucose (F: 14%). urea nitrogen (F: 33%); creatinine (F: 17%). ↑ thyroid weight (F: 55%). Thyroid: hypertrophy. C_{max} = 7.7-14 µg/mL (M-F); AUC_{0-24h}= 77-147 µg.h/mL (M-F)	TMC278-NC177 (TOX6813)/ 4.2.3.2
Rat/Sprague Dawley Main groups: 10M/10F Satellite groups:6M/6F	Oral/gavage (PEG400 + CA)	1-month	0 (vehicle), 10, 400 mg/kg/day TMC278 base	**10 mg/kg:** Thyroid: hypertrophy. C_{max} = 0.74-1.4 µg/mL (M-F); AUC_{0-24h} = 5.4-8.6 µg.h/mL (M-F) **400 mg/kg**: ↓ Hct (3%); potassium (13%); chloride (F: 2%); cholesterol F: (22%); urea nitrogen (F: 15%); ↑ albumin (M: 7%); protein (M: 5%); ↑ TSH (M: 51%, F: 15%); T_3 (M: 57%); ↓ T_4.(M: 61%, F: 52%). ↑ liver weight (M: 24%, F: 15%); ↑ thyroid weight (F: 60%). Liver: hypertrophy, Thyroid: hypertrophy. C_{max} = 7.7-13 µg/mL (M-F); AUC_{0-24h} = 90-149 µg.h/mL (M-F)	TMC278-NC117 (TOX6385)[b]/ 4.2.3.2
	Oral/gavage (0.5% w/v HPMC)		0 (vehicle), 10, 400 mg/kg/day TMC278.HCl	**10 mg/kg:** Thyroid: hypertrophy C_{max} = 0.76-1.7 µg/mL (M-F); AUC_{0-24h}= 4.5-7.8 µg.h/mL (M-F) **400 mg/kg**: ↑ RBC (F: 4%); albumin (M: 5%); ↓ cholesterol (F: 10%); triglycerides (F: 35%); urea nitrogen (M: 18%, F: 11%); ↑ TSH (M: 49%, F: 38%), T_3 (M: 56%); ↓ T_4 (M: 37%, F: 44%); ↑ thyroid weight (F: 29%). Liver: hypertrophy, Thyroid: hypertrophy C_{max} = 4.8-9.0 µg/mL (M-F); AUC_{0-24h}= 33-86 µg.h/mL (M-F)	
Rabbit/Albino New Zealand white 5F/group	Oral/gavage (0.5% HPMC)	5-day	0 (vehicle), 100, 300, 1000 mg/kg/day TMC278 base	**100 mg/kg:** ↓ body weight (8%), food consumption (23%). C_{max}: 57.3 µg/mL; AUC_{0-24h}: 1120 µg.h/mL **300 mg/kg**: ↓ body weight (14%), food consumption (62%). ↓ reticulocytes (61%); ↑ creatinine (51%). C_{max}: 124 µg/mL; AUC_{0-24h}: 2695 µg.h/mL **1000 mg/kg**: ↓ body weight (22%), food consumption (92%). ↓ reticulocytes (78%); ↑ creatinine (63%). C_{max}: 138 µg/mL; AUC_{0-24h}: 2971 µg.h/mL	TMC278-NC126 (TOX6146)/ 4.2.3.2

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2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/Beagle 1M + 1F/group	Oral/Gavage (PEG400) or (PEG400 + CA)		RD (5-day): 80 mg/kg/ day (PEG400 + CA)	See Mod2.6.7.5	TMC278-Exp.5461/ 4.2.3.2
Dog/beagle 4M/group	Oral/gavage PEG400 + CA	7-day	0 (water), 0 (vehicle), 20, 40, 80 mg/kg/day TMC278 base	**0 (water):** Vomiting, salivation, soft feces. **0 (vehicle):** Vomiting, salivation, soft feces. Focal congestion in the small intestine. **20 mg/kg**: Vomiting, salivation, soft feces. ↓ cortisol (44%); Small intestines: Focal congestion and mucosal hemorrhages. Adrenal glands: Multifocal inflammatory cell infiltration and multifocal vacuolated cells in the zona fasciculata C_{max}: 3.7 µg/mL; AUC_{0-24h}: 39 µg.h/mL **40 mg/kg**: Vomiting, salivation, soft feces. ↑ bilirubin (2-fold), glucose (7%) and cholesterol (17%); ↓ cortisol (72%); Small intestines: Focal congestion and mucosal hemorrhages. Adrenal glands: Multifocal inflammatory cell infiltration and multifocal vacuolated cells in the zona fasciculata. C_{max}: 7.7 µg/mL; AUC_{0-24h}: 159 µg.h/mL **80 mg/kg**: Vomiting, salivation, soft feces. ↑ glucose (10%), cholesterol (12%) and bilirubin (2-fold); ↓ cortisol (56%); Small intestines: Focal congestion and mucosal hemorrhages. Adrenal glands: Multifocal inflammatory cell infiltration and multifocal vacuolated cells in the zona fasciculata. C_{max}: 7.8 µg/mL; AUC_{0-24h}: 147 µg.h/mL	TMC278-Exp.5534/ 4.2.3.2

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2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/beagle 3M/3F	Oral/gavage (PEG400 + CA)	1-month	0 (vehicle), 5, 40 mg/kg/day TMC278 base	**5 mg/kg**: Uterus: increased glandular development. C_{max} = 1.4-1.1 µg/mL (M-F); AUC_{0-24h} = 23-14 µg.h/mL (M-F) **40 mg/kg:** ↓ RBC (F: 7%, F), Hct (M: 12%, F: 5%), reticulocytes (M: 61%). ↓ IP (F: 11%), triglycerides (M: 53%), ↑ bilirubin (M: 39%, F: 62%), ALP (M: 1-fold, F: 2-fold), ALT (M: 5-fold, F: 2-fold), γGT (M: 3-fold); ↑ ACTH (M: 77%, F: 92%), progesterone (M: 10-fold), 17α-OH-progesterone (M: 34-fold), ↓ cortisol (M: 47%, F: 34%); Liver: Centrilobular and periportal inflammation, brown pigmented macrophages, single cell death and oval cell proliferation. Adrenal glands: Swollen cells with densely stained cytoplasm in zona reticularis and fasciculata and presence of foamy cells in the zona fasciculata. Ovaries: ↑ in cystic luteinized follicles or tertiary follicles. Uterus: ↑ in glandular development. Testes: Prominent Leydig cells. C_{max} = 3.8-2.5 µg/mL (M-F); AUC_{0-24h}= 73-43 µg.h/mL (M-F)	TMC278-NC116 (TOX6334)[b]/ 4.2.3.2
Dog/beagle 3M/3F	Oral/gavage (0.5% w/v HPMC)	1-month	0 (vehicle), 5, 40 mg/kg/day TMC278.HCl	**5 mg/kg:** ↓ IP (F: 10%). Uterus: ↑ in glandular development C_{max} = 0.89-0.70 µg/mL (M-F); AUC_{0-24h} = 13-7.5 µg.h/mL (M-F) **40 mg/kg:** Hct (M: 15%, F: 7%), reticulocytes (M: 43%). ↓ IP (F: 8%), ↑ bilirubin (M: 17%, F:100%), ALP (M: 3-fold, F: 3-fold), AST (F: 1-fold), ALT (M: 1-fold, F: 6-fold), γGT (F: 6-fold), ↑ ACTH (M: 59%, F: 54%), cortisol (M: 54%), progesterone (M: 10-fold), 17α-OH-progesterone (M: 29-fold). Liver: Centrilobular and periportal inflammation, brown pigmented macrophages, single cell death and oval cell proliferation. Adrenal glands: Swollen cells with densely stained cytoplasm in zona reticularis and fasciculata and presence of foamy cells in the zona fasciculata. Ovaries: ↑ in cystic luteinized follicles or tertiary follicles. Uterus: ↑ in glandular development. Testes: Prominent Leydig cells. C_{max} = 4.1-5.8 µg/mL (M-F); AUC_{0-24h} = 76-81 µg.h/mL (M-F)	TMC278-NC116 (TOX6334)[b]/ 4.2.3.2

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2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies (Continued)

Test Article: rilpivirine/rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Duration of Dosing	Doses[b] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Monkey/Cynomolgus 3F/group	Oral/gavage	SD 2 weeks	**Hydrochloride salt**: SD: 2x250, 500 RD: 2 x 0 (vehicle), 2 x 100, 2 x 250 (1% HPMC + 0.5% polysorbate 20)	**SD phase** No mortality No clinical observations **RD phase** No mortality No clinical observations and no effects on BW or ECG/BP 2x 250 mg eq./kg/day: ↓ cortisol levels 8 to 12 hours after first dose of the day	TMC278-NC326 (FK6541)/ 4.2.3.2
Minipig/Göttingen 3M/3F/group	IM/injection (2mL) Negative control V1 = P338 (50 mg/mL) V2 = P338 (160 mg/mL) Nanosuspension of TMC278 LA 300 mg/mL with P338 at 50 mg/mL	6 weeks (injections on Day 1 in m. femoris alternating in contralateral muscle every 2 weeks)	600	**Negative control:** Marked ↑ CRP M/F (~24 hours postdose) **V1:** Marked ↑ CRP in M/F (~24 hours postdose) **V2:** Marked ↑ CRP in M/F (~24 hours postdose) **TMC278 LA nanosuspension:** Clinical observations: Slight, short-lasting erythema Necropsy: White deposits at injection sites; white discoloration draining lymph nodes; swelling medial iliac lymph node (1M) Histopathology: Granulomatous reaction consisting of macrophage infiltration (filled with eosinophilic material, possibly RPV) and extracellular amorphous pale eosinophilic deposits, with inflammatory infiltrate in some F. Draining lymph nodes showed infiltration of macrophages and multinucleated macrophages (with eosinophilic contents). Marked ↑ CRP in M/F (~24 hours postdose) – more pronounced, with earlier onset than control/vehicle groups. C_{max} = 0.7-1.2 µg/mL (M-F); $AUC_{0\text{-}last}$ = 51.4-52.8 µg.h/mL (M-F)	TMC278-NC368 (TOX9508)/ 4.2.3.2

[a] Dose of HCl salt is presented as base equivalents
[b] GLP compliant study

ACTH = adrenocorticotropic hormone; ALP = alkaline phosphatase; ALT = alanine aminotransferase; APTT = activated partial thromboplastine time; AST = aspartate aminotransferase; $AUC_{0\text{-}24h}$ = area under the concentration vs time curve till 24 h after dosing; BP = blood pressure; BW = body weight; BWG = body weight gain; CA = citric acid; C_{max}: maximum concentration; CRP = C-reactive protein; ECG = Electrocardiogram; F = female; FC = food consumption; γGT = gamma glutamyltransferase; Hb = hemoglobin; Hct = hematocrit, HPMC = hydroxypropyl methyl cellulose; IM = intramuscular; IP = inorganic phosphate; LA = long acting; M = male; NOEL = No Observed Adverse Effect Level; P338 = poloxamer 338; PEG400 = polyethylene glycol 400; PT = Prothrombin time; RD = repeated dose; RBC = Red blood cells; SD = single dose; T_3 = triiodothyronine; T_4 = tetraiodothyronine/thyroxine; TSH = thyroid stimulating hormone; V = vehicle; V1 = vehicle 1; V2 = vehicle 2; WBC = white blood cells

2.6.7.7A Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

Species/Strain: Mouse/SPF Swiss
Duration of Dosing: 91 days
Study No.: TMC278-NC119 (TOX6739)
Age at First Dose: 42 days
Duration of Postdose Recovery: /
Location in CTD: 4.2.3.2
Date of First Dose: [redacted]-20[redacted]
Route: Oral/gavage
GLP Compliance: Yes
Vehicle/Formulation: 0.5% w/v HPMC
Special Features:-
No Observed Adverse Effect Level: 20 mg base eq./kg/day

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (Vehicle)**		**20**		**80**		**320**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Toxicokinetics:								
No. of Animals	**M:12**	**F:12**	**M:15**	**F:15**	**M:15**	**F:15**	**M:15**	**F:15**
$AUC_{0-\infty}$ (µg·h/mL)								
Day 1	-	-	71	59	236	250	1010	707
Day 31	-	-	61[b]	74	263	313	860	1170
Day 87	-	-	80	61	210	313	665	1360
C_{max} (µg/mL)								
Day 1	-	-	14	13	28	32	63	55
Day 31	-	-	14	18	38	37	63	84
Day 87	-	-	18	19	34	42	61	90
Noteworthy Findings:								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight[a]								
Week 13/Day 91	35.3	29.1	-	-	-	-	1.110**	1.110**
Body Weight Gain[a]								
Week 13/Day 91	6.6	6.2	-	-	-	-	1.545***	1.532**
Food Consumption[a]								
Total Food Consumption	473	489	-	-	1.131**	-	1.199***	1.223**
Clinical Observations								
Abdominal Distension	0	0	-	-	-	-	5*	7**
Waste of food	-	0	-	-	-	-	-	3

(Continued)

2.6.7.7A Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (Vehicle)**		**20**		**80**		**320**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Hematology[a]								
White blood cells (10^3/µL)	7.6	-	-	-	-	-	0.526***	-
Red blood cells (10^6/µL)	9.54	9.54	-	-	-	-	0.917**	0.907**
Hemoglobin (g/dL)	14.2	14.5	-	-	-	-	0.923**	0.924**
Hematocrit (%)	46.3	47.3	-	-	-	-	0.940**	0.926***
Reticulocytes (10^3/µL)	-	256.1	-	-	-	-	-	1.369*
Thrombocytes (10^3/µL)	-	1387	-	-	-	-	-	0.882*
Neutrophils (10^3/µL)	1.28	-	-	-	-	-	0.695*	-
Lymphocytes (10^3/µL)	5.89	-	-	-	-	-	0.494***	-
Eosinophils (10^3/µL)	0.21	-	-	-	-	-	0.524*	-
Serum Chemistry[a]								
Calcium (mg/dL)	9.0	9.1	-	-	-	-	1.067*	1.077**
Inorg. Phosphate (mg/dL)	7.2	-	-	-	-	-	1.181**	-
Total protein (g/dL)	-	5.0	-	-	-	1.060*	-	1.140***
Albumin (g/dL)	3.4	3.6	-	-	-	1.083***	1.059*	1.111**
Cholesterol (mg/dL)	-	82	-	-	-	1.293*	-	1.415*
Triglycerides (mg/dL)	-	158	-	-	-	0.620*	-	0.386***
Urea nitrogen (mg/dL)	-	24.1	-	-	-	-	-	1.249**
Alk.phosphatase (U/L)	60	85	-	-	-	-	2.250***	2.976**
Alanine aminotransferase (U/L)	88	63	-	-	-	-	1.966	2.540***
Organ Weights[a,c]								
Liver (g)	1.81	1.37	-	-	1.204*	1.372**	2.066**	2.664**
Spleen (g)	-	0.103	-	-	-	-	-	1.666
Gross Pathology								
Kidneys	10	10	10	10	10	10	10	10
- Irregular surface	-	0	-	-	-	-	-	3
Liver	10	10	10	10	10	10	10	10
- Dark	0	0	-	-	-	4	10	10
- Swollen	0	0	-	-	-	-	10	10
Spleen	10	10	10	10	10	10	10	10
- Swollen	-	0	-	-	-	-	-	3

(Continued)

2.6.7.7A Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (Vehicle)**		**20**		**80**		**320**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Histopathology								
Adrenal glands (n° examined)	10	10	10	10	10	10	10	10
Swollen/dense inner cortical cells	0	-	0	-	1	-	3	-
grade 1	0		0		1		3	
Prominent X-zone	-	5	-	4	-	1	-	0
grade 1		5		3		0		0
grade 2		0		1		1		0
Degeneration brown	-	4	-	5	-	5	-	6
grade 1		3		5		4		1
grade 2		1		0		1		5
Bone marrow, femur (n° examined)	10	10	10	10	10	10	10	10
Increased M/E ratio	0	0	0	0	0	0	1	4
grade 1	0	0	0	0	0	0	1	2
grade 2	0	0	0	0	0	0	0	1
grade 3	0	0	0	0	0	0	0	1
Cellularity	-	10	-	10	-	10	-	10
grade 2		1		1		0		0
grade 3		8		9		10		6
grade 4		1		0		0		3
grade 5		0		0		0		1
Bone marrow, sternum (n° examined)	10	10	10	10	10	10	10	10
Cellularity	-	10	-	10	-	10	-	10
grade 3		9		9		9		8
grade 4		1		1		1		1
grade 5								1
Increased M/E ratio	0	0	0	0	0	0	5	4
grade 1	0	0	0	0	0	0	5	2
grade 2	0	0	0	0	0	0	0	1
grade 4	0	0	0	0	0	0	0	1

(Continued)

2.6.7.7A Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (Vehicle)**		**20**		**80**		**320**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Histopathology (Cont'd)								
Kidneys (n° examined)	10	10	-	10	-	10	10	10
Nephropathy	-	0	-	0	-	0	-	5
grade 1								2
grade 2								2
grade 3								1
Glomerulopathy	-	0	-	1	-	0	-	5
grade 1		0		1		0		3
grade 2		0		0		0		2
Inflammation capsule/adjacent tissue	-	0	-	1	-	0	-	3
grade 1		0		1		0		2
grade 2		0		0		0		1
Inflammation exudative	-	0	-	0	-	0	-	1
grade 3		0		0		0		1
Inflammation granulocytic	-	0	-	0	-	0	-	2
grade 1		0		0		0		2
Fibrosis	-	0	-	0	-	0	-	4
grade 2		0		0		0		3
grade 3		0		0		0		1
Single cell death	-	0	-	0	-	0	-	2
grade 1		0		0		0		2
Dilatation tubule	-	0	-	0	-	0	-	3
grade 1		0		0		0		3
Infiltration mononuclear cell	-	3	-	4	-	4	-	7
grade 1		3		3		4		3
grade 2		0		1		0		3
grade 3		0		0		0		1
Basophilia tubule(s)	-	4	-	7	-	3	-	9
grade 1		4		6		3		4
grade 2		0		1		0		1
grade 3		0		0		0		3
grade 4		0		0		0		1

(Continued)

2.6.7.7A Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (Vehicle)**		**20**		**80**		**320**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Histopathology (Cont'd)								
Liver (n° examined)	10	10	10	10	10	10	10	10
Vacuolization hepatocellular	0	0	0	0	0	0	2	5
grade 1	0	0	0	0	0	0	2	3
grade 2	0	0	0	0	0	0	0	2
Pigmentation Kupffer cells	0	0	0	0	0	1	3	7
grade 1	0	0	0	0	0	1	3	4
grade 2	0	0	0	0	0	0	0	3
Extramedullary hematopoiesis	-	0	-	0	-	0	-	2
grade 1		0		0		0		1
grade 2		0		0		0		1
Necrosis, single cell	3	0	3	2	2	1	9	7
grade 1	3	0	3	2	2	1	8	7
grade 2	0	0	0	0	0	0	1	0
Hypertrophy hepatocellular	4	0	2	1	8	7	10	10
grade 1	4	0	2	1	8	7	0	1
grade 2	0	0	0	0	0	0	6	6
grade 3	0	0	0	0	0	0	4	3
Kupffer cell proliferation	-	0	-	0	-	0	-	3
grade 1		0		0		0		3
Ovaries (n° examined)		10		10		10		10
CL generations		10		10		10		9
grade 1		0		0		0		2
grade 2		3		4		3		2
grade 3		7		6		7		5
CL eosinophilic		10		10		10		7
grade 1		3		2		1		0
grade 2		2		2		1		3
grade 3		0		2		1		1
grade 4		1		1		2		1
grade 5		4		3		5		2
CL basophilic		10		10		8		6
grade 1		9		10		7		6
grade 2		1		0		1		0

(Continued)

2.6.7.7A Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (Vehicle)**		**20**		**80**		**320**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Histopathology (Cont'd)								
Spleen (n° examined)	10	10	10	10	10	10	10	10
Extramedullary hematopoiesis	1	4	2	3	3	6	7	9
grade 1	1	3	2	3	3	4	5	3
grade 2	0	1	0	0	0	2	2	5
grade 3	0	0	0	0	0	0	0	1
Thymus (n° examined)		10		10		10		10
Involution	-	1	-	2	-	0	-	3
grade 1		1		2		0		2
grade 2		0		0		0		0
grade 3		0		0		0		1
Uterus (n° examined)		10		10		10		10
Infiltration granulocytic		9		10		9		6
grade 1		6		8		8		3
grade 2		3		2		1		3
Electronmicroscopy								
Liver (n° examined)	2	1					3	2
Increase of number of single membrane-bound organelles indicative of peroxisomes	0	0					2	

[a] At end of dosing period. For vehicle, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

[b] $AUC_{0\text{-}24h}$ was more than 25% extrapoled; $AUC_{0\text{-}8h}$ = 61 µg.h/mL; HPMC: hydroxypropyl methylcellulose.

[c] Both absolute and relative weights differed from controls in the direction indicated. Number indicates multiple of control for the absolute organ weights.

- No noteworthy findings; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

HPMC = hydroxypropyl methylcellulose

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: 1-month Repeated Dose Oral Toxicity Study in the Rat

Species/Strain: Rat / Sprague Dawley
Age at First Dose: approximately 6 weeks
Date of First Dose: [redacted] 20[redacted]
Vehicle/Formulation: PEG400 + CA
Special Features: -
No Observed Adverse Effect Level: 10 mg/kg/day
Duration of Dosing: 4 weeks
Duration of Postdose Recovery: 0 days
Route: Oral/gavage
Study No.: TMC278-Exp.5692
Location in CTD: 4.2.3.2
GLP Compliance: Yes

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg)	0 (water)		0 (vehicle)		10		40		160	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Toxicokinetics:										
No. of Animals					**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
$AUC_{0-\infty}$ (µg·h/mL)										
Day 1	-	-	-	-	7.5	14	23	30	52	77
Day 29	-	-	-	-	7.2	14	27	42	51	89
C_{max} (µg/mL)										
Day 1	-	-	-	-	0.94	2.0	2.0	3.2	6.5	9.6
Day 29	-	-	-	-	0.88	1.6	2.6	5.8	6.7	8.8
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Body Weight[a]	-	-	-	-	-	-	-	-	-	-
Body Weight Gain[a]	-	-	-	-	-	-	-	-	-	-
Food Consumption[a]	-	-	-	-	-	-	-	-	-	-
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Serum analysis[a]										
Glucose (g/dL)	-	0.96	-	0.88	-	-	-	-	-	0.898***
Urinalysis	-	-	-	-	-	-	-	-	-	-
Organ weights (absolute)[a]										
Liver (g)	12.08	7.16	10.87	7.30	-	-	1.149	1.105*	1.233	1.119*
Thyroid (g)	0.021	0.020	0.017	0.016	-	-	1.249	1.236	1.235	1.224
Pituitary gland (g)	0.011	0.014	0.010	0.013	-	-	1.074	-	1.162	1.136

(Continued)

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies (Continued)

Test Article: rilpivirine

Report Title: 1-month Repeated Dose Oral Toxicity Study in the Rat

DOSING PERIOD										
Daily Dose During Dosing Period (mg/kg)	**0 (water)**		**0 (vehicle)**		**10**		**40**		**160**	
No. of Animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Histopathology[a]										
Thyroid										
Follicular hypertrophy	0	0	0	0	0	0	2	0	6	4
No. of animals	**M:8**	**F:8**	**M:8**	**F:8**	**M:8**	**F:8**	**M:8**	**F:8**	**M:8**	**F:8**
PFC assay	-	-	-	-	-	-	-	-	-	-

[a] For vehicle, group means are shown. For treated groups, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

- No noteworthy findings; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

CA = citric acid; PEG400 = polyethylene glycol; PFC = plaque forming cell

2.6.7.7C Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Oral Toxicity Study with 1-Month Recovery in the Rat

Species/Strain: Rat / Sprague Dawley
Age at First Dose: 42 days
Date of First Dose: [redacted]
Vehicle/Formulation: PEG400 + CA
Special Features: -
No Observed Adverse Effect Level: /

Duration of Dosing: 186 days
Duration of Postdose Recovery: 158 days
Route: Oral/gavage

Study No.: TMC278-NC101 (TOX6142)
Location in CTD: 4.2.3.2
GLP Compliance: Yes

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg)	0 (vehicle)		40		120		400	
No. of Animals	**M:30**	**F:30**	**M:20**	**F:20**	**M:20**	**F:20**	**M:30**	**F:30**
Toxicokinetics:								
No. of Animals			**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
$AUC_{0-\infty}$ (µg·h/mL)								
Day 1	-	-	19	32	53	83	92	160
Day 83	-	-	19	41	41	100	57	184
Day 175	-	-	12	50	35	116	73	244
C_{max} (µg/mL)s								
Day 1	-	-	2.9	6.5	6.4	8.5	9.1	17
Day 83	-	-	3.4	8.2	3.4	11	3.9	15
Day 175	-	-	1.7	6.6	3.0	8.8	6.2	16
Noteworthy Findings:								
Mortality	0	0	0	0	0	0	0	0
Clinical observations								
Salivation	15	5	15	14***	14	13***	25*	17**
Wet urogenital region	7	2	-	-	-	-	13	11*
Body weight	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology[a]								
Interim analysis (3 months)								
Act. Part. Thromb. Time (sec)	25.62	-	1.116***	-	1.230***	-	1.190***	-
Protrombine Time (sec)	20.52	-	1.125*	-	1.274***	-	1.133*	-

(Continued)

2.6.7.7C Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**40**		**120**		**400**	
No. of Animals	**M:30**	**F:30**	**M:20**	**F:20**	**M:20**	**F:20**	**M:30**	**F:30**
Hematology[a] (Cont'd)								
End of dosing (6 months)								
Act. Part. Thromb. Time (sec)	20.85	-	1.148**	-	1.249***	-	1.164***	-
Protrombine Time (sec)	18.82	-	1.134**	-	1.272***	-	1.159**	-
Red blood cells (10^6/µL)	9.20	-	-	-	-	-	0.948**	-
Hemoglobin (g/dL)	16.4	-	-	-	-	-	0.951***	-
Hematocrit (%)	48.6	-	-	-	-	-	0.955**	-
Mean cell volume (fL)	-	55.1	-	-	-	0.976*	-	0.978*
Mean cell hemoglobin (pg)	-	18.7	-	-	-	0.973*	-	0.973*
Eosinophils (10^3/µL)	-	0.15	-	0.733**	-	0.733**	-	0.667***
End of recovery	**M:10**	**F:10**					**M:10**	**F:10**
Act. Part. Thromb. Time (sec)	21.61	-	-	-	-	-	1.140*	-
Protrombine Time (sec)	20.46	-	-	-	-	-	1.123*	-
Serum Chemistry[a]								
Interim analysis (3months)	**M:30**	**F:30**	**M:20**	**F:20**	**M:20**	**F:20**	**M:30**	**F:30**
Inorg. Phosphorus (mg/dl)	7.6	6.4	-	1.063*	1.026*	1.063*	1.105***	1.078***
Total protein (g/dl)	-	7.0	-	-	-	-	-	1.043**
Albumin (g/dl)	4.5	4.9	-	-	1.089***	1.041**	1.089***	1.082***
Triglycerides (mg/dl)	76	53	0.724**	0.849*	0.855	0.811*	0.632***	0.755***
Total bilirubin (mg/dl)	0.12	0.13	0.833***	0.769***	0.750***	0.692***	0.667***	0.769***
Alkaline phosphatase (U/l)	79	-	-	-	1.291***	-	1.532***	-
End of dosing (6 months)								
Calcium (mg/dl)	11.7	-	-	-	-	-	0.957**	-
Total protein (g/dl)	7.4	7.7	-	-	-	-	-	1.039**
Albumin (g/dl)	4.5	5.2	-	-	1.067***	-	1.067***	1.077***
Glucose (mg/dl)		88		-		0.886**		0.886**
Triglycerides (mg/dl)	91	83	-	-	-	-	0.681**	0.590***
Urea nitrogen (mg/dl)	18.1	-	-	-	1.099*	-	1.099*	-
Creatinine (mg/dl)	0.26	-	1.115*	-	1.115**	-	1.115*	-
Total bilirubin (mg/dl)	0.13	0.15	0.769**	0.667***	0.692***	0.600***	0.615***	0.667***
Alkaline phosphatase (U/l)	69	-	-	-	1.319**	-	1.696***	-
Urinalysis[a]								
End of recovery	**M:10**	**F:10**					**M:10**	**F:10**
Specific gravity	1.032	-					1.005*	-
Ketones (grade)	0.67	-					2.493*	-

(Continued)

2.6.7.7C Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**40**		**120**		**400**	
No. of Animals	**M:30**	**F:30**	**M:20**	**F:20**	**M:20**	**F:20**	**M:30**	**F:30**
Endocrinology[a]								
End of dosing								
TSH (ng/ml)	4.7	3.9	1.447**	1.769***	1.766*	2.410***	1.234*	2.487***
T4 (nmol/l)	44	32	0.636**	0.750*	0.432***	0.563***	0.545***	0.563***
T3 (nmol/l)	0.82	1.2	0.707*	-	-	-	1.341*	-
Corticosterone (nmol/l)	1065	1291	-	-	0.922	0.775*	0.823	0.914
Progesterone (nmol/l)	11	39	-	-	1.727	-	1.909	1.385**
ACTH (pg/mL)	-	-	-	-	-	-	-	-
End of recovery	**M:10**	**F:10**					**M:10**	**F:10**
T4 (nmol/l)	-	32					-	1.375**
Organ Weights[a,b]								
End of dosing								
Liver (mg)	13036	7883	-	-	1.207**	1.110**	1.246***	1.239***
Thyroids (mg)	24	19	1.208**	1.158*	1.375***	1.368***	1.500***	1.526***
End of recovery	**M:10**	**F:10**					**M:10**	**F:10**
Thyroids (mg)	-	18					-	1.389**
Gross Pathology								
End of dosing								
Liver (n° examined)	20	20	20	20	20	20	20	20
- dark	0	0	-	-	-	-	2	3
- yellow focus		0		-		-		3
- more pronounced lobulation	0	0	-	-	2	-	7*	5
- swollen	1	-	-	-	-	-	5	
Thyroid glands (n° examined)	20	20	20	20	20	20	20	20
- swollen	0	0	-	-	2	3	6*	9**
Histopathology								
Liver (n° examined)	19	19	20	20	20	20	19	20
- hypertrophy		3	-	3	7**	8	16***	16***
grade 1	-	2	-	3	7	8	6	10
grade 2	-	1	-	0	0	0	10	6
Lymph nodes, mesenteric (n° examined)	19	19	19	19	19	19	19	19
- swollen-vacuolated small macrophage-aggregates	-	-	-	-	-	-	5*	4
grade 1	-	-	-	-	-	-	4	4
grade 3	-	-	-	-	-	-	1	0

(Continued)

2.6.7.7C Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Mouse

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**40**		**120**		**400**	
No. of Animals	**M:30**	**F:30**	**M:20**	**F:20**	**M:20**	**F:20**	**M:30**	**F:30**
Histopathology (Cont'd)								
Pituitary gland (n° examined)	18	-	18	-	19	-	19	-
- large vacuolated cells/swollen cells (pars distalis)	2	-	11**	-	12**	-	9*	-
grade 1	2	-	10	-	10	-	9	-
grade 2		-	1	-	2	-	-	-
Thyroid glands (n° examined)	17	19	16	18	19	19	18	19
- high follicular epithelium	2	-	4	12***	15***	19***	18***	19***
grade 1	2	-	4	12	14	16	5	2
grade 2		-	-	-	1	3	13	17
- small follicles	4	3	12**	17***	18***	18***	11*	17***
grade 1	4	3	6	12	6	5	7	2
grade 2	0	-	6	5	12	13	4	10
grade 3	0	0	0	0	0	0	0	5
	M:10	**F:10**					**M:10**	**F:10**
End of recovery	-	-					-	-
Lymph nodes, mesenteric (n° examined)	9	9					6	10
- swollen-vacuolated small macrophage-aggregates	0	0					2	3
grade 1							2	2
grade 2							0	1
Thyroid glands (n° examined)	9	9					6	10
- high follicular epithelium	0	0					2	0
grade 1	0						2	
- small follicles	4	1					6*	5
grade 1	3	0					4	3
grade 2	1	1					2	2

[a] For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

[b] Both absolute and relative weights differed from controls in the direction indicated. Number indicates multiple of control for the absolute organ weights.

- No noteworthy findings; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

CA = citric acid; F = female; HPMC = hydroxypropyl methylcellulose; M = male; PEG400 = polyethylene glycol

2.6.7.7D Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: 1-month Repeated Dose Oral Toxicity Study in the Dog with 1-month Recovery

Species/Strain: Dog/Beagle
Duration of Dosing: 1-month
Study No.: TMC278-Exp.5650
Age at First Dose: approximately 6 to 8 months
Duration of Postdose Recovery: 1-month
Location in CTD: 4.2.3.2
Date of First Dose: [redacted] 20[redacted]
Route: Oral/gavage
GLP Compliance: Yes
Vehicle/Formulation: PEG400 + CA
Special Features: -
No Observed Adverse Effect Level: /

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg)	0 (control)		0 (vehicle)		5		10		40	
No. of Animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Toxicokinetics:										
No. of Animals	**M:3**	**F:3**	**ND**	**ND**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
$AUC_{0-\infty}$ (µg·h/mL)										
Day 1	-	-			13	15	22	14	51	40
Day 28	-	-			27	37	103	47	204	160
C_{max} (µg/mL)										
Day 1	-	-			0.94	1.3	1.3	1.3	2.8	2.4
Day 28	-	-			1.5	2.0	5.6	2.5	12	9.5
Noteworthy Findings										
Mortality	0	0	0	0	0	0	0	0	0	0
Clinical observations	-	-	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-	-	-
Eye examination	-	-	-	-	-	-	-	-	-	-
Body Weight (g)	12.0	10.5	12.6	10.9	-	-	-	0.917	0.976	0.917
Food Consumption (kg)	1.96	0.32	2.31	0.35	-	0.743	0.848	0.771.	0.710	0.857
Hematology										
Hematocrit (%)	50.6		47.4		-		-		0.973	
Hemoglobin (g/dL)	16.5		15.4		-		-		0.968	
Red blood cells ($10^{\wedge 3}$/µl)	7.02		6.49		-		-		0.980	
White blood cells ($10^{\wedge 3}$/µl)	8.77		9.18		-		-		1.121	
Serum analysis										
Albumin (g/dL)	3.8		3.7		-		0.973		0.973	
Total protein (g/dL)	6.2		6.0		-		0.983		0.983	
Cholesterol (mg/dL)	135		128		-		-		1.320**	
Triglycerides (mg/dL)	32		28		-		0.857		0..786	
Total bilirubin (mg/dL)	0.13		0.11		-		-		2.363***	
Alkaline phosphate (U/L)	111		138		-		-		2.210**	
Alanine aminotransferase (U/L)	47		44		-		-		2.932*	

(Continued)

2.6.7.7D Repeat-Dose Toxicity: Pivotal Studies (Continued)

Test Article: rilpivirine

Report Title: 1-month Repeated Dose Oral Toxicity Study in the Dog with 1-month Recovery

DOSING PERIOD										
Daily Dose During Dosing Period (mg/kg)	**0 (control)**		**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Urinalysis	-		-		-	-	-	-	-	-
Endocrinology										
ACTH (pg/mL.h)	965	737	521	785	-	-	1.710	1.237	3.77	2.964
Cortisol (nmol/L.h)	1386	1521	674	1256	-	-	-	0.557	-	0.581
Progesterone (nmol/L)	0.81	0.86	0.76	0.84	-	-	7.500	17.86	28.95*	11.43*
Organ weights										
Ovaries		0.826		0.771		1.326		2.597		2.384*
Gross pathology										
Swollen cervix		0		1		0		2		2
Swollen ovaries		0		1		0		2		2
Swollen uterus		0		1		0		2		2
Swollen vagina		0		1		0		2		2
Swollen mammary gland	0	0	0	0	0	0	0	1	0	0
Histopathology										
Adrenal cortex										
Reduced vacuolation diffuse (z.f.)	-	-	-	-	-	-	P	P	P	P
Largely vacuolated cells, multifocal (z.f.)	-	-	-	-	-	-	P	-	P	-
Mixed leucocytic infiltrates (z.f.)	-	-	-	-	-	-	-	-	P	-
Swollen cells (z.f./z.r.)	-	-	-	-	-	-	-	P	P	P
Female genital tract										
Prominent activation	-	-	-	-	-	-	-	P	-	P
Liver										
(sub)chronic centrilobular Perivascular inflammation	-	-	-	-	-	-	P	P	P	P
Mammary gland										
Prominent activation	-	-	-	-	-	-	P	P	P	P
Recovery	-	-	-	-	-	-	-	-	liver	liver

[a] For controls, group means are shown. For treated groups, multiples of vehicles are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

- No noteworthy findings; * - p<0.05; ** - p<0.01; *** - p<0.001

ACTH = Adreno CorticoTropic Hormone; CA = citric acid; F = female, M = male; ND = not done; P = present; PEG400 = polyethylene glycol; z.f. = zona fasciculate; z.r. = zona reticularis

2.6.7.7E Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Toxicity Study with 3-Month Interim Kill in the Dog

Species/Strain: dog/Beagle
Age at First Dose: 8 – 8.5 months
Date of First Dose: [redacted]
Vehicle/Formulation: PEG400 + CA
Special Features: -
No Observed Adverse Effect Level: /

Duration of Dosing: 182-183 days
Duration of Postdose Recovery: /
Route: Oral/gavage

Study No.: TMC278-NC115 (TOX6110)
Location in CTD: 4.2.3.2
GLP Compliance: Yes

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Toxicokinetics:								
No. of Animals	**M:0**	**F:0**	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Plasma concentration:								
AUC_{0-24h} (µg·h/mL)								
Day 1 (Day of 1st administration)			11	9.2	22	20	23	10
Day 86			21	18	28	27	41	52
Day 177			21	17	26	32	68	43
C_{max} (µg/mL)								
Day 1 (Day of 1st administration)			1.0	0.70	1.3	1.2	1.5	0.82
Day 86			1.4	1.1	2.0	2.2	2.5	3.1
Day 177			1.5	1.4	2.0	1.9	4.0	2.9
Tissue concentration (ng/g):								
Liver								
Day 92			10885	7795	22450	15950	50650	40500
Day 183 or 184			10127	7427	13897	12247	25157	25167
Adrenal								
Day 92			5460	3250	18550	8080	35300	30150
Day 183 or 184			5950	2360	6127	4273	17673	15427
Noteworthy Findings:								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Clinical Observations								
Feces, soft	6	6	6	5	6	6	6	6
Salivation	-	4	-	-	-	-	-	6
Vomiting	6	6	6	6	6	6	6	6
Feces, mucous	6	6	6	6	6	6	6	6
Feces, pale	1	0	-	-	-	-	4	4
Body Weight	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-

(Continued)

2.6.7.7E Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Toxicity Study with 3-Month Interim Kill in the Dog

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
ECG	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry[a]								
3m Interim kill ***(n = 6)***								
Cholesterol (mg/dL)	121	158	-	-	-	-	1.397	1.228
Total bilirubin (mg/dL)	0.13	0.13	-	-	-	1.462	1.308	1.769
Alk. Phosphatase (U/L)	148	101	-	1.842	1.284	1.851	1.723	3.436
6m Terminal kill ***(n = 4)***								
Cholesterol (mg/dL)	123	161	-	-	-	-	1.699	1.335
Total bilirubin (mg/dL)	0.16	0.12	-	1.333	-	1.667	-	1.667
Alk. Phosphatase (U/L)	224	144	-	1.576	-	1.667	2.304	5.438
Endocrinology[a]								
ACTH (pg/mL.h)								
Day 85 **(n = 6)**	1159	1582	-	1.139	1.601	1.881	2.323	1.409
Day 176 **(n = 4)**	583	1051	1.796	-	1.904	-	2.967	-
Cortisol (nmol/L.h)								
Day 85 **(n = 6)**	1085	1294	0..660	-	0.618	0.488	0.451	0.402
Day 176 **(n = 4)**	822	966	0.703	-	0.743	0.697	0.485	0.429
Progesterone								
Day 85 **(n = 6)**	0.76	57	-	0.965	2.763	0.930	9.474	0.246
Day 176 **(n = 4)**	1.2	1.4	-	1.214	1.917	2.000	4.333	5.929
17 α-OH-progesterone								
Day 85 **(n = 6)**	0.56	-	4.643	-	10.357	-	50.000	-
Day 176 **(n = 4)**	0.51	-	4.314	-	5.294	-	21.569	-
Organ Weights[a]								
3m Interim kill **(n = 2)**								
Ovaries		1.608		1.297		1.492		1.396
6m Terminal kill **(n = 4)**								
Ovaries		1.185		-		1.322		2.030
Gross Pathology								
6m Terminal kill								
No. of animals		4		4		4		4
Ovaries: swollen		0		0		0		3
Uterus: swollen		0		0		0		2
Vagina: swollen		0		0		0		2

(Continued)

2.6.7.7E Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Toxicity Study with 3-Month Interim Kill in the Dog

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Histopathology-3m Interim Kill								
Adrenal glands (n° examined)	2	2	2	2	2	2	2	2
Swollen cells/dense staining cytoplasm (zona reticularis)					1	1	2	2
Grade 1					1	1	1	2
Grade 2							1	
Swollen cells/dense staining cytoplasm (zona fasciculata)					1	1	1	2
Grade 1					1	1		2
Grade 2							1	
Adrenal glands (Oil red O-stain) (n° examined)	2	2	2	2	2	2	2	2
Oil red O-positive droplets (zona fasciculata)	2	2	2	2	2	2	2	2
Grade 2							1	1
Grade 3								1
Grade 4		1		2	1	1		
Grade 5	2	1	2		1	1	1	
Oil red O-positive droplets (zona reticularis)	2	2	2	2	2	2	2	2
Grade 2							1	2
Grade 3		1		2	1	1		
Grade 4	1					1		
Grade 5	1	1	2		1		1	
Epididymides (n° examined)	2		2		2		2	
Cellular debris					1		2	
Grade 1					1		2	
Liver (n° examined)	2	2	2	2	2	2	2	2
Brown pigmented macrophages							1	
Grade 1							1	
Testes (n° examined)	2		2		2		2	
Atrophic tubuli, focal							2	
Grade 1							2	
Leydig cell hyperplasia/hypertrophy					1		2	
Grade 1					1		1	
Grade 2							1	

(Continued)

2.6.7.7E Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Toxicity Study with 3-Month Interim Kill in the Dog

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Histopathology-3m Interim Kill (Cont'd)								
Thymus (n° examined)	2	2	2	2	2	2	2	2
Involution	1	1	1	1	2		2	2
Grade 1	1	1	1		1		1	1
Grade 2				1	1			1
Grade 3							1	
Ovaries (n° examined)		2		2		2		2
Atretic follicles		2		2		2		2
Grade 2				1				
Grade 4		2						
Grade 5				1		2		2
Corpora lutea (active)		1		1		1		1
Grade 1						1		1
Grade 2		1		1				
Corpora lutea (regressive)								2
Grade 2								1
Grade 5								1
Tertiary follicles		1		2		2		2
Grade 1		1		1		1		
Grade 2				1				
Grade 3						1		2
Histopathology-6m Interim Kill								
Adrenal Gland (n° examined)	4	4	4	4	4	4	4	4
Swollen cells/dense staining cytoplasm (zona reticularis)			1	1	2	2	3	3
Grade 1			1	1	2	2	3	3
Swollen cells/dense staining cytoplasm (zona fasciculata)			1	1	2	2	3	3
Grade 1			1	1	2	2	3	3

(Continued)

2.6.7.7E Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Toxicity Study with 3-Month Interim Kill in the Dog

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Histopathology-6m Interim Kill (cont'd)								
Adrenal glands (Oil red O-stain) (n° examined)	4	4	4	4	4	4	4	4
Oil red O-positive droplets (zona fasciculata)	4	4	4	4	4	4	4	4
Grade 2								1
Grade 3				1	2	2	3	2
Grade 4	3	1	3	2	2	2	1	1
Grade 5	1	3	1	1				
Oil red O-positive droplets (zona reticularis)	4	4	4	4	4	4	4	4
Grade 1								1
Grade 2			2	1	2	4	3	2
Grade 3	1	4	1	3	1		1	1
Grade 4	3		1		1			
Epididymides (n° examined)	4		4		4		4	
Cellular debris					2		3	
Grade 1					1		2	
Grade 2					1		1	
Reduced amount of spermatozoa					1		1	
Grade 1					1		1	
Gall bladder (n° examined)	4	4	4	4	4	4	4	4
Prominent bile/brown pigment (epithelial cells)					1		3	3
Grade 1					1		3	3
Liver (n° examined)	4	4	4	4	4	4	4	4
Brown pigmented macrophages					2		2	2
Grade 1					2		2	2
Testes (n° examined)	4		4		4		4	
Atrophic tubuli, focal			1		2		4	
Grade 1			1		2		4	
Leydig cell hyperplasia/hypertrophy							2	
Grade 1							2	

(Continued)

2.6.7.7E Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 6-Month Repeated Dose Toxicity Study with 3-Month Interim Kill in the Dog

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Histopathology 6m Interim Kill (cont'd)								
Ovaries (n° examined)		4		4		4		4
Atretic follicles		4		4		4		4
Grade 2		1		1				
Grade 3		2		3		1		
Grade 4		1				1		2
Grade 5						2		2
Corpora lutea (regressive)		3		4		3		4
Grade 1		1		2		1		
Grade 2		2		2		2		2
Grade 3								2
Grade 5								
Tertiary follicles				2		3		2
Grade 1				2		3		2

[a] For controls, group means are shown. For treated groups, multiples of control/baseline are shown.

- No noteworthy findings

CA = citric acid; F = female; M = male; PEG400 = Polyethylene glycol 400

2.6.7.7F Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: 12-Month Repeated Dose Oral Toxicity Study in the Dog

Species/Strain: Dog/Beagle	**Duration of Dosing:** 52 weeks	**Study No.:** TMC278-NC107 (TOX6314)
Age at First Dose: 5 or 6 months/ 6.7 – 9.3 kg for males and 6.3-8.5 kg for females	**Duration of Postdose Recovery:** /	**Location in CTD:** 4.2.3.2
Date of First Dose: -	**Route:** Oral/gavage	**GLP Compliance:** Yes
Vehicle/Formulation: PEG400 + CA		
Special Features: -		
No Observed Adverse Effect Level: /		

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Toxicokinetics:								
No. of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
$AUC_{0\text{-}24h}$ (µg·h/mL)								
Day 1	-	-	11	9.7	15	15	37	41
Day 90	-	-	18	21	29	29[a]	60	88[a]
Day 273	-	-	16	18	19[a]	31	60	51[a]
Day 363	-	-	17	19	24[b]	36	65	61[a]
C_{max} (µg/mL)								
Day 1	-	-	0.70	0.75	0.90	1.2	2.4	2.5
Day 90	-	-	1.2	1.4	2.0	2.3[a]	3.6	5.5[a]
Day273	-	-	1.1	1.1	1.3[a]	2.3	3.2	3.5[a]
Day 363	-	-	1.1	1.5	1.3[b]	2.2	4.1	5.5[a]
Noteworthy Findings:								
Died or killed prematurely	0	0	0	0	0	0	0	0
Clinical Observations								
Salivation	0	0	1	0	0	1	2	0
Feces liquid	3	4	4	4	4	4	4	4
Body weight gain (kg)[c]	2.2	3.6	-	-	-	-	0.591	0.444**
Food consumption (g)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
ECG	-	-	-	-	-	-	-	-
Hematology (Week 52)[c]								
Red blood cells (M/mm^3)	6.96	-	-	-	-	-	0.889*	-
Hemoglobin (g/dL)	16.1	-	-	-	-	-	0.869*	-
Hematocrit (%)	47.3	-	-	-	-	-	0.886*	-

(Continued)

2.6.7.7F Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 12-Month Repeated Dose Oral Toxicity Study in the Dog

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Serum chemistry (Week 52)[c]								
Calcium (mg/L)	101	106	-	-	-	-	0.901*	0.934*
Inorganic phosphorus (mg/L)	29	-	-	-	-	-	1.172	-
Creatinine (mg/L)	-	7.2	-	-	-	-	-	1.181
Alkaline phosphatase (IU/L)	220	288	-	-	-	-	2.877	1.549
Urinalysis (Week 52)[c]								
Volume	65	-	-	-	-	-	3.51	-
Endocrinology[d]								
Progesterone (nmol/L)	0.00	-	1.8	-	3.7	-	7.5	-
17α-OH-progesterone (nmol/L)	1.40	-	6.142	-	8.429	-	14.43	-
Estradiol (pmol/L)	-	-	-	-	-	-	-	-
Cortisol (nmol/L.h)	-	27	-	-	-	0.259	-	0.111
ACTH (pg/mL/h)	-	-	-	-	-	-	-	-
Luteinized hormone (ng/mL)	-	-	-	-	-	-	-	-
Testosterone (nmol/L)	-	-	-	-	-	-	-	-
Organ Weights								
Adrenal glands (g)	1.24	1.29	-	-	--	1.233	1.234	1.233
Ovaries (g)	-	1.92	-	-	-	1.396	-	2.193
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology								
Adrenal glands								
Cytoplasmic density (zona fasciculate/reticularis)	0	0	0	4	1	4	3	4
Pigment deposit in the cortex	0	0	0	0	0	0	2	2
Ovaries								
Antral follicles	-	4	-	4	-	4	-	4
Prominent early luteinized follicles	-	0	-	0	-	0	-	2
Prominent corpora lutea	-	2	-	3	-	3	-	3
Liver								
Yellow pigmentation	0	0	0	0	0	1	1	3

(Continued)

2.6.7.7F Repeat-Dose Toxicity with Recovery Period (Continued)

Test Article: rilpivirine

Report Title: 12-Month Repeated Dose Oral Toxicity Study in the Dog

DOSING PERIOD								
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)**		**5**		**10**		**40**	
No. of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Histopathology (Cont'd)								
Kidney								
Acute interstitial nephritis	0	0	0	0	0	0	2	0
Corticomedullary mineralization	0	0	0	0	0	0	0	3
Testes								
Hypertrophy of the Leydig cells	0	0	0	0	0	0	2	

[a] n = 3
[b] n = 2
[c] For controls, group means are shown. For treated groups, multiples of control/baseline are shown.
[d] Actual group means are shown as division by zero gives infinity.

- No noteworthy findings; * - p<0.05; ** - p<0.01; *** - p<0.001

CA = citric acid; ECG = electrocardiogram; F = female; M = male; PEG40 = polyethylene glycol

2.6.7.7G Repeat-Dose Toxicity: Pivotal Studies

Test Article: rilpivirine

Report Title: Endocrinological Oral (gavage) 8-week Study in the Female Sexually Immature Cynomolgus Monkey

Species/Strain: Monkey/cynomolgus
Duration of Dosing: 8 weeks
Study No.: TMC278-TiDP38-NC248 (TOX8862)
Age at First Dose: 5 or 6 months/ 6.7 – 9.3 kg for males and 6.3-8.5 kg for females
Duration of Postdose Recovery: /
Location in CTD: 4.2.3.2
Date of First Dose: [redacted] 20[redacted]
Route: Oral/gavage
GLP Compliance: Yes
Vehicle/Formulation: 1% HPMC + 0.5% Tween 20
Special Features: /
No Observed Adverse Effect Level: /

DOSING PERIOD			
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)[a]**	**200**	**500**
No. of Animals	**F:8**	**F:8**	**F:8**
Toxicokinetics:			
No. of Animals	**F:8**	**F:8**	**F:8**
Day 55			
$C_{max\ 1}$ (µg/mL)	/	0.14	0.31
$C_{max\ 2}$ (µg/mL)		0.18	0.31
$AUC_{0\text{-}24h}$ (µg·h/mL)	/	2.7	4.6
Noteworthy Findings:			
Died or killed prematurely	0	0	0
Clinical Observations	-	-	-
Body weights (kg)	-	-	-
Menses	-	-	-
ACTH (pmol/L)	-	-	-
Cortisol (nmol/L)	-		
17-hydroxyprogesterone (nmol/L) D 15	7.57	11.7	13.0
17-hydroxyprogesterone (nmol/L) D 29	7.97	17.0	18.2
17-hydroxyprogesterone (nmol/L) D 43	6.49	16.1	14.9
Progesterone (nmol/L) D15	10.6	11.3	14.5
Progesterone (nmol/L) D29	9.65	14.3	16.2
Progesterone (nmol/L) D43	8.12	13.4**	14.3**
Androstenedione (nmol/L) D15	2.64	1.94*	1.81*
Androstenedione (nmol/L) D29	2.10	1.66	1.53
Androstenedione (nmol/L) D43	2.01	1.44	1.27
DHEA (nmol/L) D15	245	264	196
DHEA (nmol/L) D29	289	296	225
DHEA (nmol/L) D43	257	270	199
Estradiol	-	-	-
LH	-	-	-

(Continued)

2.6.7.7G Repeat-Dose Toxicity: Pivotal Studies (Continued)

Test Article: rilpivirine

Report Title: Endocrinological Oral (gavage) 8-week Study in the Female Sexually Immature Cynomolgus Monkey

DOSING PERIOD			
Daily Dose During Dosing Period (mg/kg)	**0 (vehicle)[a]**	**200**	**500**
No. of Animals	**F:8**	**F:8**	**F:8**
Hematology	-	-	-
Clinical chemistry	-	-	-
Urine analysis	-	-	-
Organ weights	-	-	-
Macroscopic	-	-	-
Histopathology			
Thyroid			
Follicular cell hypertrophy	1	3	4

[a] In the control-dosed group, plasma concentrations (26 out of 80) were above the limit of quantification (0.001 µg/mL) and ranged from 0.001 to 0.003 µg/mL

- No noteworthy findings; * - $p<0.05$ (Anlysis of variance); ** - $p<0.01$ (Analysis of variance)

D = day; F = female; HPMC = hydroxypropylmethylcellulose

2.6.7.7H Repeat-Dose Toxicity

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Species/Strain: Dog/Beagle
Duration of Dosing: Days 1 and 15
Study No.: TOX10759
Age at First Dose: 8 months
Duration of Postdose Recovery: -
Location in CTD: 4.2.3.2
Date of First Dose: [redacted] 20[redacted]
Route: Intramuscular
GLP Compliance: Yes
Vehicle/Formulation: JNJ-16150108-AAA-G002 0mg/mL
Special Features: -
No Observed Adverse Effect Level:1200 mg/dog

DOSING PERIOD

Daily Dose During Dosing Period (mg/kg)	**0 (Control)**		**150 mg/dog**		**1200 mg/dog**	
No. of Animals	M:5	F:5	M:3	F:3	M:5	F:5
$AUC_{0-\infty}$ (ng·h/mL)						
Day 1 (AUC_{0-336h})	0	0	28,800 ± 3470	45,700 ± 5980	218,000 ± 110,000	204,000 ± 31,200
Day 15 (AUC_{0-264h})	0	0	34,500 ± 3520	48,500 ± 16,200	217,000 ± 60,400	206,000 ± 29,000
C_{max} (ng/mL)						
Day 1	0	0	116 ± 17.3	245 ± 77.3	1220 ± 760	1220 ± 253
Day 15	0	0	177 ± 8.96	394 ± 215	1440 ± 540	1230 ± 247
Noteworthy Findings						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight[a]	-	-	-	-	-	-
Body Weight Gain[a]	-	-	-	-	-	-
Food Consumption[a]	-	-	-	-	-	-
Clinical Observations	-	-	-	-	-	-
Dermal Observations	-	-	-	[b] (1/3)	[b] (4/5)	[b] (5/5)
Ophthalmoscopy	-	-	-	-	-	-
Electrocardiology	-	-	-	-	-	-
Haematology and coagulation[a]						
Eosinophils x 10^9/L	0.15	0.19	-	-	2.27**	1.21
Fibrinogen mg/dL	244	224	-	-	1.53**	1.46**

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	**0 (Control)**		**150 mg/dog**		**1200 mg/dog**	
No. of Animals	M:5	F:5	M:3	F:3	M:5	F:5
Clinical Chemistry	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-
Endocrinology[c]						
Cortisol	-	-	-	-	-	-
Progesterone nmol/L						
- Day 1 + 24 h	0.49	-	-	-	2.88	-
- Day 8	0.54	-	-	-	3.44	-
- Day 15	0.39	-	-	-	2.62	-
- Day 22	0.32	-	-	-	2.31	-
- Day 29	0.55	-	-	-	3.42	-
- Day 43	0.34	-	-	-	6.32	-
17α-(OH)-progesterone nmol/L						
- Day 1 + 24 h	0.90	-	-	-	3.09	-
- Day 8	0.73	-	-	-	4.21	-
- Day 15	0.61	-	-	-	3.13	-
- Day 22	0.63	-	-	-	4.14	-
- Day 29	0.69	-	-	-	5.29	-
- Day 43	0.41	-	-	-	11.78	-

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	**0 (Control)**		**150 mg/dog**		**1200 mg/dog**	
Main Study (Day 29)						
Gross Pathology						
Animals Examined	3	3	3	3	3	3
Lymph node, iliac						
- Discolouration, pale	0	0	3	3	3	3
Lymph node, lumbar						
- Discolouration, pale	-	-	-	-	1	-
Lymph node, popliteal						
- Discolouration, pale	-	-	-	-	-	1
Lymph node, renal						
- Discolouration, pale	-	-	-	-	1	-
Site, administration, 1-4, injected Day 1 and Day 15						
- Deposit(s), white	0	0	3[d]	3[d]	3	3
Recovery Study (Day 43)						
Gross Pathology						
Animals Examined	2	2	-	-	2	2
Lymph node, iliac						
- Discolouration, pale	0	0	-	-	2	2
Lymph node, renal						
- Discolouration, pale	-	-	-	-	-	1
Site, administration, 1-4, injected Day 1 and Day 15						
- Deposit(s), white	0	0	-	-	2	2

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	**0 (Control)**		**150 mg/dog**		**1200 mg/dog**	
Main Study (Day 29)						
Animals Examined	3	3	3	3	3	3
Organ Weights [e,f]						
Liver g	-	195.154	-	-	-	1.293*
Liver % of body weight	-	2.8194	-	-	-	1.203*
Prostate g	3.707	-	-	-	2.005*	-
Prostate % of body weight	0.0423	-	-	-	1.962*	-
Recovery Study (Day 43)						
Animals Examined	2	2	-	-	2	2
Organ Weights [e,f]						
Prostate g	3.690	-	-	-	2.694	-
Prostate % of body weight	0.0451	-	-	-	2.727	-
Main Study (Day 29)						
Histopathology						
Number Examined	3	3	3	3	3	3
Lymph node, iliac						
Macrophage aggregation						
Minimal	0	0	1	0	0	0
Mild	0	0	2	1	0	0
Moderate	0	0	0	2	3	3

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	0 (Control)		150 mg/dog		1200 mg/dog	
Lymph node, lumbar						
Macrophage, aggregation, moderate	-	-	-	-	1	-
Lymph node, renal						
Macrophage aggregation, moderate	-	-	-	-	1	-
Lymph node, popliteal						
Macrophage aggregation, minimal	-	-	-	-	-	1
Site, administration, 1, injected Day 1 and Day 15						
Amorphous eosinophilic deposit fascia +/- subcutaneous						
Mild	0	0	0	2	1	2
Moderate	0	0	2	1	2	1
Macrophage aggregation						
Minimal	0	0	1	0	0	2
Mild	0	0	1	1	1	1
Moderate	0	0	1	2	1	0
Inflammation, mononuclear cell						
Minimal	0	0	0	0	0	1
Mild	0	0	2	2	1	2
Moderate	0	0	1	1	2	0
Site, administration, 2, injected Day 1 and Day 15						
Amorphous eosinophilic deposit, fascia						
Minimal	0	0	-	-	1	3
Mild	0	0	-	-	1	0
Moderate	0	0	-	-	1	0
Macrophage aggregation						
Minimal	0	0	-	-	1	2
Mild	0	0	-	-	1	0
Moderate	0	0	-	-	1	0
Inflammation, mononuclear cell						
Minimal	0	0	-	-	0	3
Mild	0	0	-	-	2	0
Moderate	0	0	-	-	1	0
Inflammation, neutrophilic						
Minimal	0	0	-	-	2	0

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	0 (Control)		150 mg/dog		1200 mg/dog	
Site, administration, 3, injected Day 1 and Day 15						
Amorphous eosinophilic deposit fascia +/- subcutaneous						
Minimal	0	0	-	-	0	1
Mild	0	0	-	-	2	0
Moderate	0	0	-	-	1	2
Macrophage aggregation						
Mild	0	0	-	-	0	1
Moderate	0	0	-	-	2	1
Inflammation, mononuclear cell						
Mild	0	0	-	-	2	2
Moderate	0	0	-	-	1	1
Site, administration, 4, injected Day 1 and Day 15						
Amorphous eosinophilic deposit fascia +/- subcutaneous						
Minimal	0	0	-	-	1	1
Mild	0	0	-	-	2	2
Macrophage aggregation						
Minimal	0	0	-	-	2	0
Moderate	0	0	-	-	1	1
Inflammation, mononuclear cell						
Minimal	0	0	-	-	0	1
Mild	0	0	-	-	3	1
Moderate	0	0	-	-	0	1

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	**0 (Control)**		**150 mg/dog**		**1200 mg/dog**	
Recovery Study (Day 43)						
Histopathology						
Number examined	2	2	-	-	2	2
Lymph node, iliac						
Macrophage aggregation						
Mild	-	0	-	-	1	0
Moderate	-	0	-	-	1	2
Increased cellularity, germinal centre						
Minimal	-	0	-	-	1	1
Lymph node, renal						
Macrophage aggregation, moderate	-	-	-	-	-	1
Site, administration, 1, injected Day 1 and Day 15						
Amorphous eosinophilic deposit, fascia						
Moderate	0	0	-	-	1	2
Marked	0	0	-	-	1	0
Macrophage aggregation						
Moderate	0	0	-	-	2	2
Inflammation, mononuclear cell						
Mild	0	0	-	-	0	1
Moderate	0	0	-	-	2	1
Site, administration, 2, injected Day 1 and Day 15						
Amorphous eosinophilic deposit, fascia						
Mild	0	0	-	-	0	1
Moderate	0	0	-	-	1	1
Marked	0	0	-	-	1	0
Macrophage aggregation						
Mild	0	0	-	-	0	1
Moderate	0	0	-	-	2	1
Inflammation, mononuclear cell						
Mild	0	0	-	-	2	1
Moderate	0	0	-	-	0	1

(Continued)

2.6.7.7H Repeat-Dose Toxicity (Continued)

Test Article: rilpivirine LA

Report Title: A 4 Week Study of TMC278-LA by Intramuscular Injection in Dogs with a 2 Week Recovery Period

Daily Dose During Dosing Period (mg/kg)	**0 (Control)**		**150 mg/dog**		**1200 mg/dog**	
Site, administration, 3, injected Day 1 and Day 15						
Amorphous eosinophilic deposit, subcutaneous						
Mild	0	0	-	-	1	1
Moderate	0	0	-	-	1	0
Macrophage aggregation						
Minimal	0	0	-	-	0	1
Mild	0	0	-	-	2	1
Inflammation, mononuclear cell						
Minimal	0	0	-	-	0	1
Mild	0	0	-	-	1	1
Moderate	0	0	-	-	1	0
Site, administration, 4, injected Day 1 and Day 15						
Amorphous eosinophilic deposit, +/- subcutaneous						
Mild	0	0	-	-	2	0
Moderate	0	0	-	-	0	2
Macrophage aggregation						
Mild	0	0	-	-	2	0
Moderate	0	0	-	-	0	2
Inflammation, mononuclear cell						
Mild	0	0	-	-	2	0
Moderate	0	0	-	-	0	2

[a] At end of dosing period. For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on multiples of control)
[b] Swelling/oedema at the dose site (number of animals)
[c] For controls, group means are shown. For treated groups, multiples of control are shown
[d] Only administration site 1 injected
[e] For controls, group means are shown. For treated groups, multiples of control are shown. Statistical significance is based on actual data (not on multiples of control)
[f] Number indicates multiple of control for the absolute and relative organ weights
- No noteworthy findings; **-p<0.01; *-p<0.05

2.6.7.7I Repeat-Dose Toxicity: Pivotal Studies

Test Article: TMC278

Report Title: 9-Month IM Tox Study of a Long Acting Formulation in the Minipig

Species/Strain: pig/Göttingen
Duration of Dosing: 259 days
Study No.: TMC278-NC349 (TOX9517)
Age at First Dose: 150 days
Duration of Postdose Recovery: /
Location in CTD: 4.2.3.2
Date of First Dose: [redacted]
Route: IM
GLP Compliance: Yes
Vehicle/Formulation: aqueous buffer with P338 at 50 mg/mL/ nanosuspension of TMC278 LA at 300 mg/mL in vehicle (= G001 clinical formulation)
Special Features: /
No Observed Adverse Effect Level: /

DOSING PERIOD						
Daily Dose (mg/injection)	**0 (Group C)**		**0 (Group V)**		**600**	
No. of Animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
Toxicokinetics:						
No. of Animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
$AUC_{0\text{-}672h}$ (µg·h/mL)						
Day 0					20696	34964
Day 224					50071	44342
Noteworthy Findings:						
Died or Sacrificed Moribund	-	-	-	-	-	-
Body Weight[a]	-	-	-	-	-	-
Body Weight Gain[a]	-	-	-	-	-	-
Food Consumption[a]	-	-	-	-	-	-
Clinical Observations						
Erythema/redness	0 / 3	0 / 3	0 / 3	0 / 3	2 / 3	1 / 3
Hematology[a]						
Fibrinogen (mg/dl)	-	176	-	-	-	1.500
White blood cells (10^3/µl)	-	9.6	-	-	-	1.177
Lymphocytes (10^3/µl)	-	5.08	-	-	-	1.394
Monocytes (10^3/µl)	-	0.37	-	-	-	1.270
Eosinophils (10^3/µl)	-	0.28	-	-	-	1.321
Basophils (10^3/µl)	-	0.04	-	-	-	3.000
Serum Chemistry	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-
Gross Pathology						
Terminal sacrifice group						
Animals Examined	3	3	3	3	3	3
LYMPH N MED ILIAC						
- Discoloration	-	-	-	-	3	-
- Swollen	-	-	-	-	1	-

(Continued)

2.6.7.7I Repeat-Dose Toxicity: Pivotal Studies (Continued)

Test Article: TMC278

Report Title: 9-Month IM Tox Study of a Long Acting Formulation in the Minipig

DOSING PERIOD						
Daily Dose (mg/injection)	**0 (Group C)**		**0 (Group V)**		**600**	
No. of Animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:3**	**F:3**
INJECTION SITE(S), IM						
- Deposit[s]	-	-	-	-	3	3
LYMPH NODES						
- Discoloration	-	-	-	-	-	3
- Swollen	-	-	-	-	-	2
Histopathology						
Animals Examined	3	3	3	3	3	3
LYMPH NN POPLITEAL						
- No. Examined	2	3	2	2	3	2
- Infiltration macrophages	-	-	-	-	3	-
Grade 1					1	
Grade 2					2	
LYMPH N MED ILIAC						
- No. Examined	3	3	3	3	3	3
- Infiltration macrophages	-	-	-	-	3	-
Grade 3					2	
Grade 4					1	
INJECTION SITE(S), IM						
- No. Examined	3	3	3	3	3	3
- Infiltration macrophages	-	-	-	-	3	3
Grade 3						1
Grade 4					3	2
- Eosinophilic deposits	-	-	-	-	3	-
Grade 1					1	
Grade 2					2	
LYMPH NODES						
- No. Examined						3
- Infiltration macrophages						3
Grade 4						3

[a] At end of dosing period. For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

- No noteworthy findings

F = female; IM = intramuscular; LA = long acting; M = male; P338 = poloxamer 338

2.6.7.8A Genotoxicity: In Vitro

Report Title: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* **Test Article:** rilpivirine

Test for Induction of: *In vitro* Ames test in *Salmonella typhimurium*
Strains: *Salmonella typhimurium* strain TA1535, TA1537, TA98, TA100, TA102
Metabolizing System: Induced rat (Aroclor 1254) liver metabolic system S9-mix
Vehicles:
For Test Article: DMSO
For Positive Controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-aminoanthracene, 4-nitroquinoline-N-oxide
Treatment: 48 hours
Cytotoxic Effects: no - strong precipitation limited the dose
Genotoxic Effects: no genotoxic effects

No. of Independent Assays: 2
No. of Replicates: 3
Approximate No. of Bacteria Assayed/Dose: 10^8

Study No: TMC278-Exp.5540
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes

Metabolic Activation	Test Article	Concentration Level (µg/plate)	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
			TA98	TA1537	TA100	TA1535	TA102
Without S9	DMSO	0.00	16 ± 2.1	4 ± 1.2	112 ± 25.9	11 ± 2.1	229 ± 20.0
	TMC278	7.81	17 ± 2.0	9 ± 2.3	96 ± 3.8	12 ± 4.4	212 ± 18.8
		15.63	12 ± 2.1	8 ± 1.2	97 ± 11.5	14 ± 1.7	221 ± 16.5
		31.25	21 ± 1.4	6 ± 1.2[a]	93 ± 8.1[a]	10 ± 3.5[a]	208 ± 31.2
		62.50	13 ± 4.0[a]	5 ± 2.3[a]	79 ± 9.6[a]	14 ± 2.5[a]	187 ± 17.0[a]
		125.00	11 ± 5.3[a]	6 ± 2.0[a]	88 ± 10.1[a]	11 ± 4.4[a]	179 ± 13.5[a]
		250.00	15 ± 4.6[a]	4 ± 1.0[a]	94 ± 8.5[a]	12 ± 4.0[a]	173 ± 31.4[a]
		500.00	17 ± 1.7[a]	6 ± 2.9[a]	77 ± 7.5[a]	8 ± 1.5[a]	173 ± 10.1[a]
	2- Nitrofluorene	5.00	640 ± 68.4				
	Sodium Azide	1.00			699 ± 28.1	344 ± 76.3	
	9-Amino-Acridine	50.00		365 ± 153.1			
	4-Nitroquinoline-N-Oxide	5.00					1224 ± 251.8

(Continued)

2.6.7.8A Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
			TA98	TA1537	TA100	TA1535	TA102
With S9 (20 µL)	DMSO	0.00	17 ± 3.2	9 ± 4.4	94 ± 4.4	12 ± 1.2	344 ± 42.1
	TMC278	7.81	26 ± 5.1	5 ± 4.0	90 ± 3.0	12 ± 1.5	293 ± 9.2
		15.63	27 ± 5.3	7 ± 3.5	115 ± 12.3	11 ± 0.6	288 ± 34.9
		31.25	20 ± 5.7[a]	11 ± 0.6[a]	97 ± 12.1[a]	12 ± 1.5[a]	393 ± 49.7[a]
		62.50	17 ± 3.6[a]	7 ± 4.4[a]	84 ± 10.4[a]	10 ± 4.4[a]	356 ± 10.6[a]
		125.00	17 ± 10.6[a]	8 ± 4.0[a]	89 ± 12.0[a]	7 ± 4.0[a]	251 ± 58.0[a]
		250.00	22 ± 1.2[a]	5 ± 1.5[a]	87 ± 21.5[a]	10 ± 0.6[a]	248 ± 18.3[a]
		500.00	18 ± 4.0[a]	4 ± 1.2[a]	67 ± 7.0[a]	9 ± 6.0[a]	207 ± 35.9[a]
	2-Amino-Anthracene	1.00	837 ± 122.2	-	784 ± 71.1	144 ± 20.0	-
		2.50	-	276 ± 34.9	-	-	1000 ± 194.0

(Continued)

2.6.7.8A Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)	Assay #2 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
			TA98	TA1537	TA100	TA1535	TA102
Without S9	DMSO	0.00	18 ± 2.6	9 ± 0.6	95 ± 12.5	11 ± 2.0	248 ± 31.2
	TMC278	7.81	16 ± 9.3	8 ± 2.6	86 ± 11.1	9 ± 2.3	201 ± 16.2
		15.63	16 ± 6.6	9 ± 1.0	76 ± 16.8	10 ± 2.1	260 ± 69.4
		31.25	17 ± 5.1[a]	9 ± 5.1	94 ± 15.6	13 ± 5.5	252 ± 14.4[a]
		62.50	15 ± 3.6[a]	9 ± 5.5[a]	93 ± 8.4[a]	14 ± 3.5[a]	315 ± 36.1[a]
		125.00	15 ± 1.2[a]	7 ± 2.1[a]	96 ± 10.2[a]	8 ± 1.0[a]	284 ± 39.4[a]
		250.00	15 ± 4.2[a]	8 ± 2.6[a]	104 ± 12.0[a]	13 ± 4.7[a]	171 ± 14.0[a]
		500.00	16 ± 1.5[a]	8 ± 1.5[a]	105 ± 15.9[a]	11 ± 2.0[a]	172 ± 20.0[a]
	2- Nitrofluorene	5.00	661 ± 105.6	-	-	-	-
	Sodium Azide	1.00	-	-	837 ± 82.1	253 ± 41.1	-
	9-Amino-Acridine	50.00	-	144 ± 20.8	-	-	-
	4-Nitroquinoline-N-Oxide	5.00	-		-	-	1071 ± 208.7
With S9 (50 µL)	DMSO	0.00	19 ± 2.9	7 ± 1.2	95 ± 13.6	14 ± 3.1	337 ± 34.9
	TMC278	7.81	33 ± 6.2	5 ± 1.0	115 ± 11.5	9 ± 1.7	353 ± 26.0
		15.63	33 ± 4.6	7 ± 0.6	90 ± 20.2	8 ± 3.1	385 ± 26.6
		31.25	31 ± 1.2[a]	7 ± 2.5	108 ± 12.1	10 ± 2.1	393 ± 34.9[a]
		62.50	29 ± 3.6[a]	10 ± 3.5[a]	92 ± 5.6[a]	8 ± 1.7[a]	295 ± 58.3[a]
		125.00	34 ± 4.7[a]	6 ± 3.5[a]	94 ± 4.2[a]	11 ± 3.1[a]	261 ± 38.9[a]
		250.00	29 ± 9.6[a]	6 ± 0.6[a]	88 ± 0.6[a]	5 ± 4.0[a]	180 ± 4.0[a]
		500.00	33 ± 2.5[a]	5 ± 2.0[a]	65 ± 11.0[a]	8 ± 3.5[a]	124 ± 21.2[a]
	2-Amino-Anthracene	1.00	215 ± 112.0	-	435 ± 20.1	64 ± 5.5	-
		2.50	-	97 ± 6.4	-	-	1184 ± 284.1

[a] Precipitation at this dose level and above

DMSO = dimethyl sulfoxide

2.6.7.8B Genotoxicity: In Vitro

Report Title: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* **Test Article:** rilpivirine

Test for Induction of: *In Vitro* Mammalian Forward Mutation Test
Strains: *Salmonella typhimurium*, strains TA1535, TA1537, TA98, TA100, TA102
Metabolizing System: Induced rat (Aroclor 1254) liver metabolic system S9-mix
Vehicles:
For Test Article: DMSO
For Positive Controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-aminoanthracene, 4-nitroquinoline-N-oxide
Treatment: 48 hours
Cytotoxic Effects: Weak decrease in revertants with some strains
Genotoxic Effects: No genotoxic effects

No. of Independent Assays: 2
No. of Replicates: 3
Approximate No. of Bacteria Assayed/Dose: 10^8

Study No: TMC278-Exp.5693
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes

Metabolic Activation	Test Article	Concentration Level (µg/plate)	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
			TA98	TA1537	TA100	TA1535	TA102
Without S9	DMSO	0.00	12 ± 1.0	7 ± 3.2	108 ± 8.5	9 ± 0.6	291 ± 6.1
	TMC278	7.81	10 ± 3.8	6 ± 3.5	131 ± 22.6	13 ± 2.5	285 ± 12.2
		15.63	13 ± 4.0	10 ± 0.6	146 ± 39.1	11 ± 2.3	311 ± 41.6
		31.25	11 ± 2.6	8 ± 3.8	127 ± 18.2[a]	11 ± 0.6	313 ± 45.5
		62.50	9 ± 0.6[a]	13 ± 1.2[a]	124 ± 13.2[a]	10 ± 3.6[a]	299 ± 43.1[a]
		125.00	11 ± 3.2[a]	8 ± 1.2[a]	128 ± 22.3[a]	11 ± 1.0[a]	257 ± 36.3[a]
		250.00	10 ± 4.0[a]	8 ± 2.3[a]	105 ± 25.7[a]	14 ± 3.2[a]	275 ± 8.3[a]
		500.00	12 ± 0.6[a]	12 ± 4.7[a]	99 ± 12.5[a]	8 ± 3.6[a]	261 ± 12.2[a]
	2- Nitrofluorene	5.00	840 ± 254.0	-	-	-	-
	Sodium Azide	1.00	-	-	469 ± 60.6	207 ± 37.2	-
	9-Amino-Acridine	50.00		123 ± 35.9	-	-	-
	4-Nitroquinoline-N-Oxide	5.00			-	-	2581 ± 531.9

(Continued)

2.6.7.8B Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
			TA98	TA1537	TA100	TA1535	TA102
With S9 (20 µL)	DMSO	0.00	23 ± 4.6	9 ± 1.2	118 ± 19.5	9 ± 2.5	365 ± 20.1
	TMC278	7.81	23 ± 7.4	6 ± 2.1	124 ± 1.5	9 ± 1.5	327 ± 36.3
		15.63	25 ± 9.5	10 ± 4.6	127 ± 12.7	12 ± 7.4	249 ± 44.1
		31.25	19 ± 1.5	10 ± 0.6[a]	121 ± 6.4[a]	11	243 ± 42.4
		62.50	25 ± 4.6[a]	9 ± 3.5[a]	91 ± 8.7[a]	8 ± 5.5[a]	283 ± 10.1[a]
		125.00	20 ± 4.0[a]	8 ± 4.0[a]	80 ± 14.5[a]	9 ± 3.2[a]	412 ± 91.7[a]
		250.00	17 ± 10.7[a]	6 ± 0.6[a]	117 ± 6.5[a]	7 ± 2.0[a]	259 ± 83.9[a]
		500.00	9 ± 1.7[a]	8 ± 2.1[a]	109 ± 10.4[a]	11 ± 3.0[a]	147 ± 44.1[a]
	2-Amino-Anthracene	1.00	1161 ± 160.5		1765 ± 180.0	211 ± 26.6	
		2.50	-	344 ± 72.0	-	-	1279 ± 47.4

(Continued)

2.6.7.8B Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium* **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)	Assay #2 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)				
			TA98	TA1537	TA100	TA1535	TA102
Without S9	DMSO	0.00	13 ± 2.0	8 ± 2.6	121 ± 14.5	12 ± 4.0	228 ± 42.1
	TMC278	7.81	15 ± 3.5	8 ± 2.5	129 ± 9.2	15 ± 1.5	292 ± 72.8
		15.63	17 ± 2.5	10 ± 2.9	145 ± 24.8	17 ± 2.6	284 ± 52.0
		31.25	13 ± 5.5[a]	7 ± 1.0[a]	109 ± 5.0[a]	15 ± 4.0[a]	261 ± 36.3
		62.50	14 ± 4.0[a]	6 ± 2.1[a]	118 ± 11.1[a]	13 ± 4.9[a]	255 ± 33.3[a]
		125.00	16 ± 4.6[a]	7 ± 2.5[a]	145 ± 21.0[a]	15 ± 4.7[a]	279 ± 12.9[a]
		250.00	11 ± 4.6[a]	6 ± 2.5[a]	123 ± 5.6[a]	15 ± 2.1[a]	200 ± 6.9[a]
		500.00	14 ± 3.5[a]	8 ± 4.4[a]	113 ± 6.5[a]	14 ± 2.5[a]	225 ± 18.0[a]
	2- Nitrofluorene	5.00	805 ± 48.9	-	-	-	-
	Sodium Azide	1.00	-	-	473 ± 16.2	472 ± 28.8	-
	9-Amino-Acridine	50.00	-	84 ± 4.4	-	-	-
	4-Nitroquinoline-N-Oxide	5.00	-	-	-	-	2363 ± 352.8
With S9 (50 µL)	DMSO	0.00	20 ± 6.4	8 ± 2.1	121 ± 12.3	15 ± 2.5	340 ± 14.4
	TMC278	7.81	21 ± 7.0	9 ± 2.9	128 ± 11.2	10 ± 2.1	343 ± 76.4
		15.63	24 ± 2.0	9 ± 5.5	140 ± 12.3	12 ± 2.1	377 ± 63.8
		31.25	27 ± 6.0	9 ± 1.7	115 ± 7.5	10 ± 2.9	309 ± 32.3
		62.50	27 ± 5.0[a]	11 ± 5.0	131 ± 6.0[a]	9 ± 3.1[a]	275 ± 30.6
		125.00	18 ± 2.6[a]	12 ± 1.5[a]	115 ± 5.7[a]	8 ± 4.2[a]	328 ± 59.2[a]
		250.00	21 ± 6.0[a]	8 ± 1.5[a]	130 ± 4.2[a]	12 ± 3.5[a]	304 ± 52.5[a]
		500.00	19 ± 7.2[a]	10 ± 3.1[a]	126 ± 4.5[a]	8 ± 3.5[a]	263 ± 22.0[a]
	2-Amino-Anthracene	1.00	320 ± 32.7	-	537 ± 54.3	103 ± 14.7	-
		2.50	-	94 ± 4.6	-	-	1475 ± 20.1

[a] Precipitation at this dose level and above

DMSO = dimethyl sulfoxide

2.6.7.8C Genotoxicity: In Vitro

Report Title: *In Vitro* Bacterial Reverse Mutation Test with R314585 (TMC278.HCl) in *Salmonella typhimurium*

Test Article: rilpivirine

Test for Induction of: *In Vitro* Bacterial Reverse Mutation Test
Strains: S. Typhimurium strains TA98, TA1537, TA100, TA1535 and TA102
Metabolizing System: Induced rat (Aroclor 1254) liver metabolic system S9-mix
Vehicles:
For Test Article: DMSO
For Positive Controls: DMSO and water
Positive Controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-aminoanthracene, 4-nitroquinoline-N-oxide
Treatment: 48 to 72hours
Cytotoxic Effects: No
Genotoxic Effects: No

No. of Independent Assays: 2
No. of Replicates: 3
Approximate No. of Bacteria Assayed/Dose: 10^8

Study No: TMC278-TiDP6-NC335 (TOX8918)
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes

Metabolic Activation	Test Article	Concentration Level (µg/plate)[a]	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)									
			TA98		TA100		TA1535		TA1537		TA102	
Without S9	DMSO	0	15.7 ± 2.1		119.0 ± 18.0		11.3 ± 2.1		11.0 ± 2.6		365.3 ± 10.7	
	TMC278	3.91	11.7 ± 1.5		101.3 ± 7.6		9.0 ± 1.0		11.7 ± 3.5		382.7 ± 5.0	
		7.81	11.3 ± 3.5		109.0 ± 16.4		6.7 ± 4.7		9.7 ± 2.1		378.0 ± 9.8	
		15.63	22.7 ± 3.8		129.0 ± 4.6		8.0 ± 2.6		8.0 ± 3.0		363.3 ± 26.3	
		31.25	20.7 ± 3.2		121.7 ± 23.7		9.7 ± 3.5		6.0 ± 2.6		378.7 ± 27.9	
		62.5	13.7 ± 5.9		127.0 ± 16.7		7.3 ± 0.6		5.3 ± 1.2		357.3 ± 18.9	
		125	15.7 ± 4.7	P1	110.0 ± 11.8	P1	8.3 ± 2.1	P1	6.0 ± 2.6	P1	364.0 ± 20.3	P1
		250	19.7 ± 4.0	P1	106.3 ± 14.4	P1	9.7 ± 3.2	P1	5.7 ± 1.2	P1	303.0 ± 18.2	P1
	2-Nitrofluorene	5.00	514.7 ± 146.8*		-		-		-		-	
	Sodium Azide	1.00	-		536.0 ± 4.4*		478.3 ± 44.5*		-		-	
	9-Amino-Acridine	50.00	-		-		-		887.0 ± 177.2*		-	
	4-Nitroquinoline-N-Oxide	5.00	-		-		-		-		3309.3 ± 44.8*	

(Continued)

2.6.7.8C Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test with R314585 (TMC278.HCl) in *Salmonella typhimurium* **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)[a]	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)									
			TA98		TA100		TA1535		TA1537		TA102	
With S9	DMSO	0	16.3 ± 2.5		150.7 ± 19.0		14.3 ± 1.2		4.0 ± 0.0		415.3 ± 51.0	
	TMC278	3.91	15.7 ± 2.5		163.3 ± 31.5		11.3 ± 0.6		4.0 ± 1.7		476.0 ± 7.2	
		7.81	21.7 ± 9.6		176.7 ± 13.9		12.3 ± 4.7		5.5 ± 3.5		448.0 ± 37.5	
		15.63	19.7 ± 4.7		161.3 ± 2.5		13.0 ± 4.4		6.0 ± 2.0		456.3 ± 55.6	
		31.25	19.3 ± 5.0		180.0 ± 7.8		7.7 ± 0.6		4.7 ± 2.5		478.0 ± 32.1	
		62.5	21.0 ± 3.6		222.3 ± 15.2		11.7 ± 0.6		5.3 ± 1.2		483.0 ± 30.3	
		125	20.3 ± 3.8	P1	171.3 ± 15.5	P1	10.0 ± 4.6	P1	6.0 ± 4.2	P1	387.0 ± 67.7	P1
		250	22.0 ± 8.5	P2	163.0 ± 14.5	P2	8.7 ± 4.0	P2	4.3 ± 0.6	P2	238.7 ± 71.1	P2
	2-Amino-anthracene	2.5	1244.0 ± 79.5	*	1102.3 ± 338.5	*	116.7 ± 5.5	*	124.0 ± 16.5	*	-	
		7.5	-		-		-		-		2516.7 ± 41.0	*

(Continued)

2.6.7.8C Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test with R314585 (TMC278.HCl) in *Salmonella typhimurium* **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)[a]	Assay #2 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)									
			TA98		TA100		TA1535		TA1537		TA102	
Without S9	DMSO	0	19.3 ± 3.8		113.7 ± 3.2		7.0 ± 2.6		7.3 ± 0.6		288.0 ± 19.1	
	TMC278	3.91	14.0 ± 4.6		97.3 ± 10.1		7.3 ± 2.5		4.7 ± 1.5		303.3 ± 7.6	
		7.81	13.0 ± 7.8		96.3 ± 21.5		7.3 ± 4.0		8.0 ± 4.4		339.7 ± 12.7	
		15.63	14.7 ± 2.5		106.0 ± 18.4		5.3 ± 2.3		7.3 ± 2.5		341.0 ± 21.6	
		31.25	14.7 ± 6.5		114.0 ± 6.1		7.3 ± 3.1		6.7 ± 3.8		295.3 ± 13.4	
		62.5	15.0 ± 5.0		97.3 ± 8.4		7.3 ± 4.2		8.3 ± 1.2		292.7 ± 22.5	
		125	19.0 ± 7.0		113.7 ± 3.8		5.0 ± 2.6		5.3 ± 3.8		288.3 ± 20.5	
		250	18.0 ± 3.6		104.3 ± 7.8		6.3 ± 3.5		7.7 ± 3.5		278.7 ± 13.3	
	2-Nitrofluorene	5.00	1048.7 ± 44.8	*	-		-		-		-	
	Sodium Azide	1.00	-		415.7 ± 2.1*		313.0 ± 10.4*		-		-	
	9-Amino-Acridine	50.00	-		-		-		124.3 ± 39.5*		-	
	4-Nitroquinoline-N-Oxide	5.00	-		-		-		-		3250.0 ± 168.1*	
With S9	DMSO	0	19.3 ± 3.5		135.0 ± 11.3		9.0 ± 1.0		6.7 ± 1.5		363.7 ± 42.7	
	TMC278	3.91	29.7 ± 3.5		125.0 ± 13.5		9.0 ± 5.2		4.7 ± 3.8		385.3 ± 29.3	
		7.81	25.0 ± 5.3		129.3 ± 4.7		7.3 ± 0.6		2.7 ± 1.5		389.3 ± 25.1	
		15.63	25.0 ± 4.4		182.3 ± 26.5		8.7 ± 5.7		5.7 ± 1.5		406.0 ± 19.1	
		31.25	26.7 ± 1.2		147.3 ± 14.6		7.7 ± 1.2		3.7 ± 1.2		417.7 ± 9.9	
		62.5	30.3 ± 7.2	P1	139.7 ± 15.1	P1	9.7 ± 1.2	P1	8.3 ± 4.9		358.3 ± 17.2	
		125	23.0 ± 4.6	P1	152.0 ± 4.6	P1	9.7 ± 1.2	P1	9.7 ± 1.5	P1	268.0 ± 18.3	P1
		250	28.7 ± 5.5	P2	126.0 ± 4.6	P2	7.3 ± 0.6	P2	5.3 ± 1.5	P2	205.3 ± 32.1	P2
	2-Amino-anthracene	7.5	2417.3 ± 156.2	*	2217.7 ± 28.5	*	130.0 ± 7.0	*	242.3 ± 9.5	*	1228.7 ± 90.1	*

[a] Unless stated otherwise

*: More than 2-fold increase with TA98, TA100 and TA102 compared to the vehicle control, more than 3-fold increase with TA1535 and TA1537 compared to the vehicle control;
-: not applicable

DMSO = dimethyl sulfoxide; P1 = Slight precipitation; P2 = Moderate precipitation

2.6.7.8D Genotoxicity: In Vitro

Report Title: *In Vitro* Bacterial Reverse Mutation Test of TMC278 in *Salmonella typhimurium* with human liver S9-mix **Test Article:** rilpivirine

Test for Induction of: *In Vitro* Bacterial Reverse Mutation Test
Strains: Salmonella Typhimurium strains TA98, TA1537, TA100, TA1535 and TA102
Metabolizing System: Human S9_Mix
Vehicles:
For Test Article: DMSO
For Positive Controls: DMSO and water
Positive Controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-aminoanthracene, 4-nitroquinoline-N-oxide
Treatment: 48 to 72h
Cytotoxic Effects: Weak
Genotoxic Effects: No

No. of Independent Assays: 4
No. of Replicates: 3
Approximate No. of Bacteria Assayed/Dose: 10^8

Study No: TMC278-NC279 (TOX8086)
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes

Metabolic Activation	Test Article	Concentration Level (μg/plate)[b]	Assay #1 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)			
			TA98	TA100[a]	TA1535	TA1537
Without S9	DMSO	0	14.3 ± 1.2	99.0 ± 7.5	11.7 ± 5.0	5.7 ± 3.2
	2-Nitrofluorene	5.00	557.7 ± 64.8*	-	-	-
	Sodium Azide	1.00	-	-	411.0 ± 26.5*	-
		2.50	-	796.7 ± 26.5*	-	-
	9-Aminoacridine	50.00	-	-	-	20.3 ± 2.1*
	4-Nitroquinoline-N-Oxide	5.00	-	-	-	-
With S9	DMSO	0	25.7 ± 7.8	156.3 ± 17.6	11.0 ± 4.4	13.7 ± 3.8
	TMC278	7.81	33.3 ± 5.7	135.7 ± 17.2	11.3 ± 3.1	16.7 ± 2.1
		15.63	28.0 ± 7.5	153.0 ± 6.9	10.0 ± 4.0	13.7 ± 5.5
		31.25	31.3 ± 5.9	145.3 ± 4.7	17.7 ± 8.5	19.7 ± 3.8
		62.5	19.0 ± 10.8	158.7 ± 28.1	16.0 ± 3.6	30.3 ± 4.9
		125	18.0 ± 1.7 P	161.3 ± 25.6	15.7 ± 7.5 P	26.7 ± 10.2 P
		250	17.0 ± 7.0 P	163.3 ± 27.2 P	10.3 ± 3.1 P	33.0 ± 11.3 P
		500	15.7 ± 1.2 P	132.0 ± 6.0 P	9.3 ± 4.6 P	25.7 ± 10.0 P

* More than 2-fold increase with TA98 and TA100 compared to the vehicle control, more than 3-fold with TA1535 and TA1537 compared to the vehicle control.

(Continued)

2.6.7.8D Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test of TMC278 in *Salmonella typhimurium* with human liver S9-mix **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)[b]	Assay #2 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)							
			TA98		TA100		TA1535		TA102	
Without S9	DMSO	0	20.3 ± 0.6		141.0 ± 3.5		20.3 ± 4.9		367.7 ± 18.3	
	2-Nitrofluorene	5.00	386.0 ± 20.7*				-		-	
	Sodium Azide	1.00	-				610.0 ± 27.6*		-	
		2.50	-		1115.3 ± 40.4*		-		-	
	4-Nitroquinoline-N-Oxide	5.00	-				-		2127.3 ± 122.2*	
With S9	DMSO	0	34.7 ± 8.0		143.3 ± 16.3		24.0 ± 6.6		479.3 ± 44.2	
	TMC278	3	31.0 ± 5.0		150.0 ± 9.6		22.7 ± 3.5		467.0 ± 63.5	
		10	17.7 ± 1.5		145.3 ± 4.5		20.3 ± 5.1		445.0 ± 56.4	
		25	32.3 ± 9.0		137.0 ± 15.4		23.7 ± 3.1		480.0 ± 36.4	
		50	41.0 ± 4.4		145.3 ± 23.7		25.3 ± 4.0		477.0 ± 21.2	
		100	26.0 ± 3.6	P	141.0 ± 9.5	P	26.3 ± 1.5	P	556.7 ± 57.6	P
		200	36.7 ± 8.3	P	142.0 ± 12.8	P	28.0 ± 11.3	P	473.3 ± 33.9	P
		400	34.0 ± 10.8	P	134.3 ± 22.9	P	20.7 ± 8.1	P	443.3 ± 42.8	P

* More than 2-fold increase with TA98, TA100 and TA102 and more than 3-fold with TA1535 compared to the vehicle control.

(Continued)

2.6.7.8D Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test of TMC278 in *Salmonella typhimurium* with human liver S9-mix **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)[b]	Assay #3 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)		
			TA102		TA100
Without S9	DMSO	0	244.0 ± 7.9		82.0 ± 3.6
	Sodium Azide	2.5	-		1542.7 ± 25.8*
	4-Nitroquinoline-N-Oxide	5.00	1744.0 ± 85.1*		-
With S9	DMSO	0	273.3 ± 25.5		-
	TMC278	3	280.7 ± 33.2		-
		7.81	372.7 ± 70.3		-
		15.63	330.3 ± 43.0		-
		31.25	314.0 ± 31.4		-
		62.50	351.7 ± 17.0		-
		125	380.3 ± 11.0		-
		250	390.0 ± 13.2	P	-
		500	445.3 ± 24.8	P	-

* More than 2-fold increase with TA100 and TA102 compared to the vehicle control.

(Continued)

2.6.7.8D Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Bacterial Reverse Mutation Test of TMC278 in *Salmonella typhimurium* with human liver S9-mix **Test Article:** rilpivirine

Metabolic Activation	Test Article	Concentration Level (µg/plate)[b]	Assay #4 (Plate Incorporation) Revertant Colony Counts (Mean ± SD)		
			TA1537		TA100
Without S9	DMSO	0	5.7 ± 2.3		71.3 ± 3.2
	Sodium Azide	2.5	-		1435.3 ± 28.0*
	9-Aminoacridine	50.00	288.7 ± 38.7*		-
With S9	DMSO	0	13.3 ± 2.3		-
	TMC278	3	13.0 ± 2.6		-
		10	17.7 ± 2.5		-
		25	10.3 ± 0.6		-
		50	15.0 ± 4.4		-
		100	17.3 ± 1.5	P	-
		200	11.0 ± 5.3	P	-
		400	10.0 ± 2.6	P	-

[a] Pre-incubation.
[b] Unless stated otherwise.

* More than 2-fold increase with TA100 and more than 3-fold increase with TA1537 compared to the vehicle control.

DMSO = dimethyl sulfoxide; P = Precipitation; - = Not Applicable

2.6.7.8E Genotoxicity: In Vitro

Report Title: *In Vitro* Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique.

Test Article: rilpivirine

Test for Induction of: *In Vitro* Mammalian Forward Mutation Test
Strains: L5178Y Mouse Lymphoma Cells (TK-locus)
Metabolizing System: Aroclor 1254 induced rat liver S9 mix
Vehicles:
For Test Article: DMSO
For Positive Controls: DMN, MMS
Positive Controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-aminoanthracene, 4-nitroquinoline-N-oxide
Treatment: Microtiter® Fluctuation Technique
Cytotoxic Effects: -
Genotoxic Effects: -

No. of Independent Assays: 5
No. of Replicates: 2 10^3cells/well
Approximate No. of Bacteria Assayed/Dose: 10^8

Study No: TMC278-Exp.5539
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #1						
			SG		PE		RTG	MF	
			Total	Relative	Total	Relative		Total	Corrected
3 hour treatment	DMSO	0	32	-	106	-	-	84	74
without S9	TMC278	1	37	114	98	92	105	-	-
		2.5	36	112	116	109	122	-	-
		5	36	112	91	85	95	-	-
		10	32	99	92	87	86	79	80
		25	31	95	100	94	89	104	100
		50	32	99	101	95	94	116	111
		100	18	55	98	92	51	112	110
		250*	12	36	80	76	27	121	147
		500*	7	21	82	77	16	94	109
	MMS	15	36	111	74	69	77	211	309

(Continued)

2.6.7.8E Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique.

Test Article: rilpivirine

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #1						
			SG		PE		RTG	MF	
			Total	Relative	Total	Relative		Total	Corrected
24 hour treatment without S9	DMSO	0	31	-	95	-	-	68	66
	TMC278	20	21	67	82	86	58	116	138
		30	26	85	92	97	82	100	104
		40	25	83	80	85	70	102	121
		50	24	78	78	82	64	104	127
		60	28	91	79	83	76	93	111
		70*	26	84	78	82	69	111	137
		80*	31	100	65	68	68	-	-
		90*	28	92	79	83	77	-	-
		100*	26	84	61	65	55	-	-
	MMS	7.5	13	42	47	49	20	326	894
			Assay # 2						
3 hour treatment with S9	DMSO	0	34	-	112	-	-	72	60
	TMC278	1	31	91	123	109	100	-	-
		2.5	36	104	138	124	128	-	-
		5	37	107	105	93	100	-	-
		10	36	106	112	100	106	106	91
		15	36	106	95	85	90	110	112
		20	32	95	123	109	104	92	71
		25*	25	72	89	80	57	92	97
		30*	29	86	106	95	82	91	81
		35*	18	54	114	102	55	114	97
	DMN	400 nL/mL	35	103	82	73	75	259	366

(Continued)

2.6.7.8E Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique.

Test Article: rilpivirine

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #3						
			SG		PE		RTG	MF	
			Total	Relative	Total	Relative		Total	Corrected
3 hour treatment	DMSO	0	34	-	110	-	-	110	96
without S9	TMC278	1	36	105	105	95	100	-	-
		2.5	34	98	114	104	102	104	87
		5	34	100	91	82	82	98	103
		10	38	109	98	89	97	106	104
		25	31	91	94	85	78	138	146
		50	27	77	95	86	67	124	128
		100	12	35	94	85	29	110	113
		250*	1	3	78	71	2	-	-
		500*	1	2	60	54	1	-	-
	MMS	15	36	105	78	71	75	421	840
			Assay # 4						
24 hour treatment	DMSO	0	14	-	89	-	-	131	144
without S9	TMC278	2.5	17	120	89	100	120	-	-
		5	18	126	91	102	128	109	116
		10	20	144	92	103	149	113	119
		20	19	139	95	106	148	121	124
		30	20	141	88	98	139	98	106
		40	20	144	96	108	156	110	110
		50*	20	143	85	96	136	122	139
		60*	23	166	84	94	157	-	-
		70*	22	161	89	100	161	-	-
	MMS	7.5	8	56	47	53	30	303	788

(Continued)

2.6.7.8E Genotoxicity: In Vitro (Continued)

Report Title: *In Vitro* Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique.

Test Article: rilpivirine

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #5						
			SG		PE		RTG	MF	
			Total	Relative	Total	Relative		Total	Corrected
3 hour treatment with S9	DMSO	0	41	-	123	-	-	99	77
	TMC278	0.5	47	115	106	87	100	-	-
		1	45	109	105	85	93	102	93
		2	44	107	108	88	95	95	83
		4	47	116	101	83	95	103	97
		6	42	104	98	80	83	100	97
		8*	36	88	100	81	72	95	90
		10*	23	57	92	75	43	111	116
		15*	0	0	-	-	-	-	-
		20*	0	0	-	-	-	-	-
	DMN	400 nL/mL	43	105	91	74	78	247	309

*= Precipitation; -= Not applicable

DMN = N-nitrosodimethylamine; DMSO = dimethyl sulfoxide; MF = mutant frequency; MMS = methyl methanesulfonate; SG = suspension growth; PE = plating efficiency; RTG = relative total growth

2.6.7.8F Genotoxicity: In Vitro

Report Title: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique.

Test Article: rilpivirine

Test for Induction of: *In Vitro* Mammalian Forward Mutation Test
Strains: L5178Y Mouse Lymphoma Cells
Metabolizing System: Aroclor 1254 induced rat liver S9 mix
Vehicles:
For Test Article: DMSO
For Positive Controls: -
Positive Controls: MMS, BaP
Treatment: Microtiter® Fluctuation Technique
Cytotoxic Effects: -
Genotoxic Effects: -

No. of Independent Assays: 2
No. of Replicates: 2
Approximate No. of Bacteria Assayed/Dose: 10^8

Study No: TMC278-TiDP6-NC336 (TOX8919)
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #1	
			%RTG	MF
3 hours treatment without S9	DMSO	0	100	66.38
	TMC278	15	84	70.52
		20	89	62.17
		25	90	59.76
		30*	74	63.29
		40*	81	42.94
		50*	82	71.34
	MMS	15	47	488.91
		20	32	571.54
3 hours treatment without S9	DMSO	0	100	67.38
	TMC278	2.5	100	50.61
		5	121	50.26
		10	101	70.35
		15	85	63.63
		20	87	47.75
	B(a)P	2	47	673.77
		3	20	858.72

(Continued)

2.6.7.8F Genotoxicity: In Vitro (Continued)

Report Title: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique.

Test Article: rilpivirine

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #2	
			%RTG	MF
3 hours treatment without S9	DMSO	0	100	97.95
	TMC278	5	99	77.11
		10	100	85.77
		15	124	70.85
		20*	142	87.89
		25*	136	100.10
		30*	127	73.93
	MMS	15	75	376.88
		20	56	484.53
3 hours treatment with S9	DMSO	0	100	64.48
	TMC278	2.5	93	68.65
		5	83	68.72
		10	82	56.94
		12.5	76	68.65
		15	70	70.99
		20*	72	86.05
	B(a)P	2	15	983.93
		3	15	440.26

(Continued)

2.6.7.8F Genotoxicity: In Vitro (Continued)

Report Title: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique.

Test Article: rilpivirine

Metabolic Activation	Control, Test and Reference Articles	Concentration Level (μg/mL)	Assay #2	
			%RTG	MF
24 hours treatment without S9	DMSO	0	100	104.54
	TMC278	2.5	98	76.96
		5	95	82.34
		10	67	76.78
		20*	30	83.18
		25*	52	100.66
		30*	69	94.34
		35*	85	83.53
		40*	96	79.97
		50*	97	84.74
	MMS	5	44	712.13
		7.5	25	1210.66

*: Precipitation

B(a)P = benzo (a)pyrene; DMSO = dimethyl sulfoxide; MF = mutant frequency; MMS = methyl methanesulfonate; RTG = relative total growth

2.6.7.9 Genotoxicity: In Vivo

Report Title: *In vivo* micronucleus test on bone marrow cells of mice. **Test Article:** rilpivirine

Test for Induction of: *In vivo* micronucleus test
Species/Strain: Mouse/SPF Albino Swiss (CD1)
Age at first dose/Weight: Approximately 8 weeks
Cells Evaluated: PCEs of bone marrow
No. of Cells Analyzed/Animal: 1000 (PCEs + NCEs) to determine bone marrow proliferation and 2000 PCEs to screen for micronuclei
Treatment Schedule: polyethylene glycol (PEG) 400 solution with 100 mg/ml citric acid
Sample Time: -
Method of Administration: oral/gavage
Vehicle/Formulation: Polyethylene glycol 400 solution with 100 mg/mL citric acid
Positive Control: cyclophosphamide
Study No: TMC278-Exp.5538
Location in CTD: 4.2.3.3.2
GLP Compliance: Yes
Date of Dosing: RFS: [redacted], 20[redacted]
MNT: [redacted], 20[redacted]

Toxic/Cytotoxic Effects: No statistically significant bone marrow toxic effects were observed following treatment with 100, 400 or 1600 mg/kg TMC278 base.
Genotoxic Effects: No genotoxic effects were observed following treatment with 100, 400 or 1600 mg/kg TMC278. under the conditions described in this test.
Evidence of Exposure: -

Dose (mg./kg/day)	**0**		**100**		**400**		**1600**	
No. of animals	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Died or killed prematurely	0	0	0	0	0	0	0	0

Dose (mg./kg/day)	**0**		**100**		**400**		**1600**	
No. of animals	**-**	**-**	**M:6**	**F:6**	**M:6**	**F:6**	**M:10**	**F:10**
C_{1h} (µg/mL)	-	-	39	33	59	68	60	58
$AUC_{0\text{-}6h}$ Day 1 (µg.h/mL)	-	-	158	130	262	258	307	287

(Continued)

2.6.7.9 Genotoxicity: In Vivo (Continued)

Report Title: *In vivo* micronucleus test on bone marrow cells of mice. **Test Article:** rilpivirine

Noteworthy Findings					
Control, Test and Reference Articles	**Dose (mg/kg body weight)**	**Number of Animals and Gender**	**Sampling Time (Hours)**	**# MNPCEs per 10000 PCEs**	**Ratio PCEs/NCEs**
PEG 400	0	M/5	24	11	1.04
		F/5	24	14	1.07
		M/5	48	9	0.92
		F/5	48	22	1.27
TMC278	100	M/5	24	15	0.99
		F/5	24	12	1.34
		M/5	48	5	1.23
		F/5	48	17	1.08
	400	M/5	24	10	0.90
		F/5	24	13	1.31
		M/5	48	20	0.95
		F/5	48	28	1.16
	1600	M/5	24	8	1.24
		F/5	24	9	1.48
		M/5	48	15	0.95
		F/5	48	15	1.00
Cyclophosphamide	40 mg/mL	M/5	48	185	0.55
		F/5	48	95	0.73

F = female; M = male; MNPCEs = micronucleated polychromatic erythrocytes; MNT = Micronucleus test; NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes; PEG = polyethylene glycol; RFS = range finding study

2.6.7.10A Carcinogenicity

Report Title: Carcinogenicity Study by Oral Gavage Administration to CD-1 mice for 104 Weeks. **Test Article:** rilpivirine

Species/Strain: Mouse/Crl:CD-1™ (ICR) **Duration of Dosing:** 104 weeks **Study No.:** TMC278-NC120 (TOX7222)
Age at First Dose: 41 to 48 days **Route:** Oral/gavage **Location in CTD:** 4.2.3.4.1
Date of First Dose: [redacted], 20[redacted] **Treatment of Controls:** 0.5% w/v Methocel
Vehicle/Formulation: 0.5% w/v Methocel/Suspension **GLP Compliance:** Yes
Special Features: -
No Observed Adverse Effect Level: Not established

Daily Dose (mg/kg)	**0 (vehicle)[a]**		**20**		**60**		**160**	
Gender	M	F	M	F	M	F	M	F
Toxicokinetics:								
No. of Animals	M: 18	F: 18	M: 24	F: 24	M: 24	F: 24	M: 24	F: 24
AUC (µg.h/mL)								
Day 1 ($AUC_{0-\infty}$)	/	/	59	61	239	182	440	345
Week 28 (AUC_{0-24h})	/	/	76	51	230	278	505	766
Noteworthy findings:								
No. of Animals	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Died or Sacrificed Moribund	25	40	35	37	39	30	42	43
Body Weight[b]	48.4	37.8	1.02	1.05	1.03	1.09	1.01	1.05
Body Weight Gain[b]	15.4	13.6	1.06	1.13	1.10	1.23*	1.07	1.17*
Food Consumption[b]	35	35	1.06	1.00	1.09	1.06	1.23	1.14
Clinical Observations	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Organ Weights								
Liver (absolute)	2.334	-	1.35**	-	1.69**	-	1.18**	-
Liver (adjusted)	-	1.999	-	1.13	-	1.39**	-	1.94**
Gross Pathology								
Liver:								
Enlarged	2	7	3	7	2	10	11	30
Masses	13	4	21	9	23	13	29	32
Pale area(s)	1	6	4	9	6	10	12	33
Lungs:								
Mass(es)	23	-	21	-	26	-	30	-
Kidney:								
Left kidney								
Granular	-	6	-	6	-	4	-	15
Right Kidney								
Granular	-	6	-	6	-	4	-	14

(Continued)

2.6.7.10A Carcinogenicity (Continued)

Report Title: Carcinogenicity Study by Oral Gavage Administration to CD-1 mice for 104 Weeks. **Test Article:** rilpivirine

Daily Dose (mg/kg)	**0 (vehicle)[a]**		**20**		**60**		**160**	
No. of Animals	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Noteworthy Findings (cont'd):								
Histopathology								
Liver (n°. examined)	60	60	60	60	60	60	60	60
- M-hepatocellular carcinoma	4	0	7	0	7	2	9	7
- B-Hepatocellular adenoma	8	1	14	0	21	8	19	16
- Eosinophilic foci	0	0	0	0	1	1	3	8
- Basophilic foci	0	0	4	0	0	2	5	10
- Clear cell foci	0	0	3	1	1	1	4	4
- Generalised eosinophilic hepatocyte hypertrophy	0	1	1	0	1	11	5	42
- Brown pigmented macrophages associated with mononuclear cells	0	2	0	3	1	7	3	17
- Brown pigmented Kupffer cells/macrophages	0	5	3	3	2	17	9	17
Lungs (n°. examined)	60	60	60	60	60	60	60	60
- M-Bronchioloaveolar adenocarcinomas	6	-	5	-	0	-	10	-
Kidney (n°. examined)	-	60	-	60	-	60	-	60
Kidney (Left)								
- Glomerulonephritis	-	7	-	7	-	6	-	11
- Nephropathy		27		21		23		37
Kidney (Right)								
- Glomerulonephritis	-	7	-	7	-	6	-	11
- Nephropathy	-	22	-	20	-	20	-	36

[a] TMC278 plasma concentrations above the lower limit of quantification (0.002 µg/mL) were found in 10 out of 42 samples (ranging between 0.002 and 0.012 µg/mL).
[b] At end of dosing period. For vehicle, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

F = female; M = male
-= No noteworthy findings; * - $p<0.05$, ** - $p<0.01$

2.6.7.10B Carcinogenicity

Report Title: Carcinogenicity Study by Oral Gavage Administration to SD Rats for 104 Weeks. **Test Article:** rilpivirine

Species/Strain: Rat/Crl: CD/Sprague Dawley
Duration of Dosing: 104 Weeks (males), 98 weeks (Group 1, 3, 4 and 5 females), 97 weeks (Group 2 females)
Study No.: TMC278-NC123 (TOX7221)
Age at First Dose: approximately 42 days
Route: Oral/gavage
Location in CTD: 4.2.3.4.1
Date of First Dose: [redacted] 20[redacted]
Treatment of Controls: vehicle
Vehicle/Formulation: 0.5% w/v Methocel in purified water/suspension
GLP Compliance: Yes
Special Features: -
No Observed Adverse Effect Level: /

Daily Dose (mg/kg)	**0 (vehicle)**[b]		**40**		**200**		**500**		**1500**	
Gender	M	F	M	F	M	F	M	F		
Toxicokinetics:										
No. of Animals	M:6	F:6	M:9	F:9	M:9	F:9	M:9	F:9	M:9	F:9
AUC (µg.h/mL)[a]										
Day 1	/	/	19	17	34	40[c]	45	63	58	81
Week 27	/	/	4.5	15	11	36	14	70	20	81
Week 39	/	/	6.3	14	8.2	41	14	46	18	84
Noteworthy findings:										
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Died or Sacrificed Moribund	40	45	40[d]	46	38	35	37	33	44	34
Body Weight	-	-	-	-	-	-	-	-	-	-
Body Weight Gain	-	-	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Clinical Observations										
- Encrustations, head	-	9	-	13	-	14	-	15	-	19
- Brown staining, head	-	19	-	13	-	14	-	22	-	31
- Brown staining, dorsal body surface	7	-	9	-	6	-	10	-	15	-
- Yellow staining, ventral body surface	4	-	4	-	1	-	2	-	14	-
- Hairloss, forelimbs	1	6	6	9	2	10	4	9	10	12
- Hairloss, head	-	22	-	22	-	20	-	24	-	33
- Ungroomed	12	-	17	-	21	-	20	-	23	-
Palpable swellings	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-

(Continued)

2.6.7.10B Carcinogenicity (Continued)

Report Title: Carcinogenicity Study by Oral Gavage Administration to SD Rats for 104 Weeks. **Test Article:** rilpivirine

Daily Dose (mg/kg)	**0 (vehicle)**		**40**		**200**		**500**		**1500**	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Noteworthy Findings (cont'd):										
Serum Chemistry – Week 52 of treatment[e]										
- Alkaline phosphate U/L	53	24	1.06	1.04	1.13	1.21	1.42**	1.08	1.92**	1.29*
- Alanine aminotransferase U/L	43	-	1.02	-	1.02	-	1.49**	-	2.63**	-
- Aspartate aminotransferase U/L	74	-	1.16	-	1.09	-	1.50*	-	1.92**	-
- Gamma glutamyltransferaseGT U/L	1	-	1.00	-	2.00	-	2.00*	-	2.00*	-
- Urea mmol/L	4.10	-	0.94	-	1.01	-	1.08	-	1.14**	-
- Creatinine µmol/L	41	-	1.02	-	1.12*	-	1.22**	-	1.20**	-
- Cholesterol mmol/L	2.45	2.99	1.00	0.89	0.91	0.86*	0.84	0.80**	0.91	0.77**
- Triglycerides mmol/L	1.71	2.12	0.84	0.98	0.73*	0.78	0.80*	0.57**	0.76*	0.57**
- Chloride mmol/L	99	-	1.00	-	1.00	-	1.00	-	0.98**	-
- Calcium mmol/L	2.69	2.80	0.99	0.99*	0.99*	0.97**	0.98**	0.97**	0.97**	0.96**
- Albumin g/L	31	-	1.00	-	1.03	-	1.06**	-	1.10**	-
- a1 g/L	-	14	-	0.93*	-	0.86**	-	0.86**	-	0.93**
- a2 g/L	4	-	0.75**	-	0.75**	-	0.75**	-	0.50**	-
- Beta g/L	17	15	0.94	1.00	0.94*	0.93*	0.88**	0.93**	0.88**	0.93**
- A/G ratio	0.74	1.13	1.05	1.01	1.08*	1.07	1.19**	1.13**	1.18**	1.06**
Serum Chemistry – TERM[e]										
- Alkaline phosphate U/L	58	-	1.10	-	1.41*	-	1.81**	-	2.19**	-
- Creatinine µmol/L	33	40	1.06	1.18**	1.21#	0.98	1.21**	1.15*	1.15**	1.33**
- Glucose mmol/L	5.72	-	1.11		1.15		1.11		1.19**	
- Cholesterol mmol/L	3.42	-	0.98	-	0.88*	-	0.82*	-	0.85*	-
- Triglycerides mmol/L	-	1.27	-	1.23	-	0.86	-	0.83	-	0.72
- Potassium mmol/L	-	3.7	-	1.08	-	1.08	-	1.11*	-	1.19**
- Sodium mmol/L	143	-	1.01	-	1.01	-	1.00	-	0.99*	-
- Chloride mmol/L	100	-	1.00	-	1.02**	-	1.02**	-	1.02**	-
- Calcium mmol/L	-	2.83	-	0.98*	-	0.98	-	0.98*	-	0.98*
- Phosphorus mmol/L	-	1.41	-	1.06	-	0.96	-	0.99	-	1.15**
- Albumin g/L	30	-	1.00	-	0.97	-	1.03	-	1.07**	-
- Beta g/L	21	-	0.90*	-	0.90*	-	0.86**	-	0.81**	-
- A/G ratio	0.64	-	1.06	-	1.02	-	1.11*	-	1.22**	-

(Continued)

2.6.7.10B Carcinogenicity (Continued)

Report Title: Carcinogenicity Study by Oral Gavage Administration to SD Rats for 104 Weeks. **Test Article:** rilpivirine

Daily Dose (mg/kg)	**0 (vehicle)**		**40**		**200**		**500**		**1500**	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Noteworthy Findings (cont'd):										
Urinalysis - Week 51 of treatment[e]										
- pH	-	6.9	-	0.94	-	0.96	-	1.01	-	0.87**
- Protein g/L	-	0.27	-	0.59	-	0.89	-	0.56	-	1.11**
- Potassium mmol/L	-	160.4	-	0.94	-	0.86	-	0.86	-	0.81*
- Chloride mmol/L	45.1	31.6	1.10	1.13	1.14	1.59**	1.80**	2.75**	3.63**	4.80**
Urinalysis – TERM										
- pH	7.9	6.6	1.03	0.97	1.03	1.00	1.00	1.03	0.90**	0.91**
- Potassium mmol/L	130.7	107.2	0.98	1.06	0.81*	0.97	0.80*	0.82**	0.85*	0.85**
- Chloride mmol/L	42.2	34.9	0.95	1.01	1.29	1.47**	1.47**	1.94**	3.48**	3.46**
Organ Weights, Unadjusted (absolute)										
Thyroid and Parathyroid	0.033	0.027	-	-	-	-	-	-	1.64*	1.37**
Gross Pathology										
Number Examined:	65	65	65	65	65	65	65	65	65	65
- Lungs and Bronchi, pale area(s)	22	17	37	25	31	23	31	28	40	42
- Liver, dark area(s)	12	-	12	-	20	-	11	-	17	-
- Liver, mass(es)	-	5	-	11	-	8	-	10	-	7
- Thyroids, mass(es)	2	-	6	-	6	-	3	-	4	-
- Thyroids, enlarged	-	2	-	3	-	3	-	4	-	12
- Abnormal contents (pale/ white) of gastrointestinal Tract	6	4	9	6	18	14	15	13	32	19
Histopathology										
Liver (n°. examined)	65	65	65	65	65	65	65	65	65	65
- Hepatocelluar adenoma	0	1	1	5	3	5	3	6	3	4
- Sinuosoidal dilatation:										
Minimal	0	11	-	6	-	13	-	13	-	12
Slight	0	8	-	22	-	19	-	14	-	28
Moderate	0	6	-	2	-	4	-	4	-	3
Total	0	25	-	30	-	36	-	31	-	43
Thyroid (n°. examined)										
- Follicular cell adenoma	1	0	3	0	4	3	5	3	5	4
- Follicular cell carcinoma	0	0	0	1	2	1	1	0	2	1
- Follicular cell hypertrophy:										
Minimal	1	0	0	1	2	3	1	1	1	6
Slight	0	0	1	1	2	1	3	1	3	2
Total	1	0	1	2	4	4	4	2	4	8

(Continued)

2.6.7.10B Carcinogenicity (Continued)

Report Title: Carcinogenicity Study by Oral Gavage Administration to SD Rats for 104 Weeks. **Test Article:** rilpivirine

Daily Dose (mg/kg)	**0 (vehicle)**		**40**		**200**		**500**		**1500**	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Noteworthy Findings (cont'd):										
Histopathology (cont'd)										
- Cystic follicular cell hyperplasia:										
Minimal	3	0	1	1	3	2	0	2	5	2
Slight	3	0	4	1	2	0	2	3	1	3
Moderate	0	0	0	0	0	0	0	0	1	0
Total	6	0	5	2	5	2	2	5	7	5
Nasal turbinates (n°. examined)	65	65	65	65	65	65	65	65	65	65
- Inflammatory exudate	1	0	2	0	2	0	3	1	39	26
- Respiratory epithelium hyperplasia:										
Minimal	0	0	0	0	0	0	0	1	8	4
Slight	0	0	0	0	1	0	0	0	11	15
Moderate	0	0	0	0	0	0	0	0	5	2
Total	0	0	0	0	1	0	0	1	24	21
- Respiratory epithelium inflammation:										
Minimal	2	0	0	0	0	0	0	1	5	2
Slight	0	0	0	0	0	0	1	0	17	11
Moderate	0	0	0	0	0	0	0	0	3	4
Total	2	0	0	0	0	0	1	1	25	17
- Olfactory epithelium respiratory metaplasia:										
Minimal	0	0	0	0	0	0	0	0	0	2
Slight	0	0	0	0	0	0	0	0	5	7
Moderate	0	0	0	0	0	0	0	0	3	2
Total	0	0	0	0	0	0	0	0	8	11
Lungs (n°. examined)	65	65	65	65	65	65	65	65	65	65
- Foamy alveolar macrophages:										
Minimal	31	31	32	32	33	38	30	36	24	35
Slight	11	4	18	3	11	3	16	9	19	12
Moderate	2	0	1	2	2	1	1	0	2	6
Marked	0	0	0	0	1	0	0	0	0	0
Total	44	35	51	37	47	42	47	45	45	53

(Continued)

2.6.7.10B Carcinogenicity (Continued)

Report Title: Carcinogenicity Study by Oral Gavage Administration to SD Rats for 104 Weeks. **Test Article:** rilpivirine

Daily Dose (mg/kg)	**0 (vehicle)**		**40**		**200**		**500**		**1500**	
No. of Animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Noteworthy Findings (cont'd):										
Histopathology (cont'd)										
Lungs (n°. examined) (cont'd)										
- Alveolar epithelial hyperplasia:										
Minimal	15	9	20	9	15	8	13	16	15	17
Slight	4	2	7	2	5	4	3	3	7	11
Moderate	0	0	0	0	1	0	0	0	0	0
Total	19	11	27	11	21	12	16	19	22	28

[a] $AUC_{0-\infty}$ on Day 1 otherwise AUC_{0-24h}

[b] On Day 1, 2 samples out of 18 were above the limit of quantification (LOQ, 0.001-0.002 µg/mL), at Week 27, 17 samples out of 18 were above LOQ and at Week 39, 7 samples out of 18 were above the LOQ

[c] AUC_{0-24h}

[d] Includes one animal that was found dead during the necropsy period (Week 105)

[e] At end of dosing period. For vehicle, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline)

F = female; M = male; TERM = end of treatment period

-= No noteworthy findings; #: With exclusion of 3M, value 148 µmol/L, * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

2.6.7.11 Reproductive and Developmental Toxicity

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Dosing Period	Dose (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague Dawley 8 females/group	Oral/gavage (PEG400 + CA)	GD6-17	0 (vehicle), 40, 120, 400 mg/kg/day TMC278 base	No effects	TMC278-NC127 (TOX6147)/ 4.2.3.5.2
Rabbit/ New Zealand white 6 females/group	Oral/gavage (0.5% HPMC)	GD6-19	0 (vehicle), 25, 75, 150 mg/kg/day TMC278 base	**25 mg/kg/day**: 1 F sacrificed during the study because of abortion of its litters. **75 mg/kg/day:** 100% post-implantation loss. **150 mg/kg/day**: 1F sacrificed during the study because of abortion of it litters, ↓ fecal output in all animals, ↓ Body weight and food consumption, 100% post-implantation loss	TMC278-NC128 (TOX6235)/ 4.2.3.5.2
Rabbit/ New Zealand white 6 females/group	Oral/gavage (0.5% HPMC)	GD6-19	0 (vehicle), 5, 20, 60 mg/kg/day TMC278 base	**5 mg/kg/day**: No effects **20 mg/kg/day**: No effects **60 mg/kg/day**: ↓ Body weight, 2 F aborting prior to term, 2 F exhibiting total litter resorption (early resorptions) at term and 2 F with live litters exhibiting increased (33 and 43%) post-implantation loss	TMC278-NC129 (TOX6280)/ 4.2.3.5.2

CA = citric acid; F = females; GD = gestation day; HPMC = hydroxypropylmethylcellulose; PEG = polyethylene glycol

2.6.7.12A Reproductive and Developmental Toxicity

Report Title: Oral (gavage) male fertility study in the rat **Test Article:** rilpivirine

Fertility and Early Embryonic Development to Implantation

Design Similar to ICH 4.1.1? Yes ☐ **No** ☐
Species/Strain: Rat/Sprague-Dawley
Age at First Dose: 6-7 weeks (M, first dose), 9-10 weeks (F, mating)
Date of First Dose: [redacted] 20[redacted]
Special Features: -
No Observed Adverse Effect Level: 1600 mg base eq./kg/day
Toxicokinetics: Not determined

Duration of Dosing: **M:** 14 weeks
F: undosed
Day of Mating: F: [redacted] 20[redacted]
Route: Oral/gavage
Vehicle/Formulation: 0.5% w/v HPMC

Study No.: TMC278-NC124 (TOX6861)
Location in CTD: 4.2.3.5.1
GLP Compliance: Yes

F_0 Males

Daily Dose (mg/kg)	**0**	**100**	**400**	**1600**
No. of Animals	M: 25	M: 25	M: 25	M: 25
Noteworthy Findings				
Died or killed prematurely	-	-	-	-
Clinical Observations	-	-	-	-
Body Weight	-	-	-	-
Food consumption (g)	-	-	-	-
Copulation index (%)	-	-	-	-
Fertility index (%)	-	-	-	-
Sperm motility (%)	-	-	-	-
Sperm morphology	-	-	-	-
Mean no. corpora lutea	/	/	/	/
Pre-implantation loss (% of corpora lutea)	/	/	/	/
Post-implantation loss (% of implantation sites)	/	/	/	/
Mean gravid uterus weight	/	/	/	/
Organ weights	-	-	-	-

F = female; HPMC = hydroxypropylmethylcellulose; M = male
-= No noteworthy findings; /= Not done

2.6.7.12B Reproductive and Developmental Toxicity

Report Title: Oral (gavage) female fertility study in the rat **Test Article:** rilpivirine

Fertility and Early Embryonic Development to Implantation

Design Similar to ICH 4.1.1? Yes ☐ No ☐
Species/Strain: Rat/Sprague-Dawley

Age at First Dose: 11 weeks
Date of First Dose: [redacted] 20[redacted]
Special Features: -
No Observed Adverse Effect Level: 400 mg base eq./kg/day
Toxicokinetics: Not determined

Duration of Dosing: **M:** undosed
F: 14 days prior to pairing + 7 days of pregnancy
Day of Mating: F: [redacted] 20[redacted]
Day of C-Section: -
Route: Oral/gavage
Vehicle/Formulation: 0.5% w/v HPMC

Study No.: TMC278-NC125 (TOX6708)
Location in CTD: 4.2.3.5.1

GLP Compliance: Yes

F_0 Males

Daily Dose (mg/kg)	**0 (Vehicle)**	**40**	**120**	**400**
No. of Animals	F: 24	F: 24	F: 24	F: 24
Noteworthy Findings				
Mortality/Sacrifice	0/24	0/24	0/24	0/24
Behavior and appearance	-	-	-	-
Body weight gain	-	-	-	-
Corrected maternal weight gain	-	-	-	-
Food consumption	-	-	-	-
Weight of gravid uterus	-	-	-	-
Gross pathology	-	-	-	-
Pre-coital interval	-	-	-	-
Copulation rate	-	-	-	-
Fertility rate	-	-	-	-
Implantations	-	-	-	-
Corpora lutea of pregnancy	-	-	-	-
Pre-implantation loss	-	-	-	-
Embryos	-	-	-	-
Resorptions	-	-	-	-
Post-implantation loss	-	-	-	-

F = female; HPMC = hydroxymethylcellulose; - = no relevant changes

2.6.7.13A Reproductive and Developmental Toxicity

Report Title: Oral Developmental Toxicity Study in the Rat **Test Article:** rilpivirine

Effects on Embryo-Fetal Development

Design Similar to ICH 4.1.3? Yes ☐ No ☐
Species/Strain: Rat/SPF Sprague Dawley
Age at First Dose: about 11-12 weeks/245 - 301 g
Date of First Dose: [redacted] 20[redacted]
Special Features: -
No Observed Adverse-Effect Level: 40 mg/kg/day (maternal and embryo-fetal)

Duration of Dosing: Gestation Days (GD) 6-17
Day of Mating: GD0
Day of C-Section: GD21
Route: Oral/gavage
Vehicle/Formulation: PEG400 + CA

Study No.: TMC278-NC105 (TOX6268)
Location in CTD: 4.2.3.5.2
GLP Compliance: Yes

Daily Dose (mg/kg)	**0 (Vehicle)**	**40**	**120**	**400**
No. of Animals	F: 24	F: 24	F: 24	F: 24
Toxicokinetics:				
No. of Animals	-	F:18	F:18	F:18
$AUC_{0-\infty}$ or AUC_{0-24} (µg·h/mL)				
GD6	-	33	65	182
GD16	-	37	63	152
C_{max} (µg/mL)				
GD6	-	4.9	6.0	14
GD16	-	5.6	7.2	13
Noteworthy Findings				
Mortality	0	0	0	0
Pregnancy rate	21/24	24/24	23/24	23/24
Behavior and appearance	N	N	N	N
Body weight gain	N	N	(+)D	(+)D (GD10-13)
Corrected maternal weight gain	N	N	(+)D	(+)D
Food consumption	N	N	+D (GD10-17)	+D (GD10-17)
Weight of gravid uterus	N	N	N	N
Thyroid weight	N	N	+I	+I
Gross pathology	N	N	N	N

(Continued)

2.6.7.13A Reproductive and Developmental Toxicity (Continued)

Report Title: Oral Developmental Toxicity Study in the Rat **Test Article:** rilpivirine

Effects on Embryo-Fetal Development

Daily Dose (mg/kg)	**0 (Vehicle)**	**40**	**120**	**400**
No. of Animals	F: 24	F: 24	F: 24	F: 24
Noteworthy Findings (cont'd)				
Litter Data				
Implantations	N	N	N	N
Corpora lutea of pregnancy	N	N	N	N
Pre-implantation loss	N	N	N	N
Live fetuses	N	N	N	N
Dead fetuses	N	N	N	N
Mean litter size	N	N	N	N
Early resorptions	N	N	N	N
Late resorptions	N	N	N	N
Post-implantation loss	N	N	N	N
Body weight of live fetuses	N	N	N	N
Sex ratio	N	N	N	N
Fetal observations				
Dilated renal pelvis	N	N	+I	+I

CA = citric acid; D = decrease; F = female; GD = gestation day; I = increase; N = no relevant changes; PEG = polyethylene glycol; + = slight

2.6.7.13B Reproductive and Developmental Toxicity

Report Title: Oral Developmental Toxicity Study in the Rabbit **Test Article:** rilpivirine

Effects on Embryo-Fetal Development

Design Similar to ICH 4.1.3? **Yes** ☐ **No** ☐
Species/Strain: Rabbit/New Zealand white
Age at First Dose: between 18-21 weeks/2869 to 3794 g
Date of First Dose: [redacted]
Special Features: -
No Observed Adverse-Effect Level: 20 mg/kg/day (maternal) and 10 mg/kg/day (fetal)

Duration of Dosing: Gestation Days (GD) 6-19
Day of Mating: GD0
Day of C-Section: GD28
Route: Oral/gavage
Vehicle/Formulation: 0.5% w/v HPMC

Study No.: TMC278-NC130 (TOX6313)
Location in CTD: 4.2.3.5.2
GLP Compliance: Yes

Daily Dose (mg/kg)	0 (Vehicle)	5	10	20
No. of Animals	F: 20	F: 20	F: 20	F: 20
Toxicokinetics:				
No. of Animals	-	F:3	F:3	F:3
$AUC_{0-\infty}$ or AUC_{0-24} (µg·h/mL)				
GD6	-	95	162	219
GD19	-	105	170	232
C_{max} (µg/mL)				
GD6	-	6.4	9.7	13
GD19	-	6.7	10	15
Noteworthy Findings				
Mortality	-	0/20	0/20	0/20
Clinical observations	-	N	N	N
Pregnancy rate[a]	-	16/20	14/20	18/20
Body weight gain	-	N	N	N
Corrected mean maternal weight gain	-	N	N	N
Food consumption	-	N	N	N
Weight of gravid uterus	-	N	N	N
Adrenal weights	-	N	N	N
Gross pathology	-	N	N	N

(Continued)

2.6.7.13B Reproductive and Developmental Toxicity (Continued)

Report Title: Oral Developmental Toxicity Study in the Rat **Test Article:** rilpivirine

Effects on Embryo-Fetal Development

Daily Dose (mg/kg)	0 (Vehicle)	40	120	400
No. of Animals	F: 24	F: 24	F: 24	F: 24
Noteworthy Findings (cont'd)				
Litter Data				
Implantations	-	N	N	N
Corpora lutea of pregnancy	-	N	N	N
Pre-implantation loss	-	N	N	N
Live fetuses	-	N	N	N
Dead fetuses	-	N	N	N
Mean litter size	-	N	N	N
Early resorptions	-	N	N	N
Late resorptions	-	N	N	N
Post-implantation loss	-	N	N	N
Weight of live fetuses	-	N	N	N
Sex ratio	-	N	N	N
Fetal observations				
Left subclavian artery branches arising from the aorta	-	N	N	+I
Hypoplastic interparietal bone	-	N	N	+I

[a] Number of pregnant females/terminally sacrificed females.

F = female; HPMC = hydroxypropylmethylcellulose; I = increase; N = no relevant changes; + = slight; - = no relevant changes

2.6.7.14A Reproductive and Developmental Toxicity

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity and Juvenile Toxicity Dose Range Finding Study in the Rat. **Test Article:** rilpivirine

Effects on Pre- and Postnatal Development, Including Maternal Function

Design Similar to ICH 4.1.2? Yes ☐ No ☒
Species/Strain: Rat/Sprague Dawley
Age at First Dose: 10 weeks
Date of First Dose: [redacted] 20[redacted]
Special Features: -
No Observed Adverse Effect Level: 400 mg base eq./kg/day (maternal); 120 mg base eq./kg/day (fetal)

Duration of Dosing: GD6-LD7 (dams); D12-D25 of age (pups)
Day of Mating: GD1
Route: Oral/gavage
Vehicle/Formulation: 0.5% w/v HPMC
Litters Culled/Not Culled: -

Study No.: TMC278-NC168
Location in CTD: 4.2.3.5.3
GLP Compliance: No

F_0 Females:

Maternal Dose (mg base eq./kg/day)	**0 (vehicle)**		**0 (vehicle)**		**40**		**120**		**400**	
No. of Animals	**6**		**6**		**6**		**6**		**6**	
Toxicokinetics:										
Pup Dose (mg base eq./kg/day) LD12-D25	**0 (vehicle)**		**400**		**40**		**120**		**400**	
No. of Pups	**-**	**-**	**M: 8**	**F:8**	**M: 8**	**F:8**	**M: 8**	**F:8**	**M: 8**	**F:8**
Died or killed prematurely	ND	ND	0	0	0	0	0	0	0	0
C_{max} LD7 (µg/mL) exposure by lactation	ND	ND	-	-	0.05	0.07	0.08	0.08	0.16	0.14
C_{max} D25 of age (µg/mL) oral gavage	ND	ND	6.4	4.9	2.6	5.8	3.7	3.6	9.1	7.3
AUC_{0-24h} LD7 (µg.h/mL) exp. by lactation	ND	ND	-	-	0.62	0.74	0.94	0.91	1.9	1.8
AUC_{0-24h} D25 of age (µg.h/mL) oral gavage	ND	ND	43	39	12	18	34	28	50	53
No. Pregnant	6		6		6		6		6	
No. died or sacrificed moribund	0		0		0		0		0	
Clinical observations	-		-		-		-		-	
Gestation body weight	-		-		-		-		-	
Lactation body weight	-		-		-		-		-	
Gestation food consumption	-		-		-		-		-	
Lactation food consumption	-		-		-		-		-	
Mean duration of gestation (days)	-		-		-		-		-	
Total no of litters	-		-		-		-		-	
Mean no of pups born	-		-		-		-		-	
Mean live birth index	-		-		-		-		-	
Total litter death during LD	-		-		-		-		-	

(Continued)

2.6.7.14A Reproductive and Developmental Toxicity (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity and Juvenile Toxicity Dose Range Finding Study in the Rat. **Test Article:** rilpivirine

Effects on Pre- and Postnatal Development, Including Maternal Function

F1 litters:					
Maternal Dose (mg base eq./kg/day)	**0 (vehicle)**	**0 (vehicle)**	**40**	**120**	**400**
Pup Dose (mg base eq./kg/day) LD12-D25	**0 (vehicle)**	**400**	**40**	**120**	**400**
No. of Animals	**8M/8F**	**8M/8F**	**8M/8F**	**8M/8F**	**8M/8F**
Number of pups born	76	88	65	76	74
Pup Mortalities LD12-D25	-	--		1	2
Pup clinical signs LD12-D25					
Gestation body weight	-	-	-	-	-
Poor condition	-	-	-	-	2
Decreased activity	-	-	-	-	2
Pup body weight	-	-	-	-	-
Pup necropsy observations	-	-	-	-	-

D = day; F = female; HPMC = hydroxypropylmethylcellulose; LD = lactation day; M = male

- No noteworthy findings

2.6.7.14B Reproductive and Developmental Toxicity

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat. **Test Article:** rilpivirine

Effects on Pre- and Postnatal Development, Including Maternal Function

Design Similar to ICH 4.1.2? **Yes** ☐ **No** ☒

Duration of Dosing: GD6-LD20

Study No.: TMC278-NC131

Species/Strain: Rat/Sprague Dawley

Day of Mating: GD0

Location in CTD: 4.2.3.5.3

Age at First Dose: 9-10 weeks

Route: Oral/gavage

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Vehicle/Formulation: 0.5% w/v HPMC

Special Features: -

Litters Culled/Not Culled: -

No Observed Adverse Effect Level: 400 mg base eq./kg/day (maternal and fetal)

F_0 Females:

Dose (mg base eq./kg/day)	0	40	120	400
No. of Animals	25	25	25	25
No. died or sacrificed moribund	0	2	0	2
Clinical observations	-	-	-	-
Gestation body weight	-	-	-	-
Lactation body weight	-	-	-	-
Gestation food consumption	-	-	-	-
Lactation food consumption	-	-	-	-
Macroscopic observations	-	-	-	-
Mean duration of gestation (days)	-	-	-	-
Mean no. implantations	-	-	-	-

(Continued)

2.6.7.14B Reproductive and Developmental Toxicity (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat. **Test Article:** rilpivirine

Effects on Pre- and Postnatal Development, Including Maternal Function

F1 generation (pre-weaning):				
Dose (mg/kg/day)	**0**	**40**	**120**	**400**
No. of Animals	**25**	**25**	**25**	**23**
Mean no of pups born	-	-	-	-
Mean live birth index (%)	100	100	100	99.2*
Mean viability index (%)	99.8	99.4	97.6*	93.5**
Mean lactation index (%)	-	-	-	-
F1 generation (post-weaning):				
No. of Animals	**20**	**20**	**20**	**20**
No. died or sacrificed moribund	0	0	0	0
Pup clinical observations	-	-	-	-
Pup body weight	-	-	-	-
Pup body weight gain	-	-	-	-
Auditory acuity	-	-	-	-
Locomotor coordination	-	-	-	-
Sexual developmental observations	-	-	-	-
Fertility/mating performance	-	-	-	-
Macroscopic observations	-	-	-	-

GD = gestation day; HPMC = hydroxypropylmethylcellulose; LD = lactation day

- No noteworthy findings

* $p \leq 0.05$; ** $p \leq 0.01$ (Analysis of variance)

2.6.7.15 Studies on Juvenile Animals

Test Article: rilpivirine

No juvenile studies have been perfomed.

2.6.7.16 Local Tolerance

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
In Vitro Tests				
Bovine corneal opacity-permeability eye irritation assay	In vitro	20% w/w RPV.HCl in 0.9% NaCl (Reference: imidazole)	Increase in opacity and no increase in permeability. TMC278 considered a moderate eye irritant	TMC278-NC202 (TOX7619)/ 4.2.3.6
Phototoxicity - Balb/c mouse fibroblast assay	In vitro	0 (vehicle), 0.001 to 2.16 mg/L RPV.HCl (positive control: Chlorpromazine) In presence and absence of UVA	TMC278.HCl was not phototoxic	TMC278-NC188 (TOX7218)[a]/ 4.2.3.6
In Vivo Tests				
Primary skin irritation				
Rabbit/New Zealand White 1M + 2F	Gauze patch applied to intact skin for 4 hours	0.5 g of RPV.HCl	No skin reactions, staining of skin or corrosive effects were observed. Classified as "non-irritant" to rabbit skin.	TMC278-NC159 (TOX7555)[a]/ 4.2.3.6
Local lymph node				
Local Lymph Node Assay CBA/Ca mouse 4F/group	topical or SC (nanosuspension in P338, PS80 Vit-E-TPGS)	25 and 100 mg/mL	RPV (HCl) and nanosuspension formulations of RPV (base) fail to elicit proliferative responses higher than those observed for control groups with stimulation index values less than 3-fold for all test concentrations and routes of exposure. In all formulations and routes of exposures the test substance did not show the potential for skin sensitization.	TMC278-NC199 (TOX7453)/ 4.2.3.6

(Continued)

2.6.7.16 Local Tolerance (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
In Vivo Tests (Continued)				
HET-CAM CAM of fertilized chicken eggs	in vitro (nanosuspension in PS80)	200 and 300 mg/mL	**5 min treatment:** all vehicle and test item formulations were classified as non-eye irritant **60 min treatment:** No to very mild effects were observed **Vehicle 1 (300 mg/mL):** No effects – Vascular Irritation Index 0.0 ± 0.0 **Vehicle 2 (200 mg/mL):** No effects – Vascular Irritation Index 2.7 ± 4.6 **Vehicle 3 (300mg/mL):** No effects – Vascular Irritation Index 16.0 ± 6.9 All vehicle and test item formulations were classified as non-eye irritants under the test conditions of the experiment.	TMC278-NC301 (TOX8460)/ 4.2.3.6

(Continued)

2.6.7.16 Local Tolerance (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rabbit/New Zealand white 6M/group	IM or SC injection (1 mL/injection site) Vehicle: P338 or Vit E-TPGS Nanosuspension of RPV LA 100 mg/mL in vehicle for SC: 1 mL in flank; vehicle at contralateral site for IM: 1 mL in m. femoris; vehicle at contralateral site	0, 100	**Subcutaneous dosing:** Clinical observations: slight swelling & erythema at vehicle or active site of injection, in a few animals Histopathology: **Sites of injection**: mixed inflammatory infiltrate (moderate: 4/6; slight 2/6; vehicle: minimal in 5/6) of (sub)acute nature at 48 hours and of subacute to granulomatous nature at 72 hours **Intramuscular dosing:** Clinical observations: slight swelling & erythema at both sites of injection, in all animals. Autopsy at 48 and 72 hours: white deposits at site of injection. Histopathology: **Sites of injection**: *vehicle*: minimal to occasionally slight inflammatory changes (6/6) with minimal multifocal necrosis in 1/6; *RPV LA*: slight to moderate necrosis with inflammatory changes (6/6) with slight cyst formation (1/6), mineralized muscle fibers (2/6), and presence of minimal foreign material (1/6)	TMC278-NC237 (TOX7774)/ 4.2.3.6
Rabbit/New Zealand white 6F/group	IM injection (1 mL) vehicles: P338 P338/Na-deoxycholate P338/PEG350 PE P338/DOSS nanosuspension of TMC278 LA in vehicle	200 or 300 mg (1 mL in m. femoris; vehicle in contralateral site)	**RPV LA @ 200 mg/mL in P338:** Clinical observation: white spot on left hind leg 24 hours postdose onwards (6/6F). Gross Pathology: Intra- and perimuscular stripes of white deposits of ~6 cm (6/6F); findings comparable after 48 and 72 hours. Histopathology: Overall slight to moderate accumulation and interstitial distribution of pale eosinophilic deposits; associated with sight to moderate infiltration of granulocytes and macrophages, with local reactions largely comparable between 48 and 72 hours. Minimal to slight edema and/or hemorrhage. Focal myofiber necrosis, limited to some single fibersin some animals. **RPV LA @ 300 mg/mL in P338:** Clinical observation: white spot on left hind leg 24 hours postdose onwards (6/6F). Gross Pathology: Intra- and perimuscular stripes of white deposits of ~6 cm (6/6F); findings comparable after 48 and 72 hours. Histopathology: Overall moderate accumulation and interstitial distribution of pale eosinophilic deposits; associated with sight to moderate infiltration of granulocytes and macrophages, with local reactions largely comparable between 48 and 72 hours. Minimal to slight edema and/or hemorrhage. Focal myofiber necrosis limited to some single fibers in some animals.	TMC278-NC367 (TOX9445)/ 4.2.3.6

(Continued)

2.6.7.16 Local Tolerance (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rabbit/New Zealand white 6F/group	IM injection (1 mL) vehicles: P338 P338/Na-deoxycholate P338/PEG350 PE P338/DOSS nanosuspension of RPV LA in vehicle	200 or 300 mg (1 mL in m. femoris; vehicle in contralateral site)	**RPV LA @ 200 mg/mL in P338/Na-deoxycholate:** Clinical observation: white spot on left hind leg 24 hours postdose onwards (6/6F). Gross Pathology: Intra- and perimuscular stripes of white deposits of ~6 cm (6/6F); findings comparable after 48 and 72 hours. Histopathology: Overall moderate accumulation and interstitial distribution of pale eosinophilic deposits; associated with sight to moderate infiltration of granulocytes and macrophages, with local reactions largely comparable between 48 and 72 hours. Minimal to slight edema and/or hemorrhage. Necrosis (minimal to slight: 3/6F). **RPV LA @ 300 mg/mL in P338/Na-deoxycholate:** Clinical observation: white spot on left hind leg 24 hours postdose onwards (6/6F). Gross Pathology: Intra- and perimuscular stripes of white deposits of ~6 cm (6/6F); findings comparable after 48 and 72 hours. Histopathology: Overall moderate accumulation and interstitial distribution of pale eosinophilic deposits; associated with sight to moderate infiltration of granulocytes and macrophages, with local reactions largely comparable between 48 and 72 hours; had partly infiltrated into the deposits and were more numerous after 72 hours. Minimal to slight edema and/or hemorrhage with minimal to moderate necrosis in 4/6 F. **RPV LA @ 300 mg/mL in P338/PE PEG 350:** Clinical observation: white spot on left hind leg 24 hours postdose onwards (6/6F). Gross Pathology: Intra- and perimuscular stripes of white deposits of ~6 cm (6/6F); findings comparable after 48 and 72 hours. Histopathology: Overall moderate accumulation and interstitial distribution of pale eosinophilic deposits; associated with sight to moderate infiltration of granulocytes and macrophages, with local reactions largely comparable between 48 and 72 hours. Minimal to slight edema and/or hemorrhage. **RPV LA @ 300 mg/mL in P338/DOSS:** Clinical observation: white spot on left hind leg 48-72 hours postdose (4/6F). Histopathology: Overall moderate to marked accumulation and interstitial distribution of pale eosinophilic deposits; associated with sight to moderate infiltration of granulocytes and macrophages, with local reactions largely comparable between 48 and 72 hours. Minimal to slight edema and/or hemorrhage with minimal to slight necrosis.	TMC278-NC367 (TOX9445)/ 4.2.3.6 (Continued)

(Continued)

2.6.7.16 Local Tolerance (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Minipig/ Gottingen 3M/group	IM injection (2 mL) P338 PS80	200, 400, 500, 600	**All groups** Clinical observation: single short lasting swelling or bruise at SoI. Necropsy: Pale material at SoI, pale areas in draining lymph nodes. Histopathology: foamy macrophages, erythrocytes, brown macrophages and lymphocytes at SoI. Biomarkers: ↑ haptoglobin, CRP, CK with peak 12-24 after dosing, short lasting. **400 mg; 200 mg/mL in P338:** C_{max}: 0.098 µg/mL; $AUC_{0\text{-}648h}$: 14.5 µg.h/mL **200 mg; 100 mg/mL in P338:** C_{max}: 0.050 µg/mL; $AUC_{0\text{-}648h}$: 5.7 µg.h/mL **600 mg; 300 mg/mL in PS80:** C_{max}: 0.034 µg/mL; $AUC_{0\text{-}648h}$: 11.3 µg.h/mL **500 mg; 250 mg/mL in PS80:** C_{max}: 0.030 µg/mL; $AUC_{0\text{-}648h}$: 8.1 µg.h/mL **400 mg; 200 mg/mL in PS80** C_{max}: 0.031 µg/mL; $AUC_{0\text{-}648h}$: 9.8 µg.h/mL **200 mg; 100 mg/mL in PS80:** C_{max}: 0.013 µg/mL; $AUC_{0\text{-}648h}$: 4.3 µg.h/mL	TMC278-NC344 (FK7034)/ 4.2.2.7

(Continued)

2.6.7.16 Local Tolerance (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Minipig/ Gottingen 3M/group	IM injection (2 mL) Vehicles: P338 P338/Na-deoxycholate P338/PE PEG350 P338/DOSS; nanosuspension of RPV LA	400 or 600 (2 mL in m. femoris)	**RPV LA @ 200 mg/mL in P338:** Clinical observation: slight erythema seen 24 hours postdose (2/3). Slightly ↓ BW, BWG and FC (2/3).↑ haptoglobin, CRP. C_{max}: 0.32 µg/mL; $AUC_{Day\ 0\text{-}86}$: 95.1 µg.h/mL **RPV LA @ 300 mg/mL in P338:** Clinical observation: slight erythema 24 hours postdose (1/3 animals). ↑ haptoglobin, CRP. C_{max}: 2.25 µg/mL; $AUC_{Day\ 0\text{-}86}$: 152.0 µg.h/mL **RPV LA@ 200 mg/mL in P338/Na-deoxycholate:** Clinical observation: slight erythema (1/3) and slight to moderate swelling (1/3) seen 24 hours postdose. ↑ haptoglobin, CRP. C_{max}:1.78 µg/mL; $AUC_{Day\ 0\text{-}86}$: 69.0 µg.h/mL **RPV LA @ 300 mg/mL in P338/Na-deoxycholate:** Clinical observation: slight erythema (3/3 animals) and slight to moderate swelling (3/3) seen 24 hours postdose. Slightly ↓ BW, BWG and FC (1/3). ↑ haptoglobin, CRP. C_{max}: 1.08 µg/mL; $AUC_{Day\ 0\text{-}86}$: 100.6 µg.h/mL **RPV LA @ 300 mg/mL in P338/PE PEG 350:** Clinical observation: slight erythema (2/3) and slight to moderate swelling (2/3) seen 24 hours postdose. ↑ haptoglobin, CRP. C_{max}: 0.47 µg/mL; $AUC_{Day\ 0\text{-}86}$: 101.4 µg.h/mL **RPV LA @ 300 mg/mL in P338/DOSS:** Clinical observation: slight erythema (2/3) and slight to moderate swelling (3/3) seen 24 hours postdose. ↑ haptoglobin, CRP. C_{max}: 0.68 µg/mL; $AUC_{Day\ 0\text{-}86}$: 152.2 µg.h/mL	TMC278-NC359 (TOX9403)/ 4.2.3.6

[a] GLP compliant study

AUC = area under the concentration versus time curve; BW = body weight; BWG = body weight gain; CK = creatine kinase; C_{max} = maximum concentration; CRP = C-reactive protein; DOSS = dioctyl sodium sulfosuccinate; DSMO = dimethyl sulfoxide; F = female; FC = food consumption; HCl= hydrochloride; IM = intramuscular; LA = long acting; PE = polyethylene; PEG = polyethyleneglycol; PS80 = polysorbate 80; M = male; P338 = poloxamer 338; RPV = rilpivirine; SC = subcutaneous; SoI = site of injection; Vit-E-TPGS = Vitamin E-D-α-Tocopheryl Polyethyleneglycol 1000 Succinate

2.6.7.17 Other Toxicity Studies

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mechanistic studies					
Dog/Beagle (endocrinology study) 6M/group	Oral/gavage (PEG400 + CA)	Single dose	0 (vehicle), 20 or 80 TMC278 base	No mortality. No relevant clinical signs. No effects on body weight, cortisol, ACTH, aldosterone and CRF. **20 mg/kg**: C_{max}: 2.8 µg/mL; $AUC_{0-\infty}$: 87 µg.h/mL **80 mg/kg**: C_{max}: 2.8 µg/mL; $AUC_{0-\infty}$:105 µg.h/mL.	TMC278-Exp.5627/ 4.2.3.7.3
Guinea Pig Adrenal Cell Test (Evaluation of cortisol biosynthesis)	In vitro (DMSO 1%)	-	10^{-9} to 10^{-5}M (0.37 ng/mL to 3.7 µg/mL) TMC278 base. Positive control: Etomidate (10^{-9} to 10^{-5} M)	TMC278 inhibited cortisol synthesis in one experiment with an IC_{50} value of 6 µM but showed no significant inhibition in the second experiment. Etomidate rendered an IC_{50} value of 0.5 nM	TMC278-Exp.5653/ 4.2.3.7.3
Dog adrenal cortex (Evaluation of the cortisol in crude subcellular fraction)	In vitro	-	0.03 to 75 µM (0.011 to 27.75 µg/ mL) TMC278 base Positive control: Etomidate (0.28 µM)	**75 µM**: 39% inhibition of the conversion of pregnenolone. Progesterone and 17-α OH-progesterone concentrations increased with increasing concentrations of TMC278 whereas 11-deoxycorticosterone, 11-deoxycortisol, and corticosterone concentrations decreased with increasing concentrations of TMC278. No changes in Cortisol. Etomidate showed a 38% inhibition of the corticosteroid synthesis.	TMC278-FK4790/ 4.2.2.4

(Continued)

2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Impurities:					
Qualification of related substance A and related substance B according to ICH Q3A					
S. typhimurium TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	0 (vehicle), 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 µg/ plate TMC278 HCl spiked with 4% related substance A*	Precipitation at 55.69 µg/mL and above No evidence of mutagenic properties	TMC278-TiDP6-NC308 (TOX8901)[a]/ 4.2.3.7.6
S. typhimurium TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	0 (vehicle), 3.91, 7.81, 15.63, 31.25, 62.5, 125 and 250 µg/ plate TMC278 HCl spiked with 4% related substance B*	Precipitation at 100 µg/plate and above No evidence of mutagenic properties	TMC278-TiDP6-NC309 (TOX8900)[a]/ 4.2.3.7.6

(Continued)

2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Impurities (continued): **Qualification of** related substance A **and** related substance B **according to ICH Q3A (continued)**					
Mouse lymphoma L5178Y/tk$^{+/-}$	In vitro (DMSO)	-	**Exp 1**: 0 (vehicle), 2.5, 5, 10, 15, 20, 25, 30, 40 µg/mL 3 hours + S9 3 hours – S9 **Exp 2:** 0 (vehicle), 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 35, 40, 50, 75 µg/mL. 3 hours +S9 24 hours –S9 TMC278 HCl spiked with 4% related substance A*	Precipitation at 15 µg/mL and above No evidence of mutagenic or clastogenic properties	TMC278-TiDP6-NC311 (TOX8899)[a]/ 4.2.3.7.6
Mouse lymphoma L5178Y/tk$^{+/-}$	In vitro (DMSO)	-	**Exp1**: 0 (vehicle), 2.5, 5, 10, 15, 20, 25, 30 µg/mL 3 hours + S9 3 hours – S9 **Exp 2:** 0 (vehicle), 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 35, 40, 50, 75 µg/mL. 3 hours +/-S9 24 hours –S9 TMC278 HCl spiked with 4% related substance B*	Precipitation at 15 µg/mL and above No mutagenic or clastogenic properties	TMC278-TiDP6-NC312 (TOX8898)[a]/ 4.2.3.7.6

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Impurities (continued): **Qualification of** related substance A* **and** related substance B* **according to ICH Q3A (continued)**					
Rat/Sprague Dawley 10M and 10F/group	Gavage/Oral (0.5% w/v HPMC)	1-month	0 (vehicle), 10 TMC278 HCl, 10 TMC278 HCl spiked with 4% related substance A* 10 TMC278 HCl spiked with 4% related substance B*	No effects in any group different from the negative control group	TMC278-TiDP6-NC314 (TOX8886)[a]/ 4.2.3.7.6
related substance G* *S. typhimurium* TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	related substance G* **Exp 1 and 2**: 0 (vehicle), 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000	Increased mutation frequency in strains TA98 and TA100 in the presence of rat liver S9-mix	TMC278-NC179 (TOX6835)[a]/ 4.2.3.7.6
S. typhimurium TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	related substance J* **Exp 1, 2, 3, 4, 5 and 6**: 0 (vehicle), 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000	Increased mutation frequency in strain TA1537 in the absence of S9-mix and in strain TA98 in the presence of S9-mix.	TMC278-NC165 (TOX6616)/ 4.2.3.7.6

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
related substance G **(continued):**					
S. typhimurium TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	related substance G* **Exp 1 (2)**: 0 (0), 78.13 (93.75), 156.25 (187.5), 312.5 (375), 625 (750), 1250 (1500), 2500 (3000) and 5000 (5000)	Increased mutation frequency in the absence and the presence of S9-mix in TA98 and TA1537 and in the presence of S9-mix in TA100.	TMC278-1646_ 0016315 (TOX9643)[a]/ 4.2.3.7.6
Human lymphocytes +/- S9	In vitro (DMSO)	4 and 24 hours exposure	related substance G* 0 (vehicle), 65.22, 130.44, 260.88 and 521.75 µg/mL	Statistically significant increase in the frequency of cells with aberrations in the absence of metabolic activation (S9) after 4 hours exposure group at 521.75 µg/mL.	TMC278-TiDP6-NC184 (TOX7006)/ 4.2.3.7.6
related substance [illegible]					
S. typhimurium TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	**Exp 1 (2)**: 0 (0), 78.13 (93.75), 156.25 (187.5), 312.5 (375), 625 (750), 1250 (1500), 2500 (3000) and 5000 (5000)	No evidence of mutagenic properties with any of the tested strains both in the absence and the presence of S9-mix.	TMC278-1646_ 0015298 (TOX9645)[a]/ 4.2.3.7.6
related substance F*					
S. typhimurium TA98, TA100, TA1535, TA1537, TA102 +/- S9	In vitro (DMSO)	-	**Exp 1 (2)**: 0 (0), 78.13 (93.75), 156.25 (187.5), 312.5 (375), 625 (750), 1250 (1500), 2500 (3000) and 5000 (5000) **Exp 3**: 0, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000	No evidence of mutagenic properties with any of the tested strains both in the absence and the presence of S9-mix.	TMC278-1646_ 0015299 (TOX9644)[a]/ 4.2.3.7.6

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mouse/Swiss 12M and12F	SC/Injection (nanosuspension of RPV LA 25 mg/mL with P338 or with Vit-E TPGS)	Single dose	0 2.5 5 10 20	**2.5 mg/kg:** **Injection sites:** white deposits (F: Day 4). **Spleen:** moderate (Day 4) & marginal (Day 18) ↑ extramedullar hematopoiesis. C_{max} = 0.71-1.02 µg/mL (M-F), $AUC_{0\text{-}inf}$ = 9.3-10.7 µg.h/mL (M-F) **5 mg/kg:** Enlarged **spleen** (F), moderate (Day 4) & marginal (Day 18) ↑ extramedullar hematopoiesis; **injection sites:** white deposits (Day 4), minimal (multi)focal infiltration of inflammatory cells subcutaneous (Day 4) & in subcutis or muscle (Day 18; ↓ incidence); minimal to slight (multi)focal lymphohistiocytic infiltration in brown adipose tissue (Day 4 & Day 18; ↓ incidence); minimal to slight focal subcutaneous subacute or granulomatous inflammation (Day 4; swollen macrophages; 1M). C_{max} = 0.64-2.04 µg/mL (M-F) $AUC_{0\text{-}inf}$ = 16.8-22.6 µg.h/mL (M-F) **10 mg/kg:** Enlarged **spleen**, moderate (Day 4) & marginal (Day 18) ↑ extramedullar hematopoiesis; **injection sites:** white deposits (Day 4), minimal (multi)focal infiltration of inflammatory cells subcutaneous (Day 4) & in subcutis or muscle (Day 18; ↓ incidence); minimal to slight (multi)focal lymphohistiocytic infiltration in brown adipose tissue (Day 4 & Day 18; ↓ incidence); minimal to slight focal subcutaneous subacute or granulomatous inflammation with granulocytic infiltration & minimal to slight central necrosis or cyst formation (F) (Day 4; swollen macrophages). C_{max} = 1.43-2.77 µg/mL (M-F) $AUC_{0\text{-}inf}$ = 34.0-41.8 µg.h/mL (M-F)	TMC278-NC196 (TOX7354)/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mouse/Swiss 12M and 12F	SC/Injection (nanosuspension of RPV LA 25 mg/mL with P338 or with Vit-E TPGS)	Single dose	0 2.5 5 10 20	**20 mg/kg:** White deposits at injection sites (Days 4 and 18), ↑ spleen weight, enlarged (swollen) spleen (Day 4: 50% of animals; Day 18: 1F), moderate (Day 4) & marginal (Day 18) ↑ extramedullar hematopoiesis; **liver**: minimal to slight extramedullar hematopoiesis, **injection sites:** minimal (multi)focal infiltration of inflammatory cells subcutaneous (Day 4) & in subcutis or muscle (Day 18; ↓ incidence); minimal to slight (multi)focal lymphohistiocytic infiltration in brown adipose tissue (Day 4 & Day 18; ↓ incidence); minimal fibrosis (M; Day 4); minimal to slight focal subcutaneous subacute or granulomatous inflammation with granulocytic infiltration & minimal to slight central necrosis or cyst formation (F) (Day 4; swollen macrophages); minimal to slight focal subcutaneous granulomatous inflammation in subcutis (Day 18) with fibrosis (1F) and slight central necrosis & cyst formation (1M+1F). C_{max} = 1.61-2.16 µg/mL (M-F) $AUC_{0\text{-}inf}$ = 60.3-74.2 µg.h/mL (M-F). **20 mg/kg (in TPGS):** White deposits at injection sites (Days 4 and 18), ↑ spleen weight, enlarged (swollen) **spleen** (Day 4: 50% of animals; Day 18: 1F) **injection sites:** minimal (multi)focal infiltration of inflammatory cells subcutaneous (Day 4) & in subcutis or muscle (Day 18; ↓ incidence); minimal to slight (multi)focal lymphohistiocytic infiltration in brown adipose tissue (Day 4 & Day 18; ↓ incidence); minimal fibrosis (M; Day 4); minimal to slight focal subcutaneous subacute or granulomatous inflammation with granulocytic infiltration & minimal to slight central necrosis or cyst formation (F) (Day 4; swollen macrophages); minimal to slight focal subcutaneous granulomatous inflammation in subcutis (Day 18) with fibrosis (1F) and slight central necrosis & cyst formation (1M+1F) C_{max} = 0.96-2.85 µg/mL (M-F) $AUC_{0\text{-}inf}$ = 44.9-65.4 µg.h/mL (M-F)	TMC278-NC196 (TOX7354)/ 4.2.3.7.7 (Continued)

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 6M/group	SC or IM/Injection (nanosuspension of RPV LA @ 25 or 100 mg/mL with P338)	Single dose	5 20	**5 mg/kg, SC:** White deposits at injection sites, **injection sites**: minimal granulomatous inflammation with vacuolated macrophages sometimes with eosinophilic content, minimal fibrosis. C_{max} = 0.04 µg/mL $AUC_{0\text{-}inf}$ = 3.5 µg.h/mL **20 mg/kg, SC:** White deposits at injection sites, **injection sites**: minimal granulomatous inflammation with vacuolated macrophages sometimes with eosinophilic content, minimal fibrosis, minimal focal mononuclear cell infiltrate. C_{max} = 0.07µg/mL $AUC_{0\text{-}inf}$ = 15.5 µg.h/mL **5 mg/kg, IM:** C_{max} = 0.07 µg/mL $AUC_{0\text{-}inf}$ = 3.8 µg.h/mL **20 mg/kg, IM:** **Injection sites:** minimal focal muscle fiber degeneration, slight multifocal granulomatous inflammation in fat & epimysium, with a minimal multifocal mononuclear cell infiltrate. C_{max} = 158 µg/mL $AUC_{0\text{-}inf}$ = 0.16 µg.h/mL.	TMC278-NC244 (TOX7896)/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/beagle 6M and 6F/group	SC or IM/Injection (nanosuspension of RPV LA 100 mg/mL with P338)	Single dose	0, 200 or 400 mg/dog	**0 mg; interim (1 month and final kill)/SC:** **Thymus:** minimal macrophages with eosinophilic vacuolated cytoplasm. **200 mg/dog; interim (1 month and final kill)/SC:** **Injection sites:** white deposits, diffuse moderate inflammatory reactions, **thymus & lymph nodes**: minimal macrophages with eosinophilic vacuolated cytoplasm C_{max} = 0.11-0.19 µg/mL (M-F) $AUC_{0\text{-}85d}$ = 97.8-134 µg.h/mL (M-F) **400 mg/dog/SC:** Interim (1-month) killing: 130% ↑ weight medial iliac & axillary lymph nodes (F), 120% ↑ weight of axillary lymph nodes (M). Interim (1-month) and final killing: **Injection sites:** white deposits, diffuse moderate inflammatory reactions, **thymus & lymph nodes:** minimal macrophages with eosinophilic vacuolated cytoplasm C_{max} = 0.22-0.23 µg/mL (M-F) $AUC_{0\text{-}85d}$ = 219-234 µg.h/mL (M-F). **0 mg; interim (1 month and final kill)/IM:** **Thymus:** minimal macrophages with eosinophilic vacuolated cytoplasm, **lymph nodes:** macrophages with eosinophilic vacuolated cytoplasm (1F). **200 mg/dog/IM:** Interim (1-month) killing: 150% ↑ weight of median iliac lymph nodes (M). I**njection sites:** white deposits, multifocal moderate inflammatory reactions. Interim (1-month) and final killing: **Thymus & lymph nodes**: minimal macrophages with eosinophilic vacuolated cytoplasm C_{max} = 2.13-1.44 µg/mL (M-F) $AUC_{0\text{-}85d}$ = 148-121 µg.h/mL (M-F)	TMC278-NC234 (TOX7781)/ 4.2.3.7.7

(Continued)

2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/beagle 6M and 6F/group	SC or IM/Injection (nanosuspension of RPV LA 100 mg/mL with P338)	Single dose	0, 200 or 400 mg/dog	**400 mg/dog/IM:** Interim (1-month) killing: 200% ↑ weight of median iliac lymph nodes (M), 140% ↑ weight of median iliac lymph nodes (F), 128% ↑ popliteal lymph nodes (F). **Injection sites**: white deposits, multifocal moderate inflammatory reactions. Interim (1-month) and final killing: **Thymus & lymph nodes**: minimal macrophages with eosinophilic vacuolated cytoplasm C_{max} = 2.17-5.26 µg/mL (M-F) $AUC_{0\text{-}85d}$ = 281-265 µg.h/mL (M-F)	TMC278-NC234 (TOX7781)/ 4.2.3.7.7 (Continued)
Minipig/Göttingen 2M/group	IM/Injection (nanosuspension of RPV LA 300 mg/mL with PS80 [= F006] or RPV LA 100 mg/mL with P338 [= F004])	3 months	450 (F006) 400 (F004)	**F006:** 1 month killing: site of injection: minimal area of foamy macrophages. C_{max} = 0.017 µg/mL, $AUC_{0\text{-}696h}$ = 7.5 µg.h/mL 3-month killing: site of injection: area of foamy macrophages. C_{max} = 0.015 µg/mL, $AUC_{0\text{-}696h}$ = 5.3 µg.h/mL, $AUC_{0\text{-}2040h}$ = 8.7 µg.h/mL **F004:** 3-month killing: site of injection: area of foamy macrophages C_{max} = 0.049 µg/mL $AUC_{0\text{-}696h}$ = 13.9 µg.h/mL $AUC_{0\text{-}2040h}$ = 19.6 µg.h/mL	TMC278-NC295 (FK6407)/ 4.2.2.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Minipig/Göttingen 6sex/group Vehicle: 4sex/group Saline control group: 2sex/group	IM injection in thigh (250 µL/kg on days 1, 30, 60 and 90; 2 mL/inj. on days 120, 150, 180, 210, 240 and 270) + IM injection in m. long. dorsi (1 mL/inj. on days 120, 150, 180 and 210) Negative control (0.9% NaCl) V = PS80 + lipoid *[Nanocrystal suspension]*	9 months including 3-month interim kill	thigh: 75 mg/kg @ 300 mg/mL (up to week 13 [interim sacrifice]) + 600 mg/animal @ 300 mg/mL afterwards + m. long. dorsi: 300 mg/animal @ 300 mg/mL	**Vehicle and Saline control group** No clinical signs Slight or well-defined erythema and induration/swelling observed at injection site persistent for a few days **Test article** No clinical signs Slight or well-defined erythema and induration (notably from 5th administration) induration/swelling observed at injection site (thigh) (more pronounced in M) persistent for a few days, with a higher incidence and more induration compared to the vehicle and saline control group Haematology: slight ↑ neutrophil count (w13 M 2- 3-fold and F 2-fold) Macroscopic examination: white deposit at the injection site, sometimes accompanied by induration of the muscle Microscopic findings: Macrophage infiltration and/or amorphous pale eosinophilic deposit in the injection sites and occasionally in the draining lymph nodes. Sometimes associated with lymphohistiocytic, neutrophilic infiltration, fibroplasia, and/or muscle necrosis. These findings are expected reaction towards a particulate formulation injected IM Day 1 (M&F): C_{max} 34.4 & 74.9 ng/mL; AUC 16,943 & 33,720 ng.h/mL Day 60 (M&F): C_{max} 52.7 & 105 ng/mL; AUC 20,620 & 25,951 ng.h/mL Day 240 (M&F): C_{max} 108 & 105 ng/mL; AUC 43,410 & 35,474 ng.h/mL	TMC278-NC296 (TOX8580)/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: rilpivirine LA, P338

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rabbit/New Zealand white 6M/group	IM injection (1 mL/injection) (PS80) nanosuspension of RPV LA 300 mg/mL in vehicle [= F006]	Single dose	300 (1 mL in m. femoris; vehicle at contralateral site)	Clinical observations: at 24 or 48 hours, *vehicle*: slight/moderate erythema (2/6); *TMC278 LA*: slight erythema (1/6). At 72 hours, No signs of irritation Histopathology: **Sites of injection**: *vehicle*: minimal edema (1/6), minimal lympho-histiocytic infiltrate (1/6); *TMC278 LA*: slight to moderate inflammatory changes (6/6), hemorrhage (6/6), necrosis (6/6) and mineralization (1/6). Slight to moderate interstitial edema/fluid accumulation (6/6) and presence of polarizing crystalloid material (6/6)	TMC278-NC280 (TOX8482)/ 4.2.3.7.7
Salmonella typhimurium (TA98, TA1537, TA100, TA1535, TA102)	*In vitro* Poloxamer 338 in water (+/- S9)	NA	1st experiment: 156.25, 312.5, 625, 1250, 2500, 5000 and 10,000 µg/plate 2nd experiment: 250, 500, 1000, 2000, 4000, 8000 and 10,000 µg/plate	Not mutagenic	TMC278-NC357 (TOX9535)/ 4.2.3.7.7
Human lymphocytes	*In vitro* Poloxamer 338 in water (+/- S9)	NA	1st experiment: *-absence of S9:* 3h treatment, 18h recovery: 3600, 6000 and 10,000 µg/mL *-presence of S9*: 3h treatment, 21h recovery: 3600, 6000 and 10,000µg/mL 2nd experiment: *-absence of S9:* 21h continuous treatment: 3600, 6000 and 10,000 µg/mL *-presence of S9:* 3h treatment, 18h recovery: 3600, 6000 and 10,000 µg/mL up to 10000 µg/mL	Not mutagenic	TMC278-NC358 (TOX9523)/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: P338

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 4F/group	Oral Poloxamer 338 in water	Single dose	1600 mg/kg	Plasma concentrations were BQL in all samples, i.e., below 0.1 µg/mL	FK13157/ 4.2.2.7
Rabbit/NZW 4F/group	Oral Poloxamer 338 in water	Single dose	1600 mg/kg	Concentrations were quantifiable, i.e., above the BQL (75 ng/mL), starting from 2h to 8h. The time to reach peak plasma concentrations ranged from 48h to 72h. The decrease in plasma concentrations was mono phasic, and concentrations were still above BQL at 336h for 3 of 4 rabbits C_{max}: 0.42 µg/mL, AUC_{last}: 68.9 µg.h/mL	FK13159/ 4.2.2.7
Rat/Sprague-Dawley 6F/group	Oral/gavage of Poloxamer 338 only (aqueous solution)	14 days	0 1600 mg/kg/day	↓ body weight gain (first dosing week), (partial) recovery body weight gain (second dosing week)	1955_0016643 (TOX9648)/ 4.2.3.7.7
Rat/Sprague-Dawley 22F/group	Oral Poloxamer 338 only (aqueous solution)	GD6 to GD17	0 1600 mg/kg/day	No mortality 19 out of 22 rats were pregnant in both groups Minimal ↓ BW performance from 2nd week into post-dosing period. Fetal weight and sex ratio were considered unaffected by treatment. No test article related malformation or increased incidences of minor abnormalities were observed on external, visceral or skeletal examination. In conclusion, no effect on maternal condition apart from minimal ↓ in BW performance. Pregnancy parameters and embryo-fetal development were unaffected.	TMC278-NC347 (TOX9679)/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: P338

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 4F/group	Intramuscular poloxamer 338 (aqueous solution)	Single dose Repeated dose (D0, D3, D6 and D9) + 1 month follow/up	SD: 5 RD: 5, 10, 2x5	No mortality No test article related clinical observations nor in organs and tissues examined at histopathology SD: Transient ↓ BW (3%) C_{max} (µg/mL): 24.5; $AUC_{0\text{-}72h}$ (µg.h/mL): 679 RD: 5 mg/kg: C_{max} (µg/mL): 20.8 - 38.4; $AUC_{0\text{-}72h}$ (µg.h/mL): 705 – 987; $AUC_{0\text{-}28days}$ (µg/mL): 3832* 10 mg/kg: C_{max} (µg/mL): 49.2 – 67.1; $AUC_{0\text{-}72h}$ (µg.h/mL): 1570 - 1950; $AUC_{0\text{-}28days}$ (µg/mL): 8180 2x5 mg/kg: C_{max} (µg/mL): 48.7 – 57.4; $AUC_{0\text{-}72h}$ (µg.h/mL): 1470 - 1550; $AUC_{0\text{-}28days}$ (µg/mL): 4180*	TOX13295/ 4.2.3.7.7
Rat/Sprague-Dawley 22M and 22F/group	Intramuscular Poloxamer 338 (aqueous solution)	Every 3 days M: 4 weeks prior to mating to termination (= 9 weeks) F: 2 weeks prior to mating until GD15	0 2.5 5 10	No test article related effects on clinical condition, body weight, food consumption, mating performance, fertility and embryo-fetal development (including teratogenicity) The NOEL for gonadal function, mating behavior and reproductive performance in the male and female rat was established at 10 mg/kg/occasion for males and females The NOEL for embryonic and fetal development (including teratogenicity) was established at 10 mg/kg/occasion 10 mg/kg/occasion: Day 1: C_{max} (µg/mL): 33.1 (M), 25.6 (F); $AUC_{(0\text{-}72h)}$ µg.h/mL: 944 (M), 666 (F) Last dose**: C_{max} (µg/mL): 34.1 (M), 26.2 (F); $AUC_{(0\text{-}72h)}$ µg.h/mL: 1480 (M), 879 (F)	TOX13391/ 4.2.3.7.7
Rabbit/New Zealand white (5F/group)	Oral/gavage of Poloxamer 338 only (aqueous solution)	14 days	0 1600 mg/kg/day	No test article related mortality Soft feces (3 rabbits: slight towards end of first dosing week for 1 or 2 days; 1 rabbit: slight to moderate from Day 2 until end of dosing); ↓ body weight gain, ↓ food consumption	TMC278-NC366 (TOX9649)/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: P338

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rabbit/New Zealand white 20F/group	Oral/gavage of Poloxamer 338 only (aqueous solution)	D6 to D19	0 1600 mg/kg/day	No mortality Control: 17/20 F pregnant 1600 mg/kg/day: 19/20 F pregnant Soft feces (15/20) persisting post-dose (9/20), ↓ fecal output (6/20) ↓ BW (D9 – D19 of pregnancy) compensated but remained lower post-dose Litter parameters were unaffected by treatment, fetal weight and sex ratio unaffected by treatment No relevant malformations or increased incidences in minor abnormalities on external, visceral or skeletal examination. In conclusion, maternal toxicity was evidenced by disturbance of the gastro-intestinal tract and the effects on body weight performance and food consumption. No direct effect on the embryo-fetal development.	TMC278-NC348 (TOX9680)/ 4.2.3.7.7
Rabbit/New Zealand white 4F/group	IM Poloxamer 338 only (aqueous solution)	Single dose Repeated dose 1-month follow-up	SD: 5 RD: 5 (2 doses) 2.5 (3 doses) 2.5 (2 doses)	No mortality No test article related effects Time of the peak of concentration ranged between 7 and 24h after dosing 5mg/kg with one-week interval presented the highest C_{max} and $AUC_{0\text{-}14days}$ with high variability on the toxicokinetic parameters related to the terminal phase (CV of $AUC_{0\text{-}14days}$ = 44%) After the second dose, mean C_{trough} was 5.74 µg/mL (CV = 92%)	TOX13296/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: P338

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rabbit/New Zealand white 20F/group	IM Poloxamer 338 only (aqueous solution)	GD6 and GD12 (2 administrations)	0 2.5 5	No test article related mortality No test article related clinical signs or macroscopic findings and no effect on mean gravid uterus weight No test article related effect on embryo-fetal survival, mean fetal weight or sex ratio No external, visceral or skeletal abnormalities 18, 18 and 20 pregnant F in control, 2.5 and 5 mg/kg/occasion groups respectively NOEL for maternal toxicity is 5 mg/kg/occasion No effect of embryo-fetal survival and development or evidence of a teratogenic potential. NOEL for embryo-fetal survival and development is 5 mg/kg/occasion This dose corresponds to a mean C_{max} and $AUC_{0\text{-}tau}$ of 40.4 µg/mL and 2410 µg.h/mL, respectively, on GD6 and 49.2 µg/mL and 2670 µg.h/mL, respectively on GD12	TOX13376/ 4.2.3.7.7
Rat/Sprague-Dawley 22F/group	IM Poloxamer 338 only (aqueous solution)	One administration every three calendar days from GD6 to weaning (13 administrations)	0 2.5 5 10 mg/kg/dose	No test article related clinical signs, effects on mean BWG, FC during gestation and lactation period. 22/22F pregnant in all groups No test article related effect on gestation length, pre-birth loss, post-implantation data and pup viability from birth through weaning. No test article related maternal macroscopic findings, maternal treatment on pup observations, pre-weaning BW, sex ratio, reflex and physical development, post-weaning BW and BWG, behavioral test, sexual maturation and necropsy findings. No effect of maternal treatment on the copulation and fertility indices of the offspring.	TOX13546/ 4.2.3.7.7

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2.6.7.17 Other Toxicity Studies (Continued)

Test Article: P338

Species/Strain Sex/No. Per Group	Route (Vehicle/Formulation)	Duration of Dosing	Doses (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 22F/group	IM Poloxamer 338 only (aqueous solution)	One administration every three calendar days from gestation day 6 to weaning (13 administrations)	0 2.5 5 10 mg/kg/dose	There were 18, 19, 19 and 18 pregnant females in the control, 2.5, 5 and 10 mg/kg/dose groups, respectively, at the terminal caesarean examinations, all of which had viable embryos. There was no effect of maternal treatment on pre- and post-implantation data of the F1 females in any group. The NOAEL for embryo-fetal and peri-and post-natal development of the rat and subsequent reproductive performance of the offspring was considered 10 mg/kg/dose. GD 26: C_{max} 26.4 µg/mL; $AUC_{(0\text{-}tau)}$:807 µg.h/mL LD17 (12^{th} dose): C_{max} 25.2 µg/mL; $AUC_{(0\text{-}tau)}$:990 µg.h/mL	TOX13546/ 4.2.3.7.7 (Continued)

[a] GLP compliant study

ACTH = adrenocorticotropic hormone; AUC = area under the concentration vs time curve; BW = body weight; BQL = Below Quantifiable Limit; CA = citric acid; C_{max} = maximum concentration; CRF = corticotrophin releasing factor; CV = coefficient if variation; D= day; DMSO = dimethyl sulfoxide; Exp = experiment; F = female; GD = gestation day; h = hour; HCl = Hydrochloric Acid; HPMC = hydroxypropyl methyl cellulose; IC_{50} = The half maximal inhibitory concentration; i.e. = it est; IM = intramuscular; LA = long acting; M = male; m. long. dorsi = musculus longissimus dorsi; NA = not applicable; NaCl = Sodium Chloride; NZW = new Zealand White; NOEL = No Observed Effect Level; P338 = poloxamer 338; PEG400 = polyethylene glycol 400; PS80 = polysorbate 80; RD = Repeated dose; SC = subcutaneous; SD = single dose; TA = test article; V = vehicle; Vit-E-TPGS = Vitamin E-D-α-Tocopheryl Polyethyleneglycol 1000 Succinate; ↑ = increase; ↓ = decrease

*n=2; ** 19^{th} dose for males and GD15 (11 or 12^{th} dose) for females