MODULE 2.6 NONCLINICAL SUMMARY

2.6.6 TOXICOLOGY WRITTEN SUMMARY

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LIST OF ABBREVIATIONS

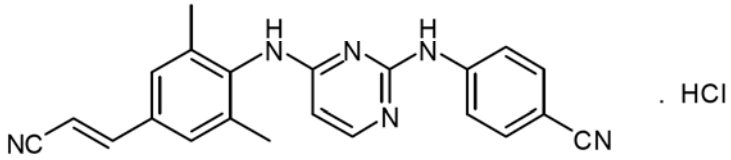
ACTH	adrenocorticotropic hormone
A/G ratio	albumin/globulin ratio
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
$AUC_{0-\infty}$	area under the time vs concentration curve from the moment of administration to infinity
BCOP	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
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
OECD	organization for economic cooperation 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
TTC	threshold of toxicological concern
Tween 20	polyoxyethylenesorbitan monooleate 20
UDPGT	T_4-uridine diphosphate glucuronosyltransferase

1 BRIEF SUMMARY

TMC278 (Figure 1) is a next generation non-nucleoside reverse transcriptase inhibitor (NNRTI) active against wild type and NNRTI-resistant human immunodeficiency virus type 1 (HIV-1).

Figure 1 Structural Formula TMC278



The following convention is applied throughout this Module: Reference is made to "TMC278" when the hydrochloride (HCl) salt was administered and to "TMC278 base" when the base was administered. The dose or concentration is always given as base equivalent. The analyt in bio-analytical determinations is referred to as "TMC278".

TMC278 base has been applied in the early phases of development and TMC278 in the later phases, after selection of the final chemical form.

The design and conduct of the safety studies were consistent with scientific and ethical principles and international guidelines. Pivotal studies have been conducted in compliance with Good Laboratory Practices (GLP) standards issued by the Organization for Economic Cooperation and Development (OECD Principles of GLP). The OECD Principles of GLP are conform GLP regulations of the United States Food and Drug Administration (FDA).

The objectives of the toxicology program were to evaluate the toxicological profile of TMC278, its metabolites, and impurities in non-clinical test systems. Furthermore, attempts were made to elucidate the mechanism of action of the effects observed. Toxicological profile and mechanism of actions were used in the assessment of a safety margin (to be presented in Module 2.4 Nonclinical Overview) between the dose and exposure needed for an efficacious and safe treatment of HIV-infected treatment-naïve men, women, and children, and the adverse effects induced in the non-clinical test systems.

All in vivo studies were done by oral administration, with the exception of the sensitization and dermal irritation studies. For oral dosing, TMC278 base was dissolved in polyethylene glycol 400 (PEG400) usually with 100 mg/mL citric acid (CA) to improve exposure, with the exception of the rabbit studies in which TMC278 base was suspended in 0.5% (m/v) aqueous hydroxypropylmethylcellulose (HPMC). TMC278 was suspended in 0.5% (m/v) aqueous HPMC in the mouse, rat, and dog studies. In the studies with cynomolgus monkeys, the vehicle was 1% (m/v) aqueous HPMC with 0.5% Tween 20.

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

Repeat dose toxicity studies were done in mice as (preparation for) a 3-month dose range finding carcinogenicity study, in rats for up to 6 months, in 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 as part of the assessment of the effects of TMC278 on juvenile animals. Juvenile rats were involved in a 2-week oral dosing study starting on lactation day 12. The dogs in studies up to 1-month duration are also considered immature. Since the animals were 6.5 to 8 months old at the start of these studies they were not yet sexually mature at the end of the dosing period. Reversibility (after a 1-month recovery period) of the effects of TMC278 on the high dose group was investigated in the 6-month rat study and the 1-month dog study.

The targets of toxicity of TMC278 identified in the repeat dose studies were: 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).

TMC278 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 TMC278 were detected.

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

TMC278 had similar targets or induced the same type of adverse effects in juvenile or sexually immature rats, dogs, and cynomolgus monkeys as in adult animals. The TMC278-related effects on the female genital tract and mammary glands in dogs at the end of the 1-month studies differed from the effects in longer duration studies with sexually mature animals at the end of the dosing period. The activation of ovaries in dogs treated with TMC278 in the 1-month studies led to secondary activation noticed in the other parts of the genital tract and mammary glands. These secondary effects occur normally in sexually mature animals during estrous cycle. Therefore, they are not noted as a difference between TMC278-treated and control animals in longer duration studies. However, in the 1-month study the control animals were still dormant. In the evaluation relative to control animals, the secondary effects were noted. Activation of ovaries was not induced during an 8-week study in immature cynomolgus monkeys at the age of approximately 18 months at the start of the study.

TMC278 tested negative for the potential to cause phototoxicity, skin irritation, and delayed-type hypersensitivity and to induce an immunotoxic effect on the challenge of rats with sheep red blood cells. TMC278 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 B※ and related substance C※, have been evaluated upon spiking to the drug substance at a level of 4%. Qualification comprised a bacterial reverse mutation Ames test, a mouse lymphoma assay, and a 1-month oral rat study. The presence of the impurities at 4% did not modify the effects of TMC278 in any of the tests. The third impurity, related substance A※, is the [redacted]-isomer of TMC278. This isomer was present in all drug substance batches involved in pivotal non-clinical studies at the level of minimally 0.61%. In view of the close structural relationship with TMC278 and the overage between the lowest non-clinical dose (5 mg/kg/day) and the recommended clinical dose of 25 mg q.d., separate qualification of related substance A※ is not considered relevant.

Three further (potential) impurities, related substance D※, related substance F※, and related substance G※, 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 HCl salt of related substance D※, related substance E※, and of related substance F※ and related substance G※ was tested in an Ames test. Only related substance E※ showed a mutagenic potential. So it is concluded that related substance D※ is a genotoxic impurity. The maximum allowable level of related substance D※ in a daily dose of 25 mg TMC278 was calculated to be 60 ppm, on the basis of the Threshold of Toxicological Concern (TTC) approach[1] with daily treatment for longer than 12 months. The level of related substance D※ in drug substance and drug product has been controlled at less than [redacted] pm.

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2 SINGLE-DOSE TOXICITY

Single dose evaluations were part of the initial oral dose range finding studies in rats and dogs. The single dose toxicity of TMC278 base on mice was evaluated in the bone marrow micronucleus study (see Section 4.3).

2.1 MOUSE STUDY

For a bone marrow micronucleus study[36,37], a single oral dose of TMC278 base dissolved in polyethylene glycol (PEG)400 with citric acid (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.

TMC278 did not cause any mortality. There were no abnormalities in clinical observations or relevant effects on body weight. At necropsy, no TMC278-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 ($AUC_{0\text{-}6h}$) 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. See Tabulated Summary 2.6.7.9.

2.2 RAT STUDY

The highest feasible concentration of an oversaturated solution of TMC278 base in PEG400 was 80 mg/mL, in terms of viscosity for administration by gavage to rats. This concentration was administered by oral gavage at a volume of 10 mL/kg, to 6 male and 6 female Sprague Dawley rats[2,3], 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.

TMC278 did not cause any mortality. All males and 2 out of 6 females showed salivation during the day of dosing. At necropsy, no TMC278-related gross lesions were recorded. The mean maximum plasma concretion (C_{max}) was 8.0 and 18.0 µg/mL and the $AUC_{0\text{-}\infty}$ was 86 and 233 µg.h/mL in males and females, respectively. See Tabulated Summary 2.6.7.5.

2.3 DOG STUDY

For dogs, the highest feasible concentration of an oversaturated solution of TMC278 base in PEG400 with or without CA was 100 mg/mL in terms of viscosity for the administration by gavage. The dogs were pre-treated 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[4,5]. The next day, the PEG400-pretreated animals were dosed with TMC278 base dissolved in PEG400 at a single dose of 40 or 80 mg/kg. The PEG400 + CA pretreated animals received TMC278 base dissolved in PEG400 + CA at a single dose of 80 mg/kg. The animals were observed for 4 days after dosing. No clinical pathology or necropsy was performed on these animals.

TMC278 did not cause any mortality. Only in the group dosed with 80 mg/kg dissolved in PEG400 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. See Tabulated Summary 2.6.7.5.

Table 1 Exposure to TMC278 (C_{max} and AUC) in an Oral Single Dose Study with TMC278 Base in Dogs

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

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

2.4 OVERALL CONCLUSION

Single dose toxicity of TMC278 is low. At an absorption-saturating dose (mice) and at the maximal feasible dose in rats and dogs, no significant toxicity was induced.

3 REPEAT DOSE TOXICITY

Systemic toxicity of TMC278 base or TMC278 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 embryo-fetal toxicity study. The studies in rats and dogs were designed to investigate the toxicity profile of TMC278 and to support clinical studies and marketing authorization. The reversibility upon repeat dosing was investigated in rats and dogs.

All studies in rats and dogs were conducted with TMC278 base dissolved in PEG400, usually with CA. In the rabbit study, TMC278 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 TMC278 base and TMC278. The studies in mice and cynomolgus monkeys were conducted with TMC278 suspended in aqueous HPMC. For an overview of all repeat dose toxicity studies, see Tabulated Summary 2.6.7.1. Quantitative mean effects are expressed as percentage or fold change from values of the vehicle control group unless stated otherwise.

3.1 STUDIES IN MICE

Oral studies in mice with TMC278 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 Non-Pivotal Pilot Oral Carcinogenicity Dose Range Finding Studies in Mice

3.1.1.1 2-WEEK STUDY WITH CD-1 MICE

In a 2-week study with CD-1 mice[6], TMC278 was administered by gavage or via diet in doses up to 2000 and 5000 mg/kg/day, respectively.

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

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

Hematology showed 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), alkaline phosphatase (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. See Tabulated Summary 2.6.7.6.

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

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

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

3.1.1.2 1-Month Study with Transgenic Mice

In a 1-month study, TMC278 was administered orally by gavage to transgenic CB6F1-nonTgrasH2 mice at 0 (vehicle), 20, 80, or 320 mg/kg/day[7].

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 TMC278-treated groups was noted and at 320 mg/kg/day this was associated with coagulative hepatocellular necrosis. Moreover,

degenerative/necrotic nephropathy (also at 80 mg/kg/day), lymphoid depletion in thymus, myeloid cell hyperplasia in bone marrow, erythro/myelopoiesis in spleen, almost complete absence of ovulation, and uterus atrophy occurred.

Systemic exposure expressed as C_{max} and AUC values are given in Table 3 below. See Tabulated Summary 2.6.7.6.

Table 3 Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Study with TMC278 in Mice

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

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

3.1.2 Pivotal 3-Month Oral Toxicity and Carcinogenicity Dose Range Finding Study with TMC278 in Mice

TMC278 was administered once daily by oral gavage for 3 months to CD-1 mice. Groups of 25 animals per sex (10 for the main study and 15 satellite animals for toxicokinetics) were given 0 (vehicle; 12 satellite animals per sex), 20, 80, or 320 mg/kg/day in 10 mL/kg[8]. 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 TMC278. The most prominent effect was hepatocellular hypertrophy with a dose-related increase in incidence and severity in the animals dosed with 80 and 320 mg/kg/day. In animals given the higher dose, this effect was associated with hepatocellular vacuolization, single cell necrosis, and pigmentation and proliferation of Kupffer cells, all to a slight to moderate degree. Electron microscopy revealed peroxisome proliferation in the animals dosed with 320 mg/kg/day.

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

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

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

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

Based on the findings in animals with 80 mg/kg/day in liver, the No Observed Adverse Effect Level (NOAEL) was considered to be 20 mg/kg/day. Systemic exposure expressed as C_{max} and AUC values are given in Table 4 below. See Tabulated Summary 2.6.7.7A.

Table 4 Exposure to TMC278 (C_{max} and AUC) in a 3-Month Oral Study with TMC278 in Mice

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

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

3.2 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 TMC278 base and TMC278 was done. Two 2-week studies with TMC278 base and TMC278 administered by gavage or via diet were done for the selection of the test article and the route of administration in the oral carcinogenicity study.

3.2.1 Non-Pivotal Pilot Oral Toxicity and Carcinogenicity Dose Range Finding, and TMC278 Base – TMC278 Bridging Studies in Rats

3.2.1.1 5-DAY ORAL TOLERANCE STUDY WITH TMC278 BASE

TMC278 base dissolved in PEG400 was administered once daily by oral gavage for 5 days at 0 (vehicle), 40 or 400 mg/kg/day in 8 mL/kg to 5 male Sprague Dawley rats/group[9,10].

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. See Tabulated Summary 2.6.7.6.

3.2.1.2 2-WEEK ORAL TOXICITY STUDY WITH TMC278 BASE

TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 2 weeks to Sprague Dawley rats at 0 (water), 0 (vehicle), 40, 120 or 400 mg/kg/day in 10 mL/kg[11,12]. All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with TMC278 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 thyroid stimulating hormone (TSH, up to 100%) in animals dosed with 120 and 400 mg/kg/day.

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

Systemic exposure expressed as C_{max} and AUC values are given in Table 5 below. See Tabulated Summary 2.6.7.6.

Table 5 Exposure to TMC278 (C_{max} and AUC) in a 2-Week Oral Study with TMC278 Base in Rats

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

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

3.2.1.3 2-WEEK ORAL STUDY WITH TMC278 BASE AND TMC278 BY GAVAGE AND VIA DIET IN RATS

TMC278 was administered once daily to Sprague Dawley rats by oral gavage suspended in 0.5% (m/v) aqueous HPMC at a dose of 400 mg/kg/day or by dietary administration at 400 or 1200 mg/kg/day[13]. TMC278 base was administered by diet at 400 mg/kg/day. The control group received unmedicated diet. All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with TMC278 base had additionally 6 males and 6 females for toxicokinetic analysis.

No mortality was noted during this study. There were no toxicologically relevant differences between groups dosed with TMC278 base or TMC278 at 400 mg/kg/day by gavage or through the diet.

With both forms, decreased 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 TMC278 by gavage were determined.

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

Systemic exposure expressed as C_{max} and AUC values are given in Table 6 below. See Tabulated Summary 2.6.7.6.

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

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

M: males, F: females

3.2.1.4 2-Week Pilot Oral Carcinogenicity Dose Range Finding Study with TMC278 in Rats

TMC278 suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage, for 2 weeks to Sprague Dawley rats at 0 (vehicle), 400, 1500, or 2000 mg/kg/day in 10 mL/kg[14]. All groups comprised 5 male and 5 female rats for the toxicological evaluations and the groups treated with TMC278 had additionally 3 males and 3 females for toxicokinetic analysis.

There were no mortalities in this study.

Increased activated partial thromboplastin time (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 TMC278 was noted in association with hypertrophy of follicular cells. In males given 2000 mg/kg/day, hypertrophy of follicular cells was associated with multifocal vacuolated cells in the pars distalis of the pituitary gland.

Systemic exposure expressed as C_{max} and AUC values are given in Table 7 below. See Tabulated Summary 2.6.7.6.

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

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

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

3.2.1.5 1-MONTH BRIDGING STUDY WITH TMC278 BASE AND TMC278 IN RATS

This study served to bridge the toxicity and toxicokinetics after administration of TMC278 base with those after TMC278[15]. Both compounds were administered once daily, by oral gavage to Sprague Dawley rats for 4 weeks at 0 (vehicle), 10, or 400 mg/kg/day in 10 mL/kg. TMC278 base was dissolved in PEG400 + CA and TMC278 was suspended in 0.5% (m/v) aqueous HPMC. All groups comprised 10 male and 10 female rats for the toxicological evaluations and the groups treated with TMC278 base and TMC278 had additionally 6 males and 6 females for toxicokinetic analysis.

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

Hematology showed decreased 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. See Tabulated Summary 2.6.7.6.

Table 8 Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Bridging Study with TMC278 Base and TMC278 in Rats

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

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

3.2.2 Pivotal Oral Toxicity Studies with TMC278 Base in Rats

3.2.2.1 1-Month Oral Toxicity Study Including Immunotoxicity Evaluation in Rats

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 4 weeks to groups of 18 male and 18 female Sprague Dawley rats at 0 (water, negative control), 0 (vehicle), 10, 40 or 160 mg/kg/day in 10 mL/kg[16]; 8 male and 8 female satellite animals per group were used for the plaque forming cells (PFC) assay. TMC278-treated groups had 4 more males and females for toxicokinetic analysis. Regular observations were made for clinical signs, morbidity and, mortality. Body weight, food consumption and body weight gain were determined throughout the dosing period. Ophthalmic examinations were performed prior to dosing and at the end of the dosing period (negative control, vehicle, and 160 mg/kg/day animals, only). All main animals were sampled for hematological, serum chemistry and urinalysis parameters at the end of the dosing period. The PFC satellites received on 4 consecutive days an intravenous injection with sheep 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 TMC278, relevant clinical signs, or effects on body weight or food consumption, no treatment-related ophthalmic effects and no effects on the direct PFC assay. 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 TMC278 has no immunotoxic potential. Systemic exposure expressed as C_{max} and AUC values is given in Table 9 below. See Tabulated Summary 2.6.7.7B.

Table 9 Exposure to TMC278 (C_{max} and AUC) in a 1-Month Toxicity Study of TMC278 Base in Rats

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

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

3.2.2.2 6-Month Oral Toxicity Study Including 1-Month Recovery in Rats

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 6 months to groups of 20 male and 20 female Sprague Dawley rats[17] at 0 (vehicle), 40, 120 or 400 mg/kg/day in 10 mL/kg. There were 6 satellite rats of each sex per TMC278-treated group for toxicokinetic analysis, and 10 rats of each sex in the control and high dose group for assessment of reversibility of the findings at 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 TMC278.

Increasing difficulties with the daily administration by gavage, morbidity and deaths started to occur after the first 2 months of dosing with comparable incidences in all groups including controls. For this reason, the dosing regimen was changed from once daily to 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 TMC278 - treated males of all groups without a dose-related trend, at all sampling times including the end of the recovery period. At the end of the dosing period, red blood cell count, hemoglobin, and hematocrit of males dosed with 400 mg/kg/day were reduced by 5%. The eosinophil count in TMC278-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 TMC278-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 TMC278-treated groups without a dose relationship) were noted at the end of treatment. All serum chemistry changes showed complete reversibility at the end of the recovery period.

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

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. See Tabulated Summary 2.6.7.7C.

Table 10 Exposure to TMC278 (C_{max} and AUC) in a 6-Month Oral Toxicity Study with TMC278 Base in Rats

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

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

3.3 STUDY IN RABBITS

3.3.1 5-Day Oral Dose Range Finding Toxicity Study with TMC278 Base in Rabbits

This non-pivotal study was part of the dose range finding studies for the embryo-fetal development study with TMC278 base in rabbits. For studies with pregnant rabbits see Section 6.2.2.

TMC278 base suspended in 0.5% (m/v) aqueous HPMC was administered by oral gavage for 5 days to New Zealand white rabbits[18]. 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. See Tabulated Summary 2.6.7.6.

3.4 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 TMC278 base and TMC278 was done.

3.4.1 Non-Pivotal Pilot Oral Toxicity with TMC278 Base and TMC278 Base – TMC278 Bridging Studies

3.4.1.1 5-DAY PILOT ORAL TOXICITY STUDY WITH TMC278 BASE

TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 5 days to groups of beagle dogs each comprising 1 male and 1 female at 0 (vehicle) and 80 mg/kg/day in 0.8 mL/kg, after a wash-out period of 7 days following a similar single dose[4,5].

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. See Tabulated Summary 2.6.7.5.

3.4.1.2 7-DAY PILOT ORAL TOXICITY STUDY WITH TMC278 BASE

TMC278 base dissolved in PEG400 + CA was administered once daily, by oral gavage, for 7 days to groups of 4 male beagle dogs at 0 (negative control, water), 0 (vehicle), 20, 40, or 80 mg/kg/day in 1 mL/kg[19,20].

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 TMC278. Multifocal inflammatory cell infiltration and multifocal vacuolated cells in the zona fasciculata of the adrenal glands were noted in all treated groups.

Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 11 below. See Tabulated Summary 2.6.7.6.

Table 11 Exposure to TMC278 (C_{max} and AUC) in a 7-Day Oral Toxicity Study with TMC278 Base in Dogs

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)	$AUC_{0\text{-}24h}$ (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

3.4.1.3 1-Month Bridging Study with TMC278 Base and TMC278 in Dogs

This study served to bridge the toxicity and toxicokinetics of TMC278 base dissolved in PEG400 + CA with those of TMC278 suspended in 0.5% (m/v) aqueous HPMC. Both forms were administered once daily by oral gavage to groups of 3 male and 3 female beagle dogs for 1 month at 0 (vehicle), 5, or 40 mg/kg/day in 1 mL/kg[21].

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 $AUC_{0\text{-}24h}$ values are given in Table 12 below. See Tabulated Summary 2.6.7.6.

Table 12 Exposure to TMC278 (C_{max} and AUC) in a 1-Month Oral Bridging Study with TMC278 Base and TMC278 in Dogs

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

M: males, F: females

3.4.2 Pivotal Oral Toxicity Studies with TMC278 Base in Dogs

3.4.2.1 1-Month Toxicity Study Including 1-Month Recovery in Dogs

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to groups of 3 male and 3 female beagle dogs at 0 (water, negative control), 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg[22]. To evaluate reversibility of the effects of TMC278 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 TMC278. Moreover, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology. Adrenal gland samples of the dogs of the negative and vehicle groups and the group dosed with 40 mg/kg/day were examined by electron microscopy.

No mortalities occurred in this study. Red vaginal discharge was noted in 2 out of 3 females dosed with 10 mg/kg/day and 1 out of 5 dosed with 40 mg/kg/day. Soft feces and vomiting occurred in all groups except the negative control group indicating this effect was vehicle-related rather than test article-related. There were no relevant effects on ophthalmoscopy, heart rate, ECGs, or hematology. Male dogs dosed with 40 mg/kg/day lost body weight during the first 3 weeks of dosing. Female dogs in this group did not gain body weight throughout the dosing period. These effects 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 TMC278. The increases were almost 30- and 10-fold 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 TMC278 may have a mild degenerative effect in this zone. The female genital tract showed increased activation in the groups treated with 10 and 40 mg/kg/day. In the ovaries, corpora lutea were detected in 2 animals dosed with 10 mg/kg/day and in 1 animal that received 40 mg/kg/day. This finding was associated with prominent high epithelium in the oviduct, increased glandular development in the uterus, cornification with increased thickness of the epithelium of the cervix and vagina and with active alveolar development in mammary glands. More prominent tertiary follicles were noted in all test article-treated animals that had not ovulated at the end of dosing period. In liver, minimal to moderate centrilobular perivascular inflammatory reaction was observed in males. Minimal increase in the number of multifocally dispersed centrilobular hepatocytes with a clear appearance was noted in the groups treated with 10 and 40 mg/kg/day. Moreover, a slight to moderate increase of MPS-aggregates, slight to minimal centrilobular hepatocellular single cell

necrosis and multifocal centrilobular perivascular fibrosis and minimal multifocal bile duct proliferation occurred in males and females dosed with 10 and 40 mg/kg/day.

All adverse effects that were seen at 40 mg/kg/day were completely reversible within a 1-month recovery period, except for the changes in the liver and the increased level of ALP in the serum. 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 TMC278 ranged from 1.3 to 3.6 without clear dose relationship. After 1 month of recovery, the concentration of TMC278 in adrenal gland as well as in plasma was below the limit of quantification. See Tabulated Summary 2.6.7.7D.

Table 13 Exposure to TMC278 (C_{max} and AUC) in a 1-Month Toxicity Study Including 1-Month Recovery with TMC278 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

3.4.2.2 6-Month Toxicity Study Including 3-Month Interim Evaluation in Dogs

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 6 months to groups of 6 male and 6 female beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg[23]. Regular observations were made for clinical signs, morbidity and mortality. Body weight and food consumption were recorded weekly. Ophthalmic examinations, ECG recordings, and samples for clinical pathology were taken prior to dosing, prior to 3-month interim evaluation, and at the end of the dosing period. Clinical pathology comprised a standard set of hematological, serum chemistry and urinalysis parameters. In addition, serum concentrations of 17α-hydroxyprogesterone, progesterone, cortisol, and ACTH were determined. For the determination of AUCs for ACTH and cortisol, 4 samples were taken over a 24 hour period from the first 3 male and 3 female animals in each group. Blood samples for toxicokinetics were taken toward the 3-month interim evaluation, and towards the end of the dosing period. In addition, on the day of necropsy, just prior to the sacrifice, a single blood sample and samples of liver and adrenal gland were taken for the determination of TMC278. After 3 months of treatment, the last 2 males and 2 females of each group were killed for the interim evaluation. The remaining animals were killed at the end of the 6-month treatment period. At both necropsies, gross lesions and organ weight were recorded and tissues and organs were sampled for histopathology.

No mortalities occurred in this study. The males and females dosed with 40 mg/kg/day lost body weight during the first 4 weeks of treatment. Thereafter, their weekly body weight gain was not different from that of the control animals. The effects on body weight are considered associated

with the reduction of food consumption noted in the animals treated with 40 mg/kg/day during the first 4 weeks of treatment. There were no relevant effects on heart rate, ECG, ophthalmology, hematology and urinalysis.

Serum chemistry towards 3 months of dosing showed 40 and 23% 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 TMC278 base was noted ranging from 100% in the animals dosed with 10 mg/kg/day to 4- to 5-fold in the animals dosed with 40 mg/kg/day. 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 TMC278. In the zona fasciculata and zona reticularis of the adrenal cortex, an increase of swollen cells with densely stained cytoplasm and a lower number of droplets stainable with Oil Red O was noted in the groups treated with 10 and 40 mg/kg/day, after 3 and 6 months of treatment. At the end of the dosing period, 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 TMC278-treated groups after 3 months and in the animals that received 10 and 40 mg/kg/day (100%) after 6 months of dosing. In the

majority of the animals treated with 40 mg/kg/day for 6 months, ovaries, uterus, and vagina had a swollen aspect. Histopathology showed a slight increase in the number of atretic follicles of animals treated with 10 and 40 mg/kg/day, for 3 and 6 months. In animals treated with 40 mg/kg/day 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 TMC278 for 3 and 6 months.

Given the changes seen in adrenals and ovaries at the lowest dose of 5 mg/kg/day, no NOAEL was established. Systemic exposure expressed as C_{max} and $AUC_{0\text{-}24h}$ values are given in Table 14 below. The mean adrenal gland to plasma ratios of the concentrations of TMC278 ranged from 4.1 to 8.1 over the groups without a clear dose relationship. Liver to plasma ratios of the concentrations of TMC278 ranged from 7.7 to 13. Adrenal and liver to plasma ratios did not change between Day 93 and Day 184/185 of treatment. See Tabulated Summary 2.6.7.7E.

Table 14 Exposure to TMC278 (C_{max} and AUC) in a 6-Month Toxicity Study with TMC278 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	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

3.4.2.3 12-Month Toxicity Study in Dogs

TMC278 base formulated in PEG400 + CA was administered once daily, by oral gavage, for 12 months to groups of 4 male and 4 female beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg[24]. 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 TMC278. 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 TMC278 base, throughout the dosing period. Evaluation of these hormones in females was hampered by interference of the estrous cycle but the serum concentrations showed an increased trend similar to what was evident in males. 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 TMC278. Mean adrenal gland weight was increased in animals treated with 40 mg/kg/day. The absolute increase was 23% in both males and females. The increase relative to body weight was 50% in males and 46% in females. This increased organ weight is probably associated with the (slight to moderate) increase in the size of the zona fasciculata in the adrenal cortex of 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 TMC278, 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 $AUC_{0\text{-}24h}$ values are given in Table 15 below. See Tabulated Summary 2.6.7.7F.

Table 15 Exposure to TMC278 (C_{max} and AUC) in a 12-Month Toxicity Study of TMC278 Base in Dogs

Dose (mg/kg/day)	Sampling days	C_{max} (µg/mL)		$AUC_{0\text{-}24h}$ (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

3.5 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 TMC278 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 with described in Module 2.6.4/Pharmacokinetics Written Summary/Section 3.1.6. For the repeat dose part of that study[25], the animals were habituated to restraining chairs for ECG recordings. TMC278 was administered for 15 days to 3 groups of 3 young mature cynomolgus monkeys at 0 (vehicle), 200 or 500 mg/kg/day. Recordings were made on Days 2

and 15. No abnormalities were noted on RR, PR, or QT (also corrected according to Fridericia[26]) 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.

3.5.1 8-Week Oral Toxicity Study with TMC278 in Juvenile Female Cynomolgus Monkeys

TMC278 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[27]. Regular observations of clinical signs, menses, morbidity and mortality were made. Weekly, body weights were determined. Blood samples were taken for clinical pathology including hematology, clinical chemistry, and urinalysis prior to the start of dosing and towards the end of the dosing period. Blood samples for endocrinology (progesterone, estradiol, and LH) were taken prior to the start of the dosing period and weekly prior to the TMC278 administration (at approximately the same time as the samples were taken during the dosing period). 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 TMC278-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. See Tabulated Summary 2.6.7.7G.

Table 16 Exposure to TMC278 (C_{max} and AUC) in an 8-Week Oral Study with TMC278 in Juvenile Female Cynomolgus Monkeys

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

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

3.6 OVERALL CONCLUSION

The repeat dose toxicity studies have identified a series of targets of toxicity of TMC278: 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.

4 GENOTOXICITY

Genotoxicity testing of TMC278 base or TMC278 comprised 4 non-mammalian (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.

4.1 IN VITRO NON-MAMMALIAN CELL SYSTEM

4.1.1 Ames Tests with Rat Liver S9

TMC278 base and TMC278 dissolved in dimethyl sulfoxide (DMSO; final concentration 0.1%) were tested in bacterial reverse gene mutation tests (Ames Test)[28,29,30]. The Ames tests were done, in triplicate, with 5 histidine-requiring strains of *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and TA102. On the basis of precipitation that limited scoring in range finding studies with plate incorporation and concentrations up to 5000 µg/plate, TMC278 base was tested in two main studies. Each of the studies comprised 2 independent repeat assays using the plate incorporation method up to 500 µg/plate. TMC278 was studied in 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, TMC278 base or TMC278 tested at the highest scorable concentration[1] 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. See Tabulated Summary 2.6.7.8.A, B, and C, respectively.

4.1.2 Ames Test with Human Liver S9

The metabolic pathway of TMC278 generating metabolites M30 and M31 (Figure 2) encompasses potentially reactive metabolites (epoxide) due to Michael addition at the α,β-unsaturated nitrile moiety[31] (see Figure 1and Module 2.6.4/Pharmacokinetic Written Summary/Section 5.4.1).

Figure 2 Metabolites M30 (Left) and M31 (Right)

[1] In the 2nd assay of the study with TMC278[33], the pre-incubation method was used. Under these conditions, no precipitation occurred at the highest tested concentration in the absence of S9.

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[32] and for that reason it was unclear whether the metabolic pathway with the potentially reactive metabolites had been adequately evaluated. In contrast, some batches of human liver S9 did yield M30 and M31. Moreover, M30 was detected in feces at 3% of the administered dose in the human metabolism study, TMC278-TiDP6-C119 (Module 2.7.2/Clinical Pharmacology Summary/Section 2.2). For these reasons, TMC278 base was tested in an Ames test in the presence of human liver S9[33]. TMC278 base dissolved in DMSO was tested in 4 independent assays up to 500 µg/plate: the highest scorable concentration due to precipitations. The concentration of M30 + M31 (incomplete separation in liquid chromatography) indicated that the human S9 metabolic enhancer was active. The validity of the test system was evaluated by the response of positive controls used in the absence of a metabolic enhancer upon plate incorporation or pre-incubation, separate from each of the 4 assays. In parallel, TMC278 metabolites M30 and M31, the carboxylic and hydroxyl metabolites of the α, β-unsaturated nitrile moiety of TMC278, respectively, were determined by means of radiolabel LC-MS. In the first parallel assay, only the presence of M30 and M31 could be established due to the low radioactive dose. In the subsequent parallel incubations, a higher specific radioactivity of ^{14}C-TMC278 was applied allowing the quantitative determination of the sum of M30 and M31.

It is concluded that TMC278, under conditions that it is metabolized via a pathway encompassing potentially reactive intermediates such an epoxide, did not induce a biologically significant increase of the mutation frequency. The experimental conditions in the Ames test yielded M30 + M31 at 1.4 – 2.6% of the dose applied. See Tabulated Summary 2.6.7.8.D.

4.2 IN VITRO MAMMALIAN CELL SYSTEM

TMC278 base and TMC278 dissolved in DMSO were tested in mouse lymphoma L5178Y cells[34, 35] 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 TMC278 base on the basis of precipitation that limited scoring was 500 µg/mL for a 3-hour exposure period without metabolic activation, 35 µg/mL for a 3-hour exposure period with metabolic activation, and 100 µg/mL for a 24-hour exposure period without metabolic activation. See Tabulated Summary 2.6.7.8.E.

TMC278 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. See Tabulated Summary 2.6.7.8.F.

TMC278 base and TMC278 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 CELL SYSTEM

To evaluate the clastogenic and aneuploidic effect on early forms of erythrocytes in bone marrow, TMC278 base dissolved in PEG400 + CA was administered to mice in an in vivo micronucleus test[36, 37]. Single oral (gavage) doses of 0 (vehicle control), 100, 400 and 1600 mg/kg in 20 mL/kg were administered to 10 male and 10 female CD-1 mice per group. The dose of 1600 mg/kg was the maximally feasible dose on the basis of the maximally feasible volume for a single dose study, 20 mL/kg, and a supersaturated and highly viscous solution of 800 mg/mL. Bone marrow was sampled from 5 males and 5 females per group, 24 and 48 hours after dosing. Systemic exposure in terms of C_{1h} and $AUC_{0\text{-}6h}$ was determined. A positive control group of 5 male and 5 female CD-1 mice received a single oral dose of 40 mg cyclophosphamide per kg body weight and was sampled for bone marrow 48 hours after dosing.

TMC278 base up to a single maximal feasible dose of 1600 mg/kg did not induce structural or numerical chromosome aberrations. At this dose, no bone marrow toxicity or signs of systemic toxicity were noted. See Tabulated Summary 2.6.7.9. Exposure data are presented in Table 17.

Table 17 Exposure to TMC278 (C_{1h} and AUC) in a Single Oral Dose Bone Marrow Micronucleus Study with TMC278 Base in Mice

Dose (mg/kg)	C_{1h} (µg/mL)		$AUC_{0\text{-}6h}$ (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

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